Alcohol and Alcohol-related Diseases

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Editors

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To Ana

Discovery consists of seeing what everybody has seen and thinking what nobody has thought.

Albert Szent-Györgyi

(Modified from Arthur Schopenhauer and his "Parerga und Paralipomena: kleine philosophische Schriften" 1851)

Preface

Alcohol—by which, unless otherwise specified, throughout this book we refer to ethanol—is one of the major risk factors for negative health outcomes worldwide. More than 60 alcohol-related diseases are known to date, ranging from addiction, through liver cirrhosis, to cancer. Collectively, these conditions account for mortality and morbidity that make alcohol use one of the leading preventable causes of disability adjusted life-years (DALYs) lost globally.

Despite its magnitude, the impact of alcohol use on public health is frequently ignored or even denied, by the public, policymakers, and health care professionals alike. Among the many reasons for this, some, such as commercial interests, ignorance, and stigma are not hard to understand. Others remain unclear. Irrespective of the causes, the result is a chronic underfunding of basic and translational research aimed at improving the understanding, diagnosis, and management of alcohol-related problems. The same applies to measures that would allow health care and other services to implement measures based on already available knowledge in order to benefit people affected by alcohol-related problems.

The silver lining is that the continuing neglect also creates unique opportunities for scientific advances with the potential to improve the understanding of alcohol-related disease mechanisms. These opportunities come with their own challenges, as alcohol has a plethora of effects. It distributes throughout all body fluids, organs, tissues, and cells and interacts with numerous lipids, proteins, and DNA. As a result, the potential interactions are so complex that a highly interdisciplinary approach is required to understand them. These complex interactions are also likely the reason why, despite the progress over the last 50 years, we still have a poor understanding of many alcohol-related disease mechanisms. Similarly, the epidemiology of alcohol use and its health consequences is lacking to a surprising extent.

This book project sprung from the first postgraduate course at the 18th European Association for Biomedical Studies on Alcoholism (ESBRA) held in 2021 in Timisoara/Romania. As ESBRA president, one of us, Sebastian Mueller, had the good fortune to organize this event together with Professor Ioan Sporea. This was also the first ESBRA congress in Romania, one of the European countries with a high alcohol consumption, and an almost non-existent health care system to cope

with the problems that result from it. The conference was held in the second year of the COVID-19 pandemic and was for many the only physical meeting during this time. We are grateful to Prof. Sporea and his team for organizing this ESBRA meeting. We would also like to thank Professor Lorenzo Leggio, who was instrumental in setting up the first CME-accredited postgraduate course with ESBRA.

In the present book that has resulted from this project, more than 100 renowned experts from 17 countries have contributed to covering various aspects of alcohol-related diseases, from those encountered in daily clinical practice to molecular mechanisms. The book aims to combine present knowledge from a diverse range of disciplines and covers both widely recognized clinical problems such as alcohol withdrawal, addiction treatment, and alcohol-related liver disease, as well as less well-known clinical entities, for example, alcoholic cardiomyopathy. Despite the multitude of contributions, the book is far from complete, and many questions remain open. Where answers are lacking, we have attempted to at least highlight the questions.

In using the book, we hope readers will find their way to areas outside their own existing expertise. We believe that an interdisciplinary understanding is essential in order to successfully address alcohol-related problems, and hope that the book helps improve patients care by fostering that kind of understanding. Ultimately, we hope the book can also provide inspiration to address the many problems that remain unresolved. This is why we have included several chapters with novel, unpublished data that we consider important and a large collection of original data from a prospective heavy drinking cohort in the Appendix. With a basis in science, we hope the book will inspire clinicians, scientists, and others to join us in the effort to combat the tremendous burden of alcohol-related disease.

We finally want to thank Springer Nature and their staff, especially Mrs. Melissa Morton, for developing and supporting the project over 2 years, and Raagai Priya Chandrasekaran, who was instrumental during the submission, editing, and production process. Special thanks also go to Johannes Mueller from Heidelberg for his assistance during the final stages.

Heidelberg, Germany — Sebastian Mueller
Linköping, Sweden — Markus Heilig
October 30, 2022

Contents

Abbreviations

1D-TE	One-dimensional transient elastography
2D-SWE	Two-dimensional shear wave elastography
4E-BP1	4E-binding protein 1
A	Antecedents
A2M	a2-Macroglobulin
AA	Acetaldehyde
AA	Alcoholics anonymous
AAF	Alcohol-attributable fraction
AAI	Acute alcohol intoxication
AAS	Atomic absorption spectroscopy
AASE	Alcohol Abstinence Self-Efficacy Scale
AAT	Alpha1-antitrypsin
AATD	Alpha1-antitrypsin deficiency
AB	Apoptotic bodies
ABCDE	Airway-Breath-Cardio-Disability-Exposure
ABIC	Age, serum Bilirubin, INR, and serum Creatinine score
ABIs	Alcohol Brief Interventions
Ac	Acetylation
ACC	Acetyl CoA carboxylase
ACC	Anterior cingulate cortex
ACD	Anemia of chronic disease
ACE	Addenbrooke's Cognitive Examination
ACE	Adverse childhood experiences
Acetyl-CoA	Acetyl-coenzyme A
ACLD	Advanced chronic liver disease
ACLF	Acute-on-chronic liver failure
ACLY	ATP-citrate lyase
ACM	Alcoholic cardiomyopathy
ACSL4	Long-chain-fatty-acid—CoA ligase 4
ACT	Acceptance and commitment therapy
ACTH	Adrenocorticotropic hormone

AD	Acute decompensation
ADAMTS 13	A disintegrin and metalloproteinase with thrombospondin type 1 motif 13
ADH	Alcohol dehydrogenase
ADH1B	Alcohol dehydrogenase-1B
ADH7	Alcohol dehydrogenase-7
ADHD	Attention deficit hyperactivity disorder
ADMA	Asymmetric dimethylarginine
AEAT	Acyl-coenzyme a:ethanol *O*-acyltransferase
AFD	Alcoholic foamy degeneration
AFLP	Acute fatty liver of pregnancy
AGDHA	Australian Government Department of Health and Ageing
AGM	Aorta-gonad-mesonephros
AGS	Acetaldehyde-generating system
AH	Alcoholic hepatitis
AHHS	Alcoholic Hepatitis Histological Score
AICF	Accelerated intravascular coagulation and fibrinolysis
AICR	American Institute for Cancer Research
AIH	Autoimmune hepatitis
AIM	College Alcohol Intervention Matrix
AJ	Adherens junctions
AKI	Acute kidney injury
ALD	Alcohol-related liver disease
ALDH	Aldehyde dehydrogenase
ALDH2	Aldehyde dehydrogenase-2
ALF	Acute liver failure
ALK 3	Activin receptor-like kinase 3
ALT	Alanine aminotransferase
AM	Alcohol mortality score
AML	Acute myeloid leukemia
AML	Angiomyolipomas
AM-LS	Alcohol mortality score including liver stiffness
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
AMUSE	Attenuation measuring ultrasound shear wave elastography
ANA	The Addictions Neuroclinical Assessment
ANKK	Ankyrin repeat and kinase domain
AP	Alkaline phosphatase
AP	Arterial pressure
APA	The American Psychiatric Association
APAP	Acetaminophen
APASL	The Asian Pacific Association for the Study of the Liver
ApoA1	Apolipoprotein A-1
APRI	Aspartate aminotransferase to platelet ratio index
aPTT	Activated partial thromboplastin time

ARBD	Alcohol-related birth defects
ARDS	Acute respiratory distress syndrome
ARFI	Acoustic Radiation Force Impulse (imaging)
ARK3	Activin receptor-like kinase 3
ARND	Alcohol-related neurodevelopmental disorder
ART	Antiretroviral therapy
ASAM	The American Society of Addiction Medicine
ASDR	Age-standardized death rates
ASE	The American Society of Echocardiography
ASH	Alcoholic steatohepatitis
ASMase	Acid sphingomyelinase
ASPD	Antisocial personality disorder
ASQ	Acoustic Structure Quantification™
ASR	Age standardized rate
AST	Aspartate aminotransferase
AT	Adipose tissue
ATGL	Adipose tissue triglyceride lipase
ATP	Adenosine triphosphate
AUD	Alcohol use disorder
AUDIT	Alcohol Use Disorders Identification Test
AUROC	Area under the receiver operating characteristics curve
AVP	Arginine-vasopressin
AWS	Alcohol withdrawal syndrome
AZT	Azidothymidine
BAC	Blood alcohol concentration
Bak 1	BCL2 antagonist/killer 1
BAP	British Association for Psychopharmacology
BASICS	Brief Alcohol Screening and Intervention for College Students
BasoE	Basophilic erythroblasts
BBB	Blood–brain barrier
BBV	Blood-borne virus
BC	Breast cancer
BCLC	Barcelona Clinic Liver Cancer staging system
BCN	Bile cast nephropathy
BCT	Behavior Couples Therapy
BD	Bipolar disorder
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BFU-E	Burst forming unit-erythroid
BHMT	Betaine-homocysteine-methyltransferase
BI	Brief Interventions
BI-RADS	Breast Imaging Reporting and Data System
BIS-11	Barratt Impulsiveness Scale 11
BIVAD	Biventricular assist device

BLEP	Bright liver echo pattern
BM	Bone marrow
BMI	Body mass index
BMIs	Brief motivational interventions
BMP	Bone morphogenic protein
BMPER	BMP-binding endothelial cell precursor-derived regulator
BPD	Borderline personality disorder
BR	Bilirubin
BrAC	Breath alcohol concentration
BSCT	Behavioral self-control training
BUN	Blood urea nitrogen
BZD	Benzodiazepines
CAB	Chromotrope-aniline-blue
cACLD	Compensated advanced chronic liver disease
CaMK	Calmodulin kinase
cAMP	Cyclic adenosine monophosphate
CAP	Controlled attenuation parameter
CAR	CXCL12-abundant reticular cells
CASA	Court Appointed Special Advocates
CASP2	Caspase 2
CAT	Hepatic catalase
CBA	Chronic binge alcohol
CBN	Causal Bayesian Network
CBS	Cystathionine-b-synthase
CBT	Cognitive behavioral therapy
CCDRFS	China Chronic Disease and Risk Factor Surveillance
CCL20	C-C Motif Chemokine Ligand 20
CCL4	Carbon tetrachloride
CCND1	Cyclin D1
CCND2	Cyclin D2
CD	Controlled drinking
CD	Crohn's disease
CD14	Cluster of differentiation 14
CDC	Centers for Disease Control and Prevention
CDH13	Cadherin 13
CDT	Carbohydrate-deficient transferrin
CeA	Central amygdala
CEBPA	CCAAT-enhancer-binding protein alpha
CET	Cue-exposure therapy
CFU-E	Colony-forming units-erythroid
CGAS	Candidate gene association studies
ChREBP	Carbohydrate responsive-element binding protein
CHRM2	Cholinergic muscarinic receptor
CIFASD	Collaborative Initiative of Fetal Alcohol Spectrum Disorders

CIWA-A	Clinical Institute Withdrawal Assessment for Alcohol scale and linked score
CIWA-Ar	Clinical Institute Withdrawal Assessment for Alcohol revised scale and linked score
CK	Cytokeratin
CK2	Casein kinase
CKB	China Kadoorie Biobank
CKD	Chronic kidney disease
CLD	Chronic liver disease
CLPs	Common lymphoid progenitors
CM	Contingency management
CMP	Cardiomyopathy
CMPs	Common myeloid progenitors
CMR	Cardiac magnetic resonance imaging
CNS	Central nervous system
CO	Carbon monoxide
CO_2	Carbon dioxide
COGA	The Collaborative Study on the Genetics of Alcoholism
COMT	Catechol-*O*-methyltransferase
COX-2	Cyclooxygenase-2
CPP	Child-Parent Psychotherapy
CRA	Community Reinforcement Approach
CRAFT	Community Reinforcement Approach and Family Training
CRC	Colorectal cancer
CREB	cAMP response element-binding protein
CRF	Corticotropin-releasing factor
CRH/CRF	Corticotropin-releasing hormone/factor
CRHR1	Corticotropin-releasing hormone receptor 1
CRN1	CB1 receptor protein
CRP	C-reactive protein
CS	Corticosteroids
CSPH	Clinically significant portal hypertension
CT	Computed tomography
cTBS	Continuous Theta-burst stimulation
CTF	Children's Friendship Training
CTGF	Connective tissue growth factor
CTQ	Childhood Trauma Questionnaire
CUP	WCRF/AICR Continuous Update Project
CVD	Cardiovascular disease
CVLT-C	California Verbal Learning Test–Children's Version
CVP	Central venous pressure
CXCL1	C-X-C motif chemokine ligand 1
CYP	Cytochrome P450
CYP27A1	Cytochrome P450 family 27 subfamily a member 1
CYP2E1	Cytochrome P450 2E1

CYP7A1	Cytochrome P450 family 7 subfamily a member 1
DAA	Direct acting antiviral
DAGLA	Diacylglycerol lipase
DALYs	Disability-adjusted life year
DAMPs	Damage-associated molecular patterns
DAT	Dopamine transporter
DBS	Dried blood spots
DBT	Dialectical behavior therapy
DC	Dendritic cells
DCM	Dilated cardiomyopathy
DD	Delay discounting
DGAT	Diacylglycerol acyl transferase
DGPPN	Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde
DIC	Dicarboxylate
DIC	Disseminated intravascular coagulation
DLGAP2	Discs large-associated protein 2
DLPFC	Dorsolateral prefrontal cortex
DLPFCX	Dorsolateral prefrontal cortex
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
DOACs	Direct acting oral anticoagulants
DR	Ductular reaction
DRD1	D1 dopamine receptor
DRD2	D2 dopamine receptor
DRD4	Dopamine receptor type 4
DRE	Digital rectal examination
DrInC	Drinker Inventory of Consequences
DRP1	Dynamin-related protein 1
DSD	Depression spectrum disease
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSMs	Dense surface models
DSM-V	Diagnostic and Statistical Manual of Mental Disorders
DT	Delirium tremens
DTI	Diffusion tensor imaging
DUS	Dried urine on filter paper
DUSP4	Dual specificity phosphatase 4
DUSP5	Dual specificity phosphatase 5
DVT	Deep vein thrombosis
E	Young's modulus
E/C	Excitation/contraction
EACA	Epsilon-aminocaproic acid
EACVI	The European Association of Cardiovascular Imaging
EASL	The European Association for the Study of the Liver

EASL-CLIF	The European Association for the Study of the Liver–Chronic Liver Failure
EBV	Epstein-Barr virus
ECA	The Epidemiologic Catchment Area
eCB	Endocannabinoid system
ECBL	Early change in bilirubin level
ECG	Electrocardiogram
ECHO	The Extension for Community Healthcare Outcomes
ECM	Extracellular matrix
ECs	Endothelial cells
ED	Emergency department
EDP	Epoxydocosapentaenoic
EEG	Electroencephalography
EEQ	Epoxydocosapentaenoic
EF	Ejection fraction
EFhd2	EF hand domain containing 2 gene
EFNS	European Federation of Neurological Societies
EFSUMB	European Federation of Societies for Ultrasound in Medicine and Biology
EFT	Episodic future thinking
EGF	Epidermal growth factor
EHD4	EH-domain containing 4 gene
EHPVO	Extrahepatic portal vein obstruction
EHS	Engelbreth–Holm–Swarm gel
EIA	Enzyme immunoassay
ELF	Enhanced Liver Fibrosis score
Elpho	Serum electrophoresis
EMA	The European Medicines Agency
EMDR	Eye movement desensitization and reprocessing
EMH	Extramedullary hematopoiesis
EMP	Erythro-myeloid progenitor
eNOS	Endothelial nitric oxide synthase
EPIC	European Prospective Investigation into Cancer and Nutrition study
EPO	Erythropoietin
EPs	Erythroblasts
ER	Emergency room
ER	Endoplasmic reticulum
ERAD	ER-associated degradation
ERCP	Endoscopic retrograde cholangiopancreatography
ERFE	Erythroferrone
ERP	Event-related potential
ES	Elasticity score
ESBRA	The European Society for the Biomedical Research on Alcoholism

ESCRT	Endosomal sorting complex required for transport
ESCs	Embryonic stem cells
ESPAD	The European School Survey Project on Alcohol and Other Drugs
ESPEN	The European Society for Clinical Nutrition and Metabolism
ET-1	Endothelin-1
ETC	Electron transport chain
EtG	Ethyl glucuronide
EtPa	Ethyl palmitate
EtS	Ethyl sulfate
EUS	Endoscopic ultrasound
EV	Esophageal varices
EV	Extracellular vesicles
EWAS	Epigenome-wide association study
FA	Fatty acid
FA	Fractional anisotropy
FAC	Ferric ammonium citrate
FA-CoA	Fatty acyl-CoA
FACS	Fluorescence-activated cell sorting
FAEE	Fatty acid ethyl esters
FAS	Facial photographic analysis
FASD	Fetal alcohol spectrum disorder
FASN	Fatty acid synthase
FDA	The US Food and Drug Administration
FFP	Fresh frozen plasma
FHVP	Free hepatic vein pressure
Fib4	Fibrosis 4 index
FKBP5	FK506-binding protein
FLD	Fatty liver disease
FLL	Focal liver lesion
FMF	Families Moving Forward program
fMRI	Functional magnetic resonance imaging
FNA	Fine needle aspiration
FOV	Field of view
FOXO1	Forkhead box protein O1
FPDD	Familial pure depressive disease
FPM	First-pass metabolism
FPSA	Fractioned plasma separation, adsorption, and dialysis
FSD	Face signature difference
G	Shear force
GABA	Gamma-aminobutyric acid
GABA-A	Gamma-aminobutyric acid A receptor
GABRB1	β1-containing GABAA receptor gene
GAD	Glutamic acid decarboxylase
GAG	Glycosaminoglycans

GAHS	Glasgow alcoholic hepatitis score
GAMT	Isoprenylcysteine carboxyl methyltransferase
GATA4	GATA-binding protein 4
GBD	Global Burden of Disease
GCL	Glutamate cysteine ligase
GCLC	Catalytic subunit of GCL
GCLM	Modifier subunit of GCL
GC-MS	Gas chromatography–mass spectrometry
G-CSF	Granulocyte colony stimulation factor
GDF15	Growth/differentiation factor 15
GE-XR	Gabapentin enacarbil extended-release
GGT	Gamma-glutamyl transferase
GHB	Gamma-hydroxybutyric acid
GHE	Global health estimates
GI	Gastrointestinal
GIRK	G protein-activated inwardly rectifying potassium
GIWA-Ar	Clinical Institute Withdrawal Assessment for Alcohol-Revised
Gli3	GLI family zinc finger 3
GLS	Global longitudinal strain
GLT-1	Glutamate transporter-1
Glu	Glutamate
GMPs	Granulocyte monocyte progenitors
GOT/AST	Glutamic oxaloacetic transaminase, see AST
GP	General practitioner
GPAM	Mitochondrial glycerol-3-phosphate acyltransferase
GPT/ALT	Glutamate-pyruvate transaminase, see ALT
Gpx	Glutathione peroxidase
GR	Glucocorticoid receptor
GSH	Glutathione
gs-MELD	Gene-signature plus MELD
GSR	Glutathione reductase
GSSG	Glutathione disulfide (oxidized glutathione)
GST	Glutathione *S* transferase
GWAS	Genome-wide association study
GWE	Gayet-Wernicke encephalopathy
H_2O_2	Hydrogen peroxide
HA	Hyaluronic acid
HABR	Hepatic arterial buffer response
Hb	Hemoglobin
HBSC	The Health Behavior in School-aged Children research project
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCs	HuH7 cells

HCV	Hepatitis C virus
HD	Hepatic decompensation
HDD	Heavy drinking days
HE	Heavily exposed
HE	Hepatic encephalopathy
HED	Heavy episodic drinking
HELLP	Hemolysis, elevated liver enzymes, and low platelets syndrome
HES	Hepatosplenic schistosomiasis
HETE	Hydroxyeicosatetraenoic acid
HF	Heart failure
HF	High frequency
HFD	High-fat diet
HFE	Hereditary hemochromatosis protein
HFEW	High FErritin
HG	Hyperemesis gravidarum
HGF	Hepatocyte growth factor
HGIN	High-grade intraepithelial neoplasia
HH	Hereditary hemochromatosis
HHCy	Hyperhomocysteinemia
HIF	Hypoxia-inducible factors
HIF-1α	Hypoxia-inducible factor-1α
HIV	Human immunodeficiency viruses
HL	Hodgkin lymphoma
HMGB1	High-mobility group box 1 protein
HMGCR	3-Hydroxy-3-methylglutaryl-coenzyme A reductase
HNF	Hepatocyte nuclear factor
HNF4a	Hepatocyte nuclear factor 4 alpha
HO	Heme oxygenase
HO-1	Heme oxygenase-1
Hp	Haptoglobin
HPA	Hypothalamic-pituitary-adrenal
HPCs	Hematopoietic progenitors
Hpx	Hemopexin
HR	Hazard ratio
HR	Heart rate
HRA	Health risk appraisal model
HR-EMA	High-resolution ecological momentary assessment
HRS	Hepatorenal syndrome
HSCIC	The Health and Social Care Information Centre (UK)
HSCs	Hematopoietic stem cells
HSCs	Hepatic stellate cells
HSD17B13	Hydroxysteroid 17-beta dehydrogenase 13
HSL	Hormone-sensitive lipase
Hsp90	Heat shock protein 90

Htc	Hematocrit
HV	Hepatic vein
HVPG	Hepatic venous pressure gradient
IAP	Intra-abdominal pressure
IARC	The International Agency for Research on Cancer
IBU	Inflammatory bowel disease
ICAM-1	Intercellular adhesion molecule-1
ICC	Intraclass correlation coefficient
ICD	The International Classification of Diseases
ICMT	Guanidinoacetate methyltransferase
ICP	Intrahepatic cholestasis of pregnancy
ICT	Inhibitory control training
ICU	Intensive care unit
IFG	Inferior frontal gyrus
IFN	Interferon
IgA	Immunoglobulin A
IGF	Insulin-dependent growth factor
IGF-1	Insulin-like growth factor 1
IL-1β	Interleukin-1β
IL	Interleukin
IM	Intramuscular
IMM	Inner mitochondrial membrane
IMS	Intermembrane space
iNOS	Inducible nitric oxide synthase
INR	International normalized ratio
IPD	Interpupillary distance
IPTs	Impulsive personality traits
IQ	Intelligence quotient
IQR	Interquartile range
IQR/M	Interquartile range/median
IREs	Iron-responsive elements
IRF3	Interferon regulatory factor 3
IRI	Ischemia/reperfusion injury
IRP1/2	Iron-responsive proteins1/2
IRPs	Iron-responsive proteins
ISI	International sensitivity index
iTBS	Intermittent Theta-burst stimulation
IV	Intravenous
IWHS	Iowa Women's Health Study
JEC	Japan esophageal cohort study
KALRN	Kalirin RhoGEF kinase
KCC	King's college criteria
KCs	Kupffer cells
KDIGO	Kidney Disease Improving Global Outcome criteria
Ki	Inhibitor affinity constant

Km	Michaelis constant
KS	Korsakoff's Syndrome
LAI	Long-acting injectable
LAMP1/2	Lysosomal-associated membrane protein 1/2
LBP	Lipopolysaccharide-binding protein
LC	Long-term negative consequences
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LD	Lieber-DeCarli diet
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LDLT	Living donor liver transplantation
LEV	Large esophageal varices
LF	Low frequency
LGE	Late gadolinium enhancement
LGIN	Low-grade intraepithelial neoplasia
LIC	Liver iron concentration
LME	Liver microenvironment
LMWHs	Low molecular weight heparins
LPC	Liver progenitor cells
LPL	Lipoprotein lipase
LPS	Lipopolysaccharide
LR	Likelihood ratio
LS	Liver stiffness
LSEC	Liver sinusoidal endothelial cells
LSM	Liver stiffness measurement
LSPS	LS–spleen diameter to platelet ratio score
LTCs	Long-term conditions
LTD	Long-term depression
LTP	Long-term potentiation
LTs	Leukotrienes
LTX	Liver transplantation
LV	Left ventricle
LVAD	Left ventricular assist device
LVP	Large volume paracentesis
LXR	Liver X receptor
M cells	Microfold cells
MA	Maximum amplitude
MAG	Monoacylglycerol
MAL	MyD88 adaptor-like
MAMPs	Microbial-associated molecular patterns
MAMs	Mitochondria-associated membranes
MAOA	Monoamine oxidase A
MAP	Mean arterial pressure
MAPK	Mitogen-activated protein kinase
MARS	Molecular adsorbent recirculating system

MAT	Methionine adenosyl transferase
MBI	Mindfulness-based interventions
MBOAT7	Membrane-bound *O*-acyltransferase domain-containing protein 7
MBOAT7/TMC4	Membrane-bound *O*-acyltransferase domain containing protein 7-Transmembrane channel-like 4
MBRP	Mindfulness-based relapse prevention
MBS	The Mind, Body, and Spirit program
MCCS	Melbourne Collaborative Cohort Study
MCP-1	Monocyte chemoattractant protein-1
MCRS	The Medical Condition Regard Scale
MCV	Mean corpuscular volume of erythrocytes
MD	Mean diffusivity
MD2	Myeloid differentiation factor 2
MDB	Mallory-Denk bodies
MDD	Major depressive disorder
MDF	Maddrey's discriminant function
MDFT	Multidimensional family therapy
MDS	Myelodysplastic syndrome
Me	Methylation
MELD	Model for end-stage liver disease
MEOS	Microsomal ethanol oxidizing system
MEPs	Megakaryocyte-erythroid progenitors
MET	Motivational enhancement therapy
MetS	Metabolic syndrome
Mfn1/2	Mitofusin 1 and 2
mGAHS	Modified GAHS
mGSH	Mitochondrial GSH
MI	Motivational interviewing
MI/MET	Motivational interviewing/motivational enhancement
MILE	The Math Interactive Learning Experience
miR	MicroRNAs
miRNAs	MicroRNAs
MLC	Myosin light chain
MLCK	Myosin light chain kinase
MMI	Multimodality imaging
MMPs	Matrix metalloproteinases
MMSE	Mini Mental State Examination
MOBC	Mechanisms of behavior change
MORE	Mindfulness-Oriented Recovery Enhancement
mPFC	Medial prefrontal cortex
MPPs	Multipotent progenitors
MR	Magnetic resonance
MR	Mendelian randomization
MR	Mineralocorticoid receptor

MRE	Magnetic resonance elastography
MRI	Magnetic resonance imaging
MRI-PDFF	Magnetic resonance imaging proton density fat fraction
MRPs	Multidrug resistance-associated proteins
MRS	Magnetic resonance spectroscopy
MS	Mass spectrometry
MS	Methionine synthase
MSCs	Mesenchymal stem cells
MSP	Mitochondria-shaping proteins
MT SAM	S-adenosylmethionine-dependent methyltransferase
mtDNA	Mitochondrial DNA
MTHF	N5-methyltetrahydrofolate
MTHFR	5,10 Methylenetetrahydrofolate reductase
mTOR	Mammalian target of rapamycin
MTP	Microsomal triglyceride transfer protein
MUP	Minimum unit price
MuRF1	Muscle ring finger 1
MVBs	Multivesicular bodies
MYC	MYC proto-oncogene
MyD88	Myeloid differentiation primary response 88
Myf5	Myogenic factor 5
NAC	*N*-acetyl-L-cysteine
NAcc	Nucleus accumbens
NACSELD	The North American Consortium for the Study of End-Stage Liver Disease
NAD+	Nicotinamide adenine dinucleotide
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NBI	Narrow-band imaging
NCOA4	Nuclear receptor coactivator 4
NCS	National Comorbidity Survey
NCTSI	The National Child Traumatic Stress Initiative (US)
NDE	Non-drinker equivalence
NE	Norepinephrine
NESARC	US National Epidemiologic Survey on Alcohol and Related Conditions
NF	Nuclear factor
NFATc4	Nuclear factor of activated T cells 4
NFS	NAFLD fibrosis score
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGS	Next-generation sequencing
NHDD	No heavy drinking days
NHL	Non-Hodgkin lymphoma
NHS	National Health Service

NIAAA	The US National Institute on Alcohol Abuse and Alcoholism
NIBS	Non-invasive brain stimulation
NICE	National Institute for Health and Care Excellence
NITs	Non-invasive tests
NLCS	Netherlands Cohort Study
NLR	Neutrophil-to-lymphocyte ratio
NLR	NOD-like receptors
NMDA	Glutamate receptor
NMDA	*N*-methyl-D-aspartate
NMDA	*N*-methyl-D-aspartic acid
NO	Nitric oxide
NOD	Nucleotide-binding oligomerization domain
NODDI	Neurite orientation dispersion and density imaging
NOP	Nociceptin
NOS	Nitrogen oxygen species
NOX	NADPH-dependent oxidase
NP	Not provided
NPV	Negative predictive value
NR3C1	Glucocorticoid receptor
Nrf-2	Nuclear factor erythroid 2-related factor 2
NSBB	Non-selective betablockers
NSCs	Neural stem cells
NSMM	Non-selective betablocker
NTA	Nano-tracking analysis
NT-proBNP	N-terminal fragment in the prohormone brain natriuretic peptide
O_2	Oxygen
OCA	Obeticholic acid
OCDS	Obsessive Compulsive Drinking Scale
OEA	Oleoylethanolamide
OFC	Occipital frontal circumference
OFC	Orbitofrontal cortex
OGC	2-Oxoglutarate, SLC25A11
ÖGPB	Österreichische Gesellschaft für Neuropsychopharmakologie und Biologische Psychiatrie
OMM	Outer mitochondrial membrane
ONS	UK Office for National Statistics
Opa-1	Optic atrophy 1
OPRL1	Opioid receptor like-1
OPRM1	U-opioid receptor
OR	Odds ratio
OrthoE	Orthochromatic erythroblasts
OUD	Opioid use disorder
OXPHOS	Oxidative phosphorylation
OXTR	Oxytocin receptor

OZALC	The Australian twin-family study of alcohol use disorder
PA	Palmitic acid
PAE	Prenatal alcohol exposure
PAFs	Population attributable fractions
PAI-1	Plasminogen activator inhibitor-1
PAMPs	Pathogen-associated molecular patterns
PATHS	Promoting Alternative THinking Strategies curriculum
PBC	Primary biliary cirrhosis
PCA	Principal component analysis
PCAP	The Parent-Child Assistance Program
PCC	Posterior cingulate cortex
PCD	Probe-to-capsule distance
PCF	Pericellular fibrosis
PCs	Principal components
PD	Personality disorder
PDE10	Phosphodiesterase-10
PDE3B	Phosphodiesterase-3B
PDE4	Phosphodiesterase-4
PDEI-5	Phosphodiesterase inhibitors type 5
PDFF	Proton density fat fraction
PDGF	Platelet-derived growth factor
PDGF-BB	Platelet-derived growth factor BB
PDMS	Polydimethylsiloxane
PE	Pulmonary embolism
PECR	Peroxisomal trans-2-enoyl-CoA reductase
PEG3	Paternally expressed gene 3
PEL	Parenchymal extinction lesion
PEMT	Phosphatidylethanolamine methyltransferase
PET	Positron emission tomography
PEth	Phosphatidylethanol
PEth-NET	The Society of Phosphatidylethanol Research
PFAS	Partial fetal alcohol syndrome
PFC	Prefrontal cortex
PFL	Palpebral fissure length
PGA	Prothrombin time, gamma-glutamyl transpeptidase and apolipoprotein AI score
PGs	Prostaglandins
PH	Portal hypertension
PHD	Prolyl hydroxylase domain-enzymes
PheWAS	Phenome-wide association studies
PHH	Primary human hepatocytes
PHLF	Posthepatectomy liver failure
PHZ	Phenyl hydrazine
PIAS1	Protein inhibitor of activated STAT 1
PIMT	Protein L-isoaspartate methyltransferase

PIN1	Peptidyl-prolyl cis/trans isomerase
PLP	Pyridoxal phosphate
PLWH/PLWHA	People living with HIV/AIDS
PMAIP1	Phorbol-12-myristate-13-acetate-induced protein 1
PMN	Polymorphonuclear neutrophils
PMP	Pyridoxamine phosphate
PNPLA3	Patatin-like phospholipase domain-containing-3 or Adiponutrin
PolyE	Polychromatophilic erythroblasts
POR	Cytochrome P450 oxidoreductase
PP	Periportal
PP1	Protein phosphatase 1
PPAC	Pooling Project on Alcohol and Cancer
PPARG	Peroxisome proliferator-activated receptor gamma
PPARs	Peroxisome proliferator-activated receptors
PPARγ	Peroxisome proliferator-activated receptor-γ
PPM1G	3′-Protein-phosphatase-1G
PPRE	Proliferator-activated receptor response element
PPV	Positive predictive value
PREMs	Patient-reported experience measures
PRMT	Protein arginine methyltransferase
PRO-C3	Precursor of Type III collagen
ProE	Proerythroblasts
PROMs	Patient-reported outcome measures
PRRs	Pattern recognition receptors
PRS	Polygenic risk score
Prx	Peroxiredoxin
PSA	Prostate-specific antigen
PSC	Primary sclerosing cholangitis
ps-MELD	Plasma-signature MELD
pSWE	Point shear wave elastography
PTSD	Post-traumatic stress disorder
PUMA	p53 upregulated modulator of apoptosis
PUP	Parents under pressure
PV	Perivenous
PVA	Polyvinyl alcohol hydrogels
PVP	Portal vein pressure
PVT	Portal vein thrombosis
PWAS	Proteome-wide association studies
PYLL	Potential years of life lost
qEEG	Quantitative electroencephalography
QIBA	Quantitative imaging biomarkers alliance
R	Response
RA	Retinoic acid
RAAS	The renin-angiotensin-aldosterone system

RASGRF2	Ras-specific guanine nucleotide-releasing factor 2 gene
RAW	Benzodiazepine-resistant alcohol withdrawal
RBC	Red blood cell
RCC	Renal cell carcinoma
RCT	Randomized controlled trials
REDD1/REDD2	Regulated in development and DNA damage responses
Reg3	Regenerating islet-derived protein 3
RF	Radio frequency
RIMP	Right Ventricular Index of Myocardial Performance
RIPK	Receptor-interacting protein kinase
RLMS-HSE	The Russian Longitudinal Monitoring Survey conducted by the Higher School of Economics
RNS	Reactive nitrogen species
ROC	Receiver operating characteristic
ROI	Region of interest
ROS	Reactive oxygen species
ROSC	Recovery-orientated system of care
RosStat	Federal State Statistics Service (Russia)
ROTEM	Rotational thromboelastometry
RP	Relapse prevention
RR	Relative risk
RRM2	Ribonucleotide reductase regulatory subunit M2
RSU1	Ras suppressor 1
rTMS	Repetitive transcranial magnetic stimulation
RTS	Room-temperature susceptometry
RV	Right ventricular
RVEF	Right ventricular ejection fraction
S6K1	S6 kinase 1
SAAF	Strong African American Families
SAGE	The Study of Addiction: Genetics and Environment
SAH	*S*-adenosylhomocysteine
sAH	Severe alcoholic hepatitis
SAHH	*S*-adenosylhomocysteine hydrolase
SALVE	The Consortium for the Study of Alcohol-related Liver Disease in Europe
SAM	*S*-adenosylmethionine
SAMHSA	Substance Abuse and Mental Health Services Administration
SAMSA	The Substance Abuse and Mental Health Services Administration (US)
SBP	Spontaneous bacterial peritonitis
SC	Short-term rewarding consequences
SCC	Esophageal squamous cell carcinoma
SCD	Sudden cardiac death
SCEs	Sister chromatid exchanges
SCN	Structural covariance network

SCs	Satellite cells
SD/M	Standard deviation/mean ratio
SDR	Standardized death rate
SE	Strain elastography
SEEDS	Strategies for Enhancing Early Development Success Program
SFS	SALVE fibrosis stages
SHT	Systemic hypertension
SI	Strain index
SIRS	Systemic inflammatory response syndrome
SIRT1	Sirtuin 1
SIV	Simian immunodeficiency virus
SKM	Skeletal muscle
SL	Spleen length
SLC1A1	Solute carrier family 1 member 1
SLC25A11	2-oxoglutarate, OGC
SLC6A3	Dopamine transporter
SLC6A4	Serotonin transporter
SMART	Self-Management and Recovery Training
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SOFA	Sequential organ failure assessment
SoHT	Society of Hair Testing
SP	Sinusoidal pressure
SP-D	Surfactant protein D
SPDEF	Sterile alpha motif/pointed domain containing the ETS transcription factor
SPH	Sinusoidal pressure hypothesis
SPM	Specialized pro-resolving mediators
SPSS	Spontaneous porto-systemic shunts
SQUID	Superconducting quantum interference device
SR	Sarcoplasmic reticulum
SR	Strain ratio
SREBP-1	Sterol regulatory element-binding protein-1
SREBP-1c	Sterol regulatory element-binding protein-1c
SREBPs	Sterol regulatory element-binding proteins
SS	Spleen stiffness
SSM	Spleen stiffness measurement
SSRIs	Selective serotonin reuptake inhibitors
StARD1	Steroidogenic acute regulatory protein 1
Stat3	Signal transducer and activator of transcription 3
STE	Speckle tracking echocardiography
SU	Standard unit
SUD	Substance use disorders
SVR	Sustained viral response

SWE	Shear wave elastography
SWI	Shear wave imaging
SWS	Shear wave speed
SWV	Shear wave velocity
TACE	Transarterial chemoembolization
TANK	TRAF family member-associated NF-κB activator
TAPSE	Tricuspid annular plane systolic excursion
TAU	Treatment as usual
TB	Temperance board
TBS	Theta-burst stimulation
TDI	Tissue Doppler Imaging
TE	Transient elastography
TEG	Thromboelastography
Tf	Serum transferrin
TF	Transcription factors
TFEB	Transcription factor EF
TfR1/2	Transferrin receptor 1/2
TG	Triglycerides
TGF	Transforming growth factor
TGF-ß	Transforming growth factor β
TGT	Thrombin generation test
THE	Time-harmonic elastography
TICs	Tumor-initiating stem-cell-like cells
TIMPs	Tissue inhibitors of metalloproteinases
TIPS	Transjugular intrahepatic portosystemic shunt
TIR	Toll-interleukin-1 receptor
TIRAP	Toll-interleukin-1 receptor domain containing adaptor protein
TJ	Tight junctions
TJLB	Transjugular liver biopsy
TkrB	Tropomyosin receptor kinase B
TLR4	Toll-like receptor 4
TLRs	Toll-like receptors
TM6SF2	Transmembrane 6 superfamily member 2
TME	Transient micro-elastography
TMREL	Theoretical minimum risk exposure level
TNF	Tumor necrosis factor
TNFAIP3	TNFα-induced protein 3
TNF-R1	Tumor necrosis factor receptor 1
TNFα	Tumor necrosis factor α
TOF	Time of flight
t-PA	Tissue plasminogen activator
TPH1	Tryptophan hydroxylase 1
TPO	Thrombopoietin
TRALI	Transfusion-related acute lung injury
TRAM	TRIF-related adaptor molecule

TRIF	TIR-domain-containing adapter-inducing interferon-β
Trp	Tryptophan
TRUS	Transrectal ultrasonography
Trx2	Thioredoxin2
TSF	12-step facilitation
TSPO	Translocator protein
TTE	Transthoracic echocardiography
UADT	Upper aero digestive tract
UAT	Upper aerodigestive tract
UC	Ulcerative colitis
UGT	UDP glucuronosyltransferase
UKAT	United Kingdom Alcohol Treatment Trial
UNODC	The United Nations Office on Drugs and Crime
UNOS	The United Network for Organ Sharing
UPP	Ubiquitin proteosome pathways
UPPS	UPPS-P impulsive behavior scale
UPR	Unfolded protein response
US	Ultrasound
USCS	Urge specific coping skills training
USE	Ultrasound-based elastography
VA	Veterans affairs
VCTE	Vibration-controlled transient elastography
Vd	Volume of distribution
VDAC	Voltage-dependent ion channel
VEGFA	Vascular endothelial growth factor A
VHL	Von Hippel-Lindau protein
VKA	Vitamin K antagonists
VLDL	Very low-density lipoprotein
VMPFC	Ventromedial prefrontal cortex
VPS4A	Vacuolar protein sorting-associated protein 4a
VR	Virtual reality
VS	Ventral striatum
VTA	Ventral tegmental area
VTE	Venous thromboembolism
VTI	Virtual Touch™ Imaging
VTQ	Virtual Touch™ Quantification
vWF	Von-Willebrand factor
WAT	White adipose tissue
WBC	White blood cells
WCRF	World Cancer Research Fund
WE	Wernicke's encephalopathy
WES	Whole-exome sequencing
WFSBP	World Federation of Societies of Biological Psychiatry
WFUMB	World Federation for Ultrasound in Medicine and Biology
WGS	Whole-genome sequencing

WHO mhGAP	WHO Mental Health Gap Action Programme
WHO	World Health Organization
WHOQOL-BREF	World Health Organization Quality of Life measure
WHVP	Wedged hepatic vein pressure
WISC IV	Wechsler Intelligence Scale for Children IV
Wisp1	Wnt-inducible signaling pathway protein 1
WKS	Wernicke-Korsakoff Syndrome
WM	White matter
ZEB2	Zinc finger E-box binding homeobox 2
ZO-1	Zonula occludens 1
ΣFAEE	FAEE concentration sum

Part I
Alcohol Consumption: Epidemiology, Policies and Legal Aspects

Chapter 1
Alcohol and Alcohol-Related Diseases: An Introduction to the Book

Sebastian Mueller

Abstract Alcohol (ethanol) is a major health risk worldwide that causes more than 60 diseases leading to three million deaths per year. This article introduces "Alcohol and alcohol-related diseases", a book that covers all aspects of alcohol and alcohol-related diseases from epidemiology to alcohol use disorders, alcohol-related liver disease or cancer. It is divided into 14 parts with contributions from more than 100 authors from 17 countries. Besides current diagnostic measures and treatment strategies, the book deals with the many underlying molecular mechanisms of alcohol toxicity including the genes that lead to addiction and disease. Novel data include first prospective data on all-cause mortality, the emerging major role of red blood cell turnover by alcohol, and fundamental links between basic energy metabolisms, alcohol, and addiction. The enormous level of complexity and interactions associated with alcohol metabolism should stimulate a very much needed interdisciplinary cooperation among clinicians, scientists, and addiction specialists. In our opinion, only such a holistic approach will allow to apply the emerging potentials of OMICS and genetics more efficiently. The world needs more institutional and societal efforts to improve and integrate not only the care of alcohol-related diseases but also the funding of topic-specific research. This book also aims at guiding policy makers to handle the topic of alcohol in our society more responsibly by pricing and legislation, which certainly are the most important measures to help decrease mortality and suffering from alcohol-related diseases.

Keywords Alcohol · Addiction · Alcohol-related disease · Alcohol-related liver disease · Alcohol-related liver disease · Addiction · Alcohol dependence · Liver cirrhosis · Mortality · Acetaldehyde · Energy metabolism

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About the Book

Alcohol is a disease agent that affects many, if not all human organ systems. These include brain and liver, which are at the center of alcohol-related disease processes. Due to genetic and environmental risk factors, alcohol use for a significant minority of users transitions from a controlled habit to what is called alcoholism, alcohol addiction, alcohol dependence or (moderate—severe) **alcohol use disorder (AUD).** Key among clinical manifestation of this condition is the emergence of continued use despite knowledge of negative consequences, or "compulsive use". This in turn feeds back on the brain itself, further promoting a progression of pathology that affects both the brain itself, and other organs.

Both in research and in clinical practice, the alcohol-related pathology of peripheral organs receives much less attention than it should given its contribution to global disease burden. In contrast, the view that alcohol addiction is a brain disease, although accepted by the majority of the neuroscience community, remains questionable. It is often claimed that a brain disease view fails to account for high rates of spontaneous recovery, places too much emphasis on a compulsive use, and has failed to identify specific neural signatures of alcohol addiction. While some of these criticisms have merit, the premise that alcohol addiction is a brain disease is no less reasonable than a disease view of other complex disorders, such as diabetes, asthma or hypertension.

Alcohol still is one of the major health risks worldwide, and it causes many diseases and cancer. Despite this major role in global morbidity and mortality, research on alcohol-related diseases is chronically underfunded. Research should be aimed at improving early diagnosis, the assessment of individual risks to develop these diseases, an understanding of their molecular mechanisms and, hence, therapeutic measures. This book project is an effort to provide an update on the health consequences of alcohol, the most important alcohol-mediated diseases, and our present understanding of the underlying mechanisms. This effort is far from being complete, despite the fact that more than 100 authors from 17 countries have contributed. Their motivation primarily originates from the devastating health consequences of alcohol, its effect on unborn life with irreversible consequences even for next generations. The idea of the book has originated from the first ESBRA post graduate course with the same name, held 2021 in Timisoara, Romania, one of the few physical conferences organized during the COVID pandemic that started early in 2020. The book covers in detail alcohol-related aspects such as epidemiology, addiction, liver disease and cancer, but also topics such as alcoholic cardiomyopathy, neurological disorders, etc.

The reasons why alcohol has evolved as a legal drug in most societies are manifold. First, it is colorless and almost tasteless. However, often overlooked, the intended effects of pleasure and relaxation are obtained immediately while most negative health effects occur usually over a long period of time and typically

without any pain. A good example is liver cirrhosis that takes 15–20 years to develop. While the slow death in cirrhotic patients is a great suffering for the families and the close environment, the patients themselves are less aware of it. The necklace of all of those reasons creates a special form of denial at the level of the individual, institutions and the society that prevents a systemic and efficient discourse about alcohol-related diseases, their research support and an efficient implementation of preventive measures. And finally, the research on alcohol-related diseases is challenging since it is confronted with a special degree of complexity that requires a tight association with patients and chronically underfinanced health care systems.

This book is also a social platform among scientists and clinicians. We also do not agree with certain claims that books may not be needed anymore in the era of internet and digital medical databases. A book is still a unique and special form of an intellectual exchange and confrontation. This is also the reason why we did not strictly homogenize content, language and conclusions but rather allowed different opinions, sometimes even contradictory views, so that a certain diversity is maintained in order to inspire for new thoughts. We especially hope that, in the long run, the book may motivate for cross-reading in order to obtain insights from other disciplines.

How Is the Book Structured?

In this book, we have enlisted leading international experts to provide a broad coverage of the scientific and clinical state-of-the-art in the field of alcohol-related diseases. Alcohol dependence, its diagnosis, therapy, and underlying mechanisms, is covered by 26 chapters. We start by introducing the brain disease view of addiction and discuss why spontaneous remission in some individuals does not negate this view, and how seemingly compulsive alcohol use can co-exist with partially preserved sensitivity to healthy rewards. We connect the dots by pointing to subsequent chapters that review behavioral as well as neuropsychopharmacological treatments, which clearly show that the brain is the biological substrate from which both alcohol addiction and the capacity for behavior change arise.

A major body of the book addresses all aspects of the liver, the major ethanol-metabolizing organ, damage to which is responsible for most of the death rates linked to alcohol. This includes diagnostic and screening aspects, pathophysiology and therapy. **Liver elastography** has certainly evolved over the last two decades as a breakthrough in the early diagnosis of liver fibrosis and cirrhosis. One part alone is devoted to the rare but often fatal alcoholic hepatitis. The book also has chapters on less frequently discussed alcohol-related diseases such as those affecting the heart and skeletal muscle or neurological disorders such as the Wernicke-Korsakov encephalopathy. There are also highly praxis-oriented chapters that discuss the

clinical management of acute alcohol-related conditioned encountered in the emergency room, alcohol detoxification, but also long-term integrative management of alcohol use disorders.

The book does not only provide state-of-the-art knowledge but also aims at identifying open questions and controversial aspects and it also includes novel, unpublished data. Consequently, it provides a platform in order to stimulate discussions and initiate novel interdisciplinary endeavors. For these reasons, an **Appendix** has been included that contains general fact sheets about alcohol and biochemical schemes that are essential to the understanding of ethanol metabolism and pathology. The Appendix also contains a patient case with questions and answers and original data from the prospective Heidelberg study cohort of heavy drinkers which not only includes patient characteristics but also mortality data and correlations analyses. We strongly believe that people and society have the right to know whether they are at risk and what the early symptoms or measures are to diagnose and prevent alcohol-related disease. The freedom to distribute and consume legally a potentially harmful drug such as alcohol should be matched with a responsibility to protect those who are at special risk for genetic reasons, or children and, especially, unborn life.

Where Does Alcohol Research Come From?

Although the ability to produce alcoholic beverages as well as the knowledge about negative side effects are known since human ancestry, scientific based mechanisms have taken a long path. The relation between alcohol and liver cirrhosis became obvious, at least to a few clinicians and scientists, in the nineteenth century. Clear statistics were established in the middle of the twentieth century. The harmful effects of alcohol have been continuously perturbated by diluting arguments of either other contents of alcoholic beverages, or other conditions such as malnutrition. However, for about 50 years, it can be considered as established that alcohol itself causes diseases such as liver cirrhosis, one of the key pathologies related to alcohol-caused premature death [1].

A blossoming of alcohol research was reached between the 1960–80ies. In some countries, institutions were founded such as the NIAAA (National Institute on Alcohol Abuse and Alcoholism) in the USA to specifically provide grant money for basic and applied studies related to alcohol. Activities later declined for reasons that are not completely clear or have not been intensively debated. One reason is, besides a decreased interest of society, most likely the enormous complexity of alcohol interactions within the human organism (Fig. 1.1). To understand alcohol related disease mechanisms, the various organizational levels have to be addressed, from the biochemical, the subcellular, cellular and the organ level and all potential interactions between these levels have to be taken into account. However, almost in parallel, biomedical science and clinical institutions have become more specialized in the 80ies. Psychiatry and internal medicine started to divide into subspecialties

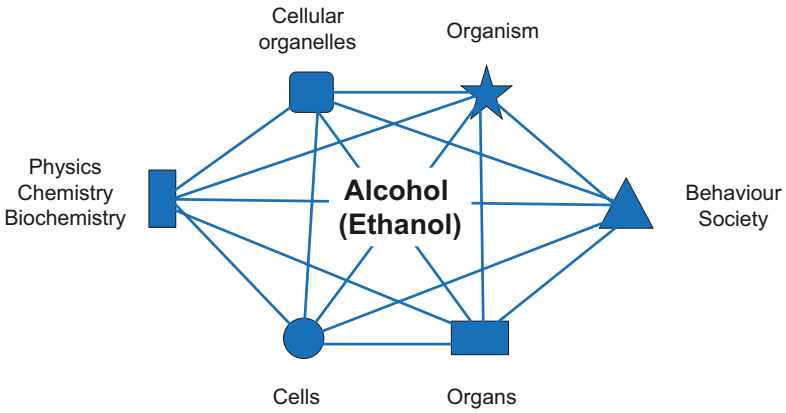

Fig. 1.1 Multiple interactions of ethanol at various organizational levels in humans

such as cardiology, gastroenterology, nephrology and so on. These structural changes started to compartmentalize medicine, making it more and more difficult to provide sufficient funding for such highly interdisciplinary research as is required for alcohol-related diseases.

One of the other specific aspects of alcohol in society is its strong association with cultural, religious, and social activities. Over centuries this has led to the development of an economy that profits from alcohol production. Moreover, for ages, governments have and still benefit through taxation from alcohol production, generating a special societal mélange of resistance and denial about alcohol-related negative sides. A famous example is the German sparkling wine tax from 1907, initiated by the German emperor at the time to finance his dream of a German fleet that could match the Royal navy. More than 100 years ago, while in Germany the governmental system changed from monarchy to chaos, from dictatorship to democracy, the sparkling wine tax still exists. Certainly, the catastrophic failure of the prohibition from 1920 to 1933 in the USA, a nationwide constitutional law that strictly prohibited the production, importation, transportation, and sale of alcoholic beverages, has engraved the certainty, that a simple stop of alcohol production is not a realistic scenario. Consequently, humans have lived, live and will live with alcohol.

A Few Words About Alcohol-Related Diseases and Standardized Terminology

There has been an intensive debate on whether certain terms such as “alcoholic” or “alcoholism” are pejorative, and discriminate patients [2]. The increasing knowledge about alcohol and addiction has shown a more diverse picture urging a need to standardize terminology to better describe pathologies both important for diagnosis and treatment, but also for research and clinical studies. Thus, the WHO stopped

using the term “Alcoholism” in 1979 because it did not describe the huge variety of alcohol-related use disorders and “Alcohol-use disorder” (AUD) was proposed instead.

However, alcohol-related terms are so deeply and long-time involved with languages in all cultures that, despite the long fight for language reform, it remains challenging. There are also serious counterarguments that should not be ignored. For instance, no terminology will survive, if it is not understood and practiced. As an example, the term “alcoholism” appears in the database “Pubmed” for the first time in 1939. It then peaks 1980 with 305 quotations, while the term “alcohol-use disorder” appears first in 1991. In 2021, “alcohol-use disorder” was used 505 times, compared to “alcoholism” with 50 times. When, however, looking at “practiced” language, e.g., in open source databases such as Wikipedia, the results are sobering. The term “alcoholism” is used by “Wikipedia” in 101 languages (August 2022) while the term “Alcohol-use disorder” has not even a single entry. Only “Substance use disorder (SUD)” is used in 10 languages with “alcohol-use disorder” only being briefly mentioned.

One could also question whether the term “alcoholism” or “alcoholic” is indeed pejorative or whether this is a phenomenon of some specific languages or cultures. We cannot uniformly share the observation that linking a “disease-causing” condition to a disease is discriminating, pejorative or not accepted by patients. Rather, it is our observation that heavy drinkers, normally subsumed within the category “alcoholic”, will have no problem in reporting their alcohol history. It can be easily agreed with the broader knowledge today that the term alcoholism is not strictly scientific, but it is also a common observation that most people in different cultures will immediately understand what “alcoholism” means. It is also quite clear that “alcoholism” may only describe a smaller cohort of heavy drinkers among “alcohol-use disorders”. More confusing, even the International Code of Diseases (ICD) still officially uses terms such as “alcoholic liver damage” (E860.0), “alcoholic polyneuropathy” (357.5), “alcoholic cardiomyopathy” (425.5), “alcoholic gastritis” (535.30, 535.31), “alcoholic fatty liver “(571.0) or “acute alcoholic hepatitis” (571.1).

Therefore, it seems that the debate will continue, and it will finally be the people and patients who will decide which language is most appropriate for respect, clarity and common use.

As a consequence, in this book, we have made efforts to mostly use the novel terminology, especially in the addiction field such as AUD and we have also tried to generally use “alcohol-related liver disease” for ALD. To avoid confusion, however, we have left the commonly used terminology for more rare entities such as “alcoholic cardiomyopathy” or “alcoholic hepatitis”.

Finally, gastroenterology or hepatology societies such as EASL, AASLD or UEG are now also pushing forward to change the term “alcoholic liver disease” to alcohol-associated liver disease or alcohol-related liver disease (ALD). The discussion is of certain relevance as ALD is the major cause of all-cause death in those who consume alcohol. Moreover, similar to the discussion above on AUD, a recent debate on non-alcohol-related liver disease (NAFLD) has emerged that considers the term

"non-alcohol" has negative stigma. It has resulted in the novel terms MAFLD (metabolic-associated fatty liver disease) [3] or **metabolic dysfunction associated steatotic liver disease (MASLD)** by AASLD. However, given the confusion and the still poor understanding of the underlying molecular disease mechanisms caused by alcohol, it is questionable whether the increased diversity of terminology really contributes to a better understanding. The initiators of the term MAFLD explicitly subsumed ALD within the new term. This is, however, not really convincing since almost all drinkers will develop fatty liver but only 20% will progress to liver cirrhosis. Normally, a new terminology should be based on a better differentiation of diagnosis, discriminative aspects or a real improvement of understanding. However, it is not even clear whether "fatty liver" is a pathology, a physiological state, or both as fatty liver can evolve in hibernating animals, under physiological conditions of fasting and conditions of transient hyperalimentation. As inflammation seems to be the main driver of "disease", steatosis should be used as neutral connotation while liver disease should be restricted to patients with liver damage, inflammation and fibrosis. The term **metabolic liver disease (MLD)** could represent such as novel term.

Is Alcohol a Disease-Causing Agent?

It is interesting to see that alcohol itself is quite well tolerated when isolated cultured human cells are exposed to it under laboratory conditions and cell membranes start to disrupt at concentrations as high as ca. 10% of ethanol. This fact already underlines that alcohol obtains its disease-inflicting level mainly in the intact organism. Moreover, it is the oxidation of alcohol that transforms it into highly toxic and carcinogenic metabolites such as acetaldehyde. In this context, it is interesting to note that many restrictions apply for acetaldehyde, when purchased officially for laboratory use, and it must be handled with certain safety measures. No such regulations apply, if alcoholic beverages are purchased, consumed and directly converted to acetaldehyde within the human body at an equimolar ratio. Nevertheless, with genetic evidence, the human body's ability to metabolize alcohol has not primarily evolved through the culture of alcohol production or uptake of fermented fruits, but rather the intestinal fermentation of carbohydrates to ethanol at significant levels during physiological food intake and digestion. In addition, during most of human history until 150 years ago, general life expectancy was below 40 years, with infectious diseases as primary cause of death, while negative effects of alcohol were considered less relevant. In the past, Roman legions were even supplied with red wine to effectively decrease the burden of gastrointestinal infectious diseases and, in the European cities of the middle age, children were routinely nourished with a low alcohol-percentage beer as drinking water was more dangerous back then. Finally, through centuries, alcohol was part of the weekly salary, and, just a couple of years ago, in some countries, alcohol was offered to hospital and medical staff at lunch time and on-call duty during the night.

Epidemiology of Alcohol-Related Diseases

We have collected an unusually high number of contributions on epidemiology in this book for several reasons (Part I). First, the data highlight the dimensions of alcohol burden in the global world. Ca. three million people die from alcohol consumption annually, not even taking into account the larger "dark" numbers due to undiagnosed or missed alcohol consumption or unrecognized liver fibrosis. With regard to other substance use, alcohol shows the highest per capita years of life lost and death rates are especially high in those aged 45–65. These different perspectives that also include a first prospective mortality study (see Chap. 7) also increasingly underline that **liver-related death** represents the major cause-specific death ranging from 35–80% of all deaths caused by alcohol. Moreover, alcohol is responsible for the majority of liver diseases globally, despite the tendency, to shift focus to other conditions, such as obesity-related liver diseases. The statistics also show that alcohol causes an impressive high number of cancers globally. These are not only primary cancers of liver, stomach, and neck, but also the very common cancers of the colon and female breast. Collectively, alcohol-related cancer cases account for 4% of all cancers globally (see also Part XII).

Some chapters impressively document that most efficient interventions to prevent this enormous burden are rather simple but effective by limiting the access to alcohol either through taxation, pricing or buying restrictions. It is frustrating to see that limited legal interventions would be able to prevent many more casualties than those that can be cured later on by even the best health care systems. Again, in confirmation of earlier success in Scandinavian countries, Russian efforts for more than two decades to implement measures such as taxation confirm how important political measures are in order not to forbid but to limit availability and affordability of alcohol. The chapter on "**Policies to reduce the burden of ALD**" by colleagues from the UK is quite elucidating.

Alcohol Use Disorders and Current Challenges for Addiction Therapy

A more integrated care for alcohol use disorders and general alcohol-related diseases is urgently needed. **Alcohol use disorder (AUD)** is a chronically relapsing disorder that involves aspects of compulsivity in alcohol seeking and taking, difficulty limiting alcohol intake, and the emergence of negative emotional states, such as dysphoria, anxiety, irritability (e.g., hyperkatifeia), in the absence of alcohol. Altogether, 26 chapters are devoted to alcohol use disorders, introduce to addiction and discuss current modes of diagnosis and treatment. Importantly, **Fetal Alcohol Spectrum Disorders (FASD)**, structural and functional central nervous system pathology in alcohol addiction and risk factors for alcohol are addressed.

Alcohol addiction encompasses a three-stage cycle that intensifies with continued alcohol use: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. These stages engage neuroadaptations in brain circuits that involve the basal ganglia (reward hypofunction), extended amygdala (stress sensitization), and prefrontal cortex (executive dysfunction). It is still not widely appreciated that the amount of drinking is not necessarily linked to dependence and vice versa. Some people can drink 60 g alcohol per day, certainly a level of increased risk for both organ-related disease and cancer, but they do not consider themselves as "alcohol-dependent" as they can stop drinking immediately for several days without medical support. Typically, such "at risk drinkers" exceed those who are really dependent, i.e., those who cannot freely discontinue drinking alcohol even so they drink, e.g., less than 40 g per day.

Altogether, very few systematic follow-ups have been performed beyond a year and numbers will be highly dependent on how the condition was diagnosed. With a broad diagnosis based on DSM-5, the number will look better, and it will also look like many people remit spontaneously [4]. Based on a "clinical" diagnosis, i.e., actual treatment seeking people, **relapse is 60–70% at 1 year**, which seems to be unchanged in almost half a century [5, 6].

Medscape also reports relapsing rates depending on the time of sobriety. Thus, 80% relapsed within 1 year, 60% for those who remained sober for 2 years while those who remained sober for at least 5 years had a less than 15% risk of relapsing [7]. According to UK rehab data, longterm rehab success rates are as low as 10% [8]. In the specific setting of heavy drinkers presenting primarily for in-hospital alcohol detoxification to an internal medicine department, we observed after a 15 year follow-up that only 66 patients (5.5%) responded to the invitation to present again for ultrasound examination and laboratory testing. During the mean observation time of ca. 5 years starting from initial detoxification, 25 (37.8%) had completely abstained from alcohol while 62.1% had resumed drinking.

With regard to dependence, genetics seems to drive at least 50%. With one well-established pathway, genetic risk manifests itself is through impaired impulse control (see Part V). So far, data show that we are in fact looking for "large swarms of little insects", i.e., a multitude of risk alleles each contributing a small proportion of risk, rather than big game, i.e., a few risk loci with large effect sizes. Metabolic genes are protective if they result in flushing, and this has been replicated in large Genome-wide association studies (GWAS). The latest GWAS found about 30 of those that were genome-wide significant. The dopamine D2 receptors is actually among them, but the meaning of it remains unclear. More details are provided in Part VI.

The book provides a more holistic view also on addiction that seems tightly to interact not only with the body's inflammatory state but also basic energy metabolism. More interdisciplinary work is certainly needed here. For instance, the novel insights about red blood cell damage, bone marrow toxicity and its relation to iron overload may also provide important links to iron deposition in heavy drinkers [9]. The book also discusses key neuroadaptations in stress systems in alcohol

dependence. These neuroadaptations contribute to negative emotional states and negative urgency that are hypothesized to powerfully drive alcohol drinking and seeking and promote relapse. These changes in stress systems, combined with the disruption of prefrontal cortex function that leads to cognitive deficits, impairments in inhibitory control, and poor decision making, contribute to the chronic relapsing nature of alcohol addiction.

Brain, Energy Metabolism and Relation to Alcohol Dependence

Brain effects of alcohol and addiction are not understandable without the fundamental, evolutionary conserved functions of the brain for organism survival and its association with food intake and basic energy metabolism (see also Fig. 1.2). The mammalian brain primarily depends on glucose as its main source of energy. In humans, the brain consumes 10 times more glucose-derived energy, than other tissues. Glucose metabolism provides the fuel for physiological brain function, neuronal and non-neuronal cellular maintenance, as well as the generation of

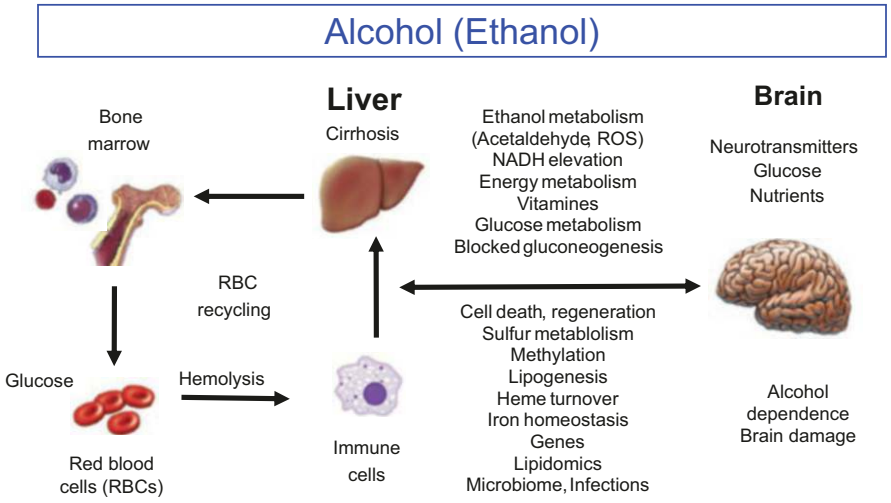

Fig. 1.2 Emerging role of hepatic alcohol metabolism, enhanced red blood cell (RBC) turnover and alcohol dependence. The brain profoundly depends on energy supply above all glucose metabolism that is primarily supplied by the liver. Alcohol not only affects neuronal circuits but impairs glucose supply by the liver through hepatic ethanol metabolism. Moreover, as shown by recent prospective all-cause mortality data (Chap. 7), enhanced RBC turnover through hemolysis and bone marrow toxicity increases the risk of iron-mediated organ damage that may also account for iron-mediated damage to the brain. Emerging data also indicate that genetic alterations and damage of the liver affect drinking behavior

neurotransmitters [10]. The brain integrates multiple metabolic inputs from the periphery and it modulates various aspects of metabolism with the hypothalamus playing a key role in the homeostatic regulation of energy and glucose metabolism [11].

On the other side, the liver is essential for neuronal glucose supply through **hepatic gluconeogenesis.** Moreover, glucose cannot be simply replaced as an energy source but only supplemented, e.g., during strenuous physical activity when blood lactate levels are elevated or during prolonged starvation with elevated ketone bodies [12, 13]. The glutamate/glutamine cycle is another example how neurons are supported energetically with glutamine by astrocytes supporting the glutamatergic neural activity using glycolysis [14].

Fascinating imaging technologies have helped to shed more light on these basal energy dependance of the brain such as nuclear magnetic resonance spectroscopy after infusion with stable glucose isotopes [15]. The most frequently used methods of brain metabolic imaging are the detection of radiolabeled glucose by **positron emission tomography (PET)** in vivo or for diagnostic imaging, and by ex vivo autoradiography using glucose analogues [16]. In addition, fluorescent deoxoglucose derivatives are being used for fluorescence imaging in animal models [17]. Based on functional PET studies, for instance, it has been concluded recently that heavy alcohol consumption facilitates the use of alternative energy substrates [18]. The study also confirmed that resting whole-brain glucose metabolism was lower in drinkers as compared to controls. Functional imaging studies have further shown that alcohol induces dopamine release and increased activity in the striatum [19]. Dopaminergic neurons respond to nutrients such as glucose. Increase in nutrient concentration causes an increase in energy state and modulates ion channel conductance to initiate the dopaminergic signaling cascade.

Biochemically, apart from the production of toxic endproducts, ethanol profoundly interferes with basic energy metabolism by shifting the balance to reduced Nicotinamide adenine dinucleotide equivalents (**NADH**) and **acetate**. This blocks the ability of the liver to provide the brain with glucose. Blood glucose concentrations decrease, but only slightly because the set point for glucose homeostasis in only slightly decreased and glucose consumption by peripheral tissue and brain is decreased [14, 20]. In contrast to other nutrients, alcohol has **access to neurons behind the blood brain barrier** and this augments the usual nutrient reward circuitry [19]. Dopaminergic projections go to the nucleus accumbens, which has a crucial role in the reward system of human brain. If chronic alcohol consumption is abruptly ended, metabolism is no longer able to respond rapidly enough to compensate. Glutamatergic neural activity adapts to chronic dysregulation of glutamate metabolism and suppression of glutamatergic neural activity by increasing excitatory and decreasing inhibitory amino acid receptors. During alcohol dependence, a point is reached where alcohol withdrawal results in significant metabolic energy depletion in neurons and other brain cells as well as hyperexcitation of the glutamatergic system. The extent and regional specificity of energy depletion in the brain, combined with hyperactivity of the glutamatergic neuronal system, largely determines the severity of withdrawal symptoms [14].

In conclusion, alcohol has an effect on the brain at multiple levels that are still poorly understood. A more interdisciplinary approach is needed to fully appreciate not only its its direct effects on brain cells and neuronal circuits but also regional and systemic energy metabolism especially those of the glucose and the liver. It also remains to be studied while heavy drinkers show slightly elevated glucose levels that further increase when cirrhosis develops (see book appendix). Of note, the book discusses that generally accepted biomarkers of alcohol such as phosphatidylethanol or glycated hemoglobin (HbA1C) are profoundly modulated during alcohol metabolism and their interpretation should be taken cautiously.

On another concluding note, for a long time, **hangover symptoms** have been linked to ethanol-mediated oxidation products such as acetaldehyde, effects on the gastrointestinal tract or dehydration, effects on glucose metabolism, sleep patterns, and biological rhythms [21]. However, till today, data of clinical studies are limited and those available lack a certain level of methodological quality [22]. The complexity of alcohol-related disease mechanisms outlined in the book, however, underline why the simple diagnosis of "hangover" is and will not be easy to fully understand, diagnose and treat.

Plaidoyer for an Integrated Patient Care for Alcohol-Related Diseases

Unfortunately, even in the highly specialized setting of alcohol-related diseases, interactions between addiction specialists and brain researchers on the one side and other organ-specialized experts on the other side are rather limited. Given the many interactions of ethanol at the subcellular, cellular and organ level and the endless interactions (Fig. 1.1), a broader perspective is urgently needed. A close interaction between addiction and other health professionals is especially required to **improve and integrate health care support for patients with AUD.** In Part XIII of the book, two chapters from UK and Romania (Chaps. 76 and 77) describe how **health care teams** have implemented a model of integrated care for patients with acute and chronic alcohol addiction and alcohol-related diseases in a resource limited setting. These chapters document how much needs to be done to both address mental health, diagnose other psychiatric disorders, and adequately treat the addiction without overlooking more common and fatal confounders of alcohol consumption, liver cirrhosis above all.

One of the major advances with regard to alcohol-related diseases has been in the area of diagnosis with the **introduction of liver elastography** (see also Part VII) [23]. As liver-related mortality accounts for the majority of alcohol-related deaths, ranging from 30–80%, an early identification of those who are at an increased risk to develop liver disease is a crucial and important step forward [23]. Since some of the techniques can be performed with little training and without dedicated

ultrasound knowledge, a broader availability will not only help to improve patient care but also close some of the gaps between the different specialties.

A closer interaction between the various medical specialties involved in the care of alcohol-related diseases in form of **virtual or physical "alcohol treatment centers"** is also justified by the many interactions of ethanol metabolism between different organs (see also Fig. 1.1).

Important Crosslinks Between Liver and Alcohol Dependence

It is less appreciated that ethanol metabolism in the liver is closely associated with alcohol dependence. Thus, the combination of a slow first oxidation to acetaldehyde and a rapid second oxidation of acetaldehyde to acetate have been shown to increase the development of dependence (see also Chaps. 50 and 75). Obviously, it is the ethanol that causes the dependence while its toxic oxidation and accumulation of these oxidation products lower the risk of alcohol addiction due to direct negative feedbacks. In this context, it is also quite interesting to note that ethanol is transformed to toxic metabolites inside the body while human cultured cells tolerate well ethanol concentrations up to 5–10%, more than 10 times of the lethal concentrations in humans. To put it in simpler terms, it seems indeed the ethanol causes the joy (and addiction) while the human body cannot escape its mandatory oxidation to toxic acetaldehyde and generation of reactive oxygen species. However, we should not forget that only a minority will suffer from these negative consequences.

There are also emerging data that brain function, alcohol consumption and liver metabolism are tightly interrelated (Fig. 1.2). Thus, as shown in the Appendix (Fig. A.85), first preliminiary data indicate that heavy drinkers who develop liver cirrhosis will reduce their drinking levels and drinking behavior. Moreover, patients with an unfavorable mutation (GG) of **PNPLA3** (see also Chap. 52), a lipid droplet associated protein which is most likely involved in triacylglycerol hydrolysis, seem to avoid high percentage alcoholic beverages. Other data show that patients with liver cirrhosis actually have higher levels of blood alcohol, ethylglucuronid and ethylsulfate despite lower daily alcohol intake [24]. And finally, as is also discussed in this book in Part VI, data are emerging that indicate that the known high variability of the alcohol biomarker phosphatidylethanol (PEth) is closely related to the alcohol-mediated red blood cell turnover. These novel observations are in line with the fact that formation of PEth requires erythrocyte phospholipase D activity and seems to be stably integrated in the RBC membrane.

Last but not least, various important **biochemical pathways are listed in the Appendix** for better illustration. Ethanol metabolism modulates various basic pathways that use vitamins such as vitamin B1 and B6 or folic acid that are important for extra-hepatic metabolic processes. For instance, vitamin B1 is essential for basic carbohydrate and energy pathways, vitamin B6 for the transsulfuration pathway

while folic acid and vitamin B12 are essential for the metabolism of methionine. Of note and as shown in the Table B.4 (original data from a heavy drinking cohort), vitamin B12 can even be elevated in patients with liver cirrhosis, most likely to compensate for relative folate deficiency.

Emerging Novel Role for Enhanced Red Blood Cell Turnover

Red blood cells have long been forgotten in the context of alcohol-related diseases. RBCs are important storage sites for vitamins such as folic acid. The fact that a prospective all-cause mortality study (Chap. 7), to the best of our knowledge the first, identifies **hemolytic anemia**, **release of heme** and **enhanced RBC turnover** as the most important prognostic factor, further sheds new light on the long-studied **hepatic iron overload** and recent observations of **brain iron accumulation** in drinkers [9]. Finally, the interactions between **hepatic encephalopathy** and liver cirrhosis require more attention as the molecular mechanisms are still not fully understood. In addition, it becomes increasingly known that the inducible cytochrome p450 system, namely the subtype CYP2E1, that oxidizes a significant amount of ethanol in heavy drinkers, is not only present in the liver, but also in macrophages and in the brain. It may also provide new links to why chlomethiazole has been used for almost one century as a sedative and hypnotic but has turned out to be a specific CYP2E1 inhibitor. There are almost no studies available on the expression and function of brain CYP2E1.

Why Can Alcohol-Related Liver Disease Be Simulated by Diabetes and Obesity in the Absence of Alcohol?

One of the major cause-specific deaths is liver related mortality, with alcohol-related liver disease being one of the famous hallmarks of alcohol consumption. In 1995, more than 25 years ago, in a book edited by Pauline Hall entitled “Alcohol-related liver disease” [25], Peter Scheuer asked “why alcohol-related steatohepatitis is so similar morphologically to **non-alcoholic steatohepatitis (NAFLD)?** [25]. Today, almost 30 years later, it is astonishing to see how relevant these questions still are. In fact, we have not seen much progress in addressing them in the the last two decades.

Especially ALD offers more unanswered questions, in addition to the above-mentioned stunning similarity between diabetes- and overweight- induced NAFLD. Almost no progress has been made in the diagnosis and treatment of the often-fatal **alcoholic hepatitis**. Modest benefits are seen in only a fraction of patients with steroids. Although the microbiome has gained great attention, simple antibiotic treatment does not seem to halt ALD. Moreover, the pathophysiology of

ALD is often explained with quite complex and methodologically challenging mechanisms, such as reactive oxygen species that require deep physical, chemical and biochemical knowledge. On the other side, enzyme systems such as the p450 system localized in the endoplasmic reticulum are attributed to mediate major disease mechanisms but both knockout animals or pharmacological blockade show less convincing effects. In other words: What are the actual mechanisms of alcohol-related liver disease and what does it have in common with NAFLD in the setting of overweight and diabetes?

Although the oxidation intermediate acetaldehyde is beyond any doubt crucial to explain alcohol-related disease mechanisms, it cannot be the major link to obesity and diabetes.

Rather, both alcohol, diabetes and overweight are characterized by an **excess of energy** and an excess of reduced nicotinamide adenine dinucleotide (NADH). Heavy drinkers, despite having access to normal nutrition, cover typically about 50% of their energy supply by ethanol (see also Table B.4). Although ethanol, just like sugars, contains only the three elements carbon, oxygen and hydrogen, it is neither chemically nor biochemically a carbohydrate, and its human metabolism is strikingly different from that of carbohydrates. Comparable to physiological energy suppliers such as fatty acids or sugars, oxidation of ethanol leads to NADH which can be further used for mitochondrial respiration (Fig. 1.3). However, in contrast to

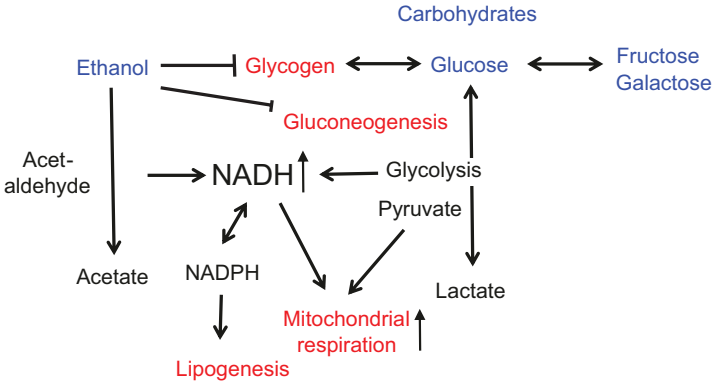

Fig. 1.3 Carbohydrate and ethanol metabolism linking brain to liver and energy metabolism (simplified scheme). Some similarities may explain why ethanol, obesity and diabetes mellitus all cause a similar hepatic steatohepatitis whether alcohol-associated or non-alcoholic. Both glycolysis and ethanol oxidation lead to **formation of NADH** which drives mitochondrial respiration and lipogenesis and may be the joint key feature in causing mitochondrial and organ damage. In difference to carbohydrates, however, **ethanol oxidation ultimately blocks hepatic gluconeogenesis** and causes rapid glycogen depletion. Hence, glucose, which is essential for energy metabolism of brain, red blood cells or muscles, becomes limiting. Sugars and ethanol also share the fate of having almost no evolutionary evolved negative feedback loops except by elimination through lipogenesis or oxidation. Not by chance, the vital energy metabolism is also closely related to dependence as seen in **food addiction**. A better molecular understanding is needed how energy and ethanol metabolism are associated with the brain and dependence. This complexity may explain the rather diverse findings in large screening studies for genes that cause addiction

glucose and fructose, ethanol metabolism **prevents gluconeogenesis** simply due to the balance shift towards NADH (see Fig. 1.3). This shifts the **lactate/pyruvate ratio** towards lactate and, consequently, away from gluconeogenesis. It should be also noted that, already at 0.4 permille of blood ethanol concentration, typical ethanol metabolism starts [26].

Ethanol also provokes a fast and efficient **depletion of glycogen stores** (see Fig. 1.3). Although fundamental to ethanol biochemistry, it is still not clear whether these changes are responsible for rapid ethanol-mediated muscular fatigue as muscles obtain glucose from the liver through the Cori cycle. As already mentioned above, it is also highly intriguing that ethanol, which does not have any biochemical feedback loop in human cells, overwhelms the mitochondrial respiratory chain and may simply cause injury by uncoupling reactions leading to release of **reactive oxygen species (ROS).** Consequently, despite providing enough energy, ethanol metabolism leads to glucose deprivation and glycogen depletion which may become limiting for cells of the brain, red blood cells or muscle cells that are highly dependent on hepatic gluconeogenesis. It is an attractive scenario that the rapid and uncontrolled "fuel burning" in mitochondria could ultimately be responsible for mitochondrial damage, e.g., through uncoupled redox reactions.

Thus, it seems that despite not being a carbohydrate, ethanol shares with sugars the immediate energy supply within the **intermediary metabolism**. They also share to some extent the **lack of a negative feedback loop**. Similar to alcohol, the human metabolism cannot really escape an excess of glucose or fructose. In this scenario, **fatty acid accumulation** is primarily the body's sole option to store the excess energy through lipogenesis. Hence, fatty liver may indeed be primarily a bystander and a metabolic consequence to quickly remove the excess of energy.

Consequently, it is the uncontrolled excess of energy that may link NAFLD with ALD. Since lipogenesis in this context would be more a solution than a problem, fatty liver may not be the actual disease but rather the mitochondrial damage and inflammation due to excess energy supply. More research on carbohydrate metabolism, its relation to ethanol, and its hormonal control is needed. Ultimately, with regard to the terminology debate, metabolic (dysfunction) associated fatty liver disease (MAFLD) [3] may not be optimal. Rather, it should be termed **metabolic liver disease (MLD)** and only include patients with signs of liver damage and fibrosis but not fatty liver itself.

Conclusion

The present book covers all aspects of "alcohol and alcohol related diseases". Alcohol is a major health risk worldwide causing more than 60 diseases and accounts for at least three million death annually and 4% of all new cancer cases. Besides current diagnostic measures and treatment strategies, the many underlying molecular mechanisms of alcohol toxicity including the genes that lead to addiction and disease are discussed. It is hoped that the full appreciation of the enormous level

of complexity and interactions created by alcohol stimulates a more interdisciplinary cooperation between clinicians, scientists and addiction specialists. In my opinion, only such a holistic approach will allow us to apply the increasingly emerging potentials of OMICS and genetics more efficiently. It is also expected that the books helps to guide policy makers in order to responsibly handle alcohol in society, certainly the most important aspect to decrease mortality and suffering from alcohol use disorder, alcohol-related diseases and cancer.

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Chapter 2
Epidemiology of Alcohol and Opioids

Jürgen Rehm

Abstract Alcohol is the most prevalent addictive substance globally, and with three million attributable deaths per year, it causes substantial burden of disease and mortality, mostly to men. While alcohol use has been causally linked to over 200 ICD three-digit categories, the majority of alcohol-attributable deaths can be found in four major categories (in the following order): injury (unintentional and intentional); digestive disease (especially liver cirrhosis); cardiovascular disease; and cancer. For burden of disease, including non-fatal outcomes, alcohol use disorders play a more important role than for fatal outcomes, but only 14% of all alcohol-attributable disability-adjusted life years are made up of alcohol use disorders. Harm per litre of pure alcohol is higher in poorer countries, and within all countries in lower socioeconomic strata. In addition to health harm to the drinker, alcohol use causes social harms such as violence and social disorder.

Opioids are a broad substance category, which includes illicit drugs and pharmaceuticals. While the estimates of prevalence and attributable harm are less reliable due to its legal status, opioid use and—use disorders are much less prevalent than

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alcohol use and—use disorders. However, its importance as a cause of death has been increasing, particularly linked to overdose deaths in high-income North America.

Keywords Alcohol · Burden of disease · Disability-adjusted life-years (DALYs) · Causes of death · Harm per litre · Harm to others · Opioids · Overdose deaths

Epidemiology of Alcohol and Opioids

Major Classes of Addictive Substances and Attributable Harm

Alcohol use has been identified as a major contributor to the burden of disease and injury [1]. In this chapter, the epidemiology of this risk factor will be laid out, in part in contrast to opioid use. Before starting, we would like to give some preliminary results of the burden of mortality caused by select behavioural risk factors potentially can causing addiction (Table 2.1).

Clearly, tobacco use is linked to the most deaths, about 3.6 times as many as alcohol use, and to 18 times more deaths than caused by drug use. The picture changes for potential years of life lost (PYLL): here the ratio between PYLL attributable to tobacco use and alcohol use is about 2.5 to 1, and the attributable PYLL ratio between tobacco use and drug use is about 10 to 1, reflecting the fact that alcohol- and drug-attributable deaths occur much earlier in the life course than deaths caused by tobacco use [3, 4]. But still, legal drugs like alcohol and tobacco cause much higher mortality than all illegal drugs combined. Within the category of illegal drugs, opioids are responsible for almost 70% of the overall mortality attributable to drug use [2].

Table 2.1 Global mortality caused addictive substances in 2019

	Deaths				Potential years of life lost			
Sex	Risk Factor	Rate per 100,000	Upper CI	Lower CI	Risk Factor	Rate per 100,000	Upper CI	Lower CI
Men	Tobacco	181	195	166	Tobacco	3839	4168	3514
	Alcohol	54	62	46	Alcohol	1620	1840	1416
	Drug	9	10	8	Drug	328	362	298
Women	Tobacco	49	54	45	Tobacco	1026	1133	925
	Alcohol	9	11	7	Alcohol	242	284	206
	Drug	3	4	3	Drug	117	132	104
Total	Tobacco	109	116	101	Tobacco	2354	2538	2181
	Alcohol	30	34	26	Alcohol	916	1033	810
	Drug	6	7	5	Drug	222	244	202

Source: Own compilation based on [2]

One shared characteristic of all three major categories of substances concerns the higher harm caused to men compared to women. However, the ratios between men and women vary with alcohol use having a much higher associated mortality burden than either tobacco or illicit drug use (Table 2.1).

Major Epidemiological Characteristics of Alcohol Use

We have started with substance-attributable harm, as this is the main reason why addictive substances are of concern. We will, of course, come back to this harm, but we would like to present some basic epidemiologic characteristics of alcohol use first.

Alcohol Use Patterns

While alcohol is the most commonly used addictive substance globally [1], the majority of adults globally abstain from alcohol in any given year [5]. More men than women use alcohol in every country of the world, and men also consume higher average volumes of alcohol and have more detrimental drinking patterns (e.g., more irregular heavy drinking occasions—for definitions and conceptualizations, see [6]; for quantitative comparisons of these indicators by country and region, see [5]).

However, while there are high-level similarities with alcohol use, such as its predominant use by men, there are differences. Figure 2.1 gives an overview on adult (defined as 15 years and older) *per capita* alcohol consumption in different countries.

Levels of average alcohol consumption are highest in Central and Western Europe, in Australasia, and select countries of Africa. They are lowest in the Muslim-majority countries in Northern Africa, in the Middle East countries, and in South-East Asia (e.g., Malaysia, Indonesia; see Fig. 2.1).

Overall, the level of alcohol consumption has been fairly stable in the past decades, but there have been changes in different regions over the past two decades (for an overview, see [7]; see also [9]): the WHO European Region had the largest declines in consumption since 2000, over the last decade driven by substantial reductions in *per capita* consumption in the Eastern part of the region [10], in particular Russia [11]. The implementation of alcohol control policies such as the "best buys" of the WHO (taxation increases, availability restrictions, ban on marketing; [12] in many countries of the region was the driving force behind these reductions [13, 14]. The Americas also had reductions in this the time span between 2000 and 2020, mainly in men, whereas both the South-East Asian and the Western Pacific Regions increased their consumption [5], in large part driven by increases in India and China [15].

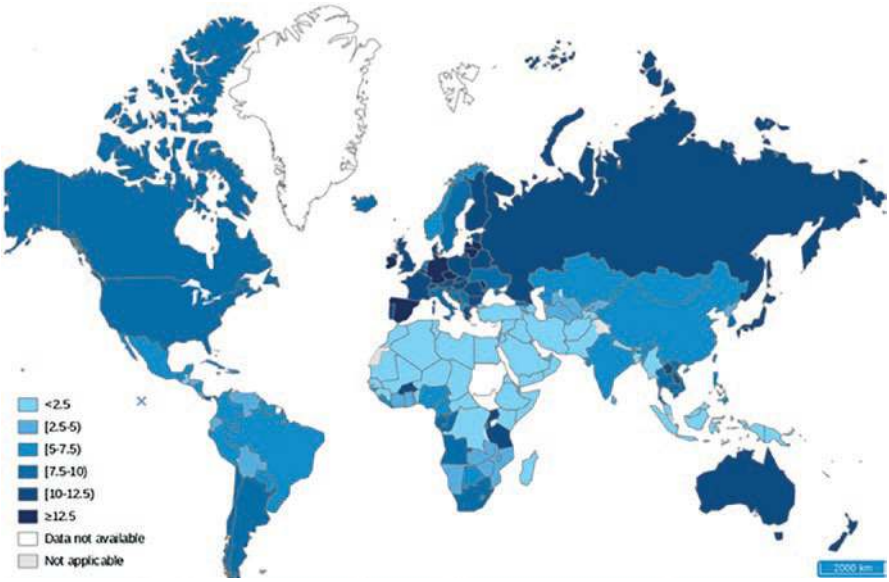

Fig. 2.1 Adult *per capita* consumption of alcohol in litres ethanol for 2019. Source: World Health Organization, World Health Statistics 2022 [7, 8]

Part of these dynamics are driven by economic growth: alcohol is no essential good, and thus lowest in low-income countries, but with increasing wealth, its use tends to increase as more people can afford it [15, 16]. Also, within upper-middle income and high-income countries, abstention from alcohol tends to be highest in low socioeconomic strata [17, 18], whereas this relationship is not so clear in low-income and lower-middle income countries [19].

Harm Attributable to Alcohol Use

Alcohol is causally linked to more than 200 three-digit ICD 10 codes [20], with about 40 conditions being 100% alcohol-attributable, meaning that without alcohol these disease categories would not exist (examples: alcohol use disorders; alcoholic liver cirrhosis). More often, the causal link is via complex pathways involving different necessary factors rather than one necessary cause. As a consequence, the attributable fractions for many alcohol-attributable disease and mortality categories are relatively low (examples from the last comparative risk assessment of the World Health Organization: 5% of all breast cancer deaths and disability-adjusted life-years (DALYs); 9% of all deaths, and 10% of DALYs due to hemorrhagic stroke; 18% of all deaths and 19% of all DALYs due to self-harm; [21]. As a consequence, alcohol consumption is a unique risk factor, which, on average, causes many different categories of death and morbidity, but most of them with comparatively low alcohol-attributable fractions.

Overall, both the Global Status Report [21] and Shield and colleagues [22] estimate that about three million deaths in 2016 could have been avoided without consumption of alcohol. The majority of alcohol-attributable mortality can be found in four major categories (in the following order): injury (unintentional and intentional; [23, 24]); digestive disease (especially liver cirrhosis, [25]); cardiovascular disease [26]; and cancer [27] (for details see Table 2.2). For DALYs, alcohol use disorders play a more important role than fatal outcomes, but only 14% of all alcohol-attributable DALYs are alcohol use disorders (all numbers from [21]).

Alcohol-attributable burden of mortality and disease thus can be characterized by injury and chronic disease outcomes, and not directly by its addictive consequences. However, some of the chronic consequences, such as alcoholic liver cirrhosis, are closely linked to chronic heavy drinking and thus to alcohol use disorders [28]. Another, in part related, important characteristic is that attributable harm is not linearly related to level of drinking. Both between and within societies, the harm per litre of pure alcohol is different for people with less wealth (general: [17]; between societies: [25]; within societies: [29]). A number of different causal explanations have been given for this (for an overview: [30]; for examples of individual-level difference within societies: [31]; between societies: [25]), most importantly drinking patterns and interactions with other behavioural risk factors such as smoking or obesity, and environmental factors such as the different absolute risks for certain disease categories and the lack of social and medical support.

Table 2.2 Number of alcohol-attributable deaths by single cause of death categories with globally more than 80,000 alcohol-attributable deaths in 2016

Alcohol-related disease	Number of death	Percentage (%)
Cirrhosis of the liver	588,100	19.8
Road injury	370,800	12.5
Tuberculosis	236,300	8.0
Haemorrhagic stroke	287,000	9.7
Ischaemic heart disease	250,800	8.5
Self-harm	147,000	5.0
Alcohol use disorders	145,600	4.9
Liver cancer	101,400	3.4
Lower respiratory infections	95,200	3.2
Colon and rectum cancers	92,600	3.1
Interpersonal violence	86,800	2.9
Oesophagus cancer	82,900	2.8
All alcohol-attributable deaths	2,967,800	100.0

Source: own compilation based on [22]

Social Consequences Attributable to Alcohol Use and Harm to Others

Alcohol causes considerable social harm in addition to health harm to the drinker, and also considerable harm to others than the drinker [17]. Examples are traffic fatalities caused by drunk driving or Fetal Alcohol Spectrum Disorders caused by alcohol use of mother, or intimate partner violence or other forms of violence [17]. But also, social outcomes such as public disorders have been causally linked to alcohol use [32].

Overall, the economic costs of alcohol in different countries which conducted cost studies have been estimated to amount on average about 2.6% (95% CI 2.0–3.1%) of the Gross Domestic Product [33]. This is likely an underestimate, as several costs (e.g., public disorder, violence) have not been included (for details cost occurring to others than the drinker, see [34]). To illustrate these numbers, we would like to give four examples of recent studies, converted into international dollars of the year 2019 [33].

In Canada, the estimating economic costs of substances is part of regular monitoring. In the last report covering the years 2015–2017, for the year 2017, it was estimated that the costs of alcohol use amounted to 470 International $ per adult 15 years and older, with a proportion of almost 60% being direct costs (largest direct category was health care; [35]). The costs attributable to alcohol were higher than the costs for tobacco or illicit drugs. In France, the costs of alcohol use for the year 2010 amounted to 2440 Int$ per adult. These substantially higher costs can be explained by higher indirect costs (90% of all costs), which are mainly derived from premature deaths [36]. A Scottish study for the year 2010 found costs of 1250 Int$ per adult. Direct costs made the majority of these costs (59%), but contrary to Canada, most direct costs were derived from the legal system (criminality attributable to alcohol and associated costs such as drunk driving, illegal production, violence; [37]). Finally, in South Africa the costs for alcohol use for the year were estimated to amount to 440 Int$ per adult, which would correspond to a higher proportion of the Gross Domestic Product than the studies cited above. Again about 60% were direct costs, again mainly from the legal sector [38]. While the results of cost studies vary based on the wealth of a country, the cost categories included [33, 39], and the drinking patterns of each country, overall, the clear message is the alcohol use incurs high economic costs with are not nearly been paid off by the taxation [33, 36, 40].

On the Comparison with Opioid Use and Opioid Use Disorders

Opioid use is much less prevalent than alcohol use. Exact numbers are not available, as opioids are an illicit substance except when given within medical treatment. The United Nations Office on Drugs and Crime (UNODC) estimate in the latest World Drug Report that in 2019 1.2% of the population between the ages of 15 and 64 used opioids at least once in that year, including people who use pharmaceutical opioids

for non-medical purposes [41]; for other estimates and a discussion of the methodological difficulties, see [1, 42]. Even given the high uncertainty of the estimates, this proportion seems considerably lower than the number of people who use alcohol (which amounted to almost 50% in the same year; see above and [5]).

The category of opioid use disorders is a bit less ambiguous, although there is a tendency to underestimate such disorders in people who use only opioids as prescribed. While the medical prescription of opioids undoubtedly fuelled the first phase of the most recent opioid crisis in North America [43, 44], most of the current overdose deaths are linked to synthetic opioids sold in the streets, especially illicitly produced fentanyl [45]. The current phase is usually labelled phase 3, with a phase 2 characterized by overdoses involving heroin between the prescription and the synthetic opioids phase.

Overall, both prevalence of use, opioid use disorders and opioid overdose mortality rates have been increasing the most in North America over the past two decades [41], and opioid overdose deaths reached their highest numbers in 2021, both in the US and Canada [46, 47]. The overall numbers have been so high during this period that they strongly contributed in both the US and in Canada to a decrease in life expectancy which began even before COVID-19 pandemic [48, 49]. Globally, age-standardized rates of opioid overdose deaths also increased over the past decades up until 2019 [2](see Fig. 2.2), albeit with much less accelerated slopes compared to North America.

Overall, as indicated in Table 2.1 above, while increasing, the burden of disease attributable to opioid use is considerably smaller than the burden of disease due to alcohol use [2]. It is also much more concentrated in people with use disorders, i.e., it is more linked to the addictive properties of the substance.

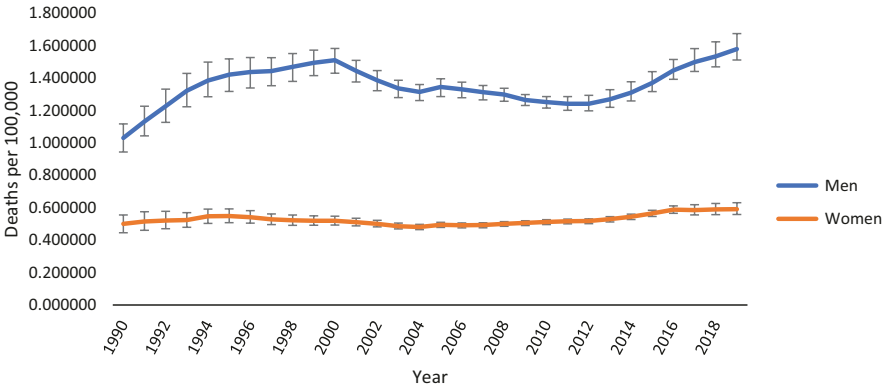

Fig. 2.2 Age-standardized rates (per 100,000) of opioid use disorders mortality. Source: own graph based on estimates of GBD-2019 [2]

Consequences for Control Policies and Prevention

The epidemiological differences specified between alcohol and opioids laid out above have clear consequences for control policies. In most countries, the most effective and cost-effective control policies for alcohol are population-based [12, 17], such as those restricting affordability and availability, as well as reducing demand for this mostly legal substance via taxation increases, restrictions on trading hours, and a ban on marketing.

For an illicit substance like opioids, control policies need to be more centred on the individual, such as an efficient treatment and harm reduction system [50]. However, for treatment and harm reduction efforts to be most effective, decriminalization of use [51, 52] is probably necessary while universal health coverage absolutely is [53]. The consequences of a change of legal status are not well researched.

As for prevention, the current efforts of educational campaigns and school-based educations seem to be of limited success in changing behaviour [17, 54, 55].

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Chapter 3
Epidemiology of Alcohol-Related Liver Disease in Europe

Peter Jepsen

Abstract This chapter describes some of the fundamentals of studying the epidemiology of alcohol-related liver disease, including different measures of burden, standardization, and age-period-cohort models. It also presents some of the source populations that may be studied, e.g., the general population, people with a hazardous alcohol consumption, or people in hospital. There is also an introduction to the Global Burden of Disease studies and the HEPAHEALTH Report published by the European Association for the Study of the Liver; both sources provide—among other things—an overview of the epidemiology of alcohol-related liver disease in Europe. Finally, the chapter presents the time-trends in the burden of alcohol-related liver disease in some European countries, primarily the United Kingdom, which has experienced an increasing burden, and Denmark, which has experienced a decreasing burden.

Keywords Epidemiology · Incidence · Mortality · Burden · Methods · Age-period-cohort models

The Burden of Alcohol-Related Liver Disease

It is common to talk about the *burden* of alcohol-related liver disease, but what do we mean by that?—the burden on whom? Is it the burden on the patients? On the patients' family? On society? This chapter is about the burden of alcohol-related liver disease on society, which can be described by the number of people living with alcohol-related liver disease (the prevalence), the number of people who develop alcohol-related liver disease (the incidence rate), the number of hospital admissions for alcohol-related liver disease (the hospitalization rate) and the number of people

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who die from alcohol-related liver disease (the mortality rate). These epidemiologic measures (prevalence, incidence rate, hospitalization rate, and mortality rate) are typically described with reference to the demographics of the underlying population, e.g., the number of new patients per 100,000 population per year.

Using Standardization to Facilitate Comparisons

When we want to compare the epidemiology of alcohol-related liver disease between countries it is convenient to standardize epidemiologic measures to a specific population. Such standardization serves to facilitate comparisons between countries by giving us the incidence in country X if the population of country X had the same age distribution (or gender and age distribution) as our standard population [1]. The standard population can be any population, and one suggestion is from Eurostat which revised the European Standard Population in 2013. It is derived from the demographics of "the EU27 plus EFTA countries, on the basis of 2010-based population projections, averaged over the period 2011-30." (https://ec.europa.eu/eurostat/web/products-manuals-and-guidelines/-/ks-ra-13-028).

What Is the Source Population?

When we describe the epidemiology of alcohol-related liver disease we are limited by the data that we have access to. Ideally, *everybody* in the population is examined for presence or absence of alcohol-related liver disease, but that is hardly ever done, at least not for a population larger than a few thousand people. Consequently, the data we have comes from selected subsets of the population, e.g., those who are in hospital, those who have a hazardous alcohol consumption, those who volunteer for a research project, those who hold a particular health insurance, or those who have signs or symptoms of liver disease.

Early or Late Alcohol-Related Liver Disease

Most of our information about the epidemiology of alcohol-related liver disease stems from hospital contacts, so it is based on people with relatively late-stage alcohol-related liver disease, e.g., cirrhosis or alcoholic hepatitis. We see the tip of the iceberg. There are also populations in which we have information from general practitioners, e.g., England [2]. Patients who see their general practitioner are, on average, less sick than those who are in hospital, and we should expect a fuller picture of the epidemiology of alcohol-related liver disease from England than from countries without information from general practitioners. Even so we still do not get the full picture because alcohol-related liver disease can be completely asymptomatic.

Prevalence of ALD

The prevalence of alcohol-related liver disease is highly dependent on the source population, e.g., the general population or a higher-risk group.

The General Population

The Dionysos study examined the prevalence of liver disease in two towns in Northern Italy in 1991 and again in 2000–2001. The towns harbored 10,151 inhabitants aged 12–65 years, all of whom were eligible for the study, and 6917 people participated in both examinations. The study reported that 30% of the population aged 12–65 years were 'inappropriate drinkers', meaning that they drank more than 30 g ethanol per day or had drunk more than 100 kg in their lifetime. In addition, 2.3% of the population were 'inappropriate drinkers with liver disease', suggesting that 2.3/30 = 7.7% of inappropriate drinkers had liver disease [3]. In this study, liver disease was diagnosed based on standard liver function tests, abdominal ultrasound examination, and possibly additional imaging and/or liver biopsy [4].

With newer modalities for noninvasive screening for liver disease, such as transient elastography, it has become more feasible to conduct studies of the general population [5]. The EU-funded LiverScreen project aims to develop a targeted screening methodology to identify persons with asymptomatic liver fibrosis and cirrhosis among the general population (https://www.liverscreen.eu/).

People with Hazardous Alcohol Consumption

A 2017 review reported that the prevalence of liver disease among people with a hazardous alcohol consumption was between 11.0% and 20.5% [6], higher than the 7.7% estimate based on the Dionysos study. Of the four reviewed studies, two examined advanced liver fibrosis defined by a stiffness ≥8 kPa on transient elastography, and they reported prevalence estimates of 17% and 18.5% [7, 8]. A third study examined the prevalence of any liver fibrosis (≥5.9 kPa) and reported a prevalence of 20.5% [9]. The fourth study reported a prevalence of probable liver fibrosis of 11%, defined by the Southampton Traffic Light, a combination of hyaluronic acid, Procollagen III N-Terminal Pro-peptide, and platelets [10].

It may be noted that the prevalence estimates using a ≥8 kPa threshold to define advanced liver fibrosis (17% and 18.5%) were higher than the 7.7% estimate from the Dionysos study. Part of the explanation is that the 8 kPa threshold is low; current guidelines from the European Association for the Study of the Liver (EASL) recommend a rule-in threshold of 12 to 15 kPa for advanced liver fibrosis [5].

People in Hospital

The Scandinavian countries have nationwide healthcare registries that were developed to monitor healthcare activity and have been used extensively for research [11, 12]. These registries rely on diagnosis codes given at hospital discharge (including outpatient visits) to determine the reason for hospitalization. The validity of these diagnosis codes is a key concern, but it is generally high enough to permit epidemiologic studies of alcohol-related liver disease [12].

A Danish study reported that the prevalence of alcohol-related liver disease was 0.22% of the population [13], just one-tenth of the 2.3% estimate from the Dionsysos study. However, this low estimate inevitably underestimates the prevalence of early-stage alcohol-related liver disease because it counts only people who are in hospital. This underestimation is emphasized by the fact that 69% of patients in this Danish study had cirrhosis [13], while in the general population only a minority of people with alcohol-related liver disease have cirrhosis.

Incidence

Determining incidence is often more difficult than determining prevalence. Not only do we need to know the size of the source population that gave rise to the cases of alcohol-related liver disease, we also need to know *when* they developed liver disease. Consequently, studies determine the incidence of symptomatic, late-stage alcohol-related liver disease, i.e., using data from hospitalized patients, assuming that the first hospitalization is the time of disease incidence.

In the population of Denmark, the incidence of alcohol-related cirrhosis was 240 per million person-years in 2018 [13]. In England, 1998–2009, it was 165 per million person-years [14]. It was lower in the Scania region of Sweden, 2001–2010, around 70 per million person-years [15]. These studies all relied on registry data from in- or outpatient hospital visits and, in the case of England, general practitioners. Some studies rely blindly on those diagnosis codes [13], but ideally they are validated through review of the medical charts [16, 17].

Hospitalization

Hospitalization rates may be based on the number of hospitalizations eliciting a discharge diagnosis code of alcohol-related liver disease within the entire population or among the subset diagnosed with alcohol-related liver disease. For example, the number of hospitalizations for alcohol-related liver disease within the general population of England or Wales in 2002–2003 was approximately 200 per million person-years [18].

Alternatively, hospitalization rates may be based on the number of hospitalizations for any reason among people with alcohol-related liver disease. As an example, a Danish study counted hospital admissions for any cause among people who had previously received a hospital diagnosis of alcohol-related liver disease [13]: It was 1.12 all-cause hospitalizations per person-year in 2018 [13].

Mortality

Mortality rates are more accessible than other epidemiologic measures of the burden of alcohol-related liver disease because more countries record causes of death than, e.g., causes of hospitalization. The number of deaths from alcohol-related liver disease within the population of England or Wales was approximately 77 per million person-years in 2005 [18]. In the Veneto region of Italy, it was around 85 per million person-years in 2008–2010. Like studies based on hospitalization registries, they rely on codes whose validity is a key concern. Causes of death are more difficult to validate than causes of hospitalization because many people die in their home, and there are no charts to validate the registration against. For additional details about mortality, see also Chaps. 2 and 7 within this book.

Survival of Patients with Alcohol-Related Liver Disease

Mortality rates from alcohol-related liver disease depend on the incidence rate of alcohol-related liver disease and the survival time of people with alcohol-related liver disease. If they live longer, mortality rates can go down despite an increasing incidence. There is some evidence that patients with a hospital diagnosis of alcohol-related cirrhosis live longer now than they used to do [19].

Age-Period-Cohort Models

Studies generally find that the incidence of alcohol-related liver disease peaks around age 55 to 60 years. This age effect is remarkably constant and probably reflects ‘years of harmful alcohol consumption’ more than ‘years since birth’. If the age effect is constant across studies, why, then, has the incidence of alcohol-related liver disease increased in England, and why has it gone down in Denmark?

A decreasing incidence in Denmark after year 2010 might be attributed to effects of events that occurred at a specific calendar time—maybe taxes on alcohol doubled on, say, 1 January 2010 (which in fact they did not). That would constitute a calendar time effect, also called a period effect. A decrease after 2010 might also be attributed to effects of events that affected a birth cohort—maybe the baby boomer generation grew up with a societal norm that it is desirable to have wine for dinner, or that drunk driving is acceptable. And maybe subsequent generations rebelled

against those norms and thought exactly the opposite, resulting in a lower incidence after 2010, when the baby boomer generation has aged past 55–60 years. These examples would constitute birth cohort effects.

Many real-life interventions involve multiple time axes (age, period, and cohort). For example, a law may be passed on 1 January 2020 saying that people born after 1 January 2000 cannot buy alcohol before age 25 years, where currently the age limit is 20 years. This would mean that people born in 1999 could still buy alcohol in 2020, whilst people born in 2000 could not buy alcohol before 2025, and people born in 2001 could not buy alcohol before 2026.

Age-period-cohort models are statistical models that can help investigators disentangle the effects of age, calendar year, and birth cohort on the incidence, prevalence, or mortality of alcohol-related liver disease. The difficulty with the class of models is that age, period, and birth cohort are interdependent in that you can compute one of them if you know the values of the other two (e.g., age + cohort = period). We cannot vary one of them and hold the other two constant, which is what we would usually do [20]. There are different types of age-period-cohort models with different assumptions about the effects of age, period, or cohort [21–23]. A further possibility with age-period-cohort models is that they can be used to predict the future epidemiology of alcohol-related liver disease [22].

Example

A 2017 study by Trias-Llimós and others used age-period-cohort models to examine time-trends in cirrhosis-specific mortality during the 1950–2011 period in eight European countries chosen for their different alcohol consumption levels, patterns, and trends: Austria, Finland, Hungary, Italy, the Netherlands, Poland, Spain and Sweden [24]. The investigators noted that cirrhosis-specific mortality mainly reflects mortality from alcohol-related cirrhosis. The cirrhosis-specific mortality rate was decreasing in all countries except Poland, and most of the countries had experienced the peak cirrhosis mortality in the 1970s (Austria, Italy, Netherlands, Spain, and Sweden) while Hungary reached its peak in the 1990s and Finland shortly before 2010.

The investigators used the age-period-cohort model to infer likely explanations to time-trends. In Italy, for example, the mortality rates over the 1950–2011 period were parallel for all age groups, and such a pattern suggests that period effects are important: Whatever happens, happens to everybody at the same calendar time. By contrast, in the Netherlands and Poland, women in different age groups had nonparallel mortality rates during the 1950–2011 period, and such a pattern suggests that cohort effects predominate [24]. For example, hypothetically, there might be a spike in mortality at age 50 in 1990, but no simultaneous increase in other age groups. Then, in 1995, there might be a spike in mortality at age 55, exclusively. Such a pattern could be explained by a higher alcohol consumption among those who were born in 1940, i.e., a cohort effect.

Next, the investigators examined the effects of age. Mortality peaked at age 60–75 years in all countries except Italy, and cirrhosis mortality peaked at a slightly older age for women than for men. The effect of age on mortality was stronger for women than for men [24].

Finally, the analysis of birth cohorts showed that mortality peaked in Sweden, Finland, and Austria with the 1940–1950 birth cohorts. In Hungary, the Netherlands, and Spain the peak was reached around the 1960 birth cohort, as in Denmark [13], and in Poland it was reached around the 1970 birth cohort. In Italy, meanwhile, the pattern was very different for men and women: For women, cirrhosis mortality peaked with the 1925 birth cohort, while for men it peaked with the 1965 birth cohort [24].

The Global Burden of Disease studies

The Global Burden of Disease concept was introduced by the World Health Organization in 1996 using data from 1990 with projections to 2020. The data have been updated several times, most recently in 2019 [25] and in 2017 before that [26]. The Global Burden of Disease studies produce a comprehensive assessment of mortality and disability for more than a hundred diseases and injuries, and its key metric is the DALY (Disability-Adjusted Life-Year) which encompasses both mortality and morbidity—it is the sum of years of life lost (mortality) and the years lived with disability (morbidity) [27]. The morbidity is a product of a condition's prevalence and its disability weight, but the disability weight for cirrhosis is so small that the DALY almost exclusively reflects mortality whilst morbidity is negligible [28, 29]. The Global Burden of Disease studies remain important for their analyses of prevalence and of mortality attributable to cirrhosis. A related strong point of the Global Burden of Disease studies is that access to data is simple (https://vizhub.healthdata.org/gbd-results/) and includes a tool to produce visualizations (https://vizhub.healthdata.org/gbd-compare/).

The Global Burden of Disease studies collect data from countries' cause-of-death and other healthcare registries, relying on verbal autopsies (interviews with family members or caretakers) of samples of decedents when nationwide registries of causes of death are unavailable [29, 30]. Modeling is used to fill in the gaps in the dataset.

Alcohol-related liver disease is defined as the alcohol-attributable portion of the following ICD-10 codes for liver disease: B18.x, I85.x, I98.2, K70.x, K71.3–K71.51, K71.7, K72.1–K74.69, K74.9, K75.8–K76.0, K76.6–K76.7, K76.9 [26, 31]. Based on the most recent data from the Global Burden of Disease, the highest age-standardized mortality rates from alcohol-related liver disease in Europe in 2019 were in the Republic of Moldova (23 per 100,000 person-years), Romania (15 per 100,000 person-years), and Ukraine (13 per 100,000 person-years), while the lowest were in Iceland (1.5 per 100,000 person-years), Norway (1.7 per 100,000 person-years), and Italy (1.9 per 100,000 person-years) (Fig. 3.1).

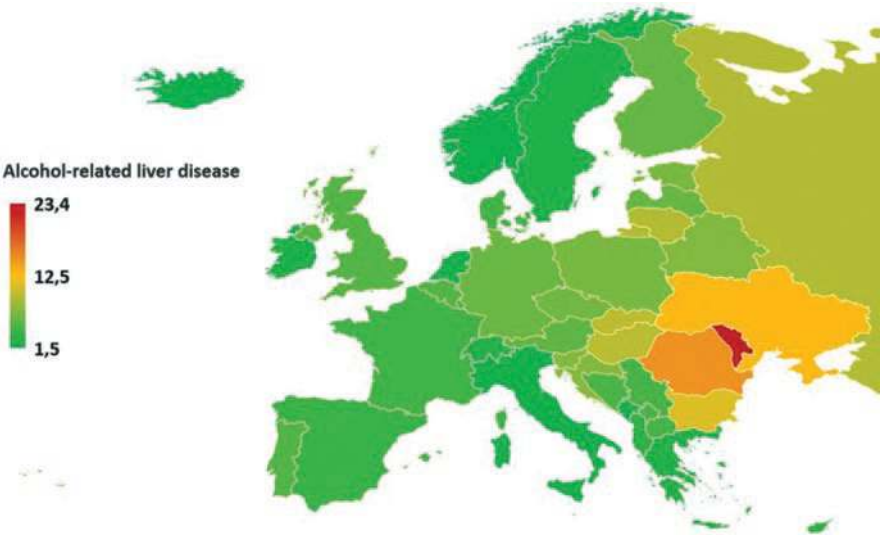

Fig. 3.1 Age-standardized mortality from alcohol-related liver disease in 2019, per 100,000 population

The mortality rate from alcohol-related liver disease in the United Kingdom in 2019 was 5.2 per 100,000 person-years (rank 19), in Denmark it was 4.5 per 100,000 person-years (rank 26), in Italy it was 1.9 per 100,000 person-years (rank 40), in Finland it was 6.0 per 100,000 person-years (rank 17), in Germany it was 6.1 per 100,000 person-years (rank 15), and in Belgium it was 5.1 per 100,000 person-years (rank 20).

Because of the strong association between alcohol use disorder and alcohol-related liver disease one would expect that the geographical patterns were the same for alcohol use disorder and alcohol-related liver disease [31]. That is not the case: Belarus ranks highest (21 per 100,000 person-years) followed by Russia (15 per 100,000 person-years), while Greece (0.3 per 100,000 person-years) and Italy (0.3 per 100,000 person-years) rank lowest (Fig. 3.2).

Belarus and other Eastern European countries have a very high ratio of deaths from alcohol use disorder to deaths from alcohol-related liver disease, suggesting that alcohol-related liver disease might be under-diagnosed or under-recorded in cause-of-death registries. Specifically, in Belarus, the ratio is 3.0, i.e., three times as many people die from alcohol use disorder than from alcohol-related liver disease, and Belarus ranks first when it comes to deaths from alcohol use disorder but tenth when it comes to alcohol-related liver disease. In the Republic of Moldova, it is the other way round; the ratio of is 0.36, i.e., three times as many people die from alcohol-related liver disease than from alcohol use disorder, and the country ranks first when it comes to deaths from alcohol-related liver disease but seventh when it comes to deaths from alcohol use disorder. These apparent inconsistencies may of course reflect the actual causes of death, and they are also seen in countries that take

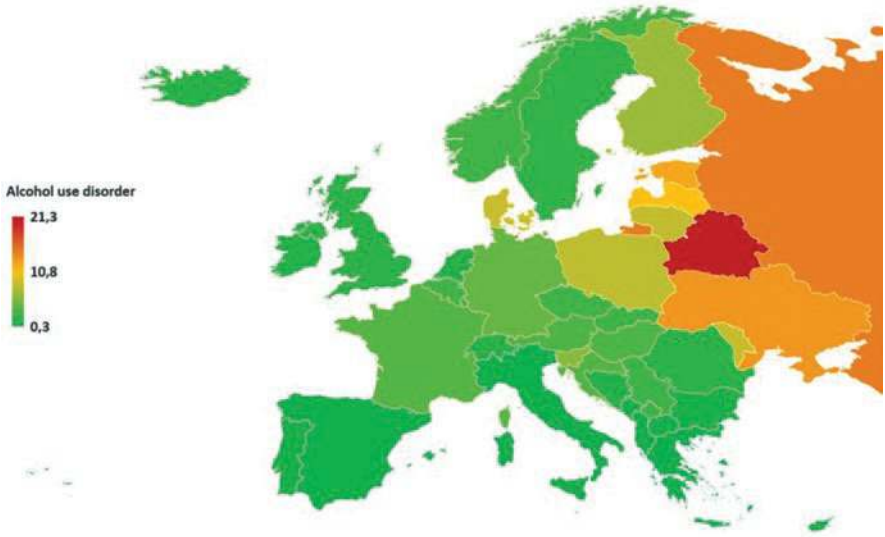

Fig. 3.2 Age-standardized mortality from alcohol use disorder in 2019, per 100,000 population

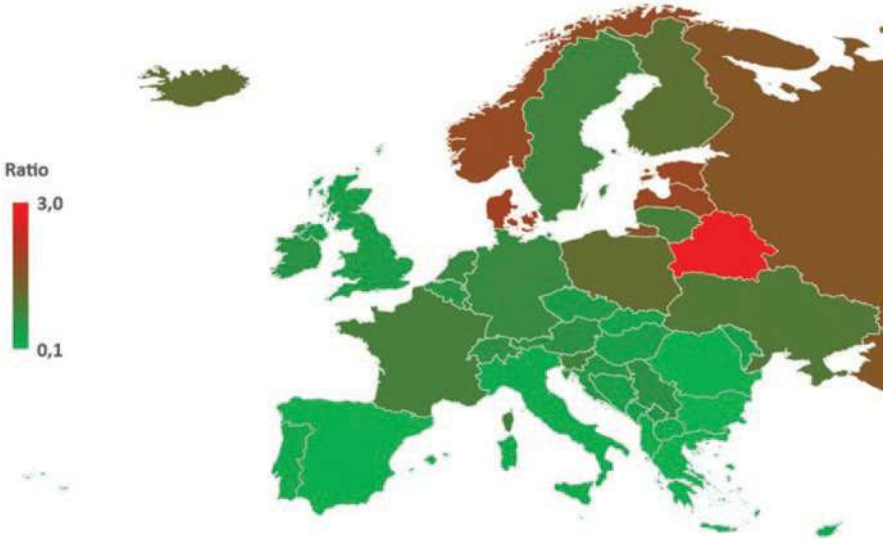

Fig. 3.3 Ratio of deaths from alcohol use disorder to deaths from alcohol-related liver disease. Countries with a high ratio may underestimate the prevalence of alcohol-related liver disease

pride in the quality of their healthcare registries. For example, Denmark ranks sixth when it comes to alcohol use disorder, but 26th when it comes to alcohol-related liver disease (Fig. 3.3).

The HEPAHEALTH Report

In 2018, EASL published its HEPAHEALTH report which described the time-trends in the mortality from liver disease in the EU region and a few additional countries [32, 33]. Mortality data were extracted from the World Health Organization's WHO European Detailed Mortality Database (https://gateway.euro.who.int/en/datasets/european-mortality-database/) combined with data on prevalence extracted from the Global Burden of Disease studies, findings from review articles, and interviews with experts.

The 34 countries under study were divided in four categories on the basis of time-trends in mortality from liver cirrhosis (of any etiology), 1970–2016. The two countries with a stable high mortality were Slovakia and Uzbekistan. The nine countries with an increasing trend were mostly in Eastern Europe, with the United Kingdom as the exception: Bulgaria, Estonia, Finland, Hungary, Kazakhstan, Latvia, Lithuania, Romania, and the United Kingdom. The 12 countries with a stable low mortality from cirrhosis were mainly in Northern Europe: Belgium, Cyprus, Czech Republic, Denmark, Iceland, Ireland, Malta, the Netherlands, Norway, Poland, Serbia, and Sweden. The remaining 11 countries were mostly in Southern or Western Europe and had a decreasing trend: Austria, Croatia, France, Germany, Greece, Italy, Luxembourg, Portugal, Slovenia, Spain, and Switzerland (Fig. 3.4).

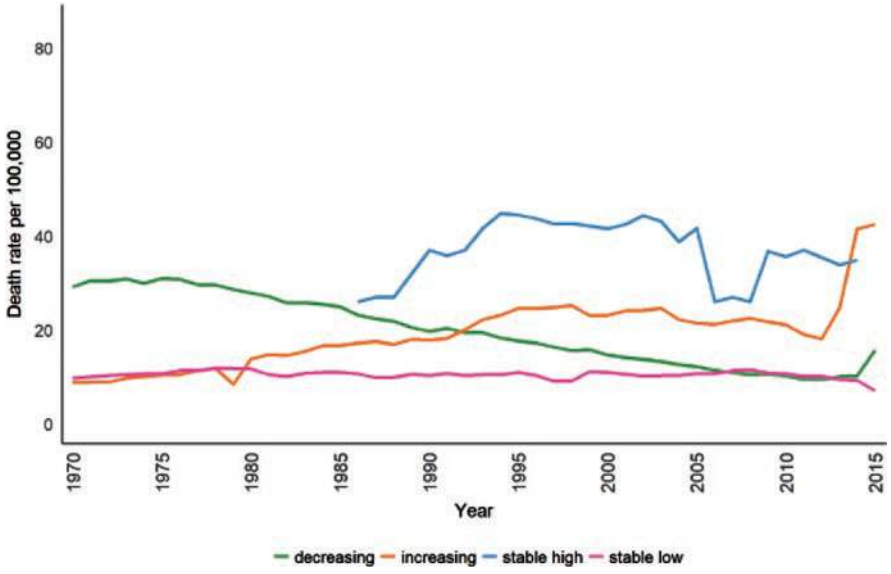

Fig. 3.4 Population-weighted average mortality rate for cirrhosis and other chronic liver diseases for countries in four trends groups (decreasing, increasing, stable low, stable high). The up-turn between the years 2012–2014 for some of the average trends are caused by only a limited number of countries providing data up to 2014/2015. For this reason, the very recent trends should not be considered, as they may be skewed by data from only one or two countries in each group

Alcohol-Related Liver Disease

The HEPAHEALTH Report also described trends by cause of liver disease, including alcohol-related liver disease. It has repeatedly been demonstrated that per capita alcohol consumption correlates with mortality from alcohol-related liver disease, and the time-trends in alcohol consumption follow the four-group pattern shown above for cirrhosis-related mortality: Countries with an increasing alcohol consumption also experienced an increase in cirrhosis-related mortality, and those with a decreasing alcohol consumption experienced a decrease in cirrhosis-related mortality [32]. The HEPAHEALTH Report noted that the Czech Republic had seen an increase in mortality from alcohol-related liver disease despite a stable alcohol consumption [32]. A similar pattern was seen in Sweden, but here the increase in mortality from alcohol-related liver disease may be attributed to a change in the type of alcohol consumed: In Sweden, wine has taken over from beer and spirits as the type of alcohol that is consumed the most [32].

Time-Trends in Selected European Countries

United Kingdom

The United Kingdom has seen a sharp increase in the burden of alcohol-related liver disease correlated with an increase in total alcohol consumption coinciding with a shift in alcohol consumption towards wine and cider rather than beer [14, 32, 34]. In 2014, The Lancet Standing Commission on Liver Disease in the UK was established in response to findings that mortality from liver diseases had increased fivefold whilst mortality from other major chronic diseases had decreased, and mortality from liver diseases had decreased in most other European countries [34]. The Commission pointed to some challenges, which are not specific to the United Kingdom:

- Patients present late, often not until they have developed cirrhosis complications, such as ascites [35]. This is unlike patients with cirrhosis from other causes [16]. Screening for alcohol-related liver disease is a promising response to this challenge.
- Complications of cirrhosis are not managed properly, resulting in high and variable mortality from complications across the United Kingdom. This problem is compounded by the stigma associated with alcohol dependence; alcohol-related liver disease is seen as self-inflicted, and treatment in an intensive care unit is often seen as futile [36].
- Treatment of alcohol dependence is not a priority in hospitals.

The Lancet Standing Commission on Liver Disease in the UK gave recommendations to combat the increasing mortality from liver disease, including interventions targeting alcohol consumption and interventions to engage primary care in earlier

diagnosis of liver disease. In 2020 the Commission's final report was published, lamenting the missed opportunities to reduce mortality from liver disease. Instead, the Commission described a continued increase in the burden of alcohol-related liver disease including an increase in the levels of hospital admissions in deprived areas [37]. To make matters worse, the COVID pandemic led to an increase in alcohol consumption in the United Kingdom [38], and Public Health England issued a press report describing a 21% increase in deaths from alcohol-related liver disease in 2021 (https://www.gov.uk/government/news/alcoholic-liver-deaths-increased-by-21-during-year-of-the-pandemic).

Denmark

In recent decades, Denmark has had a relatively high per capita alcohol consumption and mortality from alcohol-related liver disease [32]. Unlike the United Kingdom, however, Denmark has seen a decreasing burden of alcohol-related liver disease. Mortality among those who have been diagnosed with alcohol-related liver cirrhosis has gone down [19], which might have resulted in an increasing prevalence of alcohol-related liver cirrhosis because patients live longer. In reality, though, the prevalence has been stable because the incidence of alcohol-related liver disease has gone down, particularly in the younger population, i.e., ages 40–49 years and among women aged 50–59 years [13]. By contrast, the incidence has risen slightly in women aged 70 years or older. These patterns indicate a cohort effect, with lower incidence in people born after 1960.

Another contributor to the stable prevalence is the fact that patients continue to come to hospital very late, i.e., there is no screening of at-risk patients, and 69% of patients have developed cirrhosis when they receive their first hospital diagnosis of alcohol-related liver disease [13]. If screening is implemented we will likely see an increase in incidence followed by an increase in prevalence, because the screen-diagnosed patients will have a relatively long survival time because of their diagnosis in an asymptomatic, early stage of alcohol-related liver disease.

The management of Danish patients with alcohol-related liver disease has changed from primarily inpatient to primarily outpatient. Specifically, between 1994 and 2018, the number of all-cause inpatient admissions per person-year of follow-up has decreased from 2.05 to 1.12 and the number of inpatient bed days per year has fallen with it from 17.3 to 7.1. Also, the number of all-cause emergency room visits per person-year of follow-up fell from 2.59 to 0.82. Meanwhile, the number of ALD-related outpatient visits rose slightly from 0.8 to 1.3 per person-year of follow-up, and more than 50% of patients are now first seen as outpatients. This proportion was only 25% in 1994 [13]. These numbers indicate that Denmark has succeeded in transitioning from in- to outpatient care, and this is not because of screening for alcohol-related liver disease and resulting earlier diagnosis: The incidence of alcohol-related liver disease is going down, and the proportion of patients with cirrhosis is stable [13].

Alcohol Consumption in Danish Youth

Despite the decreasing incidence of alcohol-related liver disease in Denmark, there is concern over the Danish youth: Danish 15-year-olds have the highest-in-Europe prevalence of having been drunk at least twice; it is 47% for boys and 37% for girls. By comparison, the European averages are 22% and 18%, respectively, and the proportions in neighboring Sweden are 12% and 10%. The stated proportions are from the latest survey in 2018, and Denmark also held top place in the previous one from 2014 [39]. On the other hand, the Danish National Health Survey has reported a steady decline in the prevalence of high-risk drinking among survey participants aged 16–24 years from 2010 to 2013 and then again to 2017. A similar decline has occurred in older age groups [13]. It remains to be seen whether the incidence of alcohol-related liver disease will rise in the future in Denmark. The available data are consistent with a continued decline, but additional policy initiatives or norm changes might lead to further reductions in incidence.

Italy

EASL's HEPAHEALTH Report included a presentation of time-trends in Italy, an exemplar of a country with a decreasing trend in mortality from liver disease [32]. Age-standardized mortality from liver disease has dropped from 20 per 100,000 population in 1970 to 4.5 per 100,000 population in 2012. That rate is much higher for men than for women (5.7 vs. 3.4 per 100,000 population). Liver cancer is the dominant cause of death among those who die from liver disease, followed by viral hepatitis.

Mortality from alcohol-related liver disease decreased between 2003 and 2012, primarily because it decreased among 55–74-year-olds. The decline was greatest in the 2010–2012 period, coinciding with political initiatives to curb alcohol consumption with restrictions on availability and increased prices, and preceded by initiatives to engage primary care in efforts to offer primary and secondary prevention of harmful alcohol consumption [32].

The age-period-cohort study described above indicated that mortality from cirrhosis has peaked with the Italian 1965 birth cohort [24]. It is possible, therefore, that it may peak when these patients reach the peak age of cirrhosis-related mortality, which would be sometime after 2020.

Finland

Finland, too, was showcased in the HEPAHEALTH Report, as an exemplar of a country with a increasing mortality from cirrhosis [32]. Of particular concern is the high mortality among people of working age resulting in many working-life-years

lost to cirrhosis. Specifically, 45% of life-years lost to liver disease are working-life-years. Alcohol is the dominant cause of liver disease in Finland, and mortality from alcohol-related liver disease doubled from 10.3 per 100,000 population in 1996 to 19.8 per 100,000 population in 2014. The increase was particularly pronounced in 2005–2007, a likely result of Finland's lifting of restrictions on travelers' alcohol imports. This meant that alcohol was being bought in other countries where it was cheaper, Estonia in particular, and then brought back to Finland. Mortality from alcohol-related liver disease peaked in 2009, based on data through 2014 [32], as also reported in the age-period-cohort study described above [24].

Germany

In Germany, the number of admissions eliciting a diagnosis of alcohol-related cirrhosis was stable between 2005 and 2018 and constituted an average of 52% of all admissions related to cirrhosis, followed by admissions related to cirrhosis from hepatitis C (5%). The contribution from alcohol-related cirrhosis has increased in response to the introduction of treatment of hepatitis C with direct-acting antivirals, because these patients are now rarely hospitalized [40].

Belgium

A study from Belgium compared the characteristics of outpatients with cirrhosis diagnosed in two different time periods, 1995–1999 (N = 197) and 2010–2014 (N = 237) [41]. Alcohol-related cirrhosis constituted two-thirds of all patients with cirrhosis in both periods, and more than two-thirds of patients were men, but patients diagnosed in the later period were older: mean age 57 vs. 52 years. This change is consistent with the birth cohort effect seen in the neighboring Netherlands and in Denmark [24].

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Chapter 4
Epidemiology of Alcohol-Related Liver Disease in Romania

Camelia Foncea and Roxana Sirli

Abstract Alcohol related liver disease (ALD) is becoming the main cause of liver disease in Europe and worldwide. ALD is a consequence of Alcohol use-disorder (AUD), an entity quite difficult to define in terms of amount and time of drinking needed for the liver pathology to occur. Understanding the epidemiology and changing behaviors in ALD is very important for screening and prevention, since until not long-ago ALD was not even considered a disease and, from a rarely recognized condition, it is becoming a leader indication for liver transplant. This chapter aims to analyze and describe the prevalence of alcohol consumption and ALD in Romania, a country with relatively high per capita consumption where statistical data about these conditions are scarce.

Keywords Alcohol-related disease · Alcohol-related mortality · Alcohol consumption · Romania · Alcohol-related liver disease

Introduction

Harmful alcohol consumption leads to over 200 diseases and approximately 3.3 million deaths every year [1, 2], of which alcohol-related liver disease (ALD) is the most frequent. In Europe, 41% of the liver deaths are attributed to alcohol [1]. There is a correlation between alcohol consumption and prevalence of ALD in each country. In Europe, mean alcohol consumption is with 10.9 L pure alcohol per year, quite high as compared to worldwide consumption of 6.2 L/year. The average intake of pure alcohol in Europe is >25 g/day among adults, with a high prevalence of heavy drinkers (more than 60 g of pure alcohol on more than one occasion during the past 30 days) of 30.4% [3, 4]. Regarding the trend of alcohol consumption in Europe, in

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rich countries (such as the Central European ones) a slight decrease was observed during the last years, while an increasing trend was observed in the Eastern and the South-eastern parts of the WHO European Region [2–4]. More details are provided in Chaps. 2 and 3.

Definition of Alcohol Consumption

Quantification of alcohol consumption is still a challenge in clinical practice. Since it is difficult to obtain data about the precise alcohol intake in grams of pure alcohol/day, it is recommended to better record standard unit (SU) drinks/day. Unfortunately, definition of a SU varies, e.g., between Europe vs. USA from 8–16 g. In the Dietary US guidelines, one standard drink is defined as a beverage containing 14 g of pure alcohol [5]. In Romania, as in many other European countries, a standard drink is defined as a beverage containing 10 g of pure alcohol [1]. In ALD, the relationship between the intensity of alcohol consumption and liver disease severity is exponential, additional risk factors being obesity and viral hepatitis. In patients with a body mass index higher than 35 kg/m^2, the hepatotoxicity of alcohol doubles, while in patients with hepatitis C virus infection, a consumption of more than 20 g of pure alcohol per day doubles the risk for mortality [6, 7].

The European Association for the Study of the Liver (EASL) recommends that the daily alcohol intake should be limited to less than two standard drinks for women and less than three for men, as these amounts are not associated with a significant increase in cirrhosis mortality [1]. Harmful use of alcohol is defined as causing damage to the health, heavy episode drinking means consumption of more than 60 g of pure alcohol on more than one occasion during the past 30 days, while binge drinking is defined as the consumption of four or more standard drinks for women and five or more d standard rinks for men, within about 2 h [8].

Epidemiology of Alcohol Consumption in Romania

Based on European data recorded by the World Health Organization (WHO) [9], the average consumption of pure alcohol was 11.7 L/capita (individuals older than 15) in 2018 in Romania. Data are also shown in Fig. 4.1. This corresponds to a slight decrease as compared to 2016, when the average alcohol consumption was 12.6 L/capita of pure [10]. When we analyzed data from WHO [3] we could observe that Romania presented a decreasing trend from 2000 when the annual pure alcohol consumption per capita was 17.5 L, to 12.6 L/capita in 2016, reaching 11.7 L/capita in 2018.

Mortality from chronic liver disease is well documented in Europe, with the highest rates being reported for Eastern and North-eastern countries, Romania being placed on the third place. In a study from 2016, ALD was by far the most common cause for death among the alcohol-related diseases and cancers [10].

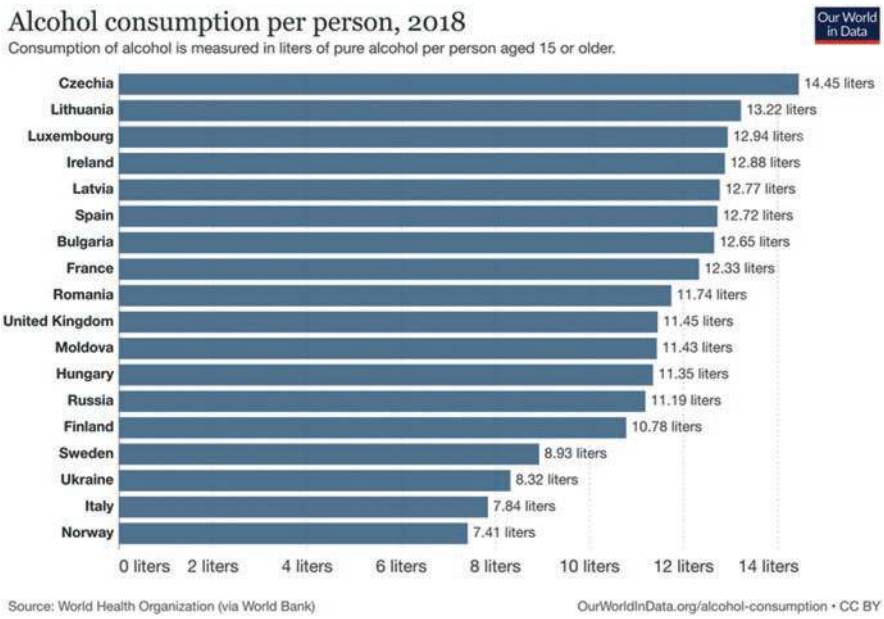

Fig. 4.1 Alcohol consumption per person in Europe 2018. Data from WHO [9]

In 2016, a global burden disease project collected data on liver disease prevalence [11]. Liver disease has been divided into chronic liver disease, cirrhosis, and liver cancer, each subdivided according to etiology: alcohol use, hepatitis B infection, hepatitis C infection and other causes. Accordingly, alcohol is the predominant cause of liver cirrhosis in most countries, especially in Eastern Europe, with a high prevalence of viral hepatitis B and C in Central and Eastern Europe. Of note, Romania also has the highest prevalence of chronic liver diseases in Europe, ALD being the most frequent etiology. The importance of ALD may be even underestimated since alcohol consumption may also contribute to other etiologies, but it is hardly recorded.

Data from the Romanian National Institute of Statistics regarding the alcohol consumption between 2000–2010 shows a decreasing trend followed by an increasing trend during 2010–2018 (Fig. 4.2) [2, 3, 12–14].

An important point of concern is alcohol consumption amongst young people. In Romania, a study evaluated 142 participants, aged between 18 and 35 years, who were questioned by AUDIT test [15]. 123 of them (86.6%) admitted an alcohol intake, while only 19 subjects (13.4%) could be considered as non-drinkers. Alcohol consumption was associated with male gender and smoking. Binge drinking was most frequent among the subjects who were considered drinkers according to the AUDIT test. The study concluded that, in Romania, alcohol consumption among young people reaches a high incidence and has reached worrying levels in comparison to other countries.

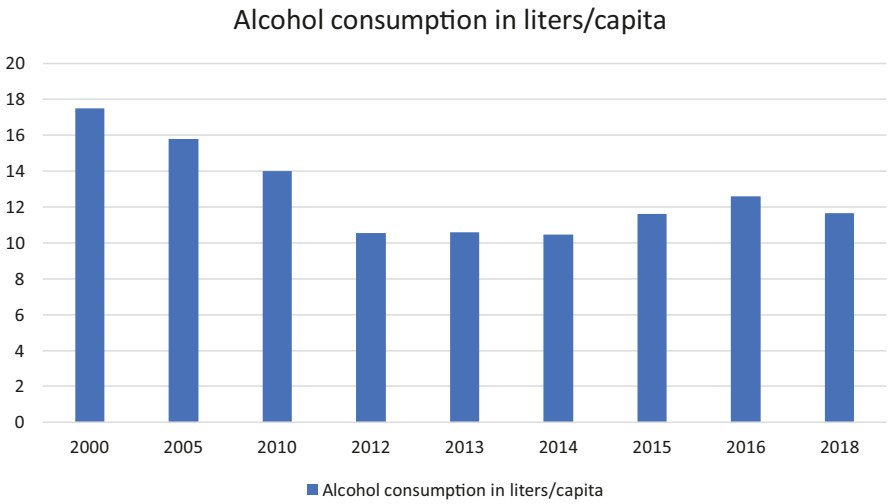

Fig. 4.2 Average per capita consumption (in liters of pure alcohol). Figure made after available data from Romanian National Institute of Statistics and WHO [2, 3, 12–14]

WHO data about alcohol consumption in young people [4] show that, in Romania, the total alcohol per capita consumption in the group age 15–19 years was 10.3 and 17.5 L in the age group 20–24 years among males, while in women it was 3.4 L in the age group 15–19 years and 5.7 in the age group 20–24 years. The prevalence of heavy drinking episodes in the age group 15–19 years was 15.5% in women and 50.7% in males, while in the age group 20–24 years, it was 24.1% for women and 62% for males.

Alcohol-Related Liver Disease in Romania

ALD is a spectrum of diseases ranging from liver steatosis to cirrhosis. Approximately 20–25% of patients who drink heavily will develop cirrhosis over a time period of 15–20 years. Chronic alcohol use of 20 to 50 g/day for women and 60 to 80 g/day for men has been shown to increase the risk for alcoholic cirrhosis [16].

Despite the high consumption levels in Romania, placing it among the European top list of AUD, no national data or registry on ALD are yet available. According to WHO, in Romania there are 6366 alcohol-attributable deaths per year due to alcohol-related liver cirrhosis for both sexes, more than due to road injuries (802/year) and cancer (4676/year) (Table 4.1) [4]. A set of indicators measuring the alcohol-attributable fraction (AAF) deaths were calculated, resulting in the proportion of deaths caused by alcohol.

A recent Romanian study analyzed the relationship between reduction of alcohol consumption by fiscal means and and its harmful effects on health. In this study

Table 4.1 Age-standardized death rates (ASDR) and alcohol-attributable fractions (AAF) in Romania in 2016

	ASDR[a]		AAF%		AAD[b] (as number)
Liver cirrhosis (males/females)	51.8	22.9	78.9	62.1	6366
Road traffic injuries (males/females)	15.7	4.2	46.9	35.9	802
Cancer (male/female)	269.1	140.2	11.8	4.8	4676

ASDR age-standardized death rates, *AAF* alcohol-attributable fractions. Data 2016 WHO
[a]Per 100,000 population (15+)
[b]Alcohol-attributable deaths, both sexes

alcohol was responsible for the top three causes of death in Romania [17]. Alcohol consumption as cause of death was divided into alcohol-related use disorder (AUD), alcohol-related cardiomyopathy, liver cancer and liver cirrhosis. During 2014–2017, the deaths caused by AUD increased in both men and women [17, 18]. Regarding the mortality by alcohol-related cardiomyopathy, an increase was observed in men, from 4/100,000 inhabitants to 8/100,000 inhabitants. In contrast, a small reduction was observed in women, from 1/100,000 inhabitants to 0.5/100,000 inhabitants. The number of deaths attributable to liver cancer due to alcohol consumption remained constant during the 4 years of observation, in both women and men (1.5/100,000 inhabitants). An increasing trend was seen among men regarding the number of cases with liver cirrhosis or chronic liver disease associated to alcohol, from 17/100,000 inhabitants in 2014 to 18/100,000 inhabitants in 2017, and a slight decrease for women (from 8/100,000 inhabitants in 2014 to 4/100,000 inhabitants in 2017) [17, 18].

As already mentioned above, statistics on the epidemiology of ALD are still limited in Romania. Despite this situation, we will present data from published studies regarding various aspects of chronic liver diseases, from which we extracted data related to alcohol abuse as cause of chronic liver disease.

A prospective multicentre study published more than 20 years ago that included 2022 patients and covered all regions of Romania, aimed at establishing the etiological profile of chronic hepatitis and liver cirrhosis in Romania [19]. The main etiological factor of chronic hepatitis was represented by infection with hepatitis viruses (90.8%). Regarding the etiological profile in liver cirrhosis, chronic infection with hepatitis viruses was responsible for 48.3% cases, ALD for 19.5% of cases, mixed etiology (viral and alcoholic) for 16.2% of cases, and unknown etiology in 11.2% cases.

In a previous study from our center evaluating the feasibility of transient elastography in a cohort of 3235 patients with chronic liver disease (CLD) from the Western part of Romania, ALD represented 7.4% of all patients [20]. However, these data may not reflect realistic ALD distribution in Romania, since alcohol could have contributed to patients with cryptogenic CLD (2.7%) and patients with mixed etiology of CLD (16.6%). Moreover, patients had been previously screened for viral hepatitis within a governmental program and were referred specifically for fibrosis assessment.

Table 4.2 Prevalence of ALD in different studies performed in Romania

Studies	Patients included	ALD prevalence (%)
Grigorescu et al. [19]	2022	19.5
Sporea et al. [20]	3235	7.4
Sporea et al. [21]	697	26.4
Barbulescu et al. [23]	839	24.1
Popoiag et al. [24]	261	52.8
Chiriac et al. [25]	446	78.8
Taru et al. [26]	231	46.3
Iacob et al. [27]	257	21
Popescu et al. [28]	815	8.6
Moga et al. [22]	499	41

In another study with the focus on elastography in predicting portal hypertension in a group of 697 cirrhotic patients, 26.4% of were related to alcohol [21].

Unpublished data from our research center in hepatology, on a period of 3 years (2018–2020) studied 499 admissions of patients with liver cirrhosis of different etiologies as compared to patients with liver cirrhosis and Covid-19 infection, in order to assess acute on chronic liver failure and especially acute decompensation. 346 patients were identified, some of them with repeated presentations, out of which the main etiologies of cirrhosis were: in 41% pure alcoholic etiology, in 32% HCV chronic infection, in 10% HBV chronic infection, in 5% mixed HBV/HCV and alcoholic etiology [22].

Data regarding the epidemiology of ALD in Romania are scarce and we present them in Table 4.2. Among cirrhotic patients, in Romania alcohol as cause of liver disease ranges between 7.4% and 78.7%, depending on the area, and on the cohorts included [20, 25]. The prevalence of ALD seems to increase in cohorts with more severe liver disease. A low prevalence of alcohol-related cirrhosis was found in a liver transplant study from Romania [28]. This raises the question about access to transplantation of patients with AUD, and underlines the need to establish a professional healthcare structure able to provide equal professional support to all patients with liver disease including those with AUD, including standardized abstinence rules and access to addiction treatment centers and social support.

Conclusions

In conclusion, data from the WHO and from the Romanian National Institute of Statistics, clearly point out that alcohol consumption in Romania is one of the highest in Europe, making it a serious risk factor for public health. AUD is one of top three causes of death, especially due liver disease involvement.

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Chapter 5
Epidemiology of Alcohol-Related Liver Disease in China

Yishu Chen, Chengfu Xu, Shijing Wang, Jiangao Fan, and Youming Li

Abstract A steady increase in alcohol production, availability and consumption in China has been noted over recent decades, which has led to rising health burdens. In this chapter, we review a multitude of epidemiological studies, both regionally and nationwide, on alcohol consumption, alcohol use disorder and alcohol-related liver disease (ALD) in China. We also collated risk factors that have been reported as relevant to ALD occurrence and development. This updated knowledge serves as a supplement to the global understanding of alcohol and health, and as a reference for the development of health response in China.

Keywords Alcohol consumption · Alcohol use disorder · Alcohol-related liver disease · Risk factor · Disease burden · Chinese population · China

Introduction

Alcohol drinking traditionally plays an important role in Chinese culture. Alcohol serves as a key component of diet and medicine and is a symbol for hospitality and festive events in China. Drinking is thought to be able to lift the spirits at celebrations and ceremonies, and social drinking is encouraged as a means to build good

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business relations [1]. Over recent decades, there has been evidence of a striking increase in alcohol consumption and related problems in China. Here we provide a brief review on the extent of alcohol use and alcohol-related liver disease (ALD) in China.

Alcohol Consumption in China

Commensurate with the thriving economy and the parallel rise in personal income, alcohol production and consumption in China have undergone a notable growth. China has a vast territory with the largest population in the world. People living in different regions share distinctive lifestyles with a diversity of drinking cultures and traditions. Currently, information about the extent of alcohol use in China remains inconsistent and dispersive.

Li et al. reported in 2003 that the proportion of regular drinkers was about 27.0% in Zhejiang Province, southeast China [2]. Between 2000 and 2016, the surveyed percentage of regular alcohol drinkers among the general adults in most regional studies ranged between 27.0% and 37.8% [3–8]. In 2014, Wang et al. reported that 42.76% of the surveyed population in Shandong Province (northeast China) had a history of excessive alcohol consumption [9]. Yan et al. investigated in 2015 that the drinking rate of the adult population in Heilongjiang, China's far-north province, was 47.58%, higher than that of most other regions during the same period [10]. In 2015, Yan et al. reported a total drinking rate as high as 66.2% in China's northwestern provincial-level administrative regions (Shaanxi, Gansu and Xinjiang) [11]. The huge discrepancy between these results suggests that apart from the confounding effects of different sampling methods and diagnostic criteria, there may be substantial differences in drinking habits across regions in China.

In the past decade, several studies have attempted to investigate on a national scale. Li et al. selected a representative sample of residents aged 15–69 years from the 2007 China Chronic Disease and Risk Factor Surveillance (CCDRFS), a continuous survey covering rural and urban districts scattered over 31 provincial-level administrative regions in China, and included 49,527 residents, of which the total current drinking rate was 35.7% (55.6% in men and 15.0% in women) [12]. Between 2010 and 2012, the Chinese Nutrition and Health Surveillance Program conducted a multi-stage cluster random sampling survey on 150 surveillance sites across China. Fang et al. included a total of 60,791 males aged 20–79 years from the program and identified the prevalence of alcohol drinking as 57.8% [13]. Another study which included 15,942 participants (7384 men and 8558 women) with the age range of 45–101 years from the 2011 baseline survey of the China Health and Retirement Longitudinal Study reported that approximately 36.42% of men and 3.73% of women had consumed alcohol in the previous 12 months [1]. The proportion of current drinkers was significantly higher in men than in women in this study. Consistently, a recent study based on the prospective China Kadoorie Biobank (CKB), which recruited 512,715 adults aged 35–74 years from 10 areas across

China from 2004 to 2008 and followed up for about 10 years until 2017, found that 33% of men enrolled at baseline drank alcohol regularly, mainly spirits, compared with only 2% of women [14].

Harmful drinking patterns such as alcohol dependence and abuse have also drawn attention from the public. Alcohol use disorder (AUD) has become a frequent problem linked to disturbances in mental and physical health and social functioning in China. In 2010, AUD was reported to be the ninth leading cause of disability, and the second most important mental disorder after depression in China [15]. The Global Burden of Disease (GBD) 2010 revealed that alcohol abuse was ranked as the sixth greatest risk factor for men in China in terms of attributable disability-adjusted life-years (DALYs) lost, contributing to more than 310,000 deaths among men each year. A recent study based on data extracted from the GBD 2019 reported that the disease burden of AUD in China was increasing from 2005 to 2019 [16].

Disease Burden of ALD in China

Long term excessive alcohol drinking is a leading cause of chronic liver disease and induces a wide range of liver pathologies from simple hepatic steatosis to steato-hepatitis, fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC). At the moment, there remains a lack of nationwide epidemiological surveys of ALD in China, though some studies are relatively large-scale (Table 5.1).

In 2000, Li et al. performed an epidemiological study in Zhejiang Province and reported that the prevalence of ALD was 4.34% [2]. For the 2000–2010 period, the

Table 5.1 Population-based surveys of alcohol-related liver disease in China

Author	Year	Province (area)	Sample size	ALD prevalence (%)	Habitual drinking (%)
Li et al. [2]	2000	Zhejiang (East China)	18,237	4.34 (M 6.36, F 0.36)	26.96 (M –, F –)
Lu et al. [3]	2000	Xi'an, Shaanxi (Northwest China)	3613	2.27 (only one female ALD)	35.15 (M 52.21, F 8.86)
Huang et al. [4]	2005	Hunan (Central China)	18,618	4.36 (M 6.00, F 0.52)	37.79 (M –, F –)
Sun et al. [17]	2007	Dehui, Jilin (Northeast China)	6043	3.98 (M –, F –)	35.03 (M 64.43, F 4.39)
Chen et al. [5]	2007	Liaoning (Northeast China)	6598	6.82 (M 9.75, F 2.00)	26.98 (M 38.33, F 5.64)
Yao et al. [18]	2011	Yuanjiang, Yunnan (Southwest China)	1690	4.97 (M –, F –)	56.27 (M –, F –)
Wang et al. [9]	2011	Shandong (East China)	7295	8.55 (M 15.76, F 1.42)	42.76 (M 74.51, F 11.32)
Yan et al. [19]	2015	Shanxi, Gansu, Xinjiang (Northwest China)	2300	8.74 (M 10.08, F 4.70)	66.22 (M 77.87, F 31.18)

ALD alcohol-related liver disease, *M* male, *F* female, – unavailable

reported prevalence of ALD across regions in China ranged from 2.27% to 6.82% [3–5, 18]. In 2011, the prevalence of ALD in Shandong Province was observed at 8.55% [9]. In 2015, Yan et al. reported that the prevalence of ALD in northwestern regions (Shaanxi, Gansu, and Xinjiang) was 8.74% [19]. Compared with other cities or regions in China, the proportion of current drinkers in Beijing is at an upper middle level (46.10%), though the ALD prevalence (1.30%) is low likely because of the relatively low ethanol intake, according to a recent report [20]. Despite regional differences, the number of patients with ALD in China has demonstrated a rising trend [21]. ALD prevalence rates with habitual drinking reported by some Chinese studies are shown in Table 5.1.

Across studies surfaced multiple notable risk factors of ALD, which have been widely discussed and validated. In the light of the *Guidelines of prevention and treatment for alcohol-related liver disease* (2018, China), risk factors relevant to ALD include dose, pattern and duration of alcohol consumption, variety of alcoholic beverages, gender, ethnicity, obesity, hepatitis virus infection, genetic variability, and nutritional conditions [22].

A threshold effect of ALD has been noted that the risk of liver injury is significantly increased when the dose or duration of alcohol consumption exceeds a limit [23, 24]. Shen et al. found through a population-based case-control study in Zhejiang Province that daily alcohol intake ≥20 g and duration of drinking ≥5 years were closely related to ALD [24], which was consistent with the finding of another study conducted in Shaanxi Province [25]. However, individual difference exists in the dose-response relationship between alcohol consumption and liver injury [24, 26, 27]. Drinking pattern also plays a role; drinking on an empty stomach is more prone to cause liver injury than drinking with meals [25]. Compared with episodic or binge drinking, drinking daily is more likely to cause severe ALD [28]. Different alcoholic beverages do harm to the liver to different degrees [25, 29]. In China, spirits make up about 70% of alcoholic beverage consumption, and it is estimated that up to 25% of the consumed alcohol is not registered [30]. Traditional distilled spirits (*bai jiu*) are the most popular unrecorded alcohols in China, of which production volume has often been underestimated by official statistics. The major health risks posed by unrecorded Chinese *bai jiu* involve not only the high concentration of alcohol but also the potential harm of toxic impurities including heavy metals and acetaldehyde [30, 31].

In China, the proportion of males with high alcohol intake is higher than that of females [32]. Compared with men, women tend to be more susceptible to alcohol-induced liver injury [33]. Moreover, a smaller dose or a shorter drinking duration could give rise to more severe forms of ALD [23, 34], alcoholic hepatitis and cirrhosis in females [35]. Blood alcohol concentration turned out to be significantly different in men and women after alcohol intake of the same dose [36]. Ethnicity [37], genetic variability [38, 39], and individual difference are also important risk factors. Several studies from Taiwan and mainland China have identified genetic polymorphisms among the Chinese Han population, which are different from those reported in the Caucasian population [39–41]. Yu et al. observed in a Zhejiang population that genetic polymorphisms of ethanol metabolizing enzymes, such as

alcohol dehydrogenase (ADH) 2, ADH3 and acetaldehyde dehydrogenase (ALDH) 2, may affect the propensity for ALD occurrence [39]. Such variations in allele frequency and genotype distribution of ALD-predisposing genes may partly account for the lower ALD incidence in heavy drinkers in China than in Western countries. The degree of malnutrition correlates closely with ALD mortality as well and vitamin A deficiency or a lower serum level of vitamin E can aggravate liver injury [42]. In addition, diets rich in polyunsaturated fatty acids have been shown to modulate ALD [43]. Obesity or overweight also leads to a higher risk of ALD progression [24].

To note, hepatitis virus infection, a major public health problem in China, may exert a synergistic effect on ALD. Drinking based on hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, and *vice versa*, could accelerate the development and progression of liver disease [22]. Zeng et al. reported that the positive rates of serum HBsAg and HCV antibody were higher in ALD patients than in alcoholics without developing liver injury [44]. Our previous epidemiological survey of ALD in Zhejiang Province found that 8.83% of ALD patients had HBV infection, of which the positive rate of serum HBsAg was 13.7% in patients with alcoholic cirrhosis and 9.8% in patients with alcoholic hepatitis, suggesting that alcoholic liver injury could increase the susceptibility to hepatitis virus in patients [45].

ALD has imposed heavy burdens on people's health as well as the healthcare system. The WHO Global Health Estimates (GHE) 2015 showed that drinking accounted for 20.0% of all deaths due to cirrhosis and other chronic liver diseases, and 35.5% of all deaths due to liver cancer in mainland China. According to WHO estimates from 2016, the age-standardized mortality from cirrhosis was 14.6 per 100,000 individuals per year in adult Chinese men and 8.3 per 100,000 per year in women, 62.6% (in men) and 41.6% (in women) of which were attributable to alcohol [46]. Data from Beijing 302 Hospital showed that ALD accounted for 3.93% of all in-patients with liver diseases between 2002 and 2013, with the ratio of patients hospitalized for ALD to all for liver diseases increasing from 1.68% to 4.59% [47]. Similarly, the proportion of patients with alcohol-related cirrhosis rose from 3.34% in 2002 to 8.40% in 2013, making alcohol the third commonest cause of liver cirrhosis in China [48]. The proportion of patients with alcoholic hepatitis in hospitalized patients for liver failure also showed an ascending trend, from 0% in 2002 to 5.2% in 2011 [49]. Between 2006 and 2010, admissions to hospital for cirrhosis caused by viral hepatitis decreased by 10%, while admissions for alcohol-related cirrhosis increased by 33% in 31 hospitals in Beijing [50]. Meanwhile, Zhu et al. reported that the proportion of alcoholic cirrhosis and acute-on-chronic liver failure in hospitalized ALD cases underwent an increase from 2007 to 2012 [51].

The nationwide CKB 2017 database showed that, among the 492,643 participants without prior cancer or chronic liver disease at baseline, 2531 cases of liver cancer, 2040 cases of cirrhosis, and 260 non-cirrhotic ALD cases were recorded after a median 10 years' follow-up. For male current regular drinkers, alcohol consumption showed positive dose-response associations with liver cancer (HR 1.44, 95% CI 1.23–1.69), cirrhosis (HR 1.83, 95% CI 1.60–2.09), and non-cirrhotic ALD (HR 2.01, 95% CI 1.77–2.28). The association with ALD appeared stronger among

men reporting flushing. Further, drinking without meals was associated with significantly greater risks of liver cancer (HR 1.32, 95% CI 1.01–1.72), cirrhosis (HR 1.37, 95% CI 1.02–1.85), and non-cirrhotic ALD (HR 1.60, 95% CI 1.09–2.33) [52]. In addition, in another large study form China, ALDH2-rs671 G > A and ADH1B-rs1229984 G > A were genotyped in 150,722 adults, enrolled from 10 areas in China during 2004 to 2008 [53]. The data support a causal effects of alcohol consumption on upper aerodigestive tract cancers, with ALDH2-rs671 AG genotype further exacerbating the risks [53].

Conclusion

Over the past three decades, alcohol production, alcohol consumption, and consequently, incident alcohol abuse and ALD have increased in China. The national alcohol-related health burden will continue to grow in the foreseeable future. Considering the notable differences in drinking patterns, composition of food, genetics, and alcohol metabolism traits across populations, nationwide epidemiological and clinical research on ALD in the Chinese population is yet to be expanded. Besides, government action is expected through a package of means including public education, regulation of alcohol production and consumption, research funding, and tax policy, to reduce alcohol abuse and related disease burden in China.

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Chapter 6
Epidemiology of Alcohol-Related Disease in Russia

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Abstract Over the past two decades a significant decrease was observed in all the indicators that are reflecting alcohol consumption by the population of the Russian Federation. These included total per capita consumption, sales of all beverage types with spirits stopping being the predominant one, unrecorded alcohol use, indicators of registered incidence and prevalence, indicator of alcoholic psychoses, as well as indicators of three main causes of alcohol-related mortality: alcohol poisonings, alcoholic cardiomyopathy, and alcoholic liver diseases. Numerous studies of changes in patterns of alcohol consumption reflected most significant decrease among the younger age groups while populations with established behavioral stereotypes, older age groups and heavy users, changed their alcohol behavior to a much lesser extent. The decisive contribution to these changes was made by the

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multiple government-enforced regulatory measures, including restrictions of physical and temporal availability, advertising restrictions, introduction of minimum price for spirits, several increases of prices and excises, countering illegal production and sales, measures against drunk driving, strict control to avoid sales to minors, as well as improvements in the field of substance use-related medical care. Measures and restrictions were gradually implemented in the Russian Federation during 2005–2016 and strictly enforced.

Keywords Alcohol-related diseases · Alcohol-related mortality · Alcohol consumption · Alcohol policy · Alcohol consumption · Russia

Introduction

During the last decades the global alcohol use was demonstrating obvious trends towards an increase of both the amounts consumed, as well as number of drinkers. Between 1990 and 2017, the proportion of lifetime abstainers decreased from 46% to 43%, global adult per-capita consumption increased from 5.9 to 6.5 L of pure alcohol, and prevalence of heavy episodic drinking increased from 18.5% to 20%. These developments put some risk in achieving global goals to reducing the harmful use of alcohol, and known effective and cost-effective policy measures should be implemented to reduce alcohol exposure [1].

For many years, the Russian Federation has been associated with the highest per capita consumption of alcohol and the most risky drinking patterns in the world [2]. For most of the territories, the combination of "vodka" and "binge" drinking was the prevailing form of harmful alcohol use (often referred to as the "Scandinavian" model, as opposed to the "Mediterranean" one). This model revived in the days of the Soviet Union, firmly taking its place in the culture of the country during the second half of the twentieth century.

Measures of the Alcohol Policy of the Russian Federation

The high level of alcohol consumption and associated social and medical consequences became the reason for governmental actions and for conducting a number of anti-alcohol campaigns in the USSR. The longest by duration and the most intensive one was initiated in 1985 and included a sharp reduction in production and sales of all types of beverages, reduction in number of retail outlets, price increases, the fight against moonshine production, as well as intensive promotion of sobriety and numerous prohibitions and restrictions. According to official estimates, during the years of the anti-alcohol campaign, alcohol sales in the country have decreased by more than 2.5 times, life expectancy increased, especially among men, birth rate increased and death rate decreased [3]. The campaign was terminated in the late 1980s due to economic difficulties, restrictive legislation was no longer enforced, the fight against illegal production and sale of alcohol became ineffective, and

alcohol consumption began to increase again. After the collapse of the Soviet Union in 1991, against the backdrop of a large-scale socio-economic crisis, the state monopoly on the production of alcoholic beverages was abolished, and the vast majority of alcohol-related production and trade enterprises (except for spirits) were privatized; there was a significant deregulation and criminalization of the alcohol market [4]. As a consequence, per capita consumption indicators did increase substantially, above levels a decade ago, and alcohol-related morbidity and mortality rates also increased substantially.

In order to influence this situation and counteract further aggravation of emerging trends, starting from the first half of the 2000s and over the next 10 years, a significant set of measures was consistently implemented to regulate activity of the alcohol industry, sales of alcoholic beverages, their availability and consumption by the population.

Concerns related to the alcohol problem have been repeatedly expressed by senior officials of the country. Of the program documents, the report of the Civic Chamber of the Russian Federation, published in 2009 should be noted, which assessed the demographic, social and economic consequences of alcohol abuse in Russia and proposed ways to address the current situation [5]. The same year, a Decree of the Government entitled "On the Concept for the Implementation of the State Policy to Reduce the Abuse of Alcoholic Products and Prevention of Alcoholism among the Population of the Russian Federation for the Period until 2020" was issued, containing the intended goals and a detailed list of upcoming actions [6].

Countering Illegal Production and Sales

Several generations of obligatory excise stamps (2000–2003) and new, difficult-to-counterfeit stamps (2005) were introduced. In addition, the EGAIS system for collecting and recording data about volumes of produced alcohol-containing products and raw materials, as well as on the import of alcohol (2006) was implemented, followed by the introduction of QR codes for the EGAIS system for registration of retail sales of spirits and wine (2016).

Advertising Restrictions

Measures aimed at limiting advertisement of alcoholic beverages were efficiently implemented: Prohibition of beer advertising on television during daytime in 2004; Prohibition of alcohol advertising on all types of public transport infrastructure in 2008; Prohibition of alcohol advertising on the Internet and electronic media in 2012 and alcohol advertising in all print media in 2013. In 2014, advertising laws were softened for domestic winemaking and some restrictions on advertising of beer and drinks until 2019 in connection with holding the 2018 FIFA World Cup in the Russian Federation.

Restriction of Physical and Temporal Availability

In 2005, sales on the territory and nearby certain types of locations were prohibited, including educational, medical, sports facilities, public transport. Another measure implemented the same year prohibited sales of alcoholic above 15% vol. in a number of public places, as well as individual sales, and sales in any establishments without appropriate licenses. In the following year, legislative measures came into force in a number of regions to restrict retail sales (with the exception of public catering) of alcoholic beverages with concentrations above 15% vol. during night hours. A ban on sales of alcoholic beverages at gas stations was implemented in 2011, followed by a ban on the retail sales of any alcoholic products (including beer) at public transport stops in 2013.

Limiting Affordability and Increasing Excise Taxes

Minimum retail prices for beverages with strength above 28% vol. were introduced in 2010 and excises were increased by 10% per year as part of an amendment to the Tax Code in the same year. This was followed by further increase in minimum retail prices of spirits (2013), increase in excise taxes on alcohol by 33% (2014), further increase of minimum prices of vodka (2014), a temporary reduction of minimum price for vodka to replenish regional budgets (2015) and, finally, a further increase of minimum price for vodka (2016).

Combating Drunk Driving

Zero tolerance for alcohol use by drivers and requirement of blood alcohol concentration of 0.0% while driving was introduced in 2010. A breathalyzer limit of 0.16 mg/L (to account for highest possible measurement error) while maintaining the "zero tolerance" policy and making the punishment for drunk driving more severe was made effective in 2013.

Prohibition of Alcohol Sales to Minors

Strict control and strengthening of administrative responsibility for selling alcoholic products to minors was implemented in 2011, followed by further increase in fines for the sale of alcohol to minors, possibility of criminal liability for repeated violations in 2014.

Measures in the Field of Medical Care

An initiative to improve the system of treatment of alcohol and drug dependency was implemented in 2011 and a "Health Development" program was initiated in 2014 to prevent harmful use of alcohol. A project of social communications "Health Factory" was initiated in 2015 aimed at eliminating risk factors for NCDs (including alcohol disorders) and focused on active people of working age. At the initiative of the WHO Office in the Russian Federation and leading country medical institutions, a manual for educating trainers in the field of screening and brief preventive counseling for risky and harmful use of alcohol was published in 2016. Most of the restrictive measures against alcohol were undertaken in 2004–2016 in the Russian Federation which can reasonably be considered as evidence-based and in line with the WHO Global Strategy to Reduce the Harmful Use of Alcohol of 2010 and the WHO Global action plan for the prevention and control of noncommunicable diseases 2013–2020. Important elements of the success implementation of these measures were commitment to the chosen goals, gradual introduction and strict implementation of alcohol control measures.

Alcohol Sales and Alcohol Use

Analysis of alcoholic beverage sales to the population of the Russian Federation provides insights for a number of observations that are important for understanding features of consumption during the reviewed period, as well as for making assumptions about the impact of specific anti-alcohol policy measures.

Data on sales of alcoholic beverages to the population is annually presented by the Federal State Statistics Service (RosStat), the Federal executive body responsible for compiling official statistical information on social, economic, demographic, environmental and other social processes in the Russian Federation [7]. For a better illustration, total ethanol content per main types of alcoholic beverages in the overall structure of consumption was compared. Figure 6.1 illustrates (with some exceptions for 2015) that spirits have been the dominant type in the structure of alcoholic beverages sold in Russia for the 2000–2018 period under review. In 2000, sales of spirits to the population corresponded to 86.4 million decalitres in terms of ethyl alcohol, and the share in the structure of consumed drinks was about 72% of the total 120.8 million decalitres. However, consumption of spirits showed a gradual downward trend. In 2013, they already accounted for less than half of the ethyl alcohol in the structure of beverages sold, and after 2017 their share was slightly below 40 million decalitres per year which corresponded to about 43% of total ethanol sold (Fig. 6.1).

In contrast, beer consumption increased, and it accounted for 25.9 million decalitres of ethanol in 2000, and for the maximum of 51.7, which corresponds to 22%

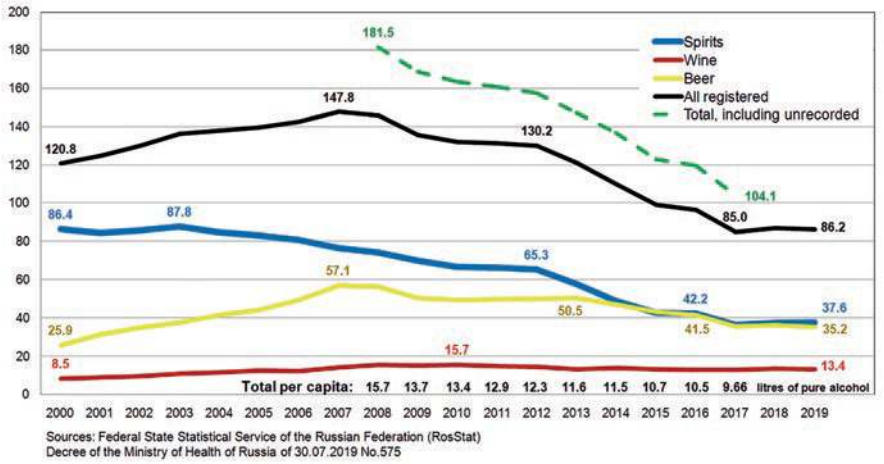

Fig. 6.1 Sales of alcoholic beverages to the population of the Russian Federation and assessment of unrecorded consumption (millions of decalitres in terms of pure alcohol) and total annual per capita consumption (litres of pure alcohol)

and 39% among all beverages sold these years. After 2014, the proportion of beer in the structure of the total ethanol consumption almost equaled with spirits and amounted to about 40–45%. Throughout the period from 2000 to 2019, wine has always been in the third place, however, its consumption has increased from 8.5 to 13.4 million decalitres in terms of ethyl alcohol content, and the share among other types of alcohol—from 7% to 15%.

Published assessments of total alcohol consumption, including recorded and unrecorded, corresponds to changes of per capita levels from 15.7 L of ethanol in 2008 to 9.7 in 2017, i.e., decrease by 38% over 10 years [8].

Unrecorded Alcohol

Unrecorded alcohol production has always been a significant part of ethanol produced and consumed in the Russian Federation. Such unrecorded alcohol includes products that contain ethanol but are not included in official sales, production or trade statistical data, or they are not taxed as beverages but are nevertheless consumed. This is a large and heterogeneous group of products, most of which have a high concentration of ethanol. Such products include illegally produced, undeclared or contraband alcohol; counterfeit alcoholic beverages in replica bottles; homemade alcohol; alcohol surrogates, such as alcohol-based cosmetic lotions and colognes, medicated formulations, and windshield washer fluid [9, 10].

During certain periods, unrecorded consumption in the Russian Federation was making up to a third of total; according to estimates, proportion of illegal production

was about 43% of unrecorded alcohol, home-made production—29%, surrogate alcohol—22%; the remaining 6% were brought from abroad by individuals. It should be noted that compared to other countries of the WHO European Region, the Russian Federation had one of the highest proportions of surrogate alcohol in total alcohol consumption [11, 12].

Alcohol-Related Disorders, Based on State-Supported Medical Facilities' Data

The data on treatment demands reported by state-supported medical facilities of the Russian Federation indicate that alcohol-related disorders predominate in the structure of chemical dependency burden. Most of the patients who were seeking treatment were those with alcoholism, alcohol psychoses and with the diagnosis of "harmful alcohol use". Over the past 20 years, proportion of such patients among all with psychoactive substances associated disorders made up around 80%.

Prevalence and incidence indicators. Over the past 15 years, rates of general and primary incidence of alcohol disorders have shown a downward trend: As compared to early 2000s with two thousand or more patients with alcohol dependency and harmful alcohol use per 100,000 total population, this indicator has continuously decreased since 2005, now reaching 1009.7 per 100,000 in 2019, i.e., a decrease by half. The primary incidence has also significantly decreased—from 264.3 per 100,000 of the population in 2004 to 79.9 in 2019, or by 3.3 times (Fig. 6.2).

In different age and gender groups—men, women, children and adolescents—the rates of decline differed, but the general downward trend can be traced quite clearly: the average rate of decline in primary incidence in general in 2003–2019

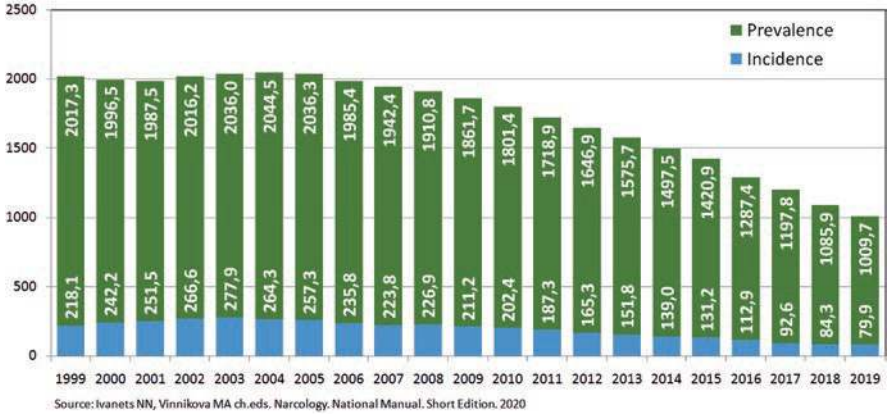

Fig. 6.2 Registered prevalence and incidence of alcohol-related disorders (per 100,000 population)

was 7.5% annually. At the same time, a high level of reduction in primary morbidity was observed in all age and gender groups: in children aged 10–14 years, the indicator decreased on average by 5.4% per year, in adolescents (15–17 years old)—by 6.1%, in adults of working age—7.4%, in men—by 5.6%, in women—by 8%.

A notable feature of changes in incidence among children, adolescents and youth populations was the decrease in the number and proportion of treatment demands related to more severe forms of alcohol abuse (dependency syndrome, alcoholic psychoses) and an increase in the proportion of patients with early forms of disorders (hazardous use of alcohol), which indicates positive changes in the structure of incidence of alcohol-related disorders. From the clinical point of view of view, identification of alcohol disorders at the stage of harmful use, i.e., before the formation of addiction syndrome, determines better prognosis and higher effectiveness of therapeutic interventions, especially among children, adolescents and youth [13, 14].

Alcoholic Psychoses

Due to the fact that alcoholic psychoses are the disorder that is most fully recorded by medical institutions, the incidence of alcoholic psychoses is often seen as an indicator to characterize overall alcohol situation.

The highest level of alcoholic psychoses-related treatment demands over the past 20 years was observed in 2003 and equaled 55.5 per 100,000 population. Subsequently, this indicator steadily decreased and in 2019 made 12.5 per 100,000 population, or decreased by almost 4.4 times (Fig. 6.3). A decrease in incidence of alcoholic psychosis is observed in all gender and age groups of the population of the Russian Federation [14].

The rates of hospital admissions also decreased: compared to 2003, the number of hospitalizations due to alcoholic psychoses decreased by three times—from

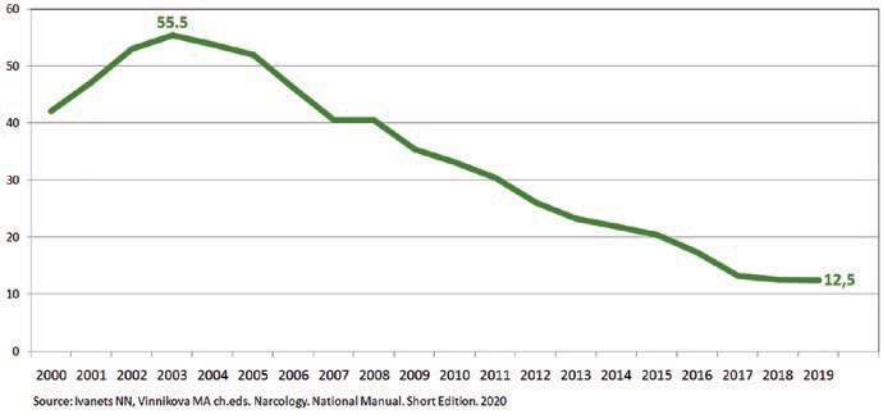

Fig. 6.3 Alcoholic psychoses registered incidence (cases per 100,000 population)

122.5 to 41.4 per 100,000 population. A similar situation was observed among adolescents: the number of hospitalizations of adolescents with alcoholic psychoses decreased from 230 in 2003 to 10 individuals in 2019.

When monitoring the alcohol use situation, comparative analysis of indicators of incidence and primary hospitalization with alcoholic psychoses was carried out. It should be noted that the indicator of incidence is reflecting outpatient referrals, and primary hospitalizations are reflecting referrals for inpatient care. Under ideal conditions, these figures are close, indicating continuity between inpatient and outpatient care. Unfortunately, the situation is far from being perfect: hospitalization rate significantly exceeds the outpatient incidence rate, which indicates that the outpatient rate could be underestimated by 1.5–two times, and for correct assessment of the alcohol situation, it is advisable to use both hospitalization indicator along with outpatient incidence. Despite these uncertainties, the dynamics of hospitalization rates for 2003–2019 also show simultaneous decline in both indicators.

Alcohol-Related Mortality

During the last 20 years, Russia has experienced a decline in alcohol-related mortality of unprecedented depth and duration. Figure 6.4 presents data of Rosstat on the dynamics of mortality in the Russian Federation from accidental alcohol poisonings, alcoholic cardiomyopathy and alcoholic liver disease, which in total account for around 85% of all alcoholic mortality since 2000.

The forced elimination from the vodka market of a notable number of small and medium-sized actors after 2006 due to a number of state regulatory measures introduced in 2005–2006 led to a significant reduction in illegal and legal sales and a

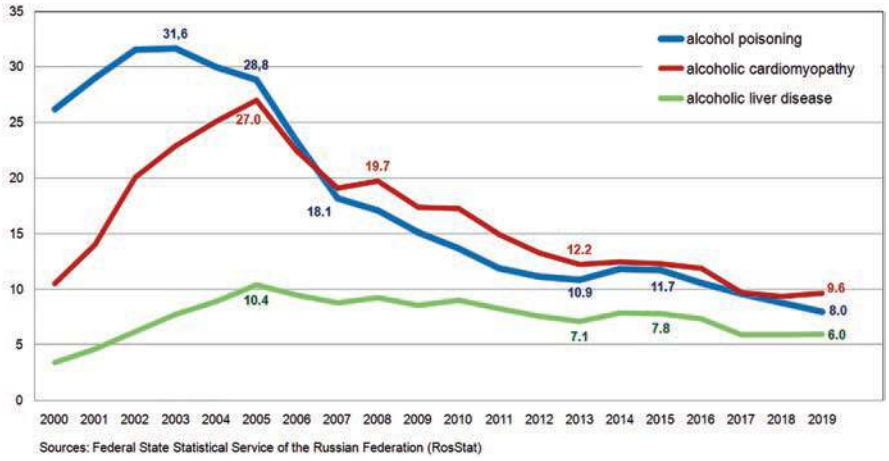

Fig. 6.4 Dynamics of mortality in alcohol poisoning, alcoholic cardiomyopathy and alcoholic liver diseases in 2000–2019 (standardized mortality per 100,000 population)

sharp decrease of both consumption of spirits and of mortality in 2006–2007 (Fig. 6.4). Additional restrictive measures on time of alcohol sales, pressure to observe age requirements, bringing up excises, introduction and gradual increase on minimal price of spirits and tight control over the legality of alcoholic products sold to the population supported a further, almost synchronous decrease in alcohol consumption and mortality from the main alcohol-related causes in 2008–2013 (Fig. 6.4). In general, between 2005 and 2019 mortality due to alcohol poisonings decreased by 78% (from 27.2 to 6.3), from alcoholic cardiomyopathy by 64% (from 27.0 to 9.6) and from liver diseases by 43% (from 10.4 to 6.0; all per 100,000 total population).

During the same years, a decrease in mortality due to somatic diseases, especially cardiovascular diseases, was observed. However, this decrease cannot be entirely attributed to the drop in alcohol use, due to the fact that in the 2000s there was a significant increase in the well-being of the population, the state carried out a number of measures aimed at protecting the health of the population, including the adoption of the national project "Health" and others. However the contribution of decreased alcohol use remains significant.

Alcohol Use Patterns' Evolution

Sociological surveys are making it possible to study evolution of alcohol use patterns among the population: changes in the frequency and quantitative characteristics, alcohol preferences by types of beverages, including in various population groups—adolescents, young adults, women, groups of high risk, etc.

Data from Roshchina [15] indicate that there were certain shifts in alcohol use patterns of Russia's population already in 2010. Since the mid-1990-s the structure of consumed beverages has changed significantly: first of all, it should be noted that by 2010, there was an increase in the proportion of beer consumers among the population: among men drinkers—from 35% to 68%, among women from 16% to 42%. Moreover, according to the author, the increase in the share of beer occurred due to the reduction in vodka: among male drinkers—from 89% to 64%, among women—from 61% to 36%. In 2010, about a third of Russian women and a fifth of men aged 16 or older did not drink alcoholic beverages at all. At the same time, the share of current non-drinkers increased between 2006 and 2010 among women from 29% to 35%, and among men from 17% to 22%. The dynamics of proportion of non-drinkers during the analyzed period differs in different age groups: the share of non-drinkers among young people under 25 increased the most, while among persons of mature age (41–60 years) and the elderly (61 years and more) the increase was either not observed or was not so significant. In 2010, the proportion of people who drank alcohol frequently (4–6 times a week and more often) was 4.4%, including 7.5% among men and 1.3% among women [15].

Changes in the prevalence of alcohol use are also confirmed by results of telephone surveys, including VTsIOM-Sputnik, whose results also revealed high rates

of current frequent alcohol use [16]. During the period from 2009–2011 the proportion of respondents who consumed alcoholic beverages several times a week, including daily consumption, made up 7–8%, and by 2018–2021, this proportion was reduced to 4–6%. Along with this, the study confirmed a considerable significant increase in the proportion of nondrinkers—from 26% in 2009 to 39% in 2021.

From the point of view of assessing the alcohol situation in Russia, large-scale RosStat studies like the "Study of behavioral factors affecting the health of the population" are of special interest. To date, two have been conducted with the interval of 5 years—in 2013 and 2018, with almost 16,000 participants aged 15 and older in 2013 and 15,000 households covered in 2018. The distribution of respondents by gender and age was representative of the population structure of the Russian Federation. The results indicate a decrease in the proportion of alcohol users among men and women, both among urban and rural residents, as well as in various age groups. The decrease in the proportion of alcohol users in the youth groups of the population is especially noticeable: for example, the proportion of individuals aged 15–19 who drank alcoholic beverages over the last 30 days decreased from 21% to 12%, at the age of 20–24 years—from 52% to 47%, 25–29 years old—from 625% to 53%. Among the older population, the reduction was not as significant. Along with this, it should be noted that the proportion of alcohol users whose high frequency of drinking is combined with the consumption of large amounts of alcohol (according to the RosStat survey, who have drunk five or more standard drinks in a row 10 times or more over the past 30 days) remains virtually unchanged: in 2013—1.9%, in 2018—1.8% of the total number of respondents [17, 18].

Interpretation of observed trends in the use of alcohol by Russia's population has also been the subject of a number of publications based on data from the Russian Longitudinal Monitoring Survey conducted by the Higher School of Economics (RLMS-HSE), a panel survey of households and individuals using multi-stage probability sampling with primary sampling units selected within geographically determined strata. The influence of the effect of gender, age, year of survey and age cohort on the consumption of alcohol and certain beverage types during the period from 1994 to 2016 was assessed. The conclusion was drawn that the downward trend in alcohol consumption observed in recent years can be explained by the predominant decrease in consumption among the younger age groups born after 1990 [19].

Another publication was devoted to the question of whether heavy drinkers changed their alcohol habits in the same way compared to light drinkers, in other words, which model of consumption reduction—polarization model or collective model—better explains the changes of alcohol use practices in the Russian Federation between 2006 and 2018. A comparative study of trends among identified percentile groups characterizing heavy drinkers, near heavy drinkers, moderate drinkers, light drinkers and nondrinkers showed that a decrease in alcohol consumption was observed in all groups, but the scale of changes was proportionately smaller among those who drank more than among those who drank less. However, the consumed amounts fell by a smaller proportion among lighter drinkers than among heavier drinkers. Interactions between the time period and the percentile groups

were significant after 2010 with trends similar for both genders. Obtained evidence failed to support polarization hypothesis and pointed towards soft collectivity hypothesis in the reduction in drinking in the Russian Federation in 2006–2018, when trends across all drinking groups have been downward, although the proportions were different for heavier or lighter drinkers [20].

Results from a number of monitoring surveys studying changes of substance use behaviors over time among certain groups of children and adolescents are also reflecting the reduction of alcohol use since the middle of 2000s.

Four waves of the European School Survey Project on Alcohol and Other Drugs (ESPAD) which compares results among students who turn 16 during the year survey is conducted, included data from the Russian Federation in the years 1999, 2003, 2007 and 2011. Although whole country-representative sample was implemented only once, all four waves of data collection provided coverage of the city of Moscow and thus allow overtime comparisons. Whilst results from 1999 and 2003 demonstrated stable indicators and even increase of some (like last 30 days wine drinking going up from 38% to 47%), the 2007 results showed an obvious decrease in all the indicators, and the trend continued in 2011. The use of any alcoholic beverages during the past 12 months went down from 86% in 2003 to 80% in 2007 and to 71% in 2011 among all students; the use of any alcoholic beverages during the past 30 days decreased from 62% to 56% and then to 37% for the same years, the proportion reporting having had five or more drinks on one occasion during the past 30 days decreased from 38% to 31% and then to 24% for the same three data collection points. The direction of changes was always the same for boys and girls [21].

The international WHO-supported research project Health Behavior in School-aged Children (HBSC) project which is a source of information on the health and well-being, social conditions and health status of 11-, 13- and 15-year-old boys and girls from more than 40 countries reflected positive changes in alcohol use during a later period. In the Russian Federation during the period between 2013/2014 and 2017–2018 data collections, proportion of 11-year-old boys who have ever used alcohol decreased from 10% to 7%, among girls from 8% to 4%; among 13-year-old boys from 21% to 12%, among 13-year-old girls from 20% to 13%. A similar trend was also observed among 15-year-olds: the proportion of boys who used alcohol decreased from 41% to 30% during the same period, and of girls from 44% to 29%. Along with this, the proportion of 15-year-olds who experienced alcohol intoxication two or more times during their lives decreased from 17% to 9% among boys and from 11% to 7% among girls. Thus, reduction in the proportion of children and adolescents who used alcohol is observed in all the age groups analyzed within the HBSC project and the results support positive changes of alcohol situation among children and adolescents [22].

In summary, the results from quite heterogeneous sources about the change in alcohol situation in the Russian Federation all indicate positive changes in alcohol use among the whole population, but to the greatest extent among children, adolescents and youth, during the years when alcohol policy control measures were implemented. To a lesser extent, the alcohol policy has affected the adult population of older age groups, among whom keeping to the prevailing stereotypes of alcohol use was more likely.

Discussion

In the last two decades, anti-alcohol policy has been actively implemented in Russia in the form of introduction and consistent enforcement of legislative measures aimed at reducing the price and time availability of alcoholic beverages, prohibiting advertising of alcohol-containing products, increasing liability for drunk driving, as well as implementing universal and selective prevention measures aimed primarily at for children, teenagers and youth.

The majority of alcohol-related monitoring indicators are reflecting an improved situation with regard to alcohol usage in the Russian Federation after 2005: there is a sharp decrease in alcohol consumption, especially for spirits—by more than two times; reduction in the level of primary and general morbidity due to alcoholic psychosis, alcoholism and harmful use of alcohol, including among children, adolescents and youth people; reduction in mortality from alcohol poisoning and somatic diseases associated with alcohol; reducing the number of crimes committed while intoxicated.

On the other side, however, along with positive trends, there are some observations that still raise concern: despite a significant decrease in alcohol sales and stabilization of death rates from alcohol poisoning at the level of 4.4–4.6 deaths per 100,000 population in 2012–2018, alcohol consumption, taking into account the illegal component, is still high and is estimated at about 10 L of absolute alcohol; the proportion spirits and unregistered alcohol stays relatively high in the structure of alcohol consumption; in the last few years (2018–2019), there has been an increase in hospitalizations of patients with alcoholic psychoses. All this may indicate the exhaustion of the resource of the anti-alcohol measures taken. Despite a downward trend after 2016, the level of alcohol-related crime remains high.

According to sociological surveys data, level of problem alcohol use remained virtually unchanged during recent years, at the level of 1.8–1.9%, and level of alcohol use had almost no decrease among the population aged 40 to 60 years. Also stabilization is observed in the proportion of population who use alcohol daily or almost daily at the level of 4–6%. Thus, against the background of the measures undertaken, alcohol consumption decreased most among the young population, while the population with established behavioral stereotypes—older age groups and heavy users—changed their alcohol behavior to a much lesser extent.

Conclusion

During the first two post-millennial decades there has been a significant decrease in all indicators that reflect alcohol consumption by the population of Russia, as well as its medical and social consequences. Although there may be various explanations for changes in some indicators, the significant number of government-enforced regulatory measures seem to be the major reason, most of them internationally recognized as cost-effective, consistently adopted during a short period of time and

strictly implemented. To consolidate this success, it appears important to continue these measures, and, in case of necessity, to further develop them. To achieve this goal, an efficient monitoring system is required in order to identify potential risks and trends in alcohol consumption and respond in a timely and adequate manner.

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Chapter 7
Alcohol and Mortality: First Preliminary Lessons from a Prospective 15 Year Follow-Up Study

Sebastian Mueller and Johannes Mueller

Abstract This book chapter introduces to all-cause and cause-specific mortality related to alcohol consumption. First preliminary data of a prospective 15 year long-term follow up study in heavy drinkers are presented and discussed. All patients received an initial abdominal ultrasound, liver stiffness (LS) measurements, routine laboratory parameters and additional information on comorbidities and morphometric data. In 803 patients (63.5%), all-cause survival status and in 786 patients (62.2%) the observation interval could be obtained. 159 patients (20.2%) had passed away during a mean observation interval of 3.8 years (1–15 years, median 3.5 years). The cause of death could be clarified in 76 of them (47.8%) and was liver-related in 34%, cardiovascular in 17%, cancer-related in 15%, followed by other causes. Taking into account elevated initial LS values, even about 50% of deaths were liver-related. The age-adjusted relative risk of death (RR) of the overall population was 3.8, with 3.9 slightly higher as for men (3.5). Highest RR was observed in heavy drinkers <40 years old with an RR of 45.0 (106.2 for women and 28.9 for men). RR did not change significantly when only looking at non-smokers. Finally, for the first time, both univariate and multivariate regression analysis identify, next to LS, hemolytic anemia has major long-term predictor of death. A long-term score to predict death in heavy drinkers is developed that includes LS, erythrocyte count, total bilirubin, alkaline phosphatase and age. It reaches AUROCs of up to 0.7 for predicting 7-year-mortality. In conclusion, preliminary data, from the first prospective long-term follow-up study with extensive initial patient characterization identify hemolytic anemia as important, hitherto unrecognized predictor of mortality in heavy drinkers.

Keywords Alcohol consumption · Alcohol-related liver disease · ALD · Mortality · Liver cirrhosis · All-cause mortality · Prospective study · Age-adjusted relative risk of death · Alcohol-related mortality · Liver stiffness

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Introduction to the Historic Background on the Relation of Alcohol, Cirrhosis and Mortality

One reason that limits our understanding of alcohol-mediated disease mechanisms is the **lack of robust prospective all-cause and cause-specific mortality data.** The reasons for it are manifold although an association between alcohol, liver cirrhosis and premature death has been noted early on. For instance, historically, the relation between alcohol consumption, liver cirrhosis and death has been well recognized in autopsy studies dating back to the middle of the nineteenth century and, as an example, Theodor Frerichs' famous "Diseases of the liver" (Klinik der Leberkrankheiten) from 1858 may be quoted here [1]. Frerichs was famous for his in-depth observations and the then state-of-the-art application of laboratory methods, combined with over 10,000 detailed and published autopsies, most of them longstanding former patients. At the beginning of the twentieth century, the relation between alcohol, liver cirrhosis and death became further substantiated. In an analysis of cases with liver cirrhosis from Vienna in 1925, the role of alcohol and even the gender-dependence was clearly identified [2]. In this study, 372 cases of cirrhosis were analyzed and linked to alcohol consumption. Daily drinking of more than 80 g of alcohol was considered as drinking at risk. In males, 14% of cirrhosis cases had never drunken alcohol, 52% drunk at risk and 34% occasionally. In women, 58% of cirrhosis cases had never drunken alcohol, 19% drunk at risk and 23% had occasionally drunken alcohol. Finally, the many autopsy- and biopsy-proven studies were carefully analyzed after the second world war, and a clear relation between dosage, time of exposure and incidence of cirrhosis, precirrhotic lesions and fatty liver was established [3].

The first causal relation between alcohol and cirrhosis was established in non-human primates by Charles Lieber [4]. The study was important since there was an ongoing debate at that time whether cirrhosis was due to caloric insufficiencies. After replacing 50% of caloric uptake with alcohol, all features of alcohol-related liver disease (ALD) could be induced in the liver including cirrhosis [4]. Importantly, many efforts were taken in this study to avoid malnutrition, demonstrating clearly, that it is ethanol that ultimately causes cirrhosis. Nevertheless, while these efforts established a causal relationship between alcohol consumption and liver cirrhosis, they did not study mortality as final endpoint and, today, we are still far from understanding the pathology of ALD, the actual molecular mechanisms, and by which ethanol causes liver disease. In addition, in former autopsy studies, although liver cirrhosis was encountered more often, there were no specific data that related alcohol consumption with all-cause and cause-specific death.

Epidemiological Evidence for Alcohol Consumption and All-Cause Specific Death Rates

Comparison of all-cause death rates, e.g., between the general population and a selected patient cohort with defined risk factors is a typical approach to shed light on the role of risk factors such as alcohol on mortality. These mortality rates can then be adjusted for gender and age. Although the general population also consumes alcohol and the causes of death are multiple, these comparisons provide a useful estimate whether a certain sample has an increased risk or not. Ideally, alcohol consumption should be recorded in a diary for many years and, over time, validated by objective parameters, and, finally, associated with death rates in comparison to other risk factors or conditions. Obviously, for many practical reasons, this has not been done so far, justifying study approaches that aim to draw conclusions from various sample cohorts. Table 7.1 lists a few studies. Table 7.1 is far from being complete but designed to exemplify the various natures of studies that have helped so far in understanding the role of alcohol for death at a statistical level, but also their limitations. First, the studies differ drastically in design, recruitment of data, definition of "alcohol". For instance, all-cause death studies need first to define the terminology "alcohol consumption". In the two quoted meta-analyses, this has been done by (a) using a prospective or historical cohort study design; (b) assessed AUD as diagnosed by a psychiatrist or physician, patients undergoing alcohol detoxification, registration at a temperance board (TB) or driving, using validated questionnaires etc. Accordingly, not all of these measures are able to assess and record the duration and amount of alcohol consumption. This becomes obvious when recruiting study participants within alcohol drinking studies in different institutions. For instance, in a recent two center drug intervention trial, in drinkers recruited through newspaper advertisment, the daily alcohol consumption was 105.5 g per day [25]. However, the consumption of patients recruited at the participating addiction department was 88.5 g per day [25] while the amount of daily drinking of patients admitted for alcohol detoxification in an Internal Medicine Department was 185.5 g per day [26]. In line with this, mean liver stiffness was ca. 10 kPa in the two first cohort, but 18 kPa in the latter, presenting for alcohol detoxification.

There are two **meta-analyses** [5, 6] on all-cause mortality and alcohol consumption. In the 2013 meta-analysis, 81 observational studies were included with 221,683 observed deaths among 853,722 people with alcohol use disorder. In men, the relative risk (RR) among clinical samples was 3.38; in women it was 4.57. Alcohol use disorders identified in general population surveys showed a twofold higher risk compared with no alcohol use disorder in men; no data were available for women. It was also noted that RRs were markedly higher for those younger or equal than 40 years old (ninefold in men, 13-fold in women) while still being at least twofold among those aged 60 years or older. In the 2018 meta-analysis, 31 studies were included out from 11,466 screened papers and 386 full text studies [6]. A total of 6768 all-cause deaths in 276,990.7 person-years of follow-up (36,375 patients) were recorded, and the pooled all-cause mortality rate was 27.7/1000 person-years

Table 7.1 Examples of mortality studies (selection)

Type and reference	Studies	Number of studies/patients/ treatment	Follow-up	Events/predictors
All-cause mortality, AUD[a] and moderate drinking				
[5]	Meta-analysis MOOSE guidelines	81 observational studies, 853,722 people		In men, the relative risk (RR) was 3.38; in women it was 4.57; Alcohol use disorders identified in general population surveys showed a twofold higher risk compared with no alcohol use disorder in men
[6]	Meta-analysis	31 studies, 36,375 patients		Pooled all-cause mortality rate was 27.67/1000 person-years (py)
[7]	125 general practice, retrospective	95,991 patients with alcohol consumption	2000 and 2014	Mortality from ONS mortality register, 25–34 units of alcohol per week (HR 1.26) and 35 units or more (HR 1.71), compared with those drinking 1–7 units per week.
[8]	Single center	1265 alcohol detox, mixed with other diseases, 1362 controls	Median 34 months	HR 12.7 mortality for AUD, predictors: age, smoking, serum creatinine, serum bilirubin, and prothrombin
[9]	Single center	909 patients, alcohol dependence (retrospective)	Median 3.8 years 2000–2010	Preselected anemia (HR 1.67), fibrinogen, and ferritin levels
[10]	Data from the MIMIC-III database	2884 patients with AUD, retrospective	28-Day Mortality	explore the predictive value of red blood cell distribution width (RDW)
[11]	Standard health-screening program	Retrospective cohort study of 430,016 adults	1994–2008	"Modest drinker" (no more than one drink a day) from "regular drinker, 23% males was modest drinker, who gained 0.94 year in life over non-drinkers and had 8% reduction in adjusted all-cause mortality (HR 0.92). regular drinkers had 43% increase in overall mortality (HR 1.43)
Cause-specific mortality, AUD				
[12]	Meta-analysis MOOSE guidelines	17 observational studies, 28,087 AUD patient	10 years of follow-up	Standardized mortality ratios were 14.8 for liver cirrhosis, 18.0 for mental disorders, 6.6 for death by injury and around 2 for cancer and cardiovascular diseases

(continued)

Table 7.1 (continued)

Type and reference	Studies	Number of studies/patients/ treatment	Follow-up	Events/predictors
[13]	Single center	23,371 patients with alcohol dependence (retrospective)	12.5-year observation 2000–2012	Identified 23 physical comorbidities contributing to hospital-based mortality in individuals with alcohol dependence: alcohol-related liver disease (33.7%), hypertension (16.9%), chronic obstructive pulmonary disease (14.1%), and pneumonia (13.3%
[6]	Meta-analysis	31 studies, 36,375 patients		most common cause of death was cardiovascular disease (CVD) (6.9/1000 py), followed by gastrointestinal deaths (5.63/1000 py), unnatural deaths (4.95/1000 py), neoplasms, respiratory diseases, and substance use disorders.
Alcoholic liver cirrhosis				
[14]	Single center	126 patients with decompensated cirrhosis	29 months	Comparison of different liver-related scores (CHILD, MELD,UK MELD, MESO index)
[15]	Single center	100 patients with alcoholic cirrhosis, 1984–1988	Till 2000 (12 years)	100 patients included (90% died, 76% with autopsy), 68 had been autopsied, cumulative mortality after 5, 10 and 15 years 71%, 84% and 90%, respectively. Causes of death were bleeding, liver failure or a combination of these two conditions in 58%, while 11% died of HCC. Using the Cox regression analysis, age, alcohol abuse and alkaline phosphatase were independent and significant predictors of mortality, but Child-Pugh class was not.
Alcohol-related liver disease				
[16]	Retrospective data from all Scottish ICUs	2463 ALD and 3590 patients with severe comorbidities from all Scottish ICU	2005–2010	Alcohol-related liver disease patients had 31% higher hazard of death (adjusted hazard ratio, 1.31).
Alcoholic hepatitis (AH) mortality				

(continued)

Table 7.1 (continued)

Type and reference	Studies	Number of studies/patients/ treatment	Follow-up	Events/predictors
[17]	Single center	71 patients Steroids vs. enteral tube feeding	1 and 12 months, death	Comparable outcome of enteral feeding and steroids in the short-term treatment, steroids have short-term complications but long-term benefits
[18]	Single center	36 patients Infliximab and steroids	1 and 2 months mortality	The study was stopped due to increased mortality in the treatment arm
[19]	Multi center	241 patients	1 and 3 months mortality	Age, serum bilirubin, blood urea, prothrombin time, peripheral blood white blood cell count
[20]		Questionnaires from 142 or 1200 hospitals in Japan, 86 patients	1998–2003	Predictors of mortality includes INR, RBC, WBC
[21]	Meta-analysis	5 studies 418 patients Placebo vs. steroids	1 and 6 months mortality	Survival was higher in corticosteroid-treated patients, survival predictors: corticosteroids, MDF, leucocytes, Lille score and encephalopathy
[22]	Multi center	1053 patients Placebo, steroid or pentoxifylline	1, 3 and 12 months mortality	no effects of pentoxifylline, OR of prednisolone was 0.72
[23]	Meta-analysis	11 studies Placebo vs. steroids	1 and 6 month mortality	HR mortality for corticosteroids was 0.64 for pentoxifylline 0.64
Wernicke-Korsakoff syndrome				
[24]	Single center	61 patients, Retrospective and prospective	2002–2011 Median 5.3 years	Cumulated mortality was 45% and death rate of 7.4 × 100 person-years causes of death included serious bacterial infections (44.5%) and cancer (33.3%).

[a]AUD alcohol use disorder

(py). Some single center studies **retrospectively** analyzed data registries from general practices with information on alcohol consumption and amount [7], used alcohol withdrawal as general criterium for AUD [8, 9], and focused on a single laboratory parameter while extracting data from a database retrospectively [10]. Another example is the retrospective follow up of a standard health-screening program in a normal population with a high sample number and quite detailed information on moderate alcohol consumption in Taiwan [11].

Other studies have tried to analyze **cause-specific mortality** with the same challenge as described above of defining inclusion criteria for alcohol consumption

retrospectively from data bases. As shown in three examples in Table 7.1, outcome differs drastically, and it underlines that careful prospective data are missing. While in one analysis (meta-analysis) [6] cardiovascular events were most common causes for death, another meta-analysis showed liver-related death as most common and cardiovascular events as lower [12]. Overall, 17 observational studies with 6420 observed deaths among 28,087 AUD patients were included. Pooled standardized mortality ratios after 10 years of follow-up among men were 14.8 for liver cirrhosis, 18.0 for mental disorders, 6.6 for death by injury and around 2.0 for cancer and cardiovascular diseases. Standardized mortality ratios were substantially higher in women, with fewer studies available. For many outcomes the risk has been increasing substantially over time [12]. The most common cause of death in the AUD population was cardiovascular disease (6.9/1000 py), followed by gastrointestinal deaths (5.63/1000 py), unnatural deaths (4.95/1000 py), neoplasms, respiratory diseases, and substance use disorders [6].

Many studies **focus on a specific clinical setting with preselected cohorts**, e.g., with alcoholic hepatitis, alcohol-related liver cirrhosis or disease. As shown in Table 7.1 a study can also focus on the diagnosis of Wernicke-Korsakoff Syndrome [24]. But again, in these studies, no detailed information is provided for the comorbidities of these patients such as liver stiffness and detailed blood parameters. Other entities such as alcohol-related cardiomyopathy are not even listed in some statistics while others estimate its incidence from 1–2% of all heavy alcohol users, representing ca. 25% of all non-ischemic cardiomyopathies. The prevalence of alcoholic cardiomyopathy in addiction units is likewise estimated around 25% [27] (see also Chap. 70). Due to the specific health burden in the UK, it has established an institution years ago, the so-called Health and Social Care Information Centre (HSCIC), to specifically record alcohol-related mortality data. Accordingly, for 2011, 6771 alcohol-related death were reported: alcohol-related liver disease ranked first (66%), followed by alcoholic liver cirrhosis (20%), psychiatric diseases (6%), alcohol intoxication (5%) and alcoholic cardiomyopathy (2%). In this context the reader is also referred to the Chap. 10. According, to the report of alcohol-specific deaths in the UK registered in 2020 [28], there were 8974 deaths (14.0 per 100,000 people) from alcohol-specific causes. This corresponded to an 18.6% increase compared with 2019 and was the highest year-on-year increase since the data time series began in 2001. Consistent with previous years, the rate of alcohol-specific deaths for males in 2020 remained more than double the rate for females. More than three-quarters (75%) of alcohol-specific deaths were caused by alcohol-related liver disease peaking at the age between 55–59 years. It should be also noted that WHO- based initiatives such as the Global Burden Disease studies do also not prospectively include routine laboratory measure or other important parameters such as abdominal ultrasound or liver elastography [29].

Taken together, studies on mortality and alcohol consumption are lacking sound prospective data, resulting in obvious contradictions, some of which are not primarily explained by geographic regions but rather the retrospective nature of data collection from various data bases. Specifically, an **ideal mortality study on alcohol consumption** should be composed the following way:

1. Objective parameters should be initially assessed and prospectively followed up with all cause or cause-specific death.
2. These parameters should contain routine laboratory parameters and non-invasive markers of the liver and spleen by abdominal ultrasound and liver elastography [30].
3. Ethnicity, nutritional parameters, clinical parameters and medical history with comorbidities should be also recorded by an educated team.
4. The last amount of daily alcohol consumption, the duration of heavy drinking and the type of alcoholic beverages should be taken also into account.
5. Finally, and ideally but almost impossible, it would be optimal to obtain a complete realistic picture of deceased patients, in the best scenario an autopsy altogether with the complete last medical records. Autopsies would be especially desirable with regard to the cause-specific death and to the development of cancer in various tissues, but also disease such as cardiomyopathy or brain alterations.

Moreover, it is recommended to mostly include heavy drinkers to increase the signal-to-noise ratio. In our opinion, confounders such as diabetes or obesity should not be excluded but rather included to determine their weight in causing death.

Preliminary Data of the 15 Year HEIDELBERG Prospective All-Cause Death Study in Patients Undergoing Alcohol Detoxification from 2007–2022

Considering the scarcity of prospective mortality data in alcohol drinkers and the still largely unknown molecular mechanisms of disease progression, in 2007, we initiated in Heidelberg a prospective follow-up study and, after 15 years, first all-cause survival data were obtained. Although we have just started to analyze the data, the novel insights justified their inclusion into this book. Extensive data are also provided in the Backmatter of the book (Tables B.1–B.39).

Study Design

The study design is shown in Fig. 7.1. In difference to the studies quoted in Table 7.1, major design elements of this prospective study were as follows:

1. Inclusion of heavy drinkers primarily presenting for alcohol detoxification, mostly through an elective and pre-planned in hospital stay of about 1 week. This approach avoids pre-selection of patients, e.g., with end-stage liver disease. With the inclusion of primarily heavy drinkers (mean 189 g/per day for 14 years) and the long observation period, a significant impact of alcohol in comparison to the general population was expected.

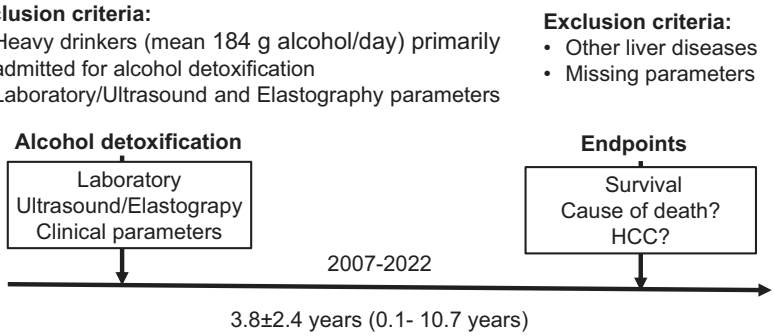

Fig. 7.1 Study design of the 15 year long-term prospective Heidelberg mortality study in heavy drinkers

2. At time of admission, a careful and standardized information sheet were obtained including, e.g., reported daily drinking, duration of heavy drinking, type of alcoholic beverages etc.
3. Furthermore, morphometric data such as weight, comorbidities such as hepatitis C infection, diabetes, smoking status were systematically obtained.
4. In addition, a complete set of routine laboratory data was taken without any preselection of parameters to remain hypothesis-free.
5. In all patients, fibrosis stage and hepatic steatosis was characterized noninvasively based on first a standardized abdominal ultrasound and, second, liver elastography. Transient elastography (Fibroscan, Echosens, Paris) was used to measure liver stiffness and controlled attenuation parameter (CAP). The inclusion of liver elastography from the very beginning guaranteed a complete recording of all fibrosis stages (F0-F4) in contrast to biopsy-based studies. As is shown in Fig. 7.4, biopsy-based studies tend to select tentatively more progressed patients. In addition, liver biopsy is known to have a 10% higher sampling error as compared to elastography with regard to fibrosis staging [30].

Limitations

Nevertheless, this prospective study approach still contains limitations and biases that have to take into consideration for the interpretation of data. Since only Caucasians were enrolled ethnicity could not be explored. Even the setting of alcohol withdrawal in a hospital may include some study bias since only a certain fraction of heavy drinkers may decide to undergo alcohol detoxification under such conditions. In addition, typically for a heavy drinking cohort, we did not manage to obtain in all patients survival status (60% with survival status) and only in 20% a cause of death was obtained with some uncertainties. The biggest challenge, also for

our study, remains the complete medical information about the deceased patients. In none of them, an autopsy was available or could be initiated.

Descriptive Data of Prospective Mortality Study in Heavy Drinkers

Initial patient characteristics at study enrolment are shown in Tables B.1 and B.4. Figure 7.2 shows the descriptive survival data. Briefly, patients were Caucasian heavy drinkers (184 ± 122 g/day) who underwent alcohol withdrawal with a mean heavy drinking duration of 14.4 ± 9.8 years. All patients had initially signed an informed consent. Mean age was 52.2 ± 11.2 years. Age distribution is shown in Fig. 7.3. Figure 7.4 shows fibrosis distribution for both the histologically (left) and non-invasively characterized patients (right panel). This figure underlines the importance of non-invasive fibrosis assessment by liver elastography. While only 4% had normal livers in the biopsy cohort, it was 10 times larger with 40% in the elastography cohort. In other words, both patients and physicians are less likely to undergo an invasive, complication-associated diagnostic procedure, if no objective or subjective signs of illness are present.

Of the initially enrolled 1264 patients undergoing alcohol withdrawal from 2007–2022, some had comorbidities such as viral hepatitis (n = 80, 6.3%) and in 107 cases (8.4%), no contact information was available. This resulted in a total number of alcohol withdrawal patients of 1077 (90.9%). Survival status on follow-up was obtained by multiple measures by a medical study team. In most cases, patients or their general practitioners were directly contacted by phone or mail. In some cases, hospital data or registry data could be used. In 803 patients (63.5%), all-cause survival status and in 786 patients (62.2%) the observation interval could be obtained. Age-related all-cause mortality is shown in Tab. 7.2. In summary, 159

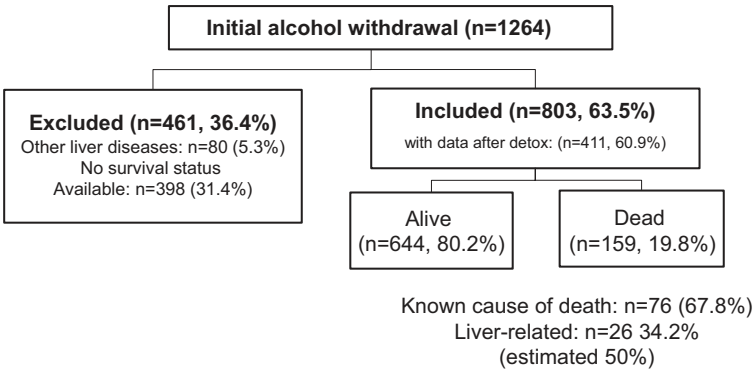

Fig. 7.2 Descriptive preliminary mortality data of the 15 year long-term prospective Heidelberg mortality study in heavy drinkers

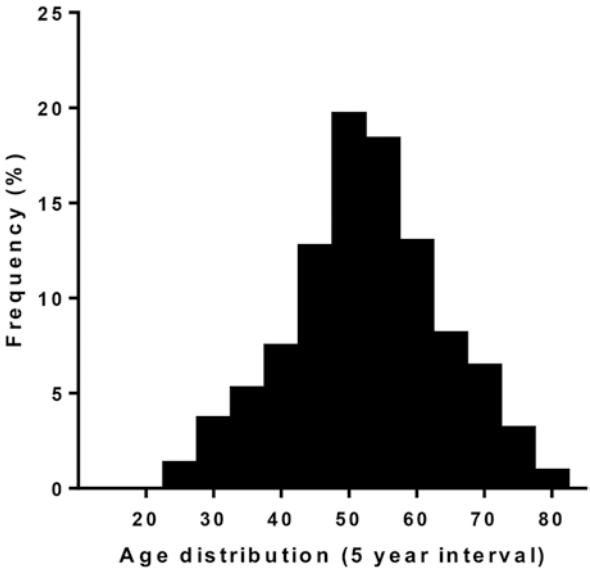

Fig. 7.3 Age distribution of the Heidelberg prospective mortality study in heavy drinkers

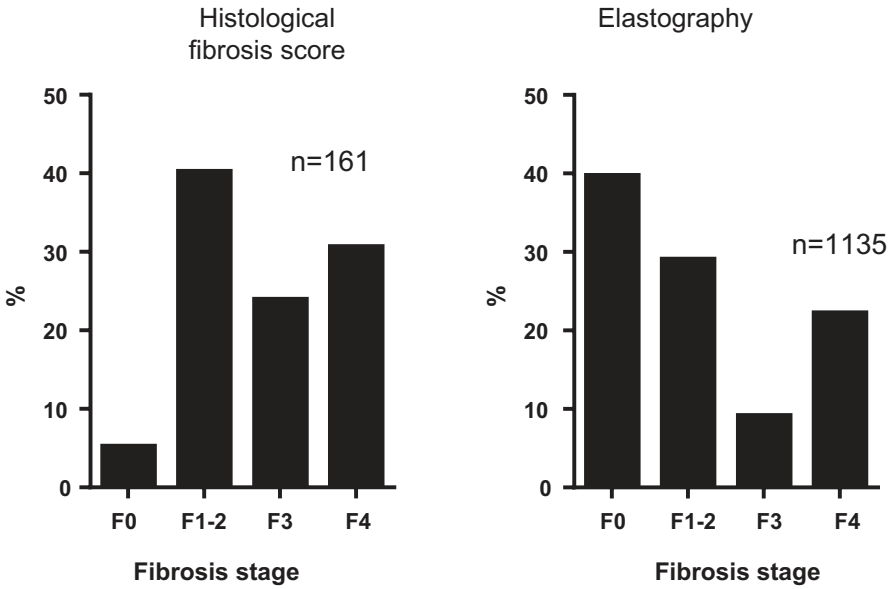

Fig. 7.4 Fibrosis distribution of heavy drinkers from Heidelberg prospective mortality study based on liver biopsy (left) and transient elastography (right). Note that the non-invasively characterized cohort contains 10 times more patients with normal livers without fibrosis

Table 7.2 Age-related all-cause mortality in comparison to the general population in heavy drinkers

Age	Number of patients	Duration of heavy alcohol drinking (years)	Total (n = 803)			Females			Males			HR	
			Death rate drinkers (%/year)	population-wide death rate (%/year)	HR	Death rate drinkers (%/year)	Population-wide death rate (%/year)	HR	Death rate drinkers (%/year)	Population-wide death rate (%/year)	HR	Women/men	Non-smokers/all (n = 226)
All	803	14.4	0.0487	0.0128	**3.8**	0.045	0.01147	**3.9**	0.050	0.01426	**3.5**	0.9	1.1
<40	81	6.3	0.024	0.00054	**45.0**	0.038	0.00035	**106.2**	0.021	0.00071	**28.9**	1.8	1.8
41–50	150	10.7	0.044	0.00167	**26.1**	0.065	0.00120	**54.2**	0.033	0.00214	**15.7**	1.9	0.5
51–60	285	15.1	0.049	0.00475	**10.3**	0.043	0.00331	**12.9**	0.052	0.00619	**8.4**	0.8	0.8
61–70	195	18.4	0.063	0.01244	**5.1**	0.045	0.00861	**5.3**	0.068	0.01660	**4.1**	0.7	1.3
>70	92	17.0	0.040	0.06609	**0.6**	0.032	0.05639	**0.6**	0.044	0.08082	**0.5**	0.7	0.8

Preliminary data (n = 803) are from the ongoing prospective study initiated in 2007 in Heidelberg. Age and gender adjusted. Please note that more patients are used in this table including those which did not have an observation time. However, these patients could not be used for Cox regression analysis

patients (20.2%) had passed away during a mean observation interval of 3.8 years (1–15 years, median 3.5 years). The cause of death could be clarified in 76 of them (47.8%) (Table 7.3).

Mortality Hazard Ratio of Heavy Drinkers in Comparison to the General Population

Mortality hazard ratio (HR) data are shown in Table 7.2. The total number of deaths of 20.2% corresponds to an annual global death rate of 4.8% and, thus, is similar to previous data from the above mentioned meta-analysis [5]. Age adjusted, in Germany, 1.2% had passed away, resulting in a relative risk of 3.8 (RR) which also corresponds well with the previous meta-analysis [5]. For women, RR is slightly higher (3.9) as for men (3.5). For the age intervals <40, 41–50, 51–60, 61–70 and >70, drastic differences were observed: 45.0, 26.1, 10.3, 5.1 and 0.6. Thus, heavy drinking has a much higher RR for mortality at a younger age shows while no differences are seen >70. Although these RRs are in line with other reports [5, 8], they are generally much higher.

Below the age of 40, 91% smoked, corresponding to only 19% at the age higher than 70. However, the mortality HR did not change significantly when only looking at non-smokers.

Cause-Specific Death

A specific cause of death could be obtained 47.8%. Cause-specific death data is shown in Table 7.3. About one third clearly died of liver-related causes due to liver cirrhosis. Initial liver stiffness (LS) was 40.1 as compared to 22.3 kPa in patients who died seemingly without a liver-related cause. The rather high liver stiffness (see Table 7.3), however, suggests that at least in some subgroups, liver may also have been contributed to death such as in the group with cardiovascular-related death (LS = 31.6 kPa) and certainly with infection-related death (53.1 kPa). Notably, rather low LS values were seen in the groups with brain- and cancer-related death (3.7 and 8.7 kPa), while interim LS values were seen (ca. 20 kPa) in the accident-, suicide and lung-related death cohorts. Notably, LS was similar (even slightly lower) in the patient's subgroup with known cause of death and those without (27.4 vs. 33.8 kPa). Importantly, initial LS was drastically lower in those who had survived (16.0 vs. 30.9 kPa). In the light of how in practice causes of death are diagnosed, we are aware that there are potential errors. In addition, there were a few overlaps such as HCC which were assigned both to liver-related death and cancer-related death. It also remains unclear whether the infection of lung-related death

Table 7.3 Cause-specific death in heavy drinkers (preliminary analysis)

Parameter	N	Percentage (%)	Age	Liver stiffness (kPa)
Survival status available	786	66.4	52.4	17.9
Alive	627	79.8	51.6	16.0
Death	159	20.2	57.5	30.9
Cause of death known	76	47.8	59.2	27.4
No cause of death	83	52.2	55.9	33.8
Liver-related	26	34.2	57.3	40.1
Cardio-vascular	13	17.1	31.6	31.6
Cancer-related	12	15.8	58.8	8.7
Suicides	6	7.9	49.4	21.5
Lung-related	6	7.9	62.1	19.5
Infection-related	5	6.6	60.9	53.15
Brain-related (stroke etc.)	5	6.6	60.1	3.7
Accidents	2	2.6	60.7	25.3

Preliminary data (n = 786) are from the ongoing prospective study initiated in 2007 in Heidelberg. Note that number of patients are slightly smaller as shown in Fig. 7.2 since only patients with known observation interval were used. Mean liver stiffness values are shown

was due to manifest liver cirrhosis. Initial liver elastography data are highly suggestive of this association.

Univariate Spearman Rho Correlation and Cox Regression Analysis with Prognostic Parameters: Hemolytic Anemia Determines Long-Term All-Cause Death in Heavy Drinkers

We next analyzed the prognostic value of routine laboratory parameters in 786 heavy drinkers for all-cause death. As is shown in Table 7.4, in **univariate correlation analysis**, besides alkaline phosphatase (AP), RBC count showed the highest and negative association with long-term survival. In other words, anemia is tightly related with long-term mortality. Among other markers of the RBC compartment, RBC count was better than hemoglobin and hematocrit. Apart from AP, markers of anemia were notably better than known other prognostic markers such as albumin, INR or bilirubin. Table B.10 in Appendix shows Spearman Rho correlation with death for all parameters including special laboratories.

Multivariate analysis confirmed that a low RBC count is an independent predictor of death (Table 7.5). Anemia in response to chronic alcohol exposure can have multiple causes ranging from iron deficiency due to blood loss up to inflammation. However, a closer look at Table B.10 suggests that the anemia rather showed typical characteristics of hemolytic anemia. Thus, levels of the hemolytic enzyme LDH, the iron marker ferritin and the end product of heme production, bilirubin, were all positively and significantly associated with long-term death. Moreover,

Table 7.4 Univariate correlation (Spearman Rho) with all-cause death in heavy drinkers

Spearman rho correlation with status dead (1 or 0)			
Parameter	Category	*r*	*p*
Liver stiffness (kPa)	Ultrasound	0.299	**6.0E−17**
Erythrocytes (/pL)	Routine laboratory	−0.281	**1.6E−15**
Signs of cirrhosis (1 or 0)	Ultrasound	0.275	**4.1E−14**
AP (U/L)	Routine laboratory	0.269	**2.4E−14**
Bilirubin indirect (mg/dL)	Special laboratory	0.258	**4.9E−03**
Transferrin (g/L)	Special laboratory	−0.257	**6.2E−11**
CD163 (ng/mL)	Special laboratory	0.256	**6.8E−04**
Hematocrit (%)	Routine laboratory	−0.252	**1.2E−12**
LDH (U/L)	Routine laboratory	0.244	**4.6E−07**
Bilirubin total (mg/dL)	Routine laboratory	0.242	**9.4E−12**
Ascites (1 or 0)	Ultrasound	0.233	**1.3E−10**
Hemoglobin (g/dL)	Routine laboratory	−0.232	**6.5E−11**
Albumin (g/dL)	Special laboratory	−0.229	**1.2E−08**
PTT (s)	Routine laboratory	0.219	**7.7E−09**
INR	Routine laboratory	0.210	**3.6E−09**
Quick (%)	Routine laboratory	−0.208	**5.9E−09**
Age (years)	General information	0.204	**1.0E−08**
Platelets (/nL)	Routine laboratory	−0.192	**6.8E−08**
MCV (fL)	Routine laboratory	0.192	**1.4E−06**
CRP (mg/L)	Routine laboratory	0.175	**1.0E−06**
LDL Cholesterol (mg/dL)	Routine laboratory	−0.170	**3.3E−05**
Cholesterol (mg/dL)	Routine laboratory	−0.168	**7.9E−06**
Glucose (mg/dL)	Routine laboratory	0.168	**6.3E−06**
Duration of heavy alcohol drinking (years)	Alcohol	0.161	**1.8E−03**
Triglycerides (mg/dL)	Routine laboratory	−0.152	**5.4E−05**
Sodium(mmol/L)	Routine laboratory	−0.131	**1.2E−03**
GGT (U/L)	Routine laboratory	0.121	**7.6E−04**
Spleen size (cm)	Ultrasound	0.117	**3.0E−03**
AST (U/L)	Routine laboratory	0.111	**1.9E−03**
HDL Cholesterol (mg/dL)	Routine laboratory	−0.103	**1.2E−02**
Hepcidin (ng/mL)	Special laboratory	−0.094	1.7E−01
Protein total (g/dL)	Routine laboratory	−0.093	**1.6E−02**
CK (U/L)	Routine laboratory	−0.091	1.0E−01
Ferritin (ng/mL)	Routine laboratory	0.076	**3.7E−02**
Haptoglobin (g/L)	Special laboratory	−0.070	1.4E−01
CAP (dB/m)	Ultrasound	0.060	1.9E−01
HbA1C (%)	Routine laboratory	0.054	2.0E−01
Liver size (cm)	Ultrasound	−0.049	2.1E−01
Hepatic steatosis (US) (0–3)	Ultrasound	0.035	3.9E−01

Parameters for analysis included routine and special laboratory, general information, medical history, comorbidities, and morphometric data. An n = 786 was included with 159 deceased patients Parameters are shown in descending order of the absolute correlation coefficient r (ignoring plus/minus signs). Please note that, in contrast to univariate Cox regression analysis, the observation time is not considered. Complete data are provided in Appendix part B Table B.10

Table 7.5 Multivariate Cox regression analysis identifying those initial laboratory parameters that predict mortality

	Univariate				Multivariate			
Parameter	HR	HR lower 95%	HR upper 95%	p	HR	HR lower 95%	HR upper 95%	p
Bilirubin total (mg/dL)	1.126	1.098	1.155	**4.5E−20**	**1.061**	1.018	1.106	**5.0E−03**
Erythrocytes (/pL)	0.407	0.329	0.503	**1.2E−16**	**0.584**	0.439	0.775	**2.0E−04**
Hematocrit (%)	0.899	0.876	0.922	**1.9E−16**				
AP (U/L)	1.006	1.004	1.007	**2.9E−16**	**1.003**	1.001	1.005	**2.2E−03**
Liver stiffness (kPa)	1.025	1.019	1.031	**5.7E−16**	**1.007**	0.999	1.015	**7.0E−02**
Hemoglobin (g/dL)	0.762	0.711	0.816	**1.1E−14**				
Quick (%)	0.978	0.972	0.984	**1.6E−12**				
LDH (U/L)	1.003	1.002	1.004	**1.1E−10**				
CRP (mg/L)	1.014	1.009	1.019	**6.0E−09**				
Protein total (g/dL)	0.467	0.358	0.608	**1.6E−08**				
Cholesterol (mg/dL)	0.991	0.988	0.995	**8.3E−08**				
INR	1.915	1.510	2.429	**8.7E−08**				
Bilirubin indirect (mg/dL)	2.891	1.959	4.268	**9.0E−08**				
PTT (s)	1.032	1.020	1.045	**2.6E−07**				
Age (years)	1.041	1.025	1.057	**2.7E−07**	**1.032**	1.016	1.048	**8.8E−05**
LDL Cholesterol (mg/dL)	0.989	0.984	0.994	**6.9E−06**				
Platelets (/nL)	0.995	0.993	0.997	**1.6E−05**				
Glucose (mg/dL)	1.007	1.004	1.010	**1.7E−05**				
Sodium (mmol/L)	0.934	0.902	0.967	**1.3E−04**				
Triglycerides (mg/dL)	0.997	0.995	0.999	**9.8E−04**				
GGT (U/L)	1.000	1.000	1.001	**5.1E−03**				
MCV (fL)	1.016	1.003	1.028	**1.2E−02**				
Ferritin (ng/mL)	1.000	1.000	1.001	**1.7E−02**				
Urea (mg/dL)	1.008	1.001	1.016	**2.9E−02**				
AST (U/L)	1.001	1.000	1.003	**3.3E−02**				
HDL Cholesterol (mg/dL)	0.995	0.989	1.000	7.0E−02				
Serum iron (ug/dL)	0.999	0.996	1.002	3.4E−01				
HbA1C (%)	1.117	0.878	1.421	3.7E−01				
CAP (dB/m)	1.002	0.997	1.006	4.9E−01				

(continued)

Table 7.5 (continued)

	Univariate				Multivariate			
Parameter	HR	HR lower 95%	HR upper 95%	p	HR	HR lower 95%	HR upper 95%	p
ALT (U/L)	0.999	0.997	1.002	5.4E−01				
Lipase (U/L)	1.001	0.998	1.003	5.7E−01				
BMI (kg/m^2)	1.010	0.973	1.049	6.0E−01				
Leukocytes (/nL)	1.007	0.971	1.045	7.0E−01				
Potassium (mmol/L)	0.964	0.641	1.449	8.6E−01				
Creatinine (mg/dL)	1.034	0.567	1.887	9.1E−01				

An $n = 733$ were include in the univariate analysis with 127 deceased patients. For the multivariate analysis, data from 677 patients could be used

death also correlated highly with large sized RBCs, as indicated by the mean corpuscular volume of RBC, also called MCV and a typical hallmark of drinkers. To further confirm the nature of anemia, we also measured the levels of unconjugated or indirect bilirubin, the end product of hemolysis and precursor of conjugated bilirubin, and the soluble hemoglobin-haptoglobin scavenging receptor CD163 that reflects macrophage-mediated erythrophagocytosis. Both markers showed the highest correlation with death ($r \sim 0.25$) only being surpassed by RBC count and AP. In conclusion, long-term follow up in our prospective cohort of heavy drinkers identifies signs of hemolytic anemia as predominant predictor of death.

Kaplan Meier Plots in Order to Prepare a Survival Risk Score for Heavy Drinkers

Figure 7.5a shows Kaplan Meier plots for major parameters from the multivariate analysis above (Table 7.5). Albumin and transferrin were also included due to their known high prognostic values in patients with ALD but number of patients was smaller (n = 400). Kaplan Meier plots were calculated based on the median of each parameter (low versus high). Figure A.86 shows separate Kaplan Meier plots with the indicated median values used for stratification. As can be seen from Fig. A.86, parameters such as LS, Albumin and transferrin show a continues spread that improves further when looking at longer time periods >5 years. RBC count, bilirubin and AP show a less pronounced spread which is fairly similar over the whole observation time. Transaminase levels ALT and AST but also GGT perform poorly. Interesting enough, total protein also performs much poorer as specific proteins such as albumin and transferrin, indicating that other proteins may compensate for decreased albumin levels. Although Fig. 7.5a is quite packed, it is clearly visible

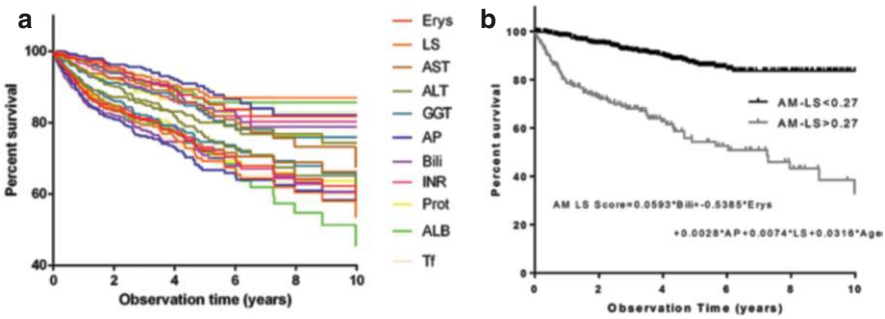

Fig. 7.5 Development of a long-term mortality risk score in heavy drinkers. (**a**) Kaplan Meier plots for the routine laboratory data identified by multivariate COX regression analysis. Although not part of the routine laboratory data, albumin and transferrin are included since they are of prognostic importance in heavy drinkers. Kaplan Meier plots for all parameters are independently depicted in Fig. A.86. (**b**) Alcohol mortality liver stiffness score (AM-LS) to predict long-term death in heavy drinkers. AM-LS score shows AUROCs of 0.7 for 7 years and 0.8 for 1 year

that low LS values are able to rule out death over the total observation time while specific parameters such as albumin stably predict death over many years. Unfortunately, albumin is not part of a routine laboratory.

Development of the Prognostic AM-LS Score

Since we lack an independent validation dataset, the development of a risk score from a multivariate model needs to be regarded with some precautions. For the development of our model, we focused on routine laboratory data, LS and age. In Table 7.5, the results of the univariate and multivariate Cox-proportional hazard model are shown. Accordingly, red blood cell parameters (bilirubin, erythrocyte count, hemoglobin, hematocrit), LS and alkaline phosphatase performed best for predicting overall mortality in this group of heavy drinkers. In contrast, transaminase and GGT levels performed poorly. To avoid overfitting, we only chose the 10 best parameters from the univariate analysis for the multivariate model. Additionally, since high correlations between parameters are problematic for multivariable analysis, we chose only the best parameter from groups of associated parameters. The selection was based either on univariate correlation analysis shown in Table 7.4 or for practical reasons. For instance, erythrocyte count was better correlated with mortality as hemoglobin or hematocrit and INR is more standardized between laboratories than Quick test or PTT. The final model included total bilirubin, erythrocytes, LS, AP, INR, LDH, cholesterol, total protein, age and CRP. With a forward stepwise approach, we identified total bilirubin (mg/dL), erythrocyte count (/pL), AP (U/L), LS (kPa) and age (years) as best and independent predictors for mortality with hazard ratios shown in Table 7.5. A risk score, here called Alcohol mortality (AM) score including LS (AM-LS) for the prediction of overall death in heavy

drinkers could be derived from those parameters. To calculate the score, the following equation can be used:

$$\mathrm{AM?LS} = 0.059 ? \mathrm{Bili\ total}\left[\mathrm{mg/dL}\right] ? 0.539 ? \mathrm{Erys}\left[/\mathrm{pL}\right] + 0.0028 ? \mathrm{AP}\left[\mathrm{U/L}\right] + 0.0074 ? \mathrm{LS}\left[\mathrm{kPa}\right] + 0.0316 ? \mathrm{Age}\left[\mathrm{years}\right].$$

The cutoff value determined through ROC analysis is 0.27. High risk patients would therefore be classified by a score value of >0.27 and low risk patients by a score value below 0.27. In our cohort, 179 patients are classified as high risk and 548 as low risk patients. 3-year survival rate for low and high-risk patients is 92.3% and 68.1% and 5-year survival rate is 86.6% and 54.2%, respectively. The relative risk of high-risk versus low-risk patients is 4.75 (3.34–6.75).

Figure 7.5b shows the Kaplan Meier plot for the AM-LS score. As compared to single parameters shown in Fig. 7.5a, it performs much better over the total observation time.

Table 7.6 shows the performance of the new AM-LS score with two other variants (logarithmized and AM score without LS) in comparison to known scores of liver cirrhosis or alcoholic hepatitis. Performance was calculated in 613 patients with all parameters ($n = 613$) and scores are sorted in descending order based on the *P* value of the Cox regression analysis. Area under the ROCs were calculated for the indicated years 1–7 using the Youden index. All three novel scores performed better

Table 7.6 Prediction of short- and long-term death in heavy drinkers by the developed alcoholic mortality score with and without liver stiffness (AM and AM-LS) in comparison to known scores of liver cirrhosis or AH

	Univariate Cox regression				AUROC				
Score	HR	HR lower 95% CI	HR upper 95% CI	p	Overall	1 year	3 years	5 years	7 years
AM	2.619	2.143	3.202	5.6E−21	0.752	0.810	0.753	0.746	0.698
AM-LS	2.508	2.061	3.054	5.0E−20	0.749	0.811	0.743	0.740	**0.704**
AM-LS LN	1.097	1.075	1.119	2.9E−19	0.757	0.810	0.761	0.747	0.719
CHILD POINTS	1.513	1.350	1.697	1.2E−12	0.624	0.745	0.672	0.641	0.653
MELD	1.129	1.091	1.168	3.1E−12	0.616	0.757	0.629	0.603	0.543
Forns index	1.282	1.189	1.381	8.0E−11	0.724	0.758	0.727	0.709	0.625
Maddrey	1.013	1.007	1.019	1.0E−05	0.601	0.698	0.607	0.569	0.530
Fib4	1.031	1.015	1.048	1.9E−04	0.708	0.740	0.733	0.704	0.650

Performance was calculated in 613 patients with all parameters (n = 613) and scores are sorted in descending order based on the P value of the Cox regression analysis. Area under the ROCs were calculated for the indicated years 1–7. Note that novel AM-LS score is especially better in predicting long-term survival (7 years) for patients without overt liver cirrhosis in abdominal ultrasound. **Scores: AM score** (alcohol mortality score without LS): Score = 0.03*Age + 0.0027*AP + 0.053 *Bili ± 0.549*Erys − 0.003*Platelets − 0.0034*Cholesterol; **AM-LS score** (alcohol mortality score with LS) = 0.0593*Bili ± 0.5385*Erys + 0.0028*AP + 0.0074*LS + 0.0316*Age; **AM-LS LN score** (logarithmized AM-LS: = 17.1 × LN(Age) + 7.8 × LN(AP) ± 18.9 × LN(Erys) + 2.1 × LN(LS) − 79.15 (for convenient calculation multiplied by 10 and subtracted by 79.15 from center around 0), Child points from the CHILD score, MELD score, Forns Index, Fib4 score and Maddrey's discrimination function were used for comparison

both for short- and long-term survival according to *P* values and AUROCs. For practical reasons, we finally asked the question how the scores perform in patients that have no signs of liver cirrhosis in abdominal ultrasound. Data are shown in Table 7.7. Again, the novel scores perform better (AUROCs 0.7–0.72) than established scores (AUROCs 0.56–0.7). The Forns Index performed best among the established scores (AUROC 0.7). Interestingly, addition of LS into the novel score improved slightly the long-term prediction of death in patients without ultrasound signs of liver cirrhosis. On the other side, the novel score without LS performed even better for the prediction of mid- and short-term death below 5 years. With the exception of the Maddrey score, conventional scores performed excellent for 1 year survival, Forns Index and Fib4 even better than the novel scores (AUROC 0.74 vs. 0.72). The rather poor performance of the Maddrey score is no surprise since it was designed for short-term prediction of death explicitly for patients with alcohol hepatitis which represent 2% of our total cohort.

Conclusion

We here present and discuss preliminary data from the first prospective long-term follow-up study in heavy drinkers with extensive initial patient characterization including abdominal ultrasound, liver elastography, and routine laboratory parameters. In 786 patients, all-cause survival status could be obtained. 159 patients (20.2%) had passed away during a mean observation interval of 3.8 years (1–15 years, median 3.5 years). The cause of death could be clarified in 47.8% and was liver-related in 34%, cardiovascular in 17%, cancer-related in 15%, followed by other causes. Based on available initial liver stiffness measurements, a liver-related death may even be as high as 50%. The age-adjusted relative risk of death (RR) of

Table 7.7 Prediction of short- and long-term death in heavy drinkers by the developed alcoholic mortality score with and without LS in patients (n = 488) without ultrasound signs of liver cirrhosis

	Univariate Cox regression				AUROC				
Score	HR	HR lower 95% CI	HR upper 95% CI	p	Overall	1 year	3 years	5 years	7 years
AM	2.852	2.043	3.981	7.5E−10	0.715	0.731	0.717	0.714	0.664
AM-LS LN	1.098	1.065	1.131	1.5E−09	0.718	0.715	0.728	0.715	0.693
AM-LS	2.565	1.858	3.539	1.0E−08	0.703	0.708	0.694	0.702	**0.669**
Fib4	1.106	1.061	1.152	1.4E−06	0.689	0.731	0.729	0.683	0.630
Forns index	1.294	1.163	1.441	2.5E−06	0.702	0.754	0.723	0.697	0.622
CHILD POINTS	1.645	1.219	2.219	1.1E−03	0.546	0.684	0.624	0.592	0.636
MELD	1.051	0.966	1.142	2.5E−01	0.500	0.629	0.534	0.506	0.460
Maddrey	0.997	0.976	1.017	7.4E−01	0.531	0.502	0.489	0.552	0.432

Note that novel AM-LS score is especially better in predicting long-term survival (7 years) for patients without overt liver cirrhosis in abdominal ultrasound

the overall population was 4.0 corresponding well with previous meta-analyses. For women, RR was slightly higher (3.9) as for men (3.5). RR was especially high in patients younger than 40, reaching there a HR 45.0 (106 for females and 29 for males). Importantly, and to our surprise, both univariate and multivariate regression analysis identify liver stiffness and hemolytic anemia has major long-term predictors of death. These findings link alcohol-related all-cause mortality data directly to red blood cell toxicity. While LS is best associated with death using Spearman rho correlation without considering the observation interval, univariate Cox regression analysis identified erythrocyte count and bilirubin as best parameters. Finally, a score was derived to predict long-term death up to 7 years in heavy drinkers based on independent parameters upon multivariate regression analysis. This **alcohol mortality score** includes liver stiffness, erythrocyte count, alkaline phosphatase and age. It remains to be addressed in future studies, how this enhanced RBC turnover is related to alcohol-related liver and bone marrow damage. See also related Chaps. 37, 38, 41, 49, 57, 58 and 64.

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Chapter 8
Legal Aspects of Alcohol Intake: A Romanian perspective

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Abstract Alcohol has been, is, and will be a serious health problem, both due to its adverse health effects and the medico-legal and legal implications. Gas chromatography combined with mass spectroscopy is the current state of the art method to assess blood alcohol levels. An analysis of blood alcohol levels from 2018–2021 in the Timisoara/Romania region demonstrated alarming levels of driving under the influence of alcohol. More than 83% of the drivers had a blood alcohol level > 0.8 g‰. A study on more than 2000 deceased people revealed elevated alcohol levels in 37–49% of all cases, some of which associated with murder, suicide, and fatal domestic violence. Complications of chronic alcohol consumption are also frequently seen in forensic autopsies including alcohol-related organ damage of liver, heart and brain.

Keywords Alcohol intake · Drunk driving · GC-MS · Liver steatosis · Cirrhosis Blood alcohol concentration · Traffic medicine

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Introduction

People have been manufacturing—and drinking—alcohol for nearly as long as the known human history. The term alcohol originally referred to a method of manufacturing a makeup and is derived from the Arabic *al-kuhul* or *al-kohl*, and it has been used with its current meaning since 1672. Chemical analyses recently confirmed that the earliest alcoholic beverage in the world was a mixed fermented drink of rice, honey, hawthorn fruit, and grape. The residues of the beverage, dated ca. 7000–6600 BCE were recovered from early pottery from Jiahu, a Neolithic village in the Yellow River Valley. Current data at the European level show that alcohol dependence remains at alarming levels (5.4% of men aged 18–64 and 1.5% of women). In Romania, there seems to be a decrease in alcohol consumption from 17.4 L (pure alcohol consumption) in 2000 to 12 L (pure alcohol consumption) in 2018 [1]. However, alcohol intake, both in drivers and in general population are worrying due to the high risk of road accidents or aggressions and also because of the long-term consequences.

Typical sources of ethyl alcohol (synonymously used with ethanol or alcohol) are alcoholic beverages with concentrations in the range between 0.5% and up to 95% (see also Appendix Fig. A.3). Alcohol concentration can vary significantly: beer 3.2–7%, wine 7.1–14%, whiskey 40–75%, vodka 40–50%, gin 40–85%, brandy 35–60%, rum 40–95% [2]. Accidental acute intoxication with ethanol, although used in massive quantities in various industries, analytical activities and therapeutic activities, is rather infrequently seen in the context of these professional activities. Most cases of acute non-professional intoxication, however, are due to alcoholic beverages used for recreational purposes.

Determination of Blood Alcohol Concentration

The determination of the alcohol concentration in the human body (blood alcohol concentration), according to Romanian legislation, is made only within the forensic institution [3]. In contrast, the assessment of the presence of alcohol in expired air is made by the traffic police using certifed devices. The respiratory elimination way is applied to new types of devices for determining the concentration of ethanol in expired air (e.g. alcohol test type Dräger). The breathalyzers can be category A (color indicators) with an accuracy of 20%, electronic devices category B with an accuracy of 5%, and breathalyzers that display the correlation ratio of breathalyzer and blood dosage. In case of elevated ethanol during a breath test, the respective person or driver must submit to the collection of IV blood sample to determine the concentration of blood alcohol concentration expressed in g‰. Biological samples are collected at the forensic institution or in other authorized medical institutions in the presence of a representative of the traffic police.

Concentrations of alcohol in the blood are usually given in grams of pure ethanol per liter [4]. Determining ethanol in biological samples by gas chromatography based on the principle of vapor space is a widely applied process worldwide, being introduced in analytical practice for over 50 years. It is also common worldwide practice to use two chromatographic columns in tandem to confirm the results in at least another column [5]. Today, gas chromatography is typically combined with mass spectrometry. At IML Timișoara, the method using gas chromatography–mass spectrometry (GC-MS) determination is applied for the biological samples collected from persons involved in various traffic incidents, from the victims of road accidents or other situations (suicides, suspicious deaths, intoxications). Figure 8.1, for example, shows a case from 2021 of proven lethal methanol intoxication as determined by GC-MS.

Many judicial decisions are influenced or completely depend on alcohol intake of accused or involved individuals. There is also a well-known association between violence and alcohol intake. Under the influence of alcohol, people may become more aggressive, resulting in family violence or domestic violence. Alcohol intake is present in many offenses of hitting or personal injury and is involved in many traffic accidents [6]. According to the current legislation in Romania, the blood alcohol concentration is determined after taking two 6 mL blood samples at an interval of 1 h [7]. Alcoholemia is determined only in the forensic toxicology laboratories within forensic medicine institutions, while alcoholemia determined in other clinical laboratories has no probative value in court. At the national level, the determination of blood alcohol is performed by the GC-MS gas chromatographic method, which is highly specific. Contamination is avoided by desinfecting the skin

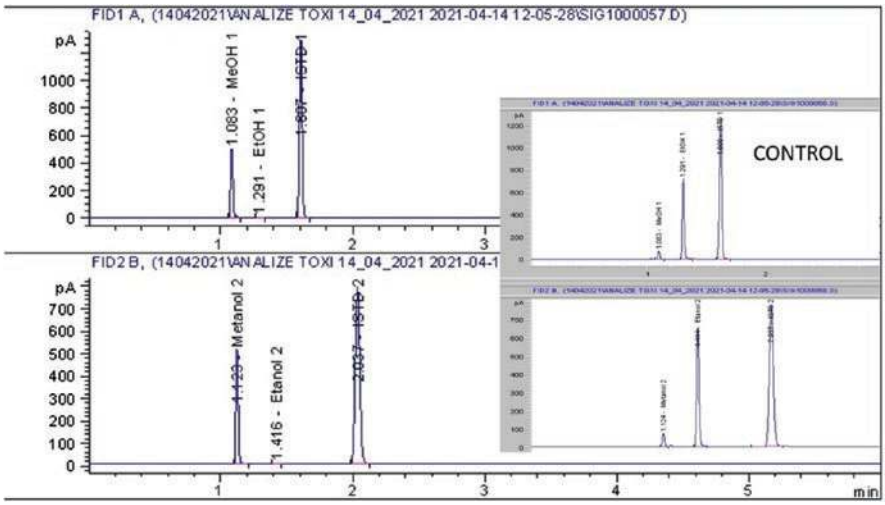


Fig. 8.1 Detection of ethanol and methanol in a blood sample using gas chromatography-mass spectroscopy. In this case, a lethal methanol concentration was confirmed

only with antiseptic substances such benzalkonium chloride or aqueous mercury chloride solution but not ethanol, ether, or benzene, respectively. In the context of crime investigations, due to postmortem transformations in the body, the alcohol concentration as measured by GC-MS depend on several factors including the elapsed time at death or concentrations of other molecules such as glucose and glycols [8].

Legislative Framework

In Romania, like in most other countries worldwide, there is a legal framework regarding the consumption and sale of alcohol. It is forbidden to sell alcohol to people under the age of 18. It is also forbidden to sell alcohol inside and near educational institutions of all grades, boarding schools, and accommodation for pupils and students, in the courtyards of these buildings and on the access roads to these units [9]. Regarding the alcohol intake by drivers, in Romania, the driving on public roads of a car or tram by a person, who has alcoholic imbibition over 0.8 g/L pure alcohol in the blood, is punished by the suspension of the driving license, fine, imprisonment from 1 to 5 years, while driving a vehicle or tram under the influence of alcohol (respectively with a blood alcohol level below 0.8 g‰) constitutes an infringement, sanctioned with a fine and suspension of the right to drive for 90 days. Thus, these Romanian legal provisions go in line with zero tolerance policy of alcohol consumption while driving. In addition, the refusal or opposition of a person who drives a vehicle on public roads, from collecting biological samples to establishing blood alcohol or expired air testing, is punishable by imprisonment from 1 to 5 years [9].

Forensic Features of Alcohol

Ethanol is a colorless, a volatile liquid with a specific smell and burning taste, has a density of 0.79 and boils at 78 °C [5, 10] (see also Appendix Fig. A.2). It is used as solvent, diluent or for synthesis in industrial laboratories, in medicine as an antiseptic and desinfectant, and in the food industry as an preservative, taste bearer or important food constituent for alcoholic beverages. Absorption begins in the oral cavity, followed by the stomach (ca. 20% of the absorption), and the rest in the upper part of the small intestine (for more details see also Chap. 50). The absorption rate depends on the amount and concentration of the drink, the fullness of the stomach, and the person's health state. Under conditions of an empty stomach, absorption is normally completed within 30 min, while 2 h are required a meal has been taking prior to consuming the alcoholic beverage. Other factors are also important for the absorption rate of ethanol: the type of drink (carbonated drinks are absorbed faster), the emotional state, concomitant drug use, and dietary measures, which can

cause oxidation of up to 20% of alcohol. Absorption is increased when the concentration in the stomach is 10–20% alcohol; above this concentration, there is irritation of the mucosa causing increased mucus secretion and slowing down absorption. After absorption, the alcohol is distributed through the circulation and diffuses passively within the various compartments. Organ concentraitons can be different to the blood alcohol. For instance, at a blood alcohol level of 1 g‰, ethanol levels in the brain will be 0.75 g‰ and in 0.85 g‰ the kidneys. Of note, higher concentrations will be found in cerebrospinal fluid and urine (1.25 g‰) due to accumulation of ethanol.

Besides hepatic metabolism under the influence of alcohol dehydrogenase (ADH), which handles about 90–95% of the total ethanol, the elimination of ethanol takes also place in the kidneys, the respiratory tract, and much less by perspiration, and tears. After ingestion, as mentioned above, alcohol reaches its maximum level within 0.5–2 h. Complete absorption occurs between 1–3 h. Blood alcohol level then start to progressively decrease at a constant rate of 0.15 g/h. Thus, the blood alcohol level can be calculated for a specific time retrospectively. The number of drinks ingested can be determined using Widmark's formula (Widmark factor reflects an estimate of the amount of water in the human body) and the value in grams of pure ethanol results [4, 10].

$$\text{Blood alcohol concentration} \times \text{G} \times \text{R} = \text{pure ethanol}\left(\text{G} = \text{body mass}; \text{R} = \text{Widmark factor}\right)$$

$$\text{Widmark's formula} = \text{quantity of ingested alcohol} \times 100 / \text{body mass}\left(\text{kg}\right) \times \text{Widmark factor}$$

For the transformation into volumes, it is divided by the density (0.79), and volumes are obtained. The maximum blood alcohol level appears 30–60 min after ingestion and is mirrored in the curve's ascending segment (1). Any element that affects the rate of absorption will affect the level of the maximum concentration of alcohol in the blood, the duration of the curve in the plateau (2), and the rhythm (3) of elimination of ethanol [4, 10, 11]. The blood alcohol level depends on the rhythm, quantity, and concentration of alcoholic drinks consumed, the time interval in which the alcoholic beverage is consumed, the degree of fullness of the stomach, the enzymatic capacity of the liver, the amount of water contained in the body [11]. Women achieve an increased concentration of blood alcohol at the same amount consumed and the same weight as men due to the difference in the amount of water in the body. Highly concentrated beverages with high sugar content slow down absorption, like beer. Certain medications may increase the blood-alcohol level or affect the rate of alcohol degradation by altering gastric absorption, inhibiting ADH, or acting on liver enzymes [10, 11].

Alcohol concentrations of the urine is used if blood samples cannot be collected both in living and deceased people. An urinary alcohol to blood alcohol ratio of 1.3:1 can be used as estimate [12]. In cases of acute ethanol intoxication, the blood alcohol levels vary from 1 to 3.5–4 g‰. Higher levels usually cause deep coma and soon death due to acute ethanol intoxication. In contrast, ethanol-mediated death

can occur in infants and young children at concentrations of alcohol as low as 0.4–0.9 g‰ [12]. Studies on the passage of alcohol through breast milk have shown that intake in infants depends on the characteristics of lactation and childs enzymatic ethanol metabolism potentially causing elevated ethanol levels. In some cases, the alcohol concentration in the vitreous humor is estimated, although the results are difficult to interpretate [13].

Clinical Examination in a Forensic Setting

In the forensic setting, clinical presentation depends on the type of consumption, duration, and the drink's characteristics. Since the psychiatric and neurological aspects of alcohol abuse are intensvely discussed in other book chapters of this book, they are only briefly mentioned here. Among forensic implications, in the context of driving drunk, we also see physical and mental aggression [12]. Between March 16 and May 15, 2020, decrees no. 195 and 240 established and from March 8, 2022, only forensic institutions were authorized to perform autopsies at the request of judicial institutions [14, 15]. During such autopsies, if prompted by the medical history or macroscopic leasionsm pneumonic changes, pneumonia, thrombi, inflammatory myocardial lesions, and other biological samples are collected. Pathological intoxication—occurs quickly after the intake of alcohol in small quantities. As expected, among alcohol users, the most common pathologies encountered during forensic autopsies are shown in Fig. 8.2 such as hepatic steatosis, liver cirrhosis, alcoholic cardiomyopathy and cerebellar degeneration. For more details the reader is referred to the respective Chaps. 38, 70 and 72 in the book on liver histology, alcoholic cardiomyopathy and and Wernicke-Korsakov syndrome. Determining the concentration of alcohol in the blood of people who have committed a crime helps to analyze and prove legal issues. Thus, acute ethanol intoxication evolves in progressive phases with typical characteristics [12] (see also chapters on addicition treatment). The phase of behavioral changes such as excitation usually occurs at blood alcohol levels of 0.30–1.20 g‰. In people with low enzyme equipment or low tolerance (infants, young children), death can occur at these levels. In the medico-legal between 1–2.5 g‰, aggressions, road accidents, and rapes can be noticed. In the coma phase, at blood alcohol levels of over 3 g‰, a person cannot normally not act anymore and most often is a victim. To specify the criminal circumstances, the consumption of alcohol is an aggravating factor in the acts committed under the influence of the drinks consumed [3, 16]. If a criminal act is committed by chance, without intention, acute alcoholism can decrease the perpetrator's liability. Consequently, drunkenness can legally decriminalize. Retrograde amnesia is essential in establishing the diagnosis, still challenging in daily practice. In late stages of alcoholism, with the onset of cognitive deficits, a person is no longer legally responsible.

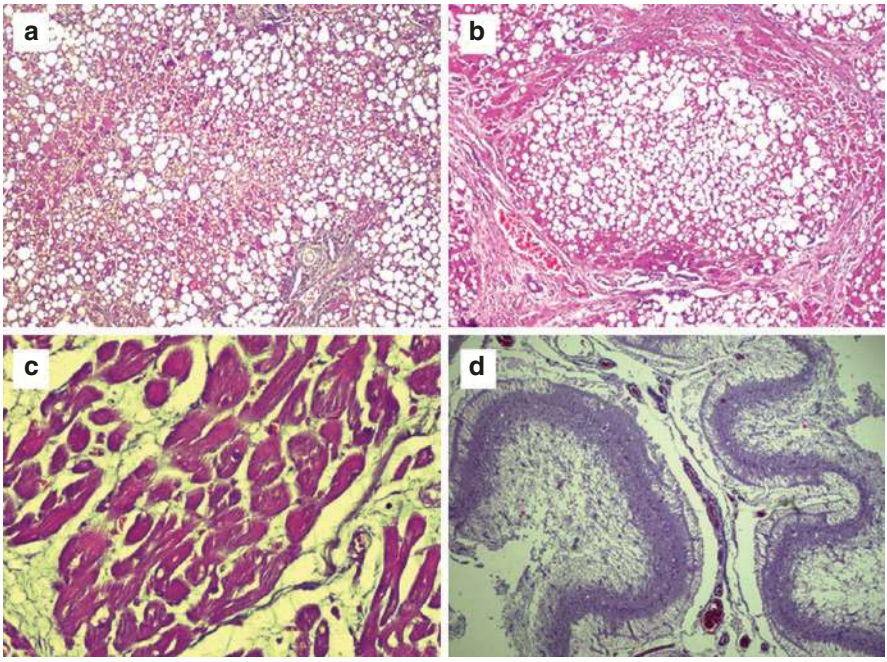
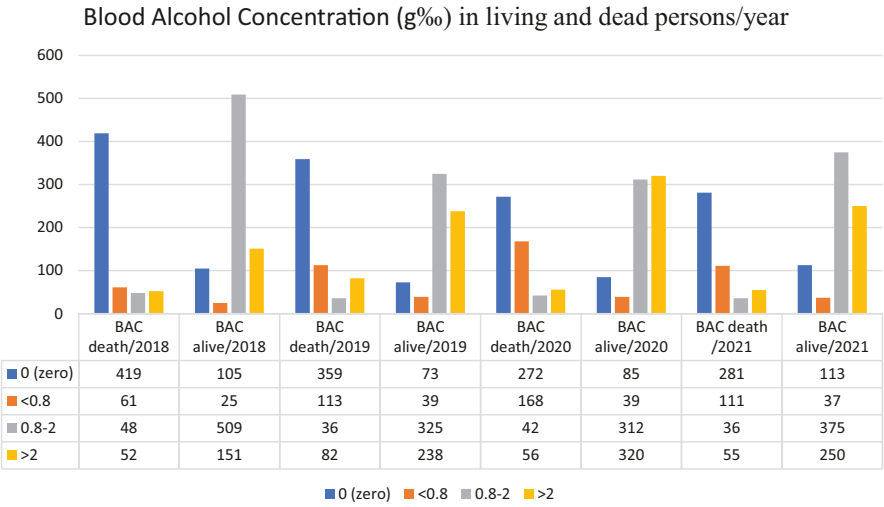


Fig. 8.2 Histological images (HE staining) of common findings during forensic autopsies: (**a**) hepatic steatosis (**b**) liver cirrhosis (**c**) alcoholic cardiomyopathy and (**d**) cerebellar degeneration

Forensic Measurements of Alcohol: A 4-Year Experience in Timisoara/Romania

We have analzyed involvement of alcohol for various forensic scenarios for the years 2018–2021. In these 4 years, at the request of the police, a total of 371 ethanol measurements were performed and calculated back to initial alcohol concentration as described above. Only in 13 drivers (3.5%), a level below the criminal limit of 0.8 g was found, while in the remaining 358 cases (96.5%) the drivers remained offenders. In the same period, our forensic department also analzyed 2716 biological samples from deceased people for ethanol using GC-MS. The results are shown in Fig. 8.3 for four different alcohol ranges: 0 g‰, below 0.8 g‰, between 0.8–2 g‰ and higher than 2 g‰. As can be seen in this figure, in the years 2018–2021, an elevated blood alcohol concentration was seen in 27–49% of all deceased people. Among the deceased people with elevated alcohol were murders, suicides but also victims of domestic violence. Figure 8.3 also shows the routine measurements from drivers. A total of 2746 samples were collected. In these 4 years, most drivers,



	BAC death/2018	BAC alive/2018	BAC death/2019	BAC alive/2019	BAC death/2020	BAC alive/2020	BAC death /2021	BAC alive/2021
0 (zero)	419	105	359	73	272	85	281	113
<0.8	61	25	113	39	168	39	111	37
0.8-2	48	509	36	325	42	312	36	375
>2	52	151	82	238	56	320	55	250

Fig. 8.3 Blood alcohol concentration (BAC) in living and deceased persons, collected from 2018–2021. Four different alcohol ranges are shown: 0 g‰, below 0.8 g‰, between 0.8–2 g‰ and higher than 2 g‰

between 85% and 89%, constantly had too high alcohol levels proving a violation of the road code. In conclusion, in the forensic setting, alcohol plays an important role either in the context of deceased people but also in the forensic setting of traffic violations.

Conclusions

In conclusion, alcohol has been, is, and will be a serious health problem, both due to its adverse health effects and the medico-legal and legal implications. Alarming levels of driving under the influence of alcohol are emerging—over 83% of the drivers had a blood alcohol are emerging level over 0.8 g‰. Our analysis also shows the importance of ethanol determination by GC-MS. Complications of chronic consumption occur in a large number of deceased cases and are associated with damage to several organs (liver, brain and heart).

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Chapter 9
COVID-19 and Alcohol Use Disorder

Miriam Gill and Jonathan Chick

Abstract Lockdown measures introduced in many countries during the COVID pandemic had the expected effect of moving sales of alcohol to sales in supermarket and via home delivery. However, other effects of lockdown—isolation, working from home, stress—appear to have led in some countries to a new sub-group of drinkers who moved from heavy to harmful drinking. Also, those already drinking harmfully tended to drink more with exacerbations in accompanying mental illness revealed in some studies. Where demographic information was available, increased drinking was reported more by women than by men. There is speculation that more drinking at home, in many countries, will have meant that a generation of children will have witnessed more parental drinking.

In communities where there were temporary total bans on alcohol, there was a surge in cases of severe alcohol withdrawal reported by some centres, whereas trauma, assaults and accidents seen by Emergency Departments reduced. Data on hospitalisations in the UK and USA showed that admission for alcohol related liver disease increased during the pandemic and there was a rise in alcohol-specific mortality but it is possible that this might in part have been a continuation of trends in both countries seen in the preceding decade. This chapter will look at how consumption behaviours changed, who were most affected, how alcohol use may have predisposed an individual to COVID-19 infection and where the pandemic left those suffering from alcohol use disorders.

Keywords Alcohol · COVID-19 · Alcohol consumption · Alcohol use disorders Alcohol related mortality · Covid restrictions · Pandemic · Coronavirus

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Introduction

Globally, harmful alcohol use is responsible for around three million deaths every year (5.3% of all deaths) and amounts to 5.1% of the global burden of disease [1]. Alcohol consumption is also linked to over 200 different diseases and other alcohol related health-harms such ranging from liver disease to mental and behavioural disorders. Importantly, drinking alcohol can predispose individuals to contracting infection [1, 2]. Alcohol consumption is an important indicator of health and many governments have struggled over the years to try and limit its damaging effects. In the UK, there are laws that include the legal age of consumption, when and where alcohol can be sold and prohibited activities when under the influence [3, 4] For more details see also Chap. 10. Scotland went one step further and introduced minimum unit pricing (MUP) in 2018 which set a base price for alcohol at a minimum of fifty pence per unit (corresponding to 8 g pure ethanol) [5]. Scotland and many other countries restrict the hours in which alcohol is sold and where it can be consumed. All these laws and restrictions were designed to try and reduce harm that could be attributable to alcohol consumption as well as having an impact on individual behaviour.

On 9 January 2020, the World Health Organisation (WHO) announced that a novel coronavirus had been identified in Wuhan City, Hubei Province, China. In February, the coronavirus was given its formal title of SARS-CoV-2 and the disease it caused was named COVID-19 [6]. On 11 March 2020 the WHO declared a global pandemic, but it was not until the 23 March 2020 that the UK Prime Minister announced strict measures to try and curb the spread of the virus [7]. Everyone was instructed to stay at home and shops and venues that did not sell essential items—such as food and medicine—were closed. However, in the UK alcohol was still available through the 'essential' shops even though entertainment venues, such as pubs and bars had closed. These 'lockdown' restrictions were rolled out across the UK and implemented for a number of months. They were reimposed in October 2020 until March 2021 [8]. Despite these measures, the UK was relatively hard hit by the virus and suffered many deaths. COVID-19 brought about change in mortality and in people's behaviour around the world.

Over the course of the pandemic, particularly through the initial stages of lockdown, there were changes to alcohol consumption. This chapter will look at how consumption behaviours changed, who were most affected, how alcohol use may have predisposed an individual to COVID-19 infection and where the pandemic left those suffering from alcohol use disorders.

Alcohol Sales

One of the most significant restrictions that was put in place in many countries in Spring 2020 was the closure of restaurants, bars, and pubs (in the UK termed on-trade). It meant that for some months following the announcement, sales of on-trade

alcohol decreased until the restrictions eased later in the year. However, that is not to say that some licensed on-trade establishments were unable to make any sales—many businesses remained open and sold through means of take-away services [9]. Despite these take-away sales, it was not enough to off-set the total loss of the closure of on-trade premises. In Scotland, research published by Public Health Scotland investigated the impact of the national lockdown and the COVID-19 pandemic on alcohol use across England, Wales, and Scotland, using an earlier study that compared the sales of on- and off- trade premises (i.e., shops and supermarkets) during lockdown to sales in the same weeks in the 2 years previous (2017–2019). The study found that the total volume of pure alcohol sales across the three countries decreased by 6%. This was attributed to the loss of sales from on-trade premises [10, 11].

However, there was an increase in off-trade sales, particularly in Scotland, where nine in every 10 units of alcohol were sold through off-licence trade in 2020 compared to seven in every 10 units in 2019 [12]. Sales in supermarkets and off-licence shops increased in Scotland by 28% and in England by 29% [11]. In 2020, 90% of all alcohol purchased was from off-trade retailers and in the same year per-adult off-trade sales in Britain were between 16–18% higher than in 2019. On-trade sales were between 59–64% lower compared to 2019 [12].

An analysis of UK household purchases during lockdown showed that households did not buy any more alcohol than would have been expected for that time of year. This is after adjusting for what would have normally been purchased from on-licence establishments [13, 14]. In the first 3 weeks of lockdown in 2020, excess purchases increased but then plateaued. This is important as although it could suggest stockpiling in the earliest part of the introduction of restrictions, it does not seem to have continued any further than the initial month. Looking particularly at what was being purchased, there seemed to be a change from buying lower strength alcoholic beverages to higher strength wines and spirits [13].

Comparing areas by social deprivation, excess purchases of alcohol were higher in the most deprived households. As the quantity of alcohol normally purchased by a household increased, so too did the number of excess purchases increase. It was estimated that within the top one fifth of households that previously purchased large amounts of alcohol, their purchases increased by over 17 times more than an equivalent number of households that purchased the least alcohol [15].

Across the Atlantic, an American study found large increases in alcohol sales from the beginning of March 2020 to the middle of April. Sales for alcohol from off-licence premises—'liquor stores'—had increased by 21% and online sales of alcohol had increased by 234% in comparison to a similar period in 2019. However, the data did not show whether these sales resulted in increased household consumption or whether it was a stockpiling measure [16].

Alcohol Consumption

Lockdown saw changes to people's behaviour, and it was hypothesised that these changes in behaviour could be reflected in changes in alcohol consumption. In a British study by Public Health Scotland, members of the public were surveyed through behavioural questionnaires and diaries that would highlight any changes in drinking habits. It was concluded that the number of drinking days had on average increased during the restrictions, however, the change was not significant [10].

A large-scale cross-sectional study looking at alcohol consumption in 21 countries in Europe suggested that there was decreased alcohol consumption during the start of the pandemic [17]. This may be due to the home being the only place people were able to drink. The closure of bars and pubs but also the restrictions of events that would previously have promoted heavier drinking, e.g., weddings, parties, concerts etc. may have had a curtailing effect. Reduced alcohol consumption may also have been due to reduced affordability from recent unemployment and financial instability. However, it was also commented that frequent and heavy drinkers tended to increase consumption rather than decrease it, while lighter drinkers tended to decrease their consumption [17].

From this European study, the UK was highlighted as being the only nation with a significant average increase in alcohol consumption. The frequency of consumption remained relatively unchanged in seven countries including Denmark and France, and the quantities of alcohol consumed remained the same in Germany and Ireland. It is interesting to note that compared to the UK, Ireland did not have any significant increases in drinking frequency or quantities consumed per occasion. Additionally, heavy episodic drinking (HED) events decreased in all countries except the UK where it stayed the same. It is worth drawing attention to the fact that the United Kingdom appears to be one of the only countries in Europe where off-licence shops (what in USA or Australia might be termed 'liquor stores') were deemed 'essential' in the list of premises allowed to stay open during the start of lockdown. One of the arguments made for recognising alcohol as being an 'essential' item was to prevent severe withdrawal in people suffering from alcohol use disorder. However, off-licences remaining open and home delivery and supermarkets also allowing the sale of alcohol probably contributed to increased at-home consumption in the UK [17].

Data from Public Health England showed that the majority of their respondents were drinking the same amount and no more frequently than they were before the pandemic. In fact, data suggested that as many respondents who had increased consumption had decreased it [18]. This data alongside the data by Public Health Scotland do appear to differ in comparison to the results published from the European wide study. In the European study only 836 respondents were from the UK out of 31,964 total respondents [17]. This suggests that it was perhaps not a fair representation of UK changes in consumption during the pandemic.

However, data from Public Health England did show that those respondents who had been drinking more during the pandemic tended to be already heavier drinkers.

Between 2020 and 2021, there was a 58.6% increase in the number of respondents drinking at higher risk levels [18].

A study by Alcohol Change UK surveyed 1555 people, 2 weeks after initial lockdown measures were imposed, and found that more than a third of participants had either stopped drinking completely or reduced how often they drank. There were around a fifth of participants who were drinking more frequently and were also drinking more per drinking day since the beginning of lockdown. This leads to a hypothesis that the pandemic has seen the development of a new sub-group of drinkers that were potentially developing harmful alcohol consumption habits [19].

This harmful pattern was further commented on by a survey carried out by the St Mary's Hospital Alcohol clinic on patients with pre-existing alcohol use disorders. The survey found that of the participants who had increased their alcohol consumption during lockdown of 2020 (24% of participants), there had been a mean weekly consumption of 82.5 units. The weekly recommended intake is no more than 14 units. These participants also had a 57.6% mean increase in AUDIT score. Overall, these studies suggest that, despite there being as many people reducing their alcohol intake as increasing it, those increasing their consumption are doing so by a significant and harmful amount [20].

In a Canadian study similar patterns emerged over the course of the pandemic. Individuals who reported decreases in alcohol consumption showed significant decreases, but individuals who reported increases in alcohol consumption showed significant increases. Data showed that there were as many participants who had increased consumption during the pandemic as had decreased. Those who reported an increase in alcohol consumption, showed a 161.5% increase in heavy drinking days (HDD). HDD was referred to as more than three drinks for females and more than four for males. A 37.2% increase in alcohol-related problems was also commented on, particularly PTSD symptoms. This suggests that within the group of those significantly increasing their drinking, more are likely to suffer from marked mental health changes [21].

Demographics of Alcohol Use

When identifying the individuals who may be most at risk of drinking harmfully, recent UK data collected by the National Health Service (NHS) found that the age group with the highest proportion of people drinking over the weekly recommended units were aged between 55 and 64. This applied to both men and women [22].

However, over the course of the pandemic, changes in consumption were found to be most evident in adults aged between 25 and 49 years, and those with middle or high incomes [13, 23]. In a survey for Alcohol Change UK, over a quarter of respondents agreed they had increased consumption over lockdown. Of those respondents the majority were in employment, from higher socio-economic groups, younger drinkers and heavier drinkers [13]. Interestingly, it was observed that women seemed to be drinking more [23]. It is surprising that women appear to have

increased their consumption during the pandemic when often those suffering from heavier intake or alcohol use disorders are typically younger males [11, 24]. An Australian study also commented on women being more likely to increase alcohol consumption compared to men. This was shown to be especially true when women were having to cope with conflicting work and family commitments. These conflicts may have become more frequent or pronounced during lockdown restrictions as schools were closed and children required more supervision and schooling at home [23, 25].

A study from the US showed 14% more alcohol had been drunk by Americans in that year. Amongst women there had been a 17% increase in consumption and 19% more amongst people aged between 30 and 60. The consumption of large amounts of alcohol amongst women—'large amounts' equating to four or more drinks within 2 h—had increased by 41% [14].

Younger drinkers were previously mentioned by Alcohol Change UK as a group that had increased consumption. Additionally, it was noted that excess alcohol purchases from off-licences were higher for younger shoppers. However, it is possible that younger people were buying for older people who were isolating or shielding at home due to greater vulnerability of contracting the virus [13].

When looking at the effects of lockdown on students and young adults, the closure of universities and many students moving from university accommodation to their parents appeared to have a significant impact on alcohol consumption. A study from the University of New Jersey found that when students moved from living with peers to living with parents, there was a significant reduction in the number of drinking days, the number of drinks per week and the number of drinks consumed in 1 day. This suggests that returning to live with parents, especially during the context of the COVID-19 pandemic, was a protective factor against heavy drinking [14, 26]. This could also be attributed to pubs, clubs, and other social spaces being closed which would have prevented socialising between young adults [26, 27].

Interestingly, in the last two decades there has been a decline in general alcohol consumption of the younger generation in the UK and other high-income countries which has translated into reduced mortality amongst adults. The same cannot be said for the 55–84 age bracket which has seen an increased mortality rate of between 23% and 49% [28]. However, the study did highlight that for students who did not change living circumstances and who remained in the same households they were living in prior to the pandemic, the frequency of consumption increased [26, 29].

In Germany, a study found there was a 6.1% increase in total revenue of alcoholic beverages compared to equivalent weeks in 2019. The study identified that the age group seemingly drinking more alcohol was between 35–44 years. The groups drinking less fell into the 18–24-year age bracket and those living with parents or alone [30]. This is interesting as it was thought that living alone may be an exacerbating factor for heavier intake [23]. In a previous study looking at drinking habits post-natural disaster, living alone was associated with increased drinking. Despite a similar hypothesis for the COVID-19 pandemic, there did not seem to be significant differences between individuals living alone and other groups. It is possible that

those living alone often went out to drink in order to socialise. Without this option during restrictions, it may have prevented an increase in drinking [23].

An additional study from Poland also commented on this by finding that most participants drank less if they were single and without children. The thought of individuals with children increasing intake more than individuals without children is potentially concerning as it could suggest a generation of children who will be more familiar with the sight of parents or guardians drinking at home. If these parents/guardians have fallen into the category of drinking significantly more than before the pandemic, it may impact the child's view on what is an appropriate amount to drink [27]. A study in Canada showed that amongst Canadian teenagers who had reported a decrease in binge drinking, cannabis use and vaping directly after lockdown restrictions were imposed, almost all reported drinking alcohol at home with their parents which was regarded as more acceptable behaviour [14].

In the German study, participants who were considered less educated—having completed less than 11 years of education—seemed to be drinking more, but unemployed participants were either drinking the same amount as pre-pandemic or less. Participants who had experienced changes in employment during the course of the pandemic either drank more or drank less. In this group there were significant differences in consumption either for better or for worse. The groups identified in having a higher risk for increasing alcohol consumption during lockdown included: middle-aged participants, participants who drank heavily before the pandemic, those under considerable stress because of the pandemic and those who were less understanding of the rationale behind stricter lockdown restrictions [30].

Causal Factors Behind Drinking Patterns

Historically, global crises in the form of mass terrorist attacks, previous epidemic outbreaks or economic adversity—such as the 2008 Great Recession—have been associated with increased alcohol use [14, 23]. This increased alcohol use is believed to be in response to psychological trauma and elevated periods of stress and anxiety [23]. During the pandemic, stress and anxiety have been identified as exacerbating factors that have led many individuals to increase their alcohol intake [31, 32]. Strict lockdown measures forced whole populations into social isolation and gave rise to fears over job security and financial stability. Consumption of alcohol in particular has been recognised as a coping strategy to help with feelings of isolation and insecurity. There are many studies that show respondents who were reporting feelings of loneliness and stress were also more likely to report an increase in alcohol consumption [13, 16, 20, 31–34]. In a study looking at mental health during the COVID-19 pandemic and substance use, women were recognised as more likely to report poorer mental health and more likely to consume alcohol in order to cope with emotional distress [32]. Responsibilities of the home are most often carried by women and having to juggle children being kept at home as well as personal affairs may have contributed to higher stress levels [23].

A study in America commented on different reasons that either drove participants to increase their consumption or to decrease it. Almost two-thirds of participants of the study reported an increase in consumption in comparison to before the pandemic. Of this group, the majority cited increased stress as a cause of drinking more and the rest cited increased availability of alcohol and boredom. Of the participants who reported to have decreased consumption, reduced alcohol availability, less free time, less money and the desire for a healthier lifestyle and concerns over the impact of alcohol on mental health were all cited as reasons for decreasing alcohol consumption during the pandemic [16, 31]. Similarly, a study from Canada reported that the main reasons for individuals increasing their drinking was the absence of normal daily routine, boredom, and stress [14]. In Greece, participants also cited isolation, changes to everyday routine and symptoms of anxiety and depression to explain why consumption was increased [14].

Mental health is an important issue when looking at the effects of increased alcohol consumption. A Polish study found that participants who were drinking more than before the pandemic were experiencing significantly poorer mental health. This was exhibited by struggling to cope with everyday function, finding less satisfaction in their daily life and suffering from depressive symptoms [27].

Alcohol-Related Hospital Admissions and Death

Data taken from national statistics from England and Wales estimated that over 8.4 million people were consuming alcohol at a high-risk level during this pandemic. This is in comparison to the 4.8 million before the first lockdown in Spring 2020. The UK Government has published data showing that the rates of unscheduled admissions to hospital for alcohol specific reasons in 2020 fell by 3.2% compared to before the pandemic [11, 35]. This is thought to be related to reduced admissions for alcohol attributed psychiatric and behavioural problems. Additionally, the most rapid decrease in admission rates corresponded with the beginning of the pandemic and the occasions of greatest restrictions in the UK [35].

However, unplanned admissions for alcohol-related liver disease (ALD) increased between 2019 and 2020 by 13.5% and have continued to increase over the course of the pandemic [35]. A tertiary liver centre in London reported a twofold increase in admissions for ALD and an additional marked increase in patients requiring high dependency or intensive care—an increase of between 11% to 24% [11, 36]. This implies that people are more seriously ill on admission. Periods of lower admission rates were at the time of greatest restriction. This was from when fewer people presented to hospital, probably through fear of contracting the virus at times where the virus was highly prevalent, or through strictly adhering to the ‘Stay at home’ messages from government and images of hospitals being overwhelmed by COVID-19 patients. The unfortunate irony of this is that patients with ALD who contracted COVID-19 were likelier to suffer worse outcomes than a COVID-19 patient who did not have similar alcohol related problems [11].

Alcohol related emergencies had also increased which included alcohol withdrawal and related suicides [37]. In Scotland, recent published data found decreases in hospital stay rates for ALD and alcohol-related mental and behavioural disorders by 9.2% and 7.5% respectively compared to 2017–2019. However, hospital stays for alcohol toxicity were 6.7% higher. The rate of alcohol-related hospital stays decreased for males by 10% but did not change for females and the most significant decreases were found in the oldest age groups and in the most deprived [38]. This is interesting as patients from lower socio-economic are often shown to have worse alcohol-related outcomes [11], however, there was not as great a reduction in hospital stays for the least deprived groups [38]. This could be potentially because patients from higher socio-economic backgrounds have greater access to secondary care and are more likely to seek such care [11].

According to the UK Office for National Statistics (ONS) in 2020 there was an 18.6% increase in deaths from alcohol-specific causes recorded in the UK compared to 2019: 14.0 deaths per 100,000 people in 2020 compared to 11.8 per 100,000 people in 2019 [39, 40]. This was the greatest year-on-year increase since 2001. Alcohol-specific deaths have also significantly increased across all four UK nations compared to relatively stable rates across 2012–2019. The term alcohol-specific death was used interchangeably with 'wholly attributable death' (due to alcohol) and encompassed three main areas: alcohol-related liver disease, mental and behavioural disorders, and external causes (e.g., accidental poisoning relating to either direct alcohol intoxication or exposure) [39]. There has been an 10.8% increase in deaths related to alcohol attributed psychiatric and behavioural disorders, and a 15.4% increase in deaths from alcohol poisoning [35]. Alcoholic liver deaths made up 80.3% of total alcohol specific deaths which equates to a 20.8% increase between 2019 and 2020. There had already been an upward trend in the number of deaths from ALD, however the pandemic seems to have accelerated this [35, 40]. In December 2020, the ALD death rates were 58.1% more than the comparable baseline month [1]. It is also worth highlighting that of liver disease in the UK, ALD accounts for 60% of all cases [40].

Scotland and Northern Ireland were seen to have the highest alcohol-specific death rates for 2020, but it was England and Scotland that showed the most statistically significant increased rates compared to 2019 [39]. In Scotland, there was a 9% increase in the number of alcohol-specific deaths recorded in 2020 compared to the average number between 2017–2019 [38]. Public Health Scotland calculated the age-sex standardised rate of alcohol-specific deaths in 2020 to be 22.0 deaths per 100,000 people. This is 8% higher than the average calculated for 2017–2019 [38].

It was noted that at the beginning of 2020, alcohol-specific death rates were below average for that seen in the same months of 2017–2019. These rates rose above average from the start of restrictions in March and continued to rise over the summer until rates peaked in October. For the same month in 2017–2019, rates were around 30% higher [38]. The female death rate was under half of the rates for males, with males making up over two thirds of the total alcohol-specific deaths. The most common age groups affected were people in their 50 s and 60 s and it was areas of

greatest deprivation that had 4.3 times the death rate compared to those in the least deprived areas [41].

Additionally, although cirrhosis of the liver due to alcohol can take at least a decade to develop, most deaths happen in response to recent alcohol consumption resulting in acute-on-chronic liver failure (ACLF) [18]. Acute-on-chronic liver failure is a syndrome defined by an acute deterioration of chronic liver disease which is associated with organ failure and mortality [35, 42]. For more details, see also book Chap. 67 on ACLF but also alcoholic hepatitis (AH) Chaps. 64, 65, and 66. Heavy drinking is strongly linked with ACLF. Over the last two decades there has been a 43% increase in liver mortality rates in England and it was recognised as the second leading cause of premature death amongst the working population. Liver mortality was already a growing issue before the pandemic however it is likely that the pandemic has exacerbated these problems [18, 35].

Across the Atlantic, research published from the US looking at alcohol-related deaths during the first part of the pandemic found that between 2019 and 2020, the number and rate of deaths attributed to alcohol increased by approximately 25%. There had been suggestions of a steady increase prior to the pandemic, however the pandemic has appeared to exacerbate these numbers. Additionally, the increased rate of alcohol-related deaths in this time period surpassed the 16.6% increase in all-cause mortality [43].

Effects of Alcohol on the Body in the Context of COVID-19

Both the long- and short-term effects of excessive alcohol consumption have been widely reported. The immediate effects of heavy drinking commonly present as acute drunkenness or alcohol poisoning while the longer-term consequences have a much more gradual progression and can damage major organs such as the liver and brain. Extended periods of excessive alcohol consumption most commonly manifest as alcohol-related liver disease (ALD) [44].

However, alcohol can itself disrupt the immune system, increasing an individual's susceptibility to infection [45]. Alcohol has been shown to suppress both innate and adaptive immunity resulting in greater viral load and spread [46, 47]. In the context of COVID-19, alcohol significantly affects many of the body's combat mechanisms against invading pathogens. The lung is the primary site through which COVID-19 is able to replicate in the body. In order to protect itself from harmful pathogens and pollutants, the lung uses physical barriers and clearance systems. The first line of defence within the airway is thought to be the mucociliary clearance system; lining the airways of the lungs are ciliated and mucous producing cells. Cilia are thin eyelash-like structures that sit on numerous cells and move in a coordinated manner to remove mucus coated particles out of the lung. Heavy alcohol consumption has been seen to not only inhibit increases in cilia movement but also affect the quality and quantity of mucus produced in the lung [48]. Cough and exhalation is also a mechanism the lung uses to rid itself of harmful toxins. Alcohol is

known to suppress coughing and is also associated with shortness of breath, or dyspnoea. Dyspnoea is a well-known symptom of COVID-19 which suggests that compounded with alcohol consumption, there would be a decreased excretion of alcohol via the lungs which may increase the damage alcohol is able to have on the lungs' innate defences [48].

If the virus has not been successfully cleared, it will enter the alveoli of the lungs. Alveoli are the main site for gas exchange and are lined with surfactant—another barrier against harmful pathogens. It was the surfactant protein D (SP-D) that was known to bind to the Spike protein of SARS-CoV-1, the causative organism of the 2002 SARS outbreak. When the Spike protein was bound by SP-D, it was unable to interact with ACE2 receptors to infect cells. ACE2 receptors are found in multiple organs in the body but in the context of COVID-19 it is important to look at the lungs. In binding to ACE2 receptors, the virus undergoes conformational changes and cleavage which assists the virus in being able to fuse into the host cell. In comparison, SARS-CoV-2, the virus responsible for COVID-19, has shown to have far stronger bonds with the ACE2 receptor following conformational changes [49]. So, it is surfactant protein D that prevents the virus from interacting with ACE2 receptors. Heavy alcohol consumption is shown to contribute to alter SP-D function and also negatively affect surfactant production [48].

As previously mentioned, alcohol consumption can also disrupt the immune system. This is primarily through changing the actions of immune cells. For example, specific cells that are responsible for destroying invading pathogens (macrophages, neutrophils, and monocytes) can be inhibited or altered by exposure to alcohol [50].

Chronic alcohol consumption has been linked to strong pro-inflammatory reactions that have been shown to contribute to disease processes in the lungs. Simultaneous to provoking these strong inflammatory responses, alcohol impairs the generation of anti-inflammatory cytokines [51]. Cytokines are signalling molecules and are responsible for coordinating the body's immune response [50]. This chaotic storm of inflammatory molecules is what contributes to respiratory and multi-organ failure through severe oxidative stress [51]. Similarly, SARS-CoV-2 infection often leads to a rapid release of pro-inflammatory cytokines and inflammatory lipid mediators, such as prostaglandins and leukotrienes. The severity of the inflammation caused by COVID-19 is thought to be a main contributor to the organ failure seen in critically ill infected patients [48]. From what we know about the inflammation caused by chronic alcohol consumption, this is likely to exacerbate the similar inflammatory response to infection.

The organ failure caused by both chronic alcohol use and COVID-19 has been attributed by some researchers to oxidative stress. In relation to heavy alcohol consumption, alveolar macrophage function is affected which leads to an inability to efficiently destroy infectious organisms. This is due to alcohol decreasing antioxidant levels by increasing alveolar barrier permeability which in turn increases the amount of free oxygen radicals. The cells are not able to rid themselves of toxic oxidants which causes oxidative stress. Severity and mortality of Acute Respiratory Distress Syndrome (ARDS) due to fluid building up in the alveoli, is increased with the increase in barrier permeability and the decrease in antioxidant levels [48].

ARDS is recognised as a severe complication of COVID-19 and carries a high mortality rate [52].

As well as the effects of alcohol in the lungs and in the immune system, patients suffering from alcohol use disorders (AUD) are also likely to have underlying medical conditions which are risk factors for severe COVID-19 infection, such as chronic obstructive pulmonary disease or liver impairment. Additionally, more patients with AUDs are male which is a recognised risk factor for greater medical intervention and higher mortality rates. It is also worth mentioning that not only can chronic alcohol use exacerbate COVID-19 infection, but COVID-19 could also be a major factor in exacerbating and worsening pre-existing liver disease [47].

During the pandemic, there were rumours that alcohol consumption could be beneficial in the prevention of contracting COVID-19. However, the evidence that has been presented challenges this, and we can conclude that alcohol consumption is a serious risk factor to contracting infections—importantly COVID-19 [51]. Chronic alcohol consumption has an adverse effect on the cells that respond to specific pathogens (T-cells) and the cells that are responsible for long term immunity (B-cells) [50]. This raises the question of what the effect of long-term alcohol use may be on an individual's response to vaccination [53].

Furthermore, aside from the serious harm alcohol can do to our bodies, it can also severely affect behaviour and impairs judgement [52]. This is significant as social distancing is considered key to preventing the spread of COVID-19 and is harder to enforce in large groups of intoxicated people [54]. In fact, in Scotland most local authorities have made it illegal to drink alcohol outdoors which was in part due to recognition that consumption of alcohol reduces compliance with social distancing rules [55]. It has also been evident that as rates of reinfection began to rise, bars and clubs became the first venues to be heavily restricted or closed again.

COVID-19 and Alcohol Use Disorders

The effect of lockdown on alcohol consumption in patients with pre-existing alcohol use disorder (AUD) has been well documented. Patients with AUD were recognised as a population that may be severely impacted by the virus and by the restrictions put in place to reduce contagion. A UK study with 182 patients with pre-existing alcohol disorders, found that of the 38% of patients who were abstinent before lockdown, 17% of this group relapsed during the restrictions. The mean AUDIT score of this subgroup had increased by 226% and the average weekly alcohol intake was 48.8 units [19]. These figures show that not only was lockdown a risk factor for relapse in those who were abstinent before the restrictions, but additionally those who relapsed increased their consumption greatly and were drinking harmfully. The WHO also recognised that hazardous and harmful drinking had increased greatly during the pandemic [56].

In China, the prevalence rate of AUD, including dependency and harmful use, was 4.4% in 2018 but had risen to 11.1% in 2020. It was suggested that social

isolation was the biggest risk factor for this increase [2, 56]. Furthermore, in Spain, patients who suffered from alcohol use disorder were identified as being a particularly high-risk group to consuming greater quantities of alcohol during the pandemic. A study found that the number of AUD patients screening positive for alcohol following outpatient treatment doubled during the 3-month period after Spain implemented lockdown in its country. Interestingly, Spain has some of the cheapest prices for alcohol compared to the whole EU which may have provided easier access to alcohol for AUD sufferers [57].

Due to the restrictions that were put in place during the beginning of the pandemic, access to help for those suffering from excessive drinking was reduced. Services including supervised consumption, detoxification, and blood-borne virus (BBV) screening and treatment were either completely stopped or significantly reduced [58]. Similarly in Scotland, Alcohol Brief Interventions (ABIs), drinkers, were curtailed. ABIs are brief discussions within Primary Care, A&E and maternity settings aimed at helping newly identified drinkers decrease their alcohol consumption to within safe standards. As face-to-face contact was severely limited during the pandemic, carrying out ABIs proved difficult as staff were redeployed to care for COVID-19 patients [59].

Telemedicine and telehealth became more common place during the pandemic as a solution to the cessation of face-to-face consults. There are many constraints to telemedicine which may have excluded disadvantaged members of the population. Telehealth is dependent on internet access which can be difficult if individuals do not have the technology or the network to support it. It is thought that those from lower socioeconomic backgrounds would be worst affected by this and yet they are the population group that often need the most support [2]. Additionally, services that were moved online were found to be less effective at preventing relapse [20]. A study from one alcohol clinic in London found that the patients who received face-to-face contact with an alcohol nurse were more likely to abstain from alcohol and less likely to relapse during lockdown compared to those patients who did not [20]. However, individuals who did have access to face-to-face appointments sometimes avoided attending due to fears surrounding leaving their homes, the possibility of contracting the virus while travelling and potentially infecting people close to them [55, 56]. This meant that often patients were only seeking care when their symptoms were most severe [55].

The lack of access to important outpatient services was not the only disruption due to lockdown. Access to leisure activities that may have been used to help negate the temptation of drinking were also severely impacted due to lockdown restrictions. Additionally, most employers were asking their employees to stay at home. This may have made it more difficult for affected individuals or individuals at risk of relapse to find reasons to leave the house or find more effective ways to occupy their time or mind with. When lockdown instructed individuals not to mix, usual support networks for affected or at-risk individuals were limited as they were deprived of the social contact they might have had access to before. Social contact is recognised to be crucial in the prevention of relapse in patients with AUDs [55]. This would have exacerbated feelings of social isolation and anxiety that many

sufferers of AUD often already face as a result of their condition [2, 55]. Counselling, relapse prevention groups and community-run organisations were also either no longer available or only available online [2, 55]. Alcoholics Anonymous (AA) is a community-run organisation that seeks out to help those with alcohol use issues to recover and continue their sobriety. Prior to the pandemic, around 5000 AA meetings were being held across Great Britain every week. However, because of government restrictions, many groups had to go online or stopped altogether [60].

The effect of COVID-19 on services was not the only concern for AUD patients. As mentioned earlier, excessive consumption of alcohol causes harm to the body which can lead to an individual's increased susceptibility to contracting viruses. Over the last decade, rates of alcohol use disorder have increased by 84% for women and by 35% for men [61]. This is particularly worrisome as women have been shown to experience worse alcohol-related health consequences compared to men. Women with AUDs have a greater risk of developing alcohol-related liver disease (ALD) [61, 62]. Patients with ALD and subsequent liver cirrhosis have worse outcomes from COVID-19 [62]. As alluded to already, patients with AUD also often have co-morbidities that will predispose them for more severe COVID-19 outcomes, for example, obesity-related metabolic syndromes or chronic obstructive pulmonary disease; being immunocompromised as a result of alcohol hepatitis therapy or liver transplantation will increase an individual's susceptibility [55]. Inpatients with AUD who were COVID-19 positive died at a significantly younger age than COVID-19 positive patients at low risk for AUD [62].

Countries Where Legislation During the Pandemic Targeted Alcohol Availability

In South Africa, there was a complete ban on alcohol sales beginning in April 2020. A study looking at the impact of the ban found a sharp reduction in patients admitted to hospital for assault, accidents and sexual assault compared to before lockdown [63]. Another two South African studies also commented on similar trends. In the periods of complete alcohol restriction, there was a significant reduction in the total number of unnatural deaths and trauma volume in hospitals [64, 65].

However, historically, a complete prohibition on alcohol can drive alcohol sales underground and into illegal markets [66]. Additionally, it could result in worse outcomes for those suffering with alcohol use disorders. India was another country that imposed for a period a ban on alcohol sales. Following the ban, there were reports of sudden escalation of hospital attendances for alcohol withdrawal seizures or delirium as dependent drinkers suffered from enforced abstinence [67].

Conclusion

As a result of the COVID-19 pandemic and the concomitant infection control restrictions, some individuals drank more but others drank less or even stopped drinking altogether. This suggests that there has been increased polarisation in the drinking habits of different groups. The groups that saw the biggest change in increased consumption of alcohol was those aged between 25–49 years old and of middle to high income. Notably, women were found to have had a larger increase in alcohol intake. Conversely, in comparison to historical periods of greater stress, there has not been a universal increase in alcohol consumption. However, for the individuals who were already in poor health and relied on alcohol before the pandemic, there were worse outcomes. On one hand, lockdown may have been protective for heavy alcohol consumers as it may have prevented greater exposure to the virus, but on the other hand, people were forced to stay at home, and it was difficult to access services that may have been critical in preventing relapse or reducing harmful intake. As a result, the UK has seen record numbers of deaths attributable to alcohol.

The next 3 years following the pandemic and associated restrictions will be especially significant in relation to alcohol use and changes in consumption. Research published in Beijing in 2008 studied alcohol abuse/dependence symptoms in hospital workers 3 years after the SARS-CoV outbreak in 2003. The findings concluded that there was a significant association between having experience of being quarantined and working in areas with high numbers of SARS patients and later symptoms of alcohol abuse/dependence symptoms [68]. Currently we can identify certain additional groups in the population that are now at risk of hazardous drinking. Additionally, individuals with pre-existing alcohol use disorders have suffered greatly because of the pandemic and there needs to be an urgent focus on providing services for these individuals. However, over the next 3 years, there could be other groups that emerge vulnerable to increased alcohol use who could suffer similar outcomes to those struggling now.

Not all countries experienced the rise in numbers of heavy drinkers and rates of alcohol-related harms that the UK experienced. It can only be speculated whether dis-allowing alcohol retail outlets as an ‘essential service’ would have prevented that. One could also speculate that tighter rules on alcohol availability might have reduced the spikes in Covid outbreaks seen in the UK when venues were allowed to open. As with many policy decisions made during the pandemic in many countries, in the end many decisions were made based on political rather than scientific decisions.

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Chapter 10
The Principles of Policies to Reduce the Burden of Liver Disease

Robyn Burton, Clive Henn, and Nick Sheron

Abstract The dose-response relationship between alcohol and alcohol-related liver disease is exponential, as a result and in contrast to many other alcohol related harms, the majority of liver mortality occurs in heavy daily drinkers who seek out cheap strong alcohol. Price elasticities for very heavy drinkers are difficult to determine as this group are not represented in population studies. Nonetheless, data from the UK shows a strong relationship between the affordability of alcohol and liver mortality clearly demonstrating that these heavy drinkers are in fact extremely price sensitive. Interventions targeted towards very heavy drinkers, such as a minimum unit price for alcohol, are highly effective and cost-effective policies that can reduce liver mortality with practically no impact on low-risk drinkers.

Keywords Alcohol · Alcohol-related liver disease · Policy · Intervention: minimum unit price

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Introduction

The liver is the largest organ in the body, the most metabolically complex, and probably the least well understood by non-liver doctors, as well as the general public. You can find the functional equivalent of the heart, lungs, and kidneys in a cheap aquarium. Simple mechanical devices can support life when these organs fail, but the same is not so for the liver which makes the building blocks of the body and detoxifies all the waste products. Prometheus was bound to a rock and visited every day by an eagle who eats half of his liver which then grew back overnight, only for the cycle to be repeated. The liver does indeed have remarkable powers of recovery. Following a single acute insult, the liver will usually regenerate. If a competent hepatobiliary surgeon removes half of the liver, then it can be expected to grow back within weeks to its full previous size and function. However, the response to repeated insults is quite different. Hepatic stellate cells are activated to secrete collagens and fibrosis, or scarring, develops as a wound healing mechanism. Nodules of liver cells surrounded by scar tissue look like a bag of marbles and regeneration is constrained. At this stage, the liver has developed cirrhosis—first described by Hippocrates in the fifth century BC. The other important disease process to affect the liver is carcinogenesis. Following repeated rounds of regeneration mutations develop with liver cells, and eventually primary liver cancer is the result.

Liver cirrhosis resulted in 223,000 deaths in the World Health Organization (WHO) European region in 2019, with a further 63,000 deaths from primary liver cancer, in total [1]. The majority of these deaths resulted from alcohol consumption (probably between 60% and 80%), but it is not possible to be exact because the coding of liver disease is poor in many European countries and aetiology is very often not recorded [2]. Liver disease causes 3% of deaths in Europe, but unlike the other major killers of the twenty-first century—the diseases related to smoking and obesity—liver disease kills people of working age. In terms of years of life lost in working age, liver disease is the second leading cause after ischaemic heart disease, killing more younger people than lung cancer, breast cancer, or diabetes (Fig. 10.1) [1].

Over the last few decades, European countries have experienced differing trends in liver mortality rates. In Fig. 10.2, we have categorised a selection of larger European nations into five groups according to their trajectory of liver mortality between 1990 and 2019 using the latest Global Burden of Disease (GBD) modelled data [3]. A small group of Eastern European countries have always had very high levels of liver mortality comprising in this selection: Moldova, Hungary and Romania. A group comprising: Sweden, Norway, Greece, Ireland, the Netherlands, and Malta have had stable low levels of liver mortality. Moldova has a standardised death rate (SDR) from cirrhosis and chronic liver disease of 74 per 100,000 population which is tenfold higher than the rate seen in Norway which is 7 per 100,000 population. There have been changes in liver mortality over the past three decades. France, Spain, Italy, and Portugal have seen marked decreases in the rate of liver deaths as a result of substantial decreases in the consumption of cheap wine [4]. Whereas in the group comprising: Ukraine, Bulgaria, Belarus, Poland, Latvia, Estonia, Finland,

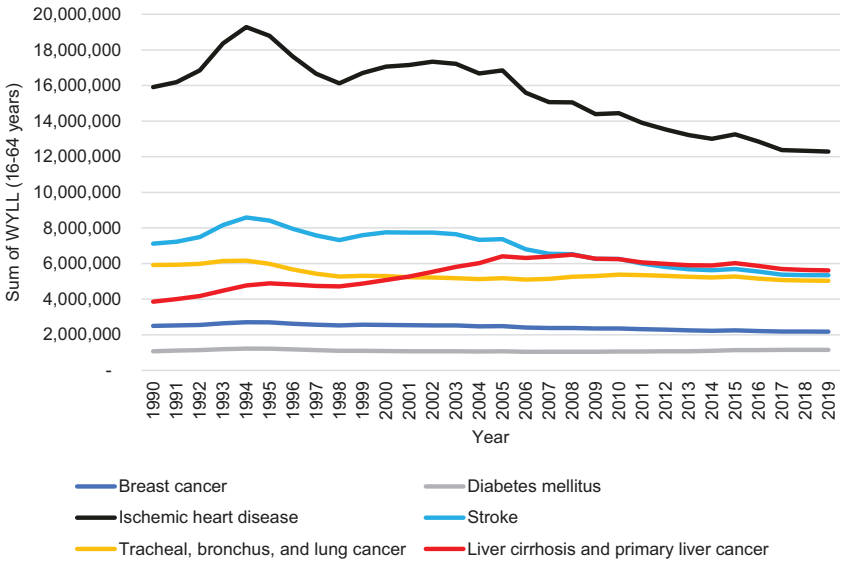

Fig. 10.1 The sum of working years of life lost (WYLL) in people aged 16-64 years) from the leading diseases in the WHO European region. Liver disease has overtaken stroke to become the second leading cause of WYLL exceeded only by ischaemic heart disease

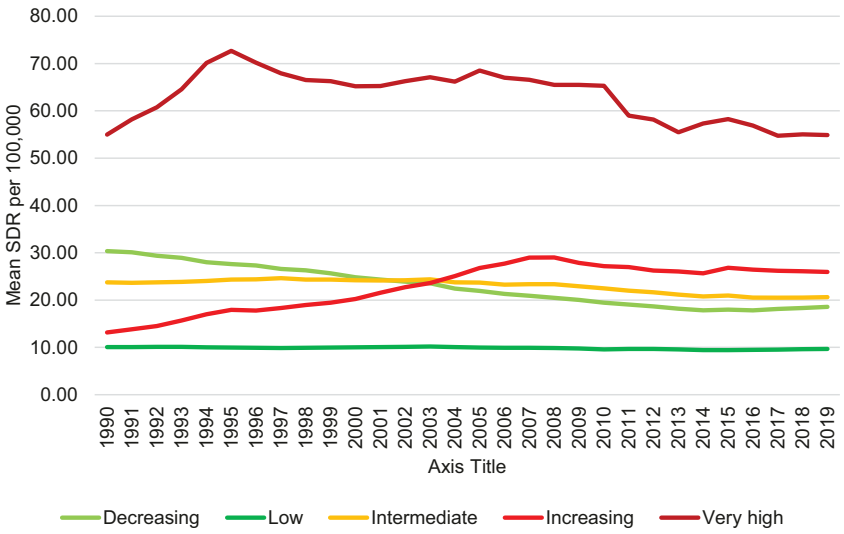

Fig. 10.2 Trends in directly standardised liver mortality rates (SDR) in WHO European region countries categorised into five groups according to the trend over time. There are marked differences between countries, but also liver death rates change considerably over time. Some countries in the Mediterranean basin have seen huge reductions in death rates, whereas other countries in including Finland and the UK have seen death rates increase substantially. The groups are as follows: Low: Greece, Ireland, Malta, Netherlands, Norway, Sweden; Decreasing: France, Italy, Portugal, Spain; Intermediate: Austria, Belgium, Croatia, Czechia, Denmark, Germany, Serbia; Increasing: Belarus, Bulgaria, Estonia, Finland, Latvia, Poland, Ukraine, United Kingdom; Very high: Hungary, Republic of Moldova, Romania

and the United Kingdom, liver mortality has risen. These data clearly demonstrate that there is nothing fixed or irrevocable about dying from liver disease. There are factors within countries that determine the 40–50-fold differences in liver mortality, and the fact that liver mortality can increase or decrease four- to fivefold over a few decades demonstrates that these factors do change and can be changed. By understanding and changing these key drivers, we could dramatically reduce liver mortality in Europe (Fig. 10.2).

The situation for liver patients in Europe is grim, but it need not be. There are simple, cheap solutions that could be implemented given a desire to improve the health of people in Europe and a degree of political will. The evidence for these solutions is well established and can be described in terms of some simple principles and relationships [5]. In this chapter we will examine the nature of these relationships.

Implications of the Exponential Relationship Between Alcohol Consumption and Liver Disease

Alcohol is a dose dependent toxin and there is a lethal dose of alcohol for anyone, however liver toxicity results in progressive liver fibrosis over 10–30 years followed by episodes of acute on chronic liver failure or variceal bleeding from portal hypertension. Liver mortality is usually related to these episodes which are strongly related to recent drinking; if alcohol consumption stops, the prognosis improves immediately. Liver disease is just one facet of health harm from alcohol and comprises around 25% of the attributable mortality with hypertension, stroke, cancer and mental and behavioural disorders comprising the remainder [6].

There are over 200 International Classification of Disease (ICD-10) codes with alcohol in their name. Some conditions are partially caused by alcohol, such as cancer, and others are entirely caused by alcohol, such as alcohol-related liver disease. For cancer, the dose response relationship is linear—the relative risk (RR) increases at levels above zero intake to 2.41 (95% confidence interval [CI] = 2.07, 2.80) at an intake of 100 g/day (Fig. 10.3) [7]. Large numbers of people are at a relatively low risk of alcohol induced cancer, but this adds up to a lot of people developing alcohol-related cancer, with an estimate of around 80,000 deaths and 1.9 million years of life lost in Europe in 2016 [9]. Because of the linear risk between alcohol and cancer, the majority of cancer cases occur in moderate drinkers, and heavy drinkers account for a minority of the overall burden. In contrast, the dose response relationship for cirrhosis is exponential, although heterogeneous, with RR between 10 and 70 at 150 g/day [10]. As a result of this relationship, the burden of liver disease is concentrated among the relatively smaller group of heavy daily drinkers with a high alcohol intake.

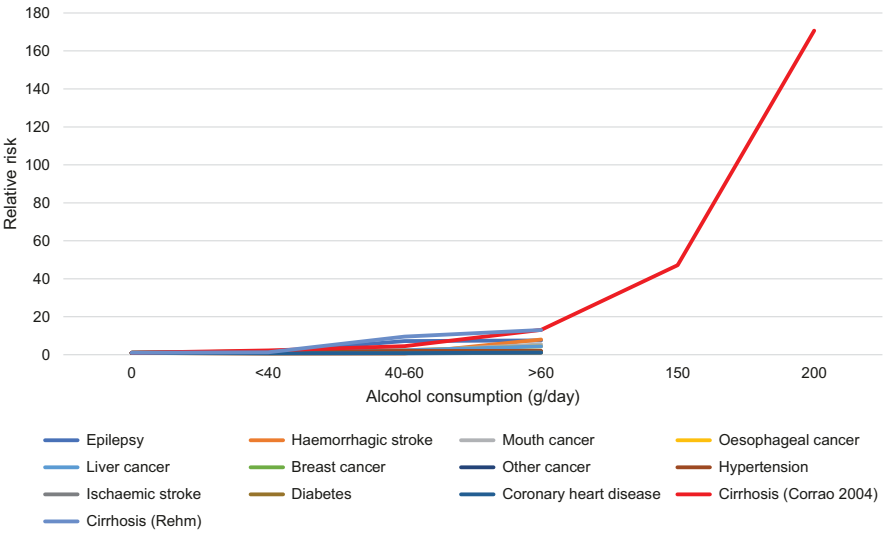

Fig. 10.3 Dose-response relationship between alcohol and 12 alcohol-related diseases in men. The figure is a simplified illustration of the various data models outlined in papers by Corrao and Rehm. For liver disease, the increase in relative risk is exponential and not linear, and as a result, the relative risk increases dramatically at very high levels of alcohol intake [7, 8]

If alcohol-related liver disease is dose-dependent at the individual-level, then it follows that it must also be dose-dependent at the population-level. A seminal paper by Milton Terris in 1967 illustrated the very close relationship between mortality from liver cirrhosis and population-level consumption of strong alcohol (wines and spirits) concluding: "The evidence strongly supports the conclusion that cirrhosis mortality is directly related to per capita consumption of alcohol from spirits and wine" (Fig. 10.4) [11]. The concept developed by Kettil Bruun and Griffith Edwards forms the basis of modern alcohol control policy. Ceteris paribus—all else being equal—the proportion of heavy and extreme drinkers remains similar. This 'population consumption theory' forms the basis of modern alcohol control policy [12–14], as the late Professor Griffith Edwards stated: "the overall level of a population's drinking is significantly related to the level of alcohol-related problems which that population will experience" [12]. This means that the most effective interventions to reduce harm are those that target the whole population. This assumption generally holds for conditions with linear dose-response curves, such as cancer, where the majority of health harm is found among the larger number of moderate drinkers. However, the dose-response curve for alcohol-related liver disease is exponential (Fig. 10.3), and as a result, cases are concentrated among a small group of heavy and extreme drinkers. Interventions targeted towards these heavy and extreme drinkers are likely to have a disproportionate benefit in terms of reducing liver mortality.

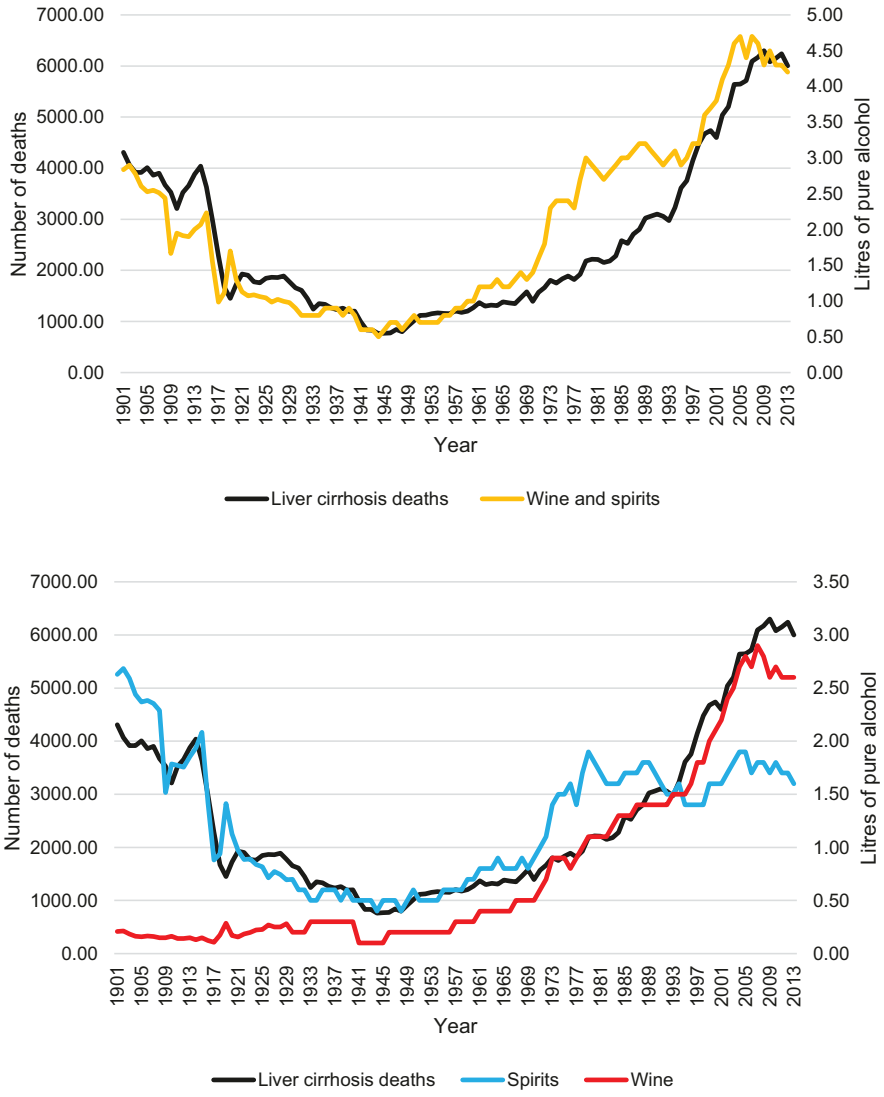

Fig. 10.4 The relationship between cirrhosis death rates and per capita consumption of wine and spirits in the UK 1901-2019. Adapted from Terris [11]

The "Unit of Alcohol" or "Standard Drink"

In academic studies alcohol is generally quantified in g/day, but alcohol is a liquid, alcoholic beverages are liquids and alcohol is sold in liquid measures. Furthermore the %ABV of alcohol is equivalent to the number of centilitres (cL) of pure alcohol in 1 L of product. With this information it becomes very easy to calculate the number of cL of pure alcohol in any drink.

In communicating health risks to the public, health agencies tend to talk about units of alcohol or standard drinks but there is no such thing. Wikipedia lists the various "standard drinks" in Europe, they vary from 8 g in UK, 10 g in France, 12 g in Finland, 17 g in Hungary and 20 g in Austria [15]. This is nonsensical. A sensible system would have a single standard measure of alcohol for public facing and academic facing communications, and the sensible choice would be to use 1 cL of alcohol as this standard measure. Many years ago we made this proposal to the European Health and Alcohol Forum, and over the life of the forum this was the single only proposal enthusiastically supported by both health and industry delegates. Establishing an EU standard alcohol measure at 1 cL is extremely low hanging fruit for a public health measure and should be prioritised.

It's Hard to Get Cirrhosis: Liver Disease Kills Extreme Drinkers

Alcohol-related liver disease is attributed to a single cause—heavy daily or near daily consumption—and the burden is concentrated among heavy and extreme drinkers. These drinkers are underrepresented across almost all routine datasets, but purposively sampled observational studies shed light on their typical consumption.

A pooled analysis of almost 3000 patients across seven countries demonstrates the very large volumes of alcohol consumed by patients with alcohol-related liver disease [16]. Almost half of the sample (45%) reported drinking >110 g of alcohol/day (96 cL/week), more than three bottles of vodka each week. A further 44% reported drinking between 80–160 g of alcohol/day (70–140 cL/week). The average daily consumption reported across the studies ranged from 65 g to 176 g/day (56–154 cL/week). Drinking an average of 84 g/day (73 cL/week) increases the risk of liver cirrhosis almost seven times in men (RR = 6.93, 95% CI = 1.07, 44.99) and over 12 times for women (RR = 12.44, 95% CI = 6.65, 23.27) [17].

In a British study the mean weekly consumption reported by patients with alcohol-related liver disease presenting to a large hospital was around 150 cL/week (Fig. 10.5) [18]. In a second sample of patients with alcohol-related cirrhosis or progressive fibrosis, the median weekly consumption was almost 75 cL/week [19]. Across a decade of household surveys in England, less than 1% of respondents reported drinking at equivalent levels to those reported in liver patients.

Only a minority of patients with alcohol-related liver disease have evidence of severe alcohol dependency [19–21], however daily drinkers with alcohol dependency have an eightfold increase in the incidence of cirrhosis [22]. In drinkers presenting to services with serious alcohol problems in Scotland, the median reported weekly consumption was almost 1500 g/week [23]. Among Scottish females drinkers presenting to these services the median was around 1200 g/week [24]. Similarly high volumes were reported in a cohort of patients undergoing medical detoxification in New Zealand at 1680 g/week [25].

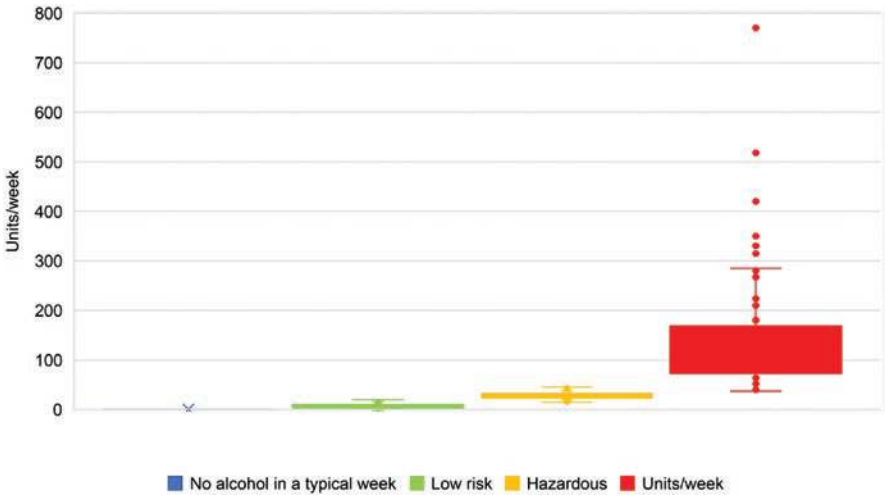

Fig. 10.5 Weekly alcohol consumption (cl or UK units) in patients with liver disease according to UK drinking grades low risk 14 cl and 35/50 cl for women / men. The mean intake in harmful drinkers with alcohol- related liver disease was 147 cl / week the median 120 cl / week [18]

The dose response relationship for alcohol-related liver disease means that heavy and extreme drinkers are at exponentially higher risk and decreases in consumption will reduce the risk of death per drink substantially more than the risk reduction experienced by lower risk drinkers.

Extreme Drinkers Buy Cheap Booze

As demonstrated in the previous section, people with alcohol-related liver disease report extremely high volumes of alcohol consumption—around 3.5 bottles of vodka per week [16]. As a result, they seek out cheaper alcohol. These extreme drinkers are underrepresented in general household surveys or consumer panels, so the limited data available to demonstrate this principle comes from observational studies specifically sampling heavy drinkers.

In England, patients with alcohol-related liver disease reported paying less than one-third of the price paid by lower risk drinkers per 10 cL of alcohol [18]. Lower risk drinkers spent an average of almost £500 on alcohol each year, which is the amount spent by higher risk drinkers in less than 2 months. In Scotland, patients with serious alcohol problems reported paying, on average, 1.7 times less per 10 cL of alcohol compared to the average price paid by drinkers in the general population [26]. When the sample was limited to those drinking at least 1600 g/week, the amount paid per 10 cL of alcohol was almost 2.5 times less than that paid by the general population.

Price Elasticity of Extreme Drinkers

The consumption of alcohol is to some extent determined by price or affordability. This relationship is described as an elasticity, which is the percentage change in consumption resulting from a 1% change in price. The recent systematic review by the Organisation for Economic Co-Operation and Development found that beer is the least price-sensitive beverage (with elasticities ranging from −0.29 to −0.83) compared to wine (−0.46 to −1.11) and spirits (−0.54 to −1.09), and that moderate consumers were slightly more price sensitive compared to heavier consumers with an additional elasticity of around 0.05% (heavier consumers were defined as ≥40 g/week and ≥20 g/week for men and women respectively) [27]. Heavier drinkers consumed around twice as much alcohol below a specific price threshold compared with moderate drinkers showing their clear preference for cheap alcohol [27].

However, the price elasticity of the very heavy daily drinkers who comprise the majority of patients with alcohol-related liver disease has never been specifically determined because they are not represented in population studies. Studies in clinical populations have confirmed patients with alcohol related cirrhosis or alcohol dependence have a clear preference for cheap strong alcohol [18, 26], and confirm that certain types of very cheap strong alcohol such as 'white cider' in the UK are consumed almost exclusively by very heavy drinkers [28].

In the UK, liver mortality rates have increased almost threefold since 1980 and the relationship between trends in mortality and the underlying changes in alcohol affordability are clear to see (Fig. 10.6).

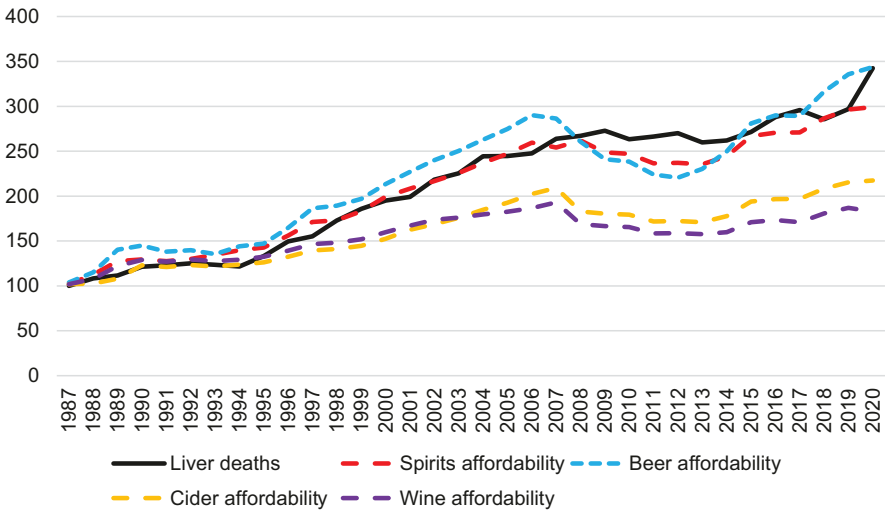

Fig. 10.6 Trends in the affordability of alcohol duty for different alcohol types in comparison to the trend in liver deaths alcohol-related liver deaths in England and Wales and duty-related affordability of alcoholic beverages. All data normalised to 100% in 1987. Affordability was calculated with the methods used by NHS digital with data sources outlined in Burton [6, 29]

Using duty receipt data from Her Majesty's Revenue and Customs and death data for England and Wales from the Office for National Statistics, the elasticity between total alcohol consumption and alcohol affordability was 0.41%, exactly what would be expected from the various meta-analyses (Fig. 10.7) [32, 33]. However, it is also possible to examine the direct relationship between alcohol affordability and mortality rates from alcohol-related liver disease, and calculate a direct elasticity, which in this case is 3.2%—six times higher than the elasticity on consumption (Fig. 10.8). This is similar to an estimate published in two systematic reviews which reported the elasticity for cirrhosis mortality to be around 3.5% (for reference, the elasticity for suicide was 0.5%) [34, 35].

Alcohol-related and liver mortality are strongly linked to health inequalities [36, 37]. The most likely explanation for this dramatic increase in elasticity is that extreme drinkers who die from alcohol-related liver disease are extremely price sensitive because they have already maxed out their spending on cheap alcohol. A price increase can be a stimulus to change behaviour, and in the case of extreme drinkers this will often be to stop drinking all together, thus reducing immediately their projected likelihood of death [38].

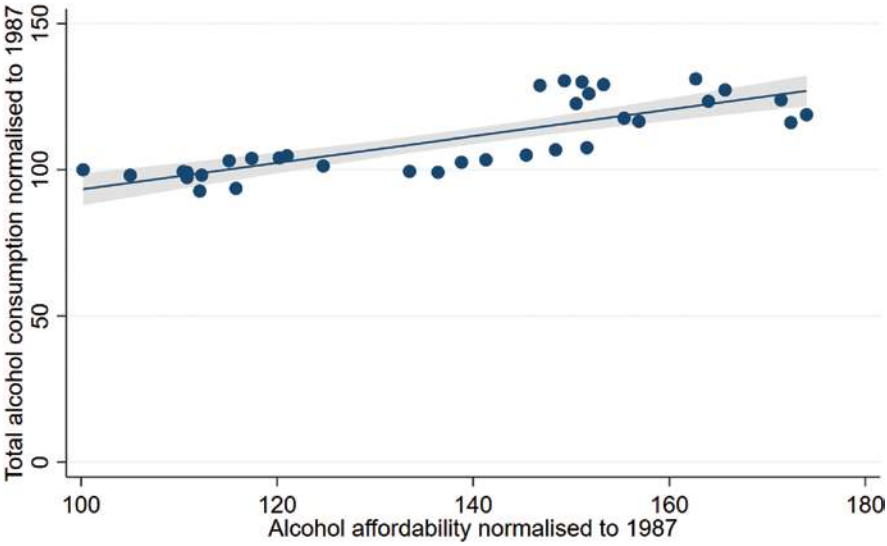

Fig. 10.7 The relationship between total alcohol consumption and the affordability of alcohol. Alcohol consumption is collated data from UK alcohol duty receipts published by the British Beer and Pub Association and affordability is from NHS Digital for the UK. All data normalised to 100% in 1980. A 1% increase in price is associated with a 0.46% reduction in alcohol consumption. Adjusted R2=0.64 [30, 31]

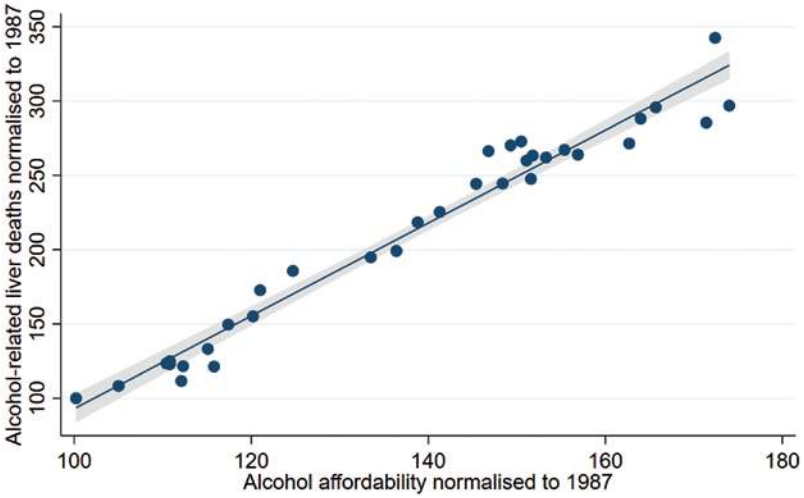

Fig. 10.8 The relationship between the affordability of alcohol and alcohol-related liver deaths. Affordability is for the UK from NHS Digital and alcohol-related liver deaths are for England and Wales. All data normalised to 100% in 1980. A 1% increase in price is associated with a 3.1% reduction in alcohol-related liver deaths. Adjusted R2=0.96 [30, 31]

Models of Policy Interventions

About 80% of deaths directly caused by alcohol are from alcohol-related liver disease, and around a further 10% are from alcohol dependency [39], so the drinking behaviour of these two groups are absolutely critical to the accurate modelling of minimum unit price (MUP).

The Sheffield model uses survey data to estimate the impact of MUP, and because heavy and extreme drinkers are substantially underestimated in these surveys, the result is a model which underestimates the policy's impact on alcohol-related liver mortality. The model reports the mean alcohol intake among harmful drinkers to be 571.2 g/week [40], which is 878.8 g less than the 1450 g/week reported in a sample of patients with alcohol-related liver disease [18]. Considering the incidence of alcohol-related liver disease in heavy drinking cohorts is between 10–20 times greater than the incidence seen in the general population [16, 41], the impact of this underestimate is likely to be substantial. The model estimates the yearly spend by higher risk drinkers to be £2862, which is £570 less than the average yearly spend on alcohol reported by the patient sample (£3432).

Minimum Unit Price in Scotland

The epidemiological, clinical and alcohol control policy evidence described previously presents a compelling case for action to prevent alcohol-related harm. Yet for many countries while levels of harm remain unacceptably high the implementation of the most impactful control policies has not materialised [42]. While the scientific evidence defines the nature and scale of a problem it is Government and politics that plays the central role in implementing effective control policies.

Health advocacy can play a key role in influencing policy change. This is more likely to be impactful if done from an informed position of the nature of policy making and which policy makers to target. Advocates will need to consider how best use to use their limited resources, their knowledge and experience to communicate the problem and solutions to policy makers and the public.

The policy making process is rarely a stable and linear process [43]. The reality is a more dynamic irrational process constructed through engagement with multiple actors often holding conflicting views on a given topic. This can be challenging for health professions and advocates to navigate as often they are used to a rational evidence-based approach. While scientific evidence derived from structured methods and subject to peer review, policy making is a "loosely organised body of precepts and positions" [44].

Policies that are aimed at preventing or reducing harm from commercial products such as alcohol that can damage health can be challenging to influence due to favoured political view on individual responsibility borne out of prevailing neoliberalism and the influence of lobbying of commercial actors [45]. Health advocates can play a key role in countering these views putting forward strategies based on equality and evidence [46].

While no framework of policy making fully captures the complexity of its reality, Kingdon's 'multiple streams approach' presents a dynamic model which can aid understanding of how some issues are addressed through policy changes and others are not [47]. It identifies three relatively independent streams or processes that when they converge create a window of opportunity through which policy change is more likely to occur (Fig. 10.9).

Advocates can influence each of the three streams and play an important role facilitating the convergence of the streams. This facilitation needs leaders, often outside of Government who can bring about policy change through the matching of problems, policy options and political support [49]. Success depends on gaining support from those in key political positions who can bring about change. Focusing on the following three areas can increase the likelihood of success:

- Making the case on the necessity to act
- Identifying the decision-making venues and procedures to pursue the proposals
- Adapting proposals to generate support and overcome opposition

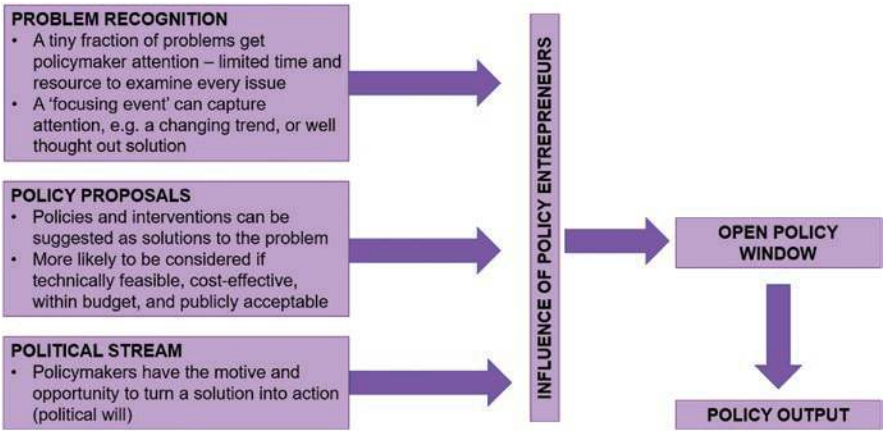

Fig. 10.9 The multiple streams approach to policy adapted from Kingdon [48]

How Policy Making Works: Does Evidence Matter?

Over recent decades, medical organisations and advocacy groups across the UK and Ireland have been vocal in their concern about health harm from alcohol. They have highlighted the burden and causes of harm, evidence-based solutions, and campaigned for Governments to do more [50–52].

Epidemiological data has been influential by identifying the burden of harm and the adverse trends. A 2006 study comparing the mortality rates from liver cirrhosis across the UK[9] has been identified as being influential in starting to change the narrative on Scotland's relationship with alcohol [53]. The study presented stark data showing mortality rates in Scotland were one of the highest in Western Europe, and had been rapidly increasing since the 1990's [54].

Evidence and expert opinion played an important role in identifying solutions to preventing and reducing alcohol-related harm including novel approaches to address the affordability of alcohol. In 2007, the advocacy group Scottish Health Action on Alcohol Problems published a report that linked alcohol's affordability with harm [52]. A key recommendation for the Scottish Government was to establish minimum prices for alcoholic drinks. A year later, a econometric study identified MUP as an effective measure to target those most at risk of harm [55].

Kingdon's policy theory suggests that problem recognition and solutions need to converge with political will to create the conditions conducive to policy change. The establishment of the Scottish Parliament in 1999 with responsibility health policy and the election of the Scottish National Party in 2007 opened up political consideration to wider prevention policies including addressing public concern about the availability and affordability of alcohol [56]. This paved the way for the Scottish Government to pursue MUP which came into force in May 2018. Ireland and Wales have now also implemented MUP, and Northern Ireland has consulted on implementation.

All fiscal policy, whether tax or MUP, is targeted because the impact is directly proportional to the amount alcohol consumed. While tax affects all drinkers, MUP is highly targeted, affecting those cheap high strength products favoured by heavier drinkers, and as such has the greatest potential to reduce health inequality [57]. Tax and MUP are therefore complimentary policies [58]. This has been confirmed by the highest courts in Europe and UK after a challenge by the alcohol industry that it have a detrimental impact on trade. The UK supreme court unanimously concluded that MUP in Scotland is *"a proportionate means of achieving a legitimate aim"* and in terms of the impact on health vs. trade impact *"That minimum pricing will involve a market distortion, including of EU trade and competition, is accepted. However, I find it impossible, even if it is appropriate to undertake the exercise at all in this context, to conclude that this can or should be regarded as outweighing the health benefits which are intended by minimum pricing"* [59].

While most producers have been against the policy, several retail bodies been in favour including supermarkets [60]. The extra income generated by MUP goes to retailers so therefore isn't a tax.

The media has a powerful role in agenda-setting and influencing public opinion this in turn can influence policymaker's and shape a government's response. Inaction by politicians to address an issue may in part be influenced by concerns relating to media backlash from legislative changes. An unpublished study suggests these concerns are unfounded and since the implementation of MUP in Scotland the majority of media discourse has been supportive of MUP (Robyn Burton unpublished data). Studies of media and political impact showed no adverse political impact (ibid). The fact that MUP is a highly targeted measure that does not affect the low-risk consumers that do not purchase the cheapest strong alcohol was central to the court judgements and hugely important to the favourable public and media response to MUP. Perhaps it is time for the alcohol policy community to re-evaluate their aversion to targeted alcohol policy measures.

The divergence of policy in relation to MUP across the UK nations and Ireland has created a controlled natural experiment with England as the no treatment control. This will produce important evidence on the impact of MUP on health harm and identify any unintended consequences.

Conclusions

Alcohol-related liver disease is a substantial public health burden but need not be. There are factors between countries that determine 40–50-fold differences in liver mortality across Europe [3]. Liver mortality within countries can increase or decrease four- to fivefold over a few decades demonstrating that these factors change and can be changed. There are simple cheap solutions that could be implemented given a desire to improve the health of people in Europe and a degree of political will. The underlying principles are straightforward. The dose-response relationship between alcohol and liver fibrosis is exponential, as a result, the

majority of liver mortality occurs in heavy daily drinkers who seek out cheap strong alcohol. This group are not represented in population studies and price elasticities for alcohol consumption are undetermined. However, data from the UK shows a strong relationship between the affordability of alcohol and liver mortality clearly demonstrating that these heavy drinkers are in fact extremely price sensitive. Interventions targeted towards very heavy drinkers, such as a minimum unit price for alcohol, are highly effective and cost-effective policies that can reduce liver mortality with practically no impact on low-risk drinkers. The solutions for alcohol related liver disease, the largest cause of liver death in Europe by a country mile, do not lie in expensive hepatology units or with Big Pharma. The solution is simply to set realistic minimum prices per centilitre of pure alcohol across Europe. As the results of the ongoing controlled natural experiments in alcohol policy become clear the political imperatives to do this will become overwhelming. See also other related Chaps. 2, 3 and 7.

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Part II
Alcohol Addiction: Introduction and Diagnosis

Chapter 11
Medical Treatment of Alcohol Use Disorder: A Multidisciplinary Approach

Julia Sinclair and Sarah Welch

Abstract There is a wide range of factors at the societal (macro), health system (meso), and individual (micro) level that will have a significant impact on the expectations and experiences of treatment for people with alcohol use disorder (AUD). These factors have a complex, dynamic interplay, and as they are less amenable to exploration using traditional biomedical research methods their impact is often overlooked.

Stigma, both felt and enacted, is one manifestation which will have an impact on how and when people who need medical treatment for AUD present, as does the lack of training for health professionals in the identification and management of alcohol related harm.

For people who recognise their need for treatment, there may be many obstacles to accessing the correct care. It may be helpful to consider AUD as a long-term condition, requiring a personalized treatment plan and active involvement of the patient and their circle of concern. Integrated pathways, and evidence-based interventions specifically for patients with alcohol related liver disease, and co-morbid mental health conditions are an essential component of any modern evidence-based treatment system to ensure people with AUD have the best chance of achieving and sustaining recovery.

Keywords Alcohol use disorder · Multidisciplinary · Stigma · Long-term conditions · Recovery · Recovery orientated systems of care

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Introduction

Most of this textbook's focus is on the evidence base for individual modes of intervention for patients with alcohol use disorder (AUD). In this chapter we will consider some of the broader, complex, and interacting latent factors that will have an impact on how these are delivered and received. These predisposing factors are less amenable to a randomised control trial design and therefore frequently overlooked within the biomedical context, but we believe are essential to consider when translating the evidence-base from one healthcare system to another.

We start with an overview of potential factors at the macro (societal), meso (health system), and micro (individual) level. These all have an impact on who accesses treatment, their duration of untreated AUD, the severity and complexity of any alcohol related harm, as well as their expectations of care.

See Fig. 11.1 *Overview of potential factors affecting the delivery, experience of, and outcomes from evidence-based interventions for AUD.*

All complex systems function using dynamic feedback loops and so while the flow is presented as moving from macro to micro (left to right) and from global to specific (top to bottom) the reality is that the impact on the system will be multidirectional. The relative impact of any particular factor or process will differ across societies and health systems, but all are relevant.

The components in Fig. 11.1 are not presented as an exhaustive list, but rather a conceptual map of processes that need to be considered when implementing an evidence-based treatment system.

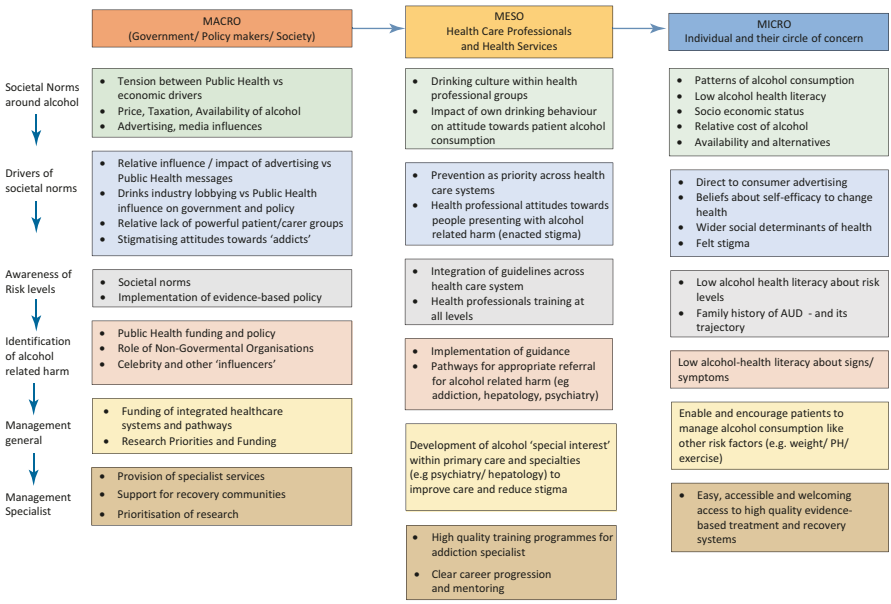

Fig. 11.1 Overview of potential factors affecting the delivery, experience of, and outcomes from evidence-based interventions for alcohol use disorder

Figure 11.1 helps in considering the broad systemic change that is needed to help with both prevention, self-identification and early identification of AUD and alcohol-related harm. These are all essential prerequisites to an optimal modern, evidence based and comprehensive treatment system.

Stigma at Macro, Meso and Micro Levels

When thinking about how the process map might be used, one approach may be to consider the impact of stigmatisation operating at different levels in communities and across health systems.

International research on the public stigmatization of people with AUD indicates that stigmatization is high in comparison with other mental disorders and has remained substantially unchanged over a 20-year period [1]. This is manifest in reluctance to consider alcohol use as a mental health problem; attribution of responsibility and blame; prevalence of negative stereotypes; and relative public acceptance of structural discrimination. If we start with the "micro" level, we can learn much from the reasons people give for not accessing treatment, or for significant delay before they do so. In a study of people identified as having AUD by screening in primary care settings in six European countries [2], the most common reasons given for not seeking treatment were "lack of problem awareness" and "stigma or shame". Given this message, what are the factors at macro and meso levels that can have a positive or negative impact?

At a "macro" level, if the priority given to alcohol health literacy and prevention of alcohol-related harms by national and local public health systems is low in proportion to the scale of alcohol-related harm, this constitutes a form of stigma by neglect. Further complications arise from tension between economic drivers and health concerns, and the relative influence of (for example) lobbying by the alcohol industry [3, 4]. Where evidence-based public health interventions (e.g., minimum unit pricing, taxation on alcohol, reduced access to alcohol) may be unpopular, it can be politically convenient for government departments to portray alcohol use as a matter of personal choice, therefore further stigmatising those who develop AUD.

In contrast, clear political leadership, and evidence of commitment to public health strategies to prevent and reduce alcohol-related harm can help to influence societal health literacy and attitudes towards alcohol use. Similarly, dedicated funding for high-quality, person-centred specialist treatment services, and prominence in priorities for research funding to develop the range of interventions available, indicate a commitment to people with AUD and to their circle of concern, as a public good rather than for a marginal group.

At a "meso" level, training of health professionals is a key factor with impact. Without training, staff in healthcare settings will bring to their roles the attitudes to alcohol use of their family background and wider culture. These will vary widely and not all attitudes will be stigmatising, but training should help to reduce overall stigma by providing staff with a sound understanding of the relationship between

alcohol use, alcohol-related harm, and AUD. Healthcare staff are particularly well-placed to have conversations about alcohol, and to help with prevention and early detection of AUD. For example, if discussion about alcohol use and screening for AUD is routine in a wide range of healthcare settings as for other risk indicators such as blood pressure, public understanding can grow. Healthcare staff who are comfortable with initiating conversations about alcohol and have positive and hopeful attitudes towards recovery can help people to feel positive both about their own potential for change and about their entitlement to seek help.

In contrast, where training is lacking, healthcare staff may feel powerless to help. When this happens, there may be a "collusion of denial" [5] where the problem is not spoken about; or the staff member may see the problem as the responsibility of others or of the person themselves. Therapeutic nihilism resulting from underfunded treatment services, and the lack of high-profile patient advocacy groups (as seen for cancer diagnoses in many countries) also has a stigmatising impact.

For people who have already realised that they are in difficulty with alcohol use, and who would like help, there may be many obstacles to accessing the correct care. These may include difficulty in finding out where and how to seek help, understanding what that would entail, and how feasible that may be for them based on resources required (including time, transport and other practical factors). Current alcohol treatment systems often appear complex to those not working within them, and people require a significant amount of agency to navigate their way successfully through them [6]. A further barrier noted by Probst and colleagues [2] in their primary care-based study was the wish not to stop drinking completely: therefore it is important that healthcare services welcome people who have concerns about their alcohol use but are undecided about their long-term goals [7]. To be effective, service planning at "macro" level and service delivery at "meso" level need to take into consideration these and other barriers that impact local populations.

Specialist alcohol treatment systems need to offer high quality person-centred care, to be acceptable and effective [8]. This includes integration with sustainable recovery communities (see below), which link in with health systems as part of a dynamic pathway for individuals to enter and exit treatment [9]. In most societies, funding for alcohol-related research does not reflect the burden of harm from this substance. Thus, a proactive co-ordinated and responsive system, which could influence policy makers could also stimulate a more active and productive research environment.

With the broad-ranging vision described above, the hope would be that:

- alcohol is used more safely in the population in general
- fewer people are at risk of alcohol-related harm
- where harm is experienced, it is detected quickly (by improved early self-identification due to increased levels of alcohol health literacy)
- people are more willing to present to health professionals (reduced stigma)
- Integrated health systems and training of health professionals ensure early detection in all settings
- effective help is available at the "early intervention" stage

- fewer people will develop severe AUD
- of those who develop severe AUD, fewer will progress to a long-term condition with an inherent pattern of remission and relapse.

However, there will always be individuals whose vulnerabilities (biological, psychological and social) will require the specialist interventions described in section three of this text. The rest of this chapter therefore considers the conceptualisation and framework of a multi-disciplinary, modern, and comprehensive treatment system with which people with severe AUD may best engage. If we consider severe AUD as a long-term condition, it will require a holistic and personalized response to people's needs which will change over time. A recovery-orientated system of care offers a helpful lens through which to consider this [9–11].

AUD as a Long-Term Condition and Recovery Systems

Definitions of Recovery

The concept and parameters of what constitutes 'recovery' have changed over the years and there still remain significant variations in how it is used [12–15].

However, over the last two decades the concept of recovery has evolved from being almost uniquely associated with the 12-step process where the terms 'recovery' and 'abstinence' were essentially synonymous to a much broader concept. The United Stated Substance Abuse and Mental Health Services Administration (SAMHSA) defined recovery broadly for addictions and other mental disorders as having four dimensions [16]:

- Health
- Home (having a stable and safe place to live)
- Purpose (meaningful daily activities)
- Positive relationships and networks

The lack of a clear consensus on the definition of recovery has inevitably limited the development of a body of evidence that could assist in understanding its role in terms of improving outcomes for patients with severe AUD within treatment systems. However, the US National Institute on Alcohol Abuse and Alcoholism (NIAAA) [17] has recently published an alcohol specific recovery research definition based on DSM-5 AUD criteria, which they hope will address these limitations and facilitate recovery-related science. In this definition, as well as being an 'outcome', recovery is also recognised as a 'process' through which individuals 'pursue' remission from DSM-5 AUD criteria, as well as cessation of heavy drinking, both of which they also define [17].

This categorical definition may help improve the clarity of research in this area, but globally definitions of recovery are likely to remain highly variable, and potentially contentious. It is also worth noting that given that stigma towards 'alcoholism'

from which the 12-step recovery movement emerged, for some the term 'recovery' is similarly stigmatised and other less value-laden terms such as living 'alcohol-free' are preferred [18]. As in other areas of medicine and society people may choose to self-identify as being 'in recovery', despite never having had a diagnosis of AUD or any treatment for it. Given that the significant majority of people with severe AUD do not access any formal treatment [2, 19–21], this process of 'natural recovery' represents an important and significant number of individuals. Understanding that people with AUD may reach stable recovery through a range of 'natural' as well as 'assisted' routes, enables an exploration of how and why this may vary between different individuals [22]. This in turn could inform policy at the macro and meso level to reduce stigma, improve access to appropriate treatment at the appropriate time, as well as impact on societal norms around alcohol.

AUD as a Long-Term Condition

The historical divisions between addiction and broader psychiatric services have been well described [10], and sadly remain in many places. This continued lack of integration despite overwhelming evidence of comorbidity between disorders [23] needs to be addressed as well as the importance of treating both disorders if positive outcomes for patients are to be achieved [24, 25].

The concept of recovery-oriented systems of care evolved from the advocacy of people who had overcome addictions or mental ill health for provision of care to be more than the reduction of symptoms or substance use, and to focus more holistically on the longer term goals of individuals living well in their communities [9]. Whilst this requires the engagement of the individual, active consideration is also needed of the wider determinants of health and social inequalities (e.g., childhood adversity, poverty, social networks, educational disadvantage, poor housing, race, gender etc.), which may reduce the agency needed by an individual to access and engage with treatment services, and sustain that engagement over time [6]. Whilst the effectiveness of Recovery orientated systems of care have not been systematically tested, there are many similarities with the well-developed evidence base for management of long-term conditions from which evidence can be extrapolated.

From a health system perspective considering AUD as a long term condition (or within a chronic disease model) is reasonable given its natural history, spectrum of severity, relapse and remission pattern, and frequent co-morbidities [26]. The effective management of long-term conditions requires a range of interventions: evidence-based medical interventions, targeted to the stage and severity of illness; patient education to enhance self-management; and the development of 'systems of support' (including non-health professionals, voluntary and community groups, and immediate circle of concern) [27]. As part of this, the role of collective efficacy is recognised as important in supporting sustained behavioural change and improving outcomes [28].

The joint working between patients ('experts by experience') and clinicians ('experts by training') is increasingly recognised as essential for optimal outcomes in long term conditions and for patients with AUD. This includes the development of patient reported outcome measures (PROMS) and patient reported experience measures (PREMS), which focus on health-related quality of life, and aspects of the 'humanity of care' from a patient perspective [29, 30]. One such PROM has been developed with people in recovery (but not specifically for AUD), focussing on patient perspective both in its development and intended use. SURE –(substance use recovery evaluator) has been shown to be psychometrically valid, and easy to complete [31]. PROMS combined with other longer-term quality of life measures used in research and routine care [32] should help to develop services which are more personalised and responsive to patient needs.

So what might modern, evidence based and comprehensive treatment for patients with AUD look like?

First, we need to be acutely aware that any specialist service for people with AUD will only ever be a marginal aspect of the societies in which we live, where the majority of people drink alcohol and where alcohol production and consumption may be a significant economic driver. This causes tension for national and regional policy makers, structural stigma within health systems, and challenges for patients to know how best to access care at earlier stages [33]. It could be argued that an essential aspect of clinical leadership within addiction services is to play a role in challenging this, and developing the competencies required within the non-specialist workforce [34].

Second, if we consider AUD as a long-term condition, we need to continue to improve and refine our evidence-based treatments, so that differences in age, gender, stage of illness, and associated co-morbidities can be factored into a personalized treatment plan. Patients' pathways are rarely linear (see Fig. 11.2) most will

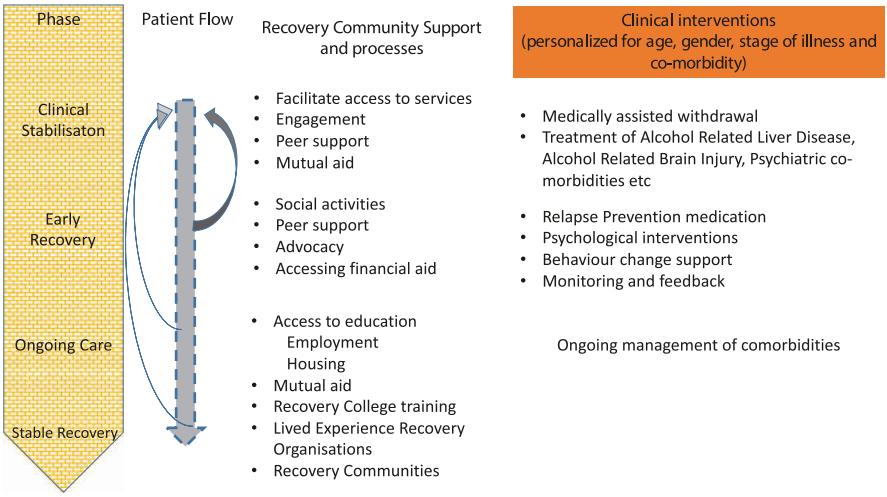

Fig. 11.2 Framework for a modern comprehensive system of care for severe AUD

have spiral trajectory through any system of care, and each new presentation may require a different approach. Therefore, health systems need to be set up to be able to offer a range of modalities of treatment as appropriate (e.g., harm minimization, medically assisted withdrawal, relapse prevention interventions etc.) rather than these being seen as separate entities. In addition, the pathway through services and the importance of systems of support in developing collective efficacy is likely to be significantly enhanced by integration with recovery communities providing peer support, facilitating access to housing and education, as well as encouraging an early return for clinical treatment after relapse.

Finally, given the significant interdependencies of AUD with alcohol related comorbidity and mortality, and the wider health and social inequalities experienced by people with AUD [33, 35] the structural barriers to accessing appropriate personalized care need to be reduced. Integrated pathways, and evidence-based interventions specifically for patients with alcohol related liver disease [36, 37], and co-morbid mental health conditions [24, 25] are an essential component of any modern evidence based treatment system to ensure people with AUD have the best chance of achieving and sustaining recovery.

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Chapter 12
Diagnosis of Alcohol Use Disorder and Identification of Unhealthy Alcohol Consumption

Anne Lingford-Hughes and Alexander Vale

Abstract Correct identification of individuals that misuse alcohol and robust, comprehensive assessment of their symptoms, circumstances and complexity is essential for making effective decisions about treatment. This chapter discusses how definitions of harmful levels of alcohol consumption differ between nations and when compared to World Health Organisation (WHO) guidance. It also outlines the two principal frameworks that exist for diagnosis of alcohol related disorders, the fifth version of Statistical Manual of Mental Disorders (DSM-5), and the eleventh revision of the International Classification of Diseases (ICD-11). Classification of alcohol disorders within the DSM-5 has undergone significant changes from its predecessor. Assessment of patients with alcohol problems is best achieved by taking a comprehensive psychiatric history, adapted for and focussed on the patient's history with alcohol. This chapter will outline the kind of comprehensive assessment undertaken in a specialist alcohol service that can be adapted depending on the setting and presentation of the individual. Alcohol history taking is demonstrated in view of the diagnostic criteria set out earlier in the chapter. Several other considerations during the assessment process are described to allow for better understanding of the backgrounds and complexities of those who misuse alcohol. The chapter describes the relevance of social and personal circumstances to the drinker, the physical health and neurological complications alcohol drinkers may experience and the interface with co-morbid mental health problems.

Keywords Alcohol · Diagnosis · Assessment · Addiction · Dependence · Physical health · Mental health

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Introduction

Correct identification of individuals that misuse alcohol and robust, comprehensive assessment of their symptoms, circumstances and complexity is essential for making effective decisions about treatment, and to establish a therapeutic alliance. Consideration of alcohol use disorders should be made in all clinician settings, ergo all clinicians should have an awareness of alcohol use disorders and when to seek support and specialist intervention, always acting within their own competencies. This chapter will examine the frameworks that exist for diagnosis, before moving onto considerations a clinician should make when assessing patients. Breakdown of the assessment process is comprehensive but not exhaustive and directs the reader to further topics when required.

Harmful Levels

To contextualise the diagnosis and assessment of patients with alcohol problems, it is imperative to understand the description of alcohol use and safe drinking levels. There is no single international standard. While many countries have adopted the "standard drink" to include 10 g of pure alcohol as dictated by the World Health Organisation (WHO) [1] and its Alcohol Use Disorders Identification Test (AUDIT), this varies widely, ranging between at least 8–20 g in different countries.

There is an even wider variation in how countries define low risk alcohol consumption. Some define low risk consumption both per day and per week, while others do not. In addition, some countries chose to make separate recommendations for men and women. Table 12.1 [2] below illustrates this, and for comparison, the

Table 12.1 Governmental standard drink definitions and low-risk consumption guidelines in grams of pure ethanol from selected European countries compared with the United States adapted from [2]

Country	Standard drink (g)	Guidelines for men		Guidelines for women	
		Per day	Per week	Per day	Per week
USA	14	56	196	42	98
Austria	20	24	168	16	112
Croatia	10	12	–	10	–
Denmark	12	–	168	–	84
France	10	30	210	20	140
Germany	12	24	–	12	–
Ireland	10	–	170	–	110
Italy	12	36	–	20	–
Poland	10	40	280	20	140
Spain	10	–	170	–	110
Sweden	10	20	–	10	–
United Kingdom	8	24–32	–	16–24	–

WHO defines low risk consumption on a single drinking day as 1–40 g for men, and 1–20 g for women. Comparisons to the United States are of particular importance given the proportion of clinical trials that emerge from the country.

Diagnosis

The reader will likely be aware of the two principal frameworks for mental illness classification used globally. Firstly, we have the Statistical Manual of Mental Disorders (DSM) published by the American Psychiatric Association (APA). The fifth and latest version of the DSM was published in 2013 and it will henceforth be referred to as DSM-5 [3]. Secondly, the International Classification of Diseases (ICD), published by the WHO that is currently on its 11th revision, was ratified in May 2019. It will henceforth be referred to as ICD-11 [4]. Each publication differs in origin and scope which will not be discussed in this book chapter. The diagnostic criteria set out in these frameworks underpin the assessment of patients who misuse alcohol. Of note, it is convention for the DSM-5 to be the principal framework used in research studies. This is important as there are differences between DSM-5 and ICD-11, particularly in regard to 'alcohol dependence' as described below [5].

DSM-5

From DSM-IV to DSM-5 there were bigger changes compared to the relatively incremental changes between the latest versions of the ICD [3, 6]. In the DSM-IV, there were two distinct disorders (alcohol abuse and alcohol dependence) which have been collated into a single disorder with sub-classifications to denote severity for the DSM-5. The new diagnostic category, Alcohol Use Disorder (AUD), represents a single continuum between abuse and dependence, therefore AUD would not always be synonymous with alcohol dependence going forward. Of note is that DSM-5 removes a criterion referencing legal problems, including arrests and criminal convictions directly resulting from alcohol use.

Previously in DSM-IV, over a 12-month period, meeting 1 or more abuse criteria would denote an alcohol abuse diagnosis. If over the same 12-month period, a patient met 3 or more dependence criteria, they would receive the diagnosis of alcohol dependence. Now in DSM-5 when a patient meets 2 or more of the following criteria within a 12-month period, a diagnosis of Alcohol Use Disorder can be made as shown in (Table 12.2):

Table 12.2 AUD is subject to the following sub-classification; Mild AUD, 2–3 Criteria met, Moderate AUD, 4–5 Criteria met, Severe AUD, 6 or more criteria met

Criteria	Alcohol use disorder (DSM-5)
1	Alcohol is often taken in larger amounts or over a longer period than was intended
2	There is a persistent desire or unsuccessful efforts to cut down or control alcohol use
3	A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects
4	Craving, or a strong desire or urge to use alcohol
5	Recurrent alcohol use resulting in a failure to fulfil major role obligations at work, school, or home
6	Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of alcohol
7	Important social, occupational, or recreational activities are given up or reduced because of alcohol use
8	Recurrent alcohol use in situations in which it is physically hazardous
9	Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol
10	Tolerance, as defined by either of the following:
	(a) A need for markedly increased amounts of alcohol to achieve Intoxication or desired effect
	(b) A markedly diminished effect with continued use of the same amount of alcohol
11	Withdrawal, as manifested by either of the following:
	(a) The characteristic withdrawal syndrome for alcohol
	(b) Alcohol (or a closely related substance, such as a benzodiazepine) is taken to relieve or avoid withdrawal symptoms

ICD-11

ICD-11 describes two distinct diagnostic categories that will be especially clinically relevant to the reader, Alcohol Dependence and Harmful Pattern of Use of Alcohol. Previously in the ICD-10 they were referred to as Alcohol Dependence Syndrome and Harmful Alcohol Use respectively [7]. The Table 12.3 indicates the definition of each diagnosis using slightly formatted but close to exact language from the ICD-11.

The definition of alcohol dependence has changed incrementally between ICD-10 and ICD-11. The diagnosis performed well psychometrically and showed high concordance with its DSM-IV equivalent, meaning that fundamental changes were unnecessary as described by Saunders et al. [8]. With regard to Harmful Pattern of Use of Alcohol, the paragraph regarding harm to the health of others was added to the ICD-10 counterpart to reflect the collateral damage experienced by those around the alcohol user [9].

Table 12.3 Alcohol dependence and Harmful Pattern of Use of Alcohol as described by ICD-11

Alcohol dependence	Harmful pattern of use of alcohol
A disorder of regulation of substance use arising from repeated or continuous use of substance. The characteristic feature is a strong internal drive to use substance, which is manifested by impaired ability to control use, increasing priority given to use over other activities and persistence of use despite harm or negative consequences	A pattern of alcohol use that has caused damage to a person's physical or mental health or has resulted in behaviour leading to harm to the health of others
The diagnosis requires two or more of the following three central features to be evident over a period of at least 12 months, but the diagnosis may be made if alcohol use is continuous for at least 1 month.	The pattern of alcohol use is evident over a period of at least 12 months if substance use is episodic or at least 1 month if use is continuous.
1. Impaired control over alcohol use—in terms of the onset, level, circumstances or termination of use, often but not necessarily accompanied by a subjective sensation of urge or craving to use alcohol.	Harm to health of the individual occurs due to one or more of the following:
2. Alcohol use becomes an increasing priority in life—takes precedence over other interests or enjoyments, daily activities, responsibilities, or health or personal care. Alcohol use takes an increasingly central role in the person's life and relegates other areas of life to the periphery; continues despite the occurrence of problems.	1. Behaviour related to intoxication
3. Physiological features (indicative of neuroadaptation to the alcohol) as manifested	2. Direct or secondary toxic effects on body organs and systems
By; (i) tolerance, (ii) withdrawal symptoms following cessation or reduction in use of that substance or (iii) repeated use of the substance (or pharmacologically similar substance) to prevent or alleviate withdrawal symptoms. Withdrawal symptoms must be characteristic for the withdrawal syndrome for alcohol and must not simply reflect a hangover effect.	3. A harmful route of administration.
	Harm to health of others includes any form of physical harm, including trauma, or mental disorder that is directly attributable to behaviour related to alcohol intoxication on the part of the person to whom the diagnosis of Harmful Pattern of Use of Alcohol applies.

There is no DSM-5 equivalent for Harmful Pattern of Use of Alcohol. The ICD-11 also added a new category entitled Hazardous Use of Alcohol. This is included in the health risk factors section and does not constitute a diagnosis. It is not discussed in full during this chapter other than to define it as not yet reaching the level of having caused harm to physical or mental health of the user or others around the user, which distinguishes it from previously discussed diagnostic categories (Fig. 12.1) [4].

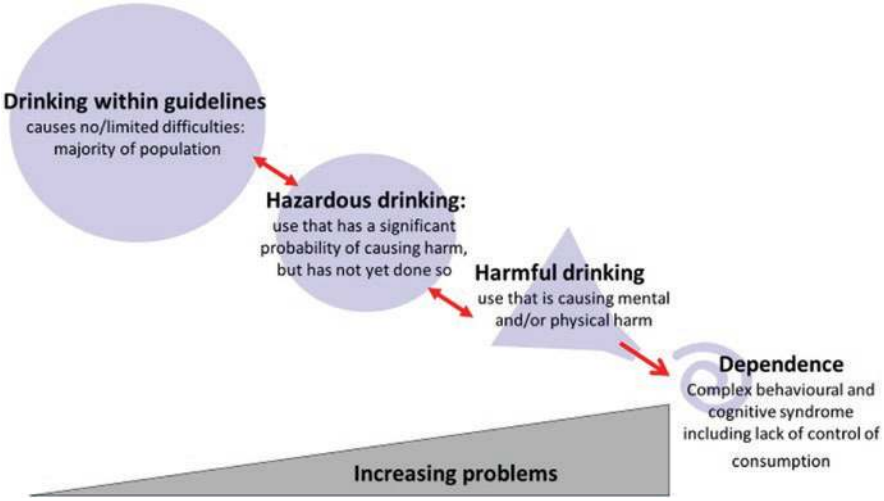

Fig. 12.1 Progression of categories of drinking behaviour according to ICD-11. Harmful drinking is depicted as a funnel as without change, the risk of progression to the 'spiral' of dependence is high. Likely movement between categories is depicted as bidirectional arrows, with one-way arrow to dependence as recovery to other categories of drinking behaviour is unlikely

Binge Drinking

There is no universally accepted definition of binge drinking and therefore no diagnostic criteria. In the United States, binge drinking is defined as a pattern of drinking that brings a person's blood alcohol concentration (BAC) to 0.08 g/dL or above. This is typically achieved when men consume 5 or more standard drinks (14 g of pure ethanol in the USA) and women 4 or more drinks in a 2-h period [10]. In contrast, the U.K. [11] defines binge drinking as males who exceeded 8 "units" of alcohol on their heaviest drinking day, and females who exceeded 6 "units" on their heaviest drinking day. (A "unit" is a term used in the U.K. to denote 8 g of pure ethanol).

The initial description of binge drinking is centuries old, and with time the term has moved from describing a period of heavy drinking lasting several days, toward often describing a single episode or day. Somewhat confusingly, both definitions continue to be used when describing the problems of those that misuse alcohol [12]. In view of this, when drinkers use binge drinking to describe their own relationship with alcohol, it can refer to a variety of patterns. A clinician should always clarify the exact details with focus on the degree of control they exhibit.

Dependence vs. Addiction

Before moving on to assessment of patients with alcohol disorders, an important point needs to be made about the difference between addiction and dependence. During the meetings and subsequent debates between experts that resulted in the creation of the DSM-III-R [13], published in 1987, a vote decided on diagnostic language used. Many favoured "addiction", citing its association with a behavioural compulsion and distinguishing it from physical dependence. The counterargument for "dependence" declared it could more easily be applied to all substances and would avoid unnecessary stigmatisation. As the reader may have inferred, "dependence" won the vote [14].

The result has created linguistic confusion for clinicians, and the reader may have also noticed that "dependence" was removed from a diagnosis for DSM-5. In biology and pharmacology, dependence refers to a physical adaptation to a substance including, for example, tolerance and withdrawal. Like opioids, alcohol and benzodiazepines, other prescription drugs can cause physical dependence but are not necessarily associated with broader complex behaviours typically seen in 'addiction', for example drug-seeking, primacy of drug-related behaviours etc.

It is important to communicate with patients in language they both understand and feel is relevant to establish both rapport and a therapeutic relationship. Furthermore, a misunderstanding between clinicians may lead to mismanagement of the patient's illness. For example, if a patient is admitted to a medical setting for a pharmacological detox to treat alcohol dependence, they could be discharged as soon as the detox is completed, without an aftercare plan. This fails to acknowledge that addiction often follows a relapsing-remitting pattern, and that long-term disease management that includes psychological support and treatment is essential in sustained abstinence and recovery.

Assessment of Patients with Alcohol Related Problems

The assessment of patients with alcohol problems is best achieved by taking a comprehensive psychiatric history, adapted for and focussed on the patient's history with alcohol. This chapter will outline the kind of comprehensive assessment undertaken in a specialist alcohol service in order to demonstrate the gold standard. Patients' needs and priorities will vary widely, meaning assessments may be more focused on different areas, depending on the patient. It is likely that the information will be gathered over more than one visit and involve contact with a range of members of a multidisciplinary team.

It is important to remember that although this is likely to be an interaction between a clinician and a patient, it is also a discussion between two people [15].

The patient is likely to share deeply personal and possibly traumatic information when describing their history with alcohol. Creating the right environment for the patient to feel comfortable doing so is therefore integral. Assessment alone can help change the drinker's attitudes towards drinking [16].

It is also important to make a few further points about language. If the assessor is familiar with the vernacular of individuals who misuse alcohol, for example the measures in which they consume drinks (e.g., pints, cans, a half of whisky) or even brands and preparations of alcohol available it is likely to increase rapport and set the tone for a better assessment. Some people with alcohol disorders may not even wish to be known as "patients". It is commonplace in the U.K. to refer to people accessing alcohol services as "clients". In practice, it is best to clarify preference in this regard with the person directly [17]. This chapter refers to people with alcohol use disorders as patients for consistency.

Referral

If a patient has been referred to an alcohol service, it is important to note who made the referral, which can be done before seeing the patient. They may have self-referred after doing their own research, or be referred there by another clinician or service. Alternatively, they may have been referred directly by another professional. One common route of referral to draw attention to is those from criminal justice systems, for whom referral to treatmens is sometimes also part of a probation arrangement. A self-referral may indicate a greater degree of motivation, whereas a history of non-attendance would naturally raise concerns about engagement and indicate that a more assertive approach may be required. Ideally, referrals should be well triaged by an experienced or the lead clinician.

Alcohol History

It is most important to establish a current pattern of drinking, in part to inform the clinician about the nature and severity of the patients' alcohol problems, and whether they are physiologically dependent. Some patients will have a very regimented and consistent pattern of consumption. At the other extreme, some patients will vary the quantity and type of alcohol daily. Below are important lines of enquiry and some examples of common effective questions [18]. The reader should note that an aim of the alcohol history is to ensure all domains in the diagnostic criteria set out earlier in the chapter are discussed.

1. **"Talk me through a typical drinking day".** This alone can sometimes elicit enough information to determine quantity, type of alcohol and drinking pattern. This can be followed with closed clarifying questions if required. It is also good

practice to ask the patient to complete a simple drink diary over 1 week, especially if further clarifications are required, or the patient is unable to describe their drinking effectively. If possible, the exact nature of alcohol consumed should be determined, including the brands and preparations.

2. **Salience.** One must determine if other aspects of the patients' life have been neglected in order to facilitate the procurement and consumption of alcohol. Suggested domains for inquiry would be employment ("have you missed work due to your drinking?") and relationships (has your relationship with your partner/children been affected by your drinking?). The clinician should directly enquire about the impact of recovery from heavy drinking episodes on the patients' responsibilities.
3. **Control.** A lack of control over alcohol consumption is a central feature of alcohol use disorders. Patients should be asked if they often drink more or for longer than initially intended. Another useful question is "Who has more control, you or the alcohol?" [15] A week or two of abstinence interspersed with weeks of heavy continuous drinking or another harmful pattern use, is not evidence of control. The patient may perceive such patterns as controlled and should be sensitively challenged accordingly [15]. Furthermore, the patient should be asked if they have an unfulfilled desire, and made unsuccessful attempts to reduce or stop drinking. This is also highly relevant to treatment planning, since in the absence of motivation to stop or reduce drinking, treatment is not likely to be successful [19].
4. **Compulsion.** The patient should be asked directly about the strength of their craving or urge to drink. This should be separate from features of withdrawal discussed below. The clinician should also ask if this compulsion continues in the presence of knowledge that drinking is harmful to their physical health, mental health and social well-being.
5. **Tolerance.** Consuming progressively more alcohol without reaching intoxication is an important feature of dependence. A simple question could be "Have you noticed that you need to drink more to become drunk?" or "Are becoming less drunk with the same amount of alcohol?"
6. **Withdrawal.** The patient must be asked about what happens if they do not drink. If the patient is physically dependent, he or she will experience the classic alcohol withdrawal syndrome. Symptoms to be specifically asked about are agitation, restlessness, sleepiness, shakiness, sweating, nausea, withdrawal seizures, and hallucinations in visual and tactile modalities. A clinician may enquire about hallucinations by asking "do you ever see or sense things that aren't there?". Alcohol withdrawal is dangerous, potentially fatal and often poorly understood by the drinker. For example, many patients who are alcohol dependent are unaware that withdrawal exists beyond tremor and agitation. The identification and management of withdrawal is discussed in a separate chapter. It is important to differentiate alcohol withdrawal from blackouts, defined by the inability to recall events that occurred during a drinking episode. These can be complete or fragmentary and are associated with increased harm. Patients can also be scared the following day upon realising they have no recollection of events [20].

7. **Timeframe including periods of abstinence**. Alcohol Use Disorders often follow a relapsing-remitting pattern. Enquiring about the timeframe of a patient's alcohol use is necessary per the diagnostic criteria, and helps the clinician better understand the patient's journey and circumstances. Carefully document the patient's timeline, and include treatment they received, if any, at the time. Previous treatment may include detoxification (inpatient or outpatient), rehabilitation (inpatient or outpatient), previous engagement with specialist drug and alcohol services, psycho-social interventions and experience with mutual aid organisations, for example, Alcoholics Anonymous.
8. **Risks.** Enquiry into and assessment of risk is an important part of a psychiatric history, and this remains true when adapted for alcohol use disorders. Important discussion points include:

   – Risky behaviours associated with acute intoxication; For example, intoxication may result in unprotected sexual intercourse, increasing exposure to sexually transmitted viruses. Other common situations that could lead to harm include physically violent altercations, drunk driving, operating machinery, or walking alone at night in darkened areas. A degree of disorientation occurs in acute intoxication leaving the person at risk of accidents and misadventure of varying consequence [15, 18].
   – Deliberate self-harm and suicide attempts; both are of higher prevalence in those with alcohol disorders and must be enquired upon. The clinician should determine whether there are suicidal thoughts, intent, or specific plans. If the latter, the nature of those plans must be explored, with the understanding that plans involving active methods (e.g., hanging, use of firearms) are associated with the highest risk. This information must be integrated with an understanding of other key risk factors for suicide, such as male sex, and lack of social support (see below).
   – Additional risk to others; the clinician should enquire about the welfare of dependents in the patients' life for example, their children and anyone else they formally care for.

9. **Treatment Goals.** It is important to establish the goals of the patient in order to inform future treatment. A patient who drinks daily may wish for a 'quick fix' of more "controlled drinking", which is difficult to achieve. Alternatively, they may wish for abstinence and plan to do this immediately which is unwise and dangerous. More suitably, a patient that drinks daily may wish for abstinence via gradual reduction in consumption or a pharmacologically assisted detoxification programme, or a patient that occasionally drinks to excess may wish for less frequent episodes where less alcohol is consumed. Establishing patient goals allow for an open discussion and expectation setting.

Screening Questionnaires

There are several validated questionnaires to assess alcohol use disorders. They should not replace the comprehensive framework of assessment illustrated in this chapter, but can identify those needing onward referral to specialist services. They are useful in settings where assessment of alcohol related problems was not the primary goal of the conversation, for instance in outpatient clinics, inpatient psychiatric wards and psychological therapy services.

The most used screening questionnaire is the Alcohol Use Disorder Identification Test (AUDIT), developed with support of the WHO [21]. The standard version comprises of 10 questions each scoring 0–4 points, and can form part of a structured interview or be self-administered. A score of 1 to 7 suggests low-risk consumption. Scores from 8 to 14 suggest hazardous or harmful alcohol consumption, and a score of 15 or more indicates the likelihood of alcohol dependence (moderate-severe alcohol use disorder according to the DSM-5). Thus, a score of 8 or higher denotes drinking warranting further assessment. The Fast Alcohol Screening Tool (FAST) and Paddington Alcohol Test (PAT) are two relatively recent questionnaires that are brief and intended for use in emergency room settings [15].

Polysubstance Use Involving Alcohol

Polysubstance use has become increasingly common and has been exacerbated by increased availability of prescription drugs of abuse such as opioids and benzodiazepines. This chapter will not discuss the prevalence of other substance use disorders with alcohol in detail but will include vital information. The most common substance use combination with alcohol is nicotine and it has been shown that each can act as a conditioned cue for the other [22]. The clinician should ask about tobacco use when assessing patients with alcohol use disorders, and should be aware of important associations. For example, alcohol dependent smokers have higher rates of tobacco related disease, and are more likely to die form tobacco related disease than alcohol dependence [15]. Furthermore, head and neck cancers, cirrhosis and pancreatitis are more common in those that misuse alcohol that also smoke [23].

The clinician should ideally use an open question such as “Do use any other drugs?”, before asking a series of clarifying closed questions if needed about individual drugs. If not mentioned in the discussion, the clinician should be sure to ask about use of the following drugs and their derivative products; cannabis, stimulants including cocaine and amphetamines, opioids, benzodiazepines and “Z-drugs”, novel psychoactive substances and club drugs including ketamine, gamma hydroxybutyrate (GHB), MDMA and mephedrone. This list is not exhaustive, hence there is

value in asking “Do you use anything else?” to ensure substances are not missed. This question could also reveal the misuse of prescription drugs that may not necessarily be illegal. Another question that can be of relevance is” do you buy anything over the internet?” as drugs of abuse are increasingly being procured online.

Of particular importance are drugs that can create a dangerous and fatal level of central nervous system and therefore respiratory depression in combination with alcohol, with opioids, benzodiazepines and GHB being important examples. Furthermore, when cocaine and alcohol are taken together, they form an active metabolite called cocaethylene. This enhances the euphoric effects of cocaine and increases the risk of cardiotoxicity. If a patient with polysubstance use is encountered, a referral to a specialist drug and alcohol service is strongly recommended.

Social Circumstances and Personal History

This broad area of the assessment process allows for a better understanding of the patient, their strengths and vulnerabilities, and how this may shape their relationship with alcohol [15]. Information gathered from this conversation can also give the clinician insight into the patient’s support network, which can be crucial in achieving and maintaining abstinence. The clinician should gather information on patients’ childhood, interaction with their parents, and educational attainment. The patient should also be asked about family history of alcohol use disorders, especially amongst first degree relatives.

The clinician should enquire about past and current employment history, and their current financial situation and sources of income, including how much they are spending on alcohol per week. One should also ascertain current and historical romantic relationships, and access to a healthy and supportive friendship network with particular focus on how alcohol might impact the relationships. Finally, it is good practice to enquire about forensic history, and any interactions the patient has had with the police force, especially in the context of acute intoxication. It is worth noting the association between alcohol and domestic violence, and drinkers can be both the perpetrators and the victims [24]. The clinician should aim to sensitively enquire about domestic violence, especially if suspicion is raised.

Physical Health and Neurological Complications

Discussion and assessment of physical health is an integral component of the approach to treating individuals with drinking problems. The World Health Organisation estimates that 4% of all worldwide deaths and 4.5% of the global burden of disease and injury are attributable to alcohol [25]. At the point of pharmacological dependence, one can be close to certain that it presents danger to the individual’s body tissue and biochemistry.

The clinician should question the patient on their past medical history, identifying current and past diagnoses and the extent of specialist involvement. For example, are they treated in a hepatology clinic? To fully investigate all potential physical complications, a patient would require several interventions. The list below is not exhaustive but should allow the reader to consider the comprehensive nature of physical complications in those who misuse alcohol. It is essential that a clinician acts within their own competence and seeks advice and onward referral, when necessary, which is likely to be often. An advanced nurse practitioner or doctor should be involved in assessment and clinical decision making at this juncture.

1. **Physical examination**. An abdominal examination in view of the association with liver disease, acute and chronic pancreatitis, gastrointestinal ulceration and other gastrointestinal tract disease.

   Alcohol is primarily metabolised by the liver, making this organ an important site of alcohol related pathology. Accordingly, alcohol is the biggest cause of liver damage in the U.K., USA, Europe and Australia [15]. Patients may not develop or notice symptoms of alcohol-related liver disease until it is advanced, resulting in jaundice, encephalopathy or gastric bleeding, so early identification of those who are at risk is crucial. There are three broad categories; fatty liver (reversible with abstinence), alcoholic hepatitis (may be fatal but can be reversible with abstinence), alcoholic cirrhosis (progressive and can be fatal but can stabilise with abstinence). Some alcohol services may have access to simple ultrasound methods to screen high risk patients for fatty liver disease and generate appropriate referral to a hepatologist. Non-alcoholic fatty liver disease is associated with obesity alone.

   Other considerations would include cardiac examination and electrocardiogram (ECG), due to risk of arrythmia and cardiomyopathy; respiratory examination, due to increased risk of lung infections secondary to impaired immunity; head and neck examination, due to the association with related malignancy.
2. **Neurological examination**. Alcoholic cerebellar degeneration classically presents insidiously with ataxia and incoordination. Wernicke's encephalopathy is a medical emergency occurring secondary to thiamine deficiency in those that misuse alcohol. Presentation is usually acute, and a classic diagnostic triad of confusion, ataxia and abnormal ocular movements is described. Practically, all three are present in only 10 percent of cases. There should be a low threshold for seeking emergency medical care, especially with new confusion. Individuals with alcohol use disorders may also experience peripheral neuropathy, likely secondary to vitamin B deficiency, and poor diet is also linked to cerebellar degeneration. Therefore, a basic assessment of diet and comment upon nutritional status of the patient is essential. Another neurological consideration is the risk of acute or chronic subdural hematoma. These result from tears in bridging veins that cross the subdural space, and are more likely to occur in patients with a long history of heavy alcohol use that has resulted in gray matter loss and concomitant increase in subdural space. Subdural hematomas are often secondary to falls when intoxicated, but can also occur in the absence of any traumatic brain injury.

3. **Cognitive Assessment**. There should be a low threshold for assessment if cognitive impairment is suspected, particularly in older patients and those that have been drinking heavily for long periods. Common methods of assessment include the Mini Mental State Examination (MMSE) and Addenbrooke's Cognitive Examination (ACE).

   Korsakoff's syndrome can emerge following an acute episode of Wernicke's Encephalopathy or occur insidiously. It is characterised by a deficiency in recent memory, a striking lack of insight, and a disturbance in the sense of time. Personality changes including apathy and self-neglect may also occur, as can confabulation (fabrication of experiences to fill memory gaps). Other cognitive functions usually appear intact unless examined in careful detail.

   Autopsy in heavy drinkers shows a significantly decreased brain weight. CT scanning shows cortical shrinkage and ventricular enlargement in two thirds of alcohol dependent people compared to age matched controls [26]. Similar results have been seen with MRI studies with changes in both grey and white matter [27].

   If a patient is cognitively impaired, it is important to establish a timeline of symptom onset in order to determine whether changes are directly alcohol related. Personality changes resulting from other mechanisms of cognitive impairment could initiate alcohol misuse. It is important to note whether performance on neuropsychology testing improves with abstinence.
4. **Cardiovascular disease risk factors**. The latest evidence suggests that alcohol consumption increases coronary heart disease amongst all drinkers. Previous observational evidence indicating a J-shaped relationship and that light to moderate drinking has a cardiovascular protective effect was oversimplified and oversold [15]. The clinician should consider other risk factors not previously discussed, i.e., body mass index, age, gender, hyperlipidaemia and smoking status.
5. **Other disorders to consider**. Excessive alcohol consumption is associated with several other dermatology, endocrine and metabolic disorders, for example osteoporosis and gout. Excessive alcohol consumption is associated with the development of type II diabetes, which may resolve with abstinence. If a type II diabetic ceases to drink, one must be mindful that a patient's diabetic medications could be more likely to cause hypoglycaemia.
6. **Relevant serological investigations**. Biomarkers are discussed in a separate chapter, but clinically relevant blood tests should be considered depending on clinical findings and further discussion. Serial liver function tests are likely to be required in alcohol-related liver disease.
7. **Imaging**. Brain imaging is required if cognitive impairment or cerebellar degeneration is suspected, preferably by Magnetic Resonance Imaging (MRI). Radiological examination of the liver is often required, typically by ultrasound or computed tomography (CT).

Pregnancy

There is no clear evidence constituting a safe level of alcohol consumption in pregnancy.The consensus is that ideally, pregnant women should abstain from alcohol altogether, due to both increased risk during pregnancy and delivery (including spontaneous abortion, premature delivery and stillbirth) and to the foetus (including foetal alcohol spectrum disorders). It is not advisable to have a baby whilst alcohol dependent due to the physical and emotional consequences. If this situation nevertheless arises, immediate integration with a specialist drug and alcohol service is necessary. Unsupported, abrupt cessation of alcohol would remain dangerous.

Co-Morbid Mental Illness

Alcohol misuse is associated with several co-morbid psychiatric disorders. Many trials examining the treatment of those with alcohol dependence do not include patients with psychiatric co-morbidity. It is important that the clinician recognises when to signpost to other specialist mental health services. This chapter will not describe the diagnostic criteria of other mental disorders, the reader should examine the DSM-5 or ICD-11 for further information as required. Depression and anxiety are the most important to be aware of and it can be difficult to discern whether they were a factor in the development of an alcohol disorder, or a consequence of it. It is good practice to undertake a mental state examination as part of the assessment process. It can provide information on the status of co-morbid mental illness, or even provide clues to undiagnosed mental disorders. If any concerns emerge it is advisable to seek clarification from experienced professionals where necessary.

Depression

Depression is the most important mental illness to ask about and must be distinguished from normal feelings of sadness following adversity. Onset of depressive symptoms should be cross referenced with the timeline of a patient's drinking history, in order conclude which came first if possible. Failure to make these determinations leads to the overdiagnosis of depressive illnesses in those with alcohol use disorders. It can take a significant amount of time to develop an understanding of a patient's story, but it can be essential to ensure effective treatment [15].

This differential diagnosis is not trivial, and needs to be carefully pursued on a case-by-case basis. On one hand, co-morbidity of independent depressive illness and alcohol use disorders is higher than expected from their respective prevalence. On the other hand, depressive symptoms can occur as part of an alcohol withdrawal syndrome, but have been shown to commonly dissipate as a patient achieves

abstinence. Indeed, for most patients' depressive symptoms improve as they reduce or stop drinking but in some, symptoms can persist or even emerge in abstinence. Therefore continual, dynamic assessment of the patient is required if they are receiving on-going treatment from an alcohol service, and the clinician should be aware of how to perform this effectively.

Anxiety Disorders

Symptoms of anxiety disorders are common in patients with alcohol disorders. As with assessing depressive symptoms, the clinician must determine the timeframe of symptoms in relation to the patients' alcohol history and periods of abstinence in order to direct management. Anxiety is a central feature of alcohol withdrawal and often follows heavy drinking, so assessment should distinguish these periods from formal anxiety disorders for example, generalised anxiety and panic disorder when forming diagnoses. Post-Traumatic Stress Disorder (PTSD) is an important anxiety disorder to make a point of. One third of individuals with PTSD are estimated to have had a diagnosis of alcohol dependence in their lifetime [28].

Deliberate Self-Harm and Suicide

Deliberate self-harm and suicide are associated with alcohol use disorder. Thoughts of and intent to carry out each should therefore be asked as part of the risk assessment. Various studies have demonstrated the increased risk of suicide attempts and completed suicide, and remarkably, alcohol consumption precedes or is part of an episode of deliberate self-harm half the time in all of those that then seek hospital treatment [29]. Those with alcohol problems may experience a lack of social support and isolation; unemployment and financial difficulty; co-morbid mental and physical health problems, all of which are risk factors for completed suicide [29, 30].

Bipolar Affective Disorder

Up to 45% of patients with Bipolar Affective Disorder have an alcohol use disorder [31], and there is greater association with manic than hypomanic states. The clinician should determine whether periods of heavy alcohol use are historically associated or causative of manic episodes. If a patient with an alcohol disorder is manic, disinhibition and seeking relief from uncomfortable situations may increase drinking. Acute mania is usually apparent during routine history taking, however, more subtle symptoms may emerge during formal psychiatric examination.

Schizophrenia and Other psychoses

Lifetime prevalence of alcohol use disorders in schizophrenia is estimated at 20% [32]. As with other psychiatric co-morbidity, the clinician should determine the relationship between alcohol and the pattern their psychotic illness follows. Current symptoms of psychosis should be enquired upon in the psychiatric examination, although as with mania, they may be self-evident. Obtaining information from independent informants can be helpful when symptoms are difficult to evaluate. In the event acute psychotic symptoms are present in the context of no past history of psychotic illness, they should be differentiated from alcoholic hallucinosis. This is a rare disorder characterised by auditory or visual hallucinations occurring acutely and in clear consciousness either during or after heavy drinking. Differentiation from schizophrenia can be difficult and may need specialist advice. Psychotic symptoms are also present in withdrawal states (delirium tremens) discussed in a separate chapter.

Other Psychiatric Disorders

Several other disorders can influence the assessment of individuals with alcohol problems. There is a strong association with personality disorders (most commonly anti-social and borderline as per the DSM-5 criteria), attention deficit hyperactivity disorder (ADHD) and eating disorders (most commonly binge eating and bulimia nervosa). As with all psychiatric co-morbidity, it may be that the degree of complexity requires assessment within a specialist alcohol service.

Medication History

It is important to take a detailed list of the patients' current prescribed and over the counter medication, ideally directly from their prescription to ensure accuracy. This can further inform the clinician on the past medical and psychiatric history in the event a patient has forgotten to disclose a condition. It may also present an opportunity to give information on dangerous combinations with alcohol, for example with anti-coagulant medications, or identify potential diminished treatment response, for example lithium in bipolar affective disorder. It can be useful for a clinician to confer with a pharmacist on such matters.

Conclusion

Alcohol is a commonly used drug and unfortunately many individuals consume alcohol at such levels as to impair their mental and physical health. Alcohol impacts on every organ in the body and individuals may not realise the harm their consumption is causing them. Therefore, clinicians should question every patient about their alcohol consumption and to probe further, as necessary, to determine if criteria for harmful use or dependence are met. Direct questioning should also establish if there are any impacts on their health. If someone is drinking at a level causing harm, simple interventions such as making them aware may change their consumption, prevent progression to dependence and improve their health. Once dependent on alcohol, a comprehensive assessment is essential in order to develop an appropriate treatment plan accounting for any biopsychosocial risks eg complications from alcohol withdrawal, safe-guarding, suicidality etc. and recovery capital eg previous periods of abstinence. Not completing an alcohol assessment may result in a patient having unnecessary investigations and/or treatment and should therefore never be missed.

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Chapter 13
State Markers of Alcohol Use and Their Application

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Abstract Alcohol-related disorders are widespread and often underdiagnosed. They are associated with substantial costs, not only for the individual drinking alcohol but to the society as a whole. Questionnaires and biomarkers are useful to facili-

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tate screening, diagnosis, and treatment of alcohol-related disorders and thus prevent later complications. The analytical spectrum offers a wide portfolio of direct and indirect alcohol markers which can be investigated. Indirect state markers such as gamma-glutamyl transpeptidase (GGT), carbohydrate deficiency transferrin (CDT), and the mean corpuscular volume (MCV) are influenced by age and sex, various substances, and non-alcohol-related diseases. Furthermore, they do not cover the entire timeline for alcohol consumption. Direct state markers such as ethyl glucuronide (EtG), phosphatidylethanol (PEth), and fatty acid ethyl esters (FAEE) have gained enormous interest in the last decades as they embed the consumed ethanol within their chemical structure. As biomarkers with high sensitivity and specificity covering different windows of detection, they are recommended in guidelines, should be routinely applied, and contribute to new perspectives in the prevention, interdisciplinary cooperation, diagnosis, and treatment of alcohol-related disorders.

Keywords Alcohol biomarker · Ethanol metabolites · Traditional biomarkers · Ethyl glucuronide · Phosphatidylethanol · Gamma-glutamyl-transferase

Introductory Paragraph
State markers of alcohol use offer the opportunity to objectively assess alcohol intake. According to their specific characteristics, they provide different types of information: from abstinence monitoring to assessing heavy use over time. If appropriately applied and used in conjunction with self-reports and questionnaires, they become an indispensable tool in the assessment (including screening) and treatment of many conditions, such as alcohol use disorders (AUD), evaluation of liver transplant candidates, forensic evaluations, and others.

Introduction

Alcohol-related disorders are among the 10 most common diseases worldwide. The point prevalence for alcohol dependence, e.g., in Germany, as in other comparable countries, is 5%, and the lifetime prevalence is 10%. Worldwide, approximately 4% of deaths are attributable to alcohol. This is greater than deaths caused by HIV, violence, or tuberculosis [1]. The yearly costs attributable to alcohol in Europe are approximately 270 billion €. Costs produced by alcohol include both direct costs (i.e., costs in which goods or services are being used or delivered such as medical care) and indirect costs (those stemming from lost productivity due to illness, death, or accidents). Another relevant source of costs attributable to alcohol are intangible costs (costs that are not related to any material loss: e.g., emotional suffering). Importantly, many of these costs are born not only by the individual drinking alcohol but by society as a whole (the so-called social costs) [2].

It is estimated that only about 10% of all alcohol-dependent individuals receive specific treatment, most of them (about 80%) by their general practitioner and only a minority in specialized settings or general hospitals [3]. Thus, alcohol-related disorders are common, expensive in their entire course, and often underdiagnosed. To facilitate screening, diagnosis, and treatment of alcohol-related disorders and thus prevent later complications, questionnaires like the CAGE questionnaire or the Alcohol Use Disorders Identification Test (AUDIT) are useful and recommended in various guidelines. Biomarkers also play an important role in many of the stages of alcohol use disorders, from screening and early detection to treatment monitoring, especially in abstinence-oriented settings [4, 5].

Indirect state markers, as well as direct state markers, are routinely used to detect alcohol use. The indirect state markers like gamma-glutamyl transpeptidase (GGT), mean corpuscular volume (MCV), and carbohydrate deficiency transferrin (CDT) are influenced by age and sex, various substances, and non-alcohol-related diseases. Furthermore, they do not cover the entire timeline for alcohol consumption and in some cases, they need prolonged ingestion of relatively high amounts of alcohol to become elevated [6].

Direct state markers have gained enormous interest in the last decades as they are metabolites of alcohol that become detectable in or after the presence of alcohol. As biomarkers with high sensitivity and specificity that also collectively can cover a more complete timeline following alcohol use, they should be routinely used, and can contribute to new perspectives in the prevention, interdisciplinary cooperation, diagnosis, and treatment of alcohol-related disorders.

Direct Ethanol Metabolites

Direct ethanol metabolites available for routine use are:

- Ethyl glucuronide (EtG), in serum, urine, and hair
- Ethyl sulfate (EtS), in urine and serum
- Phosphatidylethanol (PEth) in whole blood
- Fatty acid ethyl ester (FAEE) especially in hair

Ethanol metabolites are detectable in serum for hours, in urine for up to 7 days, in whole blood for more than 2 weeks, and in hair over months.

Ethyl Glucuronide

Ethyl glucuronide (EtG) is a phase II metabolite of ethanol, with a molecular weight of 222 g/mol, and metabolized by the UDP-glucuronosyl transferase, see Fig. 13.1. Although only about 0.5% of all the ethanol ingested undergoes this degradation

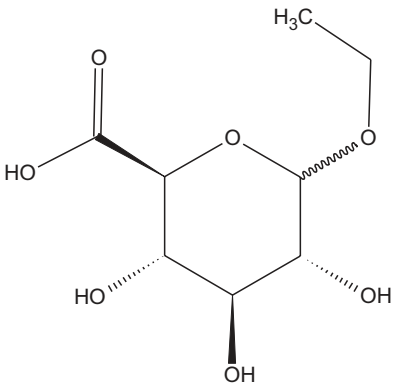

Fig. 13.1 Molecular structure of ethyl glucuronide

pathway, EtG can function as a biomarker that is detectable in the presence of ethanol. Moreover, EtG is non-volatile, water-soluble, stable in storage, and can, depending on the amount consumed and time spent for consumption, still be detectable in the body for a prolonged period after alcohol has been eliminated [7]. It can be detected for up to 90 h in urine. There is no difference in the elimination rate between a healthy population and heavy alcohol consumers at the beginning of detoxification treatment [8]. EtG can be detected in post-mortem body fluids and tissues like gluteal and abdominal fat, liver, brain, and cerebrospinal fluid, and also in the bone marrow and muscle tissue.

Compared with traditional biomarkers, EtG displays a high sensitivity. Even small amounts like 0.1 L champagne can be detected up to 27 h when measuring EtG. Experiments with 1 g ethanol (champagne, whisky) as well as the use of mouthwash and hand sanitizer gels might yield positive ethyl glucuronide concentrations, but usually with values of less than 1 mg/L in urine [9]. Measurable concentrations in urine can be found up to 11 h after the ingestion of alcohol. This aspect is of relevance regarding unintentional exposure to alcohol: Pralines, nonalcoholic beer, pharmaceutical products, fruit juice, sauerkraut, mouthwash products, and hand sanitizer gels may contain small amounts of alcohol. Even the intake of 21–42 g yeast with approximately 50 g sugar leads to measurable EtG and EtS concentrations in urine.

Therefore, a patient's claim of not having consumed alcohol may be trustworthy, even when EtG is detectable in urine. Since patients in withdrawal treatment should avoid even the smallest amount of alcohol, they have to be informed of such hidden sources of ethanol to avoid unintentional intake. A differential cut-off of 0.1 mg/L in cases where total abstinence is the goal, and 1.0 mg/L if small amounts of alcohol intake are tolerated, have been recommended for practical reasons. Applying these cut-offs, the probability of false-positive results is very low [10].

Low positive EtG values can reflect unintentional intake of ethanol. However, by informing the patient about potential pitfalls and using appropriate cut-offs this issue can be handled appropriately.

Selected Applications for the use of EtG:

1. Outpatient Addiction treatment programs

   Regular alcohol screening is a frequent intervention in many outpatient addiction treatment settings. Urine EtG has shown a higher sensitivity compared to traditional screening methods in this population, providing more accurate feedback to both patients and professionals. In a former study, the percentage of ethanol-positive samples was less than 2%, whereas screening for ethyl glucuronide reported positive in ~22% of the cases [11].

2. Specific high-risk groups

   In opioid-maintenance treatment, numerous patients are suffering from Hepatitis C (HCV) infection. Alcohol consumption, especially in large amounts, can lead to the progression of cirrhosis. Furthermore, alcohol potentiates respiratory depressant effects of methadone. Previous studies showed the usefulness and necessity of the determination of ethyl glucuronide in patients in opioid-maintenance treatment. For example, one study showed that of all EtG-positive patients 42% (n = 8 of 19) would have not reported alcohol consumption [12]. Therefore, the use of direct ethanol metabolites in high-risk groups permits further possibilities for therapeutic interventions, consequently leading to improvement in the quality of life.

3. Monitoring Programs

   Ethyl glucuronide is successfully used in monitoring programs like the Physician Health Programmes in the USA. They provide a therapeutic program for physicians with impairing health conditions such as substance-related disorders. Being in the monitoring program, physicians with substance-related disorders are allowed to maintain their licensing on the condition that regular proof of abstinence is shown. Measuring EtG in urine, Skipper and colleagues showed that of 100 random samples collected, no sample was positive for alcohol using standard testing; however, seven were positive for EtG (0.5–196 mg/L), suggesting recent alcohol use. EtG testing can provide additional information and consequently, may lead to further treatment and improvement for the patient [13].

4. Pharmacotherapeutic studies

   As an objective outcome parameter, EtG testing has shown to be useful in pharmacotherapeutic studies [14].

5. Liver transplantation

   Up to 30% of liver transplantations are related to alcohol. Post-operatively, 20–25% of patients lapse or relapse to alcohol intake. In 18 patients with alcohol liver disease (ALD), Erim et al. found no self-report of alcohol consumption. One out of 127 tests for breath alcohol was positive, whereas 24 of 49 urine samples were positive for EtG. Comparable results were reported by another

study, which found self-reported alcohol consumption in 3% in contrast to 20% positive urine EtG and EtS tests [15].

6. Pregnant Women

   Alcohol intake during pregnancy is a well-established risk factor for developmental impairments, especially fetal alcohol spectrum disorders (FASD). It is also known that a relevant proportion of pregnant women drink alcohol. Therefore, alcohol screening during pregnancy should be routinely conducted. Alcohol intake during pregnancy can be investigated in maternal (including hair, blood, and urine) and fetal specimens (meconium). Similar to other groups, self-reports and questionnaires tend to underestimate alcohol intake. Ethyl glucuronide both in urine and meconium has been shown to improve the detection of drinking [16].

   The applications mentioned above show that EtG tests complement self-reports and questionnaires, yielding valuable information on alcohol consumption that is relevant to diagnosis and treatment.

Irrespective of the setting and population where it is applied, EtG always displays a significantly greater sensitivity for the detection of alcohol drinking when compared to questionnaires, self-reports, and traditional biomarkers. However, the information it provides must always be considered together with that obtained from other sources, and the final assessment and decision is a clinical one.

Methodological Aspects

The gold standard for the determination of EtG is liquid chromatography-tandem mass spectrometry (LC-MS/MS), which remains the only valid method of analysis in inquiries with medico-legal relevance [17]. However, availability and cost of this methodology have been issues in the past. Therefore, a semi-quantitative EtG Enzyme Immunoassay (EIA) became commercially available a decade ago. It has a clinical cut-off of 500 ng/mL and offers a low and clinically relevant analytical range (15.3–2000 ng/mL). Its validity is very similar to that of the gold standard. A more recent methodological development has been the appearance of point-of-care EtG immunoassay dip cards, which are commercially available at a very low cost. A recent study suggests the validity of the method to be high [18].

Limitations

Among the limitations of EtG analyses are false-negative results produced by urinary tract infections, especially E. Coli, as certain strains may be able to degrade EtG rapidly. This degradation does not appear to affect the direct alcohol biomarker

ethyl sulfate (EtS) within the urine sample, which remains stable despite the infection. This leads some authors to recommend simultaneous analysis of both biomarkers when a false-negative result is suspected for EtG. Interestingly, a study reported that the bacterial degradation of EtG by E. Coli can be prevented by the use of dried urine on filter paper (DUS). Furthermore, the addition of bacterial growth inhibitors into liquid urine containers (sodium azide sampling tubes) is feasible [19].

Given the impact alcohol has on liver function, it is important to note that liver disease does not influence the validity of EtG, as has been shown in studies conducted on patients with liver disease, including cirrhosis. Similarly, previous studies also suggest that race, nicotine consumption, body mass index, and body water content do not influence EtG concentrations. Not even the presence of Gilbert's syndrome, a glucuronidation disorder, shows any EtG formation impairment [20]. Conversely, a reduced renal function seems to prolong the elimination time of EtG. Other potential factors affecting EtG levels are age, sex, and cannabis consumption.

Clinical Impact of EtG

Traditional biomarker validation studies rely on cross-sectional designs where sensitivity and specificity are the cornerstones of the evidence-gathering process. Accordingly, EtG has shown a high sensitivity and also high predictive values, both positive and negative, both in experimental and clinical settings. However, cross-sectional validation prevents linking biomarker properties to patient outcomes on a longitudinal basis. A recent publication of a diagnostic randomized clinical trial where EtG was compared to ethanol in a randomized design [21], enabled the linking of biomarker properties and diagnostic performance to patient outcomes, demonstrating that implementing EtG in the routine screening of alcohol-dependent outpatients leads to decreased drinking and increased rates of abstinence over time.

> Recent evidence suggests that the application of a highly sensitive biomarker such as EtG has therapeutic properties, leading to reduced drinking and increased abstinence rates, probably due to increased feedback to both professionals and patients about their drinking.

Ethyl Sulfate

Ethyl sulfate (EtS), like EtG, is the product of a secondary elimination pathway for alcohol, see Fig. 13.2. EtS is usually measured in urine, but can also be assessed in plasma. An immunochemical detection test for EtS is currently not commercially available. For the combined detection of EtS and EtG, the use of rapid LC/MS-MS

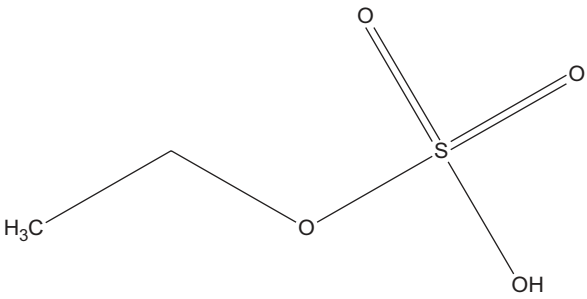

Fig. 13.2 Molecular structure of ethyl sulfate

procedures is routinely applied. The formation is affected by sulfotransferase and the breakdown by sulfatase. The molecular weight is 126 g/mol and the molecular formula $C_2H_5SO_4H$. Currently, an LC-MS/MS method with penta-deuterium EtS as internal standard and two ion transitions can be used in forensic and medico-legal cases as well as in clinical routine. The fact that the formation route of EtS is different from that of EtG offers the opportunity for increased sensitivity when analyzing the two biomarkers together. However, the discrepancies between the two are usually low [22].

In summary, a cut-off of 0.05 mg/L for repeated alcohol intake has been suggested. As for EtG, there is evidence of prolonged elimination in patients with reduced renal function. Importantly, bacterial degradation of EtS has not been described. Therefore, EtS should be analyzed in conjunction with EtG, when degradation of this biomarker is suspected, to prevent the appearance of a false-negative result.

EtS is rarely analyzed alone. Usually, both, EtG and EtS, are assessed to complement each other. EtS might help to prevent false-negative results due to bacterial degradation of EtG, especially by *E. Coli* and it might also increase the sensitivity of EtG alone.

Fatty Acid Ethyl Esters

Fatty acid ethyl esters (FAEE) are non-oxidative metabolic products of ethanol that can be detected in blood, hair, and various organs with a reduced or deficient capacity to oxidize ethanol after consumption, see Fig. 13.3. Since these esters were proven to cause damage to sub-cellular structures, they were postulated to be mediators of organ damage. FAEE are formed in the presence of ethanol from free fatty acids, triglycerides, lipoproteins, or phospholipids. Thereby, two enzymes catalyze their formation: acyl-coenzyme a:ethanol o-acyltransferase (AEAT) and fatty acid ethyl ester-synthase. FAEE-synthase can be isolated from rabbit myocardium, human brain, and rat fat tissue. Two of these FAEE-synthases were shown to be

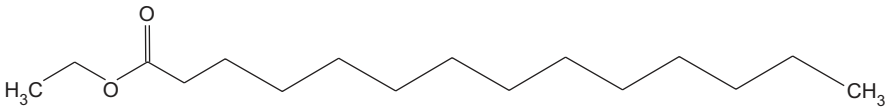

Fig. 13.3 Molecular structure of a fatty acid ethyl ester, ethyl myristate

identical to rat liver carboxyl esterase. Furthermore, pancreatic lipase, lipoprotein lipase, and glutathione transferase were shown to possess FAEE-synthase activity.

Detectable FAEE levels are found in blood shortly after alcohol consumption, and remain positive for more than 24 h. Maximum FAEE concentrations in blood are found in samples taken 2 or 4 h after the start of drinking and remain in the ng/mL range [23]. Of 15 different FAEE in hair, the sum of four (ethyl stearate, ethyl oleate, ethyl myristate, and ethyl palmitate) have been demonstrated to function as a marker in hair analysis. With a cut-off of 0.5 ng/mL, sensitivity and specificity of 90% were reported. A differentiation between abstinent, social, and excessive drinkers appears possible [24]. However, the complex GC/MS method commonly used for this analysis renders it of little practical utility for routine use.

Phosphatidylethanol

Phosphatidylethanol is a phospholipid formed in the presence of alcohol via the action of phospholipase D, see Fig. 13.4. The precursor is the naturally existing lipid-phosphatidylcholine. PEth consists of a glycerol backbone, which is substituted at positions sn1 and sn2 with two fatty acids and is esterified at position sn3 with phosphoethanol. Due to variations in chain length and saturation of the fatty acids, various homologs of PEth are present. In 2010, 48 PEth homologs were described in the blood of a deceased alcohol-dependent individual [25]. The PEth homologs 16:0/18:1 and 16:0/18:2 are the two most prevalent, and are often measured together. Upon initiation of abstinence, the elimination half-life of PEth ranges from 3.5–9.8 days for total PEth, 3.7–10.4 days for PEth 16:0/18:1, and 2.7–8.5 days for PEth 16:0/18:2, see Fig. 13.5 for an example on PEth elimination [27].

Upon regular alcohol consumption, PEth concentrations in human blood reach an equilibrium (plateau) between formation and degradation, at a level that is representative of drinking habits [28, 29]. A drinking experiment with healthy individuals by drinking up to a target alcohol concentration of 1 g/kg (1‰) once daily on each of 5 consecutive days yielded PEth values up to 237 ng/mL [30]. Measurements were performed with LC-MS/MS. Various studies found no false-positive results for PEth, and a linear relationship between consumed amounts of alcohol with phosphatidylethanol values has been described [31].

Thanks to ongoing refinements in analytical methods, the sensitivity and specificity of today's PEth analysis appear to be extremely high [32]. Also relevant is the fact that, besides its already known potential for abstinence monitoring, current

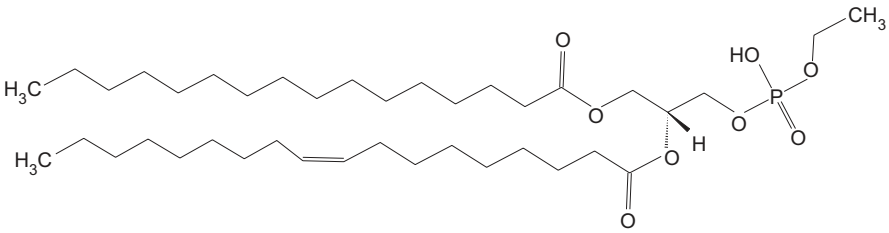

Fig. 13.4 Molecular structure of phosphatidylethanol (PEth) 16:0/18:1

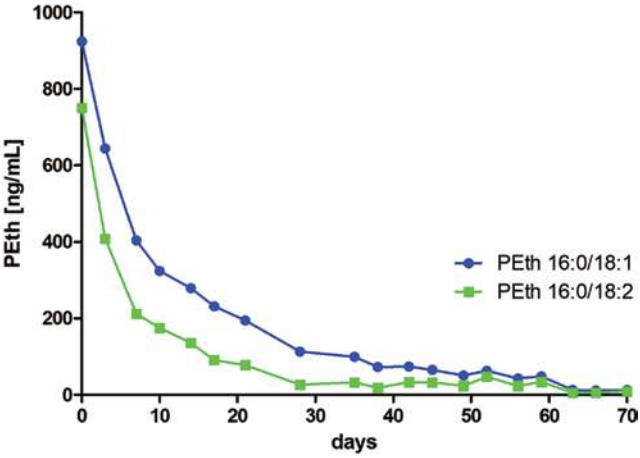

Fig. 13.5 Elimination of PEth in an alcohol withdrawal and early relapse prevention treatment setting [26]

evidence suggests PEth could be a suitable biomarker for the differentiation between light and heavy drinking, a feature not offered by other direct alcohol biomarkers [33]. To grade drinking behavior, a lower and an upper cutoff for PEth 16:0/18:1 is applied. According to the 2022 Consensus of Basel by the Society of PEth Research, a PEth 16:0/18:1 concentration <20 ng/mL (<0.0285 µmol/L) is compatible with abstinence or low alcohol consumption. ≥20 ng/mL but <200 ng/mL (<0.285 µmol/L) represents alcohol consumption, and a concentration ≥200 ng/mL is strongly suggestive of chronic excessive alcohol consumption [34]. Importantly, PEth values seem not to be influenced by liver diseases and hypertension [35].

Among the new direct alcohol biomarkers, PEth seems to be the only one capable of assessing abstinence and also differentiating between light, moderate and heavy drinking. It is recommended in evidence and consensus-based guidelines for many settings, including the assessment of alcohol consumption during pregnancy. Emerging data suggest, however, that the so far poorly understood strongly varying half-life of PEth between heavy drinkers seems to be related to alcohol-mediated red blood cell turnover (Bartel et al, 2023, in preparation). For more details, Chaps. 7, 37, 57 and 58 are recommended.

Methodological Aspects

PEth is most frequently determined using LC-MS/MS. It facilitates the detection and quantification of single analogs if a reference is available. Everyday practical use is facilitated through the possibility of sampling the specimen using dried blood spots (DBS). DBS have proven to deliver results comparable to whole blood measures. Furthermore, there is no difference between venous or capillary blood [26]. Obtaining a specimen using DBS is simplified since non-medical staff can obtain capillary blood from a finger prick, the risks for HIV and hepatitis C infections are decreased, and storage and transport are possible at room temperature in an envelope. Additionally, the possibility to use DBS sampling in combination with DBS autosamplers permits fully automated analysis of PEth, within 5 min per sample. This represents a significant advantage when compared to the laborsome hair analysis or urine/blood-based analytical methods that involve liquid handling, as large batches of samples can be processed in a fully automated manner, 'from card to chromatogram, with no hands-on' [36, 37].

Clinical Applications

Similar to what has been described for EtG, PEth has shown a high degree of sensitivity and specificity for the detection of drinking, see Fig. 13.6. It has been applied to various populations and clinical settings, such as HIV populations, pregnant women, and alcohol-dependent patients [33]. Interestingly, PEth shows good correlations with AUDIT-C scores [38]. Therefore, PEth is a useful biomarker of heavy alcohol consumption over time. Finally, some authors suggest that PEth might have a role in patients who are tested for EtG and EtS, have low positive values and deny alcohol consumption, as a further evaluation with an even more sensitive biomarker.

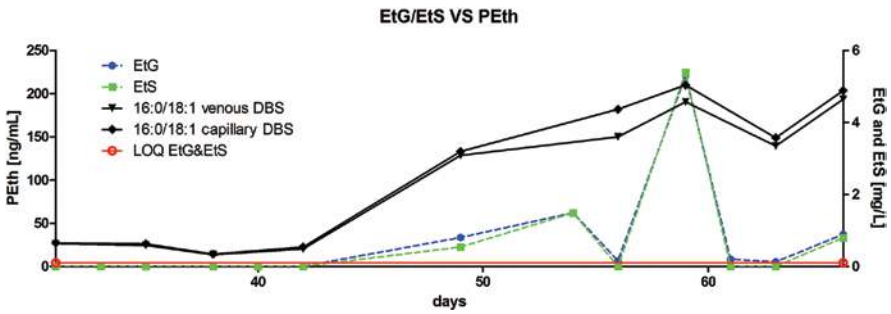

Fig. 13.6 Comparison between the measurement of EtG/EtS and PEth during several relapse situations (at least 4) in an alcohol withdrawal and early relapse prevention treatment setting [26]. While EtG/EtS returns to low concentrations after drinking, PEth stays elevated due to accumulation and a slower elimination

Limitations

In blood samples containing ethanol, post-sampling formation of PEth can be an issue, depending on the choice of sampling strategy [39]. Without influencing the PEth levels, whole blood samples can be stored frozen at −80 °C to avoid post-sampling degradation [40]. PEth concentrations in DBS were found to be stable for more than 6 months at room temperature. One reason for this improved stability in DBS is that any enzymatic activity is stopped in the process of drying [41]. Experimental studies in rats showed that ceramide can block the activity of phospholipase D and inhibits the synthesis of PEth. Also, sodium metavanadate ($NaVO_3$) eliminated post-sampling formation during the storage and drying of DBS, and is currently applied in some commercial DBS sampling devices [39].

Importance of Reference Standard Purity

For PEth measurements the purity and pathway of synthesis for the used reference standard is of high importance: When measuring PEth in whole blood from patient samples, the calibration and quality control samples are usually prepared by spiking PEth reference standard solution (reference material from a commercial supplier) into blood from a person with no alcohol use. The patient samples are then referenced to these artificially prepared samples with known PEth concentrations, based on a calibration curve. Dependent on the route of synthesis, the artificially prepared reference standard may not only contain the targeted PEth 16:0/18:1, but also its regioisomer PEth 18:1/16:0. The artificially prepared samples might therefore not necessarily reflect the naturally occurring PEth composition in human blood, which only contains PEth 16:0/18:1, see Fig. 13.7. If the reference standard contains a

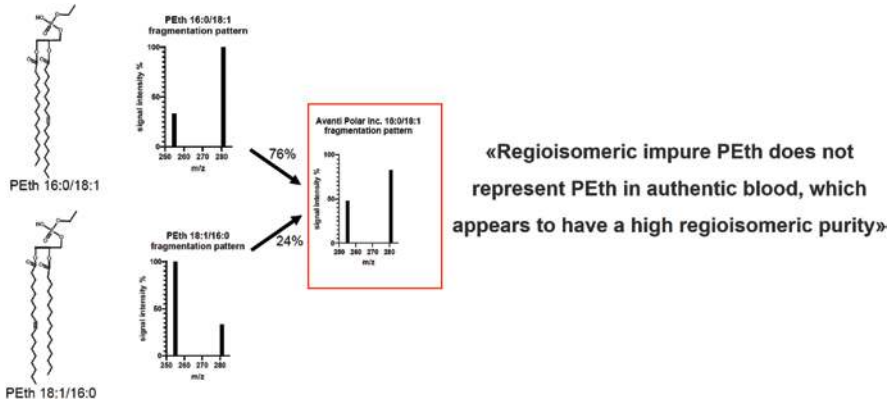

Fig. 13.7 The impact of PEth 16:0/18:1 reference material purity on the fragmentation pattern of PEth, depicted based on reference material with a isomeric purity of only 76% PEth 16:0/18:1 and 24% PEth 18:1/16:0 [42]

significant fraction of the PEth isoform 18:1/16:0, a significant quantification deviation might occur, due to different fragmentation patterns within the mass spectrometer [42].

Hair Analyses

Hair analysis is well established to assess ethanol intake. FAEE and ethyl glucuronide, are mainly used as long-term alcohol markers in hair. The time frame for detection of alcohol consumption is longer in hair compared to blood or urine. Due to head hair growth of 1 cm per month, depending on the hair length, evidence of alcohol consumption can be found for the respective period. Since hair must grow out of the scalp to be cut or shaved for analysis, the proximal ~0.5 cm (representing approximately 2 weeks) is not accessible. The deposit of lipophilic FAEE in hair occurs in sebum, the oily product of microscopic exocrine glands that lubricates hair and skin, whereas hydrophilic EtG is incorporated through perspiration and/or from blood.

Measurement of FAEE and EtG allows differentiation between chronic excessive and moderate alcohol consumption as well as abstinence or very low levels of alcohol consumption. Nowadays, the concentration of ethyl palmitate (EtPa) alone is used for interpretation instead of the concentration sum (ΣFAEE) of the four esters: ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate, as previously applied. The use of EtPa with these cut-offs in place of ΣFAEE for alcohol intake assessments produces only a minor loss in discrimination power, leading to no essential difference in the interpretation concerning chronic excessive alcohol consumption, and is suitable to confirm EtG results in abstinence assessments.

The Society of Hair Testing (SOHT) published the following guideline within their 2019 consensus [43]: The EtPa cut-off for abstinence assessment was defined at 0.12 ng/mg for the 0–3 cm segment and at 0.15 ng/mg for the 0–6 cm segment. The cut-off for chronic excessive drinking was set at 0.35 ng/mg for the 0–3 cm segment and at 0.45 ng/mg for the 0–6 cm segment. An EtG concentration ≥30 pg/mg in the proximal hair with a length of 3–6 cm strongly suggests chronic excessive alcohol consumption (>60 g EtOH per day). An EtG concentration of more than 5 pg/mg in the proximal hair with a length of 3–6 cm strongly suggests repeated alcohol consumption. If samples less than 3 cm or greater than 6 cm are used, the results should be interpreted with caution. The analysis of FAEE alone is not recommended to determine abstinence from ethanol.

For abstinence assessments, EtG should be the first choice, and the analysis of EtPa alone is not recommended to determine abstinence from ethanol. A negative EtPa result does not disprove abstinence but indicates the need for further monitoring. For the assessment of chronic excessive consumption, the combined use of FAEE and EtG can be recommended to increase the validity of hair analysis [44].

In a prospective controlled alcohol-dosing study with three groups (no alcohol, daily 20 g and 30 g pure alcohol) (n = 30), the following median EtG in hair

concentrations were found: group no alcohol: 0.5 pg/mg, group 20 g EtOH daily:5.6 pg/mg and group 30 g EtOH daily:11.3 pg/mg. The authors concluded that differentiation is possible between abstinence and repeated low to moderate amounts of alcohol consumed [45].

In an alcohol dosage study using retrospective alcohol consumption evaluated by timeline follow-back interview EtG in hair was measured in 130 non-excessive alcohol consumers (<60 g pure ethanol/day) varying between < LLOQ and 29.8 pg/mg hair [46]. In another alcohol dosage study using retrospective alcohol consumption evaluated by timeline follow-back interview EtG in hair was measured in 36 alcohol-dependent patients (25 males/11 females) starting an alcohol detoxification program. In this study, EtG in hair concentrations varied between 32 and 662 pg/mg [47].

Other Influencing Factors

Whereas a false-positive result for EtG in hair after use of EtG-containing shampoo has only been reported in one case, regular use of alcohol-containing hair tonic can lead to false-positive FAEE results. No such false-positive results in combination with ethanol-containing products have been reported for EtG. Impaired kidney function may lead to higher EtG levels, as indicated by preliminary results. BMI has also been shown to have an influence on the EtG concentration in hair (higher EtG concentrations in participants with high BMI) [48]. False-negative results for both alcohol markers can also be caused by the use of hair cosmetics, like alkaline hair cosmetics for FAEE, oxidative treatment for EtG, and hair straightening for EtG. Cleansing shampoos may also alter EtG and FAEE concentrations in hair [49]. Prolonged incubation with water does affect EtG (washout effects due to the polarity of the analyte), but not the apolar FAEE [50].

The hair color and melanin content in hair does not affect the result. In segmental investigations of hair samples, a chronological correlation to drinking or abstinent phases with FAEE is not possible, while this has been shown to be feasible for EtG. Altogether, hair analysis for FAEE or EtG is currently a potentially useful tool to clarify past alcohol consumption.

Practical Use

Hair analysis for FAEE or EtG is applicable in several contexts including evaluating driving ability and forensic psychiatry. Another clinical use of alcohol metabolites measures in hair is the screening for alcohol use in medication-assisted treatment of opioid-dependent subjects as mentioned above.

Summary Regarding Direct Alcohol Biomarkers

Specific direct ethanol metabolites are available, that permit to distinguish between short-term intake of small amounts and long-term use of large amounts of alcohol, see Table 13.1. Cut-off values and influencing factors are summarized in Tables 13.2 and 13.3. Appropriate methods of analysis and pre-analytical considerations are crucial for a valid and reliable detection of markers. Currently, the best detection methods are LC-MS/MS-based, and with every new generation of mass spectrometers, the sensitivity of these instruments increases. Commercial solutions for direct implementation are available for most of the described analytes.

EtG is detectable in urine using LC-MS/MS even after ingestion of low amounts of alcohol (1 g), which may occur by consuming certain food, drugs, or using disinfectants. Individuals with the motivation or obligation for abstinence have to be informed about these "hidden contents" to avoid involuntary intake of alcohol. For forensic purposes, the current cut-off value of 0.1 mg/L should be adapted to exclude cases of involuntary alcohol use. Concerning differences in formation and degradation, EtG and ethyl sulfate (EtS) are preferentially analyzed together. In the absence of known influencing factors, EtG in hair can be recommended as a marker for alcohol intake for the last 3 months.

For the assessment of drinking habits, phosphatidylethanol (PEth) is an ideal alcohol marker. It permits distinguishing between abstinence/very low consumption, moderate alcohol consumption, and excessive consumption. While positive urine values of EtG and EtS can be caused by unintentional alcohol intake, positive values of PEth are related to previous intoxications of 0.5‰ or higher. Using finger pricks and dried blood spots for PEth, the sample collection, as well as the sample storage and distribution, are simplified. Advantageously, with today's methods,

Table 13.1 Clinically relevant options for the determination of direct biomarkers, concerning the amount and duration of alcohol intake (modified according to Thon et al. [51])

Duration of consumption	Amount of consumption	
	>1 g/day	>40–60 g/day
<1 day	serum, urine: EtOH, EtG, EtS	Serum and urine: EtOH, EtG, EtS
		PEth in whole blood and dried blood spots (LC-MS/MS)
>1 day	serum, urine: EtOH,EtG, EtS	Serum and urine: EtOH, EtG, EtS
		PEth in whole blood and dried blood spots (LC-MS/MS)
>14 days	serum, urine: EtOH, EtG, EtS	Serum and urine: EtOH, EtG, EtS
		PEth in whole blood and dried blood spots (LC-MS/MS)
Weeks to months	serum, urine: EtOH, EtG, EtS	Serum and urine: EtOH, EtG, EtS
		PEth in whole blood and dried blood spots (LC-MS/MS), EtG and FAEE in hair

EtOH ethanol, *EtG* ethyl glucuronide, *EtS* ethyl sulfate, *PEth* phophatidylethanol, *FAEE* fatty-acid ethyl esters

Table 13.2 Clinically relevant options for determination of direct biomarkers, concerning the amount and duration of alcohol intake (modified according to Thon et al., PEth-NET and SOHT [34, 43, 51])

Biomarkers	Amount of consumption	Cut-off
EtG in hair	Does not contradict self-reported abstinence	≤5 pg/mg
	Social consumption (20–40 g/day)	5–30 pg/mg
	Strongly suggests chronic excessive alcohol consumption (>60 g/day)	≥30 pg/mg
EtPa in hair	Strongly suggests repeated alcohol consumption	>120 pg/mg (0–3 cm)
		>150 pg/mg (0–6 cm)
	Strongly suggests chronic excessive alcohol consumption	≥350 pg/mg (0–3 cm)
		≥450 pg/mg (0–6 cm)
EtG in urine	Total abstinence	0.1 mg/L
	– unintentional intake	0.1 – 0.5 mg/L
	– recent alcohol use	
	– longer back-dated alcohol intake in larger amounts	
	unintentional intake unlikely, but possible, active alcohol intake probable	0.5–1 mg/L
EtS in urine	Total abstinence	0.05 mg/L
PEth 16:0/18:1	Compatible with abstinence or low alcohol consumption	<20 ng/mL (<0.0285 µmol/L)
	Alcohol consumption	≥20 ng/mL but <200 ng/mL
	Strongly suggestive of chronic excessive alcohol consumption	≥200 ng/mL (≥0.285 µmol/L)

EtG ethyl glucuronide, *EtPa* ethyl palmitate in hair, *EtS* ethyl sulfate, *PEth* Phosphatidylethanol

Table 13.3 Detection of direct biomarkers, concerning the amount and duration of alcohol intake (modified according to Thon et al. [51])

Direct bio-markers	Potential influencing factor	Influence
EtG in urine	E.coli, when using dried urine spots	No
	Grade of liver disease, smoking, BMI, body water content reduced kidney function	No
EtS in urine	E.coli, when using dried urine spots	No
PEth	Liver disease	No
	Hypertension	No
	Storage of ethanol blood samples	No
	Refrigerator temperature, −80 °C	
EtG in hair	Hairsprays with ethanol, hair colour, melanin content, age, sex, BMI	No

Table 13.3 (continued)

Direct bio-markers	Potential influencing factor	Type of influence
EtG in urine	E. coli, C. sordelli	decrease
	Reduced kidney function	Longer detection
	Chloral hydrate	False-positives
EtS in urine	Reduced kidney function	Longer detection
	Closed Bottle test (OECD 301 D)	28 days stable detection, depletion after 6 days
	Manometer Respiratory Test (MRT)	
FAEE in hair	Aggressive alkaline hairsprays	False-negative
	Hairsprays with ethanol	False-positives
PEth	Ethanol-containing blood samples, Storage of ethanol blood samples at RT and −20 °C	Increase
EtG in hair	Hairspray with EtG	Increase
	Reduced kidney function	Increase
	Bleaching, hair styling products	False-negative

EtG ethyl glucuronide, *FAEE* fatty-acid ethyl esters, *EtS* ethyl sulfate, *PEth* phosphatidylethanol, *BMI* body mass index, *RT* room ambient temperature, *E. coli* Escherichia coli, *C. sordelli* Clostridium sordelli

PEth can be analyzed fully automated within a runtime of 5 min. Different guidelines for the interpretation of values are available from international societies such as the Society of Hair Testing (SOHT) and The Society of Phosphatidylethanol Research (PEth-NET).

Traditional Biomarkers for Alcohol Consumption

Many routinely used clinical chemistry parameters show pathological changes as evidence of the biochemical burden of ethanol metabolism. None of these conventional indicators show 100% sensitivity or specificity. Nonetheless, evidence of long-term alcohol consumption can be obtained from these state markers, especially a combination of several individual indicators. The currently used and further potential alcohol biomarkers are shown in Table 13.4.

Blood Alcohol Content Calculation

A mathematical estimation of blood alcohol content is useful when blood alcohol is not currently detectable, or for the prediction of alcohol level. While there are several ways to calculate it, the simplest is Widmark's equation:

$$\mathrm{Co} = \mathrm{A} / \left[\mathrm{p} \times \mathrm{r}\right]$$

Table 13.4 Diagnostic characteristics of several conventional and potential alcohol biomarkers (adapted from Topic and Djukic [52])

Alcohol biomarker (abbreviation)	Window of assessment	Specificity/ Sensitivity	Type of alcohol consumption	Application	Specific comments
Conventional alcohol biomarkers					
Ethanol (EtOH)	6 h	High/high	Recent alcohol consumption/ alcohol intoxication	In traffic safety/ emergency department	In combination with clinical observation—monitoring alcohol habits
Gamma-glutamyl transferase (GGT)	2–8 weeks	Moderate/ moderate	Chronic, heavy	Screening/ monitoring	More specific for alcoholics ages 30 to 50 years
Aminotransferases ratio AST/ALT ≥2	unknown	High/low	Heavy	Alcoholic hepatitis/ relapse	Less frequently used than GGT for screening heavy alcoholics
Mean corpuscular volume (MCV)	Up to several months	Moderate to high/low	Chronic	Screening/fetal alcohol effects	Show dose-dependent response to the intensity of alcohol intake
Carbohydrate deficient transferrin (CDT)	2–3 weeks	High/ moderate	Heavy	Screening/ monitoring/relapse	No elevation in single episodes of acute alcohol intoxication
GGT-CDT	2–3 weeks	High/high	Heavy	Abstinence/relapse	Useful for diagnostic alcohol-liver damage due to excessive and prolonged intake
Potential alcohol biomarkers and devices					
Ethyl glucuronide (EtG) and ethyl sulfate (EtS)	Several days	High/high	Recent alcohol consumption	Abstinence/relapse	High inter-individual variations
Phosphatidylethanol (PEth)	1–2 weeks up to several weeks	High/high	Abstinence monitoring, social/ moderate alcohol consumption, and heavy, chronic alcohol consumption	Abstinence/relapse, consumption habits	Promising in differentiating alcohol—from non-alcohol induced liver disease
Fatty acid ethyl esters (FAEE)	24 h (after ingestion)	High/high	Recent heavy	Distinguishing social drinkers from heavy	Combination of FAEE and EtG in meconium—markers for fetal alcohol exposure

Alcohol biomarker (abbreviation)	Window of assessment	Specificity/ Sensitivity	Type of alcohol consumption	Application	Specific comments
Whole blood associated acetaldehyde assay (WBAA)	unknown	High/high	Chronic/heavy	Monitoring abstinence	IgAs against acetaldehyde protein adducts- promising in differentiating alcohol—from non-alcohol induced liver disease
Total sialic acid (TSA)	Unknown	Between GGT and ALT	Chronic, heavy	relapse	Increased in high alcohol consumption and reduced during abstinence, especially among women
5-HTOL/5-HIAA	1 day	High/high	Recent alcohol consumption	Evaluation of treatment/relapse	5-HTOL/5-HIAA >20 marker of recent drinking
Transdermal devices (SCRAM, WrisTAS)	–	High/high	Records continuous alcohol consumption	By court monitoring of abstinence	Need product improvements

Co is the theoretical maximum concentration of alcohol in blood (mg/g).

A is the amount of alcohol in the body (g).

p is the body weight (kg).

r is the correction factor corresponding to the ratio of total body water and blood water (0.6 for females and 0.7 for males).

Sex plays an important role in the total amount of water in the body. In general, men have less fatty tissue and a higher percentage of water (58%) than women (49%), and thus the volume of distribution (Vd) for ethanol is higher in men. According to its partition coefficient (Poct/water is 0.1), ethanol is 10 times more soluble in water than in lipids. Thus, upon ingestion of the same amount of ethanol, the BAC will be higher in females than in males. The Widmark's equation has been improved subsequently by introducing individual r, based on the multiple linear regression equations:

$$\text{for females : rFI} = 0.31223 - 0.006446 \times \text{body weight}\left(\text{kg}\right) + 0.004466 \times \text{body height}\left(\text{cm}\right).$$

$$\text{for males : rMI} = 0.31608 - 0.004821 \times \text{body weight}\left(\text{kg}\right) + 0.004632 \times \text{body height}\left(\text{cm}\right).$$

There is no absolute accurate blood alcohol calculator because numerous factors influence the BAC, such as male versus female sex, rate of metabolism and elimination, health status, medications that might be taken, drinking frequency, amount and the type of food in the stomach and small intestine, the time when food was eaten, and others [52].

Gamma-Glutamyl-Transferase (GGT)

GGT is a membrane-bound glycoprotein enzyme that occurs ubiquitously in the organism, but mainly in the liver, pancreas, and renal proximal tubules. GGT detectable in serum arises mainly from the liver so that an increase in serum enzyme activity would be a sensitive indicator for hepatobiliary diseases. Chronic alcohol consumption induces an increase in enzyme synthesis and, through direct activation of the enzyme from membrane binding, leads to an increase of GGT in serum. The release of enzymes through liver parenchymal damage also presents a secondary mechanism in chronic alcoholic hepatitis. To exceed the normal values (4–18 U/L in women and, 6–28 U/L in men) requires chronic, daily alcohol intake over at least 4–6 weeks. A short-term, higher alcohol burden causes no such increase. Nevertheless, drinking intensity has more influence on GGT than drinking frequency. In absolute alcohol abstinence, normalization of the values occurs within 3 weeks to 60 days.

The sensitivity of GGT to detect heavy alcohol use varies, according to age, sex, and body weight, from 35% to 85%. GGT increases with age in heavy as well as moderate drinkers. In contrast, in young adults aged less than 30 years, even when these are alcohol dependent, the sensitivity of the markers is very low. In addition, the higher vulnerability of women to alcohol-associated liver diseases is well

known. Other studies have shown that the relationship between being overweight or obese (BMI > 25 or 30, resp.) and having an increase in GGT are related. GGT levels can also be increased by various other causes, for example, the effects of many medications and teratogens, diabetes, and cholestatic or inflammatory liver diseases. Accordingly, the specificity of 63–85% is not satisfactory [53]. Despite its attraction and ease of use, GGT is not suitable as a solitary indicator of chronic alcohol misuse and current liver diseases [54]. More detailed information is also provided in Chap. 37 and the Appendix Tables B.1–B.4.

Mean Corpuscular Erythrocyte Volume (MCV)

Measurements of MCV are common in standard clinical investigations, and an increase occurs in 4% of the general population and 40% to 60% of patients with alcohol misuse. Koivisto et al. reported definite evidence for a significant dose-dependent relationship between MCV and the intensity of alcohol consumption [55]. The mechanism responsible for increasing MCV is hitherto unclear. Direct hematotoxic damage or interaction of ethanol and its metabolites, especially acetaldehyde, with the erythrocyte membrane, has been suggested [56]. An increase in MCV can be expected in long-term alcohol consumption. MCV values then normalize slowly during abstinence over 2–4 months. The sensitivity of MCV in screening for alcohol misuse is inferior to GGT, at least in men. In interpreting MCV values, other causes such as Vitamin B12 or folic deficiency, non-alcohol-related liver diseases, reticulocytosis, and hematologic diseases should be considered. More information on MCV, especially its relation to bone marrow toxicity and RBC turnover, is provided in Chaps. 7, 37, 57 and 58.

Carbohydrate Deficient Transferrin (CDT)

Transferrin is the most important iron transport molecule in humans. Its synthesis and glycosylation occur in hepatocytes. Depending on the iron load as well as the number and breakdown of carbohydrate chains, different isoforms can be detected. Differentiation occurs through measurements of isoelectric points (pl), whose values depend on the load of bound iron ions and the number of sialic acid residuals in carbohydrate chains. Abnormal isoforms with much-increased pl-values over 5.65 in the cerebrospinal fluid and serum of alcohol-dependent patients were found and were traced back to small levels of bound sialic acid residuals.

In subsequent investigations, more precise differentiation into mono-, di- and asialotransferrin was made, and all abnormal isoforms were sub-grouped under CDT [57]. All abnormal transferrin molecules increase in chronic alcohol consumption. Measurements with HPLC showed that increased alcohol consumption leads to increased disialotransferrins, while increases in asialotransferrin occur in chronically increased alcohol consumption only. A variety of methods and respective

reference levels for the detection of CDT is available. To date, measurements of CDT using HPLC are the reference standard, but various enzyme immunoassays are also in use. For confirmation analyses, immune electrophoresis is employed, while a direct CDT detection method using specific antibodies is still under development [58].

The underlying mechanism for CDT development is not exactly known. Inhibition of intracellular transmission of carbohydrates to change through toxic effects from ethanol or acetaldehyde is presumed. Ethanol's influence on the activities of membrane-bound sialyltransferase and plasma sialidases in hepatocytes has been discussed, in which an imbalance in favor of sialic acid reduction enzymes occurred.

There has been no agreement in previous studies concerning the correlation between CDT concentrations in serum and the absorbed amounts of alcohol. Although an increase in CDT with daily consumption of 60–80 g alcohol over 7 days has been shown, other studies have reported contradicting results. Additionally, contradicting results on the effect of moderate drinking (<40 g alcohol) are found. In alcohol-dependent patients, CDT is, however, sensitive enough for detecting relapses and monitoring sobriety [59].

The clinical strengths of CDT as a biomarker vary depending on sex, BMI, age, nicotine use, and anorexia [60]. Previous studies showed that CDT in men is a more sensitive indicator for alcohol-related diseases compared to women. CDT values in women might be increased under natural conditions but not much in increased alcohol use. Furthermore, hormonal factors appear to play a role, and CDT values are increased in pregnant women, but reduced in postmenopausal women. In females, CDT in serum also depends on age.

Among conventional alcohol markers, CDT is currently considered the most useful and significant indicator [61]. Information on sensitivity and specificity varies, since no methodical standardization exists. Further, the heterogeneity of test populations concerning age, sex, alcohol consumption, duration of abstinence before serum extraction as well as current liver diseases makes the comparison with other traditional markers difficult. In selected, clinical patient groups, various test methods with specificity between 90% and 100% with high sensitivity (50–90%) have been reported].

Serum Transaminases (ASAT/ALAT)

Increases of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) in serum are unspecific signs of hepatocellular damage. While ASAT is produced in the liver, skeleton, and cardiac muscle tissues, ALAT is a liver-specific enzyme. ASAT also occurs in red blood cells as is discussed in Chap. 41. Thus, an increase in ALAT indicates liver diseases (fatty degeneration, tumors, metastases, cirrhosis, cholangitis). By contrast, measurements of ASAT must differentiate between alcohol-sensitive, mitochondrial (m-ASAT), and cytoplasmic isoform

(c-ASAT). Conclusions on alcohol-induced liver damage can only be drawn from increased m-ASAT/c-ASAT quotients [62]. Increased ASAT values would be found in alcohol-dependent patients from 39–47%. In the WHO/ISBRA-Study, the sensitivity of ASAT was between 23% and 45% (women vs. men). The toxic effects of ethanol on mitochondria lead to increased release of ASAT compared to ALAT [63]. Thus, measurements of the de-Ritis-Quotient (ASAT/ALAT) increase the alcohol specificity of both markers—a quotient over 1 and even 2 would offer strong indications for an ethanol toxicity to be the etiology.

In summary, the sensitivity and specificity of both enzymes as indicators for alcohol misuse are considered variable so that an interpretation of an increased serum activity is mainly meaningful in the context of other liver values (Bilirubin, Alkaline phosphatase, GGT).

HDL Cholesterol and Apolipoprotein

Increases in HDL cholesterol and apoprotein I/II are described in many studies as specific and sensitive indicators of chronic alcohol strain; by contrast triglycerides and total cholesterol are nutritionally influenced. Alcohol leads to an increase in the concentrations of cholesterol and phospholipids within the HDL particles, and causes a shift to a higher proportion of phospholipids HDL_2-particles [64]. Studies have shown this phenomenon to be the basic principle for the observed cardioprotective effects of moderate alcohol consumption [65]. Chronic alcohol load causes an increase in HDL over 50 mg/dL, after withdrawal and with continuing abstinence, the values normalize within 1–4 weeks. The pathogenic cause for alcohol-related HDL- and apoprotein increases is postulated to be an enzyme induction as well as increased lipoprotein lipase activity.

Increased HDL levels without alcohol use can occur under the influence of medication (sedatives, lovastatin), pronounced underweight and physical strain. Still, the specificity of this marker is highly esteemed. Moreover, it proved itself to be practicable. Particularly in patients without liver damage, HDL and apoprotein I/II can be used for monitoring abstinence since changes in alcohol consumption would be accurately reflected.

Combination of Individual State Markers

Since individual conventional alcohol markers were found to be insufficiently sensitive and/or specific for the recognition of alcohol misuse, combinations of parameters have been investigated. The better-known combinations comprised CDT, GGT, MCV, and ASAT. In general, if chronic alcohol consumption should be detected, it is recommended to use a combination of indirect markers (e.g., gamma-glutamyltransferase and mean cell volume and carbohydrate-deficient-transferrin,

antilla index, alc index) to increase sensitivity and specificity in different contexts (family practice, inpatient admission, emergency admission, preoperative screening, and intensive care unit (German S3-Guidelines alcohol-related disorders, 2021, [66]).

Gamma-Glutamyl Transferase and Carbohydrate-Deficient Transferrin

Some studies showed that the combined use of GGT and CDT resulted in higher sensitivity and specificity compared to the use of either one alone. Sillanaukee et al. reported in 2001 a sensitivity of 75% and specificity of 93% for CDT from 257 alcohol-dependent patients and 362 occasional drinkers. γ-CDT is estimated using the formula [**γ-CDT = 0.8 ln(GGT) + 1.3 ln (CDT)**]. Compared to CDT and GGT alone, ASAT, ALAT, or MCV showed the logarithmic transformation from GGT and CDT to have the best predictive value to differentiate between alcohol-dependent patients and occasional drinkers. Values for γ-CDT correlate to current amounts of consumption, regardless of whether a heavy alcohol-dependent individual or an occasional drinker was tested. γ-CDT can thus be used to monitor abstinence, although in continuing abstinence the values normalize within 2–3 weeks.

Alc-Index

By combining Methanol, Aceton/Isopropanol, GGT, and CDT in a logistic regression formula Brinkmann et al. [67] developed the so-called "Alc-index "to differentiate between alcohol-dependent patients and non-drinkers [67]. The basic principle for the investigations was the hypothesis that each of these alcohol markers shows overlap in values in the collective with none or low alcohol consumption and alcoholics. From the results, an Alc-index of 1.7 as a cut-off was defined with a specificity of 100% and a sensitivity of 90% to differentiate between alcohol-dependent and non-alcohol-dependent individuals. The advantage of this index is the single cut-off point instead of four for individual markers, which can prevent false conclusions with elevated values of an isolated marker.

Early Detection of Alcohol Consumption: Test (EDAC)

EDAC uses results from a series of routine lab parameters to identify heavy drinkers and light drinkers in study groups. Attempts were made already in the 80 s, to differentiate between light alcohol misuse and heavy alcohol dependence through

multivariate statistical analysis of blood samples from patients with conspicuous alcohol use. The multiple chemical and clinical parameters extracted should reflect alcohol's influence on various organs and organ systems. Subsequent to these studies, the procedure was abandoned because of impractical, partly costly statistical analysis. Harasymiw et al. [68] developed EDAC test to be an established procedure, in which a form of "mathematical fingerprint" for each tested patient could be created from 10 out of 30 routine lab values through a linear discrimination analysis [68]. The fingerprint of an individual could possibly be that from a heavy alcoholic and presented as P-positive, representing the degree of concordance to the stereotyped alcoholic lab profile. In general, a P-positive value of over 50% showed current heavy alcohol consumption, whereas values below or equal to 50% showed evidence of light alcohol misuse. The EDAC test was successfully used as screening for alcohol misuse and to identify heavy or risky alcohol consumption in various studies on different study populations. A higher sensitivity of 34–65% (women/men) for EDAC test compared to 23–30% for GGT in a population of 1605 heavy drinkers or probands with risky alcohol consumption has been reported. The specificity is 89% (men) and 98% (women). Sensitivity and specificity of over 80% each were reported in the identification of alcohol misuse in heavy male and female drinkers.

GGT-ALT Combination

Another combination of enzymes, γ-GT-ALT (alkaline phosphatase) can potentially be useful for clinical diagnostic validation of liver damage related to excessive and prolonged intake of alcohol. If the ratio of γ-GT/ALT exceeds the value 1.4, there is a probability of 78% that the liver impairment is due to alcoholism. This particular combination was not shown to be a biomarker when used for monitoring the treatment of alcohol dependent patients with disulfiram.

Although the suspicion of heavy alcohol use behind hepatotoxicity may be supported by several lines of clinical and biochemical data, the specific role of alcohol in many cases is difficult to distinguish in individuals who deny alcohol use, see Fig. 13.8. Clinical symptoms related to heavy drinking may originate from virtually any tissue. Unexpected abnormalities in liver enzymes or blood cell count in health screening programs may also reveal alcohol abuse.

Subsequently, efforts should be focused on objective confirmation of alcohol use and rule out other aetiologies. Non-alcoholic fatty liver disease (NAFLD) is the most common non-alcoholic aetiology behind liver dysfunction and the workup should consist of evaluating metabolic co-morbidities with measurements of body mass index, waist circumference, and oral glucose tolerance. Specific tests are available to rule out viral hepatitis and several genetic diseases, such as hemochromatosis. In alcohol consumers, ethanol may be present even at the time of the clinic visit. Measurements of ethanol metabolites or other laboratory parameters sensitive to ethanol may provide confirmatory data. Liver enzymes give information on the

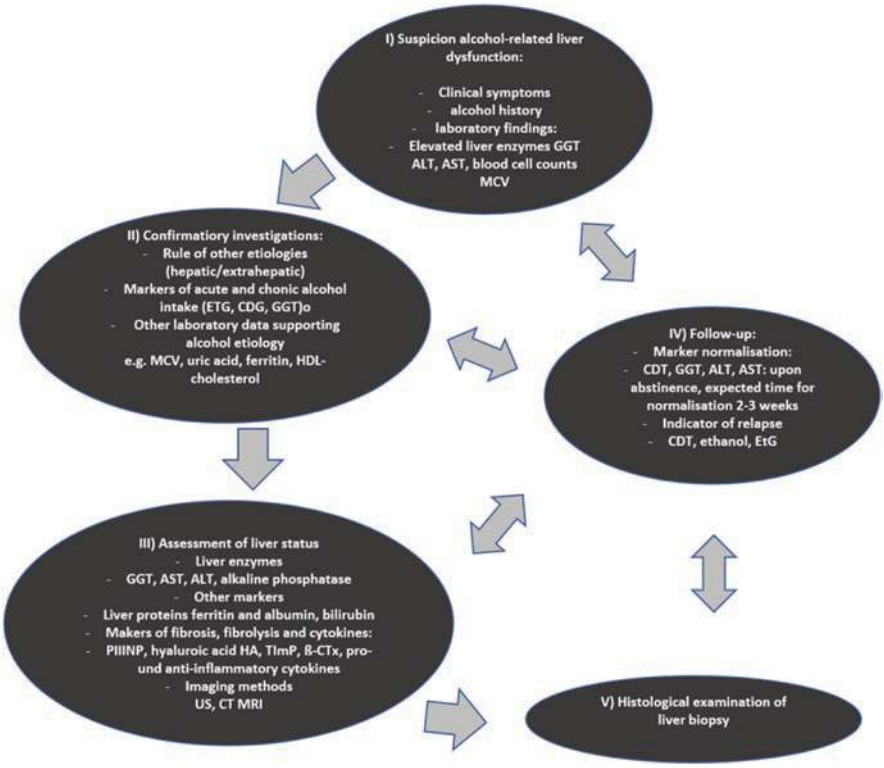

Fig. 13.8 Schematic representation of the clinical assessment of liver dysfunction in alcohol consumers (Niemela and Alatalo [69])

nature of liver pathology. An isolated abnormality in GGT is usually reversible and related to an increased oxidative stress burden. Increased ALT is commonly a result of fat deposition in the liver and can be reversible. Elevated ferritin and albumin may occur in the early phases of liver disease, whereas in patients with advanced alcohol-related liver disease (ALD) the rates of albumin synthesis decrease and correlate with poor prognosis. ALD status is also associated with both the collagen and cytokine markers. Markers of collagen synthesis and degradation as well as pro- and anti-inflammatory cytokines may follow inverse kinetics, which may help to differentiate alcoholics at risk for cirrhosis. US (ultrasonography), CT, computed tomography, MR, and proton magnetic resonance spectroscopy are commonly suggested. Follow-up of laboratory values is an integral part of the comprehensive assessment and treatment of patients with signs of liver dysfunction. If excess alcohol consumption (or obesity) is suspected, normalization during abstinence (or weight loss) is confirmatory. If initial GGT levels return to normal after abstention, the patient is likely to have recovered from liver disease. Liver enzyme activities

repeatedly above twice the upper reference limit are used as a decision-making point for liver biopsy to rule-out severe liver diseases, and to distinguish patients needing the closest monitoring. Follow-up by markers of ethanol consumption, such as CDT, can be used to assess the degree of alcohol dependence and to detect relapses, which increase the probability of subsequent severe liver problems. The current clinical gold standard for the diagnosis and grading of hepatic disease is liver biopsy. It is, however, an invasive procedure with sampling variability and therefore is not suitable for screening or repeated measurements.

Summary Regarding Indirect Alcohol Biomarkers

Traditional biomarkers may appear to be practical and cost effective, but have considerable limitations. Combinations of routine lab parameters, such as CDT und GGT, may allow some level of inference regarding regular, long term (days, weeks) alcohol consumption. The diagnostic sensitivity of individual parameters, like ASAT or ALAT, is low, the specificity is moderately high, except for CDT which shows moderate sensitivity and high specificity to differentiate between alcohol-dependent individuals and control persons. Additional related information can also be found in Chaps. 37, 39, 40 and 41.

Conclusions

Together, direct and indirect alcohol biomarkers cover a wide range of intervals between consumption and detection. Especially the direct alcohol biomarkers are currently vastly underutilized, and their broad implementation offers new opportunities for prevention, interdisciplinary cooperation, diagnosis, and treatment of alcohol-related disorders. This, for instance, is reflected by the assessment of evidence and consensus-based German S3 guideline “Screening, Diagnosis, and Treatment of Alcohol Use Disorders“, where the following recommendations regarding the use of ethanol metabolites are made with high and highest levels of evidence and recommendation [5]:

- For acute alcohol intake, ethanol in breath or blood and/or ethyl glucuronide (EtG) and ethyl sulfate (EtS) in urine are recommended
- For chronic intake the recommendation is for PEth in blood and/or EtG and/or Ethyl palmitate (EtPa) in hair, respectively.
- Since the database in the last years significantly has increased especially for phosphatidylethanol (PEth), a new recommendation for screening during pregnancy recommends EtG in urine and/or FAEEs in hair, and especially PEth in blood. If implemented, this has the potential to prevent FASD risk.

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Chapter 14
Addictions Neuroclinical Assessment

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Abstract Alcohol use disorder (AUD) is a common, complex condition with substantial heterogeneity that has confounded the understanding of its etiology, diagnosis, and outcomes. The Addictions Neuroclinical Assessment is a clinical framework that seeks to understand the etiology and heterogeneity of AUD and other substance use disorders based on three neurofunctional domains: incentive salience, negative emotionality, and executive functions. These domains are aligned with the three stages of the cycle of addiction—binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation—and are supported by our current understanding of the neuroscience of substance use disorders. The ANA includes a battery of measures to assess these neurofunctional domains, consisting of readily available neuropsychological and behavioral tasks, as well as clinical and self-report measures. Ancillary measures, such as genetic, epigenetic, and neuroimaging markers, as well as measures of the environmental and social determinants are recommended to provide additional information not otherwise captured by the three domains. This review summarizes the current empirical work on the ANA framework, and highlights important directions for future research. The ANA aims to serve as a critical tool for both researchers and clinicians to provide a common framework to aid in the understanding of the etiology and heterogeneity of substance use disorders.

Keywords Alcohol use disorder · Neurofunctional domains · Incentive salience · Negative emotionality · Executive function · Etiology · Heterogeneity

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Introduction

Alcohol is one of the most widely used psychoactive substance in the world. Globally, 80% of adults reported using alcohol at some point in their lives, with 52.3% reported using within the past year [1]. A significant minority of those who consume alcohol will develop alcohol use disorder (AUD), which is characterized by an impairment in the ability to stop or control alcohol use despite negative social, occupational, or health consequences. Global lifetime and 12-month prevalence of AUD were 8.6% and 2.2% respectively [1].

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) outlines 11 diagnostic symptom criteria for AUD [2]. An individual who meets two criteria qualifies for a mild AUD diagnosis, four for moderate, and six for severe AUD. This symptom-based approach to diagnosing AUD offers a considerable degree of diagnostic reliability [3]. However, it provides little information about the etiology and pathophysiology of the disease. Two individuals may present completely different symptom profile with divergent medical histories, and yet receive the same diagnosis of AUD, and possibly the same severity. Thus, the current nosology for AUD belies its heterogeneity.

Heterogeneity among individuals with AUD is not a new issue. Researchers have previously recognized and attempted to identify subtypes of AUD (see [4] for a review). In 1960, Jellinek described five different classifications of alcohol use based on etiological elements (e.g., psychological and physiological vulnerability), alcoholic process elements (e.g., level of tolerance or loss of control), and consequences of drinking (e.g., physical/mental, socioeconomic) [5]. In 1981, Cloninger and colleagues identified two types of individuals with AUD, characterized by age of onset of problematic drinking, heritability, severity, and personality factors [6]. In 1992, Babor and colleagues extended this classification system by including genetic, biological, psychological, and sociocultural traits [7]. Recognizing the cross-sectional limitations of previous works, Lesch and Walter described four subtypes of AUD based on biological, sociological, and psychological information using data from their prospective, longitudinal study [8]. Employing more modern statistical techniques, Moss and colleagues conducted a latent class analysis using data from the National Epidemiologic Survey on Alcohol and Related Conditions and identified five subtypes of alcohol dependence, based on family history of drinking, age of alcohol dependence onset, DSM-IV AUD criteria, and co-occurring psychiatric and substance use disorders [9]. Most recently, Mann and colleagues used factor mixture models to identify four latent subgroups of drinkers based on their reward and relief drinking patterns [10].

Despite these attempts, there is little agreement in the field regarding the different subtypes of AUD. This lack of consensus may be rooted in the limitations of the measures that has thus far been employed. The Addictions Neuroclinical Assessment (ANA) attempts to address this limitation by establishing a neuroscience-based framework and rationale for understanding the heterogeneity of AUD [11, 12]. The ANA framework is composed of three main neurofunctional domains relevant to

AUD and addictions in general: Executive Function, Incentive Salience, and Negative Emotionality. Assessing these three neurofunctional domains, both in patients and individuals at risk for AUD, using established neuropsychological assessment tools and clinical measures will allow for better understanding of the heterogeneity of AUD and its etiology, ultimately improving the nosology of AUD. The ANA framework goes beyond AUD and is an important step towards understanding substance use disorders in general, providing both clinicians and researchers with a common framework to integrate findings from basic neuroscience research into clinical practice.

Addictions Neuroclinical Assessment: A Neuroscience-Based Framework

Advances in neuroscience research have shed light on the neurobiological underpinnings of substance use and substance use disorders [13, 14]. Addiction can be understood as a three-stage cyclical process of binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation [13, 15, 16]. Individuals may start using drugs in a controlled manner, driven primarily by the positively reinforcing effects of the drug. Continued drug use results in homeostatic dysregulation causing changes in the brain's reward, stress, and executive function systems, which ultimately leads to a compulsive pattern of drug use—characterized by a loss of control and use despite negative consequences. The three domains of ANA are based on these three stages of addiction cycle [11]. The incentive salience domain captures the binge/intoxication stage, with processes related to reward, motivational salience, and habit formation. The negative emotionality domain encompasses the withdrawal/negative affect stage, which include stress and negative affective states as a result of drug withdrawal and long-term drug use. The executive function domain corresponds to the preoccupation/anticipation stage, with processes related to planning towards future goals, impulsivity, and working memory. As described below, ANA aims to provide both clinicians and researchers with a common, standardized set of measures by leveraging both neuroscience-based assessments with clinical measures to provide a comprehensive assessment of the three neurofunctional domains.

These neurofunctional domains, informed by our current understanding of the neurobiology of addiction, allows for the development of deeper insights into the processes related to the etiology and pathophysiology of AUD and improve our understanding of the heterogeneity of the disease. This can ultimately lead to better treatment interventions. For example, naltrexone, an FDA-approved medication for AUD, is an opioid antagonist that produces its effects by blocking the rewarding effects of alcohol [17, 18]. Meanwhile, acamprosate, another FDA-approved medication for AUD, acts primarily as a functional glutamate antagonist and is thought to address the cravings associated with acute and protracted alcohol abstinence

[19–21]. While these medications were not developed using the ANA framework, their hypothesized mechanisms of actions each target specific domains of ANA. Naltrexone may be especially efficacious for individuals for whom alcohol has a high incentive salience, while acamprosate might be more efficacious for individuals with executive function deficits who may be more vulnerable to craving-induced relapse. Identifying each individual's level of incentive salience, negative emotionality, and executive functioning would thus be key in helping individuals "break" the cycle of addiction. These neurofunctional domains would also provide a framework for researchers to develop novel therapeutics that target specific addiction-related processes.

Similar Frameworks in Addiction and other Psychiatric Research

The use of transdiagnostic domains to understand the etiology and heterogeneity of psychiatric disorders is not new. Before ANA, the National Institute of Mental Health put forth the Research Domain Criteria (RDoC) project [22]. The goal of RDoC is to create a research framework to facilitate the integration of clinical, genomics, and neuroscience research to inform classification schemes of psychiatric disorders. During its inception, RDoC is composed of five neuroscience-based systems: Negative Valence Systems, Positive Valence Systems, Cognitive Systems, Systems for Social Processes, Arousal and Regulatory Systems. Since then, a sixth domain, Sensorimotor Systems has been added. Each system is composed of distinct constructs, and is further organized by units of analysis (genes to paradigms). Since its inception, the RDoC project has had a significant impact on mental health research over the past decade and continues to play an influential role [23]. The RDoC constructs have been used to understand addictive behaviors [24]. In 2015, the US National Institute on Alcohol Abuse and Alcoholism (NIAAA) first proposed the Alcohol Addiction Research Domain Criteria (AARDoC) as an extension of the RDoC framework to the alcohol field [25]. Subsequently, Kwako et al., [11] further expanded and operationalized this framework as the Addictions Neuroclinical Assessment (ANA). The three-domain framework of ANA is consistent with and can be embedded within the larger RDoC framework.

There have been other models and frameworks proposed for understanding the underpinnings of addiction to alcohol and other drugs. Drawing insights from the three-stage cycle of addiction and findings from neuroimaging research, Goldstein and Volkow proposed the *Impaired Response Inhibition and Salience Attribution* (I-RISA) syndrome of drug addiction model, whereby addiction is a cycle characterized by drug reinforcement (intoxication), craving, bingeing, and withdrawal [16, 26]. This model postulates that disruptions in the neural circuits underlying response inhibition and salience attribution in the prefrontal cortex underlie the development and maintenance of addictive disorders. This framework bears similarities and is relevant to the three neurofunctional domains of ANA.

The Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) is an initiative to identify the cognitive systems and component processes in schizophrenia and develop measurement and treatment approaches to target these systems. Constructs included in these systems include working memory, long-term memory, executive control, social/emotional processing, attention and perception. This initiative has identified and selected a set of social, cognitive and affective measures, behavioral tasks, and imaging biomarkers to assess these constructs [27–30]. The Cognitive Neuroscience Test Reliability and Clinical Applications for Schizophrenia (CNTRACS) is a consortium that has developed out of CNTRICS with the goal of testing the application and psychometric properties of the identified measures. The ANA framework shares many overlapping features with CNTRICS and provides a useful blueprint in applying a similar framework to the addiction field.

Domains

Incentive Salience

Incentive salience is the cognitive process in which a reward and its associated cues are conferred motivational salience, creating a strong drive in the individual to pursue the reward [31]. In natural settings, incentive salience motivates the individual to pursue rewards that are necessary for survival (i.e., food, water, sex). This process is mediated by the mesocorticolimbic system, a circuit that includes the ventral tegmental area, striatum, and nucleus accumbens [13, 32]. Initially, exposure to a novel reward causes midbrain dopaminergic neurons that project to the basal ganglia to release dopamine [33]. However, after repeated exposures to the reward, these midbrain dopamine neurons cease firing during the reward itself, but rather activate when cues predictive of the reward are presented [33]. Ingestion of misused drugs, including alcohol, also result in phasic release of dopamine in the mesocorticolimbic system, and repeated use results in drug-associated cues to be imbued with incentive salience [34]. These neuroadaptations underlie the formation of habitual drug taking [35]. Following chronic drug use, exposure to drug-associated cues can result in an intense desire for the drug (i.e., craving) and subsequently lead to compulsive use [13]. It is important to note that incentive salience only confers the motivation to pursue the reward (‘wanting’) and can be dissociated from the hedonic aspect of the reward itself (‘liking’) [31]. Thus, individuals who are drug tolerant may report an intense desire to use the drug yet perceive little pleasure when using the drug.

Additionally, while the use of these drugs can lead to substance use disorders, most individuals who use drugs do not develop the disorder [36, 37]. Indeed, when given the choice between saccharin and alcohol, only 12.5% of rats showed a strong preference for alcohol, mirroring rates found in human population [38, 39]. Studies on the behavioral economics of drugs suggest that it is the relative value of rewards,

rather than the absolute value, that drives individuals to choose drugs over non-drug alternatives [40]. Therefore, the incentive salience of a reward may be relative rather than absolute, and may prove to be less powerful in the presence of alternative rewards. Incentive salience can be assessed with measures that capture the motivational salience for the drug and drug-associated cues. Individuals with AUD show altered neural responses to alcohol- and non-alcohol related cues [41–43], and exposure to alcohol-related cues induce cravings in these individuals [44, 45], suggesting that cue-reactivity paradigms may be useful probes of incentive salience. Additionally, individuals with AUD show impairments in reward learning which may be indicative of disrupted reward processing [46], and measures that tap into this construct may prove useful. Finally, behavioral economic indices of alcohol demand are also useful indicators of alcohol motivation and incentive salience [47].

Negative Emotionality

While most individuals begin drinking due to the positive reinforcing effects of alcohol, some individuals drink to alleviate negative emotional states, such that drinking behavior is maintained through negative reinforcement [48, 49]. The rise of negative affective states can also be a result of chronic alcohol use itself. Long-term heavy drinking results in allostatic changes such that cessation of alcohol consumption causes the manifestation of withdrawal symptoms, including *hyperkatifeia*, defined as an increase in the intensity of negative emotional and motivational sign and symptoms from withdrawal of misused drugs [50]. Alternatively, existing comorbidities, history of trauma, and exposure to high levels of distress can also result in negative emotional states that increase the likelihood of chronic alcohol consumption [51, 52]. These processes are not mutually exclusive, and most likely co-occur. Individuals with AUD generally report elevated levels of dysphoria, [53] and also display increased negative emotional responses to stress and alcohol cues [54]. Among individuals with AUD who are trying to abstain, these negative emotional states often precipitate relapse [55, 56].

The neurobiology underlying negative emotional states involves the interactions between multiple brain systems, including the reward circuitry (mesolimbic dopamine system) and the brain stress and anti-stress systems (hypothalamic-pituitary-adrenal axis and extrahypothalamic systems) [13, 57]. Chronic alcohol use results in neuroadaptive changes in these systems. During acute withdrawal, the brain reward circuitry becomes less sensitive to stimulation by natural rewards [58, 59], which may be reflected behaviorally as loss of interest towards non-drug rewards. Chronic alcohol use also result in upregulation of the brain stress system, mediated by neurotransmitter systems such as corticotropin-releasing factor (CRF), dynorphin, norepinephrine, orexin, substance P, and vasopressin, and a downregulation of the brain anti-stress system, which involves neuropeptide Y, nociception, endocannabinoids, and oxytocin, all of which are hypothesized to be involved in the manifestations of negative emotional states [13].

Negative emotionality can manifest as symptoms of depression, anxiety, aggression, alexithymia, and anhedonia, and as such clinical measures of these constructs may be useful indicators. Negative and positive affect, typically assessed using self-report questionnaires, may reflect negative emotionality. Behavioral tasks that assess responses to negative emotional stimuli, such as ostracism and presentation of negative emotional faces, as well as tasks that assesses distress tolerance and amotivation for rewards can be useful objective markers of negative emotionality. Specific aspects of personality (such as low conscientiousness, low agreeableness, and high neuroticism) may also be useful in capturing trait levels of negative emotionality.

Executive Function

The executive function domain refers to a collection of mental processes involved in the cross-temporal organization of thoughts and behaviors. This organization lends itself to the organization of behavior towards a future objective [60–63]. While the general domain of executive function is broad (see [60] for a review), the focus here is on executive functions related to the addiction process, which include attention, response inhibition, planning, working memory, behavioral flexibility, and valuation of future events [11]. Deficits and alterations to these processes can result in the loss of top-down control of thoughts and behavior. Individuals may thus have difficulty controlling thoughts and impulses related to drug use, and behaviors may end up being dictated primarily by bottom-up processes related to incentive salience. Impaired executive functions act as both a risk factor and a consequence to chronic alcohol and drug use. Individuals with impaired executive functions are more susceptible to use drugs, develop a use disorder, and relapse during drug abstinence [64, 65], and chronic alcohol and drug use also impairs executive functions [66]. This creates the potential for a vicious cycle: individuals with deficits executive control are prone to engage in alcohol and drug use, which subsequently cause further impairments in executive control.

Related to the above classical executive functions, research has begun to show the importance of interoceptive and metacognitive processes in the etiology of substance use disorders [67]. Interoception, referring to the receiving, processing, and integrating of internal and external stimuli to modulate behavior, is important in the regulation of the individual's homeostatic state. Interoceptive awareness forms an internal representation of the individual, and is integral for a wide range of human behaviors [68]. The individual's awareness of their current and future internal states can modulate the individual's likelihood in engaging in approach and avoidant behavior towards drugs and drug-related stimuli [67, 69]. Individuals with AUD display lower levels of interoceptive awareness, and among this population, interoceptive awareness was inversely associated with alcohol craving [70]. Metacognition, referring to processes related to the appraisal, monitoring, and regulation of cognition [71], is also recognized to play a role in the maintenance of substance use

disorders [72, 73]. Exposure to drug-related stimuli can elicit strong cravings and negative emotional states, and how an individual responds to these thoughts and negative affect will determine the behavioral outcome of that encounter [73].

The neurobiological correlates of executive functions consist of networks involving the frontal cortex [13]. Connections between the frontal cortices and basal ganglia are involved in the regulation of cue-induced cravings and impulsive actions primarily through glutamatergic projections, while projections between the frontal cortices and extended amygdala through glutamatergic projections are critical in regulating negative emotional states [13]. The anterior cingulate cortex is postulated to underlie interoceptive awareness [68]. Additionally, individuals with AUD and individuals with obsessive-compulsive disorder showed reductions in gray matter volume in the anterior cingulate cortex and insular, which may suggest a neurobiological correlate of obsessive thoughts (i.e., cravings) [74]. The default mode network (DMN), a large-scale brain network primarily composed of the medial prefrontal cortex, posterior cingulate cortex/precuneus and angular gyrus, also plays a critical role in interoceptive and metacognitive processes. Interactions between the DMN, executive control, and salience networks are disrupted in individuals with substance use disorders [69, 73, 75, 76].

Ancillary Assessments

While the three neurofunctional domains form the core of the ANA framework, leveraging ancillary measures related to the etiology of AUD will lead to a better understanding its heterogeneity. Examples of ancillary measures include genetic and epigenetic markers, neuroimaging markers, agent use history, environmental and social variables, as well as relevant consequences of drug use.

Substance use disorders are strongly heritable, with heritability estimates of ~50% for AUD, and ranging from 0.39 to 0.72 across substances [77]. Genome-wide association studies (GWAS) of AUD have identified relevant genes that are alcohol-specific (e.g., alcohol metabolizing genes), and others that may be relevant to addictions in general (e.g., SLC39A8, DRD2) [78]. Individual genetic variants are unlikely to be significant predictors of the disease state. As such, genetic and genomic information may be ancillary to the main ANA domains. However, they may be helpful in elucidating the functional and molecular mechanisms underlying the heterogeneity and etiology of AUD. Thus, collection of genomic data as an ancillary assessment is recommended in ANA studies. Polygenic scores, a method of summarizing the total contributions of multiple genes related to a particular disease [78, 79], together with genomic structural equation modeling [80, 81], may address some of the predictive limitations of single-gene approaches. Changes in the transcriptome due to chronic and heavy substance use, such as microRNAs and epigenetic changes, are also important features to be measured [82, 83]. Together, these approaches will elucidate the genetic and epigenetic factors underpinning the addiction process, and provide an integrative approach to understanding the disease and related comorbidities.

Employing neuroimaging methods will also be an important aspect of ANA. Positron emission tomography (PET) has been successful in understanding the role of specific neurotransmitter systems involved in substance use disorders [84, 85]. Functional magnetic resonance imaging (fMRI) techniques can also capture differences in brain morphology [86], resting state functional connectivity [87], and neural responses to alcohol and other cues [88] as well as emotional stimuli and executive function tasks, as a function of alcohol use. These approaches can be integrated with both genetic and clinical measures as part of a multimethod approach in assessing the ANA domains [89].

Substance use history, environmental and social variables, and consequences of drug use are important dimensions related to substance use that should be captured with ancillary assessments. These assessments may be important in capturing processes and outcomes of substance use that are not otherwise included within the three domains of ANA. Since the ANA domains are transdiagnostic, substance-specific measures can serve as important markers that contribute unique information about the individual's substance use. Measures assessing frequency and quantity of consumption of the substance also serve as vital clinical outcomes that can be used to monitor progression and recovery. The environmental and social milieu of the individual also modulates their risk of substance use, and are important variables for consideration [11].

Empirical Research

Since its introduction by Kwako and colleagues [11], multiple groups have independently validated and applied the domains of ANA. Most of these studies used similar methodologies, employing a combination of factor analytical techniques and structural equation modeling to identify the key latent variables and their associations with sociodemographic variables, risk factors, and drinking-related outcomes.

Initial validation of the three neurofunctional domains was conducted by Kwako and colleagues using a deeply phenotyped sample consisting of 454 individuals across the spectrum of alcohol use who underwent the National Institute on Alcohol Abuse and Alcoholism (NIAAA) Natural History Protocol [90]. In this retrospective analysis, measures relevant to the three neurofunctional domains were selected and subjected to exploratory and confirmatory factor analyses. A three-factor solution provided the best fit to the data, consistent with the three neurofunctional domains of ANA. The three factors showed significant cross-correlations (r's = 0.76 to 0.90). Trait anxiety showed cross loadings to both the negative emotionality and incentive salience factors, while depression loaded on to the incentive salience but not negative emotionality factor. This may reflect negative reinforcement (alleviation of negative mood states) driving incentive salience processes. Multiple indicators, multiple causes (MIMIC) analysis identified early life stress and sociodemographic variables as significant predictors of the three latent factors. To characterize the ability of the latent factors in distinguishing individuals with and

without AUD, receiver operating characteristics (ROC) curves of the true positive rates (sensitivity) versus false positive rates (1-specificity) were examined. The areas under the ROC curves for the latent factors ranged from 0.85 to 0.96, indicating that the ANA factors showed a remarkable ability to identify individuals with AUD [90]. This study provides the first empirical evidence of the three-domain structure of ANA and highlighted its application to understanding AUD etiology.

Votaw and colleagues replicated the findings of Kwako et al. by retrospectively analyzing data from a multisite naturalistic prospective observational study in 563 individuals seeking treatment for AUD [91]. The researchers focused specifically on the negative emotionality domain, using measures of depression, anxiety, anger, and drinking-related unhappiness, guilt and anhedonia, as indicators in a confirmatory factor analysis. A single-factor solution for the negative emotionality domain provided an excellent fit to the data, consistent with the findings of Kwako and colleagues [83]. Additionally, the researchers found that the latent factor was invariant across time and across genders (i.e., factor structure, loadings, and means did not differ between baseline, 6- and 12-month follow-up, and between males and females). Higher negative emotionality was associated with more frequent and heavier drinking, and drinking to regulate negative affect, providing evidence of the construct validity of the negative emotionality factor [91].

In a more recent extension of their work, Votaw and colleagues examined the predictive validity of the negative emotionality factor using a latent growth curve mediation model [92]. Using data from 263 treatment-seeking individuals, the researchers tested whether drinking motives at 6 months mediated the relationship between negative emotionality at baseline and drinks per drinking day at 7–12 months. Baseline negative emotionality, estimated using the same indicators as their previous study [91], was found to be indirectly associated with greater alcohol consumption 12 months following treatment initiation through higher coping motives at 6 months. Baseline negative emotionality, however, was not related to changes in coping motives, nor were changes in coping motives related to drinking outcomes at 12 months [92]. This was the first study to test the predictive validity of the ANA domains.

While the previous two studies focused primarily on the negative emotionality domain, Stein and colleagues sought to replicate and validate the factor structure of the incentive salience domain [93] using data collected from the same participants in Votaw et al. [91]. Measures of incentive salience include items from the Alcohol Dependence Scale, Impaired Control Scale, Situational Confidence Questionnaire, and Marlatt Relapse Interview. A one-factor model of incentive salience provided good fit to the data. The model was also invariant across sex. Incentive salience was negatively associated with percent days abstinent, and positively associated with drinking quantity, frequency, and heavy drinking days, as well as measures of testing personal control, urges, social pressure to drink, and family history of AUD, providing evidence of convergent validity. The factor was not associated with religiosity or family social support, thus demonstrating discriminant validity. Finally, the researchers established predictive validity by demonstrating that incentive salience at baseline was associated with heavy drinking days at 12-month follow-up [93]. Thus, this study provided independent replication and validation of the incentive salience domain proposed in the ANA.

Nieto and colleagues pooled data from six clinical and experimental psychopharmacology studies to assess the ANA framework in a sample of 1679 heavy drinkers with and without AUD [94]. Measures of harmful drinking, AUD severity, alcohol-related problems, alcohol craving, anxiety, depression, attention, working memory, impulsivity, and delay discounting, were subjected to exploratory factor analyses to elucidate the underlying latent structure of the measures. Four factors emerged, corresponding to the three domains proposed in the ANA framework, and an additional factor capturing alcohol-related consequences. Being male was associated with higher levels of alcohol-related consequences and incentive salience. Age was positively associated with alcohol-related consequences, incentive salience, and negative emotionality, and negatively associated with executive function. Those who had their first drink at a younger age was associated with greater alcohol-related consequences and incentive salience, and a family history of alcohol problems was associated with higher levels of alcohol-related consequences, incentive salience, and lower executive functioning. Both drinking frequency and quantity was positively associated with alcohol-related consequences and incentive salience, but only drinking frequency was associated with greater negative emotionality. Finally, individuals with AUD exhibited greater scores on all factors except executive function.

Demartini and colleagues sought to replicate the findings from Kwako and colleagues in a sample of non-treatment seeking low and high risk drinkers [95]. Data from 335 individuals were collected, including measures of impulsivity, depression, drinking motives, alcohol expectancies, obsessional and compulsive behavior, and habit. Factor analyses indicated a three factor solution, corresponding to the three neurofunctional domains. A history of childhood trauma and AUD were predictors of impairments across all three domains. Earlier age of first drink, and family history of AUD were associated with greater incentive salience. No sex differences were found across the three domains [95]. This work provides an important replication and extension of the original study by including unique indicators that was not previously used, while still arriving at the same factor solution.

Using the AARDoC framework, Al-Khalil and colleagues sought to identify the neural correlates underlying the incentive salience and negative emotionality domain in a sample of non-treatment seeking heavy drinking adults [96]. Participants were presented with alcohol, negative-valenced, and neutral cues while undergoing a functional magnetic resonance imaging paradigm. Changes in functional connectivity in the nucleus accumbens and amygdala were hypothesized to reflect incentive salience and negative emotionality related processes, respectively. Changes in functional connectivity between the nucleus accumbens, cerebellum and prefrontal cortex, and functional connectivity between the amygdala and occipital, parietal, and hippocampal regions were detected in response to alcohol cues relative to neutral cues. Additionally, functional connectivity changes between the nucleus accumbens, lateral temporal, occipital, and parietal regions, and changes in function connectivity between the amygdala, fusiform, and lingual gyri were observed in response to negative cues relative to neutral cues. Some of the functional connectivity changes also differed as a function of AUD severity [96]. Thus, functional connectivity between these regions may underlie levels of incentive salience and negative emotionality in heavy drinkers. This study provides important information

about the neurobiological correlates underlying the incentive salience and negative emotionality domain. Future work would be needed to replicate and extend these findings to delineate the neural correlates of the ANA framework across individuals with and without AUD.

Extending beyond AUD, Nieto & Ray applied the ANA framework to methamphetamine use disorder [97]. One hundred and eighty-five non-treatment-seeking frequent methamphetamine users were recruited and completed a deep phenotyping battery that consisted of measures of methamphetamine withdrawal, craving, anxiety, depression, risk attitudes, behavioral inhibition, attention, working memory, impulsivity, and delay discounting. The researchers conducted factor analyses and arrived at a three-factor solution, corresponding to incentive salience, negative emotionality, and executive functioning. Age was negatively associated with executive function, and methamphetamine symptom count was positively associated with negative emotionality and incentive salience. Past 30-day methamphetamine use was associated with higher incentive salience, consistent with what was found among the treatment-seeking individuals. This work demonstrates the implementation of the ANA framework to other substance use disorders, and underscores some of the common neurofunctional pathways underlying addiction to alcohol and other misused substances.

Taken together, the studies summarized above provide important validation and extensions of the original ANA framework. While these studies are generally consistent with the three neurofunctional domains, some differences across studies were present. For example, Kwako and colleagues found sex differences for the negative emotionality domain [90], but this was not replicated by Votaw et al. [91] and Nieto et al. [94]. These inconsistencies may be due to differences in the samples used in the studies. The latter two studies consisted of individuals on the higher end of the AUD severity spectrum; those in Votaw et al. were composed exclusively of treatment-seeking individuals [91, 92], while participants in the study by Stein and colleagues were primarily heavy drinkers enrolled in psychopharmacological studies with strict exclusion criteria [93]. Kwako and colleagues had the most diverse sample with the broadest inclusion criteria, which included both treatment- and non-treatment-seeking individuals with and without a diagnosis of AUD, which may be more representative of the broader population of individuals across the spectrum of alcohol use and risk for AUD [90].

Addictions Neuroclinical Assessment Battery

Building upon the proposed ANA framework, the NIAAA intramural clinical research program has developed the ANA testing battery, a collection of neurocognitive and behavioral tasks and self-report questionnaires that assesses the three neurofunctional domains of ANA. These measures were selected based on a review of the addiction literature and through consultations with relevant experts in the field. The goal of this assessment battery is to develop a set of

standardized measures for clinicians to assess the neurofunctional domains of patients, and for researchers to study the etiological processes of AUD using the ANA framework. The list of assessments, organized by domains, can be found in Table 14.1. A prospective clinical study using this assessment battery is currently underway (NCT04946851, clinicaltrials.gov). Due to the breadth of measures employed, the full battery takes approximately 4–5 h to complete

Table 14.1 List of measures in the ANA assessment battery

	Incentive salience	Negative emotionality	Executive function
Behavioral Tasks	• Alcohol Approach Avoidance Task (Approach-Avoidant Bias) • Drinking Identity Implicit Association Task (Implicit Association) • Alcohol Stroop (Cue-reactivity) • Implicit Choice Task (Alcohol preference)	• Paced Auditory Serial Addition Task (Distress Tolerance) • Cyberball (Ostracism) • Effort Expenditure for Rewards Task (Motivation for rewards) • Probabilistic Reward Task (Reward learning) • Penn Emotion Recognition Task (Emotion recognition)	• Stop Signal Reaction Task (Response Inhibition) • Digit Span—Backwards (Working Memory) • Beads in a Jar (Inference) • Trail Making Test (Task Switching) • Manikin Test of Spatial Orientation (Mental Rotation) • Continuous Performance Task (Attention) • Wisconsin Card Sorting Task (Cognitive Flexibility) • Balloon Analog Risk Task (Risk-taking)
Self-report measures	• Hypothetical Purchase Task (Alcohol Demand)	• Snaith-Hamilton Pleasure Scale (Anhedonia) • Connor-Davidson Resilience Scale (Resilience) • Inventory of Socially Supportive Behaviors (Social Support) • Experiences in Close Relationships Scale (Attachment Anxiety/Avoidance) • Positive and Negative Affect Scale (Affect) • Toronto Alexithymia Scale (Alexithymia)	• Metacognitions Questionnaire (Metacognition) • Multidimensional Assessment of Interoceptive Awareness (Interoception)
Ancillary measures	• Self-Rating of the Effects of Alcohol Questionnaire (Alcohol sensitivity) • Obsessive-Compulsive Drinking Scale (Compulsive drinking) • Penn Alcohol Craving Scale (Craving)	• Montgomery-Asberg Depression Rating Scale (Depression) • State-Trait Anxiety Inventory (Anxiety) • Buss-Perry Aggression Questionnaire (Aggression) • NEO Personality Inventory (Personality)	• Barratt Impulsivity Scale (Impulsivity) • UPPS-P Impulsivity Scale (Impulsivity) • Monetary Incentive Delay (Delay Discounting)

(including breaks). A major objective of this ongoing work is to characterize the underlying latent factor structure of the individual neurofunctional domains and how these latent sub-factors may relate to each other. Figure 14.1 shows a hypothesized model of latent factors in each domain as the domains relate to each other along the cycle of addiction. Importantly, the study will examine associations between these domain factors and sub-factors as a function of AUD diagnosis and other indicators and outcomes. Work is currently being done in evaluating the psychometric properties of the ANA battery, to use these domain factors to identify AUD subtypes, and to understand how proximal and distal factors impact drinking outcomes and confer risk to the development of AUD. Potential differences in factor scores and associations between individuals with and without AUD may reflect some of the etiological processes of AUD and help identify potential mechanistic underpinnings of the neurobiology of the addiction cycle. Detailed descriptions of the assessment battery and measures can be made available upon request to the corresponding author.

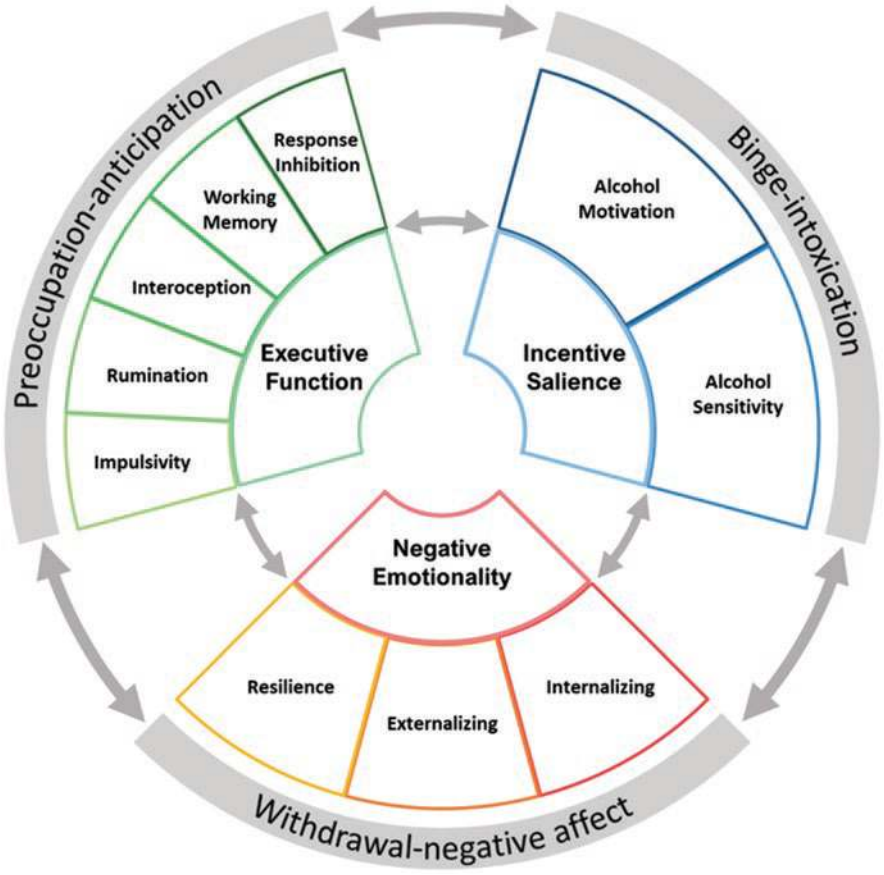

Fig. 14.1 Conceptual framework of the addictions neuroclinical assessment (ANA) domains and underlying latent factors

Future Directions

The goal of ANA is to create a common framework for both clinicians and researchers to understand the etiology and heterogeneity of AUD and substance use disorders. The work thus far has focused on empirically validating the theoretical model of ANA. Establishing the foundational validity of the model is critical before hypothesis-driven research can be undertaken. It is important, however, that the ANA framework must not be rigid in its conceptualization and be flexible to new research findings. Continual, incremental improvements in face of new information will determine the success of ANA. While the framework thus far has primarily been applied to understanding AUD, the theoretical foundation of ANA is not tied to specific substances and thus should be broadly applied to other addictions. An additional future direction for researchers to consider is how the ANA framework can serve as a bridge between preclinical and clinical research, with the development and application of models that can aid in both translational and reverse-translational approaches to understand addictions [12, 98].

The imperative aim of the current research is to use these neurofunctional domains to elucidate homogenous subtypes of AUD. Utilizing mixture modeling approaches such as latent profile analysis are important next steps in identifying these subgroups [99]. Understanding the biological correlates of the ANA domains using genetic, epigenetic, and neuroimaging methods will also be key in understanding the specific molecules, brain regions, and neural networks that underlie the ANA domains. Much of the work on ANA thus far has employed retrospective, cross-sectional samples that limit our understanding of the causal relationship between and across domains. Future research that employ longitudinal designs will be critical in our understanding of how these domains change as a function of alcohol use, alcohol use disorder progression, and importantly, as a function of recovery [100]. Equally critical is the use of diverse samples to understand how the etiology and heterogeneity of AUD might differ across gender, racial, and ethnic groups. Socioeconomic factors [101] and sociocultural factors [102] are known to be strong predictors of alcohol use, and differences in these factors across racial and minority groups may contribute to the health disparities seen within AUD by modulating the etiological process of the disease. Finally, a shorter assessment battery will be necessary for the successful application of the ANA framework in the clinical setting to aid in diagnosis and characterization of individuals with AUD. Thus, future research will need to identify the most efficient measures for the three domains and rigorously test the validity and reliability of the assessment battery.

Conclusions

In summary, the Addictions Neuroclinical Assessment is a clinically-directed, neuroscience-based framework to understand the etiology and heterogeneity of AUD and other substance use disorders. ANA consist of three neurofunctional

domains relevant to addictions: incentive salience, negative emotionality, and executive functions. These domains are based on the three stages of the cycle of addiction and are supported by our current understanding of the neuroscience of substance use disorders. Measuring these neurofunctional domains will require the use of neuropsychological and behavioral tasks, as well as clinical and self-report measures. Ancillary measures, such as genetic, epigenetic, and neuroimaging markers, are recommended to supplement the assessment of the three domains. This review includes a summary of the current empirical work that has utilized the ANA framework. Current limitations of the research, and important directions for future research are highlighted. The ANA aims to serve as a tool for both researchers and clinicians to provide a common framework to aid in improving the understanding of the etiology and heterogeneity of AUD, and substance use disorders in general.

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Part III
Treatment of Alcohol Addiction

Chapter 15
Evidence-Based Behavioral Treatments

Anders Hammarberg and Stina Ingesson

Abstract Behavioral treatments for of alcohol use disorders (AUD) include numerous interventions based on the theories of behaviorist learning, which assume that alcohol related behaviors are learned according to the principles of classical and operant conditioning. Behavioral treatment for AUD aims to alter these behaviors and replace them with non-alcohol related behaviors, with the aim of accomplishing reduced consumption or abstinence from alcohol. The aim of this chapter is to give an overview of evidence based behavioral treatments for AUD, including Brief Interventions, Motivational Interviewing/Motivational Enhancement Therapy, Cognitive Behavioral Treatment (CBT), Behavioral Couples Therapy (BCT), Community Reinforcement Approach (CRA), Contingency Management (CM), Cue Exposure Therapy (CET), Mindfulness Based Interventions (MBI) and 12-step based treatments. Further, the aim is to focus on some specific areas of interest, these being the availability of treatments for controlled drinking, behavioral treatments combined with pharmacotherapy, technology-based treatments and common mechanisms of behavior change. The chapter ends with some suggestions for future directions in research on behavioral treatments for AUD, where the most tangible challenges may be not the question of which treatments are efficacious, but instead how to increase availability to treatment and how to make treatment for AUD more attractive to those in need.

Keywords Alcohol · Alcohol use disorder · Behavioral treatments · Randomized controlled trials · Mechanisms of behavior change

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Introduction

Behavioral treatments for of alcohol use disorders (AUD) include numerous psychological interventions, which are based on, or partly influenced by, behaviorist learning theory. In the context of AUD, behavioral interventions are based on the assumption that behaviors related to problematic alcohol consumption are learned according to the same basic principles as all other behaviors, i.e., classical and operant conditioning. In Fig. 15.1, we give a schematic overview over how alcohol behaviors are learned. According to the theory of classical conditioning, behaviors that we repeat become associated (conditioned) with stimuli present in those situations. Using the example of alcohol consumption, stimuli associated with drinking may be exteroceptive, for example other individuals (e.g., best friends or partner), places (the favorite bar, at home while cooking dinner, the country house) or events (while watching sport events, listening to music). Stimuli may also be interoceptive, for example drinking when in a certain emotional state. As conditioning takes place, the stimuli become antecedents (A) to alcohol consumption, i.e., stimuli that trigger future alcohol consumption when the individual is exposed to them.

According to the theory of operant conditioning, rewarding behaviors tend to be repeated (reinforced) more than others. A specific behavior (alcohol consumption); termed "response" (R), will be reinforced by its short-term consequences (SC). The short-term consequences may be either positively reinforced, as it results in rewarding consequences. Alcohol consumption is typically positively reinforced by increased pleasure and positive emotions, as well as increased sociability. Alcohol consumption may also be negatively reinforced, as alcohol may reduce unpleasurable experiences. Alcohol consumption is thus, negatively reinforced by creating a relief from, e.g., negative emotions such as stress, anxiety or withdrawal symptoms.

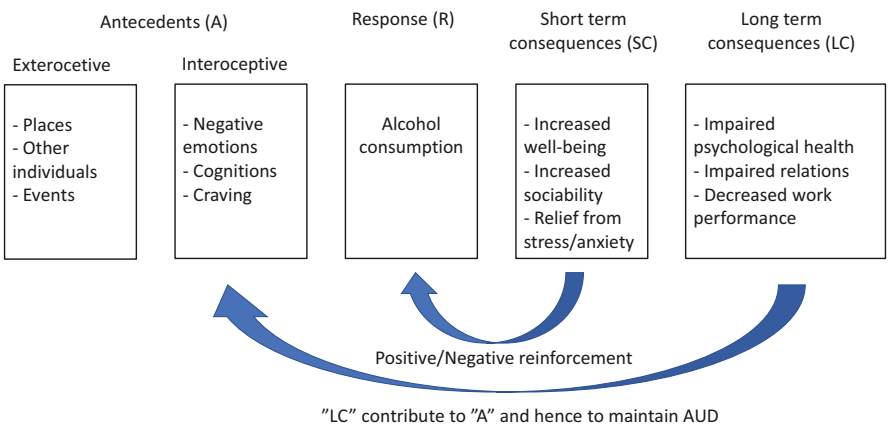

Fig. 15.1 Schematic overview of basic learning theory based on the example of alcohol use disorder

Alcohol consumption also have well known long-term negative consequences (LC), such as impaired psychological health, impaired relations and decreased work performance. The long-term negative consequences function as antecedents to future alcohol consumption and hence contribute to the maintenance of an AUD. Again, this can be explained by the reinforcing properties of alcohol mainly due to that alcohol consumption is negatively conditioned, as it in the short-term may alleviate negative emotions and cognitions related to the long-term consequences of previous alcohol consumption.

As will be apparent in this overview, behavioral treatments target different aspects of this behavior chain. For example, cognitive and behavior treatment (CBT), Community Reinforcement Approach (CRA), Behavior Couples Therapy (BCT) and Contingency Management (CM) focus on reinforcing alternative behaviors to alcohol consumption, or coping skills training in order to curb the drinking response while cue-exposure therapy (CET) focus on decreasing the association between antecedents and response.

Behavioral treatments vary considerably in content, intensity, and length, and are more or less applicable in different contexts. Some have been developed for use in general health care (e.g., Brief Interventions (BI)) while others are more suitable for use within specialized addiction treatment. In this chapter, we will give an overview of evidence based behavioral treatments, along with a brief summary of their empirical support. In addition, we will focus on some specific areas that we consider to be of key interest for the development of the field of AUD from a clinical and research perspective; the availability of treatments for controlled drinking (CD), the development and validation of technology-based treatments, and research on common mechanisms of behavior change.

Overview of Evidence Based Behavioral Treatments

A considerable number of behavioral treatments for AUD have been developed mainly from the 1980's and onwards. However, there is a large variation regarding the empirical support for these treatment approaches [1]. By evidence-based we mean interventions that have been empirically supported through randomized controlled trials (RCTs), and also preferably have been evaluated in systematic reviews and/or metanalyses. Treatment approaches covered by this overview are briefly summarized in Table 15.1.

A challenge in summarizing behavioral treatments in AUD, is the large variation of target populations in studies on this heterogenous disorder. In this section, we will provide an overview of behavioral treatments for individuals ranging in problem severity from heavy drinkers to those who fulfil diagnostic criteria for AUD. The focus will be on adult patients without comorbid psychiatric diagnoses. Interventions for adolescents and young adults as well as for patients with comorbid psychiatric conditions are covered in other chapters in this book.

Table 15.1 Brief overview of behavioral treatments

Treatment	Primary content
Brief Intervention (BI)	Assessment of alcohol use by and its consequences by self-report and biomarkers together with personalized feedback from healthcare personnel.
Motivational Interviewing (MI)/Motivational Enhancement Therapy (MET)	Person-centered, process-based collaborative treatment, focused on eliciting and strengthening motivation for and self-efficacy to change.
Cognitive behavioral treatment (CBT)	Treatment components include, e.g., functional analysis of risk-situations, goal setting, self-monitoring of consumption, identification of alternative behaviors and coping skills training.
Behavioral Couple Therapy (BCT)	CBT-based intervention in which a partner actively participates in treatment. Focus on relapse prevention, increasing communication skills and to identify common activities that can serve as alternative to alcohol consumption.
Community Reinforcement Approach (CRA)	Aims to change individual contextual factors which are identified as important to support change, including behavioral skills training, job skills training, social and recreational counseling, relapse prevention and relationship counseling.
Contingency Management (CM)	Reduction in alcohol consumption is reinforced by reimbursements in cash or vouchers for goods or services which are found rewarding. Continuous rewards for alcohol-reduction or abstinence generate operant conditioning to goal behaviors.
Cue Exposure Therapy (CET)	The individual is repeatedly exposed to alcohol-related cues with the aim to attenuate alcohol craving responses in situations in which alcohol consumption is a learned behavior. With prolonged exposure, alcohol-related stimuli will gradually lose their reinforcing properties.
Mindfulness Based Interventions (MBI)	Practice of mindfulness skills aims to increase awareness of alcohol-related triggers in order to target the individual's response to alcohol cravings and intrusive automatic alcohol-related thoughts in risk-situations. Skills reduce the risk of relapse into problematic drinking.
12-step based treatments	The overall purpose is to facilitate abstinence from alcohol, increase psychological well-being and quality of life, improve interpersonal skills and the ability to cope with stressful life events.

Brief Interventions

Brief interventions (BI) include an assessment of alcohol use and personalized feedback, with the aim of providing the patient with different options for behavior change. BI most often contain screening for hazardous alcohol consumption by the Alcohol Use Disorder Identification Test (AUDIT) [2] or some other short self-report instrument, combined with blood tests for alcohol biomarkers. In conjunction with the assessment, counseling is provided in which the patient will be offered advice based on the results of the assessment. BI is typically delivered in a single session by a health care professional within primary care [3] and is carried out

according to the principles of Motivational Interviewing (MI), which will be more thoroughly described in a following section. Meta-analyses have shown that BI is efficacious in reducing alcohol consumption for non-treatment seeking heavy drinkers and for individuals with mild AUD [4–6]. However, BI is considered less efficacious for individuals with moderate to severe AUD [7]. To address the need for more extensive support for patients with greater needs, BI may be incorporated within a stepped care model (see Fig. 15.2). In such a model, the treatment dose is adjusted based on a clinical evaluation of the patient's needs [8–10]. Patients who do not benefit from a BI, are offered extended assessment with feedback (step 2) and—if needed—treatment (step 3), that may contain a psychological program based on the principles of cognitive behavioral therapy (CBT) and MI [11] and/or pharmacological treatment. Only patients who require more extensive treatment are referred to specialized addiction treatment.

Despite the evidence supporting the efficacy of BI, these methods have not been successfully implemented within the regular health care system [12]. It is estimated that in primary care, less than one in five of individuals with hazardous consumption are identified and less than one in four with alcohol dependence are offered treatment [13].

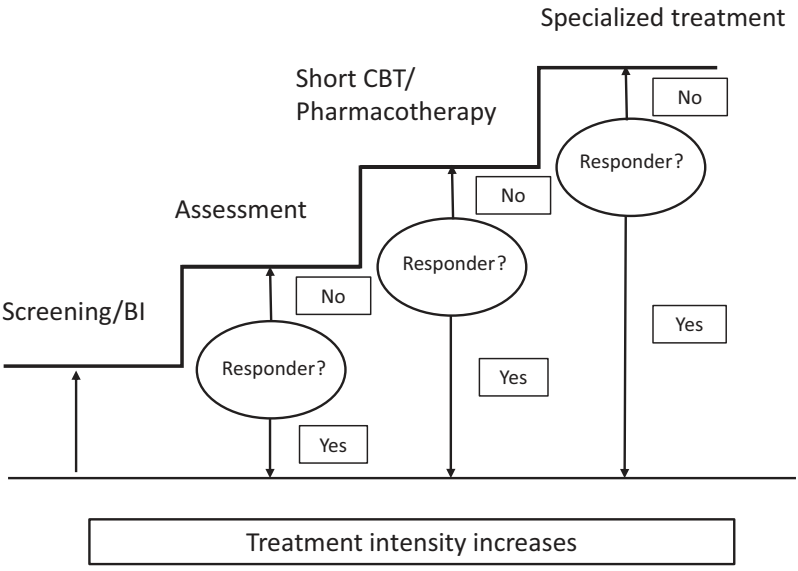

Fig. 15.2 Schematic description of a stepped care intervention. Modeled after Wallhed Finn et al. [8], Grothues et al. [10] and Sobell and Sobell [9]

Motivational Interviewing (MI) and Motivational Enhancement Therapy (MET)

MI is commonly defined as a brief, empirically validated, person-centered collaborative treatment, focused on eliciting and strengthening motivation for change [14]. MI was originally developed for targeting motivation for change in individuals with substance use disorders [15]. It has evolved into a widely accepted method used in different clinical and research settings associated with goals of a healthier lifestyle, such as smoking cessation, weight loss, or the reduction of alcohol consumption [16–18]. Rather than being a treatment with fixed themes every session, MI is a process-based approach that aims to explore and strengthen the individual's engagement to the identified goal, to resolve ambivalence, and to alter behaviors that maintain status quo [19]. Motivational Enhancement Therapy (MET) is an extension of MI, which includes components with the aim of further strengthening commitment to change [20]. In the first MET-session, patients go through assessments (questionnaires and biomarkers) in conjunction with a taking a history. This provides the patient and therapist with material for a deepened understanding of the patient's alcohol problems and related consequences, including severity of AUD, alcohol related harm, and psychological health. The second session is dedicated to a structured feedback on the initial assessment, with the aim of strengthening the patient's motivation for change. Further, the patient is encouraged to invite a significant other who can function as an additional reinforcer of the change process. The following 2–3 MI-sessions aim at further building motivation and consolidate commitment to change. Lastly, MET includes an option for the patient to formulate a written change-plan, including a formulation of goals together with defining activities and needs for support required to achieve the specified goals. As in MI, the role of the therapist in MET is to support the patient's change in a collaborative, empathetic, and non-confrontational manner.

Several RCT: s have supported the efficacy of MI and MET in reducing alcohol consumption, promoting abstinence and improving social functioning [20–22]. In a meta-analysis, Vasilaki et al. [23] found that, compared to no treatment, MI/MET was superior regarding reduction of alcohol consumption (d = 0.18), with a steep increase in effectiveness if follow-up period was restricted to 3 months (d = 0.60).

Cognitive Behavioral Therapies (CBT)

As mentioned in the introduction to this chapter, CBT is based on theories of learning according to behaviorist theory, and also rely on cognitive—and emotion theory. CBT models for AUD are based on the assumption that alcohol-related behaviors and the related negative consequences, may be changed by reinforcing alternative behaviors to alcohol consumption [24]. Alternative behaviors may be, e.g., reconnecting with friends, or engaging in physical exercise instead of spending time

drinking. CBT models also emphasize the acquisition of specific alcohol-related coping strategies, such as seeking social support or turning down drink offers [25]. CBT treatment manuals typically comprise specific components including goal setting, self-monitoring of alcohol consumption and a functional analysis, which is a structured way of identifying antecedents, as well as short- and long-term reinforcers of the problem behavior. This tool supports the patient in the identification of triggers associated with drinking (the "A" in Fig. 15.1), and specific skills training to cope with high-risk situations. Other key components of CBT are the emphasis on an active patient, a collaborative work alliance with the therapist, and in-between session assignments as a means of facilitating the generalization of behavioral and coping skills. Typical examples of CBT interventions are Relapse prevention (RP) [24–26] and Behavioral Self-Control Training (BSCT) [27].

Numerous studies, reviews, and meta-analyses corroborate the efficacy of CBT for treating AUD [28, 29]. A recent meta-analysis of CBT for SUD reported significant effects with different contrasts and type of outcomes [29]. Studies with the comparator being; minimal intervention, waitlist, or assessment only as control conditions showed a pooled effect size of g = 0.58 for frequency outcomes (e.g., number of abstinence days) and g = 0.67 for quantity outcomes (e.g., drinks per drinking day) at early follow-up. CBT in contrast to nonspecific therapy showed an effect size of g = 0.18 for frequency outcomes and g = 0.42 for quantity outcomes. In comparison with other specific therapy, the effects of CBT were nonsignificant for both types of outcomes. To summarize, CBT has strong support as an efficacious method for the treatment of AUD, albeit there is no evidence to support that CBT is superior to other specific behavioral treatments for the treatment of AUD.

Behavioral Couples Therapy (BCT)

Behavioral Couples Therapy (BCT) is a CBT-based intervention in which a partner actively participates in the treatment sessions together with the patient [30, 31]. According to BCT, a partner may work as a reinforcer of the behavior change in treatment due to several factors. First, the partner may act as a coach in the process of changing cognitions and behaviors related to problematic alcohol consumption. Second, the negative alcohol-related consequences which affect the partners as individuals and as a couple, can be addressed and resolved more effectively when the two parties meet together with a therapist. Lastly, general relationship functioning can be discussed and improved [32].Together, these factors are theorized to be helpful for the patient in order to accomplish and maintain a reduced alcohol consumption. BCT interventions also typically include psychoeducation, communication skills, behavioral activation to increase positive shared activities, negotiation of sobriety contracts, and relapse prevention with the goals of improving mutual coping and decreasing use [33]. Many components resemble those of other approaches such as individual RP and also Community Reinforcement Approach and Family Training (CRAFT, [34]) a method which addresses the partner exclusively. In a

meta-analysis [35], it was concluded that BCT is efficacious in reducing negative consequences of alcohol use and in increasing relationship satisfaction. For the reduction of alcohol consumption, the results were mixed and dependent on timing of measurement. BCT was not superior to comparators at post treatment follow-up, but in a longer perspective, (6- and 12-months post treatment), participants in BCT reported lower alcohol consumption and higher rates of abstinence. Noteworthy is that studies have identified a decrease in domestic violence following participation in BCT [36]. More recent studies have among others focused on investigating the efficacy of BCT in group format [37].

Community Reinforcement Approach (CRA)

The main goal of Community Reinforcement Approach (CRA) is to reinforce a reduced consumption or complete abstinence from alcohol by changing important contextual factors in the patient's life [38]. CRA shares its theoretical basis with CBT, in its aim to alter the antecedents and reinforcers of alcohol consumption. What is specific to CRA, is its emphasis on the surrounding community in this change process, e.g., family, and social network, recreational activities and occupational status [39, 40]. CRA and CBT share common treatment components. For example, CRA procedures commence with a functional analysis of drinking behavior in order to map antecedents, short- and (most often) negative long-term consequences to the problem behavior. This is followed by the identification of which areas would be most helpful to target for the reinforcement and maintenance of abstinence or a reduction in alcohol consumption. Thereafter, a treatment plan is outlined, based on the "Happiness Scale" which indicates what areas of life are the most important in achieving behavior change. The components may contain behavioral skills training, job skills training, social and recreational counseling, relapse prevention and relationship counseling. Evidence for CRA is generally favorable for AUD although there is a lack of recent metanalyses or systematic reviews. A meta-analysis from 2004 included two studies with a total of 343 participants [41]. The studies evaluated CRA compared with treatment as usual (TAU). Regular disulfiram treatment was not included in the treatment groups, but a relatively large proportion of patients used disulfiram during the treatment period. The meta-analysis showed a greater reduction in the proportion of days with alcohol consumption in the groups that received CRA compared to the control groups (weighted mean difference = −0, 94).

Contingency Management (CM)

Contingency Management (CM) stems from the principles of operant conditioning theory, in which problematic alcohol behaviors can be modified by altering the value of alcohol consumption relative to other incentives [42, 43]. In a CM program,

the planned behavior—which in this respect is moderation of or abstinence from alcohol consumption—is reinforced by cash or by vouchers for goods or services that the participant considers rewarding, for example food coupons [44]. Most studies using contingency management have focused on substances whose metabolites are easily measured and have a large window of detection, e.g., cannabis [45], nicotine [46] and cocaine [47]. There is support for CM also among heavy drinkers and individuals with AUD although since the window of detection for ethanol is shorter, CM used in these populations requires a more frequent monitoring of breath, blood, or urine biomarkers. More recently, CM programs have used new technologies that allow continuous monitoring of alcohol use, including transdermal alcohol monitoring [44] and ethyl glucuronide [48].

A concern regarding CM has been that the method may not result in prolonged behavior change post treatment [43]. However, a recent meta-analysis concluded that reduction in substance use post CM exposure can be traced at long term follow-up [49]. Lastly, A systematic review suggests that CM as a stand-alone intervention provides equal effects compared to CM as an add-on intervention to other treatment programs [50].

Cue-Exposure Therapy (CET)

Cue-Exposure therapy (CET) is based on learning theory and can generally be described as a method for extinguishing conditioned responses through exposure to stimuli combined with response prevention [51, 52], where the learned/conditioned response is either, e.g., initiation of drinking, or a heavy-drinking episode. In a laboratory-like situation, the patient is exposed to alcohol-related stimuli (cues) with the aim to attenuate alcohol craving responses in situations in which alcohol consumption is a conditioned response. With prolonged exposure, alcohol-related stimuli will gradually lose their reinforcing properties. CET may include exteroceptive cues (visual, e.g., pictures of alcoholic beverages or a tray with beverages, tactile, taste, smell or auditory) [51, 53]. Cues may also be interoceptive, where the patient is instructed to refrain from further consumption when exposed to stimuli after having consumed a fixed amount of his/her preferred beverage. This is often called a priming dose paradigm [54]. Sessions are typically administered over a period of several weeks, in which the intensity of exposure is gradually increased. Theoretically, the gradual exposure, will lead to a reduction of alcohol craving, alcohol consumption and an increased sense of self-efficacy in coping with high-risk situations.

CET has received mixed results in systematic reviews and meta-analyses. In the most recent meta-analysis, Mellentin et al. found small effect in favor of CET (Cohen's $d < 0.10$) compared to control conditions on drinking outcomes post treatment or at long-term follow-up points [55]. However, most studies included in this meta-analysis involve control conditions which are considered efficacious for the treatment of AUD (most often CBT). As proposed by Mellentin et al. [55], there are

indications that the efficacy of CET may increase, if combined with interventions specifically targeting coping skills in relation to craving responses, termed urge specific coping skills training (USCS).

Third Wave of Cognitive Behavioral Therapies

Third wave CBT is a group of emerging treatment methods that could be defined as an evolution and extension from the first wave of behavior therapy and the second wave of CBT [56]. These interventions share cognitive and behavioral theories but introduce an emphasis on how to relate to inner experiences, metacognitive perspectives and contextual factors compared to traditional CBT [57]. Typically, the third wave CBT umbrella construct includes mindfulness-based interventions (MBI) [58], Acceptance and commitment therapies (ACT) [59], Dialectical Behavioral Therapy (DBT) [60] and Metacognitive Therapy [61]. Shema therapy is occasionally included in reviews on third wave CBT and is thus included in this overview.

Among third wave CBT approaches, Mindfulness-based interventions (MBI) has the strongest empirical support. MBI originates from Buddhist practice [62] and includes awareness techniques which involve skills for the practice of being in the present and observing thoughts and emotions in a neutral and non-judgmental way [63]. As an intervention for AUD, mindfulness techniques typically aim to increase awareness of alcohol-related triggers and learned non-helpful behaviors. The aim is to target the individual's response to alcohol cravings and intrusive automatic alcohol-related thoughts in risk-situations, and thus reducing the risk of relapse into problematic drinking.

Several well-designed controlled trials have been conducted with MBI as only treatment or as add-on treatment for AUD [58, 64]. The most frequently evaluated forms of mindfulness-based treatments are Mindfulness-Based Relapse Prevention (MBRP) [65] and Mindfulness-Oriented Recovery Enhancement (MORE) [66]. Several recent systematic reviews and/or meta-analyses have been conducted. For example, Li et al. [67] found small-to-large effects of MBI on reducing substance use, craving and stress compared to alternative treatments (e.g., TAU, CBT, and support group). Corroborating this conclusion, Goldberg et al. found that mindfulness-based interventions were superior to no treatment ($d = 0.55$), minimal treatment ($d = 0.37$), non-specific active controls ($d = 0.35$), active controls ($d = 0.23$), and that MBT did not differ in outcome compared to evidence-based AUD treatments [68].

For the remaining treatment approaches included in third wave CBT, the empirical evidence for the treatment of AUD is weak. Although occasional studies exist, the empirical evidence for the efficacy of both ACT and ST for AUD is still limited [69], while DBT has mainly been evaluated in patients with substance use disorders and comorbid borderline personality disorder [70]. Metacognitive beliefs have been strongly connected to addictive behaviors and targeting these is suggested to be a potentially effective component in treatments for AUD [61]. To our knowledge,

Metacognitive therapy has only been tried in a systematic case series with promising results, but there are yet no randomized controlled trials among AUD patients performed for this method [61].

12-Step Based Treatments

In a strict sense, 12-step-based treatments may not be regarded as a behavioral treatment as they do not explicitly rest on a behavioral theory foundation. At the same time, 12-step based treatments involve components such as reinforcement of non-alcohol related behaviors and model learning through sponsorship and are thus included in this overview.

12-step based treatments contain components with the overall purpose to facilitate abstinence from alcohol, increase psychological well-being and quality of life, improve interpersonal skills and the ability to cope with stressful life events [71]. Three main forms of treatments can be distinguished in the abundant flora of 12-step based interventions.

The first form is peer-led Alcoholics Anonymous (AA) mutual-help groups with the goal of long-term, complete abstinence [72]. A typical group meeting lasts for 60 to 90 min, with the aim of providing members an opportunity to share their experiences of alcohol problems as well as their recovery process, and to support one another in practicing the different steps that constitute the 12-step program. Further, members are encouraged to obtain a 'sponsor'—a recovery mentor well-established in a sober lifestyle. A sponsor functions as a personal supporter and also enables model learning of functional coping strategies to members in their goal to abstain from alcohol. Members can attend meetings as often as they deem necessary. The efficacy of AA has been debated over the years. The evidence is inconsistent, due to a lack of well-designed RCT:s and systematic reviews conflicting on the degree of support for the efficacy of AA in maintaining long-term abstinence [73, 74].

Secondly, 12-step facilitation (TSF) is therapist-led program delivered in individual or group format with the aim of facilitating entry to and/or improving involvement in AA. Originally developed as one of the treatment arms in Project Match [20], TSF includes the basic components of the AA program as mentioned above, as well as interventions designed to link individuals to AA groups [75]. In the literature, TSF varies considerably in session length, format, and duration of treatment, ranging from a single session lasting a few minutes [76, 77] to multiple sessions delivered over several months [20]. In regard to treatment efficacy, it is difficult to disentangle the effect of TSF in itself from the effect of AA attendance among participants. A recent meta-analysis suggests that there is proof for the efficacy of TSF/AA in increasing abstinence rate compared to active comparisons (RR = 1.21) [74].

The third form of 12-step treatment consists of outpatient or residential treatment programs, extending for up to a month in time [78]. Programs may include additional programs for significant others as well as AA attendance. In many countries,

intensive treatment programs of this kind have historically represented one of the most common available treatment programs for people with AUD. However, the empirical evidence for this form of intervention is surprisingly weak, with a lack of RCT:s evaluating the efficacy of this intensive form of treatment [79, 80].

Behavioral Treatments in Conjunction with Pharmacotherapy for AUD

The question whether there is an additive treatment effect of a psychological treatment to pharmacological treatment for AUD has been debated since the new generation of pharmacotherapy for AUD emerged in the 1990's. In theory, there are good reasons to expect an additive effect when psychological treatment is given in conjunction with pharmacotherapy. The reduction in alcohol consumption due to anti-craving effects of, e.g., naltrexone, nalmefene and acamprosate could be further enhanced by components in a behavioral treatment, such as coping skills training. The results from controlled studies are, however, mixed.

Most trials investigating concomitant treatments have involved acamprosate and/or naltrexone in combination with a specific behavioral treatment and/or TAU [81, 82]. For acamprosate, no differences in outcome for an add-on behavioral treatment has been found in controlled trials [83–87]. In the case of naltrexone, results have been mixed. Anton et al. [88] found an additive effect of CBT and MET to naltrexone treatment. Similarly, Balldin et al. [89] found an additive effect of CBT in combination with naltrexone relative to naltrexone + TAU, placebo + naltrexone and TAU + CBT. However, other studies have shown no additive effect of behavioral treatment to naltrexone. For example, in the COMBINE study, by far the largest among studies carried out to address this question (n = 1383), no additive effects of any combination of pharmacological and psychological treatment were found [90].

In a meta-analysis covering CBT in combination with pharmacotherapy for addictive disorders in general (including several different types of substance use disorders), the authors concluded that there is empirical evidence supporting that adding specific behavioral treatment modalities to TAU + pharmacotherapy provides an additive effect on treatment outcome (post treatment and at follow-up), but that CBT did not show superior outcome compared to, e.g., MET and CM in this respect [91].

In a majority of these trials, extensive medical management programs have been carried out in conjunction to the pharmacological treatments. Also, study designs comprising frequent follow-up visits for research purposes are common, which altogether may dilute additional effects of behavioral interventions to pharmacotherapy [90]. Despite the mixed results, there is a consensus among most researchers within the field of AUD, that treatment approaches should include the opportunity of being treated with both behavioral and pharmacological treatment alternatives [92].

Behavioral Treatments for the Goal of Controlled Drinking

The question if individuals with AUD can achieve and maintain a non-abstinent treatment goal has been controversial for decades [9]. Controlled drinking (CD) generally refers to when an individual who previously exhibited out-of-control drinking returns to a stable pattern of alcohol consumption (below levels of hazardous drinking) with a significant reduction of the risks involved [93]. Extensive research has proven that controlled drinking (CD) may be a viable treatment goal for those with mild to moderate levels of AUD [15, 94, 95]. In 2021, Henssler and colleagues presented a systematic review and meta-analysis for the efficacy of psychological treatments with abstinence- and controlled drinking outcomes [94]. The analyses showed that there was no significant difference between groups for the proportion of individuals who had significant improvements regarding drinking reduction. Further, the analyses showed no significant differences between abstinence- and non-abstinence paradigms regarding the proportion of individuals who reached low risk drinking (OR = 1.32, 95% Confidence interval = 0.51–3.39). In addition, social functioning was improved equally between groups.

The behavioral treatment supporting a goal of CD which has gained the strongest empirical evidence is Behavioral Self-Control Training (BSCT), which is based on the principles of CBT [27]. BSCT involves goal setting, functional analysis of high-risk situations, and the acquisition of skills both for improved control over drinking rate and coping strategies to increase number of days with abstinence. In the only conducted meta-analysis of BSCT [96], 17 randomized controlled trials investigating the efficacy of BSCT were identified. This meta-analysis showed that BSCT was superior to no treatment (d = 0.94), to active comparators (d = 0.20), and compared to abstinence-oriented approaches (d = 0.28) although the latter did not reach a level of statistical significance.

To summarize, CD methods have shown to be equally efficacious to abstinence-oriented methods [97] for patients with mild to moderate levels of AUD. It has been suggested that an increased availability of treatments for the treatment goal of CD would lower the threshold for treatment seeking, which in turn would contribute to reducing the treatment gap for AUD [98]. There are indications of an increased acceptance towards these methods and non-abstinence outcomes among policy makers and in research. One example is the introduction of risk drinking levels as a structured assessment for the reduction of risk and improvement of functioning by the World Health Organization (WHO) [99] (Chap. 20). Still, the implementation of these methods is scarce.

Technology-Based Interventions

Rather than being a specific type of treatment in itself, technology-based interventions generally involve new approaches for providing existing treatments, e.g., BI or more extended treatments such as CBT or MET. Common forms of technology-based

interventions are by smartphone, computer or tablet via a dedicated program, application or through a web browser [100]. The development of digital interventions for AUD has been significant over the last 20 years as a consequence of the high global access rates to the Internet and smartphones. Along with this, people's preferences for seeking information on health-related issues have also changed drastically, as has the preference for seeking care for lifestyle-related problems. To illustrate this, a recent global survey showed that people in English speaking countries with moderate alcohol problems prefer getting help from web-based treatment applications [101]. Several benefits have been identified alongside with this development, e.g., a possibility to overcome barriers for seeking treatment e.g., perceived stigma and fear of negative consequences from being registered with having alcohol problems [102]. Web-based solutions may also increase access to treatment for people who have difficulties getting time off work, leave home or live in rural areas [103]. From a research perspective, technology-based interventions can facilitate rapid clinical innovation, facilitate recruitment to studies, achieve larger sample sizes and increase retention in clinical trials [104]. New technologies are also commonly used in current alcohol and drug prevention research with the aim of targeting at-risk groups, such as adolescents and young adults, e.g., in student environments. These interventions are typically brief, use text messages and information on negative consequences of alcohol consumption to affect excessive drinking behaviors [105].

Regarding the empirical evidence for the efficacy of technology-based interventions for AUD, several systematic reviews and meta-analyses have been published [106–108]. For example, Kiluk et al. investigated the efficacy of CBT-based digital interventions in contrast to either minimal intervention, to TAU only or as an add-on to TAU for studies including at-risk or heavy drinkers [108]. Digital interventions as a stand-alone treatment in contrast to a minimal treatment showed a positive (though small) effect (g = 0.20). Digital interventions in comparison to TAU showed no differences in efficacy between interventions. Digital interventions as an add-on intervention to TAU showed superiority compared to TAU only (g = 0.30).

In general, most technology-based interventions have targeted at-risk drinkers and/or heavy drinkers, with fewer studies including clinical population samples. Hence, from the meta-analyses referred to, it is difficult to draw any conclusions regarding the efficacy of technology-based interventions on more severely affected patients fulfilling criteria for AUD. However, recent studies have targeted patients with a confirmed AUD-diagnosis. For example, Johansson et a found only small differences in drinking outcomes in favor of Face-to-Face CBT compared to internet-delivered therapist supported CBT in a treatment seeking sample fulfilling DSM 5 criteria for AUD (mean number of AUD-criteria = 6.3) [109].

Lastly, a challenge in technology-based studies have been high attrition rates and low levels of engagement in treatment programs [110]. Efforts have been made to investigate how to address these challenges in future technology-based programs. In short, interventions with a personal contact, normative information or feedback on performance, tailored treatment content, multimedia delivery of content and follow-up reminders may increase retention to treatment and efficacy of treatment and increase follow-up rates in studies [111].

Mechanisms of Change in Behavioral Treatments

Mechanisms of behavior change (MOBC) in treatment have gained considerable attention during the last 20 years of research in the field of AUD [112]. One reason for this increased attention is the fact that controlled studies consistently have failed to find differences in outcomes between active treatments, even when these are outwardly different in treatment content. As an explanation for these negative findings, it has been proposed that efficacious interventions may comprise common factors for change in alcohol consumption. This idea stresses that causal relationships behind intervention and change can be more thoroughly understood by identifying the mechanisms, or what can be defined as the basis for the treatment effect, that is responsible for the change [112, 113]. A factor that has further contributed to the increased interest in MOBC is the development of more advanced statistical methods in recent years, providing researchers with more powerful tools to investigate common factors in treatments [114]. In the field of AUD treatment goal-direction, increasing self-efficacy, a structured way of working with problem behaviors and self-monitoring of consumption, as well as more specific aspects such as therapeutic alliance or technical skills by therapists have been suggested as general MOBC [115–117].

CBT models propose that drinking related coping skills and self-efficacy of abstinence are key features for positive outcomes in AUD treatment [112, 115]. These skills may be, e.g., handling urges, seeking adequate social support or engaging in positive non-alcohol related reinforcing activities, skills that also have been suggested to be MOBC in CBT treatment [115, 117]. Other identified MOBC are the quality of the therapeutic alliance, self-regulation, and emotional regulation [118, 119]. Altogether, there is some promising evidence supporting the suggested MOBC in CBT, but the collective empirical evidence is mixed, and more studies are warranted. One contributing factor to this may be limited study designs which generate lack of data that would allow for longitudinal statistical methods [115].

In the field of MI, studies on MOBC have focused on both technical and relational aspects of therapist skills to promote behavior change in patients [120]. The most well-studied MOBC in MI is change talk [121]. Change talk refers to the verbalization of arguments for change, and is an indicator of the patient's readiness for change according to theory of MI, as opposed to ambivalence. Consistent therapist techniques of MI, e.g., questions and complex reflections in combination with MI spirit, are theorized to evoke change- and commitment talk in session [122]. On the contrary, sustain talk; the verbalization of arguments for the maintenance of status quo has been found to predict negative outcomes [123, 124]. Further, it has been suggested that not merely frequency of change talk, but also the strength of change talk is an MOBC in MI [122, 125]. Still, although technical aspects of change have been more emphasized as MOBC, it is suggested that these technical skills are efficient primarily when relational skills, such as expressed empathy and MI-spirit is present in session [120].

Future Directions in Research on Behavioral Treatments

The future holds several challenges for the field of behavioral treatments for AUD. The first may be the obvious and well-known fact that only a small proportion of individuals with AUD receive treatment for their condition [98, 126, 127]. One of the major contributing factors—which specifically has to do with behavioral treatment—is the lack of access to non abstinence-oriented evidence-based treatments within addiction services [128, 129]. A promising approach that may be one key to increasing treatment seeking, is if a patient-centered approach were practiced to a higher degree within addiction care [130]. A patient-centered approach resembles the shared decision approach in which the patient and the therapist (e.g., a physician or psychologist) together decide on a treatment goal and suitable treatment, which should allow a goal of CD [131].

In order to increase access to treatment for addiction treatment such as short-term treatments within psychiatric care, primary—or occupational care [8, 132, 133], and through continued development of web- and telehealth alternatives should be viable options in addition to traditional treatment modalities in specialized addiction care. A larger variation of treatment options may increase the probability of individuals seeking treatment in this highly heterogenous population and thereby decrease the treatment gap.

One big challenge is the low level of implementation of novel treatment methods within addiction services. The lack of efficacious behavioral treatments calls for more research studies with longitudinal designs. Further, treatments need to be evaluated during their implementation in ecologically valid clinical settings, supported by structured models for implementation [134].

AUD is a highly heterogenous disorder, thus one treatment approach cannot be expected to be equally efficacious within the disorder. In the light of this, efforts have been made to explore phenotypes of addictive disorders, e.g., within a common theoretical framework; The Addictions Neuroclinical Assessment (ANA) [135] (Chap. 14). Within the ANA framework, three neuroscience-based dimensions have been identified that are associated with the development and maintenance of addictive disorders [136, 137] being negative emotionality, executive function, and incentive salience. The three domains may also serve as future treatment targets in potentially new interventions for AUD. Potentially, these three domains may be addressed as primary or secondary targets by behavioral treatments within the context of third wave CBT. We consider relatively new approaches, such as metacognitive therapy and mindfulness-based interventions as promising for these purposes [61, 138–141]. Also related to the ANA dimensions, the paradigm of cognitive bias modification training, in which alcohol related cues are paired with an avoidance reaction, have shown promising results [142, 143]. However, the preliminary results need to be verified in well-powered RCTs.

Conclusion

Behavioral treatments for AUD is a broad umbrella construct comprising treatments both for the given individual, as well as treatments involving a partner and significant others. Further, as shown in several reviews and meta-analyses, there is scientific evidence for the efficacy of these interventions, although there are also important knowledge gaps, e.g., regarding third wave treatment approaches.

When summarizing the research field of behavioral treatments, we conclude that the future challenge is not primarily the question of which treatments are efficacious, but instead how to increase availability to treatment, and perhaps most importantly—how to make treatment for AUD more attractive to those in need.

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Chapter 16
Approved, Promising, and Experimental Medications for Treatment of Alcohol Use Disorder

András H. Lékó, Mehdi Farokhnia, and Lorenzo Leggio

Abstract Alcohol use disorder (AUD) is a chronic relapsing disease with significant, medical, psychosocial, and economic sequelae. A limited number of medications are approved for AUD treatment. Disulfiram, naltrexone (oral and long-acting injectable), and acamprosate are approved by the US Food and Drug Administration (FDA), and used in many other countries, to treat patients with AUD. In addition to these medications, the European Medicines Agency (EMA) has also approved nalmefene. Currently approved medications for AUD are underutilized and have shown suboptimal effect sizes, mainly due to the heterogeneity of the target patient population. Therefore, further research is needed to expand the armamentarium of medications for AUD. In addition to the medications approved by the FDA and/or EMA, here we review other promising and experimental medications, specifically: topiramate and gabapentin, which are recommended for AUD treatment by practice guidelines of the American Psychiatric Association; baclofen which has approval in France; varenicline, doxazosin, and prazosin which have shown efficacy in some clinical trials; and some newer medications under investigation, such as zonisamide, ibudilast and samidorphan. Finally, we provide some final remarks regarding challenges and opportunities related to medication development in the AUD field.

Keywords Alcohol · Alcohol use disorder · Pharmacotherapy · Medication development · Clinical trial

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Introduction

Although we have a better understanding of the molecular and neurobiological basis of alcohol use disorder (AUD), a limited number of medications have been approved for AUD. In the United States, less than 9% of patients who need treatment for an AUD receive a single prescription of any FDA-approved pharmacotherapy, indicating that available medications are considerably underutilized [1]. The effect size of current pharmacotherapies for AUD is suboptimal, which is at least partially due to the heterogeneity of AUD, and subgroups of patients may respond differently to a certain medication. More recently, research has focused on new drug targets, with more personalized approaches, and alternative measures of efficacy, paving the way for potential new medications. Since not only abstinence, but also reductions in drinking can be clinically meaningful, the U.S. Food and Drug Administration (FDA) now accepts both abstinence and no heavy drinking days as a primary outcome for phase 3 trials of AUD pharmacotherapy, in which a heavy drinking day is 4/5 ≤ drinks in a day for women/men [2]. The European Medicines Agency (EMA) also includes non-abstinent endpoints, such as reductions in total alcohol consumption and heavy drinking days, in their guidelines [3]. Outcomes of clinical trials are diverse and may measure number of heavy drinking days per month, mean number of drinks per day, self-reported craving, quality of life, etc. A 2-level decrease in World Health Organization (WHO) risk levels (from very high to medium or from high to low) is also a strong indicator of reduction in alcohol consumption, providing another standardized measure of efficacy in AUD clinical trials [4], and also accepted by the EMA as a non-abstinent endpoint [3]. For an in-depth discussion of study outcomes, see Chap. 20.

In this chapter, we will briefly review: (1) medications which are already approved by FDA and/or EMA for AUD treatment; (2) medications with approval for other indications which have been found to be effective in clinical trials of AUD and are sometimes used off-label for this condition; and (3) promising new medications and drug targets with preliminary findings. For all medications reviewed in this chapter, we will concisely describe the neurobiological background and their mechanism(s) of action, as well as clinical results on their efficacy, side effects, and factors that may moderate their effects. This information is summarized in Table 16.1.

Table 16.1 Medications approved for treatment of AUD

Medication (dose)[a]	Putative mechanism of action	FDA/EMA Approval	Alcohol-related effects	Common side effects
Medications approved for treatment of AUD				
Disulfiram (125–500 mg/day)	Inhibition of alcohol metabolism (acetaldehyde dehydrogenase enzyme)	AUD	Tachycardia, headache, nausea, and vomiting after alcohol consumption. Medium effect in open-label trials, no effect in blind designs.	tachycardia, headache, nausea, vomiting
Acamprosate calcium (666 mg TID)	Putative antagonism of NMDA receptors	AUD	Improves probability of maintaining abstinence. Reduces the number of drinking days.	diarrhea
Naltrexone (50 mg/day)	Antagonism of μ-, κ-, and δ-opioid receptors	AUD Opioid use disorder (OUD)	Reduces craving, number of heavy drinking days, risk of return to drinking, and risk of binge drinking, and improves quality of life.	nausea, headache, dizziness, and sleep problems
Naltrexone LAI (380 mg/4 week) i.m.	Antagonism of μ-, κ-, and δ-opioid receptors	AUD OUD	Reduces number of drinking days and number of heavy drinking days. Compared to oral naltrexone, time to relapse is longer.	injection site related inflammation and infection
Nalmefene (18 m/dg)	Antagonism of μ- and δ-opioid receptors; partial agonism of κ-opioid receptor	Reversal of opioid overdose, Approved for AUD in Europe	Reduces number of heavy drinking days and total alcohol consumption.	nausea, vomiting, fatigue, insomnia, and dizziness

(continued)

Table 16.1 (continued)

Medication (dose)[a]	Putative mechanism of action	FDA/EMA Approval	Alcohol-related effects	Common side effects
Additional effective medications based on clinical trials				
Topiramate (100–300 mg/day)	Antagonism of glutamatergic AMPA and kainate receptors, and facilitation of GABA activity; blocks L-type calcium channels, reduces voltage-dependent sodium channel activity, inhibits carbonic anhydrase	Adjunct for partial and tonic-clonic seizures and migraines Topiramate ER in combination with phentermine for weight management in obesity	Reduces craving, percentage of heavy drinking days and drinks per drinking days.	paresthesia, taste abnormalities, anorexia, difficulties with concentration
Gabapentin (600–1800 mg/day)	Blocks voltage-gated calcium channels and enhances permeability of voltage-gated potassium channels. Indirectly modulates GABA receptors.	Adjunct for partial seizure and postherpetic neuralgia	Reduces the number of drinks and percentage of heavy drinking days, especially in patients with history of alcohol withdrawal symptoms.	dizziness, somnolence
Gabapentin enacarbil extended-release (GE-XR) (1200 mg/day)	Blocks voltage-gated calcium channels and enhances permeability of voltage-gated potassium channels. Indirectly modulates GABA receptors.	Restless leg syndrome and postherpetic neuralgia	Reduces the number of drinks and drinks per heavy drinking days in patient subgroup with higher baseline heavy-drinking days per week, impulsivity, and lower levels of anxiety, depression, and mood disturbances.	fatigue, dizziness, somnolence

Table 16.1 (continued)

Medication (dose)[a]	Putative mechanism of action	FDA/EMA Approval	Alcohol-related effects	Common side effects
Baclofen (30–80 mg/day)	Agonism of $GABA_B$ receptors	Muscle spasticity	Improves probability of maintaining abstinence; decreases craving. More effective in patients with alcohol-associated liver disease.	sedation, drowsiness, dizziness, headache
Varenicline (2 mg/day)	Partial agonism of α4β2 nicotinic acetylcholine receptors	Smoking cessation	Decreases craving in smokers and non-smokers. Reduces mean number of drinks in smokers. Better response in less-severe AUD, those with depressive symptoms, and males.	Nausea, abnormal dreams, constipation
Ondansetron (8 μg/kg/day)	Antagonism of 5-HT3 serotonin receptor	Nausea and vomiting	Reduces the amount and frequency of drinking in early-onset AUD.	headache, constipation
Prazosin/ Doxazosin (16 mg/day)	Antagonism of α1 receptors	Hypertension and benign prostatic hyperplasia	Reduces number of heavy drinking days and drinks per week. Better response in patients with higher blood pressure and AUD family density.	drowsiness, dizziness, fatigue

(continued)

Table 16.1 (continued)

Medication (dose)[a]	Putative mechanism of action	FDA/EMA Approval	Alcohol-related effects	Common side effects
Promising new medications				
Zonisamide (500 mg/day)	Blocks voltage-sensitive sodium channels and T-type calcium channels, enhances synaptic inhibition by facilitating GABAergic, dopaminergic, and serotonergic transmission. Indirectly attenuates glutamatergic transmission.	Adjunct treatment for epilepsy	Reduces number of heavy drinking days, drinks per week, and alcohol craving	drowsiness, difficulties with concentration, decreased appetite, abdominal pain
Mifepristone (600 mg/day)	Antagonism of glucocorticoid receptors	Pregnancy termination	Reduces alcohol craving.	nausea, vomiting, diarrhea
LY 2940094 (40 mg/day)	Antagonism of nociceptin (NOP) receptors	Not approved	Decreases percentage of heavy drinking days and increases percentage of abstinent days.	insomnia, anxiety, vomiting
Samidorphan (1–10 mg/day)	Antagonism of μ-opioid receptor, mixed agonism-antagonism of κ, and δ-opioid receptors	Not approved	Reduces craving and average daily alcohol consumption.	nausea, vomiting, somnolence, dry mouth, dizziness
Ibudilast (100 mg/day)	Inhibits phosphodiesterase-4 (PDE4) and −10 (PDE10), and macrophage migration inhibitory factor (MMIF). Reduces neuroinflammation and supports neurotrophin expression.	Not approved	Reduces craving, percentage of heavy drinking days and alcohol-cue elicited activation in the brain. Better response in patients with comorbid depressive symptoms.	headache, nausea

[a] Oral administration, unless otherwise specified

Approved Pharmacological Treatments

Only three drugs are currently approved by the FDA for use in AUD. **Disulfiram**, a drug targeting the metabolism of alcohol, was the first medication approved for the treatment of AUD in 1949. During alcohol metabolism, alcohol dehydrogenase first oxidizes alcohol into acetaldehyde, then acetaldehyde dehydrogenase (ALDH)

metabolizes acetaldehyde into acetate. Disulfiram irreversibly blocks the ALDH enzyme, leading to the accumulation of acetaldehyde after alcohol consumption. Acetaldehyde is a toxic metabolite, causing flushing, tachycardia, headache, nausea, and vomiting. These highly unpleasant symptoms occur within 5–15 min after alcohol intake, and deter alcohol consumption [5]. After consumption of large amounts of alcohol, the disulfiram-alcohol interaction may result in a medical emergency, with severe cardiovascular reactions [6]. Therefore, disulfiram should only be used in patients who are seeking to maintain total abstinence and is not useful if reducing drinking is the treatment goal. Its use should be limited to patients who are already abstinent and are seeking to maintain abstinence.

The efficacy of disulfiram largely depends on patient motivation and compliance, which is usually low (around 20%). This is mostly because disulfiram's mechanism of action is based on producing unpleasant disulfiram-alcohol reactions that deter alcohol drinking, without targeting key mechanisms that promote alcohol drinking, such as craving and negative affective states in the withdrawal phase [7]. Presumably related to this mechanism of action, a meta-analysis of disulfiram trials did not support the efficacy of this medication in general, but provides robust support for efficacy when disulfiram is administered under supervision [8]. Disulfiram, therefore, continues to have clinical utility, but primarily in special settings, e.g., when the patient has family support that allows for supervision of disulfiram administration, when the treatment goal is to achieve a certain duration of sobriety in order to evaluate potential psychiatric co-morbidities, etc.

The aversive reaction to disulfiram limits the design and interpretation of double-blind, placebo-controlled trials. If the placebo does not have an aversive effect upon intake of alcohol, it is easy for the patients and the researchers to become unblinded, while if it does, it may not be a real placebo. In open-label trials, disulfiram was found to have a medium effect size and was superior to acamprosate and naltrexone, in terms of no relapse and time to first heavy drinking day; by contrast, disulfiram was not superior to placebo in studies with a blind design [9]. The effect size variables were total abstinence, proportion of abstinent days to treatment days, mean days of alcohol use, no relapse, time to first heavy drinking day, or three or more weeks of consecutive abstinence.

There are reports of other putative pharmacodynamic actions of disulfiram. Among these, rodent studies have shown that inhibition of dopamine β-hydroxylase results in reduced synaptic norepinephrine and increased dopamine levels and thereby prevents drug-primed reinstatement [10, 11], but it is unclear whether these effects translate to primates [12]. More recently, it has been suggested that inhibition of the cytoplasmic FROUNT protein (a common regulator of chemokine receptor CCR2 and CCR5 signaling) by disulfiram leads to anxiolytic effects [13], but this effect has not been observed in humans at clinically used doses [14].

Acamprosate was approved for the treatment of AUD in 2004. Although its exact mechanism of action is unclear, evidence suggests that acamprosate counteracts increased glutamatergic activity at N-methyl-d-aspartic acid (NMDA) receptors that occurs during alcohol withdrawal, and may also have indirect effects on γ-aminobutyric acid (GABA) type A receptors [15]. The efficacy of acamprosate

has been assessed in numerous randomized, double-blind, placebo-controlled trials and meta-analyses. Large, multi-center, randomized, controlled trials (RCTs) in the United States and Germany did not observe a significant effect of acamprosate, neither on percentage of abstinent days, nor on time to return to heavy drinking [16, 17]. However, recent meta-analyses, including large numbers of studies, suggest that acamprosate significantly improves the probability of maintaining abstinence and lowers the number of dropouts during a 12-month follow-up, compared to placebo. Overall, 9% fewer patients returned to drinking, and percentage of drinking days decreased by 8.8% with acamprosate treatment, compared to placebo, while the number of drinks per drinking day did not change. These findings suggest that acamprosate is more useful in promoting abstinence and relapse prevention than in reducing alcohol intake [18–20]. The only adverse event which occurred more frequently with acamprosate than placebo was diarrhea. Other less common side effects may include nausea, vomiting, gastrointestinal discomfort, headache, and dizziness, although the causality is unclear. Acamprosate is not metabolized, which is advantageous for AUD patients with impaired liver function [21, 22]. However, as a disadvantage, acamprosate needs to be administered 3× times per day, which limits its clinical utility.

Naltrexone was approved for AUD treatment in 1994, and its long-acting injectable depot form in 2006. Naltrexone is a nonselective antagonist with high affinity for μ-, and lower affinity for κ- and δ-opioid receptors. It reduces mesolimbic opioidergic activity, and in turn modulates dopamine-mediated rewarding effects of alcohol [1]. A large-scale RCT and various meta-analyses have found naltrexone to reduce craving, number of heavy drinking days, return to drinking, and binge drinking, as well as improving quality of life, compared to placebo [16, 19, 20]. A meta-analysis of several RCTs showed that with oral naltrexone, 9% fewer patients returned to heavy drinking, and the number of drinking days decreased by 5.4%, compared to placebo [20]. An interesting approach is targeted naltrexone use for problematic drinkers, that is, taking the medication in anticipation of a potential high-risk drinking situation. This type of naltrexone administration effectively lowered proportion of patients relapsing to heavy drinking [23], mean drinks per day, and number of drinks per drinking day [24]. Common side effects include nausea, headache, dizziness, and sleep problems. Based on early reports, naltrexone was thought to be contraindicated in patients with liver disease. However, as naltrexone has been now used in clinical practice for a few decades, the risk of hepatotoxicity has proven to be less concerning, and naltrexone can be used, albeit with caution, in patients with non-advanced liver disease. Naltrexone should be discontinued in the event of signs/symptoms of acute hepatitis and should not be used in patients with more advanced liver disease [22, 25].

Depot naltrexone, similar to depot formulations of antipsychotics, is beneficial, because of two main reasons: (1) the monthly administration ensures compliance and (2) the extended release during a 4-week period reduces the risk of side effects and maintains a stable plasma level in the therapeutic range. Depot naltrexone effectively reduces the number of drinking days and heavy drinking days per month [20, 26]. Compared to oral naltrexone, time to relapse is longer and, as expected,

adherence is higher [27, 28]. A rare side effect of the depot formulation is injection site-related necrosis, while infection or inflammation are somewhat more common. Both oral and depot naltrexone are contraindicated in individuals who take opioid analgesics [29]. Of note, unlike oral naltrexone, injectable naltrexone does not undergo first-pass metabolism in the liver. Therefore, the systemic dose needed to achieve adequate central receptor occupancy is lower, which can be expected to reduce the potential risk for hepatotoxicity. One significant disadvantage of depot naltrexone is its significantly higher cost, compared to the other approved medications; this is likely the main reason why depot naltrexone is used less often compared to the other medications for AUD.

Another opioid medication, **nalmefene,** is approved for the treatment of AUD by EMA. Nalmefene has high and similar affinity for μ- and κ-opioid receptors, where it acts as an inverse agonist at the former, and a weak partial agonist at the latter, in both cases rendering it a functional antagonist of endogenous opioid peptide signaling. Its affinity for δ-opioid receptors is 25–200× lower, and thus not relevant in vivo at clinically used doses. Based on results of clinical trials and meta-analyses, nalmefene and naltrexone are more efficacious in reducing heavy drinking than maintaining abstinence [30]. Although a direct comparison of naltrexone and nalmefene has not been performed, preliminary evidence from a meta-analysis comparing placebo-controlled studies of the two drugs suggests a potential advantage of nalmefene over naltrexone in reducing quantity of drinking [31]. The effect of nalmefene on quantity of drinking is useful for harm reduction in patients who do not endorse abstinence as a treatment goal, by allowing targeted administration in anticipation of high-risk situations [32]. RCTs in Europe and Japan, including AUD patients with medium, high, or very high drinking risk levels, found that nalmefene effectively reduced the number of heavy drinking days and total alcohol consumption with a small effect size [33, 34]. It is, however, important to keep in mind that the number of studies and the clinical experience with nalmefene is significantly limited, compared to naltrexone, limiting the possibility of a direct comparison.

Additional Effective Medications Based on Clinical Trials

Although not approved by the FDA for the treatment of AUD, topiramate and gabapentin are approved for other indications, and their off-label use for AUD is recommended in the 2018 practice guidelines of American Psychiatric Association (APA). APA suggests that these two medications be offered to patients with moderate to severe AUD who have a goal of reducing alcohol consumption or achieving abstinence; are intolerant to or have not responded to naltrexone and acamprosate; and have no contraindications to the use of topiramate and gabapentin [35]. **Topiramate** is approved for the treatment of seizures and migraines. Furthermore, since 2012, it is also approved, in an extended-release formulation together with phentermine (a norephinephrine releasing agent), for chronic weight management in adults with overweight or obesity. Topiramate blocks L-type calcium channels, reduces

voltage-dependent sodium channel activity, and inhibits carbonic anhydrase. In addition, topiramate antagonizes glutamatergic AMPA and kainate receptors, and facilitates GABA activity through a nonbenzodiazepine site of $GABA_A$ receptor [36].

A placebo-controlled study shed light on topiramate's neurobiological mechanism of action in relation to AUD, showing attenuation of alcohol cue-elicited activation in the ventral striatum (VS) and orbitofrontal cortex (OFC). This inhibition leads to a reduction in alcohol craving, which appears to be the primary effect of topiramate [37, 38]. A first single-site large RCT and a subsequent larger, multisite, randomized controlled trial found 300 mg of daily topiramate being more efficacious than placebo in reducing percentage of heavy drinking days and drinks per drinking days from baseline to week 14 [39, 40], and this effect diminished rapidly after discontinuation [41]. Several clinical trials have supported the efficacy of topiramate [20, 42]. Furthermore, indirect comparisons by a network meta-analysis suggested topiramate being superior to nalmefene, naltrexone, and acamprosate on alcohol consumption outcomes, although the results of this network meta-analysis must be taken with caution and further examined by future larger RCTs directly comparing these drugs [42].

Topiramate treatment is frequently associated with side-effects, some of which may be clinically relevant and require dose reduction or discontinuation. As expected, topiramate often leads to a decrease in appetite and weight. Other common side effects include paresthesias, dizziness, sedation, taste abnormalities, and cognitive dysfunction (difficulties with concentration and short-term memory) [36, 39]. Gradual dose titration to 300 mg is suggested to minimize these adverse effects. Less common but notable side effects include metabolic acidosis, nephrolithiasis, and precipitation of acute angle-closure glaucoma [35].

Gabapentin is an oral anticonvulsant drug that is indicated for the treatment of epilepsy and postherpetic neuralgia, with doses ranging from 300 to 1800 mg/day. While being a structural analogue to GABA, gabapentin has no apparent activity at $GABA_A$ receptors, and some inconsistent results suggest activation of $GABA_B$ receptors. $GABA_B$ receptors are metabotropic, G-protein coupled receptors that can activate inward rectifying potassium channels and inhibit voltage-gated calcium channels both pre-, post-, and extrasynaptically, are also expressed by astrocytes, and can regulate both synaptic and extrasynaptic GABA levels. Gabapentin also blocks voltage-gated calcium channels containing the α2δ-1 subunit and enhances permeability of voltage-gated potassium channels, which collectively decreases neuronal excitability [43]. In rats, gabapentin normalizes the increased GABAergic neurotransmission in the central amygdala (CeA) that results from chronic alcohol exposure, and accordingly reduces alcohol self-administration in alcohol dependent rats upon intra-CeA infusion [44]. A human magnetic resonance spectroscopy study showed that, within the dorsal anterior cingulate cortex, gabapentin-related decrease in GABA levels and increase in glutamate levels are positively associated with percentage of abstinent days [45].

A single-center, outpatient, randomized, double-blind, placebo-controlled trial with gabapentin found that 28 days of treatment with 600 mg/day gabapentin

reduced both the number of drinks per day and percentage of heavy drinking days, and increased the percentage of abstinent days [46]. This study, together with initial human laboratory studies [47–49], were followed by a longer, placebo-controlled, randomized, double-blind trial with a larger sample size and two dosages of gabapentin: 900 or 1800 mg/day. Both doses of gabapentin were found to be safe, but only the higher dose was effective, as compared to placebo. Specifically, the higher dose of gabapentin increased the abstinence rate and reduced the number of drinks and heavy drinking days per week, as well as relapse-associated symptoms of insomnia, dysphoria, and craving [50]. Based on these studies, **gabapentin enacarbil extended-release (GE-XR)** 2 × 600 mg/day, the prodrug formulation of gabapentin, was tested in a large, multisite clinical trial. However, GE-XR group did not differ from placebo on the primary outcome, percentage of individuals with no heavy drinking days, and no clinical benefit was found for other drinking-related outcomes, alcohol craving, or sleep problems [51].

A recent meta-analysis of seven gabapentin RCTs showed evidence of benefit, with a medium effect size, on percentage of heavy drinking days [52]. Just like any other medication, it is conceivable that gabapentin may work for some patients with AUD and not others. Specifically, a recent study supports the efficacy of gabapentin, but only in patients with a history of alcohol withdrawal symptoms [53]. Also, a re-analysis of the abovementioned GE-XR clinical study revealed a subgroup of likely responders, where GE-XR was superior to placebo on percent of heavy drinking days and drinks per week. These likely responders were characterized by higher baseline heavy drinking days per week, cognitive and motor impulsivity, and lower levels of anxiety, depression, and mood disturbances [54]. Although potentially useful to identify responsive patients, predictors identified through such post-hoc analyses need to be examined and replicated prospectively to draw a conclusion.

Gabapentin appears to be safe and well tolerated in individuals with AUD. In the RCTs that have been carried out, no serious adverse events have been reported. Higher doses of gabapentin were associated with dizziness, somnolence, and peripheral edema, but these side effects were all transient and reversible [5].

Baclofen is an orthosteric $GABA_B$ receptor agonist approved by the FDA to reduce muscle spasticity associated with neurologic disorders such as multiple sclerosis. In France, baclofen also has regulatory approval since 2018 for the treatment of AUD at a maximum daily dosage of 80 mg. Originally, the maximum approved dosage was 300 mg/day, but it was reduced in 2017 to the current 80 mg/day, because of increased risk of hospitalization and fatalities at higher doses [55]. $GABA_B$ receptors are metabotropic receptors; activation of presynaptic $GABA_B$ receptors increases potassium conductance, which in turn decreases neuronal excitability and GABA release from nerve terminals, thereby reducing extracellular GABA concentration [56]. Increased GABAergic neurotransmission in the central amygdala seems to be a key player in the process and progression of alcohol dependence. In rats preferring alcohol over a high-value reward, a model that resembles some aspects of AUD in humans, GABA transporter expression in the CeA is lower compared to non-alcohol preferring rats, indicating decreased GABA clearance and increased extracellular GABA. Furthermore, knockdown of GABA transporter

reversed the preference of non-alcohol preferring rats from high-value reward to alcohol [57]. Baclofen may also suppress alcohol-stimulated dopamine release by activating $GABA_B$ receptors on dopaminergic axon terminals in the mesolimbic circuit and ventral tegmental area (VTA), thereby potentially normalizing the disturbed functional connectivity in the reward network [58, 59]. Baclofen dose-dependently decreases alcohol self-administration in both non-dependent and dependent rats, but dependent rats are more sensitive to baclofen's effects [60]. In Sardinian alcohol-preferring rats, baclofen reduced and delayed responding to alcohol reinforcement [61]. Findings from human fMRI studies showed that baclofen reduces alcohol-cue elicited neural activation in the OFC, amygdala, and VTA, and decreases cue-related functional connectivity between VTA and anterior cingulate cortex (ACC), as well as between VTA and medial prefrontal cortex (mPFC) [62, 63]. These effects on alcohol cue-related and reward networks are thought to disrupt the induction of alcohol intake induced by consuming a first drink (priming), as for example shown in a behavioral human laboratory study that did not obtain fMRI data [64]. Another proposed mechanism is that baclofen may act as a replacement medication, mainly through modulating the subjective and physiological responses to alcohol drinking. For example, in the context of alcohol use, in heavy drinking individuals with AUD, baclofen leads to feeling more intoxicated and more 'high'; and strengthens the decrease of heart rate and increase of diastolic blood pressure [65, 66].

After several promising preclinical studies [67] the first preliminary double-blind RCT, showed that low-dose (30 mg/day) baclofen can help maintain abstinence, by reducing craving and obsessive thoughts about alcohol [68]. The efficacy of high-dose (270 mg/day) baclofen was first described in a self-case report of a French physician in 2004. Baclofen reduced his craving and helped him remain abstinent for more than 9 months [69]. After these preliminary results, numerous clinical trials were conducted with mixed results [70–72]. A meta-analysis from 13 RCTs showed that baclofen was superior to placebo in lengthening time to lapse (drinking any amount of alcohol) and increasing the likelihood of abstinence [73]. Level of alcohol intake at study inclusion was found to be a moderator: higher daily alcohol use at baseline was associated with a larger baclofen treatment effect [74].

The alcohol-related outcomes targeted by baclofen seem to be dose-dependent, with higher doses decreasing craving, while lower doses promote abstinence [73, 75]. Of note, *Garbutt et al*. recently showed that higher dose of baclofen was superior to both placebo and lower dose, by reducing percent of heavy drinking days and increasing abstinent days during treatment period. They also revealed a potential role of sex in baclofen's effects: in women, lower dose showed superiority to placebo, while higher dose did not, but the opposite was observed in men [76]. It has been suggested that baclofen may be more effective in patients with higher severity of AUD who also have comorbid alcohol-associated liver disease (ALD) [21, 22, 72]. In fact, while a multi-site study in patients with hepatitis C, which was conducted at the US Department of Veterans Affairs (VA), did not indicate a significant difference between baclofen and placebo [77], a first RCT in patients with AUD and cirrhosis [78], and a subsequent multisite, double-blind RCT in patients with AUD

and ALD, found that baclofen was more effective compared to placebo in maintaining abstinence among patients with liver problems [79]. This observation and its lack of hepatotoxicity make baclofen an attractive medication for patients with comorbid AUD and ALD.

A meta-analysis, including 12 RCTs, found that baclofen increases the occurrence of the following adverse events compared to placebo: dizziness (RR 2.16), sedation/drowsiness (RR 1.48), paresthesia (RR 4.28), and muscle spasm/rigidity (RR 1.94) [80]. Other less common side effects include headache, confusion, excessive perspiration, itching or pruritus, abnormal muscle movements, numbness, and slurred speech [77].

An international consensus statement reconsidering multiple meta-analyses [73, 81, 82] pointed out the lack of consistent results in support of baclofen's efficacy, although it also emphasized its utility for specific sub-populations, e.g., patients with severe AUD and ALD [72]. A different yet related arena in medication development for AUD are positive allosteric modulators of the $GABA_B$ receptor ($GABA_B$ PAMs), which exert no intrinsic activity and only potentiate signaling when the endogenous ligand is also present. In rodent experiments, $GABA_B$ PAMs suppress excessive drinking, binge-like drinking, operant alcohol self-administration, reinstatement of alcohol seeking, stress- and cue-induced relapse-like drinking, and *c-Fos* expression in brain regions involved in stress-induced relapse [83–85]. $GABA_B$ PAMs were effective in suppressing alcohol-related outcomes at lower doses than those inducing sedation, an important aspect in terms of the potential for human translation, given the wider therapeutic window that this pharmacological approach offers [85].

Although **varenicline** is approved by the FDA only to help with smoking cessation, it is sometimes prescribed off-label for AUD, based on its promising efficacy in reducing alcohol craving and consumption. Varenicline is a partial nicotinic agonist, with high affinity and selectivity for α4β2 nicotinic acetylcholine receptors. It activates the receptor with about half of nicotine's maximal efficacy, while at the same time preventing the binding of nicotine [86]. This partial agonism mechanism helps maintain dopaminergic tone in the absence of nicotine, while reducing nicotine-induced dopamine release, therefore promoting smoking cessation. A similar mechanism of action was hypothesized against alcohol positive reinforcement and craving. However, in a recent preclinical experiment, this mechanism seemed to be specific to nicotine, and not directly applicable to alcohol, morphine, or natural rewards [87]. Another study in rats found that varenicline microinfusions into the accumbens, but not into the VTA, reduced context-induced relapse to alcohol-seeking [88].

Attenuation of alcohol's reinforcing effects and craving after varenicline pretreatment (2 mg/day for 7 days) was shown in an RCT examining 2-h alcohol self-administration in non-alcohol-dependent, heavy-drinking smokers, where reduction of alcohol intake was also observed [89]. The first multisite RCT established that varenicline (2 mg/day for 13 weeks) decreased percent of heavy drinking days, drinks per drinking days, and alcohol craving [90]. AUD severity and age were found to be significant moderators: better treatment response was observed in older

patients and those with less severe AUD at baseline [91, 92]. Patients experienced mild to moderate adverse events; nausea, abnormal dreams, and constipation had significantly higher rates in the varenicline than the placebo group [90]. Later, a multisite RCT, conducted in Sweden, showed no effect of varenicline over placebo on the proportion of self-reported heavy drinking days, but the highly specific objective biomarker of alcohol consumption, phosphatidylethanol (PEth) demonstrated a significant reduction in alcohol use, and self-reported cravings were also reduced by varenicline [93]. A recent meta-analysis of 10 studies, with an aggregate sample of 731 participants, suggested that varenicline significantly decreases alcohol craving in patients with AUD, but did not support a significant effect on drinking-related outcomes (percentage of heavy-drinking days, number of drinks per drinking day, or percentage of abstinent days) [94]. In conclusion, varenicline has been consistently found to reduce alcohol craving, but its effects on drinking-related outcomes remain unclear and most likely limited to a sub-population (e.g., people with AUD who are also smokers).

Varenicline can also improve working memory in heavy drinking individuals [95] and may be more effective in patients with comorbid depressive symptoms [96]. Numerous human studies have investigated the efficacy of varenicline in patients with comorbid alcohol and nicotine dependence. Varenicline both promoted smoking abstinence and reduced mean number of drinks per drinking day in a clinical trial [97]. Varenicline's efficacy does not seem to differ between smokers and non-smokers, but in smokers, varenicline improved drinking-related outcomes only among those who reduced their cigarette consumption [91, 92]. Sex appears to moderate varenicline's effects in this population with alcohol-nicotine dependence comorbidity: in a different phase 2 RCT, varenicline treatment (2 mg/day for 16 weeks) resulted in decreased heavy drinking only among men, whereas smoking abstinence was prolonged in the overall sample [98]. A recent clinical trial compared varenicline to varenicline-naltrexone treatment for smoking cessation and drinking reduction among heavy-drinking smokers. Varenicline alone was significantly more effective in smoking cessation. Both treatments reduced drinks per drinking day, compared to baseline. Although the main medication effect did not meet the study significance threshold, it favored the varenicline plus naltrexone condition across the entire trial [99].

The noradrenergic system plays an important role in the neurobiology of alcohol reinforcement and relapse. Alcohol withdrawal and stress responses lead to excessive noradrenergic activation, producing anxiety and hyperarousal, which is strongly linked to alcohol seeking behavior and relapse. Not only withdrawal, but also acute alcohol administration increases norepinephrine release [100]. Inhibition of norepinephrine synthesis was shown to reduce alcohol intake in rats and the euphoric effects of alcohol in humans [101, 102]. The α1 adrenergic receptor antagonists **prazosin and doxazosin** have been found to be potential medications to treat AUD. Their suggested mechanism of action is blocking α1 receptors in key areas involved in the transition to dependence such as the CeA. The expression of these α1 receptors is elevated in AUD and promotes GABA release associated with

increased alcohol intake [103]. In preclinical experiments, prazosin reduced alcohol intake in dependent rats [104].

In humans, a 12-week RCT found that prazosin (16 mg/day) effectively reduced alcohol consumption, expressed by number of drinks and number of heavy drinking days per week. Drowsiness and edema were reported more often in the prazosin group, compared to placebo [105]. In another clinical trial, greater rates of reduction in drinks per week was observed under prazosin in patients with high, but not low, diastolic blood pressure, which might be associated with lower tolerability of the drug in the latter subgroup [106]. Prazosin also showed efficacy in reducing stress-induced alcohol craving and anxiety during early abstinence [107, 108].

Severity of alcohol withdrawal, often measured by the Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar), appears to be a significant moderator of the treatment response to prazosin. Prazosin reduced percent of heavy drinking days and percent of drinking days in patients with higher CIWA-Ar scores, whereas no such benefit of prazosin was observed in patients with low or no withdrawal symptoms [109]. Doxazosin, another α1-antagonist, has a longer half-life and a more favorable pharmacokinetic profile than prazosin. A 10-week RCT showed that doxazosin (16 mg/day) did not reduce drinks per week and number of heavy drinking days per week in the full sample, but two moderators were found to influence doxazosin's effects: patients with higher family history density of alcohol problems (*a priori* moderator) or with higher baseline blood pressure (moderator selected *post hoc*) had a better response to doxazosin. As for side effects, dizziness, depression, urinating troubles, and headache were more frequent in the doxazosin than the placebo group [110, 111].

Promising New Medications

Like topiramate, **zonisamide** is also approved by the FDA as an adjunct treatment for epilepsy. Zonisamide blocks voltage-sensitive sodium channels and T-type calcium channels, and enhances synaptic inhibition by facilitating GABAergic, dopaminergic, and serotonergic transmission and indirectly attenuating glutamatergic transmission [112]. In an initial placebo-controlled human laboratory study in people with hazardous alcohol use (during 90 days prior to study entry: more than 3/4 drinks per day on any day or more than 7/14 drinks per week for women/men, respectively), a single-dose of 100 mg zonisamide reduced alcohol craving during a priming drink challenge session and alcohol intake during two consecutive 1-h self-administration segments [113]. In a 12-week RCT, zonisamide (500 mg/day) was found to be effective in decreasing the number of heavy drinking days, drinks per week, and alcohol craving [114]. Zonisamide showed an effect size similar to topiramate in reduction of alcohol consumption (percent of drinking days per week, drinks per day, and heavy drinking days per week), while having a more favorable side effect profile. Zonisamide's cognitive side effects (modestly reducing verbal

fluency and working memory) are similar to those of topiramate, but unlike topiramate, zonisamide does not cause irritability, paresthesia, or erectile dysfunction [115].

The nociceptin (NOP) receptor belongs to the opioid receptor family and is widely expressed throughout the mesolimbic reward pathway. Targeting this system modulated alcohol intake in preclinical experiments, oddly enough both when agonists and antagonists were used. Central NOP administration prevented stress- or reinforcement-induced alcohol-seeking behavior, and reduced alcohol self-administration [116, 117]. Systematic application of a brain-penetrant NOP receptor agonist reduced alcohol intake and withdrawal-induced anxiety and prevented cue- and stress-induced relapse [118]. Paradoxically, the NOP receptor antagonist LY2817412 also dose-dependently reduced voluntary alcohol intake and cue-induced relapse in rats; CeA and VTA were identified as target regions for this effect in the brain [119, 120]. Another **NOP antagonist, LY 2940094**, also attenuated alcohol self-administration, alcohol-seeking behaviors, and alcohol-induced dopaminergic transmission in the brain reward pathways [121].

The reason why both NOP receptor antagonists and agonists have produced the same effect on alcohol intake remained unclear. It was hypothesized that agonists may produce desensitization and internalization of NOP receptors, therefore reducing expression of NOP receptors on the cell membrane [121]. An RCT evaluating the efficacy of the NOP antagonist LY 2940094 (40 mg/day, for 8 weeks) found that it decreased the percentage of heavy drinking days and increased percentage of abstinent days, compared to placebo. These promising self-reported outcomes were associated with significant reduction of gamma-glutamyl transferase (GGT) serum levels, which is an indirect objective biomarker of alcohol use. The frequency of side effects (mainly insomnia, anxiety, and vomiting) was not different between the active drug and placebo groups [122].

Samidorphan also targets the opioid system. It is a μ-opioid receptor antagonist and a mixed agonist-antagonist of κ- and δ-opioid receptors [123]. Samidorphan is FDA approved for the treatment of psychotic or bipolar illness, in a formulation together with the antipsychotic olanzapine, a combined pharmacological approach shown to prevent some of the weight gain associated with olanzapine use [124]. Samidorphan has also been formulated together with buprenorphine, and this combined formulation has shown some promise for the treatment of depression, presumably through the κ-opioid receptor blockade by buprenorphine, while preventing the addiction-liability of this compound due to samidorphan's μ-antagonistic effects [125]. Compared to naltrexone, samidorphan has a longer half-life, increased oral bioavailability due to decreased hepatic metabolism, and higher binding affinity to μ-opioid receptors. In a 12-week RCT, samidorphan treatment (1, 2.5, 10 mg/day), compared to placebo, was associated with a more pronounced reduction of alcohol craving and average daily alcohol consumption. The latter was measured by percentage of patients who achieved ≥2-category downshift in World Health Organization (WHO) risk levels of drinking [126]. However, development of samidorphan as a standalone medication has been discontinued.

Chronic alcohol use disrupts the hypothalamic-pituitary-adrenal (HPA) axis, which leads to deficits in reward function that contribute to anhedonia/dysphoria and craving in AUD. Alcohol intake acutely elevates cortisol levels. While most sites, such as the pituitary, hypothalamus, and hippocampus are under glucocorticoid-receptor (GR) mediated negative feedback control by cortisol, frequent HPA axis activation leads to a GR mediated increase of corticotropin releasing factor (CRF) signaling in the CeA and BNST [127, 128]. In the CeA, CRF promotes withdrawal-induced alcohol drinking, escalation of alcohol intake during transition to dependence, and binge-like drinking. Given that alcohol use results in elevated GR activation and, in turn, increases pro-drinking CRF signaling in the CeA, antagonism of the GR appears to be a promising approach for AUD treatment [126]. Both systemic and intra-CeA administration of GR antagonists, **mifepristone** and the more selective CORT113176, reduced alcohol intake in dependent rats. Based on these preclinical results, a randomized, double-blind, placebo-controlled, human laboratory study was conducted in non-treatment seekers, where mifepristone (600 mg/day for 7 days) reduced cue-elicited alcohol craving and the number of drinks per day, both during treatment and 1 week follow-up period after study drug discontinuation. Its side effects are mainly gastrointestinal, such as nausea, vomiting, diarrhea, but no adverse event was reported in the human laboratory study. The findings suggest that brief (1 week) treatment with mifepristone immediately following acute withdrawal may be beneficial in conjunction with a course of psychosocial treatment [129].

Prolonged consumption of alcohol leads to a systemic inflammatory state with increased inflammation in the CNS and elevated peripheral levels of proinflammatory cytokines. In the brain, microglia cells show signs of activation *in vivo* in response to chronic alcohol use, and *in vitro*, alcohol administration results in elevated cytokine levels in microglia cultures [130]. Peripheral levels of pro-inflammatory cytokines positively correlate with craving and are increased in response to acute alcohol administration, while anti-inflammatory cytokine levels show opposite changes [131, 132]. In turn, alcohol-induced neuroinflammation contributes to increased alcohol-seeking behavior, neurotoxicity [133], cognitive and behavioral impairment, and brain damage [134]. **Ibudilast** inhibits phosphodiesterase-4 (PDE-4), −10 (PDE-10), and macrophage migration inhibitory factor (MMIF), thereby reducing neuroinflammation and supporting neurotrophin expression. Ibudilast reduced alcohol intake in three different preclinical models [135]. A randomized, crossover, double-blind, placebo-controlled human laboratory study then showed that ibudilast treatment (100 mg/day, for 7 days) reduced tonic craving for alcohol and improved mood during stress exposure. However, compared to placebo, ibudilast did not modify subjective effects of alcohol, such as alcohol cue-induced craving, alcohol-induced sedation, or positive mood increased by alcohol infusion. A secondary analysis suggested that among individuals with more depressive symptoms, ibudilast attenuated the stimulant and mood-altering effects of alcohol [136]. In a later outpatient RCT, ibudilast (100 mg/day, for 14 days), compared to placebo, reduced percentage of heavy drinking days and attenuated alcohol cue-elicited activation in the VS [137]. A secondary analysis of this study re-evaluated

the results of the earlier human laboratory study, describing a decreased alcohol-cue induced craving with ibudilast [138]. A recent neuroimaging study established that ibudilast reduces not only VS activation in response to alcohol cues, but also alcohol cue-elicited functional connectivity between VS-OFC and VS-ACC. Furthermore, both abovementioned measures of VS activation and functional connectivity correlated positively with the number of drinks per drinking day [139].

Numerous other compounds and pharmacological targets have been tested during the past years. Some showed promise in preclinical research but failed in translation to humans. Examples of medications/targets which seem promising based on preclinical studies and preliminary human results include (but are not limited to) vasopressin receptor V_{1b} antagonism [140–142], N-acetylcysteine [143–146], spironolactone [147–151], oxytocin [152–156], ghrelin system [157–162], and glucagon-like peptide-1 analogues [163–168].

Final Remarks

At the time of writing this chapter, only three medications are approved by the FDA for AUD. These are underutilized and have limited efficacy. Therefore, discovery of novel and more effective pharmacological treatments is a high priority. The fact that no new medications have been approved in the past two decades is frustrating and calls into question whether the entire approach currently used in the field should be revised or even challenged. It certainly does not help that the private sector does not invest in medication development for AUD. This is a serious problem that puts medication development in this field at a major disadvantage compared to other fields of biomedical research, despite the fact that AUD is among the leading causes of mortality and morbidity worldwide. There is however also cause for optimism. Rapidly developing basic neuroscience and neuroimaging have started to discover new mechanisms behind development and maintenance of addiction, including AUD. These advances in neuroscience continue to identify novel targets to explore preclinically and then clinically.

In this chapter, we reviewed the approved and additional clinically tested medications from the last few decades. We can conclude that none of these medications work as a general remedy for all patients with AUD. Hence, personalized medicine approaches are key. AUD is a heterogenous medical disorder, and different medications may work best or exclusively for specific subgroups of patients. Comorbidity (e.g., gabapentin in patients with history and symptoms of withdrawal; baclofen in ALD; varenicline, ibudilast in depressive symptoms; prazosin/doxazosin in patients with family history of AUD, higher blood pressure and more withdrawal symptoms) and severity of AUD (e.g., varenicline in less, and baclofen in more severe AUD) are mere examples of potential moderators of pharmacological treatment effects.

Different RCTs have used different outcomes to define efficacy. Standardization of outcomes is critical, especially from a regulatory standpoint, but these standards

need to evolve. Reduction in WHO drinking risk level seems to be a useful outcome, since growing evidence shows that non-abstinence-oriented outcomes may be beneficial. On the other hand, it is important to match the medication with the treatment goal. For example, acamprosate and baclofen seem to work better in maintaining abstinence, while naltrexone, topiramate, and varenicline seem to be more effective to reduce heavy alcohol use.

Continued efforts and new frontiers in medication development for AUD are critical to move the field forward in the years to come [169]. This chapter attempts to offer a broad spectrum of approved and non-approved, but promising, pharmacotherapies to the readers, no matter if the reader is a student, physician, scientist, patient, or someone simply interested in learning more about AUD.

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Chapter 17
Comorbidity of Alcohol Use Disorders with Substance Use Disorders and Psychiatric Disorders

Justyna Zaorska and Marcin Wojnar

Abstract Prevalence of psychiatric disorders in patients with alcohol use disorder (AUD) is significantly higher than in individuals without AUD diagnosis. For this reason, it is important to screen all patients with AUD for other mental disorders as well as to evaluate alcohol use in patients with psychiatric disorders. Dual diagnosis implicates more severe course of comorbid disorders, poorer treatment outcomes, higher risk of suicide, and worse social functioning. There are several explanations proposed for high comorbidity of AUD and mental disorders. Among them are shared neurobiological mechanisms and joint genetic background, self-medication hypothesis or substance-induced mental disorders. Providing health care for individuals with such comorbidity may be particularly challenging. Treatment of AUD, including pharmacotherapy, should be performed simultaneously with treatment of coexisting mental disorder, including use of antidepressants, mood-stabilizers or antipsychotics. First-line treatment for depression comorbid with AUD requires SSRIs, SNRIs or mirtazapine. For bipolar disorder and AUD, lithium in monotherapy or combined with valproate should be considered first, while in psychotic disorders and AUD, clozapine and long-acting injectable antipsychotics may be recommended.

Keywords Comorbidity · Alcohol use disorder · Psychiatric disorder · Substance use disorder · Depression · Neurobiological mechanisms · Neurotransmission · Self-medication · Antidepressants

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Epidemiology

Alcohol use disorder (AUD) is a highly prevalent disorder worldwide and has a high comorbidity with other mental disorders. In the US National Epidemiologic Survey on Alcohol and Related Conditions Wave III (NESARC-III) carried out in 2012–2013, the 12-month prevalence of DSM-5-defined AUD was 13.9%, while lifetime prevalence was 29.1% [1]. In the National Survey on Drug Use and Health 2020 [2] among people aged 12 or older, 10.2% had past year AUD with the highest prevalence in adults aged 18–25 (15.6%) followed by adults aged 26 or older (10.3%) and by adolescents aged 12–17 (2.8%).

General comorbidity in AUD has been the subject of several epidemiological studies. In the Epidemiological Catchment Area (ECA) Study on the US population, among those with AUD, 36.6% had a comorbid mental disorder and 21.5% had another drug use disorder (DUD) in their lifetime. Prevalence of psychiatric disorders in the group of patients with AUD was significantly higher than in the group without AUD diagnosis ($p < 0.001$), and equaled 3.8% for schizophrenia, 13.4% for any affective disorder, 19.4% for anxiety disorder and 14.3% for antisocial personality disorder (ASPD) [3]. National Comorbidity Survey (NCS) demonstrated that in last 12 months more than 25% of individuals with AUD met criteria for a major depressive episode, and 37% of them met criteria for anxiety disorder [4, 5]. AUD was associated with over two times higher incidence of post-traumatic stress disorder (PTSD), almost four times higher incidence of major depressive disorder (MDD), over four times higher incidence of general anxiety disorder and over six times higher incidence of bipolar disorder (BD) [5, 6]. More recent data from the Wave III of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC III) revealed significant associations between DSM-5 AUD in the past 12 months and increased odds of many psychiatric disorders. Particularly, past year AUD increased the probability of any DUD (without nicotine) with an odds ratio (OR) of 3.3, MDD with OR 1.2, ASPD with OR 1.6 and borderline personality disorder (BPD) with OR 1.9. Lifetime prevalence of AUD increased the odds of any anxiety disorder by 1.3. All associations were controlled for sociodemographic characteristics and other disorders [1].

A relationship between psychiatric morbidity and alcohol use has been a subject of research as well. In Sanchez-Pena study [7], the percentage of patients meeting criteria for concurrent AUD was: 30% for individuals with personality disorder (PD), 24% in subjects with adjustment disorder, 22% with depressive disorder, 18% with anxiety disorder, 11% with schizophrenia, and 9% with BD. The National Survey on Drug Use and Health 2020 [2] demonstrated that adults with any mental illness in the past year were more likely than adults with no mental disorder to be past month binge alcohol users (28.5% vs. 22.8%) and heavy alcohol users (8.8% vs. 6.4%) (Table 17.1).

In epidemiological studies on comorbidity of MDD and AUD, lifetime prevalence of those diagnoses varied from 27% to 40% [8–10]. Past year prevalence was investigated in the National Survey on Drug Use and Health 2020 [2]. In that study,

Table 17.1 Prevalence of alcohol use disorder in subjects with psychiatric disorders—data sourced from the results of ECA study [3]

General population	6%
Schizophrenia	34%
Major depressive disorder	17%
Bipolar disorder	44%
Anxiety disorders	18%
Antisocial personality disorder	74%

adults with past year major depressive episode were more likely to be dependent on or to abuse alcohol than those without major depressive episode (17.0% vs. 7.0%). Metanalysis by Hunt et al. indicates that in patients with bipolar disorder prevalence of AUD was higher than for any other substance use disorder (SUD) and reached 42% [11].

In patients with PD, according to metanalysis of Guy and colleagues [12], lifetime prevalence of AUD in persons with ASPD was 77%, in individuals with BPD was 52%, and 39% in subjects with other PD. Use of alcohol by patients with Attention Deficit Hyperactive Disorder (ADHD) is also a common occurrence. According to different studies, prevalence of AUD in this group of patients goes up to 43% of adults with ADHD [13].

In conclusion, co-occurrence of AUD and psychiatric disorders is commonly observed, and the comorbidity seems to be bidirectional—the prevalence of major psychiatric disorders in individuals with AUD is higher than in the general population, and the prevalence of AUD in patients with psychiatric disorders is higher than in the general population. The strongest association seems to be with ASPD and BD. Importantly, both externalizing and internalizing conditions seem to be associated with AUD, which may indicate different reasons and motivations for alcohol use.

Significance of Psychiatric Comorbidity

Psychiatric comorbidity in AUD has a negative impact on the course and prognosis of both AUD and comorbid psychiatric disorders, and on patients' general functioning. Patients with MDD and AUD have more comorbid mental disorders and more suicidal behaviors than AUD patients without comorbidity. Patients with depression and AUD also have worse treatment outcomes—they spend more time being depressed, have lower global functioning and higher life dissatisfaction [14, 15]. Results of AUD treatment in individuals with comorbid mood or anxiety disorder appear to be poorer than in AUD alone. For example, in a study of Kushner et al. [16], persons with mood or anxiety disorder were more likely to return to drinking within 4 months following treatment. On the other hand, recent review of studies on patients with Social Anxiety Disorder (SAD) and AUD supports the thesis that this comorbidity worsens treatment compliance, but it also indicates that there is not enough data to consistently state that SAD comorbidity impacts treatment response in patients with AUD [17].

Table 17.2 Impact of psychiatric comorbidity in AUD [20, 21]

• More severe course of coexisting disorders
• Higher risk of attempted and completed suicide
• Poor prognosis and worse treatment outcomes
• Lower compliance
• Lower treatment retention
• Higher risk of relapse
• More somatic disorders
• Lower quality of life
• Worse family, social, and occupational functioning
• More conflicts with law
• Increased mortality

In individuals with BPD, co-occurrence of AUD was associated with higher unemployment, poor school performance and promiscuity [18]. According to Margolese et al. study, patients with schizophrenia and AUD suffer from greater severity of psychopathology [19]. Other studies on schizophrenia and AUD suggest that individuals with such comorbidity have neurocognitive dysfunctions, which entail deterioration of working memory, episodic memory, and verbal learning (Table 17.2) [20, 22, 23].

Relationships Between AUD and Coexisting Mental Disorders

Several explanations for the co-occurrence of AUD/SUD and mental disorders have been proposed. They include shared neurobiological mechanisms and risk factors, vulnerability for a second disorder caused by the primary disorder, "self-medication" hypothesis, substance-induced mental disorders (especially depression) and the impact of social problems caused by AUD/SUD on mental health.

Neurobiological Mechanisms

Physiologically, under the influence of stress, signals from the centers in the limbic system reach the hypothalamus via serotonin and noradrenaline pathways. As a result, corticotropin-releasing hormone (CRH; also referred to as "factor", CRF) is released, which in turn stimulates the pituitary gland to release corticotropin (ACTH). Increase of ACTH concentration causes the adrenal glands to produce cortisol. As a result of alcohol intoxication, there is an additional release of CRH in the extended amygdala, a cytoarchitectonically related set of structures that include

central amygdala, nucleus accumbens shell, sublenticular substantia innominate and bed nucleus of stria terminalis), and activation of the hypothalamic-pituitary-adrenal (HPA) axis cascade. This demonstrates one of the mechanisms by which alcohol increases stress hormone levels. Sustained and excessive release of stress hormones results in a progressive adaptation of the central nervous system (CNS), and the emergence of a new, allostatic state, thought to be one of the important components of addiction [24]. Although the exact role of the HPA axis in the pathophysiology of mood and anxiety disorders remains unclear, its involvement has been well documented for many years. For instance, chronic stress and the accompanying increase in stress hormone levels (CRH, ACTH, cortisol) tend to damage some brain structures (e.g., hippocampus) and contribute to the development of anxiety and depressive symptoms [25].

Alcohol intoxication and alcohol withdrawal are conditions where neurotransmission homeostasis is severely disturbed. In general, acute alcohol administration increases gamma-aminobutyric (GABA), dopaminergic, opioidergic, and serotonergic (5-HT) neutrotransmission, and decreases glutamatergic neurotransmission. The stimulating effects of alcohol also relate to changes in brain derived neurotrophic factor (BDNF), which promotes activation of its high affinity receptor, Tropomyosin receptor kinase B (TrkB), and subsequent signaling pathways. Alcohol withdrawal in turn is associated with attenuated GABA, 5-HT and DA neurotransmission, and increased CRH concentration, with a major imbalance between inhibitory GABAergic and excitatory glutamatergic neurotransmission. Merlo Pich and colleagues [26] found that dialysate CRH levels in the amygdala are elevated during alcohol withdrawal in rat brain, and dialysate DA and 5-HT levels in the Nucleus Accumbens are decreased. The directionality of all these neurotransmitter changes is consistent with hypothesized changes in neurotransmission in depression [27, 28].

It is also worth mentioning that alcohol consumption may decrease the availability of tryptophan (Trp) to the brain as it activates liver Trp pyrrolase, the rate-limiting enzyme of the kynurenine pathway of Trp degradation. This may be a mechanism through which alcohol intake may lead to depletion of 5-HT synthesis and, consequently, to altered regulation of emotions, mood, arousal, sleep, eating, pain, aggression, and impulsivity [29–31]. In animal studies, reduced serotonin neurotransmission has been demonstrated to be associated with relapse [32], while in both human and animal studies dysregulation of noradrenergic transmission (as a response to arousal, stress, and withdrawal) was indicated as an important component of AUD pathophysiology [32]. Reduction in dopamine release is considered to be associated with dysphoria, malaise and depression during alcohol withdrawal [30]. In addition, alcohol intoxication stimulates endogenous opioid system (including peptide transmitters such as endorphin and enkephalin, which take part in controlling pain, mood, and stress responses) and increase opioid blood levels. In AUD and chronic alcohol use, levels of endogenous opioids are reduced, which may contribute to negative emotional states [30].

Common Pathogenesis

Shared Genetic Vulnerability with Different Phenotypic Pictures

In 1992, Winokur proposed a division of primary unipolar depression into familial subtypes. One of those subtypes was depression spectrum disease (DSD), which he defined as a depression in a person having a family history of AUD and/or ASPD (and may have family history of primary depression). A second subtype was familial pure depressive disease (FPDD)—a depression in a person without a family history of AUD and/or ASPD, but with family history of depression. Unstable personality characteristics were observed in DSD depressive patients compared to FPDD [33, 34]. Also, a twin study suggests that familial factors strongly impact the association between depression and AUD. In a sample of 1874 monozygotic male twins, individuals with depression had 2.8 times higher odds of AUD than twins without history of depression [35]. In AUD genetics studies, most data support the importance of genetic variation (the role of genetic polymorphisms) in genes related to alcohol metabolism (*ADH1B* and *ALDH2*), susceptibility and resistance to stress (*COMT* and *SLC6A4*), the reward system (*OPRM1*, *DRD2* and *DRD4*), and cognitive functions and behavior control (*COMT* and *MAOA*). Furthermore, studies on gene-environment interaction suggested that comorbidity of AUD and depression may arise from common environmental causes in patients with specific genetic risk variant (i.e., *SEMA3A*) [36, 37]. The recent meta-analysis of genome-wide association studies (GWASs) identified several risk genes associated with AUD. Consistent with above-mentioned data, it underlines the importance of genes related to alcohol metabolism (*ADH1B*, *ADH1C*, *ALDH2*) and neurotransmission (*DRD2*). However, it also identified other genes such as *GCKR*, *SIX3*, *KLB*, *SLC39A8*, *FTO* and several novel ones, for instance, *PDE4B*, *THSD7B*, *CADM2*, *DPP6*, *SLC39A13*, *TMX2*, *ARID4A*, *C14orf2*, *TNRC6A,* and *FUT2*. This meta-analysis does not support the role of genes involved in behavior control (*COMT* and *MAOA*), though it revealed significant genetic correlations with other psychiatric disorders, with the strongest relationship with MDD [38].

Typology of Cloninger, which was related to his theory of personality, divided alcohol-dependent individuals into two types. Type I is characterized by a later onset of drinking (after the age of 25), ability to abstain from time to time, at least intermittently, men and women alike. The personality profile of this group is dominated by risk avoidance, low need for stimulation and high need of social approval. These people tend to deal with problems with the help of alcohol. Type II includes people with an early onset of drinking (before 25 years of age), almost exclusively men, burdened with addiction in the family and inheriting addiction from their fathers, often using other psychoactive substances. In the personality profile there is dominance of antisocial traits with a high need for stimulation. Type II AUD individuals consume alcohol for their enjoyment, not to alleviate anxiety or tension. It

is supposed that subjects with Cloninger's Type I have more evident impairment in dopaminergic transmission, while those with Type II have a significant deficit in serotonergic transmission and normal dopaminergic transmission [39–42].

Mental Disorders Lead to Alcohol Use: A "Self-Medication" Hypothesis

Alcohol is commonly perceived as a substance that "elevates mood", as it may produce stimulant-like effects. Concurrently, due to sedative properties it reduces tension and anxiety. It is widely observed that individuals with AUD/SUD have poor emotion regulation skills—they have difficulties in regulating affect in a context of their own emotional state as well as in social contacts. Hence, they may feel overwhelmed with emotions or do not feel their emotions at all. *Self-medication* with alcohol or other substance helps to reduce negative emotional and mental symptoms [43]. This phenomenon seems to be more frequent in individuals with primary deficit of central activity of NA, DA and 5-HT (as alcohol intake increases activity of these neurotransmitters) and in depressed women [20].

Substance/Alcohol-Induced Mood Disorder

While analyzing background of comorbidity of depression and AUD, the occurrence of alcohol-induced depression should also be considered. Depression that began before the onset of AUD or during uninterrupted abstinence should be considered as independent depression. Alcohol-induced depression occurs during or shortly after alcohol intoxication or withdrawal and lasts up to 4 weeks [44]. The differences in course and family history of independent versus alcohol-induced depression are presented in Table 17.3.

Table 17.3 Differences in course and family history of independent vs. alcohol-induced depression [45]

Independent depression	Alcohol-induced depression
Onset of depressive symptoms before patient started using alcohol	Intensive alcohol use preceded incidence of a first episode of depression
Depressive symptoms occurred or maintained during long-term abstinence (at least 4 weeks)	Depressive symptoms remitted during abstinence lasting at least 4 weeks
Family history positive for recurrent depression in a first-degree relative	Family history negative for depression

Comorbidity of Selected Psychiatric Disorders and AUD

Mood Disorders

Depression is the most common co-occurring psychiatric disorder in individuals with AUD. Given the high prevalence of depression in the general population, a high rate of co-occurrence in AUD could be expected and may suggest over-representation. However, current studies indicate that the prevalence of depression is nevertheless higher in AUD than in the general population. Comorbidity of depressive disorder and AUD is associated with greater severity of symptoms, worse treatment response, worse prognosis, and increased risk for suicidal behavior [46]. There is a lower likelihood of remission of depression, and it takes more time to achieve response to treatment in patients with comorbid disorders [47]. Occurrence of alcohol-induced depression is associated with worse alcohol-related treatment outcomes and with a greater risk for later MDD [48, 49]. According to a recent meta-analysis, lifetime prevalence of AUD reaches 35% in patients with BD. Use of alcohol in this group may contribute to poor compliance and worse treatment outcomes. Both poor treatment adherence and alcohol use are important risk factors of suicide in patients with BD and AUD [50, 51].

Schizophrenia

About 1/3 of patients with schizophrenia meet the criteria for diagnosis of AUD. A recent meta-analysis indicates a lifetime prevalence of AUD in subjects with schizophrenia at 24.3% [52], another study estimates this prevalence at 33.7% [3]. One US study reported that 36.4% participants met criteria for AUD before the first episode of psychosis [53]. Some significant predictors of this comorbidity can be identified: male sex, severity of negative symptoms, severity of depression, low education, previous violent offending, and family history of substance use disorders [54, 55]. The comorbidity of AUD in patients with schizophrenia is associated with greater severity of psychopathology, higher suicidality, medication nonadherence, high rates of hospitalization, aggressive behaviors, and socioeconomic problems such as homelessness and incarceration [56].

Neurocognitive dysfunction (impairment of working memory, episodic memory and verbal learning), which is observed in many patients with schizophrenia, may contribute to a particularly high risk of relapse to alcohol use. Furthermore, disorganized memory and the inability to inhibit craving may be risk factors for inducing alcohol-seeking and subsequent alcohol-dependence [19, 23, 57, 58]. Based on recent studies, there is evidence for certain genetic factors that may lead to co-occurrence of AUD and schizophrenia. It was noted that SUD (including AUD) and schizophrenia may share genetic liability [59] and it was observed that several

polymorphisms of the brain-derived neurotrophic factor gene (*BDNF*) may be associated with a shared genetic vulnerability for co-occurring schizophrenia and alcohol dependence [60]. Another explanation of high comorbidity of AUD and schizophrenia is the self-medication hypothesis. Alcohol may be used to reduce discomfort arising in the initial stages of psychosis or to decrease anxiety or mental tension accompanying hallucinations and delusions. It can be also used to improve mood or self-esteem [61].

Anxiety Disorders

Cloninger's typology of AUD emphasizes heritable personality traits as a key factor for developing type I or type II alcoholism. Type I includes individuals that are believed to drink to cope with negative affect associated with self-consciousness or anxiety, while type II includes persons having relatively less fear and guilt and demonstrating antisocial behaviors. According to the NESARC study, drinking to cope with symptoms of anxiety disorder increases the risk of AUD, and persons with anxiety disorder who drink to cope with anxiety have five times higher risk for developing AUD in 3 years and a transition from initial alcohol use to AUD is faster [62]. Expectedly, AUD treatment outcomes are poorer in this group of patients as well [63]. There is growing evidence supporting the hypothesis that the common co-occurrence of anxiety disorder and AUD is a consequence of overlapping neurodysregulations. Amygdala is a key structure in physiological and behavioral response to stress and anxiety. In addition, it is a crucial structure in the neurocircuitry of addiction. Brain imaging studies demonstrated abnormal central amygdala function in individuals with anxiety disorder and in individuals with AUD [64] as well as it is postulated that chronic alcohol use results in neuroadaptations within the central amygdala, which are analogous to the neuroadaptations that occur after chronic stress [65, 66].

Personality Disorders

In the NESARC study 42% of participants who met the diagnostic criteria for any PD were also diagnosed with DSM-IV alcohol dependence, with the most pronounced co-occurrence with Cluster B PDs [67]. It is clearly noticeable that AUD is related to several personality traits, like low agreeableness and impulsivity, which are consistent with traits typical for ASPD and BPD. Furthermore, personality traits like impulsivity, aggressive or neurotic tendencies, which are constitutional for ASPD and BPD, may contribute to externalizing behaviors, such as alcohol or other substance misuse. Cloninger in his studies on type II alcohol dependence and on ASPD noticed that both conditions are marked by high

novelty-seeking, low harm avoidance, and low reward dependence [68]. Slutske and colleagues verified this observation using genetic methods. They found that genetic variance associated with behavioral undercontrol (including traits like impulsivity, novelty-seeking, aggression) accounted for 40% of the genetic variance in alcohol dependence [69]. According to current data, it can be assumed that AUD patients with co-occurring PD are less likely to remain in treatment, likely to drink more alcohol per day, and—in patients with ASPD—have earlier onset of AUD. It is not thoroughly clear if, and how, personality disorder impacts an outcome of AUD treatment since there is little reliable research on this topic. As reported by recent literature, treatment outcomes may not substantially differ in those groups of patients [70, 71].

Comorbidity of AUD and Other SUDs

Alcohol is frequently used together with other substances. According to SAMHSA, in the US 5.6% of adults have used both alcohol and another illicit drug within the past year and 1.1% have met diagnostic criteria for both AUD and another SUD [2]. The most commonly reported substances co-used with alcohol are cannabis (10%), opioids (2.4%), cocaine (2.5%), and amphetamine (1.2%). Polydrug use, that includes alcohol, is associated with additional comorbidities, including higher prevalence of mood disorders and anxiety disorders. Furthermore, AUD is more severe in patients with other substance use disorder (AUD-SUD) than in individuals with AUD only. Moreover, individuals with SUD are likely to drink alcohol both during drug-craving episodes and during drug-use episodes [72, 73].

Use of alcohol with other substances, especially with opioids and benzodiazepines, increases rates of adverse events, overdose, and death [74]. It was observed that in the US between 1999–2008 there was a large increase in overdoses caused by combination of alcohol with opioids (76% increase), which is particularly dangerous because of both having suppressive effect on the brain respiratory center. About 20% of overdoses were caused by combination of alcohol and other substances [75]. Also, patients using more than one substance are likely to have poor outcomes from behavioral treatment [76].

There is an unquestionable relationship between alcohol drinking and tobacco use, with about 40% of adults who drink alcohol also being current smokers [77] as compared to about 23% in the general population. It is observed that with increasing rate of smoking the amount of alcohol consumed increases monotonically, indicating that the relationship is dose-dependent [78]. AUD patients are more likely to use tobacco (according to studies, up to more than 80% of patients) and use it heavier than non-AUD individuals [79]. Importantly, alcohol and tobacco potentiate the risk for head and neck cancers [80].

Treatment of Comorbid AUD and Mental Disorders

To provide a comprehensive care for patients with dual diagnosis a few principles of treatment might be framed. Due to strong association of AUD and other mental disorders in course and prognosis, as improvement of one disorder facilitates treatment of another, simultaneous treatment of both disorders should be performed. Best is if treatment is delivered by the same interdisciplinary team in the same treatment center, preferably dedicated to therapy of patients with dual diagnosis. When introducing pharmacotherapy, it is worth remembering that effect size of antidepressants or antipsychotics might be moderate, and placebo effect might be particularly pronounced [81–84].

Treatment of Depression Coexisting with AUD

All patients with depression should be screened for alcohol use and, if needed, diagnosed for AUD. Intervention for AUD should be set up at early stages of treatment, as maintaining abstinence improves effectiveness of antidepressant medication. While initiating treatment, symptoms of alcohol withdrawal should be assessed and detoxification should be initiated, if necessary. All patients should be proposed an effective medication for AUD as well as psychosocial therapy. Symptoms of alcohol-induced depression may go away with abstinence, so it is important to get the patient abstinent or at least encouraged to substantially reduce alcohol use. It is not presently known how long to optimally wait before initiating treatment after achieving abstinence. Four-week observation with standard interventions supporting abstinence may allow avoiding unnecessary pharmacotherapy, which might involve a risk of potential drug interactions and adverse effects [85]. Other recommendations [83] suggest that a week of observation is enough to state that depression is likely independent. It might be concluded that if there are other indicators for independent depression (such as prior episodes or family history), time of observation may be shortened, and starting antidepressant treatment early should be considered.

If a patient cannot achieve abstinence, it is worth investigating past episodes and considering treatment depending upon obtained information. Mild depressive symptoms may be treated with Cognitive-Behavioral Therapy (CBT) and interventions supporting abstinence. Moderate and severe symptoms should be treated with antidepressant medication. Choice of antidepressant may be made according to local standard or clinical guidelines, though some suggestions are put forward below. In case of treatment-resistant depression standard guidelines should be followed. CBT or other therapeutic techniques may be provided at any stage of treatment [44, 82–84, 86]. A recent meta-analysis of studies on clinical interventions for patients with AUD and depression revealed no certain evidence for effects of CBT, Selective Serotonin Reuptake Inhibitors (SSRIs) or Tricyclic Antidepressants

(TCAs) on remission from depression, but it proved that CBT may likely reduce depressive symptoms (moderate confidence), SSRIs are likely to improve functional status (moderate confidence), and TCAs may reduce depressive symptoms (low confidence). For alcohol use outcomes, there was no evidence for effect of CBT, SSRIs or TCAs on remission, but it was revealed that CBT and SSRIs may reduce alcohol use (low confidence) [87].

Pharmacological Treatment of Depression Coexisting with AUD

SSRIs are first-choice drugs as they are well-tolerated, safe, cause mild sedation and have low risk of alcohol-drug interactions. Effectiveness of SSRIs is modest, with stronger evidence in independent depression [44] and a combination of CBT and SSRI provides better treatment effect. Studies support combined pharmacological treatment for depression and AUD (for example, with sertraline and naltrexone) [88]. There is evidence that combined pharmacotherapy provides better outcomes—maintained abstinence, delayed relapse to heavy drinking, fewer serious adverse events, remission of depressive symptoms. If SSRIs fail (or there is a history of SSRI failed attempt), medication with noradrenergic or mixed mechanism: SNRIs (venlafaxine, duloxetine) or NASSA (mirtazapine) should be introduced. Providing that the patient maintains abstinence, pharmacological treatment of depression might be discontinued after 6 months of remission. However, in case of MDD standard long-term pharmacological treatment preventing relapse should be continued. In those who start drinking again or drink continuously, it is not necessary to withdraw medication. While choosing antidepressant it should be remembered that fluoxetine, bupropion, and venlafaxine might be abused [44, 81–84, 86].

Treatment of Bipolar Disorder Coexisting with AUD

For now, there are not enough data to provide a solid recommendation for treatment of patients with comorbid BD and AUD, but some guidance might be proposed to simplify clinical decision making. Current literature supports the use of lithium in monotherapy or combined with valproate as a first line treatment for patients with BD and AUD. Initiating medication for AUD should always be considered by clinicians to reduce alcohol consumption. Available data support the effectiveness of naltrexone for reducing alcohol use in BD patients. Disulfiram, even though included in guidelines, has not much evidence specific for BD patients. Aripiprazole, lamotrigine, gabapentin, and topiramate may be considered as well, but there are not enough data published to recommend them as a first line treatment. They may nonetheless be considered as an adjuvant therapy together with medications with support in the evidence, such as lithium and valproate [51, 89]. Quetiapine has been studied extensively for the treatment of comorbid BD and AUD as an agent that may

influence both mood symptoms and alcohol consumption. For now, results of studies are mixed. There was a positive effect on mood and alcohol drinking reported in some studies, but it has not been replicated in others. When used as adjunctive therapy to valproate or lithium in a randomized controlled trial, no significant effect on AUD-related outcomes was reported [90]. Altinbas and Evren proposed an algorithm for the treatment of comorbid BD and AUD [91], see Table 17.4 below.

Treatment of Schizophrenia and Psychotic Disorders with AUD

The choice of antipsychotic medication should be made with caution because it may have implications for alcohol consumption. It is observed that first-generation antipsychotics do not decrease the amount of alcohol used in individuals with schizophrenia and AUD, and even may increase alcohol use and craving [6]. There is support for use of clozapine in patients with schizophrenia and AUD. It was postulated that clozapine may alleviate dysfunction of brain circuits that appear in individuals with schizophrenia and substance use disorder by its weak blockade of D2 receptor coupled with noradrenergic effects [92]. Recent research demonstrated that individuals receiving clozapine are more likely to achieve remission from AUD and have lower rates of relapse in comparison to individuals taking another atypical antipsychotic [93]. Long-acting injectable antipsychotics should be taken into consideration, since they may improve treatment adherence, something that is particularly relevant in a dual-diagnosis population. In a randomized controlled trial comparing oral risperidone and long-acting injectable risperidone in patients with schizophrenia and AUD, drinking-related outcomes were poorer in patients receiving oral formulation of risperidone [94]. There are studies supporting the superiority of long-acting injectable paliperidone as well [56, 95]. Among medications for AUD, disulfiram and naltrexone have been studied in patients with schizophrenia. Despite previous concerns about pro-psychotic effect of disulfiram, current studies demonstrate that disulfiram may be effective in this group of patients as it is well tolerated and does not worsen psychosis. Similarly, naltrexone decreases alcohol consumption and does not affect symptoms of schizophrenia [56, 81].

Table 17.4 Treatment of comorbid AUD and BD [91]

Mood state	Depression	Mania/mixed	Euthymia
Evidence-based choice	1. Quetiapine	1. Valproate	1. Valproate
	2. Lithium	2. Quetiapine	2. VPA + naltrexone
	3. Valproate		3. VPA + disulfiram
Expert opinion	1. Lamotrigine	1. Lithium	1. Lithium
	2. Aripiprazole	2. Carbamazepine	2. CBZ/Oxcarbazepine
			3. Acamprozate

Psychotherapy can be applied to the appropriate patients during all phases of treatment

Treatment of Personality Disorders with AUD

Treatment of patients with AUD and PD may be challenging for clinicians. On one hand, addressing PD and AUD independently may be needed, as an active use of substance disrupts treatment process. On the other hand, it may be unworkable to focus on one issue at a time, while both conditions closely influence each other. For individuals with BPD in therapeutic process, there may occur behaviors that potentially make further AUD treatment impossible (such as suicidal thoughts or attempts associated with personality disorder). Integrated treatment, using key components from multiple therapies and targeting specific traits (such as impulsiveness and emotion dysregulation) are considered an effective approach for patients with AUD and PD [68].

Summary

AUD frequently coexist with other substance use and mental disorders. Dual diagnosis leads to more social and medical complications—patients with comorbid AUD and other psychiatric disorders have a higher risk of suicide, more severe course of both diseases, poorer treatment outcomes and prognosis, have more comorbid somatic disorders, and show worse social and occupational functioning. For this reason, it is recommended to screen all patients with mental disorders for alcohol and other psychoactive substance use. The risk of recurrence is higher in subjects with comorbid AUD and psychiatric disorders, independently of relapse to alcohol use. Psychiatric patients treated for a psychiatric disorder with residual symptoms get back to use of alcohol or other drugs earlier and more frequently than those with complete remission of symptoms.

Diagnosing and treating patients with comorbid disorders is particularly difficult and challenging for health care professionals. Treatment of AUD should be performed concurrently with antidepressant medication and there is no need to withdraw antidepressant medication because of alcohol drinking. Reasonable use of antidepressants alleviates depressive symptoms and may increase likelihood of reducing alcohol consumption. Recommended antidepressants are SSRIs, SNRIs, and mirtazapine, as they have a low risk of alcohol-drug interactions and alcohol use should not affect antidepressant treatment. For bipolar disorder and AUD, lithium in monotherapy or combined with valproate should be considered as first-line treatment, while in psychotic disorders and AUD clozapine and long-acting injectable antipsychotics are better choice than first-generation antipsychotics. In patients with AUD and PD integrated therapy targeting specific traits might be effective, though therapeutic process may be challenging and complex care should be provided.

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Chapter 18
Treatment of Adolescents

Anna E. Kirkland, Kathryn S. Gex, Brittany E. Bryant, and Lindsay M. Squeglia

Abstract Adolescence is a critical phase of psychosocial and neural development that oftentimes overlaps with the initation and escalation of substance use, particularly alcohol use. Adolescent alcohol use is not innocuous, and endorsing a single symptom of alcohol use disorder (AUD) during adolescence predicts AUD diagnosis in adulthood. Further, up to 5% and 15% of adolescents and young adults, respectively, meet critertia for AUD. The age of intervention for AUD is a critical predictor of treatment outcomes. Therefore, intervening during adolescence could lead to better outcomes across the lifespan. Currently, there are several available treatments for adolescent AUD, as well as possible adjunctive treatments and alternative modalities to existing interventions. Currently available treatments include: family-based therapy, cognitive behavioral therapy (CBT; including 3rd wave CBT), motivational interviewing/motivational enhancement therapy, multicomponent psychosocial therapy, and brief alcohol interventions. Possible adjunctive treatments include 12-step programs, pharmacotherapy, exercise-based therapies, goal setting, and progress monitoring. Unfortutanetly, the effect size for existing interventions are small-to-moderate, leaving much room for future improvement. Digital strategies and culturally based programs may help increase the effectiveness of existing treatments. This chapter will provide a guide through the current standalone, possible adjunctive, and new modalities as well as discuss the obstacles surrounding adolescent AUD interventions.

Keywords Adolescent · Alcohol use disorder · Intervention · Development · Family-based therapy · Cogntive-behavioral therapy · Brief interventions · Pharmacotherapy

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Introduction

Adolescence is a critical phase of maturation within physiological, cognitive, and psychosocial systems. The timeframe of adolescence has multiple definitions, ranging from 10–19 years old to 10–24 years old according to the World Health Organization (WHO) and Center for Disease Control and Prevention (CDC), respectively. Two important, and often overlapping, occurrences happen during adolescence: the final phases of neurodevelopment [1, 2] and initiation/escalation of substance use [3], particularly alcohol use [4, 5].

Brain Development Overview. The brain is maturing until around the age of 25, with significant structural and functional maturation occurring during adolescence. Structural changes include distinct maturation patterns within gray matter (e.g., neuronal cell bodies, dendrites, glial cells) and white matter (i.e., myelinated axons). Recent lifespan curves [6] show a white matter trajectory with a nearly linear increase almost to the age 30 followed by a steady decline with age. Gray matter shows a different trajectory with an increase in gray matter until approximately age 6, followed by a linear decrease throughout adolescence and into late adulthood (i.e., inverted-U shape). Further, regional gray matter trajectories support differential maturation timelines across brain systems, with sensory regions maturing first and fronto-temporal association regions (e.g., cognitive control and executive functioning) maturing later [5]. Due to the biological make-up of of gray and white matter, the decrease of gray matter over adolescence is thought to at least partially correspond with the pruning of weak synaptic connections resulting in increased regional specificity and plasticity [7], while the increase in white matter is thought to represent greater global connectivity [5, 8]. Additionally, neurotransmitter systems, like dopamine (reward, motivation, approach), glutamate (excitatory), and GABA (inhibitory), mature during adolescence [9].

Alcohol Use During Adolescence. In concordance with these neural changes, adolescent behaviors shift towards an increase in novelty and sensation seeking [1, 2, 10] and the development of higher-order cognitive abilities [11]. Importantly, the novelty and sensation seeking systems (e.g., reward pathway modulated by dopamine) develop before the higher-order cognitive systems (e.g., prefrontal systems) [8, 10]. This has led to the "imbalance hypothesis" of neurodevelopment, which proposes that enhanced motivation for novel and rewarding stimuli, coupled with immature cognitive control, can lead to initiation of substance use during adolescence [5, 11]. For other hypotheses of adolescent substance use initiation, see Lees et al. [5].

Adolescent substance use levels are heterogenous, ranging from low or normative use to heavy or pathological use, and alcohol is the most used substance during this time of development [3, 4]. According to the WHO, 27% of

adolescents (ages 15–19) worldwide endorsed currently drinking alcohol, defined as consumption of an alcoholic beverage in the previous 12 months [4, 5]. There is variability of adolescent (ages 12–15) alcohol use within regions of the world. For example, in Africa the lowest rates of alcohol use are found in Senegal (1.7% for girls, 5.8% for boys) and the highest rates are in Seychelles (56.2% for girls, 59.7% for boys), while in Asia the lowest rates are in Myanmar (0.6% for girls, 3.0% for boys) and the highest rates are in Thailand (9.2% for girls, 21.0% for boys) [12]. Across the world, the highest rates of adolescent alcohol use are reported in the European region (44% past month use), and the lowest rates of use are in the Eastern Mediterranean region (1.2% past month use) [4]. The type of alcohol use is also of importance during this age, with 45% of adolescents using alcohol worldwide endorsing binge drinking (defined as 60+ grams of pure alcohol or approximately four standard drinks in one drinking occasion) [4, 5, 13], indicating that high levels of drinking are common within this age group.

While alcohol use during adolescence is sometimes considered a "normal" exploratory behavior, it can have lasting adverse consequences. Problematic alcohol use during this life stage is related to psychosocial problems, including comorbid psychopathology [14, 15], poorer academic success [16], and detrimental neurocognitive consequences [5, 17, 18]. It has also been the leading cause of global premature death (ages 15–49) [19]. Early alcohol initiation increases the risk of subsequent alcohol use disorder (AUD) and related problems [20, 21]. Adolescents who endorse subthreshold levels of AUD (even just one symptom) have a higher risk (odds ratio: 2.0) of transitioning into meeting full AUD criteria by early adulthood [22]. Alarmingly, almost 15% of youth meet the diagnostic criteria for AUD by age 18 [23], and half of individuals that meet the criteria for lifetime AUD do so by the age of 21 [24]. Genetic factors, the environment (e.g., cultural acceptance of alcohol use, parenting styles, peer influence), and their interaction have been associated with early initation and escalation of alcohol use [25–28]. Additionally, adolescent alcohol use has been associated with worse outcomes than adult alcohol use, including being more likely to transition into an AUD or other substance use disorder (SUD) [20, 29] and having more detrimental brain aberrations [5].

It is clear that adolescent alcohol use is not innocuous, and adolescents are not spared from developing an AUD. In fact, the age of onset for AUD is a critical factor for treatment outcomes [22]; thus, decreasing problematic alcohol use at an early stage could have significant long-term implications for persistent AUD [30]. This creates an urgent need for efficacious adolescent-focused AUD treatments. This chapter will be focused on: (1) the currently available treatments for adolescent AUD, (2) possible adjunctive interventions to existing treatments, and (3) alternative intervention modalities (see Fig. 18.1 for overview).

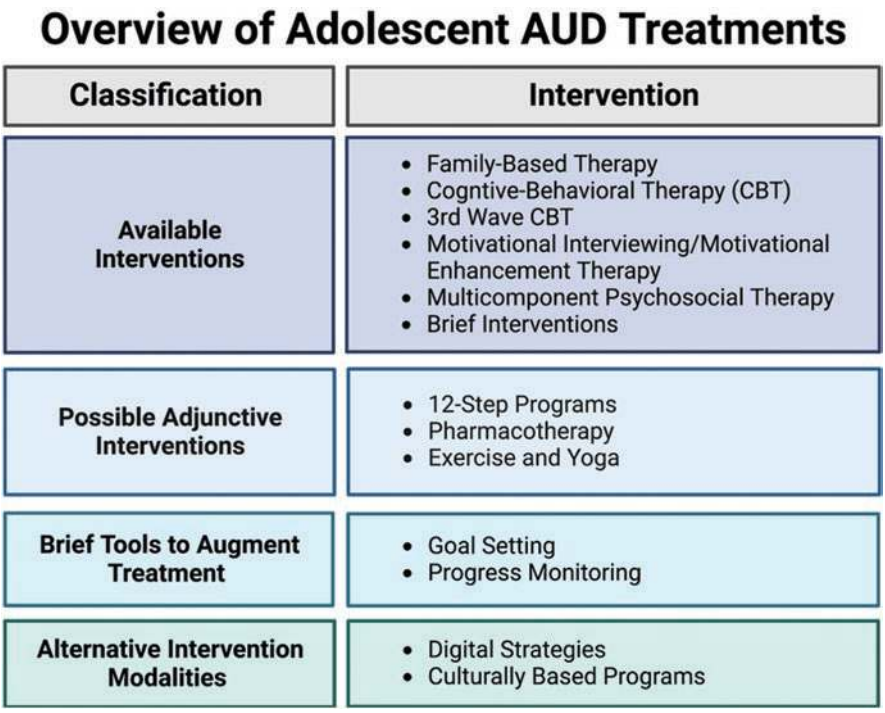

Fig. 18.1 Overview of Adolescent AUD Treatments covered in this chapter. Interventions range from available, possible adjunctive, and alternative modalities. Image made with BioRender

Available Treatments

Family-Based Therapy

Even though adolescence is often a time dedicated to branching away from the family unit and placing more importance on an individual's social circle, family-based therapies are considered first-line standalone interventions for adolescent AUD. Family-based therapies involve the parents or caregivers, as well as siblings. They include Multisystemic Therapy, Multidimensional Family Therapy (MDFT), Functional Family Therapy, Brief Strategic Family Therapy, Ecologically Based Family Therapy, Family Behavior Therapy, Culturally Informed Flexible Family Treatment for Adolescents, and Strengths Oriented Family Therapy [31].

Family-based interventions are effective at increasing attendance and therapeutic alliance [32]. Across all adolescent SUDs, family-based therapy programs were more effective than behavioral therapy, Cognitive Behavioral Therapy (CBT), motivational interviewing/motivational enhancement (MI/MET), psychoeducational therapy, or group counseling [33]. Several systematic reviews and meta-analyses of family-based therapies have been conducted in adolescent alcohol use and

AUD. Universal family-based programs have been shown to be effective (in 9 out of 12 studies) at reducing alcohol use in youth, as compared to control or other interventions, with the post-intervention duration lasting from 2 months to 8 years [34]. This finding was corroborated by a later meta-analysis [35], with both reports indicating small but persistent effects. Conversely, another review of 46 studies found no intervention effect for family-based therapies in comparison to no intervention or standard care on prevalence or frequency of alcohol use in adolescents, nor was there a difference when comparing family/parent interventions to youth-alone interventions [36]. This could be due to the low-quality of evidence included or heterogeneity across studies, either of which will require more studies to parse out any possible effects from family-based interventions on adolescent alcohol use.

Additionally, there are parent-based interventions that focus specifically on the parent as the agent of change [37]. Modifiable parental risk factors (parental provision of alcohol, favorable parental attitudes towards alcohol use, and parental drinking) and protective factors (greater parental monitoring, parent-child relationship quality, parental support, and parental involvement) are longitudinal predictors of alcohol initiation and levels of problematic alcohol use in adolescence and adulthood [38]. Parent-based therapies come in various forms, including programs dedicated to only the parents, joint parent-child programs, or parent programs in association with school-based interventions.

For parent-based therapies, a meta-analysis of 20 alcohol use prevention randomized controlled trials (RCT) in adolescents (up to age 18) found a reduction in their child's overall alcohol use, binge drinking, and drinking intention, with interventions targeting both general and alcohol-specific parenting behaviors having the most effect [37]. However, this meta-analysis excluded studies if the majority of the youth had an AUD as it was focused on preventing or reducing alcohol use. This demonstrates the potential for parent-based therapy to limit early alcohol use, which may be critical for adolescent AUD prevention efforts. More research on the effects on adolescent AUD specifically is needed.

While family- or parent-based interventions may have small effects, there is some data indicating that they may not be superior to individual treatment. Several studies conducted in adolescent SUD have shown well-established efficacy for family-based interventions [32, 39, 40], yet there is little data within adolescent AUD specifically; thus, more evidence is needed within this area before establishing the efficacy of family- or parent-based interventions for adolescent AUD.

Cognitive-Behavioral Therapy (CBT)

CBT is a psychosocial treatment, delivered in either an individual or group format, that is focused on teaching skills (e.g., self-monitoring, identifying triggers, managing cravings, developing communication, and establishing alternative reinforcement contingencies) to modify problematic thoughts and behaviors. Since its emergence in the 1960s, CBT remains a pillar of psychotherapeutic intervention for several mental

health conditions and has some of the strongest empirical support of all evidenced-based psychotherapeutic interventions [41]. The effectiveness of CBT has been examined within adolescent SUD [42], with several studies suggesting CBT is well-established at reducing alcohol and other substance use in adolescents [43]. The strongest evidence thus far for CBT's therapeutic action within adolescent SUD was in combination with brief individual motivational enhancement [44]; however, this study was focused on cannabis use and has not been followed-up in adolescent AUD. Within alcohol use specifically, most of the data assessing the efficacy of CBT within adolescent alcohol use are from older studies (>20 years). CBT targeting personality risk-factors for alcohol misuse found a reduction in drinking rates, drinking quantity, and problematic drinking symptoms as compared to the no-treatment control group in high school students not diagnosed with an SUD [45]. While effects of CBT do not seem to be persistent (<6 months), one study included an integrated family component which sustained the effects on alcohol and cannabis use through the 6-month follow-up period [46]. Overall, there is a dearth of studies specifically investigating CBT as an intervention for adolescent AUD; however, CBT has been generally effective in reducing substance use during adolescence.

3rd Wave CBT

Third wave CBT interventions build on the principles of CBT while focusing on the individual's relationship with thoughts and feelings rather than the content. These methods focus on mindfulness, acceptance and commitment therapy, dialectical behavior therapy, mindfulness-based cognitive therapy, functional analytic psychotherapy, meta-cognitive therapy, and more [47]. Observationally, mindfulness qualities, such as urgency, are associated with reduced lifetime use of alcohol and cannabis in high school students [48]. Mindfulness interventions and their specific components, like urge surfing [49] and brief breath work [50], have improved alcohol outcomes in adolescents and young adult college students [49, 51]. Additionally, three facets of mindfulness (describing, nonjudging of inner experience, and acting with awareness) were negatively related to alcohol outcomes in college students [52]. The reduction of stress may be a mediating factor between mindfulness interventions and alcohol and other substance use [50, 51]. Mindfulness has also been combined with more traditional CBT methods in adolescents, showing a reduction in alcohol consumption as compared to assessment-only control groups. However, adding mindfulness was not superior to CBT alone [53]. Acceptance and commitment therapy (ACT) is another form of CBT that focuses on teaching individuals how to accept difficult emotions and other challenging situations, which has shown efficacy in mental health disorders and adult SUD [54]. Two adult studies have reported ACT as an effective treatment for adult AUD [54], but no studies have been conducted in adolescent AUD. Mindfulness, ACT, and other 3rd wave CBT practices may be useful lines of future research for both adolescent and adult AUD.

Motivational Interviewing/Motivational Enhancement Therapy

Motivational interviewing (MI) elicits behavioral change by targeting ambivalence and enhancing internal motivation, with the goal of helping the patient to recognize their alcohol use problems and encourage self-directed use/problem reduction and/or treatment seeking. Motivational Enhancement Therapy (MET) is similar, but it is delivered in a more structured and manualized manner [39, 55]. MI/MET can be delivered as standalone or adjunctive treatments in primary care or emergency room settings, school-based interventions, or other acute settings. However, these methods may be best suited for younger and less severe populations [39]. This could be due to the source of ambivalence, where younger and less severe populations may have ambivalence towards reducing their substance use, while older and more severe populations may have ambivalence towards engaging in treatment. An older review of MI in adolescents and young adults with substance use problems found an advantage of MI to standard or other care in 29% of studies (5/17), where the person-centered nature of the intervention was identified as a key component for treatment success [56]. Unlike some other treatment interventions, MI/MET have been more thoroughly assessed in adolescent alcohol use specifically. MI has been associated with alcohol-related harm reduction and alcohol consumption [57–60]. One study reported that MI increased motivation and self-efficacy, which in turn decreased the amount an individual intended to drink in the future and increased cognitive dissonance related to heavy drinking [59]. However, a meta-analysis of adolescent SUD treatments found small-to-no effects of MI [30]. The effectiveness of MI/MET may be driven by fidelity [61]. In general, MI/MET may be a useful standalone or adjunctive treatment since it is relatively brief, empathic, and focuses on enhancing motivation and self-efficacy.

Multicomponent Psychosocial Therapy

Multicomponent psychosocial therapy combines treatments, including family therapy, CBT, MI, and contingency management (i.e., using reinforcement and punishment to manipulate substance use) [62]. Currently, MET/CBT and MET/CBT with family-based therapy are the most efficacious combinations for adolescent SUD [32, 39, 40]. The Adolescent-Community Reinforcement Approach (A-CRA), which is a combination of individual therapy, family therapy, and case management, has been found to be comparable in effectiveness as MET/CBT; however, it is more expensive [63]. Combining MI with CBT was effective at increasing the drive to reduce alcohol use, reduce the frequency of alcohol use, and improve the general knowledge surrounding alcohol and its effects, as compared to a control group [64]. Another study looked at the addition of abstinence incentives (contingency management) to individual MET/CBT with weekly behavioral parent training in adolescents with AUD. While the addition of abstinence incentives did not affect the main

outcome of abstinence, it did lower the percentage of days using alcohol as compared to the control group [65]. Contingency management has been popular in the adult SUD literature as a standalone or adjunctive treatment, but, apart from the above study, the current literature in adolescents has been focused on cannabis and nicotine use [62, 66]. Multicomponent therapies may also be beneficial for targeting other consequences of adolescent substance use, like sleep quality and emotional regulation [67]. Taking this multi-pronged approach to treatment may increase the likelihood of treatment success, but it may incur a greater time and monetary cost.

Brief Interventions

Brief interventions, specifically brief motivational interventions (BMIs), have been investigated as a treatment option for emerging adults (ages 18–25) as they are short, flexible, and address motivation to change drinking in a population that may not be aware of their problematic alcohol use or inclined to change such behaviors. They can be administered as standalone or adjunctive treatments. Currently, BMIs are considered a Tier 1 Approach (categorized based on empirical evidence, cost, and relevance to college student drinking patterns) by the National Institute of Alcohol and Alcoholism (NIAAA) according to their College Alcohol Intervention Matrix (AIM) [68]. BMIs include the Brief Alcohol Screening and Intervention for College Students (BASICS) program, which includes normative, personalized feedback on drinking patterns and behaviors and are often delivered using MI style [69]. BASICS and other BMIs have been found to be more efficacious at changing drinking behaviors in college students than no or minimal treatment [70], albeit with small to moderate effect sizes [71, 72]. A scoping review aimed to identify efficacious novel components (e.g., focus on substance-free activities, mindfulness of alcohol cues, or relaxation training) that could be added to BMIs to increase these effects and found that approaches that enhance health and wellness, as an addition to BMIs, may lead to increased help-seeking behaviors in emerging adults [73]. Brief interventions may help to decrease the likelihood of transitioning into problematic alcohol use patterns or future AUD by changing beliefs about and attitudes towards alcohol, and their ease of delivery make them a prime area for future research.

Possible Adjunctive Interventions

12-Step Programs

12-step programs are peer-based mutual-help organizations that aim to provide substance use treatment that is free and community-based. Within adolescent SUD, 47% of treatment programs require participation in a 12-step program during treatment, and 85% of programs recommend Alcoholics Anonymous (AA) or Narcotics

Anonymous (NA) as continuing care options [74]. Attending AA or NA meetings may be a useful addition to other treatment options, with an 8-year prospective study finding an average of 2 days of abstinence associated with each AA/NA meeting attended in addition to inpatient care [75]. A randomized clinical trial tested an adolescent-specific version of an integrated 12-step facilitation treatment (iTSF) against 10 sessions of MET/CBT. There were no differences in percent of days abstinent between treatment groups, but there was greater attendance to iTSF (only at the beginning of the study) which was related to greater abstinence at follow-up [74]. 12-step programs may be best suited in combination with other evidence-based interventions and may exert the most benefit early in treatment and when meetings are specifically designed for adolescents and young adults.

Pharmacotherapy

Adjunctive pharmacotherapies may help increase the effectiveness of psychosocial interventions and improve treatment outcomes. To date, there are no Food and Drug Administration (FDA)-approved medications for adolescent SUD, other than buprenorphine, which has been approved down to 16 years of age for opioid use disorder. Despite alcohol being the most used substance during adolescence, only three medications (i.e., naltrexone, n-acetylcysteine, and disulfiram) have been tested among adolescents who use alcohol, with fairly low sample sizes. See Table 18.1 for an overview of the candidate adolescent AUD medication RCTs (see [81] for a detailed review). More studies are needed to understand how adjunctive medications may improve outcomes for youth struggling with AUD.

Exercise and Yoga

Physical interventions, like general exercise or yoga, have had some recent attention as a possible prevention or adjunctive treatment for adolescent AUD. Exercise may help reduce factors that are barriers to treatment (e.g., lack of social support, poor mental health, stress, boredom) and improve protective factors (e.g., creating a routine, providing alternative activities) [82]. A review of high school students surveyed from 1991 until 2009 found that higher levels of exercise were negatively associated with alcohol, nicotine, and cannabis use; however, higher athletic team participation was related to higher alcohol use [83]. These results should be interpreted with caution as they were observational in nature, leaving room for reverse inference and confounding factors. Another review found exercise was related to improvements in actual alcohol consumption and in intentions to use, knowledge about, and attitudes towards alcohol, but these outcomes dampen with length of follow-up [84]. The majority of these studies were in school settings, with single

Table 18.1 Pharmacotherapy candidate medications investigated with RCT for adolescent AUD

Medication	Reference	Sample	Dosing & comparator	Results
Naltrexone	Niederhofer et al. [76]	N = 30 adolescents with alcohol dependence (ages 15–19)	50 mg/day	Higher rates of abstinence
			Control: placebo	
	Miranda et al. [77]	N = 22 alcohol using adolescents (ages 15–19)	50 mg/day	Decreased drinking & drinking days, craving, subjective response to alcohol
			Control: placebo	
	O'Malley et al. [78]	N = 128 heavy drinking youth (ages 18–25)	25 + 25 mg targeted daily	No effect on heavy drinking days or % days abstinent
			Control: placebo	Decreased number of drinks/drinking day and % of drinking days with BAC >0.8
			Platform: psychosocial	
N-Acetylcysteine (NAC)	Squeglia et al. [79]	N = 116 youth with cannabis dependence (ages 15–21)	2400 mg/day	NAC was associated with less alcohol use
			Control: placebo	
Disulfiram	Niederhofer et al. [80]	N = 26 adolescents with alcohol dependence (ages 16–19)	200 mg/day	Higher rates of abstinence v. placebo
			Control: placebo	

session or multi-session (7–24 weeks) designs. For yoga specifically, one randomized trial found a trend towards decreased alcohol use after 20-sessions of mindfulness yoga as compared to control [85]. The evidence is currently weak for general exercise or yoga as an adjunctive treatment for adolescent AUD. Further, while most of the studies reported would be considered standalone interventions by design, the evidence suggests that this would not be recommended for adolescent AUD treatment and should only be considered as adjunctive at this time.

Brief Tools to Augment Treatment

Goal Setting

Goal setting is meant to promote self-efficacy and effort towards achieving goals, which may translate to positive treatment outcomes. This technique shows that individually selected goals predict long term outcomes in adult AUD [86]. There has only been one study in adolescent substance use, which found goal setting at treatment admission predicted treatment outcomes up to the 24 month follow-up, and

predicted drinking outcomes at 12-months [86]. More research is needed to better understand how this simple tool may impact adolescent AUD treatment outcomes.

Progress Monitoring

As with goal setting, progress monitoring is a relatively simple intervention that relies on periodic and reliable assessments of progress to evaluate and inform treatment. This progress allows clinicians to adapt treatment in real time, which can be beneficial to the adolescent [39]. There is not much research on this approach, but a pilot study showed a decrease in self-reported behavioral and emotional symptoms measured by the Youth-Outcome Questionnaire [87]. Progress monitoring could be easily integrated into various adolescent AUD interventions, which could possibly yield higher treatment effect sizes.

Alternative Intervention Modalities

Digital Strategies

Digital interventions for mental and behavioral health treatments are those designed or adapted to be administered over the computer, web, smartphone app, text-message, or other computerized technology. These strategies, often termed broadly as *mHealth*, *eHealth*, or *telehealth*, are characterized by the rapid or immediate transfer of health information due to the mobility, direct communication, and instantaneous access afforded [88]. Rapid technological advancement has played a key role in expanding the number and type of digital modalities available to deliver intervention content and, thus, the potential to reach broader audiences all over the world [88]. Smartphones in particular have become largely ubiquitous with over 95% of adolescents owning or having access to a smartphone [89].

Relative to traditional in-person approaches, digital-based interventions are perceived as more private and less stigmatizing [39]. This perception is key as concern about stigma has been shown to interfere with substance use treatment seeking [90], which is already low among young people. However, despite these low rates, young people are more likely to agree to participate in remotely delivered interventions than in face-to-face interventions [91]. There is also evidence to support that digital interventions facilitate self-efficacy, as well as motivation, social support, and relapse prevention [39].

Research on the efficacy of digital-based interventions for adolescent alcohol use is mixed, but promising. Methodological differences, such as intervention design, setting, length, outcome measures, and participant characteristics, are a limiting factor in the ability to draw conclusions about digital intervention efficacy for

adolescents with AUD. Additionally, relatively few digital intervention strategies have been developed for and tested with this population. Of those that have been evaluated, there are no clear features that seem to predict efficacy. For example, several interventions incorporate tailored messaging, however, not all are efficacious in decreasing alcohol consumption or related harms [92]. Ultimately, it is not yet clear which modalities or interventions designed or adapted for mHealth may be most efficacious for adolescents, though text-message, or SMS, seems to be the most preferred modality [92].

There are notable challenges to using digital interventions. Although young people are more likely to agree to participate in these types of interventions and some show efficacy in reducing alcohol consumption, face-to-face interventions are more efficacious overall [93, 94]. This may be due, in part, to the level of interaction with intervention material. Most digital interventions are entirely automated. While automation frees-up counselor resources considerably, there is evidence that participants do not fully attend to remotely delivered intervention material and are often preoccupied [95]. Notably, intervention interactivity significantly affects drinking outcomes and this association is mediated by information retention [96]. Thus, this can be a problem for "static" web-based interventions and automated text-message interventions alike if the intervention requires low levels of interaction, regardless of the level of tailoring.

Culturally Based Programs

Marginalized adolescents (including, but not limited to adolescents of color, youth in the LGBTQIA^{+} community, and linguistically minoritized youth) are faced with unique challenges related to substance use treatment, including: the lack of culturally relevant interventions, the short-term effectiveness of various treatment modalities, and the sustained financial and systemic barriers to adolescent treatment. Research and clinical experience suggest that there are meaningful racial differences in treatment response, engagement, and retention regarding substance use treatment [97, 98]. Additionally, there are racial and ethnic differences in specific drugs used, health and legal consequences of drug use, and substance use attitudes for marginalized communities [97, 99, 100]; thus, existing substance use treatments validated with homogenous, predominantly white U.S. samples are inadequate and cultural adaptations of substance use interventions are needed.

Cultural adaptation is defined as systematic modifications to an evidence based intervention by introducing culturally relevant components, while maintaining the core components of the generic intervention [97, 101]. In short, changes are made to an intervention to accommodate the needs of a target population [101]. During the cultural adaptation process, values, norms, and attitudes of the target population are considered or incorporated into the culturally adapted version of the evidence-based intervention, making treatment more relevant to marginalized populations. Culturally adapted interventions also provide necessary opportunities to address the

social, cultural, and contextual issues associated with substance use that may often be experienced by marginalized populations. There are various frameworks and extents to which modifications can occur, ranging from preferred language and clothing to culturally meaningful metaphors and matching clinicians with patients based on race [98].

Successful culturally adapted substance use interventions include Strong African American Families (SAAF), a preventive intervention to deter alcohol use among rural African American adolescents [102]. SAAF's structure was informed by the Strong Families Program [103]. During the design process, SAAF authors used data collected from their research with rural African American communities in the lower southern region of the United States to design a culturally and ecologically sensitive program. Data from this study demonstrated that fewer SAAF participants initiated alcohol use compared to the control group and those who used alcohol increased their use at a slower rate over time in areas where there are few mental health, substance use, and prevention programs. Strengthening Families Program (SFP), an evidence-based family skills training intervention developed and found efficacious for substance use prevention in the 1980s, has been culturally adapted across the U.S. for African, Hispanic, Asian, Pacific Islander, and Native American populations. Since 2003, the intervention has been culturally adapted for use in 17 countries and has been found to be twice as effective as school based alcohol prevention programs [104]. Without the culturally adapted version of various evidence-based interventions, there would likely be a large population of diverse and marginalized youth whose unique challenges with substance use would go unseen and unaddressed.

Discussion

As reviewed within this chapter, there are many available interventions, possible adjunctive treatments, and alternative intervention modalities for adolescents with AUD. A vital step in tackling adolescent problematic alcohol use and AUD is to find quality, evidence-based interventions. In the United States, this can be done through visiting the NIAAA treatment navigator (https://alcoholtreatment.niaaa.nih.gov) or by reading the Substance Abuse and Mental Health Services Administration (SAMHSA) document on this topic [105]. SAMHSA lists the five signs of quality as follows: accreditation, medication (applicable to adult SUDs with FDA-approved pharmacotherapy options), evidence-based practices, inclusion of family, and ongoing support.

There are several obstacles to adolescent AUD treatments. First, alcohol and other substance use is considered a normal part of adolescence in many cultures around the world. This can make it difficult for individuals to recognize when their drinking behaviors may be entering into problematic use or AUD territory, which will reduce the likelihood of receiving help and subsequently increase their likelihood of a lifelong struggle with AUD [22]. MI/MET and brief interventions that incorporate personalized feedback may be the most useful tools in this

situation, as they target ambivalence and provide normative drinking data for comparison. Further, the brief and non-judgmental nature of MI/MET may make it more appealing to adolescents and emerging adults [56, 73]. Second, there is a lack of agreement surrounding the appropriate outcomes for research studies. Specifically, there is concern over whether abstinence-based outcomes are in fact the best goals [106]. Abstinence-based outcomes may conflict with some newer interventions, such as personal goal setting, which allows the adolescent to be in control of their treatment goals. Previous research has shown that a reduction in the WHO risk level for alcohol consumption is a valid and reliable non-abstinent based target for adult AUD, and a reduction of at least two levels is now an acceptable European Medicines Agency (EMA) endpoint for adult AUD pharmacotherapy trials [107]. A study of adolescents with a SUD and comorbid ADHD found that a two-level reduction in the WHO drinking risk level after treatment with CBT and pharmacotherapy (specifically for ADHD) was associated with improvements in general functioning and ADHD symptoms [107]. Thus, even a reduction in alcohol consumption during adolescence could have positive lifelong implications for SUD and other comorbid symptoms. Third, co-morbidities (e.g., anxiety and depression) and substance co-use (e.g., cannabis and nicotine) are the rule, not the exception, in adolescent AUD [14, 15, 108]. Effective interventions will need to take an integrative approach to provide treatment for and beyond adolescent AUD. Fourth, there is a large gap between the number of adolescents who need care and those who receive care, with SAMHSA reporting that 5% of teenagers and 15% of young adults need treatment for AUD but less than 1% receive it. Compounding this problem is access to care, where those who did receive care were more likely to be older in age and white [109]. Location is also a contributing factor to access to care. Rural adolescents are more likely to report alcohol and other substance use than their urban peers [110], yet such regions have fewer health providers and intervention options. Other barriers to treatment include traditional clinic hours that may limit treatment for families that do not have flexibility during the day or (in countries without universal healthcare) trouble with insurance coverage (which may be worsened by other co-occuring disorders). Digital strategies may be useful for overcoming some of these barriers, as they reduce some of the burden to receiving evidence-based and high-quality treatment [111].

There is still much room for improvement as the psychosocial interventions have only been modestly effective, with one-third to one-half of youth returning to alcohol or other substance use within 12 months of treatment [30, 33]. While several medications have been efficacious in treating adult AUD, pharmacotherapy research focused on adolescent alcohol use has been sparse [77]. As such, evaluation of alternative and more efficacious treatments for adolescent AUD is warranted.

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Chapter 19
Transcranial Magnetic Stimulation in Addiction Therapies

Angela Sanna and Marco Diana

Abstract Alcohol use disorder (AUD) is a chronic, relapsing disease, associated with high morbidity and mortality, and a high prevalence worldwide. Pharmacological and behavioral interventions to treat AUD are not satisfactory and the majority of patients display poor adherence to therapy and a high rate of relapse. Among the non-pharmacological treatments for AUD, repetitive transcranial magnetic stimulation (rTMS) has shown therapeutic potential in promoting abstinence due to its ability to modulate neural plasticity. Preclinical and clinical evidence has shown that a boost of dopamine transmission plays an important, but not exclusive role in mediating the long-lasting effect of rTMS. Nevertheless, despite several reports showing the efficacy of rTMS in treating AUD, a standard protocol that may be desirable in clinical practice is still lacking, due to heterogeneity of protocols and variability in response to rTMS. Ongoing research is exploring new neurophysiological biomarkers to further elucidate the mechanism of action of brain stimulation and to better select patients who may benefit from rTMS.

Keywords Alcohol use disorder · Transcranial magnetic stimulation · Addiction

Introduction

Substance and behavioral addictions are chronic medical illnesses causing important social and health harm [1]. Among these, alcohol use disorder (AUD) has a high prevalence worldwide which varies depending on socioeconomic and cultural

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factors, reaching high rates in European countries, and is associated with high morbidity and mortality [2]. AUD has a multifactorial etiology including a genetic predisposition [3] and is comorbid with other psychiatric disorders, such as antisocial personality disorders, major depression, anxiety, and post-traumatic stress disorder [4]. As for other addictions, AUD may be explained by the so-called "addiction cycle" [5], characterized by compulsive alcohol seeking and intake, the failure to limit alcohol intake, and the emergence of a negative emotional state when alcohol is not available. These behavioral features have been described for different addictions, regardless of the specific mechanism of action of the drug. It reflects complex neuroplastic changes in the addicted brain that lead to an excessive function of limbic areas, along with reduced activity of the prefrontal cortices, together sustaining the addiction cycle [6].

In this composite framework, dopamine is considered a *top player* in each step of the addiction cycle [7, 8]. The hypodopaminergic hypothesis of drug addiction states that an altered dopamine transmission is a hallmark of the addicted brain [8]. Indeed, preclinical and clinical evidence shows an altered mesocortical limbic dopamine transmission during binge intoxication, withdrawal, and anticipation in SUD and AUD [8–10]; in addition, preclinical evidence showed that dopamine can reverse morphological and functional changes in the brain of alcohol-abstinent rats [11]. Likewise, in humans, PET studies have shown that alcohol dependence is associated with a blunted dopamine transmission [12] and that dopamine levels remain low in the brain of detoxified alcoholics [13], which may be considered a marker of vulnerability to relapse. Along with PET studies, quantitative electroencephalography (qEEG) and functional magnetic resonance (fMRI) have further clarified the complex maladaptive changes in the addicted brain during the last decade. Neurophysiological measures such as event-related potential (ERP), brain rhythm oscillation, and brain graph analysis have revealed altered connectivity in the brain of AUD patients, and have been proposed as biomarkers of vulnerability to drug addiction and as tools to monitor the progression of the disorder and the efficacy of therapy [14]. Moreover, fMRI studies in AUD confirmed an altered connectivity in the fronto-striato-parietal network and showed the need to recruit a larger neuronal activation to promote an inhibitory response to alcohol-related stimuli [15, 16]. Taken together, these observations support the concept that AUD, and SUD in general, are chronic disorders characterized by an altered functionality of large interconnected brain networks modulating several behavioral features.

Treatment of AUD is challenging and the pharmacological armamentarium is limited. Disulfiram, acamprosate and naltrexone are currently approved for patients with moderate-to-severe AUD. Other medications, such as gabapentin and topiramate, are approved for other indications, but can be used off-label in moderate to severe AUD [17]. Drugs acting on dopamine transmission such as aripiprazole have been evaluated, but efficacy has not been supported [18]. Behavioral interventions are currently employed for for treating AUD [19] but, as with other SUD and BA, AUD patients display poor adherence to therapy and a high rate of relapse.

Among the non-pharmacological treatment for AUD, repetitive transcranial magnetic stimulation (rTMS) holds a therapeutic potential in promoting abstinence, presumably due to its ability to modulate neural plasticity.

TMS Fundamentals

TMS is a non-invasive brain stimulation (NIBS) technique, introduced by Barker in 1985 to probe motor cortex functionality [20], through the electromagnetic induction of an electric field in the brain. Applied in this manner, it continues to be a valuable diagnostic tool, e.g., in neurosurgical practice to allow preoperative evaluation. When delivered in a repetitive fashion, rTMS can modulate neural plasticity producing objective neurobiological changes and therapeutic effects in several psychiatric and neurological disorders [21]. Indeed, rTMS may exert excitatory or inhibitory effects in the underlying cortex; for the so-called conventional protocols frequencies ≥5 Hz (high frequency, HF) are considered excitatory, while frequencies ≤1 Hz (low frequency, LF) produce inhibitory effects. Further, the relatively new "*patterned*" protocols apply short rTMS bursts at a high intraburst frequency interleaved by short pauses of no stimulation [22, 23]. Among these, theta-burst stimulation (TBS) protocols are characterized by short bursts of 50 Hz (within bursts) repeated at 5 Hz (between bursts) as a continuous (cTBS), or intermittent (iTBS) train [24]. TBS protocols, inducing long-term potentiation (LTP) and long-term depression (LTD) at the synaptic level, are commonly used to probe motor cortex excitability [25]. Moreover, rTMS protocols typically have a short duration, and are widely used in clinical practice for therapeutic purposes in several psychiatric and neurological disorders to reduce patient discomfort [21, 25]. The mechanism of action of rTMS, not completely elucidated, involves neurotransmitter release, modulation of gene expression, synaptic long-term potentiation, and depression, acting together to induce persisting neuroplastic changes in the stimulated spot and distant anatomically interconnected areas, thus modulating the activity of entire brain networks [26, 27].

rTMS in SUD and AUD

Despite several reports showing the efficacy of rTMS in treating addiction [28, 29], heterogeneity of rTMS protocols among different studies has hindered the attainment of a standard protocol that may be desirable in clinical practice. An international group of investigators with expertise in neuromodulation and addiction research (international network of tES/TMS trials for addiction medicine-INTAM) has been assembled to provide ongoing review of the evidence for NIBS in SUD and to provide appropriate guidelines regarding protocols, measures of outcome, and future directions [28]. According to the aforementioned addiction cycle, rTMS

approaches are directed either to enhance prefrontal cortex activity with LTP enhancing protocols or to decrease the excessive functionality of the limbic system with LTD-inducing ones [30]. Indeed, for different purposes, brain areas are chosen as a target for rTMS according to their accessibility (cortical areas), their role in modulating a specific behavioral and/or cognitive function, and their connectivity with other brain networks [31]. From this point of view, prefrontal cortical areas, and dorsolateral prefrontal cortex (DLPFCX) in particular, are common targets of rTMS due to their role in modulating executive functions and their connections with temporal, parietal, and deep limbic areas, involved in cognitive and behavioral functions [32, 33].

LTP-inducing protocols (HF and iTBS) have been applied to either right [34–40] or left [41–44] DLPFCX (Fig. 19.1) showing efficacy in reducing alcohol craving and consumption as well as ameliorating behavioral features and comorbid depression, but the results are inconsistent and controversial, parameters of stimulations vary among different studies and no superiority has been found between left or right DLPFC stimulation.

An interesting approach is the use of deep TMS, which employs a Hesed-coil (H-coil) able to provide a bilateral, deeper non-focal stimulation of the PFC [45]. The rationale for using H-coil derives from the view that addiction may be considered a *whole brain* disease, with plastic alteration in different brain areas of both hemispheres, influencing several behavioral features involved in drug dependence [7, 46]. Indeed, dTMS has shown efficacy in reducing alcohol craving and consumption [47–51] and reducing associated depressive symptoms and anxiety [50, 51].

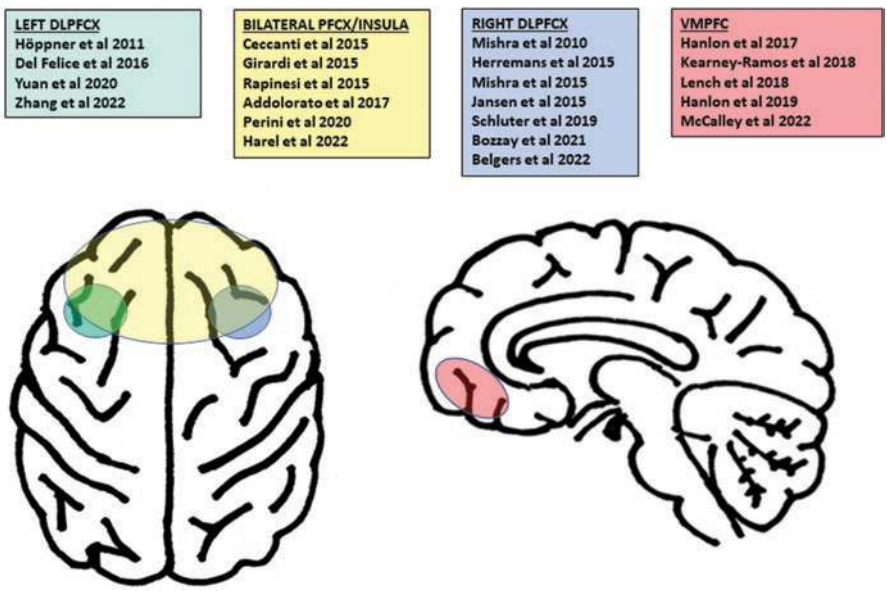

Fig. 19.1 Schematic representation of target regions and related studies for the application of rTMS to treat AUD

Another approach in neuromodulation employs LTD-inducing protocols (LF and cTBS) targeting the ventromedial prefrontal cortex (VMPFC) to inhibit the excessive limbic activation in addiction [30]. Indeed, the inhibitory stimulation of VMPFC can decrease cue-induced craving in CUD and AUD [52–56] while, if applied to DLPFCX, would impair inhibitory control and subsequently increase alcohol drinking [57, 58].

Additional brain areas are currently studied as brain targets for neuromodulation, with particular interest to those involved in the so-called *salience network* (i.e., insula), due to its role in regulating behavioral responses to external stimuli [57]. Early observations have shown that patients carrying an insular lesion quit nicotine addiction [59]; moreover, recent resting state [60] and brain lesion [61] functional connectivity studies have shown an altered functionality of insula in addiction which suggests its use as a target in neuromodulation techniques. Nonetheless, while early studies have shown encouraging results for using insula as a potential target for stimulation in nicotine [62] and cocaine dependence [55], no effect was observed in alcohol craving and consumption with respect to sham stimulation when an HF protocol was applied targeting bilateral insula with dTMS [63]. It is noteworthy that authors of this study employed the same protocol used for nicotine addiction [62] that targeted bilateral PFC and insula; indeed, although no difference was found in alcohol craving and intake between real and sham rTMS, a significant effect induced by rTMS on resting state insula connectivity emerged, suggesting that a concomitant stimulation of PFC and insula may be required to obtain a behavioral effect on alcohol consumption. Moreover, since both LTD and LTP-inducing protocols have been used with controversial results, the role of the insula as a target for neurostimulation is still an object of debate and needs further research [57].

The Role of Dopamine in the Therapeutic Effect of rTMS in AUD

Despite evidence supporting the therapeutic potential of rTMS in several neurological and psychiatric conditions [21, 28], the precise neural mechanism underlying its efficacy is not fully elucidated. As mentioned before, the effect is pleiotropic, involving modulation of synaptic plasticity and neurotransmission [64], which ultimately influence the activity of different brain networks thought to modulate specific brain functions [65]. Preclinical and clinical studies have shown that rTMS applied to frontal and prefrontal areas can induce a release of dopamine in other cortical and subcortical areas [66–69]. These data suggest the ability of rTMS to exert its effects, not only in the stimulated area, but also in distant interconnected areas (see [70] for further details); furthermore, this evidence pinpoints to the role of dopamine as an important effector of rTMS action.

Considering the aforementioned pivotal role of the blunted dopamine transmission in the genesis and maintenance of the addiction cycle, the increase of dopamine induced by rTMS may be considered a biomarker to be subjected to rigorous

experimental scrutiny [7, 71]. This assumption has been confirmed by preliminary SPECT studies showing a decrease in dopamine transporter (DAT) availability in striatal regions following rTMS in pathological gambling [72] and AUD [48], which would reflect an increased dopamine transmission in the mesolimbic-cortical circuits. Another study by Ceccanti and coauthors [47] has shown a decrease in serum prolactin and cortisol along with a reduction in craving and number of drinks per day, following HF deep rTMS applied bilaterally to the DLPFCX of patients with AUD. Prolactin and cortisol serum levels, in turn, reflect the hypothalamic-pituitary-adrenal axis activation, which is over-functioning during withdrawal [6, 8], and prolactin is an indirect marker of dopamine transmission [73]. Accordingly, reduction of prolactin serum levels suggests increased brain dopamine levels. These data, although preliminary, support the hypothesis that a boost of dopamine transmission is one of the core mechanisms of the rTMS therapeutic effect [70]. Importantly, however, dopamine should not be seen as a single player accounting for the therapeutic effect of rTMS in addiction, and other neurotransmitters such as glutamate, are equally involved in inducing the long-lasting plastic changes exerted by rTMS [74, 75].

Future Directions and Conclusions

Evidence on rTMS therapeutic potential in alcohol addiction is encouraging, but results are controversial and the heterogeneity of methods and stimulation areas does not allow for extrapolating a standardized protocol that may be used in clinical practice. Response to rTMS is variable [76], making it necessary to find reliable biomarkers to monitor the neuroplastic changes underlying the therapeutic effect. In the last decade studies on brain connectivity with fMRI and qEEG have shown that behavioral responses to rTMS correlate with changes in brain EEG rhythms [44], resting-state connectivity [77], and grey matter volume [78]. These ongoing observations will help to further elucidate the mechanism of action of brain stimulation and to increase the arsenal of available suitable biomarkers to select patients who may benefit from brain stimulation.

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Chapter 20
The Clinical Benefits of Non-abstinent Outcomes in Alcohol Use Disorder Treatment: Evidence from Clinical Trials and Treatment Implications

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Abstract Abstinence from alcohol has historically been the focal endpoint in clinical trials evaluating alcohol use disorder treatment, yet more recent work has provided compelling evidence that drinking reductions, short of total abstinence, are achievable, stable, and associated with improvements in how individuals with alcohol use disorder feel and function over time. A growing body of literature has examined the achievability, sensitivity to treatment effects, stability, and clinical benefits of the non-abstinent outcomes suggested by the Food and Drug Administration and European Medicines Agency. This chapter aims to review this evidence from clinical trials and discuss the implications for alcohol use disorder treatment. The reviewed body of literature indicates that non-abstinent goals, including no heavy drinking, reduction in World Health Organization risk drinking levels, and non-consumption outcomes (e.g., craving), are more achievable than abstinence, sustainable over long periods following treatment, and associated with clinical benefits, such as reductions in the physical and psychosocial consequences of drinking and improved mental health and quality of life. Overall, the reviewed literature supports the feasibility of drinking reduction goals and broader non-consumption functional outcomes in clinical practice.

Keywords Alcohol use disorder · Reductions in drinking · Clinical trials · World Health Organization risk drinking levels · Quality of life

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Introduction and Background

Abstinence from alcohol has historically been the focal endpoint in clinical trials evaluating alcohol use disorder (AUD) treatments [1, 2], which reflects the broader discourse surrounding AUD etiology and treatment. The dispositional disease model of addiction suggests that addiction is a "disease" confined to a small proportion of the population who are "powerless" over alcohol, thus necessitating complete abstinence to prevent excessive alcohol consumption and consequences [3–5]. The disease model of addiction had a pervasive influence on AUD treatment in the United States (U.S.) and Europe to a somewhat lesser extent [6]. Most notably, the disease model of addiction influenced the 12-Step Model of Alcoholics Anonymous (A.A.), which is readily accessible and free worldwide [7]. There are some benefits to abstinence as a primary outcome in AUD clinical trials. For example, abstinence is easy to quantify, potentially less prone to recall bias, and sensitive to biomarkers of alcohol consumption, such as phosphatidylethanol (PEth), a direct alcohol biomarker, and the liver enzyme γ-glutamyltransferase (GGT) [8]. Nevertheless, requiring alcohol abstinence in AUD treatment is based on an outdated understanding of AUD etiology, heterogeneity, and diagnosis [1, 9], and there are also costs of a sole focus on abstinence as an outcome in AUD treatment and clinical trials with respect to efficacy of interventions (most reduce drinking and few achieve abstinence) [10] and patient goals (most prefer drinking reduction) [11].

The purpose of clinical trials for AUD treatment is often to evaluate novel interventions but evaluating abstinence alone might preclude the identification of potentially effective interventions. If an endpoint required by regulating bodies is too challenging to meet, the pharmaceutical industry might be unwilling to spend resources pursuing medications development [9, 12]. Furthermore, mechanisms of action of select psychotherapies and pharmacotherapies might be better suited, or equally well suited, to drinking reduction than abstinence. For example, cognitive-behavioral treatments for AUD often provide skills to reduce excessive alcohol consumption once drinking has been initiated (e.g., setting drink limits) [13, 14]. Specific pharmacotherapies (e.g., naltrexone) have also demonstrated reductions in drinking intensity, but not abstinence, which is consistent with the mechanisms of these medications, to reduce the reinforcing properties of alcohol, including during acute alcohol consumption [15–17].

Not only is abstinence often inconsistent with the proposed mechanisms of AUD treatment, but it is inconsistent with patients' goals for treatment. There is a significant treatment gap for those with alcohol use disorder (AUD) in the U.S. and Europe [18, 19], and not being ready to stop drinking is a reason for not entering treatment

[20, 21]. Indeed, high rates of individuals entering treatment report drinking reduction as a goal [5, 22]. Surveys of individuals in recovery indicate that non-consumption outcomes, such as quality of life, substance use reduction, and meeting basic needs, are the most important outcomes [23]. Providers who treat patients with AUD are also more concerned with quality of life as an outcome than abstinence [24].

There is also growing acceptance of non-abstinent (i.e., moderated) drinking outcomes among various stakeholders, including the scientific community and treatment providers. Guidelines for AUD treatment in several countries support drinking reduction goals [25, 26], and the NIAAA definition of recovery emphasizes remission from AUD, cessation of heavy drinking, and functional outcomes [27]. Recent surveys with treatment providers indicate that greater than half reported moderated drinking as an acceptable treatment outcome [28], with greater support in Australia and Europe than in the U.S. and Canada [29]. These shifting viewpoints on acceptable AUD treatment outcomes extend to AUD clinical trials. A recent review of AUD pharmacotherapy trials indicates more recent trials were far more likely to report reduced consumption as an outcome than prior trials [30].

The increased acceptance of non-abstinence outcomes is mirrored in recent changes to endpoints supported by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) to approve medications for AUD. In their approval of Vivitrol® (i.e., extended-release injectable naltrexone) in 2006, the FDA defined the percent of subjects with no heavy drinking days (4+/5+ for women/men; [31]) as the primary endpoint of Phase 3 alcohol pharmacotherapy trials to receive approval for a New Drug Application [32, 33] and this was solidified in later reports [34]. Recently, the European Medicine Agencies [35] also explicitly outlined the following endpoints, including non-abstinent endpoints: i) Continued abstinence after detoxification or the medication "grace period" (i.e., the time over which the medication is expected to begin exerting effects), ii) reductions in total alcohol consumption (grams of ethanol) and heavy drinking days (40+/60+ grams of alcohol for women/men), and iii) the proportion of subjects with a significant shift in World Health Organization (WHO) risk drinking levels, which is defined as a 2-level shift or greater (see Table 20.1 for a description of WHO risk drinking levels).

A growing body of literature has examined the achievability, sensitivity to treatment effects, stability, and clinical benefits of the non-abstinent outcomes suggested by the FDA and EMA and other non-abstinent outcomes. This chapter aims to review this evidence from clinical trials and discuss the implications for AUD treatment.

Table 20.1 Overview of the advantages and disadvantages of consumption, non-consumption, and composite outcomes used in alcohol use disorder clinical trials

Outcome		Definition	Advantages	Disadvantages
Consumption outcomes	Abstinence	Continued abstinence from alcohol over a specific period, including the entire treatment period, the last month of treatment, or following a medication's "grace period" over which it is expected to exert effect	• Easy to quantify	• Difficult to attain and pharmaceutical companies might be unwilling to pursue medications development
			• Potentially less prone to recall bias	• Inconsistent with the mechanisms of some medications and psychosocial treatments that target reduced drinking
			• Sensitive to biomarkers	• Inconsistent with the goals of many patients interested in drinking reduction, reduced consequences, and improved quality of life
				• May serve as a barrier to treatment seeking
	No Heavy Drinking Days	Absence of heavy drinking days (4+/5+ drinks for men/women) over a specific period, including the entire treatment period, the last month of treatment, or following a medication's "grace period" over which it is expected to exert effect	• Higher achievement rates than abstinence	• Does not perform better than other arbitrary drinking cutoffs
			• As sensitive to active treatments as abstinence and other commonly assessed outcomes (e.g., drinking consequences)	• Ignores the linear nature of the association between level of alcohol consumption and psychosocial functioning/findings that any reductions in drinking are associated with improved functioning
			• Appears to be sustainable up to 1 year following treatment	• Less sensitive to active treatments than continuous outcomes, such as percent heavy drinking days
			• Concurrently and prospectively associated with lower alcohol consumption/ consequences, greater physical/mental health, and lower healthcare costs than any heavy drinking	• Ignores body weight and different effects of differing number of drinks depending on body weight
				• International comparisons are difficult given differing standard drink definitions across countries

	WHO Risk Drinking Levels	A 1- or 2-level decrease in the following risk levels from baseline to the last 4 weeks of treatment:	• An interpretable framework for clinicians and regulation agencies to evaluate drinking reductions	• Cutoffs limit variability in outcomes and continuous outcomes (e.g., percent heavy drinking days) may be more sensitive to treatment effects
		• Abstinence: 0 g of ethanol or standard drinks[a]/day for both males and females	• Higher achievement rates than abstinence and no heavy drinking days	• Sensitive to treatment effects has primarily been examined for medications/unclear if WHO risk levels are sensitive to active psychosocial treatments
		• Low risk: 1–40 g ethanol/day or > 0–2.86 standard drinks/day for males; 1–20 g ethanol/day or >0–1.43 standard drinks/day for females	• Sensitive to active treatment effects; in some cases, more sensitive than abstinence and no heavy drinking days	• Validation studies are needed in more diverse patient populations, including those outside of the U.S. and the U.K. and those with co-occurring psychiatric disorders
		• Medium risk: 41–60 g ethanol/day or 2.87–4.29 standard drinks/day for males; 21–40 g ethanol/day or 1.44–2.86 standard drinks/day for females	• High stability rates up to 3 years post-treatment	
		• High risk: 61–100 g ethanol/day or 4.30–7.14 standard drinks/day for males; 41–60 g ethanol/day or 2.87–4.29 standard drinks/day for females	• Reductions in risk levels are concurrently and prospectively associated with improved mental health outcomes, quality of life, physical health outcomes, and reduced alcohol consequences	
		• Very high risk: ≥101 g ethanol/day or ≥7.15 standard drinks/day for males; ≥61 g ethanol/day or ≥ 4.30 standard drinks/day for females	• Findings consistent across those with mild/moderate/severe alcohol dependence and those with any drinking and abstinence at the end of treatment	

(continued)

Table 20.1 (continued)

Outcome		Definition	Advantages	Disadvantages
Non-Consumption outcomes	Alcohol Temptation/ Craving	Various measures of the strength and/or frequency of alcohol temptation/craving have been used, including the Alcohol Abstinence Self-Efficacy Scale, the Obsessive-Compulsive Drinking Scale, and a single item measure (not at all tempted to drink vs. a little, somewhat, considerable, extremely tempted to drink), during treatment and post-treatment	• Consistent with conceptualizations of the etiology of AUD and DSM-5 AUD criteria	• Limited research on sensitivity to active treatment effects
			• Sensitive to change from pre- to post-treatment	• Less sensitive to change from pre- to post-treatment than alcohol consumption outcomes
			• Alcohol temptation/craving during and post-treatment predicts long-term alcohol consumption/consequences	
			• Demonstrated validity of a practical, single-item, binary measure	
	Alcohol Consequences	Measured with the Drinker Inventory of Consequences at post-treatment, which assesses the frequency of physical, interpersonal, intrapersonal, impulse control, and social responsibility consequences from alcohol use	• Reflected in DSM-5 AUD criteria	• Inconsistent associations with longer-term consumption outcomes
			• Consistent with patients' desires to reduce harmful alcohol use in treatment	• Some evidence indicating poor psychometric properties of the Drinker Inventory of Consequences
			• Consistent with the mechanisms of treatment (e.g., a treatment might promote safer drinking, without necessarily reducing consumption)	
			• Sensitive to active treatment effects	
			• Demonstrate similar pre- to post-treatment effect sizes as consumption outcomes	
	Quality of Life	Many measures of quality of life are available, though the World Health Organization Quality of Life measure (WHOQOL-BREF) at post-treatment was used in the reviewed literature	• Of all outcomes, most aligned with patients' and providers' goals in AUD treatment	• Mixed findings on sensitivity to active treatments—has not been sensitive to topiramate treatment effects
			• Sensitive to improvements from pre- to post-treatment	• Limited research on the predictive validity for long-term alcohol use and functioning outcomes
			• Sensitive to active treatment effects in naltrexone and nalmefene trials	• Many measures of quality of life available and used, rendering comparisons across studies difficult

Composite outcome	A composite of alcohol consumption and consequences (measured with the Drinker Inventory of Consequences), resulting in four categories: (1) abstinent, (2) moderate drinking without problems, (3) heavy drinking or problems, and (4) heavy drinking and problems; categories 1 and 2 are considered remission	• Higher achievement rates than abstinence	• Limited research on the predictive validity for long-term alcohol use and functioning outcomes
		• Concurrently associated with alcohol consumption, mental health, social support, employment, and purpose in life, with those in the remission categories reporting better outcomes than those with heavy drinking and/or problems	• Limited research on sensitivity to active treatment effects
			• Evidence that group 3 (heavy drinking or problems) is not reliably different than those in the remission categories on functioning

[a]Standard drinks = 14 g of alcohol

Non-abstinent Endpoints in AUD Clinical Trials

No Heavy Drinking Days ("Low-Risk Drinking")

Given that the FDA has identified the percentage of participants with no heavy drinking days (NHDD) at the end of treatment, also called "low-risk drinking," as an endpoint for Phase 3 alcohol clinical trials, recent research has examined the achievability, sensitivity to treatment effects, stability, and clinical benefits of this outcome.

Advantages

In an initial examination, Falk and colleagues [32] combined data from two clinical trials—the COMBINE study, in which participants in the U.S. were randomized to nine treatment conditions (i.e., placebo, acamprosate, and/or naltrexone in combination with Medical Management, with or without a combined behavioral intervention) and a placebo-controlled trial of topiramate in combination with motivational enhancement—and showed that NHDD was substantially more achievable than abstinence. In COMBINE, which required a period of abstinence before randomization, 44.0% of participants who received naltrexone reported NHDD during the last 2 months of treatment, and 31.1% reported abstinence. Abstinence was not required before randomization in the topiramate trial, and 13.6% of participants who received topiramate reported NHDD during the last 2 months of treatment, compared with 5.6% who reported abstinence. The effect sizes for active interventions compared to control conditions on NHDD were similar to the effect sizes for abstinence and other commonly-assessment outcomes (e.g., drinking consequences, percent days abstinence) [32].

The NHDD outcome also appears to be relatively stable across long-term follow-ups. Another analysis conducted by Witkiewitz and colleagues [36] used data from the COMBINE study to identify two latent groups of participants during treatment, those with a high probability of NHDD and those with a high probability of at least some heavy drinking days, and the stability of these groups over three times: during treatment, the 3 months immediately following treatment, and 1 year after treatment. This analysis showed transitioning from the NHDD group to the group with some heavy drinking days was the highest in the first month of treatment (14.3%), but after treatment more than 90% of individuals remained in the NHDD group up to 12 months following treatment.

Achieving low-risk drinking has also demonstrated clinical benefits. In the COMBINE trial, participants with NHDD during the last 2 months of treatment reported significantly lower alcohol consumption and consequences immediately and 1-year post-treatment than those with any heavy drinking [32]. In the same

study, abstainers reported significantly lower drinking intensity and consequences post-treatment than low-risk drinkers (i.e., non-abstinent and NHDD), but there were few differences between these two groups in drinking intensity and consequences over the 1-year follow-up period [32]. Additionally, low-risk drinking during treatment and 6 months following treatment was prospectively associated with better mental and physical health outcomes over long-term follow-ups (i.e., 1–3 years post-treatment) than heavy drinking in several clinical trials, including COMBINE, Project MATCH (i.e., U.S. trial with three psychosocial intervention conditions), the United Kingdom Alcohol Treatment Trial (UKATT; i.e., trial with two psychosocial intervention conditions), and two trials conducted in a large, private, nonprofit integrated health care delivery system in the U.S [37–39]. Lastly, those with NHDD during the last month of treatment have been found to have lower healthcare costs over the year following treatment than those with any heavy drinking [40]. Those with NHDD 6 months following treatment also have lower inpatient and emergency department utilization over the 5 years following treatment than those who engaged in some heavy drinking [41].

Disadvantages

The NHDD outcome also has several limitations. Perhaps most notably, there is strong evidence for a linear association between alcohol consumption and functioning, indicating that any reductions in alcohol consumption represent improvements [2, 42, 43]. Indeed, individuals above "low-risk drinking" cutoffs still demonstrate substantial reductions in drinking [36], and there is significant heterogeneity concerning psychosocial functioning within individuals with some heavy drinking, who are typically considered a homogenous group of "failures" [44]. Similarly, some heavy drinking during treatment is still associated with better long-term alcohol consumption, physical/mental health outcomes, and healthcare costs than persistent heavy drinking [37, 38, 40]. Given these concerns, previous analyses have compared alcohol consumption cutoffs and shown that various cutoffs do not perform better than each other in predicting end-of-treatment and 1-year post-treatment alcohol consumption and consequences, indicating the 4+/5+ definition is arbitrary [45] and that the "ideal" cutoff for maximum drinks for greatest sensitivity and specificity in predicting drinking consequences was substantially higher than current heavy drinking definitions [46]. NHDD also ignores differences in body weight (e.g., five drinks for a man who weighs 300 lbs. or 136 kg versus a man who weighs 150 lbs. or 68 kg will have considerably different effects on health and functioning) and makes international comparisons difficult given differences in standard drink sizes across countries (8 g in the UK to 20 g in Austria). Lastly, NHDD is less sensitive to active treatment effects and more susceptible to inaccuracies in standard drink estimations during data collection than continuous outcomes, such as percent heavy drinking days [47].

World Health Organization Risk Drinking Level Reductions

Reductions in drinking intensity (e.g., drinks per day/drinks per drinking day, total alcohol consumption) are commonly reported in alcohol clinical trials, but the WHO risk levels attempt to formalize these reductions to provide an interpretable framework for clinicians and regulation agencies [42]. In recognizing these formalized cutoffs, the EMA recently recognized a 2-level reduction or greater (e.g., from very high risk to medium risk; see Table 20.1) as an endpoint in alcohol clinical trials for medication development. Accordingly, there has been a proliferation of recent research validating the WHO risk level reductions in both epidemiological samples [48–51] and clinical samples [52–56]. These recent analyses have found clinical benefits of both 2-level and 1-level reductions, consistent with research indicating any reduction in heavy drinking is associated with improved functioning.

Advantages

Multiple analyses of large clinical trials, including pharmacotherapy and psychosocial treatment trials, indicate that 1- and 2-level reductions in WHO risk drinking levels are more achievable than abstinence and NHDD. For example, rates of achievement for each outcome during the last month of treatment range from 0% to 41.6% for abstinence, 1.9% to 51.0% for NHDDs, 11.5% to 77.1% for WHO 2-level reductions, and 37.2% to 87.6% for WHO 1-level reductions [10, 57–59]. These reported rates are across active and control conditions and treatments targeting abstinence and reduced drinking. However, WHO risk drinking level reductions are sensitive to active treatment effects, including naltrexone, nalmefene, varenicline, and topiramate [10, 59, 60]. In several cases, WHO risk drinking level reductions have been more sensitive to treatment effects than abstinence and NHDD [10, 59].

WHO risk reductions appear to be relatively stable across long-term follow-ups. Among individuals in the COMBINE study who achieved at least a 1-level reduction at the end of treatment, 85.5% reported maintaining this reduction at the 1-year follow-up [54], and 84.9% maintained this reduction at the 3-year follow-up [57]. Similarly, among those who achieved at least a 2-level reduction at the end of treatment, 77.8% and 77.3% maintained this reduction at 1 and 3 years post-treatment, respectively [54, 57].

Several studies indicate that WHO risk reductions capture clinically meaningful benefits across several categories of functioning and patient groups. Specifically, reductions in WHO risk levels over treatment are associated with improved mental health outcomes, quality of life, and physical health outcomes (e.g., systolic blood pressure, liver enzyme levels) and reduced alcohol use consequences post-treatment, 1 year following treatment, and 3 years following treatment [52–54, 57]. Findings suggest that even a 1-level reduction is associated with better outcomes,

though greater drinking level reductions correspond to greater improvements in mental health and reductions in drinking consequences [52]. Furthermore, these clinical benefits are consistent across those with mild, moderate, and severe alcohol dependence and when excluding those who were abstinent at the end of treatment [58].

Disadvantages

WHO risk drinking levels empirically demonstrate advantages over abstinence and NHDD and are likely more aligned with patients' goals to reduce drinking and improve functioning. However, issues that plague any cutoff extend to WHO risk drinking levels. Cutoffs limit variability in outcomes, and therefore continuous outcomes, such as percent heavy drinking days and alcohol consequences, may be more sensitive to treatment effects [59]. Although the stability and clinical benefits of WHO risk drinking level reductions have been evaluated in both pharmacotherapy and psychosocial treatment trials [57, 58], it is unclear if these outcomes are sensitive to active psychosocial treatment effects [61]. Similarly, future research is needed to validate WHO risk drinking level reductions among various client groups, including additional samples outside the U.S., Europe, and the U.K., and among treatment-seeking individuals with co-occurring psychiatric disorders [61].

Non-Consumption Outcomes

Although recent research has advanced the validity and application of non-abstinent consumption outcomes, there are several disadvantages to all consumption outcomes. Non-consumption outcomes, such as craving, quality of life, alcohol consequences, and social support, are more aligned with patients' goals [18], biopsychosocial models of AUD [62], and DSM-5 AUD criteria [63]. Although the FDA and EMA only consider consumption outcomes in the approval of AUD medications, guidance from the FDA explicitly states that consumption is a surrogate endpoint for how a patient thinks and feels, given assumptions that clinical trials would need to be impractically long and large to identify an effect on psychosocial outcomes [34] and EMA guidance includes many non-consumption outcomes as secondary endpoints [35]. Accordingly, it is essential to examine how non-consumption outcomes compare to consumption outcomes in AUD clinical trials. Of note, most clinical trials for AUD examine non-consumption secondary outcomes, and therefore we aim to present a non-exhaustive review of studies that directly compared commonly evaluated non-consumption outcomes to consumption outcomes.

Alcohol Temptation/Craving

Craving is considered a central feature of AUD, reflected in its inclusion as a DSM-5 AUD criterion [64], and is often a target in psychosocial and behavioral treatments for AUD due to its strong association with alcohol use following treatment [65]. Analyses of data from COMBINE and Project MATCH indicate that various measures of temptation to drink/craving, including a single-item measure of temptation to drink, temptation measured via the Alcohol Abstinence Self-Efficacy Scale (AASE) [66], and craving measured via the Obsessive Compulsive Drinking Scale (OCDS) [67] are sensitive to change from baseline to post-treatment [68, 69]. A single-item measure of temptation to drink at the fourth treatment session in Project MATCH was associated with drinking frequency, quantity, and consequences 1 year following treatment and drinking quantity 3 years following treatment. A dichotomized version of this temptation item (i.e., not at all tempted to drink versus a little/somewhat/considerable/extremely tempted to drink) had more sensitivity for long-term drinking outcomes than alcohol consumption during treatment [69]. Similarly, an analysis of both COMBINE and Project MATCH showed that post-treatment alcohol temptation and craving, measured via the AASE and OCDS, respectively, predicted 1-year post-treatment consumption outcomes [68].

The strengths of craving as an outcome include its strong concurrent and predictive association with alcohol consumption, relevance to AUD diagnosis and recovery, and evidence that craving can be modified with AUD treatment and is sensitive to the effects of AUD medications, such as naltrexone [62, 65, 68, 69]. Alcohol temptation can also be measured via a practical, single-item, binary measure that has demonstrated predictive validity [69]. Nevertheless, there is the need to determine the extent to which alcohol temptation/craving is sensitive to active treatments instead of purely pre- to post-treatment change. This future direction is particularly important given that alcohol temptation/craving may be less sensitive to change during treatment than alcohol consumption outcomes [68].

Alcohol Consequences

Like craving, alcohol consequences are also reflected in DSM-5 AUD criteria (e.g., social/interpersonal problems and physical/psychological problems caused or exacerbated by alcohol; [64]) and consistent with patients' desires to reduce harmful alcohol use [23]. Furthermore, it is feasible that a patient receiving AUD treatment could reduce consequences without reducing drinking, given treatments often target reducing harms associated with alcohol use, rather than focusing on consumption [46]. Indeed, drinking consequences, measured via the Drinker Inventory of Consequences (DrInC), are often a secondary outcome in AUD clinical trials and are sensitive to treatment effects (e.g., nalmefene, topiramate) [46, 47] and demonstrate similar pre- to post-treatment effect sizes as consumption outcomes [47]. However, post-treatment drinking consequences have demonstrated inconsistent associations with longer-term consumption outcomes, which may be due to poor psychometric properties of the DrInC [68].

Quality of Life

Of all non-consumption outcomes reviewed, improved quality of life in treatment is the most aligned with patients' and providers' goals [23, 24]. An evaluation of quality of life as an outcome in COMBINE, using the World Health Organization Quality of Life measure (WHOQOL-BREF), found that quality of life was sensitive to improvements from the baseline to 10-week post-treatment follow-up assessment [70]. Previous analyses have also found that naltrexone and nalmefene improve quality of life compared to placebo conditions [17, 71]. An analysis of two topiramate trials found a small, albeit non-significant, effect of topiramate versus placebo on quality of life [59]. Other limitations of quality of life as an outcome include limited research on its long-term predictive validity and the preponderance of measures used to assess quality of life, which make comparison across studies difficult [72]. Given the relevance of quality of life to patients' treatment goals, future research validating this outcome, including patient-centered approaches to measurement development [73], is needed.

Composite Outcome of Consumption and Non-consumption Outcomes

To balance the strengths and weaknesses of both consumption and non-consumption outcomes, some have suggested using a composite outcome that combines information on alcohol consumption and consequences. Specifically, using this composite outcome, patients are classified into four categories: (1) abstinent, (2) moderate drinking without problems, (3) heavy drinking or problems, and (4) heavy drinking and problems [74, 75], with categories 1 and 2 considered as being in remission. Of note, the designation "without problems" includes problems that are not recurrent (i.e., never happening or happening only happening once or twice over the assessment period), as opposed to the complete absence of consequences, consistent with DSM-5 AUD criteria that consider recurrent problems [64]. Findings from Project MATCH indicate that remission measured with the composite outcome is more achievable than abstinence alone, with approximately 50% of patients categorized as being in remission over post-treatment and long-term follow-ups (up to 1-year post-treatment), compared with 21% to 36% who reported abstinence [74, 75]. Concurrent validity analyses indicate that the composite outcome is associated with alcohol use frequency and quantity, consequences, and psychosocial outcomes (e.g., mental health, social support, employment, purpose in life) at 1-year post-treatment, with those in remission reporting better outcomes than those with heavy drinking and/or alcohol problems.

However, the predictive validity of this outcome has not been examined, and additional examinations of category 3 (heavy drinking or problems) indicate that this group is not reliably different from those in the remission categories on measures of functioning, which appears to be driven by high functioning individuals who report heavy drinking [74, 75]. The composite outcome is also prone to the weaknesses of both consumption and non-consumption outcomes, including

limited research on the predictive validity and sensitivity to treatment effects (consistent with non-consumption outcomes) and the arbitrary nature of heavy drinking cutpoints and evidence for individuals with some heavy drinking who are high functioning (consistent with consumption outcomes).

Applying Research on Non-abstinent Endpoints to Clinical Practice

The reviewed body of literature indicates that non-abstinent goals, including no heavy drinking, reduction in drinking risk levels, and non-consumption outcomes, are more achievable than abstinence, sustainable over long periods following treatment, and associated with clinical benefits, such as reductions in the physical and psychosocial consequences of drinking and improved mental health and quality of life. Accordingly, non-abstinence goals should be routinely discussed and targeted in AUD treatment. Figure 20.1 provides a schematic of steps for selecting a treatment goal and treatment modality and monitoring treatment goals for non-abstinent and abstinent outcomes.

Selecting a Treatment Goal

Clinicians treating AUD should openly and non-judgmentally ask patients about their goals in treatment and explicitly communicate that both non-abstinent and abstinent outcomes are achievable in treatment and associated with clinical benefits. Based on the reviewed clinical trials, potential treatment goals to discuss with

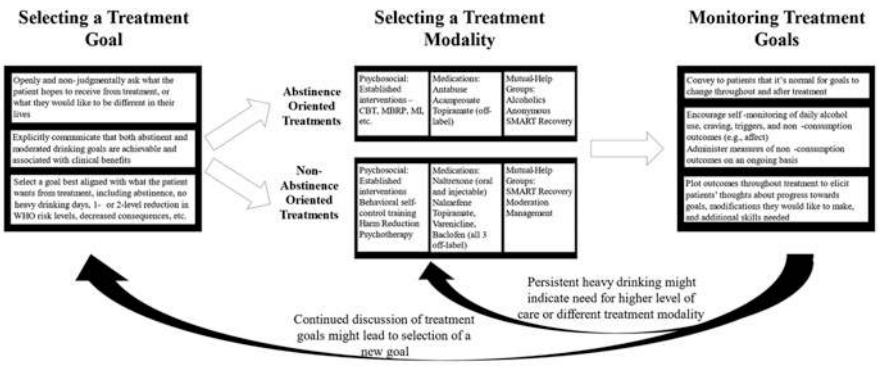

Fig. 20.1 Steps for selecting a treatment goal and treatment modality and monitoring treatment goals for non-abstinent and abstinent outcomes. CBT, cognitive-behavioral therapy; MBRP, mindfulness-based relapse prevention; MI, motivational interviewing; SMART Recovery, self-management and recovery training

patients might include reductions in drinking intensity, including no heavy drinking days or reductions in WHO risk level, or in drinking consequences. Such conversations might improve treatment engagement, rapport, motivation, and hope for a good outcome in treatment, given the high rates of individuals who desire [5, 22] and achieve non-abstinent outcomes [5].

Some treatment providers might have concerns about whether a non-abstinent treatment goal is a good fit for a given patient. Prior research indicates that individuals who can successfully achieve non-abstinent outcomes tend to have lower AUD severity and alcohol consumption at treatment entry, fewer negative mood symptoms, fewer heavy drinkers in their social network, and higher confidence and commitment to change [76–78]. However, a recent meta-analysis and meta-regression found that overall AUD severity within a clinical trial did not predict better outcomes in abstinent-focused or non-abstinent-focused treatment [79].

Interestingly, the patient characteristics that predict a lower likelihood of achieving moderated drinking (e.g., greater drinking intensity and consequences, poorer mental and physical health) are aligned with who is most likely to self-select an abstinence goal [22]. Drinking goal is also related to the achieved treatment outcome. Patients who select an abstinence goal are more likely to report abstinence following treatment, and those preferring non-abstinence are more likely to report a "non-problem" drinking outcome [80]. These findings indicate that providers should support free goal choice among patients instead of selecting a treatment goal for patients based on their characteristics. Clinical trials encouraging free goal choice produce similar outcomes to trials that require abstinence or moderated drinking [79]. Most importantly, evidence and theory suggest that patients prefer to choose their goals, are more committed and motivated when choosing their own goals, and are more likely to achieve their goals when they have free goal choice [5].

Selecting a Treatment Modality

Patients' drinking goals should be considered when selecting and delivering AUD treatment. Appropriate interventions for non-abstinent drinking goals will briefly be described below, and see Paquette and colleagues for a more detailed overview [5].

Psychosocial Interventions

Most psychosocial interventions for AUD were developed and evaluated with abstinence endpoints, but non-abstinence goals can be incorporated into most, if not all, established interventions, including cognitive-behavioral therapy (CBT) [81], motivational interviewing (MI) [82], and mindfulness-based relapse prevention (MBRP) [83], among others [5]. For example, CBT for AUD significantly impacts alcohol use frequency *and* quantity outcomes [14], and all skills presented in CBT (e.g., engaging in non-substance use reinforcing activities, planning for high-risk

situations) can be applied to moderated drinking goals. Meta-analyses of MI have also shown impacts on reduced alcohol consumption [84], and the spirit of MI, including collaboration between the therapist and client, evoking the client's ideas about change, and supporting a client's autonomy, is fundamentally at odds with mandating abstinence [82]. MBRP has also demonstrated impacts on reduced drinking intensity among individuals with both abstinent and non-abstinent treatment goals [85]. Given this widespread evidence that psychosocial interventions for AUD can be applied to non-abstinent outcomes, perhaps the most significant consideration when administering such treatments to a client with a moderated drinking goal is to ensure that examples and suggestions are tailored to non-abstinent drinking.

Several psychosocial interventions have been developed explicitly for moderated drinking, though the evidence base for these interventions is sparse compared to the interventions discussed above. Behavioral self-control training, a treatment that helps patients reduce drinking intensity by pacing drinks, identifying high-risk situations for heavy drinking, and goal setting, demonstrates better outcomes than no intervention, with outcomes similar to abstinence-oriented treatments [86]. Harm reduction psychotherapy, which takes a biopsychosocial approach to modify a patients' emotional, family, social, and medical problems without requiring abstinence, has been extensively described but has not received much empirical investigation [5, 87].

In conclusion, many psychosocial interventions are appropriate for patients interested in reducing drinking, if treatment providers are cognizant of providing appropriate examples and suggestions. Further supporting this conclusion, a recent meta-analysis showed that achievement of "successful" alcohol treatment outcomes did not depend on whether psychotherapies are targeted to abstinence or moderated drinking [79].

Pharmacological Interventions

Unlike psychosocial treatments, some pharmacological interventions may be better suited to either non-abstinent or abstinent treatment goals. Most notably, disulfiram (FDA and EMA approved for AUD) is only indicated for individuals who plan to abstain from alcohol use. This is due to the medication's mechanism of action, blocking the conversion from acetaldehyde to acetic acid during alcohol consumption, resulting in an upsurge of acetaldehyde and making an individual ill if they consume alcohol [88]. There is also evidence that acamprosate (FDA and EMA approved) may be more effective in promoting abstinence than reducing heavy drinking [89]. Acamprosate targets glutamatergic neurotransmission, may have larger effects on abstinence than heavy drinking, and appears to be more effective when patients have undergone alcohol detoxification before initiating acamprosate treatment [89].

Concerning pharmacotherapies appropriate for non-abstinence treatment goals, a relatively large body of literature indicates that oral and injectable naltrexone (FDA and EMA approved) effectively reduces heavy drinking [15–17, 88, 89].

Evidence also suggests the utility of targeted oral naltrexone to reduce drinking intensity during situations that are high risk for heavy drinking [90, 91]. These findings are consistent with naltrexone's mechanism of action as an opioid antagonist, which may decrease reinforcing properties of alcohol, thus decreasing drinking intensity during alcohol consumption [88]. Similar findings have been demonstrated for nalmefene, an opioid antagonist approved by the EMA [88].

Several medications used off-label for AUD have also demonstrated efficacy for reducing heavy drinking and, therefore, may be appropriate for non-abstinent drinking goals. Of these, topiramate has the most substantial evidence base [11, 16, 92]. Topiramate is an anticonvulsant drug that is FDA and EMA approved and has a complex mechanism of action, including state-dependent blocking of voltage sensitive sodium channels, increased $GABA_A$ neuronal activity, and antagonism of glutamate [88, 93]. Meta-analyses indicate that topiramate has significant impacts on abstinence and heavy drinking [92] and might be more effective in reducing total alcohol consumption than active FDA and EMA-approved medications [16]. Varenicline, an alpha4beta2 nicotinic receptor partial agonist, baclofen, a $GABA_b$ agonist, and gabapentin, which binds voltage-sensitive calcium channels to modulate GABA and glutamate activity, have low quality or mixed results [11, 88, 94, 95] but may be appropriate for off-label use among patients with non-abstinence drinking goals [10, 11, 16]. Concerning populations who may respond best to these medications, varenicline may be appropriate for heavy drinking smokers and heavy drinking males [96], baclofen may be most effective for heavier drinkers [97], and gabapentin may be most effective for those with more alcohol withdrawal symptoms [95].

Mutual Help Groups

Treatment providers might also encourage engagement in mutual-help groups to enhance social support for reducing alcohol use [98]. However, providers might be ambivalent about recommending participation in 12-step mutual-help groups, such as A.A., given that these groups target abstinence and are based on the dispositional disease model of addiction, which assumes individuals are "powerless" over any alcohol consumption [5, 98]. Indeed, prior A.A. engagement predicts an abstinence drinking goal compared to a controlled drinking goal, indicating that the theoretical basis of 12-step groups may be incompatible with a moderated drinking goal [99, 100].

Self Management and Recovery Training (SMART) Recovery or Moderation Management groups may be more appropriate for patients with non-abstinence goals. SMART Recovery teaches tools and techniques for drinking reduction, including enhancing motivation for change, coping with cravings, problem-solving, and engaging in reinforcing activities, including meetings [98, 101]. SMART Recovery acknowledges that individuals are on their own recovery pathways, does not exclude individuals who are actively drinking, and encourages the use of pharmacotherapies for AUD [98, 102]. Moderation Management is a mutual-help group

that more explicitly targets moderated drinking through increased awareness of alcohol consumption and consequences, setting limits, and skill-building to reduce drinking [98, 103].

Monitoring Treatment Goals

Given the benefits of drinking reduction and non-consumption outcomes, as reviewed, it is important to assess these outcomes throughout treatment. First and foremost, it is crucial to convey to patients that it is normal for goals to change throughout treatment [5, 79]. Treatment providers should encourage patients to self-monitor and record daily alcohol use, cravings, triggers for alcohol use/cravings, and other non-consumption outcomes (e.g., negative mood), and providers may also use repeated PEth testing to provide feedback to patients about effects of drinking on blood PEth levels over time. If indicated, providers might also administer measures of non-consumption outcomes during treatment, including measures of craving, consequences, and quality of life. When discussing patients' reported outcomes each session, treatment providers should openly elicit patients' thoughts about progress toward goals, potential modifications to goals, and additional skills needed to meet goals. Monitoring might also inform any need for additional treatment. For example, persistent heavy drinking and/or lack of drinking reductions may indicate the need for a higher level of care (i.e., detoxification, inpatient treatment, intensive outpatient treatment) or another treatment modality to support reductions in alcohol consumption and positive long-term outcomes.

Conclusions

Drinking reductions in alcohol treatment are achievable, sensitive to treatment effects, sustainable over long-term follow-ups, and associated with clinical benefits. Accordingly, drinking reduction outcomes should be assessed in clinical trials and drinking reduction goals should be routinely discussed with patients presenting to AUD treatment. Although more research is needed to operationalize non-consumption outcomes and develop assessments of these outcomes that are easy to measure and interpret, initial evidence suggests that craving, alcohol consequences, and quality of life demonstrate improvements following AUD treatment and are associated with other clinical benefits. Future studies might also develop patient-centered outcome measures [72] and personalized assessment tools responsive to individual goals [43]. Nearly all established psychotherapies and several pharmacotherapies for AUD support non-abstinence and non-consumption outcomes, indicating the feasibility of drinking reduction goals and broader non-consumption functional outcomes in clinical practice.

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Chapter 21
Laboratory Based Approaches to Medications Development for Alcohol Addiction

Steven J. Nieto, Suzanna Donato, Artha J. Gillis, and Lara A. Ray

Abstract Human laboratory models have been used in medication development for alcohol use disorder (AUD) for decades. Paradigms such as alcohol cue-exposure and alcohol administration provide initial efficacy of pharmacotherapies for AUD and co-administration of medication and alcohol is an important step in establishing safety. More recently, the medication development pipeline has included human laboratory testing as a recommended step in safety and initial efficacy testing of novel compounds. Nevertheless, the degree to which the effects of pharmacotherapy, versus placebo, are (un)detected in the human laboratory may or may not predict their likelihood of efficacy in a randomized controlled trial (RCT). The present review discusses the history and multiple paradigms available for medication development in AUD, then discusses the optimal role of human laboratory models in AUD medication development, next it reviews simulation studies comparing signal detection in the human laboratory versus RCTs, and finally, it reviews empirical data comparing the observed signal for various AUD medications in the human laboratory and in RCTs. Together, these sections provide historical, theoretical, and empirical data pertaining to how best leverage human laboratory models for AUD medication development.

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Introduction

Medication development for alcohol use disorder (AUD) is a costly and time-consuming endeavor. It is estimated that the average time from discovery to market for a given pharmaceutical in the United States is 13.5 years and costs roughly $1.8 billion dollars [1, 2]. Further, less than 1 in 10,000 compounds that are screened will successfully reach the pharmaceutical market [3]. Only four medications have passed this process and are approved by the FDA for the treatment of AUD (i.e., disulfiram, acamprosate, nalmefene, and naltrexone, the latter two medications being available in oral and injectable formulations). While the availability of pharmacological treatments for AUD indicates progress over the past 25 years [4], the therapeutic uptake of these medications is limited. This may be due to the high heterogeneity of AUD and limited effect size of any one medication. As is common when treating other psychiatric illnesses (e.g., depression), a cache of medication choices is optimal to enhance treatment outcomes [3]. However, the current process for developing and testing AUD medications limits the development of novel pharmaceutical agents.

The National Institute on Alcohol and Alcohol Abuse's (NIAAA) Medication Development Team was established with the key long-term goal of making the drug development process more efficient. The current model for testing pharmacotherapies places heavy emphasis on the clinical trial phase of testing, which requires considerable resources and often results in null findings. Clinical trials may always be the gold standard for establishing the safety and efficacy of medications, however, developing new ways to accelerate the process prior to reaching testing in patients may be the key to advancing clinical care for AUD. Laboratory models carry several unique advantages in relation to examining medication effects [5]. Firstly, they are less costly than clinical trials and offer more efficient approaches for screening (i.e., informing go-no go decisions). These models also involve a high level of experimental control, allowing for greater confidence in the therapeutic mechanisms of action and informing promising potential molecular targets. Human laboratory models also capture important between-person variability in medication response, permitting researchers to better predict subgroups of target responders. In sum, human laboratory studies are able to not only test *whether* these drugs are able to elicit desired therapeutic outcomes but also *how* they elicit their pharmacological effect [6] and in *whom*.

In addition to human laboratory approaches representing an important precursor to the clinical trial phase of medications development, they also are crucial for the translation of preclinical findings to clinical samples. While a detailed review of animal models for AUD pharmacotherapy development is outside the scope of this chapter (see [7] for an in-depth review of translational animal models), it is

important to note the significance of preclinical models in demonstrating early efficacy and exploring potential treatment mechanisms. The successful translation between early preclinical studies and later phase trials is critical for the medication development process [8]. Human laboratory models have the potential to act as important intermediaries of this process, facilitating the link between early preclinical studies and later clinical trial testing through congruent modeling [9, 10].

It is clear that human laboratory approaches represent a critical step in the medication development process due to their ability to pinpoint therapeutic mechanisms of action and fill an important translational gap between preclinical findings and large-scale clinical trials. Importantly, co-administration of the novel medication with alcohol in a controlled environment also provides necessary safety data. In line with the importance of this work, NIAAA started the Human Laboratory Program (HLAB), an initiative to standardize human laboratory paradigms for the purpose of reducing methodological variability and refining the screening process for promising medications. By establishing both reliable and valid screening models, the field of medications development can improve the timing and selection of promising compounds to move to next phase clinical trials.

In summary, the medications development process is a high priority, yet the current sequence of moving novel compounds to market encompasses many challenges. Further work is needed to refine the process and better predict which compounds hold the highest probability of success. Human laboratory models represent a critical tool for predicting key clinical outcomes and translating important work from the preclinical phase. This chapter will discuss key findings on the theoretical and observed value of human laboratory approaches in the medication development pipeline. While a comprehensive review of the range of human laboratory models available for medication screening is beyond the scope of this chapter, consideration will be given to recent novel contributions that have promising implications for human laboratory approaches. In their final remarks, the authors discuss critical gaps that still exist and future directions for the application of laboratory approaches in the medication development model.

Main Body

The Theoretical Predictive Value of Human Lab in Medication Development

General Overview of Theoretical Value

Human laboratory models were originally developed for the study of alcoholism etiology and were later adapted for the study of treatment efficacy mechanisms [5, 11]. These paradigms encompass a wide array of methods for collecting data on central features of addiction under well-controlled experimental conditions. In several areas of psychiatry, human laboratory models are used to advance medication

development and serve as a critical translational bridge between preclinical investigations and randomized clinical trials.

There are several human laboratory paradigms that have been developed to model addictive disorders (Table 21.1). Most of these have been applied to examine medication effects on intermediate AUD phenotypes. Some of the most common paradigms used in AUD medication trials include alcohol cue-reactivity and alcohol administration studies. The cue-reactivity paradigm involves passive exposure to alcohol-related cues that triggers the underlying motivational processes that contribute to alcohol drinking [27]. Reactivity to cues is usually assessed using subjective (e.g., self-reported craving) and physiological indicators (e.g., heart rate) alone or in combination. In the typical in vivo cue-reactivity paradigm, participants complete baseline physiological and subjective measures prior to initiating experimental

Table 21.1 Examples of laboratory paradigms applied to pharmacotherapy development for alcohol use disorder

Laboratory paradigm	Phenotype/ mechanism of action	Pharmacotherapy	Outcome	Citation
Positive and negative affective drinking cues	Cue-induced craving	Gabapentin	Decreased positive affect-induced craving	[12]
Delayed discounting task	Inhibitory control	Naltrexone	Reduced impulsive responding	[13]
Alcohol demand	Alcohol reinforcing value	Naltrexone	Reduced alcohol demand	[14]
Alcohol self-administration in a naturalistic setting	Priming dose craving	Naltrexone	Decreased self-administration, slower drinking progression	[15]
Alcohol administration	Subjective responses to alcohol	Naltrexone	Decreased stimulation	[16–19]
Stress Induction	Stress-induced craving	Lofexedine	Increased sedation	[20, 21]
		Ibudilast	Rapid recovery in positive mood post-stress	
Initial priming and self-administration of alcohol	Low self-control	Aripiprazole	Break in alcohol-induced stimulation leading to increased consumption	[22]
Alcohol cue-exposure	Negative affective withdrawal symptoms	Acamprosate	Reduction of autonomic nervous system reactivity, no decrease in craving	[23]
Alcohol cue-exposure in fMRI scanner	Neural alcohol reward signal	Ibudilast	Attenuated alcohol cue-induced activation in the ventral striatum	[24]
Priming dose of alcohol and cue-exposure	Cue and priming dose-induced craving	Olanzapine	Reduction in craving, mediated by DRD4 polymorphisms	[25, 26]

procedures. After completing baseline assessments, participants are instructed to interact (i.e., smell, touch) with a glass of water. After water cue exposure, participants are instructed to interact with a glass containing the participant's most commonly consumed alcoholic beverage. After both exposures (water and alcohol), participants rate their subjective craving and physiological measurements are taken. In addition to demonstrating a high level of reproducibility [28], the cue-reactivity paradigm is sensitive to medication effects. For example, alcohol cue-induced craving is blunted by FDA-approved medications for AUD, such as naltrexone [15, 29, 30], as well as several other pharmacotherapies including D-cylcoserine [31], gabapentin [12], mifepristone [32], olanzapine [33], prazosin [34], and quetiapine [35].

Passive (i.e., controlled administration or alcohol challenge) and active (i.e., alcohol self-administration) alcohol administration paradigm have also been used in medications development for AUD [36]. Passive administration paradigms consist of controlled alcohol infusions under laboratory conditions wherein subjective response to alcohol are the primary endpoints. Subjective responses to alcohol are typically assessed across four domains: stimulation, sedation, negative affect, and alcohol craving. Self-administration models that include fixed and progressive ratio schedules of reinforcement have also been used in translational AUD research [7]. Under the traditional progressive ratio schedule, the response requirement for an alcohol infusion gradually increases throughout the session.

Human laboratory models serve as an important step along the medication development continuum for AUD. In early phases, these models establish initial evidence of safety and tolerability of a candidate compound. This is a crucial step because safety concerns and negative side effects can hamper the drug from moving forward in the process towards FDA-approval. Equally important, human laboratory paradigms are used to examine potential medication and alcohol interactions. In the case of alcohol, it is important to assess for potential sedative effects of a medication, as sedative effects in combination with alcohol use can have severe health implications.

Human laboratory studies provide crucial means of exploring treatment targets ("early efficacy endpoints" also called "surrogate endpoints") for specific components of the AUD cycle without the need for time consuming and expensive RCTs. For example, human laboratory paradigms can provide a valuable opportunity to assess the feasibility of an approach prior to carrying out a costly and time-consuming clinical trial with actual efficacy endpoints (i.e., drinking outcomes across a 12-week trial [4].

There are a number of advantages that human laboratory models have over randomized clinical trials. Human laboratory studies can be well powered with a smaller sample size and consequently be less costly, thus making them better positioned to engage in hypothesis testing. However, this is under the important assumption that the laboratory outcomes being tested correlate with clinical efficacy (not always supported/tested in the literature). Human laboratory models are conducted under well-controlled experimental conditions, which affords researchers greater sensitivity to detect a medication signal on a target variable. This enhanced sensitivity may be due to the surrogate outcomes of craving and subjective response to alcohol having less variability than real-world drinking outcomes. Additionally, the

shorter duration of the human laboratory testing is often associated with higher medication compliance and lower attrition rates than 12-week RCTs. Given the multidimensional nature of addictive disorders, human laboratory models can provide more granular assessments of medication effects on intermediate phenotypes. Human laboratory methods can help elucidate the neurobiological bases by which a pharmacotherapy exerts its therapeutic effects. Of course, these paradigms are not without limitations. One notable limitation is that the outcomes tested in human laboratory models may not be robustly associated with the clinical outcomes of interest for pharmacotherapies. The better a paradigm can approximate drinking/clinical outcomes, the more likely it is to provide valuable information regarding the initial efficacy of a novel compound [10]. To that end, it is crucial that the field strives to identify and refine screening models.

Qualitative Reviews of the Consistency Between Behavioral Pharmacology and RCT Results

The positive translational value of human laboratory paradigms has been highlighted in qualitative reviews. In many instances, a positive medication signal on a human laboratory endpoint is related to a positive medication signal in clinical trials [36]. However, this viewpoint must be tempered considering the publication bias toward positive medication findings, particularly in the behavioral pharmacology literature. There are also more clinical trials, compared to human laboratory studies, conducted on AUD medications. This may suggest that more emphasis is being placed on clinical efficacy outcomes versus a more nuanced understanding of the medication's mechanism of action.

Human laboratory studies have the capacity to test various mechanisms of AUD pharmacotherapies on clinically relevant translational phenotypes, thus furthering research in medications development [37]. In many cases, a positive medication signal using cue-reactivity and self-administration paradigms seem to be related to medication efficacy in clinical trials [38]. Cue-reactivity had better predictive validity for medications that are hypothesized to address consequences related to abstinence, such as alcohol craving, negative emotionality, and sleep disturbances. However, some models of cue-reactivity testing pre-select participants who are "cue-reactive" and exclude those who are not [39], which impacts generalizability. Alcohol administration paradigms had better predictive utility for drugs that decrease/blunt the rewarding effects of alcohol. Thus, understanding a candidate drug's mechanism of action may inform the optimal human laboratory paradigm that should be used to predict efficacy in clinical trials [38]. When these paradigms are standardized, as in the NIAAA's Human Laboratory Program (HLAB), they can help inform Go/No Go decisions early on in the medication development process [4].

Another common critique of human laboratory paradigms for AUD is a lack of generalizability due to artificial laboratory settings. However, controlled experimental conditions can be an important complement to randomized clinical trials.

For example, a meta-analysis by Hendershot et al. [40] examining the effects of naltrexone on alcohol self-administration and craving found that naltrexone effects sizes on human laboratory endpoints dovetailed naltrexone effect sizes observed in clinical trials. While the experimental setup in human laboratory paradigms differs significantly from clinical trials, the fact that both yield similar medication effect sizes may suggest that the medication signal is generalizable across research settings. A similar pattern was reported in a meta-analysis by our group examining the effects of naltrexone on subjective response to alcohol in the human laboratory [41].

These extensive reviews by experts in the field, while qualitative in nature, provide theoretical insights into the consilience between human laboratory studies and clinical outcomes. However, quantitative estimates of predictive utility are needed.

Empirical Evidence on the Value of Human Lab Models in Medication Development

Probing the Predictive Value of the Human Lab Models Using Simulations

Our laboratory has leveraged simulation methods to differentiate between lab and clinical trials approaches in finding medication signals [10]. Specifically, we conducted a Monte Carlo simulation study to further explore the relative efficiency of pilot trials versus laboratory studies in screening medications for AUD. The simulation study tested the following parameters: (1) average medication effect size (Cohen's d = 0.2, 0.5, and 0.8 representing small, medium, and large effects), pilot study sample size (N_{pilot} range 6 to 36), the multiplicative increase in sample size associated with typically less expensive and quicker laboratory studies (Lab Multiple = 1, 2, and 4), and lastly the correlation between clinical and laboratory effect sizes ($\rho_{Lab\text{-}Clinic}$ = 0.3, 0.6, 0.9, representing relatively poor, moderate, and strong correlation between clinical and laboratory effects). Ten thousand clinical effect sizes were simulated for each of these parameter combinations, half from a null distribution N(0, 0.2) and half from the specified mean effect size N(d, 0.2). Laboratory effect sizes were simulated based on population correlation $\rho_{Lab\text{-}Clinic}$ with equivalent distributions to the clinical effect sizes. We then calculated the probability of a significant and positive trial (i.e., statistical power with $\alpha = 0.025$, one-tailed) for each of the simulated effect sizes in both a pilot study of sample size N_{pilot}, and a laboratory study of sample size $N_{pilot} \times$ Lab Multiple. By summing these powers we calculated the expected number of positive trials for medications with true and false effects (i.e., drawn from distribution N(d, 0.2) or N(0, 0.2) respectively). Using simulation methods, it was feasible to calculate the sensitivity or the probability that a medication with a true effect would screen positive.

The findings from the Monte Carlo simulation suggested that sensitivity increased with greater sample size, greater average effect size, and greater correlations between laboratory and clinic effect sizes. This simulation study also found evidence to suggest that pilot study is superior in terms of sensitivity only when a

laboratory study confers no advantage in terms of sample size. When the sample size of the laboratory study was double that of the pilot study, or larger, the laboratory study exhibited greater sensitivity than a pilot trial across the range of sample sizes and medication effect sizes tested. In brief, the utility of human laboratory models over pilot RCTs hinges on the association between the human laboratory outcome and the RCT outcomes. When the human laboratory outcome is distal to the RCT drinking outcome, the benefits of the human laboratory model are null. However, as the human laboratory model better approximates the clinical outcome of interest, then the human laboratory model is of maximal utility. In sum, human laboratory models and/or pilot randomized controlled trials should serve as intermediaries in the transition from preclinical studies to large, and costly, randomized controlled efficacy trials. The selection of a human laboratory model as an intermediate step in efficacy testing should be informed by the association between the clinical outcome and the human laboratory outcome as well as the putative mechanism of action of the novel treatment. Specifically, considering the purported mechanism of action of a given medication should guide the selection of surrogate measures (i.e., early efficacy endpoints) in a human laboratory study. Considering a single mechanism (e.g., reductions in cue-induced craving) for multiple (and novel) pharmacotherapies can lead to false negatives. Therefore, we suggest that a combination of established mechanisms as well as mechanisms uniquely associated with the novel therapeutic be targeted in human laboratory testing.

Probing the Predictive Value of the Human Lab Models Using Observed Data

While medication effects on human laboratory endpoints are hypothesized to predict treatment outcomes, this hypothesis has remained largely hypothetical or tested in simulated fashion (as described above). Our laboratory recently implemented a novel meta-analytic method to empirically test whether human laboratory tests of medication effects predict medication outcomes in RCTs [42]. After a comprehensive literature search for AUD medications tested in the human lab and in RCTs, medication effects were computed on relevant endpoints. For the human laboratory studies, we computed medication effects on stimulation, sedation, and craving during the alcohol administration ($k = 51$ studies, 24 medications). For RCTs, we computed medication effects on any drinking and heavy drinking ($k = 118$ studies, 17 medications). Medication was the unit of analysis and Williamson-York bivariate weighted least squares estimation was applied to preserve the errors in both the independent and dependent variables. Results revealed a significant and positive relationship between medication effects on alcohol-induced stimulation, sedation, and craving in the laboratory, and drinking outcomes in RCTs, such that medications that reduced stimulation, sedation, and craving during the alcohol administration were associated with better clinical outcomes. A leave-one-out Monte Carlo analysis examined the predictive utility of these laboratory endpoints for each

medication. The observed clinical effect size was within one standard deviation of the mean predicted effect size for all but three pharmacotherapies. This proof-of-concept study demonstrated that behavioral pharmacology endpoints of alcohol-induced stimulation, sedation, and craving track medication effects from the human laboratory to clinical trial outcomes. These results apply to alcohol administration phenotypes and can be expanded to other paradigms, such as cue-exposure and stress-exposure. Further, given that alcohol administration models are not employed in treatment-seeking samples, it should be noted that the aforementioned meta-analytic study found a relationship between medication effects in the human laboratory among non-treatment seekers and clinical trial effects among treatment-seeking individuals. Lastly, these novel methods and overall approach can be applied to a host of clinical questions and can streamline the process of screening novel compounds for AUD.

Recent Advancements: Novel Compounds and Novel Human Lab Models

Recent approaches emphasize using established medications for AUD to evaluate validity of animal and human models to advance translational research for AUD. Those models can then serve as a springboard for the development of new pharmacotherapies. This approach is being employed in our laboratory to test a novel human laboratory model to screen AUD medications. Below we describe two recent approaches from our laboratory aimed to apply novel human laboratory models to study AUD as well as leveraging human laboratory paradigms to assess the efficacy of a novel compound.

Practice Quit Attempt Model

The practice quit attempt model used by Perkins and colleagues initially tested FDA-approved smoking cessation medications in order to validate their model [43–45]. These studies found that in individuals with high motivation to quit, there was a significant increase in number of days abstinent when on active medication compared to placebo; the same pattern of findings was not evident in individuals with low motivation to quit [43–45]. Further, when modafinil, a medication that has not shown efficacy as a smoking cessation aid, was added as a study arm, they demonstrated model specificity, as bupropion was effective at improving abstinence, while modafinil was not [44]. More recently, Perkins and colleagues have moved to testing novel medications using their practice quit attempt model [46, 47]. They did not find a significant reduction in days abstinent when testing a novel fibrate medication [46] or a novel positive allosteric modulator of nicotinic receptors [47], which provides a critical “no-go” signal for both medications. The development of the practice quit attempt model for medication development in nicotine dependence

provides strong "road map" for the development of a similar approach modified to AUD.

To address this central limitation in the AUD medications development field, we are expanding the traditional laboratory methodology to more closely mirror actual treatment processes [48]. Through combining a well-established laboratory paradigm (i.e., cue-reactivity), with an abbreviated "practice" quit attempt in intrinsically motivated individuals with AUD, our human laboratory model reduces the conceptual distance between the lab and the clinic, thus reducing the possibility for laboratory effects to "fade" when applied to the clinical context.

Our ongoing study (ClinicalTrials.gov Identifier: NCT04249882) aims to develop and validate a novel early efficacy paradigm, informed by smoking cessation literature, to screen novel medications for AUD. As an established AUD medication, naltrexone serves as an active control to test both the practice quit attempt model and the efficacy of a promising AUD pharmacotherapy, varenicline. Individuals with current AUD reporting intrinsic motivation to change their drinking complete a week-long "practice quit attempt" while on study medication. Participants are randomized in a blinded manner to either naltrexone, varenicline, or placebo. During the practice quit attempt, they complete daily visits over the phone and fill out online questionnaires regarding their drinking, alcohol craving, and mood. Additionally, participants undergo two alcohol cue-reactivity sessions. The successful completion of this study will advance medications development by proposing and validating a novel early efficacy model for screening AUD pharmacotherapies, which in turn can serve as an efficient strategy for making go/no-go decisions as to whether to proceed with clinical trials.

Ibudilast Efficacy in the Human Laboratory

The identification of novel treatment targets and the development of rigorous laboratory paradigms to screen and optimize novel therapeutics represents a research priority. Ibudilast is a neuroimmune modulator that inhibits phosphodiesterase −4 and −10 and macrophage migration inhibitory factor. Recently in an AUD sample, ibudilast was shown to decrease reactivity to a psychological stressor. Furthermore, ibudilast was effective in blunting alcohol reward among participants with greater depressive symptoms, a hallmark symptom of protracted withdrawal. Preclinical research in opiates has demonstrated that drug withdrawal is necessary for microglia activation and neuroinflammation in reward networks, suggesting that ibudilast may be most effective among patients who experience withdrawal-related dysphoria.

Based on the aforementioned work, our laboratory recently completed a laboratory study that examined withdrawal-related dysphoria as a moderator of IBUD efficacy in the natural environment measured using Daily Diary Assessment (DDA) approaches [24]. More specifically, we examined the efficacy of ibudilast to improve negative mood, reduce heavy drinking, and attenuate neural reward signals in individuals with AUD. Fifty-two nontreatment-seeking individuals with AUD were randomized to receive ibudilast (n = 24) or placebo (n = 28). Participants completed a

2-week daily diary study during which they filled out daily reports of their past day drinking, mood, and craving. Participants completed a functional magnetic resonance imaging (fMRI) alcohol cue-reactivity paradigm half-way through the study.

The results of the study demonstrated that ibudilast did not improve negative mood on drinking or non-drinking days, indicating that its mechanism of action may be non-mood dependent in non-treatment-seeking individuals. Ibudilast reduced the probability of heavy drinking days over 2 weeks for non-treatment-seeking individuals relative to placebo. Ibudilast also attenuated alcohol cue-elicited activation in the ventral striatum (VS), potentially through a dopaminergic-related mechanism. This is a critical proof-of-mechanism whereby modulation of neuroimmune signaling via ibudilast reduced the incentive salience of alcohol cues in the brain. Exploratory analyses indicated that VS activation to alcohol cues was predictive of subsequent drinking in the ibudilast group, such that individuals who had attenuated VS activation and were treated with ibudilast had the fewest number of drinks per drinking day in the week following the scan. Overall, we applied a human laboratory model to support the efficacy of ibudilast for the treatment of AUD and suggest a potential biobehavioral mechanism through which ibudilast acts.

Conclusions

This chapter discusses a host of human laboratory models of alcohol use disorder and reviews their application to medication development. As can be seen in Table 21.1, these models range from alcohol cue-exposure, stress-exposure, alcohol administration, alcohol self-administration, delayed discounting, to name a few. Each model has its own strengths and weaknesses as well as methodological variation in how they are implemented. One of the key conclusions from the review of various human laboratory models is that there is ample opportunity to standardize methods across studies. A recent meta-regression from our laboratory has shown that variability in methods for alcohol administration in the laboratory, such as target BrAC and drinking characteristics of the study sample, predict the likelihood of detecting a medication effect, compared to placebo [49]. As such, opportunities to refine and standardize methods for human laboratory testing and to conduct multisite studies of novel medications should receive a high priority. To that end, the HLAB initiative by NIAAA has recently reported the results of a 3-site human laboratory study of varenicline's effects on alcohol cue-reactivity [39], an important step towards standardizing early efficacy testing through human laboratory protocols.

Next, this chapter reviews a series of simulations conducted by our group comparing signal detection in the human laboratory versus RCTs [10]. As show in Figs. 21.1 and 21.2, results of our Monte Carlo simulation found that a laboratory study with a paradigm well-calibrated to capture meaningful clinical effects is better able to detect true positive medication effects than pilot RCTs. Calibration refers to the degree to which the endpoint in a human lab study is associated (or correlated) with an RCT endpoint. These results argue for the careful "calibration" of

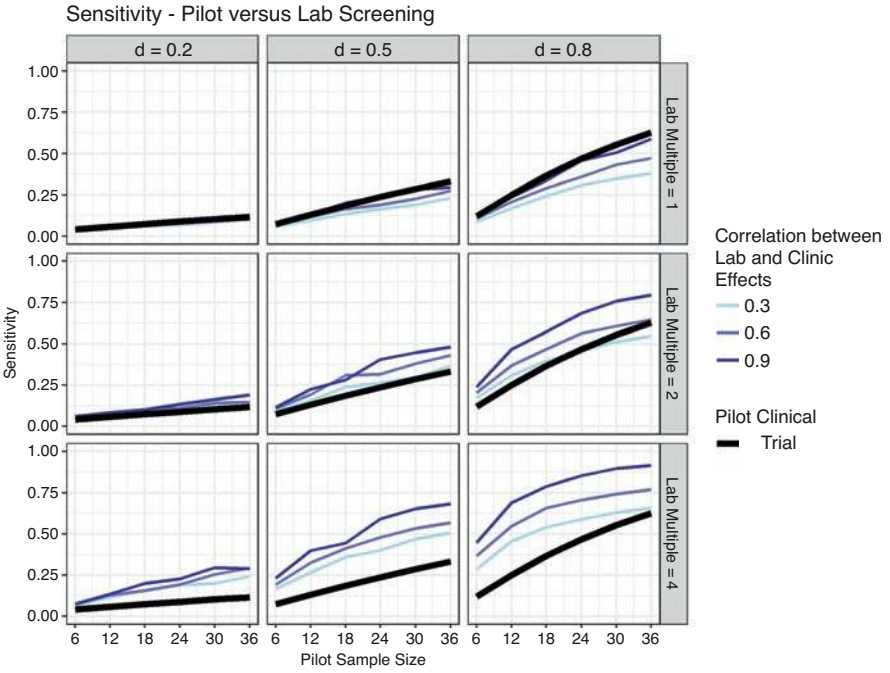

Fig. 21.1 Sensitivity results for pilot versus laboratory screening of novel medications. These sensitivity analyses are the result of a Monte Carlo simulation study (n = 10,000 per specification) examining the effect of pilot study sample size, mean medication effect size (Cohen's *d*), multiplicative increase in laboratory versus pilot clinical trial (Lab Multiple), and correlation between laboratory and clinic effect sizes. Sensitivity was defined as the expected number of positive screens as a proportion of the total number of true positive medications. This figure was adapted from Ray et al. [10]

human laboratory paradigms as a critical step in overcoming the "valley of death" in medications development for AUD and ultimately promoting the translation of novel compounds to clinical populations. In other words, these simulations argue that the degree to which human laboratory models capture "shared variance" with alcohol consumption endpoints in a clinical degree determines the utility of the human laboratory paradigm for early efficacy detection. A key conclusion from this line of research is the need to empirically examine the association between laboratory and RCT outcomes in AUD medication studies.

To that end, this chapter turns to a study in which we use meta-analytic principles to empirically test the degree to which medication effects in the human laboratory (i.e., effects on subjective responses to alcohol during alcohol administration in the lab) are predictive of the same medication effects in phase II trials, or RCTs [42]. For behavioral pharmacology, we computed medication effects on alcohol-induced stimulation, sedation, and craving during the alcohol administration (k = 51 studies, 24 medications). For RCTs, we computed medication effects on any drinking and heavy drinking (k = 118 studies, 17 medications). We used medication as the unit of

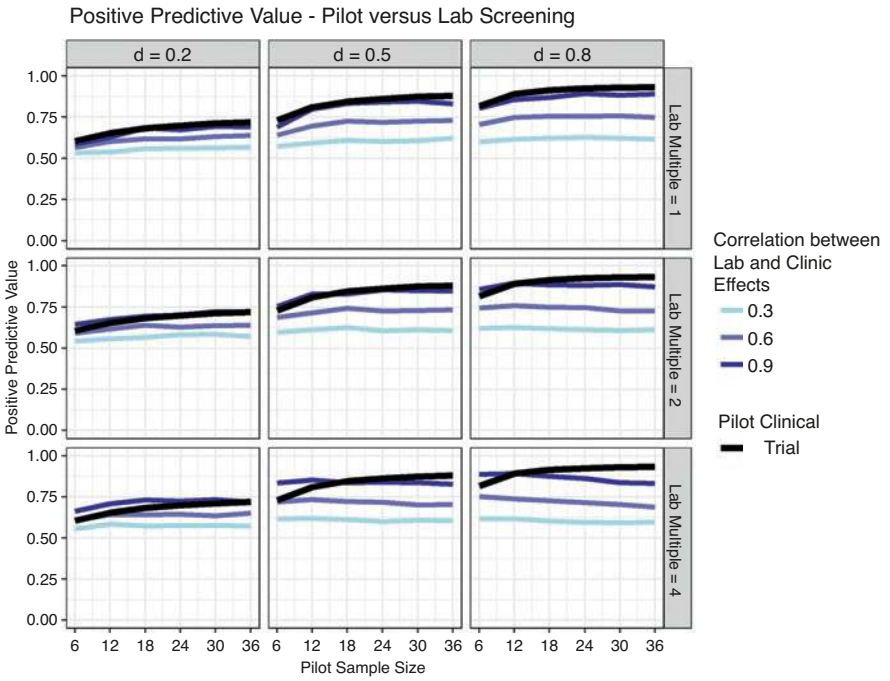

Fig. 21.2 Positive predictive value results for pilot versus laboratory screening of novel medications. These positive predictive values are the result of a Monte Carlo simulation study (n = 10,000 per specification) examining the effect of pilot study sample size, mean medication effect size (Cohen's *d*), multiplicative increase in laboratory versus pilot clinical trial (Lab Multiple), and correlation between laboratory and clinic effect sizes. Positive predictive value was defined as the proportion of positive screens that represent true positives. This figure was adapted from Ray et al. [10]

analysis and applied the Williamson-York bivariate weighted least squares estimation to preserve the errors in both the independent and dependent variables. Results revealed a significant and positive relationship between medication effects on alcohol-induced stimulation, sedation, and craving in the laboratory, and drinking outcomes in RCTs. The effects were such that medications that reduced stimulation, sedation, and craving during the alcohol administration were associated with better clinical outcomes. A leave-one-out Monte Carlo analysis (shown in Fig. 21.3) examined the predictive utility of these laboratory endpoints for each medication. The observed clinical effect size was within one standard deviation of the mean predicted effect size for all but three pharmacotherapies. This proof-of-concept study demonstrated that behavioral pharmacology endpoints of alcohol-induced stimulation, sedation, and craving track medication effects from the human laboratory to clinical trial outcomes. These methods and results can be applied to a host of clinical questions and can ultimately streamline the process of screening novel compounds for AUD. This approach can be used to quantify the predictive utility of

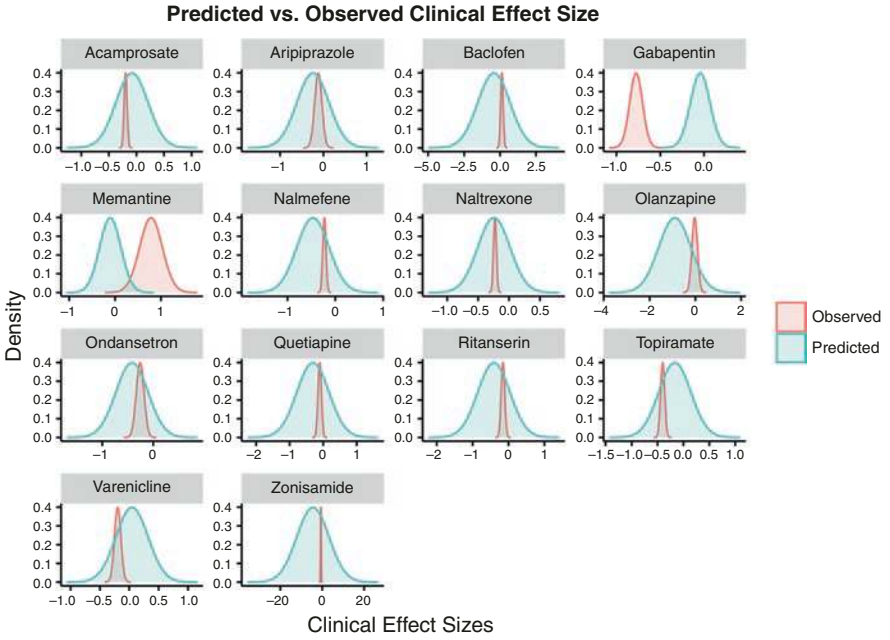

Fig. 21.3 Predicted and observed clinical effect size distributions. Predicted effect size distributions were computed for each medication across laboratory effect sizes using a leave one-out Monte Carlo simulation. This figure was adapted from Ray et al. [42]

cue-reactivity screening models and even preclinical models of medication development. Hence one of the key conclusions from this line of research is that methods and data are available to provide empirically-driven analyses of the relationship between human laboratory and clinical trials endpoints so as to inform their optimal application.

Finally, the chapter discusses ways in which we can improve the consilience between human laboratory models and clinical trials endpoints by (a) developing more ecologically valid models to bolsters the association between lab and clinical endpoints, and (b) combining alcohol use data collection in human laboratory studies through micro-longitudinal designs. For the first effort, we discuss a new model for which testing is underway [48]. This model consists of a practice quit attempt in which individuals with AUD who are motivated to change their drinking agree to engage in a 7-day practice quit attempt. If supported as a sensitive method for medication screening, this model could be used widely as an ecologically valid approach to testing early efficacy of novel medications, and/or combination pharmacotherapies. Further, we recommend testing multiple medications, against a placebo arm, simultaneously as a way to expedite the transition from preclinical to human testing of promising compounds. Nevertheless, the concomitant test of multiple medication arms versus a placebo condition increases the sample size requirement and overall effort towards the human laboratory endeavor. For the second effort, we demonstrate

the application of the micro longitudinal design in which daily drinking is reported, including circumstances surrounding the drinking episode and responses to alcohol [24, 50, 51]. This approach has been undertaken successfully with a novel neuroimmune modulator and has the potential to be applied broadly in pharmacotherapy development for AUD.

In closing, this chapter discusses a series of studies applying human laboratory paradigms to medication development for AUD, from qualitative reviews, to empirical studies, to simulation studies, meta-analytic reviews, and perspective/opinion pieces. A common theme across this chapter is the need to optimize the clinical utility of human laboratory model in medication development for AUD. To that end, a central tenant of the chapter is the need to design and test human laboratory models with the highest level of consilience with real-world alcohol consumption. As the field continues to progress towards standardizing and optimizing medication development, careful attention to the role of human laboratory testing is warranted to close the translational gap from preclinical to clinical testing and to effectively deliver novel compounds to individuals suffering from AUD.

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Chapter 22
Emergency Room: Acute Alcohol Intoxication and Other Alcohol-Related Acute Problems Including Alcohol Withdrawal Syndrome

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Abstract Alcohol consumption is widespread around the world. It is a leading cause of disability and mortality, in which the Emergency Department plays a key role since it is often the first contact between the patient with an alcohol use disorder (AUD) and the health care system. Acute alcohol intoxication is the most common disorder in the context of alcohol-related emergencies, and can lead to severe life-threatening conditions if not treated early. Since diagnosis requires the exclusion of other causes, the only validated therapy is methadoxine in addition to support of vital functions. Alcoholic hepatitis is a clinical syndrome characterized by jaundice and liver impairment. The treatment depends on severity, evaluated by Maddrey's score: if above 32 points, alcoholic hepatitis is severe, and needs to be treated with corticosteroids (methylprednisolone 40 mg IV daily) and liver transplantation should be consider early. Patients who abruptly discontinue or reduce alcohol consumption are at risk of the alcohol withdrawal syndrome. Symptoms follow a scale of severity, and the most sever form is represented by Delirium Tremens, defined by

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hallucinations and disorientation, until seizures and coma. The cornerstone of pharmacological treatment is the administration of long-acting benzodiazepines.

Keywords Alcohol use disorder · Acute alcohol intoxication · Alcohol withdrawal · Acute alcoholic hepatitis

Introduction

Alcohol consumption is widespread throughout the world, but alcohol use and abuse differ between countries: For instance, Europe has the highest level of alcohol consumption in the world (about 10.9 L of alcohol per person per year), while the global average consumption is 6.2 L/year, as pointed out by World Health Organization (WHO) [1–3].

Alcohol is a leading cause of disability and mortality, and is the main risk factor for preventable death in subjects aged between 25 and 49 [4]. Several specific alcohol-related clinical problems are recognized, both acute (e.g., acute alcohol intoxication, alcohol withdrawal syndrome and acute alcoholic hepatitis) and chronic (e.g., liver cirrhosis, neoplasm, neurological or cardiovascular diseases). Moreover, excessive alcohol use increases the risk of intentional and unintentional injuries, including car accidents, aggressions, domestic violence, murders and suicides. According to WHO, alcohol use could cause about 3.3 million deaths per year [3]. The main causes of death are related to liver cirrhosis, cancers (liver, upper gastrointestinal tract, upper respiratory tract, etc.) [5] and cardiovascular diseases [1, 2, 6].

The amount of alcohol intake is quantified in alcohol units, referring to 10–12 g of pure alcohol [7]. This measure is equivalent to a glass of wine (125 mL), a bottle of beer (330 mL) or a glass of liquor. Mostly, one drink (i.e., one alcohol unit) corresponds to a blood alcohol concentration (BAC) of 15–20 mg/dL, which requires 1 h to be metabolized [8]. The emerging phenomenon of “binge drinking”, which entails the intake of at least four drinks for women and five drinks for men on a single occasion, is an important risk factor for acute alcohol intoxication and its complications, especially in adolescents, since BAC can reach levels of 80 mg/dL in about 2 h [9, 10]. In adolescents, binge drinking increases the risk of developing diseases, related to reduced expression of alcohol-degrading enzymes, and the risk of developing alcohol use disorder (AUD) [11, 12].

The number of emergency room admissions for acute or chronic alcohol-related diseases are underestimated and underreported. The emergency room plays a key role, because it is often the first contact between the patient and health care professionals. However, it is necessary to recognize.

these conditions in order to refer the patient to the appropriate local alcohol services, which reduces cost, crowding, and correctly refers the patient to the right care [13, 14].

Acute Alcohol Intoxication

Acute alcohol intoxication is a clinically harmful (although typically transient) condition that usually follows the ingestion of a large amount of alcohol [8]. Alcohol is absorbed through the small intestine, primarily by the stomach (70%), to a lesser degree by the duodenum (25%), and only 5% by the remaining intestine [15]. Its metabolism begins at the time of ingestion, and 10% of degradation occurs already through local metabolism in the stomach. In this mechanism, a part of the ingested alcohol is oxidized by alcohol dehydrogenase (ADH) present in the stomach [16], rather than being absorbed into the systemic circulation. There is no specific transport protein that binds alcohol, and thus the remaining alcohol is absorbed through membrane passage, and distributed in the total body water. From the stomach, alcohol reaches the duodenum and jejunum, with a progressive reduction of local metabolism. The remaining metabolism (90%) takes place in the liver, where the main enzymatic systems responsible for the oxidation of alcohol are present (90% constituted by ADH, 8–10% by the microsomal alcohol oxidant system (MEOS) and 0–2% by catalase [17]. When the alcohol consumption exceeds the metabolic capacity of the liver and sufficient blood alcohol levels accumulate, the intoxication symptoms begin [8].

The Diagnostic and Statistical Manual of Mental Disorders (DSM-V) diagnostic criteria for alcohol intoxication include [18]:

1. recent ingestion of alcohol;
2. clinically significant problematic behavioral or psychological changes (e.g., inappropriate sexual or aggressive behavior, mood lability, impaired judgment) that developed during, or shortly after, alcohol ingestion;
3. one (or more) of the following signs or symptoms developing during, or shortly after, alcohol use: (1). slurred speech. (2). incoordination. (3). unsteady gait. (4). nystagmus. (5). impairment in attention or memory. (6). stupor or coma;
4. the signs or symptoms are not attributable to another medical condition and are not better explained by another mental disorder, including intoxication with another substance

The diagnostic criteria are reported in Table 22.1.

Epidemiology

Acute alcohol intoxication is a common cause of admission to the Emergency Department: in the USA, it accounts for over 600,000 visits each year [19], while in Europe, it represents around 1.2–5% of all Emergency Deparment visits annually [20]. Of these admissions, about 1% of intoxicated patients who do not present other complications receive treatment in the Intensive Care Unit (ICU), in Emergency Departments specialized in acute alcohol intoxication [21]. In the United States, an increase in Emergency Department admissions between 2006–2014 has been

Table 22.1 Diagnostic criteria for diagnosis of Acute Alcohol Intoxication according to the Diagnostic and Statistical Manual of Mental Disorders fifth edition (American Psychiatric Association [2013] Diagnostic and statistical manual of mental disorders: DSM-5, fifth ed. The American Psychiatric Association, Washington, DC)

Diagnostic criteria for Acute Alcohol Intoxication
1. Recent ingestion of alcohol
AND
2. Clinically significant problematic behavioral or psychological changes (e.g., inappropriate sexual or aggressive behavior, mood lability, impaired judgment) that developed during, or shortly after, alcohol ingestion
AND
3. One (or more) of the following signs or symptoms developing during, or shortly after, alcohol use
(a) slurred speech
(b) lack of coordination
(c) unsteady gait
(d) nystagmus
(e) impairment of attention or memory
(f) stupor or coma
AND
4. Signs or symptoms are not attributable to another medical condition and are not better explained by another mental disorder, including intoxication with another substance

estimated to result in an increase in alcohol-related health care costs by 272%, reaching $ 15.3 billion in 2014 [22]. Furthermore, also due to the rise of binge drinking, it has been estimated that about 17% of patients who are admitted to the Emergency Department for alcohol-related problems are adolescents <14 years [14], with BAC values > or =80 mg/dL [10].

There are several risk factors for developing either mild or severe acute alcohol intoxication (i.e., severe enough to require an Emergency Department visit). These risk factors can be genetic and environmental, and include [11]:

- sex: differences in BAC between men and women may vary, depending on gastric metabolism of alcohol and how BAC is measured (whether by body mass or by g per h or g per L of liver volume) [23–25];
- age: liver enzymes are not fully expressed in younger subjects;
- race: some studies have shown that Native Americans may have an increased degradation of alcohol compared to Caucasians, who in turn show equal metabolic capacity of alcohol compared to Chinese [26];
- nutrition: fasting increases the risk of alcohol intoxication because of reduction in gastric ADH levels [27];
- biological rhythms;
- exercise;
- alcohol use disorder (AUD): as a result of enzyme induction, heavy drinkers show an increased alcohol degradation;
- drugs.

Patients suffering from AUD are often difficult to treat in the Emergency Department, because they often present complicated physical and psychosocial situations, aggressive behaviors and poor compliance. This can cause delays and failures in the treatment of this type of patient [28].

Signs and Symptoms

The clinical presentation of acute alcohol intoxication can vary in relation to BAC levels. Acute alcohol intoxication can therefore be divided into three levels of severity, mild, moderate, or severe. BAC below 50–100 mg/dL (10.9–21.7 mmol/L) corresponds to mild symptoms, including relaxation, euphoria and/or mild impairment in coordination and attention [29]. In the moderate form, which is associated with a BAC between 100 and 200 mg/dL (21.7–43.4 mmol/L), symptoms may include ataxia, hyperreflexia, lack of coordination, slurred speech, prolonged reaction time, nystagmus, and behavioral changes. The most severe form of AAI can finally appear when the BAC increases over 200 mg/dL and the clinical features worsen with the onset of nausea/emesis, amnesia, diplopia, dysarthria. At a BAC higher than 300 mg/dL (65.1 mmol/L), there is an increased risk of respiratory depression, coma and cardiac arrest. The signs and symptoms are reported in Table 22.2.

In subjects with low alcohol tolerance (a mechanism induced by alteration of the GABA and glutamate neurotransmitter systems at the level of the central nervous system), however, severe symptoms may occur even at lower BAC levels. Conversely, in chronic drinkers with AUD who present higher alcohol tolerance, BAC levels can be markedly increased before severe symptoms occur [8, 31].

In acute alcohol intoxication, multiple organs and systems can be affected by the toxic action of alcohol. Multiple metabolic derangements, including hypoglycemia, hyperlactatemia, hypokalemia, hypomagnesemia, hypocalcemia, and hypophosphatemia, can also occur [8].

Neurological symptoms are usually the first to become noticable to health professionals. The most frequent neurological complications in acute alcohol intoxication are represented by convulsions. These are usually tonic-clonic, and can transition into a status epilepticus. This is a life threatening condition in which generalized seizure activity lasts >5 min, or multiple seizures occur within that period, potentially resulting in cardiac arrhythmia, cardiac damage because of a catecholamine surge, respiratory failure, hypoventilation, hypoxia, aspiration pneumonia, and pulmonary edema. Secondary to convulsions, patients may develop rhabdomyolysis, which generally manifests itself through the onset of asthenia, myalgia and flaccidity, and can lead to acute renal failure and hyperkaliemia in severe forms, with an increase in blood creatine phosphokinases [29]. The causes of neurological symptoms in acute alcohol intoxication include a lack of thiamine, electrolyte derangement, alterations in the levels of GABA and glutamate, or an acute damage of the blood brain barrier [32]. Other serious forms of neurological involvement also exist and should be remembered: acute alcoholic encephalopathy, also called

Table 22.2 Main clinical symptoms in acute alcohol intoxication according to blood alcohol concentration (BAC)

Symptoms	BAC	Drinks
Motor impairment	BAC < 50 mg/dL (10.9 mmol/L)	
Mild euphoria/dysphoria		1–2 drinks
Relaxation		
Social disinhibition		
Altered perception of the environment	BAC > 100 mg/dL (21.7 mmol/L)	3–5 drinks
Ataxia,		
Hyper-reflexia		
Impaired judgment		
Lack of coordination		
Mood, personality, and behavioral changes Nystagmus		
Prolonged reaction time		
Marked slurred speech		
Amnesia	BAC > 200 mg/dL (43.4 mmol/L)	10–20 drinks
Nausea/vomiting		
Diplopia		
Dysarthria		
Hypothermia		
Hypoventilation		
Cardiac arrhythmia		
Coma	BAC > 400 mg/dL (86.8 mmol/L)	>20 drinks
Respiratory arrest		
Death		

Modified from Vonghia et al. [8] and Jung et al. [30]

[a]A standard single drink contains about 10–12 mg of ethanol, which is estimated to increase the blood alcohol concentration of a 70-kg (155-lb) man by 15–20 mg/dL

Gaye-Wernicke encephalopathy, involves the metabolism of thiamine, and is characterized by subacute hemorrhagic encephalopathy and development of oculomotor disorders, cerebellar ataxia, memory impairment, hyperkinesia and disorders of the autonomic system, leading to chronic Korsakoff's syndrome;

- central pontine myelinolysis, characterized by an acute cerebral demyelination, with consequent development of myosis, tetraplegia, aphonia, impaired ocular motility in the horizontal plane;
- Marchiafava-Bignami syndrome, a demyelination of corpus callosum, with manifestations ranging from memory alterations to coma.
- Finally, patients with AUD or repeated episodes of alcohol intoxication have a higher risk of developing dementia [33].

The cardiovascular system can be affected through the action of alcohol on arterial vessels. Vasodilation can occur, resulting in an increase in heart rate, and causing hypotension and heat loss [15]. Furthermore, alcohol can directly induce arrhythmias, such as atrial or ventricular fibrillation, or alterations of QRS or QTc, which

can be observed through an ECG. Direct damage to the myocardium can also take place, as evidenced by increases in troponin values [34–36]. These disorders can also arise in young subjects, in the absence of underlying heart disease [14]. Furthermore, through echocardiography, an alteration of diastolic function (E/E' ratio) was detected in subjects with acute alcohol intoxication (indicating an acute diastolic impairment), which can be used as an echocardiographic marker of early alcoholic cardiomyopathy [37].

From a respiratory perspective, patients with acute alcohol intoxication have an increased risk of developing aspiration pneumonia. This can be due to dysfunction of the clearance of the ciliary mucosa, due to alcohol-related immune dysfunction, or both [38]. There is also a risk for respiratory depression secondary to neurological impairments.

Gastrointestinal effects of alcohol can be highly variable. Nausea and vomiting are common, due to pyloric spasm. They are often accompanied by diarrhea, due to increased transit of intestinal contents, but there can also be abdominal pain, and pancreatitis [39, 40]. Alcohol intake can also irritate the esophagus and stomach mucosa, leading to esophagitis, gastritis and gastric ulcers [30]. The predominant gastrointenstinal insult from acute alcohol intoxication affects the liver, the organ primarily involved in the metabolism of alcohol. Alcohol exposure can lead to acute alcoholic hepatitis, a serious condition associated with a high mortality already at the first episode, and described in detail below [41]. Acute alcohol intoxication can also lead to the development of the Zieve syndrome, characterized by hemolytic anemia, jaundice, and hypertriglyceridemia, even though it is not a common occurrence [42].

Finally, acute alcohol intoxication is associated with an increased risk of injury, trauma and death, which can be further increased in patients with comorbid psychiatric disorders [43].

Diagnosis

The diagnosis of acute alcohol intoxication can be made only after having ruled out other and potentially more serious conditions with a similar clinical presentation, such as sepsis, meningitis, trauma with intracranial bleeding, intoxication from other substances, hypoxia, hypoglycemia or other metabolic disorders. It's important to avoid minimizing issues or rapidly discharging patients because of disruptive behaviors, as this can lead to a life-threatening condition remaining undiagnosed [44]. The first step is to collect an accurate alcohol history. Although this can often be difficult, the history should be focused on the amount of recent alcohol intake, type of beverage consumed, and the time course of symptoms [8].

The physical examination is based on the ABCDE (Airway-Breath-Cardio-Disability-Exposure) pattern, systematically analyzing respiratory, cardiac and the neurological systems, state of hydration, AUD-related signs, including poor nutritional status. Lab tests should include BAC, together with an analysis of liver function, electrolytes (sodium, potassium, etc.) and toxicological screening aimed at

detecting the presence of other intoxicants that may interact with alcohol and further complicated its effects. Depending on the BAC value, it may be important to increase the blood degradation of alcohol in order to prevent possible organ damage [8, 14]. Arterial or venous blood gas analysis is also a quick test that can be very useful, and can immediately give information about the metabolic and respiratory status of the patient. Electrocardiogram and chest radiography must also be performed. If the neurological state is altered, and/or the patient is suspected to have had head trauma, Computed tomography (CT) or MRI of the brain should also be performed.

Treatment

There are three main treatment goals: patient stabilization, acceleration of alcohol elimination and, if necessary, patient sedation until sobriety has reached. Maintaining a free airway is essential to prevent aspiration, and the lateral safety position is recommended. Oxygen may need to be provided, preferably guided by pulse oxymetry. Antibiotics may be required if respiratory infection is suspected or confirmed. In patients with heavy alcohol use, serious infections may be present with surprisingly little symptoms, and chest rays should be ordered liberally.

Hydration is mandatory and intravenous access should be obtained in order to administer fluids [15], and correct hypoglycemia as well as electrolyte balance. A fluid therapy protocol has been suggested: 500 mL of 10% dextrose +500 mL of 0.9% sodium chloride with 2 g of magnesium sulphate +100 mg thiamine +1 mg folate. It is essential to administer thiamine before glucose loading, to reduce the risk of precipitating Wernicke's encephalopathy [45]. Bicarbonates should only be used in cases of metabolic acidosis. Nausea and vomiting can be treated with antiemetic drugs and electrolyte solutions, which avoids prolonged gastric fluid losses [46]. Moreover, in case of hypothermia that is unresponsive to fluid therapy, hemodialysis or peritoneal dialysis are therapeutic options [47]. Should the patient psychomotor agitation, haloperidol is the drug of choice, because it minimizes the potential for negative effects on circulation [44].

The strategies reviewed above are supportive. The only validated therapy in acute alcohol intoxication is **methadoxine,** a pyrrolidone carboxylate of pyridoxine, (pyridoxol L-2-pyrrolidone-5-carboxilate) that enhances the plasma clearance of alcohol, thus reducing toxic consequences of the alcohol exposure. Pyridoxine increases the synthesis of glutathione and the activity of acetaldehyde dehydrogenase, thus increasing in the urinary elimination of ketones [48–50].

A bolus of 900 milligrams IV, (commonly three vials of 300 mg each), doubles the rate at which alcohol blood levels decrease compared to placebo, obtaining a faster rate of alcohol elimination and an accelerated recovery. It appears to be manageable and safe [48, 49]. In some recent trials, methadoxine has also been tested as a drug to stop the chronic consumption of alcoholic beverages in patients with AUD, with encouraging preliminary results [51]. Moreover, having an antioxidant action,

methadoxin appears to improve the condition of patients with alcohol associated liver diseases (ALD) [52]. Also, methadoxine in association with cortisone proved to be more effective than cortisone alone in the treatment of acute alcoholic hepatitis [53].

Biomimetic nanocomplexes containing alcohol oxidase and catalase could be possible future treatments for acute alcohol intoxication, as suggested by *in vivo* and *in vitro* studies, with the aim of reducing alcohol levels through the encapsulation of the same enzymes inside a thin polymer shell [54]. In this way, these nanocomplexes simulate the action of cellular organisms with internal subcellular compartments which are normally able to transform and eliminate toxic metabolic waste. These data, although preliminary, deserve to be explored infurther clinical studies.

Alcohol Withdrawal Syndrome

Patients who abruptly discontinue or reduce alcohol consumption are at risk of developing an alcohol withdrawal syndrome (AWS). Considering that the prevalence of AUD among adult patients presenting to Emergency Departments is about 20–25% [55], a significant proportion of patients hospitalized for medical or surgical problems are at risk of developing AWS. Indeed, the prevalence of AWS among patients admitted to an ICU reaches 40%. AWS is associated with an increased risk of infections and death [56, 57]. Although withdrawal symptoms are common among AUD patients (up to 50%) [58, 59], only a minority of these reach medical attention.

AWS is a clinical condition characterized by symptoms of central nervous system hyperexcitability and autonomic hyperactivity, manifested, e.g., as agitation, tremors, irritability, anxiety, hyperreflexia, confusion, hypertension, tachycardia, fever and diaphoresis). The onset of these symptoms is usually within 6–24 h after the abrupt discontinuation or marked decrease of alcohol consumption in patients suffering from AUD [60]. The spectrum of disease ranges from mild-moderate forms characterized by tremors, nausea, anxiety and depressed mood, to severe forms characterized by hallucinations, seizures, delirium tremens and coma [61]. While mild–moderate AWS is often self-managed by patients or dissipates within 2–7 days from the last drink [59, 62], the more severe forms require medical treatment [58, 59].

Pathophysiology

Acute alcohol ingestion produces CNS depression due to an increased GABAergic neurotransmission (stimulation of GABA-A receptors) [63] and to a reduced glutamatergic activity (inhibition of N-methyl-D-aspartate—NMDA—receptors) [64,

65]. Chronic CNS exposure to alcohol produces adaptive changes in multiple neurotransmitter systems, including GABA, glutamate and norepinephrine [66], which compensate for the alcohol-induced dysregulation. This causes a new neurochemical equilibrium to be established [67], in which there is a reduction of alcohol's effects on the CNS such that excitation—inhibition balance is maintained despite the suppressant effect of alcohol, a condition known as tolerance [64, 68]. Abrupt discontinuation of alcohol use under these conditions leads to a rebound, in which excitation—inhibition balance is shifted toward increased excitation. The reduction in GABA and increase in glutamate neurotransmissions that occur with acute alcohol intake are now reversed, and lead to a generalized CNS hyperexcitability and AWS symptoms [67]. Moreover, upregulated dopaminergic and noradrenergic pathways are also involved in the development of hallucinations and autonomic hyperactivity [61].

Symptoms

Any patient presenting with symptoms of hyperarousal should be evaluated for AWS. The patient's drinking habits, i.e., duration, amount and frequency of alcohol consumption, should be evaluated. This is particularly appropriate if symptoms occur after some hours of alcohol abstinence, e.g., on awakening in the morning. Clinical questionnaires, such as CAGE or AUDIT [69] can help the physician identify an underlying AUD. Patients unable to collaborate due to an altered mental state should be monitored for the appearance of AWS symptoms, in particular if the altered state appears following a major trauma or road accident [70]. The clinical manifestations of AWS are usually progressive, such that initial mild symptoms progressively become more severe. However, AWS can abruptly begin with the onset of a delirium tremens (DT; see below), particularly in patients who have experienced prior episodes of DT, and those with repeated AWSs. The latter is thought to result from 'kindling', a phenomenon originally described for seizures, in which repeated episodes of delirium tremens or AWS result in a progressive lowering of the threshold for at which the next episode occurs [60].

Initial AWS symptoms include tremor, diaphoresis, nausea/vomiting, hypertension, tachycardia, hyperthermia and tachypnoea, typically start 6–12 h after cessation of alcohol consumption, and disappear if alcohol intake is resumed [71]. It is important to keep in mind that several medications patients may be receiving, such as beta-blockers, clonidine, or calcium-channels blockers, could mask changes in blood pressure and heart rate, vital signs otherwise useful to monitor progression of withdrawal severity.

A second stage of AWS symptoms can start appearing approximately 24 h after the last drink, and consists of visual and tactile symptoms. At this stage, a quarter of AWS patients can also experience hallucinations, which are most commonly visual, but can also be auditory or tactile [71]. These disturbances are also called withdrawal hallucinosis, or pre-delirium. Before abnormal perceptions reach the level of true hallucinosis, i.e., a condition in which the patient loses touch with reality, the sensorium can be clear, and the abnormal sensations can be perceived as unreal, classifying them as illusions [71].

The third stage, and the most severe manifestation of AWS, is represented by DT, which affects about 5% of patients with AWS [61]. DT usually appears 48–72 h after the last drink, but could begin up to 10 days later, in particular in patients who combine heavy alcohol use with non-medical used of bensodiazepines. Unless treated aggressively, symptoms of DT normally last 5 to 7 days [71, 72]. The emergence of severe DT in a hospitalized patient is typically the result of a failure to initiate treatment of AWS in time, providing insufficient treatment, or both [61]. DT is a clinical condition characterized by a rapid fluctuation of consciousness and cognition, combined with severe autonomic manifestations such as sweating, nausea, tachycardia and tremors), and psychiatric symptoms (anxiety) [61]. The typical DT patient shows anxiety, agitation, hallucinations and disorientation. The lack of orientation differentiates delirium from withdrawal hallucinosis. Delirium, psychosis, hallucinations, hyperthermia, malignant hypertension, seizures and coma are common manifestations of DT [18, 71, 72]. DT could be responsible for injury to patients and staff, or for other medical complications, such as aspiration pneumonia, arrhythmia or myocardial infarction, which can lead to death in about 1–5% of patients [61]. After the treatment of acute AWS, some symptoms can persist from weeks to months following the 5–7 days of acute detoxification period, representing the 'protracted AWS' [61].

Alcohol withdrawal seizures are a separate, common and important manifestation of the hyperexcitability that emerges in the central nervous system during AWS, and affect about 10% of patients [72]. Seizures precede the emergence of DT, frequently occur in the absence of other AWS symptoms, and occur in 90% of cases within 48 h from the last drink. Seizures are tonic-clonic, diffuse, with little or no postictal period [72]. Although most commonly self-limiting, they can be difficult to manage, and in almost one-third of patients, alcohol withdrawal seizures progress to DT [61]. In more than half of the cases, seizures recur, and in up to 5%, they may progress to a status epilepticus [73]. More than 50% of withdrawal seizures are associated with risk factors such as prior seizures, structural brain lesions, or use of other drugs [74]. Seizures that occur later than 48 h after the last drink should prompt the clinician to consider other causes, such as head trauma or combined drug withdrawal effects [74].

Diagnosis

The diagnosis of AWS is based on the observation of signs and symptoms of withdrawal in patients who have experienced an abrupt reduction or cessation of alcohol consumption [18]. At least two of the following symptoms should be observed [18]:

- autonomic hyperactivity (sweating or tachycardia);
- increased hand tremor;
- insomnia;
- nausea or vomiting;
- transient visual, tactile or auditory hallucinations or illusions;
- psychomotor agitation;
- anxiety;
- tonic–clonic seizures.

In addition, symptoms related to acute or chronic alcohol abuse or withdrawal should be differentiated from those related to other psychiatric disorders [18].

The Clinical Institute Withdrawal Assessment for Alcohol (CIWA-A) scale, and particularly its 10-item revised form (CIWA-Ar) [75], is useful for assessing the severity of AWS (Table 22.3). Scores <8 indicate mild withdrawal, 8–15 indicate moderate withdrawal (marked autonomic arousal) and >15 indicate severe withdrawal and are also predictive of the development of seizures and delirium. Pharmacological treatment is not necessary if CIWA-Ar is <8–10, may be appropriate with scores ranging 8–15 to prevent progression to more severe forms of AWS, and is strongly indicated in patients with CIWA-Ar scores >15.

The Alcohol Withdrawal Scale represent another useful tool to assess AWS's severity in patients who cannot cooperate due to severe withdrawal [76]. However, from a practical point of view, the need is to predict the probability of a patient developing severe AWS in order to start a more aggressive treatment despite presenting symptoms. It should be emphasized that severe medical illnesses, such as

Table 22.3 Clinical Institute Withdrawal Assessment for Alcohol—revised (CIWA-Ar) scale

Clinical Institute Withdrawal Assessment for Alcohol revised	
Symptoms	Range of scores
Nausea or vomiting	0 (no nausea, no vomiting): 7 (constant nausea and/or vomiting)
Tremor	0 (no tremor): 7 (severe tremors, even with arms not extended)
Paroxysmal sweats	0 (no sweat visible): 7 (drenching sweats)
Anxiety	0 (no anxiety, at ease): 7 (acute panic states)
Agitation	0 (normal activity): 7 (constantly thrashes about)
Tactile disturbances	0 (none): 7 (continuous hallucinations)
Auditory disturbances	0 (not present): 7 (continuous hallucinations)
Visual disturbances	0 (not present): 7 (continuous hallucinations)
Headache	0 (not present): 7 (extremely severe)
Orientation/clouding of sensorium	0 (orientated, can do serial additions): 4 (disorientated for place and/or person)

pneumonia, coronary heart disease, alcohol liver disease and anemia, have been reported to precipitate AWS and to increase the risk of severe AWS [60]. In these patients, prophylactic treatment could be useful, regardless of CIWA score.

Treatment.

Goals of Treatment

The primary objective of AWS treatment is to prevent withdrawal seizures, DT, and other serious medical complications. In addition, symptoms of AWS are uncomfortable, and patients may be unwilling to stop drinking in part due to a fear of developing withdrawal. Consequently, treatment should aim at reducing symptom's severity, preventing progression to more severe forms and improving the patient's quality of life [61, 77]. In addition, treatment of AWS should help patients engage with a multidisciplinary alcohol relapse prevention program [61, 77, 78]. Patients with mild-to-moderate AWS (e.g., CIWA <15) can be managed as outpatients, while those presenting with more severe forms should be monitored and treated in an inpatient setting [79].

General Treatment and Supportive Care

Non-pharmacological interventions should be aimed at providing a low stimulus environment. A quiet room without dark shadows, noises or other excessive stimuli (i.e., bright lights) is recommended [60]. General supportive care should correct dehydration, hypoglycemia and electrolyte disturbances, and should include hydration and vitamin supplementation. In particular, thiamine supplementation is essential for the prevention of Wernicke's Encelopathy (WE) [8].

Specific Treatment

The general principle of AWS treatment is to reduce CNS hyperexcitability using medications that show cross-tolerance with alcohol, and then taper these at a rate that allows the pathological excitation—inhibition balance that has been established during prolonged heavy drinking to normalize. Although this can essentially be achieved using any medication that potentiates GABA-ergic transmission, benzodiazepines (BZDs) are the gold standard treatment of AWS [80, 81]. This is the only pharmacological class with robust evidence supporting their ability to prevent the progression to complicated AWS and to reduce the incidence of seizures (84%), DT and mortality [60, 78, 79]. BZDs mediate their therapeutic effect through positive allosteric modulation of GABA receptors, thus mimicking the effects of alcohol [82]. Although evidence on the superiority of any BZD over another are lacking, the

most robust data are available for long-acting agents (e.g., chlordiazepoxide and diazepam) [60, 83], promoting a smoother withdrawal [60, 83]. Several BZDs have active metabolites generated by phase I liver oxidation and inactivated by phase II liver glucuronidation and excretion [60]. As a consequence, patients with reduced liver metabolism (e.g., elderly, those with advanced liver disease) could benefit from short-acting agents, such as oxazepam or lorazepam, in order to prevent excessive sedation, ataxia resulting in a risk of falls and fractures, and respiratory depression [83]. Besides BZDs, clomethiazole is also widely used in Europe [60].

AWS can be treated using different treatment regimens, specifically called 'fixed-dose', 'loading dose' and 'symptom-triggered':

In the fixed-dose approach, a predetermined administration schedule is used, independent of patient's symptoms, with a tapering over 4–7 days. An example of such a regimen is diazepam 10 mg four times a day for 1 day, then 5 mg four times a day for 2 days, then tapering off, typically combined with the possibility to administer extra doses if sufficient symptom control is not achieved. This represents the preferred approach to patients with, or at risk of, severe AWS. The potential disadvantage of this approach is a risk that the administered doses are either insufficient to prevent the emergence of a DT, or unnecessarily high, resulting in excessive sedation [60, 83]. Most treatment providers therefore have 2–3 different fixed order sets for these tapers, at different dose levels. It can, however, be difficult to select the right order set for an individual patient based on the initial evaluation of AWS severity. This approach therefore requires careful monitoring, so that dosing can be properly adjusted.

The loading-dose scheme can be combined with the approach above. A moderate-to-high dose of a long-acting BZD, corresponding to 15–30 mg diazepam is administered, and the response is monitored. If withdrawal severity is adequately lowered within appr. 1 h, treatment can transition to a fixed taper. Otherwise, bolus doses are repeated until patient is sedated. In rare cases, repeated loading doses are insufficient to obtain an adequate response. This identifies the few patients who are in need of particularly aggressive treatment to prevent emergence of DT, potentially including i.v. administration of BDZs (diazepam or midazolam) under strict monitoring at an intermediate care or intensive care unit.

The use of a symptom-triggered schedule requires the administration of the chosen drug (e.g., diazepam 5–20 mg, chlordiazepoxide 50–100 mg or lorazepam 2–4 mg) whenever the CIWA-Ar score is >8–10. Symptoms are evaluated hourly and the chosen drug (i.e., 5–10 mg of diazepam) is administered in order to reduce the CIWA-Ar score below 8 points. Although there is no evidence of a clear superiority for any of the three regimens [60, 83], the symptom-triggered seems to reduce total BZD consumption and treatment duration [80].

It should be emphasized that the administration of BZDs represents the cornerstone of the management for any severity of AWS, and is effective for prevention of both seizures and DT. In particular, treatment of DT requires the use of BZDs as primary drugs, with the possible use of antipsychotic medications to control symptoms of psychosis (see below). However, the use of BZDs also has limitations. It is associated with an increased risk of excessive sedation, motor and memory deficits and respiratory depression, particularly among patients with liver impairment [81,

84]. Moreover, the risk of abuse and dependence [60, 83] limits BZD use in AUD patients if withdrawal is managed in an outpatient setting [81].

Considering these limitations, several other medications have been tested for the treatment of AWS in research studies. Among these, α2-agonists and beta-blockers are useful for the management of neuro-autonomic manifestation such as tachycardia and hypertension; neuroleptics should be used to control hallucinosis and delirium. However, these medications can mask underlying core withdrawal symptoms of CNS hyperexcitability, thus increasing the risk of seizures and DT. These medications should therefore not be used as monotherapy, but rather as adjunctive drug on BZDs.

Although used in the management of mild-to-moderate forms of AWS, the clinical utility of antiepileptics (e.g., carbamazepine, valproic acid, gabapentin, pregabalin and topiramate) is limited by a potential for liver toxicity associated with som of some of these agents [60, 82], and a lack of efficacy when used as first-line treatment [85, 86]. Sodium oxybate, with its alcohol-mimicking effects represents a useful option in the treatment of both AWS and long-term treatment for alcohol relapse prevention [60]. Finally, baclofen, a GABA-B agonist, represents a promising drug in the treatment of both AWS and post-withdrawal [87], or at least a valid alternative [88]. The lack of any significant side effects and of liver toxicity [89] makes it possible to use this drug for the treatment of AUD patients suffering from liver disease [90].

Alcoholic Hepatitis

Alcoholic hepatitis is a clinical syndrome characterized by acute liver inflammation, jaundice and liver function impairment, that occurs in patients with a history of acute heavy and/or prolonged alcohol use. More details are provided in Chaps. 64, 65, 66, 67 and 68 of Part X. The laboratory profile reveals neutrophilia, moderately high AST levels (generally <300 IU/L) with an AST/ALT ratio greater than 1.5–2 and in severe forms a total serum bilirubin level greater than 85 micromol/L (5 mg/dL) [3, 91].

Pathogenesis

Heavy drinkers (60 g/day of alcohol consumption) develop hepatic steatosis, a condition that is reversible if alcohol intake is reduce or discontined. This is an asymptomatic condition, which can be associated with hepatomegaly and a mild alteration of AST and GGT [92, 93]. If heavy alcohol use continues, steatosis can progress to an inflammation of the liver, i.e., a (chemical) hepatitis. Factors such as obesity or viral infections (most importantly Hepatitis C), in association with other genetic or environmental factors, increase the risk for progression and severity of alcoholic hepatitis.

Diagnosis

Symptoms associated with alcoholic hepatitis are mainly malaise and painful hepatomegaly. In many cases, the acute inflammation occurs against a background of chronic cirrhotic damage (see below), potentially resulting in hepatic decompensation and encephalopathy. The diagnosis of an alcoholic hepatitis is mainly suggested by a careful alcohol history taken by the physician. This must assess both previous and current consumption, evaluating any quantitative alterations in alcohol consumption [94]. It is not uncommon for alcoholic hepatitis to occur in patients who have been abstinent in the weeks prior to diagnosis [91].

In the absence of jaundice, alcoholic hepatitis can be asymptomatic at the presentation. In the presence of a history of heavy alcohol use consistent with the onset of alcoholic hepatitis, blood tests can confirm the diagnosis in most cases: the rise in bilirubin>3 mg/dL, AST/ALT ratio > 1.5 but <400 IU/mL in acute can support the hypothesis. Serologic tests for viral hepatitis (Hepatitis B and C) should be performed, as well as liver autoimmunity and Wilson's disease screening. Abdominal ultrasound can be helpful to rule out mechanical causes of jaundice.

In the symptomatic forms, although alcoholic hepatitis is a frequent cause of jaundice, histological confirmation should be performed if possible, as jaundice could be linked to other causes such as drug hepatotoxicity, infection, the occurrence of hepatocellular carcinoma (HCC), end-stage liver failure or following a complication, in particular gastrointestinal bleeding [95, 96].

Moreover, at the time of diagnosis of symptomatic alcohol hepatitis, more than 50% of patients have underlying advanced liver disease and almost all patients with severe alcohol hepatitis have already developed cirrhosis [93, 97].

Assessment of Prognosis

The most used score to assess the severity and the short-term prognosis of alcohol hepatitis, both in randomized trials and in clinical practice, is conventionally Maddrey's discriminant function score (MDF), based on the levels of serum bilirubin and prothrombin time. If MDF is above 32 points, alcohol hepatitis is severe and treatment with corticosteroids (methylprednisolone 32–40 mg IV daily) should be administered. Otherwise, the disease has a poor prognosis, with mortality of 20 to 30% within 1 month after presentation and 30 to 40% within 6 months after presentation [98, 99]. The early improvement in liver function observed in the first week of treatment is a predictor of short-term survival.

The Lille score integrates patient characteristics at the initiation of corticosteroid treatment such as age, serum albumin, serum creatinine, prothrombin time and change in bilirubin at the end of the first week of corticosteroid therapy. This score is calculated at the **seventh day** of medical and drug treatment and is used to assess the therapeutic response to corticosteroid therapy. Patients with a Lille score **above**

0.45 are non-responders to corticosteroid therapy and have a very low 6-month survival, (around 20% to 30%). Patients with a Lille score lower than 0.45 are responders to corticosteroid therapy and have an excellent 6-month survival, around 70–80%. Discontinuation of corticosteroid therapy is recommended in "complete non-responders" (defined as Lille score of 0.56) because of the risk of severe infections, because in this subgroup corticosteroid therapy is not more effective than placebo. If the Lille score is between 0.45 and 0.56, the arduous challenge to continue corticosteroid therapy should be considered on a case-by-case basis [100].

Treatments

The cornerstone of treatment is to maintain total alcohol abstinence. Abstinence is associated with a markedly improved 5-year survival. Anticraving drugs are strongly suggested after the first episode of alcoholic hepatitis to maintain alcohol abstinence [91, 101].

In most cases of alcoholic hepatitis, especially in severe forms, malnutrition should be recognized and treated, potentially requiring nutrition via a nasogastric tube, or parenteral nutrition [102]. A recent trial confirmed that adequate calory intake associated with cortisone treatment in severe alcoholic hepatitis reduces the 6-month risk of mortality.

Corticosteroid therapy is recommended in severe alcoholic hepatitis [103]. The European Association for the Study of the Liver (EASL) has identified prednisone as the first-line treatment, to be started as early as possible [104]. If prednisone is contraindicated, pentoxifylline is the drug of choice. Consequently, in an Emergency Department, it is advisable to detect alcohol hepatitis, to establish if it is a severe form (with calculation of MDF), and then to start corticosteroid therapy.

The prognosis of severe acute alcoholic hepatitis is related to the response to corticosteroid therapy and to the absence of infectious complications. An infection is observed in 20–30% of patients at admission and develops in 25% in the first month of corticosteroid treatment. At admission, if an infection is diagnosed, corticosteroid therapy should be initiated after a proper infection control.

In case of non-response to corticosteroid therapy, highly selected patients should be considered for early liver transplantation [105, 106].

Trauma and Injuries

It's estimated that between 5% and 40% of trauma patients attending emergency departments are alcohol-related cases [107]. In fact, alcohol use, in particular intoxication, plays a major role in a wide range of injuries, some of which are readily classifiable as alcohol-related, for example road injuries or violent assault, and others which are less so (e.g., falls, injuries in the workplace) [108, 109]. The amount

of alcohol consumption is directly related to the risk of injuries, and even the consumption of two drinks (=24 g of alcohol) doubles the odds of injury. The risk then increases sharply with higher consumption [110].

It's important to emphasize that alcohol use often plays an important role in interpersonal violence, in particular domestic violence perpetrated by male, where it can be considered a causal factor. Worldwide, governments are implementing new laws and restrictions in order to reduce any kind of alcohol-related injury, particularly due to road accidents and interpersonal violence [109].

Conclusion

Alcohol-related emergencies such as acute alcohol intoxication or alcohol withdrawal syndrome are currently a widespread problem and occur very frequently in the Emergency Department. Since in most cases these are reversible conditions, if recognized and treated early, the onset of life-threatening complications can be avoided. Systematic screening should aim at identifying AUD in patients admitted to the emergency room for alcohol-related symptoms, and relapse preventions treatments is essential to initiate in order to to prevent new acute episodes. See also related Chap. 48.

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Part IV
Fetal Alcohol Spectrum Disorders (FASD)

Chapter 23
Fetal Alcohol Spectrum Disorders: An Introduction

Eileen M. Moore and Edward P. Riley

Abstract Alcohol is a teratogen, a substance that can disrupt prenatal development and cause malformation. It is now widely accepted that alcohol has the potential to produce adverse outcomes, including dysmorphic features, growth retardation, birth defects, and neurobehavioral deficits. While there are arguably references to the adverse consequences of gestational alcohol exposure in ancient works, modern research on fetal alcohol spectrum disorders (FASD) did not begin until the 1970s. It is estimated that about 10% of pregnancies are alcohol exposed and about 1 in 13 of these offspring will have FASD. Early identification and diagnosis along with appropriate services and interventions is associated with improved outcomes for individuals with FASD. This chapter provides a general introduction to FASD, with emphasis on epidemiology, diagnosis, and alterations in brain and behavior resulting from prenatal alcohol exposure. It also includes a discussion of the potential interventions being developed for individuals with FASD.

Keywords FASD · FAS · Prenatal · Fetal · Alcohol · Pregnancy

Introduction

Prenatal alcohol exposure (PAE) has the potential to produce a range of adverse outcomes, impacting a variety of organs and systems, most notably the brain. This impact on the brain can result in a wide array of cognitive and behavioral deficits that impact quality of life. Additionally, PAE may also result in deficits in growth as well as specific craniofacial features. Collectively, the array of outcomes produced by PAE are referred to as fetal alcohol spectrum disorders (FASD). Here we present an overview of FASD, including its history, epidemiology, diagnostic considerations, brain and behavioral manifestations, and interventions.

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History

Warren [1] and Brown et al. [2] provide excellent reviews of the history of PAE and FASD beginning with possible references to the adverse effects of alcohol consumption during pregnancy noted by ancient Greek scholars as well as in the Bible. However, the first clear writings on this topic came from the London College of Physicians in 1724 noting a link between the consumption of distilled spirits and adverse effects on the progeny. In the early twentieth century, Stockard [3] wrote on the impact of alcohol use on developing offspring, citing both experimental animal research on a variety of species, as well as human observational studies. The animal research noted that alcohol treatment resulted in death, slowed growth, and/or malformations. Using fish eggs Stockard noted that "alcohol…showed a peculiar affinity for the developing nervous system…" By 1900 it was shown that alcohol freely enters embryonic tissues in guinea-pigs and dogs resulting in fetal alcohol blood concentrations similar to that of maternal blood alcohol concentrations [4]. At about the same time Sullivan [5] described the outcomes of children born to women from Liverpool Prison who had histories of chronic alcohol misuse that were "uncomplicated by other degenerative factors." He analyzed 600 children, born to 120 of these women and compared them to 138 children born to 28 cases of female relatives who did not use alcohol. The death rate of children under age two (including still births) was nearly two and a half times higher in the alcohol exposed offspring than controls (55.2% vs. 23.9%, respectively). Among surviving children, there appeared to be an increased rate of epilepsy (4.1%) as compared to the population estimates in England (0.1–0.6%). Sullivan also described a few cases of mothers who were imprisoned during a significant portion of their pregnancy, and therefore did not have access to alcohol, whose children were less affected. Based on these observations, Sullivan noted alcohol's "toxic influence exercised on the developing embryo throughout pregnancy" and concluded that alcohol exposure during gestation is "peculiarly unfavorable to the vitality and to the normal development of the offspring. Its gravity in this respect is considerably greater than that of paternal alcoholism" [5]. Alcohol was even included as a cause of intrauterine disturbances in Adami's "Principles of Pathology" [6]; although, he does comment that while it is "well founded" that the children of parents with alcohol use disorders are "of lowered intelligence and vitality with unstable self-control," it is difficult to disentangle the impact of alcohol intoxication during fetal development from other factors, including heredity, malnutrition, and neglect.

Another important factor influencing the history of FASD was that prohibition of alcohol was legislated in a number of countries in the early twentieth century [1]. With prohibition interest in researching PAE declined and previous findings appeared to be ignored, reinterpreted, dismissed, and/or forgotten. In 1939, Mapother [7] summarized the literature regarding the impact of alcohol on offspring. In terms of experimental work in animals, he acknowledged that some effects of alcohol were found in non-mammalian and non-human mammalian species but noted that the research was more supportive that alcohol would have an

"eventual production of an improved strain through wholesale elimination of the more weakly progeny in pre-natal or early post-natal life." He did not discuss any of the prior research that had been conducted in humans. Rather, he stated: "All this, however, is very academic and has little relation to anything at all probable in man; the concentrations used in producing experimental effects are far greater than anything that ever occurs in the human." The view that alcohol could not produce adverse effects upon the developing embryo/fetus in humans came to be dominant for the next few decades. In fact, alcohol even came to be considered somewhat beneficial. For example, the use of high-dose intravenous alcohol for patients at risk for preterm delivery was advocated [8] and most obstetric textbooks from before the 1970s actually recommended that women consume alcohol while pregnant [9].

A few studies describing relations between PAE and poor outcomes continued, although they did not receive much attention. In 1957, a thesis was completed in Paris describing poor outcomes in children who were exposed to alcohol *in utero* [10]. In 1968, Lemoine, a French physician, published his observations of 127 children who exhibited similar physical features and behavioral deficits and who were all born to mothers who had an alcohol use disorder [11]. Unfortunately, these results were not widely disseminated, perhaps because they were published in French. The modern history of FASD began in the early 1970s, with the publication of two papers in the *Lancet* by Jones and Smith and colleagues describing the fetal alcohol syndrome [12, 13]. Despite these papers being met with skepticism and some backlash [14], additional case studies, clinical research, and animal studies, confirmed the teratogenicity of alcohol. In June 1977, the first health advisory was released in the United States in the Food and Drug Administration Drug Bulletin and the Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report [15].

Epidemiology

Determining the true prevalence of FASD is difficult for a number of reasons, including challenges related to recognition, screening and diagnosis and stigma, among other barriers. Active case ascertainment methods are considered the "gold-standard" for prevalence estimation [16] and have been conducted in several countries to estimate the prevalence of FASD. In the US, 13,146 children in first grade were systematically assessed in four communities and the conservative estimate for FASD was between 1–5% of children [17]. In Canada 2555 elementary school students were assessed in the Greater Toronto Area and the prevalence of FASD was estimated to be nearly 2% of children [18]. The estimated prevalence of FASD in the Greater Manchester region of the United Kingdom is 2–4%, in communities near Rome, Italy it is 2–6%, in rural Croatia ~7%, and in Poland at least 2% [19–22]. Much higher prevalence estimates are observed in other specific areas. For example, prevalence of FASD in a community in the Western Cape Province of South Africa, a wine growing region with a history of utilizing the 'Dop system'

(see Williams [23] for a history), are estimated at 16–31% [24]. FASD is also more prevalent among children in foster care, in the correctional system, and among populations in special education.

Active-case ascertainment studies are not available for the majority of countries. However, it is estimated that about 10% of women report consuming alcohol during pregnancy [25]. Not every woman who drinks during pregnancy will have a child with FASD, given that there are many factors that influence risk and resilience. Overall, it is estimated that 8 out of 1000 children in the general population have an FASD and 1 in 13 alcohol-exposed pregnancies will result in a child with FASD [26]. However, regional differences exist in prevalence of alcohol use during pregnancy and FASD. Popova and colleagues identified the available studies reporting prevalence in the general population of each country and used advanced statistical techniques to predict the prevalence for countries where empirical data were not available [25–28]. The European region had the highest rates of alcohol drinking (at any level) during pregnancy (25.2%), although Africa had the highest rates of binge-drinking during pregnancy (3.1%). In the Americas, alcohol use during pregnancy was estimated at 11.2%, and binge drinking at 2.8%. Alcohol use and binge drinking during pregnancy was estimated at 8.6% and 1.8%, respectively, in the Western Pacific region. Comparatively low levels of alcohol use were estimated in the South-East Asian (1.8%) and Eastern Mediterranean (0.2%) regions (it was not possible to estimate binge-drinking in these regions). The rates of FAS and FASD roughly corresponded to the estimated drinking levels. The European region was estimated to have the highest rates of FAS and FASD (37.4 and 198.2 per 10,000, respectively), followed by the region of the Americas (16.6 and 87.9 per 10,000, respectively), the African region (14.8 and 78.3 per 10,000, respectively), and the Western Pacific region (12.7 and 67.4 per 10,000, respectively). The regions with the lowest estimated prevalence of FAS and FASD were the South East Asian (2.7 and 14.1 per 10,000) and Eastern Mediterranean (0.2 and 1.3 per 10,000) regions.

Diagnostic Considerations

Screening

The majority of alcohol-affected individuals are missed or misdiagnosed. In a clinical sample of 156 children who met criteria for an FASD, 125 had never been recognized as having been affected by PAE [29] and in a large active case ascertainment study [17], only 2 of 222 children diagnosed with FASD had a previous FASD diagnosis. A number of factors may contribute to this problem, including unknown maternal alcohol use histories, particularly in foster and adopted children; high rates of co-occurring mental health disorders, concern about stigmatization, poor recognition and/or a lack confidence among health care practitioners in making an FASD

diagnosis, as well as a lack of multidisciplinary FASD diagnostic clinics [30–37]. Compounding these barriers, the age at which an individual is assessed can impact the likelihood of a diagnosis, with diagnosis being especially difficult in infancy and adulthood [38]. Additionally, a number of different diagnostic schemas have been proposed (see Table 23.1), which can be confusing for clinicians, especially given that evaluations of the same individual using different diagnostic schemas can result in different diagnoses being made under the FASD umbrella [42].

Of the children born with FASD each year, it is estimated that less than 1% will receive a diagnosis [43]. This is unfortunate, as early identification and diagnosis coupled with appropriate interventions and services can mitigate adverse outcomes for individuals with FASD [37, 44]. Screening tools are essential for the identification of at-risk individuals. Several screening tools are available for use [45]. Caregiver questionnaires have been developed, such as the Neurobehavioral Screening Tool, which includes 12 items from the Achenbach Child Behavior Checklist that have been shown to significantly differentiate FASD from control groups [46]. However, the reported sensitivity and specificity of this tool varies, with some studies reporting 94% sensitivity and 96% specificity in children age 4–6 years and others reporting 72% sensitivity but only 34% specificity in children and young adults age 3–22 years [47, 48]. Other questionnaires have been developed for specific high-risk populations, such as youth and adults in correctional facilities [49].

Other approaches that have been developed for screening individuals include evaluating markers of PAE. For example, one of the earliest markers of PAE was a pattern of craniofacial features, which along with growth retardation and central nervous system dysfunction, led to the recognition of FAS. These features can be detected either by manual measurement or facial analysis software [50]. Figure 23.1 depicts the facial features of FAS. While most individuals who are affected by PAE do not display the full dysmorphic facial features required for a diagnosis of FAS, research suggests that even among children who do not meet criteria for FAS or partial FAS, analysis of 3-dimensional (3D) facial photography is capable of detecting characteristics consistent with PAE [50]. This suggests that 3D facial analysis screening may have potential for identifying children at risk for FASD. Another marker that is well studied and shows promise for screening is the evaluation of oculomotor control. Eye-tracking to measure saccades, or the rapid movement of eyes that shifts the center of gaze across our visual fields, reveals that children with FASD have longer saccadic reaction times, increased variability, and increased saccadic errors during tasks as compared to controls [52]. Eye-movement was 90% accurate in distinguishing children with FASD from ADHD [53], which is very relevant clinically, given that as many as 95% of children with heavy PAE meet diagnostic criteria for ADHD [31]. Serum protein analyses, dermatoglyphics, functional near-infrared spectroscopy, olfactory testing, and DNA methylation [54–57] have also been investigated as potential screening tools. While many show promise, there is limited research to date for these methods.

Diagnosis

The seminal paper by Jones, Smith & colleagues [12] described a distinct pattern of craniofacial features, growth retardation, as well as developmental delay or intellectual disability and subsequently coined the term FAS [13]. However, it soon was recognized that not all the individuals affected by PAE displayed the features required for a diagnosis of FAS, yet still may have functional deficits that affected their quality of life. Indeed, the range of physical, cognitive, and behavioral outcomes associated with PAE is quite variable. Research has revealed several factors that can influence outcomes associated with PAE, either conferring risk or resilience, including, but not limited to, the dose and timing of alcohol exposure, nutritional status of the mother, genetics of both the mother and the embryo/fetus, co-exposures to other substances, maternal stress, etc. The term FASD became broadly adopted as it refers to the full range of adverse effects caused by PAE [40].

There are a number of existing diagnostic schemas that attempt to capture the wide-ranging outcomes within FASD. In the United States, common diagnostic schema include those proposed by the Institute of Medicine (IOM) and the FASD diagnostic clinic at the University of Washington (4-Digit Code) [39, 58]. Additionally, the *Diagnostic and Statistical Manual of Mental Disorders, fifth Edition* included Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE) in the appendix as a "condition for further study" [41]. Canada, Australia, and Scotland also have their own diagnostic schemas, which are rather similar to one another [59–61]. The criteria for the IOM, 4-Digit Code, and Canadian schemas are listed in Table 23.1.

Comparisons between the different diagnostic schemas have revealed fairly high discordance. Patient records from 1392 individuals evaluated for FASD at the University of Washington were used to apply the 4-Digit Code, IOM, Canadian, and Australian diagnostic systems and while 82% were diagnosed with an FASD by at least one system, only 11% were diagnosed by all four systems [42]. For many of the diagnostic schemas, a distinction between individuals who have the sentinel facial features associated with PAE and those who do not is made. Additionally, they may allow for a diagnosis to be made even if alcohol-exposure status is unknown when the seminal facial features are present. With one exception, central nervous system involvement is a requirement of every diagnosis. The brain has been noted to be particularly vulnerable to alcohol's teratogenic effects, largely due the fact that it is developing over the entire course of the embryonic and fetal development.

Table 23.1 Different diagnostic schemas of FASD

	4-digit diagnostic code (3rd ed.) [39]	IOM updated criteria [40]	Canadian criteria [41]
FAS	Categories A & B		Not proposed
Alcohol exposure	Confirmed or not confirmed	Confirmed or not confirmed	FASD with sentinel facial features is roughly equivalent to FAS in either the 4-Digit Code or IOM diagnostic schemas
Facial features	Simultaneous expression of all three facial features: small palpebral fissure lenght (≤2 SD), smooth philtrum and thin upper lip (ranks 4–5 using the UW Lip-Philtrum Guides)	≥features: short palpebral fissures (≤10th percentile), thin vermillion border (ranks 4–5 on racially normed lip/philtrum guide), & smooth philtrum (ranks 4–5 on racially normed lip/philtrum guide)	
Growth retardation	Prenatal or postnatal height and/or weight ≤10th percentile with age and gender adjustments	Prenatal or postnatal height and/or weight ≤10th percentile on racially or ethnically appropriate growth curve.	
CNS involvement	Head circumferences ≥2 SD below norm or significant abnormalities in brain structure or evidence of hard neurological findings or significant impairment in ≥3 domains of brain function (≥2 SD below the mean) as assessed by validated and standardized tools	Deficient brain growth, abnormal morphogenesis, or abnormal physiology, including ≥1 of the following: head circumference ≤10th percentile, structural brain anomolies, recurrent nonfebrile seizures. For children ≥3 years, cognitive impairment (general conceptual ability ≥1.5 SD below mean or performance IQ, verbal IQ, or spatial IQ ≥1.5 SD below the mean; or cognitive deficit in ≥1 domain ≥1.5 SD below the mean [executive functioning, learning, memory, or visual-spatial] or behavioral impairment (behavioral deficit in ≥1 domain ≥1.5 SD below the mean in impairments of self-regulation [mood or behavioral regulation impairment, attention deficit, or impulse control). For children <3 years of age, evidence of developmental delay ≥1.5 SD below the mean is required.	

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	4-digit diagnostic code (3rd ed.) [39]	IOM updated criteria [40]	Canadian criteria [41]
Partial FAS	Category C		
Alcohol exposure	Confirmed	Confirmed or not confirmed; if no documentation of PAE exists, additional criteria are required (see growth retardation and CNS involvement sections below)	Not proposed
Facial features	Short palpebral fissures (≤2 SD) and either a smooth philtrum or thin vermillion border (ranks 4–5 on UW Lip-Philtrum Guides) with the other being normal, or palpebral fissure (≤1 SD) and both a smooth philtrum and thin vermillion (ranks 4–5 on UW Lip-Philtrum Guides)	Same as FAS	
Growth retardation	Not required	Not required for children with documented PAE. For children without documented PAE, growth deficiency or deficient brain growth is required; the criteria for growth deficiency are the same as for FAS.	
CNS involvement	Same as for FAS	For children with documented PAE, only evidence of neurobehavioral impairment is required; using the same criteria as FAS. For children without documented PAE, growth deficiency or deficient brain growth, abnormal morphogenesis, or abnormal neurophysiology is required; using the same criteria as FAS.	
ARND		This diagnosis cannot be made in children under 3 years.	

	4-digit diagnostic code (3rd ed.) [39]	IOM updated criteria [40]	Canadian criteria [41]
Alcohol exposure	SE/AE and ND/AE combined are roughly equivalent to ARND in the IOM or FASD without sentinel facial features in the Canadian guidelines.	Confirmed	FASD without sentinel facial features is roughly equivalent to ARND in the IOM diagnostic schema or the combination of SE/AE and ND/AE in the 4-Digit Code.
Facial features		Not required	
Growth retardation		Not required	
CNS involvement		Evidence of global impairment (general conceptual ability ≥1.5 SD below the mean, or performance IQ, verbal IQ, or spatial IQ ≥1.5 SD below the mean; or cognitive deficit in ≥2 neurobehavioral domains ≥1.5 SD below the mean (same domains as FAS) or behavioral deficit in ≥2 domains ≥1.5 SD below the mean (same domains as FAS).	
Other		This diagnosis cannot be made in children under 3 years.	
ARBD			
Alcohol exposure	Not proposed	Confirmed	Not proposed
Facial features		Not required	
Growth retardation		Not required	
CNS involvement		Not required	
Other		≥1 specific major malformations demonstrated in animal models and human studies to be the result of PAE; cardiac: atrial septal defects, aberrant great vessels, ventricular septal defects, conotruncal heart defects; skeletal: radioulnar synostosis, vertebral segmentation defects, large joint contractures, scoliosis; renal: aplastic/hypoplastic/dysplastic kidneys, “horseshoe” kidneys/ureteral duplications; eyes: strabismus, ptosis, retinal vascular anomolies, optic nerve hypolasia; ears: conductive hearing loss, neurosensory hearing loss.	

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	4-digit diagnostic code (3rd ed.) [39]	IOM updated criteria [40]	Canadian criteria [41]
FASD			Two diagnoses: FASD with & without sentinel features
Alcohol exposure	FASD, in these schemas, is used as a non-diagnostic term refering to the full range of deficits resulting from PAE.		FASD with sentinel facial features allows for confirmed or unconfirmed; FASD without sentinel facial features requires confirmation of PAE with estimated dose at a level known to be associated with neurodevelopmental effects
Facial features			FASD with sentinel facial features requires: Simultaneous expression of all three facial features: small palpebral fissure length (≤2 SD), smooth philtrum and thin upper lip (ranks 4–5 using the UW Lip-Philtrum Guides)
Growth retardation			Not required
CNS involvement			Both diagnoses require evidence of pervasive brain dysfunction, defined as severe impairment (≥2 SD below the mean*) in ≥3 domains: motor skills; neuroanatomy/ neurophysiology; cognition; language; academic achievement; memory; attention; executive function, including impulse control and hyperactivity; affect regulation; adaptive behavior, social skills or social communication

	4-digit diagnostic code (3rd ed.) [39]	IOM updated criteria [40]	Canadian criteria [41]
SE/AE	Categories E & F	Not proposed	Not proposed
Alcohol exposure	Rank 3–4 exposure. Rank 4: confirmed PAE & exposure pattern is consistent with medical literature placing fetus at high risk (i.e., high peak BAC at least weekly in early pregnancy). Rank 3: confirmed PAE but the level of alcohol is less than Rank 4 or the specifics of the exposure level is unknown	SE/AE and ND/AE, together, are roughly equivalent to ARND in the IOM and FASD without sentinel facial features in the Canadian schemas.	
Facial features	Not required		
Growth retardation	Not required		
CNS involvement	Rank 3–4 CNS involvement. Rank 4: ≥1 significant structural abnormalities of the brain or neurological findings of presumed prenatal origin (i.e., microcephaly, hydrocephaly, heterotopias, change in shape or size of brain regions, siezures, other hard neurological signs, etc.). Rank 3: problems across ≥3 domains as assessed by standardized, validated psychometric assessments including but not limited to executive function, memory, cognition, social/adaptive skills, academic achievement, language, motor, attention or activity level that are likely due to underlying brain damage rathn than adverse postnatal environments		

(Continued)

	4-digit diagnostic code (3rd ed.) [39]	IOM updated criteria [40]	Canadian criteria [41]
ND/AE	Categories G & H		
Alcohol exposure	Same as for SE/AE	ND/AE and SE/AE, together, are roughly equivalent to ARND in the IOM and FASD without sentinel facial features in the Canadian schemas.	
Facial features	Not required		
Growth retardation	Not required		
CNS involvement	Rank 2 CNS involvement: ≥1 domain with at least mild to moderate delay or impairment but <3 domains with significant (≥2 SD below mean) impairment or the appropriate testing has not occurred, i.e., if children are <6 years of age (in these cases, children should be re-assessed when old enough to determine if they meet Rank 3 criteria)		
Notes	The 4-digit code provides an assessment of effects in four areas (growth, facial features, CNS involvement, and alcohol exposure) that results in 256 different codes and 22 diagnostic categories (A-V). Categories A-C & E-H are included in this table; categories I-J include cases where PAE is confirmed but only sentinal physical findings were present (I) or no physical findings or CNS abnormalities were detected (J); the remaining categories (D, K-V) include categories for when PAE is unknown or there is confirmation that PAE did not occur.	Consideration of FASD is a complex diagnostic process requiring a multidisciplinary approach, sound clinical judgment, and the consideration of the biological parents when available to account for the heritibility of head circumference, growth, cognitive and behavioral characteristics. Differential diagnoses must include genetic disorders or other teratogenic conditions.	A designation of "at risk for neurodevelopmental disorder and FASD, associated with prenatal alcohol exposure" is used to describe individuals with confirmed PAE and some indication of neurodevelopmental concerns, but who do not meet the criteria for either of the FASD diagnostic categories

Please refer to the original text for full diagnostic criteria. *IOM* Institute of Medicine, *UW* University of Washington, *CNS* central nervous system, *FASD* fetal alcohol spectrum disorders, *FAS* fetal alcohol syndrome, *SE/AE* static encephalopathy/alcohol exposed, *ND/AE* neurobehavioral disorder/alcohol exposed

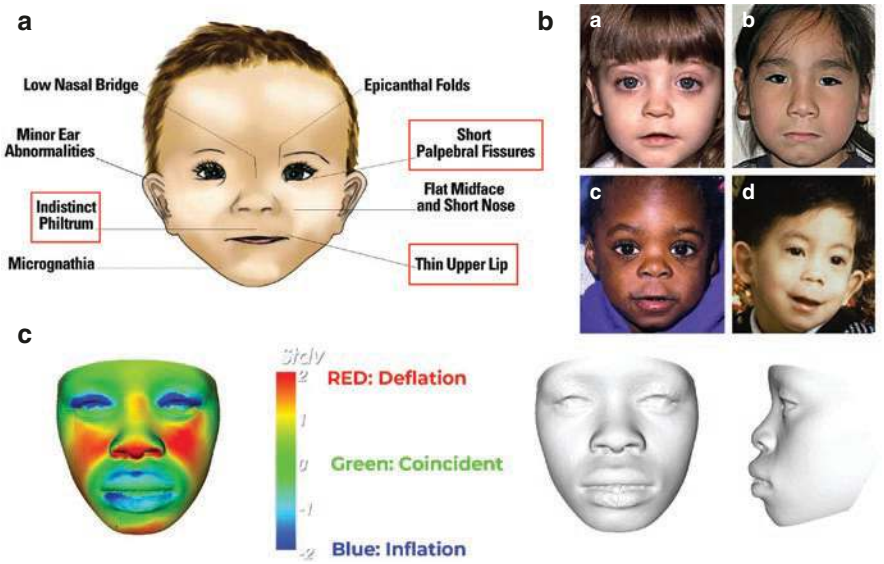

Fig. 23.1 (**a**) Diagram of common dysmorphic facial features in children with FASD. Note the three cardinal facial features of FAS in the red boxes (Source: Warren et al. [51]). (**b**) Examples of the FAS facial phenotype (small eyes, smooth philtrum, and thin upper lip) across four races: (*a*) Caucasian, (*b*) Native American, (*c*) African American, (*d*) Mexican American. Copyright 2022, Susan Astley Hemingway PhD, University of Washington. (**c**) A 3D comparison of facial features between a child with FAS and a composite control face (image courtesy of Michael Suttie, Ph.D.)

Brain, Cognition, and Behavior

Brain. PAE has the potential to adversely impact any organ systems development; however, the brain is the most studied. The brain has a large window of development, effectively spanning the entire period of the embryonic and fetal stage (and beyond). Thus, alcohol exposure at any point during gestation has the potential to impact brain development and, consequently, cognition and behavior.

Although gross abnormalities in brain are not overly common, magnetic resonance imaging (MRI) studies have found that PAE is associated with a variety of changes to the brain. The most commonly reported finding is smaller brain volume [62]. Several brain regions have been identified as being smaller than one would expect given the overall smaller brain size (i.e., disproportionately smaller). These include the corpus callosum, cerebellum, and basal ganglia [62, 63].

The corpus callosum is the most frequently affected brain region in FASD [64]. Abnormalities were noted in some of the first brain autopsies in children with FAS, and cases of full or partial agenesis of the corpus callosum were reported in some early MRI studies [65]. The shape, location, and size of the corpus callosum may be affected and have been documented in newborns through adulthood [62, 66]. Diffusion tensor imaging (DTI) studies have also reported microstructural changes

to the corpus callosum, including lower fractional anisotropy, higher mean diffusivity, and higher radial diffusivity in a pattern that suggests that myelination or axonal density/caliber is affected [61]. The more anterior (genu) and posterior (isthmus-splenium) regions appear to be the most affected, in terms of macro and microstructure [61]. It should also be noted that, in addition to the corpus callosum, white matter macrostructure (smaller size and displacement) and microstructure (lower FA in several tracts) across the brain is affected by PAE [67–69].

The cerebellum is also frequently affected [64]. It has been shown to be disproportionately smaller, malformed, and displaced in FASD [70] and the volume of the cerebellum relates to the quantity of PAE [71]. The more anterior lobules and the vermis appear to be affected the most, while the more posterior and inferior regions are relatively spared [72]. The most severe effects on the cerebellum are observed among those with FAS, although deficits are also observed in individuals with PAE who do not exhibit the sentinel facial features [73].

The entire basal ganglia volume is disproportionately smaller. Regionally, the caudate is most consistently noted as disproportionately affected by PAE [67]. Further, shape analysis shows that the head and tail regions of the caudate bilaterally are deformed and that the extent of the deformation is related to PAE [74]. However, other basal ganglia regions are also reported to be disproportionately affected by PAE, including the putamen and pallidum [62, 75, 76].

Other regions have also been reported to be disproportionately affected, including limbic, diencephalic, brainstem, and cortical regions. Effects appear to be depending on a number of factors, including the dose of alcohol exposure, diagnostic severity, sex, and age. For example, the volume of diencephalic structures has been reported to relate to the degree of facial dysmorphology in children with PAE [76]. Additionally, the regional effects of PAE on brain structure may differ across development. Longitudinal studies show that children with heavy PAE have differing developmental trajectories of cortical volume, thickness, and gyrification [77]. Generally speaking, as children age into adolescence, a larger discrepancy in brain morphometry emerges between those with PAE and controls.

Functional MRI (fMRI) studies show an overall pattern of broad, widespread brain activation and/or recruitment, in a variety of tasks, of brain regions that differ from those activated in unexposed controls [61, 78]. The activation patterns are often reminiscent of what is observed in younger children, perhaps suggesting delayed maturation. Alternatively, the differences in activation patterns could indicate that the individuals with FASD are utilizing different strategies to complete the task than are controls, and exert greater effort; or that compensatory mechanisms are needed to complete the task. Resting-state fMRI, a task-independent approach to examine neural networks, indicates that PAE is associated with network disruption in neonates that may continue until at least young adulthood [79, 80].

Cognition and Behavior. Consistent with the brain findings, global cognitive deficits are observed in those with FASD, evidenced by generally lower intelligence; however, specific deficits in cognitive domains are also apparent [81].

Attention. Attention deficits are among the most well-documented effects of PAE and have been called a hallmark feature of FASD [82]. Children with PAE are less

efficient at processing visually presented information, have slower reaction times, lower accuracy rates, and make more omission errors than controls [82]. Auditory attention, however, is less impacted than visual attention [82, 83]. Indeed, rates of attention deficit/hyperactivity disorder (ADHD) are as high as 95% in FASD [30]. However, studies comparing children with FASD to nonexposed children with ADHD demonstrate that these two groups differ from one another in a number of ways. As compared to children with ADHD, children with PAE generally have lower IQ scores, more difficulty with encoding information as well as focusing, sustaining, and shifting attention, and greater deficits in working memory, planning, fluency, and set-shifting [81].

Executive functioning. This cognitive construct encompasses a number of interrelated cognitive processes important for goal-directed behavior [81]. Children with PAE have been shown to display deficits across executive function domains, including verbal fluency, response inhibition, problem solving and planning, concept formation, cognitive flexibility, and working memory [81]. Age may be an important moderator of some of these effects. In a cross-sectional study of spatial working memory, an interaction between age and performance was noted such that the children with PAE appeared to improve at a slower rate than controls [84]. Executive function deficits have also been documented in adults with FASD [85].

Learning & Memory. The majority of studies have examined verbal learning and memory. Children with FASD have slower word-list learning, have difficulty recalling words after a delay, and problems discriminating the correct words from distractors [81]. However, once the verbal information is encoded it is retained at the same rate as in controls [86, 87]. Fewer studies of nonverbal memory exist and their results are inconsistent. Some studies report encoding and retrieval deficits for nonverbal information and that deficits in in nonverbal learning and recall persist after accounting for IQ deficits, while others indicate that the long-term retention of visual information is spared [81].

Visual-spatial. Animal studies suggest that PAE produces impairment in the visuo-spatial domain, especially in spatial learning and memory [88]; however, less is known about humans with FASD. Children with PAE have been shown to have impairments of visual construction and copying figures tasks [87]. However, their difficulty may be due, at least in part, to motor deficits rather than completely attributable to perceptual deficits. When asked to recall hierarchical figures, children with PAE had deficits in the recall of local but not global features of the stimuli that were not due to the size of the stimuli or memory deficits [89].

Motor function. Both fine motor and gross motor skills are affected by PAE; however, complex fine motor skills tend to be more severely affected than basic motor skills [90, 91]. Deficits in oculomotor control [50], hand-eye coordination, bimanual coordination, regulating isometric and isotonic force, and sensorimotor ability as well as longer reaction times, difficulty maintaining postural balance, and atypical gait characteristics have all been documented in children with FASD [81]. Some motor deficits have also been shown to persist into adulthood [85].

Language and communication: Speech production and language processing deficits have been observed in children with heavy PAE [81]. Expressive language skills

appear to be somewhat more affected than receptive (impressive) language ability [92]. Parents report that young children with FASD have deficits in communication skills, including trouble maintaining conversation, answering questions, and staying on topic [93], which appear to worsen with age [94].

Secondary conditions: Secondary conditions are problems that develop from the difficulties a person experiences as a consequence of FASD. Secondary conditions encompass a wide array of problems that can impact an individual's quality of life. Individuals with PAE are reported to have deficits in a number of interpersonal domains, adaptive functioning as well as higher rates of mental health problems [30, 43, 94]. Children with PAE are more likely to be described as hyperactive, disruptive, impulsive, or delinquent [95]. Higher rates of inappropriate sexual behavior, disrupted school experiences, delinquency, alcohol and other substance use problems have been observed in adolescents and adults with FASD [43]. Rates of ADHD, oppositional defiant disorder, conduct disorder, mood disorders, and specific phobias are elevated in children with FASD as compared to controls [30, 96, 97]. Of course, PAE cannot fully account for these poor outcomes given that there is a high degree of heritability for many of these behaviors. However, a meta-analysis comparing the risk for externalizing disorders due to PAE versus genetics found that there was increased risk of ADHD due to PAE beyond that due to either parental alcohol use disorder or genetic liability [98]. Regardless of cause, these poor outcomes can be mitigated if children with FASD are identified and diagnosed early, receive appropriate services, and are raised in a stable and caring home environment [43].

Interventions

There are three main points at intervention (see Fig. 23.2). FASD is preventable if no PAE occurs. Preconception interventions focus on increasing awareness of harms of drinking during pregnancy, preventing risky drinking, and education about utilizing contraception effectively. If PAE has occurred, FASD can be mitigated or prevented with drinking cessation interventions. Nutritional interventions show promise as well. Lastly, postnatal interventions include nutritional supplements, exercise, cognitive and behavioral interventions. Psychotropic medications are also often used, however, there are no specific medications approved for the treatment of FASD.

Pre-conception Interventions

Preventing an alcohol-exposed pregnancy will ensure that a child is not born with FASD. Universal interventions target large audiences to increase awareness about the harms of PAE and/or provide education about contraception and prenatal care.

Fig. 23.2 Diagram of the three points of intervention to prevent or mitigate FASD. Interventions after birth may occur at any point over an individual's lifetime. Silhouette images, from left to right, are credited to Chipmunk131/shutterstock.com, Neboisa Kontic/shutterstock.com, and KatarinaF/shutterstock.com

The media, billboards, pamphlets in healthcare facilities, and other health promotion education strategies are used [99]. Warning labels on alcoholic drinks are one example; however, these labels have had only modest effects on drinking during pregnancy, primarily affecting behavior in women who were light drinkers while behavior remains unchanged in the women who drank more heavily [100]. A multimodal universal intervention was implemented in two provinces in South Africa with high prevalence of FASD [101]. After the development of media highlighting FASD, implementation of regular health talks on FASD to patient groups at healthcare facilities, training on FASD for health providers and social workers, and referral of women at high risk for having a child with FASD to existing intervention programs in the community, knowledge about FASD increased and prevalence of FASD decreased from 8.9% to 5.7%.

Worldwide, it is estimated that about 10% of women drink while pregnant [25]. Interventions targeted at women of childbearing age have the potential for reducing alcohol-exposed pregnancies [102]. Six months after an intervention that focused on increasing commitment to change risky drinking and/or ineffective contraception use behaviors via motivational interviewing, 68.5% of women who were previously deemed high-risk for having an alcohol-exposed pregnancy were no longer at risk [103].

Women who have previously had a child with FASD are also at risk of having additional children with FASD. The Parent-Child Assistance Program is an intensive case-management intervention for pregnant or parenting mothers who have alcohol and drug use disorders [104]. This program has been implemented in Washington State for several decades. At-risk pregnant and parenting mothers are enrolled in the case-management program for 3 years and receive outreach, assistance, structured goal-setting, problem solving, coaching, and linkage to recovery supports. The program has reduced future births of children exposed to alcohol and

drugs, increased rates of recovery from substance use in mothers, improved parenting of birth mothers, and increased rates of safe and stable child placement. The benefits of investing in the health and safety of at-risk women and children are clear: this program has demonstrated its cost-effectiveness in terms of decreased welfare costs and foster care costs [105].

Interventions During Pregnancy

Once an alcohol-exposed pregnancy has occurred, drinking cessation or reduction interventions can mitigate the impact of alcohol on the developing embryo/fetus. A meta-analysis found that single-session, face-to-face brief interventions appeared to have some positive benefits on the maintenance of alcohol abstinence during pregnancy [106]. Even as little as a 10-min educational session in combination with providing a nine-step self-help manual was effective in fostering alcohol cessation in economically disadvantaged pregnant women [107]. Drinking cessation interventions can also improve some birth outcomes. In a group of non-dependent women who were screened for alcohol use and either recommended to stop drinking or provided with a brief, multi-session intervention during pregnancy, the women in the intervention group were five times as likely to be abstinent by the third trimester [108]. For the women who were consuming two or more drinks/occasion, the intervention resulted in longer birth length in their infants. Additionally, a larger number of non-viable outcomes were noted in the assessment only group (2.9%) as compared to the intervention group (0.9%; [108]).

Alcohol can interfere with the absorption of nutrients, which, during pregnancy, can potentially exacerbate the teratogenicity of alcohol [109]. Supplementation may improve the nutritional status of the mother and embryo/fetus and confer some resiliency against FASD. Prenatal choline supplementation has been shown in animal studies to mitigate behavioral deficits caused by prenatal alcohol [110]. In a prospective cohort study in the Ukraine, women were randomly assigned to receive either a daily multivitamin and mineral supplement, with or without an additional 750 mg of choline supplementation, or standard care in which prenatal vitamins were recommended but not provided. While choline did not appear to confer any benefit in infants, small but significant positive effects of the multivitamin supplement were observed in the offspring, including better problem solving and pre-linguistic skills at 6-months [111]; however, there was little evidence of benefit in preschool children [112]. In contrast, another study found benefits of choline in a South African cohort of women who were recruited mid-pregnancy and randomly assigned to receive either 2 g choline or placebo [113, 114]. Infants born to the choline-treated mothers had larger volumes of several brain regions [114], were more likely to meet criterion on an eyeblink conditioning task at 6.5 months, showed greater catch-up growth in weight and head circumference at 6.5 and 12 months, and had better visual recognition memory at 12 months [113]. The choline dosage in this study was much larger than in the

study conducted in the Ukraine, suggesting a dose threshold may exist for effectiveness.

Postnatal Interventions

Nutrition: In a double-blind, randomized, placebo-controlled pilot trial to test the effectiveness of daily treatment with 500 mg choline for 9-months, there were some modest treatment effects on memory in the youngest children [115]. In a 4-year follow-up, choline-treated children had fewer behavioral problems, higher non-verbal intelligence, visual spatial, working memory, and verbal memory skills [116]. In a separate study that administered 625 mg choline daily for 6 weeks to children between 5–10 years, no cognitive improvements were observed [117]. The null effects may be related to the age of the children at choline administration or the relatively short timeframe of the study. More studies of choline treatment are needed to determine the parameters of its effectiveness. Animal studies suggest effectiveness of choline in addition to other nutritional and antioxidant supplements, including vitamins E and C, omega-3, resveratrol, and epigallocatechin gallate [118]; however, most of these have not been studied in humans.

Exercise: In a physical activity program for children with FASD that consisted of 1.5-h sessions that occurred twice per week for 8 weeks, improvement in executive functioning was observed in children from the pre-program to the 3 month post-intervention assessments [119]. The program included a warm-up activities, 15-min sessions that targeted three of the following: speed and agility, strength, balance, bilateral coordination, upper-limb coordination, and fine motor skills; a 20-min "choice" period, where a skill that was chosen by either the child or their caregiver was targeted; and a cool down period. The average preprogram score was in the mildly impaired range, while the average score at the 3-month follow-up was in the below average range [119]. This study indicates that a clinically relevant improvement in executive functioning may be achieved as a result of a physical activity training, consistent with a number of animal studies that have found that exercise can mitigate some symptoms induced by PAE [120].

Habilitation: Wells et al. [121] conducted a randomized, controlled study of cognitive habilitation in children with FASD who resided in foster or adoptive homes. Children with FASD between the age of 6–11 were assigned to receive either the intervention or no-treatment. The intervention utilized components of the Alert program [122], which teaches self-regulation skills, and additionally targeted memory, emotional awareness, and cause-and-effect reasoning skills. Occupational therapy and family psychoeducation were also included in the intervention. Seven months after enrollment (2–3 months after treatment concluded) children in the intervention group had significantly improved executive function and emotional problem-solving skills as compared to the no-treatment group [121].

Children with FASD experience significant academic difficulties and behavioral problems. Difficulties with mathematical concepts are common [123]. The Math

Interactive Learning Experience (MILE) program was developed to improve pre-math and mathematical skills in children age 3–10 years [124, 125]. It is a 6-week, comprehensive intervention that involves active-learning math instruction using manipulables and visual aids, immediate feedback, and experience mediation to support learning and improve integration of concepts in an individually placed curriculum; caregiver education; school consultation; and as needed case management and psychiatric consultation [124]. Children were randomized to either the MILE program or standard psychoeducational care groups. Children in the MILE program demonstrated greater gains on standardized testing and fewer behavioral problems than the comparison sample, persisting for 6 months [124]. The benefits of the MILE program were replicated in a separate sample of children with FASD [125].

Friendship Training: O'Connor and colleagues [126] evaluated the effectiveness of a friendship training intervention in school-age children with FASD who had social skills deficits. Children were assigned to receive either the child friendship training or they were placed in a delayed treatment group. The intervention involved 12 weekly, 90-min sessions. Parents attended separate concurrent sessions where they were provided psychoeducation on the social skills being taught to their children. Children who received the friendship training demonstrated significantly improved social knowledge as well as parent-rated social skills and problem behaviors, effects that were maintained over a 3-month follow-up period. While the children benefitted from the program, unfortunately, no treatment effect was noted on teacher-rated behaviors. The adaptation for the manualized behavior treatment is described in Laugeson et al. [127].

Families Moving Forward (FMF): Children with FASD commonly have behavioral problems and the parents of these children are often stressed [128]. The FMF program was designed to modify parenting attitudes and responses towards their child's problem behaviors [129]. It integrates several child-management and parent training techniques and teaches caregivers the skills to promote a positive behavior support approach to dealing with problem behaviors, emphasizing antecedent-based behavior strategies and developing and advocating for accommodations for their child [130]. The FMF intervention, implemented for 9–11 months with at least 16 every-other-week, 90-min sessions, resulted in caregivers reporting increased parenting self-efficacy, self-care, and reduced child disruptive behaviors. On a standardized parent-reported behavior questionnaire, a significant and clinically relevant change in Problem scores occurred from pre- to post-intervention. The intervention group reported that their children's problem behavior were, on average, in the clinical range at the pre-test, while the post-test scores were in the borderline range. The FMF program is being translated to a mobile health intervention app that allows for caregiver self-delivery, called FMF Connect [131]. The app will help address some barriers to care, including limited access to services.

Alcohol Intervention: Studies estimate that between 35–60% of adolescents and adults with FASD have an alcohol or substance use problem [85]. An adapted version of Project Step Up, an alcohol harm-reduction program, was evaluated in adolescents with FASD [132]. This intervention focused on practical knowledge and skills to empower adolescents with FASD to make safe decisions regarding alcohol.

It incorporated motivational enhancement techniques, normative feedback, education, risk assessment, coping and alcohol refusal skills training in a group setting. Adolescents were assigned to either receive the Step Up intervention or were provided with written materials on alcohol misuse and stress reduction. Before the intervention, 33% of the teens reported light/moderate drinking, while the remainder reported abstinence or infrequent drinking. For the light/moderate drinkers, those in the intervention group reported a significant decrease in in alcohol risk and harm, which was partially sustained at the 3-month follow-up [132]. The adapted Step Up intervention may help prevent some alcohol-misuse and harm in adolescents with FASD.

Summary and Conclusion

The impact of PAE has been documented throughout history, although our modern understanding of FASD did not commence until the 1970s. Despite public health prevention efforts, the prevalence of PAE is estimated at 10% globally and it is thought that 1 in 13 alcohol-exposed pregnancies results in a child with FASD. Children with PAE may exhibit brain, cognitive and behavioral deficits. Common sites of alcohol teratogenesis in the brain include the corpus callosum, cerebellum, and basal ganglia. Brain network function is disrupted during completion of cognitive and motor tasks as well as during resting conditions. The impact of PAE on the brain translates into a number of cognitive and behavioral deficits, including deficits in general cognitive ability, attention, learning and memory, visuospatial ability, motor skills, language and communication, executive functioning as well as secondary conditions. Early identification and diagnosis and referral for appropriate services and interventions can mitigate adverse outcomes.

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Chapter 24
Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure

Michael Suttie

Abstract Fetal alcohol syndrome was first described in the literature over 50 years ago and is widely recognized as a devastating public health problem. Prevalence in the U.S. is estimated to be as high as 2.0–7.0 per 1000 individuals in school-age populations. However, prevalence dramatically increases when we consider the full spectrum of prenatal alcohol-associated effects, with estimates of fetal alcohol spectrum disorders (FASD) ranging as high as 2–5% in the U.S. and Western Europe. The diagnosis of FASDs relies upon identifying a range of physical defects and specific neurocognitive and behavioral profiles. FAS is at the most severe end of the spectrum with multiple diagnostic criteria stating reliance on the recognition of at least two of the three cardinal facial features; a smooth philtrum, a thin upper lip vermillion and reduced palpebral fissure length. Alcohol exposed individuals who lack the required criteria for a FAS diagnosis are inherently challenging to identify and subsequently suffer high rates of missed diagnoses and misdiagnosis. In this chapter, we review the 2D and 3D imaging based methods for identifying the range of FASD associated facial dysmorphism across the FASD spectrum, and discuss the identification of and relationships between the face, brain and neurocognitive performance.

Keywords Facial dysmorphism · FAS facial recognition · Face-brain interactions in fetal alcohol spectrum disorders · Prental alcohol exposure · Fetal alcohol spectrum disorders

Introduction

Alcohol's teratogenic insult can be severe at crucial developmental stages. It predominantly damages the central nervous system, adversely affecting growth, behavior, and cognitive development in childhood when affected individuals are typically

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brought to the attention of care professionals. Amongst many other detriments, a child prenatally exposed to alcohol can be permanently afflicted with impaired learning, dysfunctional behavior, and growth deficits. The detrimental impact of in-utero alcohol exposure has long been known, with the first formal report documented in the late 1960s [1]. However, this article was in French and did not significantly impact medical literature. There was little interest until 5 years later when the Lancet published a report by Dr. Kenneth Jones & Dr. David Smith [2]. Jones and Smith identified a set of clinical diagnostic criteria; specifically, they described a unique set of facial characteristics associated with prenatal alcohol exposure (PAE). Since this publication, a spectrum of associated PAE effects has been recognized and has given rise to the term fetal alcohol spectrum disorders (FASDs). There is considerable variation in the combination and severity of effects, convoluting clinical diagnoses and making the task of identifying those affected clinically challenging. A variety of factors are thought to influence this range and severity, although the timing of exposure [3], exposure patterns [4] and genetic predisposition [5] appear to be the primary modifying factors that influence the outcome for individuals with PAE.

Diagnostic Criteria

At the most severe end of the FASD spectrum are fetal alcohol syndrome (FAS) and partial fetal alcohol syndrome (PFAS), characterized by a set of distinctive facial features, cognitive and behavioral deficits, and reduced growth. Three cardinal facial features form part of the FAS/PFAS diagnosis; palpebral fissure length (distance between inner and outer corners of the eye openings) < tenth percentile, a thin upper lip-vermillion border (thin upper lip), and a smooth philtrum (smoothness of the midline groove in the upper lip) [6]. Several diagnostic systems are in circulation, which differ in criteria to categorize the effects of PAE clinically, but there is universal agreement amongst these systems on the significance of the three cardinal facial anomalies [7]. Additionally, across diagnostic guidelines, there is often a necessary combination of criteria for FAS/PFAS, such as a small occipital frontal circumference (OFC; head circumference), a history of maternal drinking during pregnancy, structural brain anomalies, growth deficiency, and behavioral or cognitive deficits. Phenotypic facial features are a hallmark of FASDs and play a crucial role in identifying individuals with PAE. However, a patient may often present to a clinician with cognitive and/or behavioral deficits, confirmed or suspected PAE, but lack the identifiable facial criteria for a FAS/PFAS diagnosis. Such individuals may have been exposed to alcohol in utero at levels similar to those diagnosed as FAS/PFAS and present a vast array of other physical and neurological deficits. Depending on the diagnostic guidelines used for clinical assessment, classification without facial features will rely on other criteria requiring confirmed alcohol exposure, central nervous system impairments, and neurocognitive and behavioral deficits [6, 8] Hoyme et al. [6] provide an updated set of clinical diagnostic guidelines for

diagnosing FASDs, including two categories for confirmed PAE without facial features: alcohol-related neurodevelopmental disorder (ARND), which requires neurobehavioural impairment, and alcohol-related birth defects (ARBD), which require one or more major malformations. This is just one example of several diagnostic guidelines with criteria for identifying individuals with PAE without facial features. Most diagnostic guidelines have some methodology for identifying these individuals [7]. However, the heterogeneous and indistinctive nature of presentation means they are often misdiagnosed or undiagnosed. Furthermore, and most concerning, the vast majority of individuals with PAE fall into this clinically challenging category [9].

Prenatal Alcohol Exposure and the Developing Brain

Perhaps the most significant effect of PAE is the potential damage caused to the developing brain. Some brain regions are particularly vulnerable, and few remain unharmed by alcohol's teratogenic insult. Magnetic resonance imaging (MRI) enables a highly accurate assessment of brain shape, size, spatial displacement, and even function. The corpus callosum, the most extensive white matter tract primarily responsible for the interhemispheric connection between the left and right sides of the brain, appears disproportionately affected by alcohol teratogenesis. Significant variations in this structure have been observed and include complete or partial agenesis [2], localized shape differences [10, 11], displacement [12] and localized volumetric reductions [13]. These findings are representative of the agreed vulnerability of midline structures in PAE. Deep grey matter structures are also consistently reported to be disproportionally affected, and findings include a reduced volume of the hippocampi [14, 15] and basal ganglia [16, 17], with many studies focusing specifically on the caudate nucleus [15, 18, 19]. The neural structures affected by PAE are responsible for memory, sensorimotor, behavior, and impulse control, among many other cognitive functions.

The disruption to developing brain structures causes extreme variability in cognitive dysfunction, although some common functional deficits are apparent in both FAS and in less severe impairments. For example, there are significant declines in overall cognitive ability determined by intellectual quotient (I.Q.) scores, memory and recall, and executive function (higher-level cognitive functions such as abstract thinking, problem-solving, and planning ahead). Additionally, psychosocial deficits and problem behaviors such as hyperactivity, impulsivity, poor socialization, and poor communication skills are apparent at a young age, interfering with a child's education, home life and social environments. A combination of these factors puts affected children and adolescents at a higher risk of delinquent behaviors, trouble with the law, psychiatric disorders, and drug and alcohol abuse, which inevitably follow into adulthood. As with many childhood conditions, early and accurate diagnosis of FASDs is crucial to introducing appropriate educational, psychological, and welfare interventions. Providing the necessary support as early as possible

reduces the risk of social and behavioral problems negatively affecting the individual later on in life. Clinical diagnosis requires a multidimensional approach, considering minor physical anomalies, facial dysmorphology and neurocognitive and behavioral profiles. There is a distinct lack of clinical expertise specifically for FASDs. An individual will often need to seek a referral to a specialist clinic where they will typically undergo examination by both dysmorphologists and clinical psychologists. Only a small part of the clinical assessment involves facial analysis, and due to the nature of FASD facial features being mostly observational, the examination is necessarily subjective, with the accuracy being dependent on the skill and experience of the clinician.

Clinical Recognition of FASD-Associated Facial Features

Identifying Cardinal FAS Features

Standard clinical assessment of physical FASD features by trained dysmorphologists uses manual anthropometry and visual assessment. Palpebral fissure length is typically measured using a clear ruler, and philtrum smoothness and the thinness of the upper lip vermillion are assessed using Likert scales [6, 20]. Alternatively, 2D Commercial off-the-shelf software for the identification of the FAS facial phenotype is currently available. Initially developed in 2003, the FAS Facial Photographic Analysis (FPA) Software uses 2D photographs in combination with the 4-Digit Diagnostic Code [20–22] which aims to identify the magnitude of expression of the FAS phenotype. The software determines metrics to represent the three cardinal facial features; palpebral fissure length, lip thickness, and philtrum smoothness from a series of three 2D images using manually guided procedures. The image acquisition protocol requires three images; a frontal view, lateral view, and ¾ view to be taken with a standard digital camera. For dimensionality, the image is scaled using a visual guide (small sticker) physically placed on the subject's forehead. Using the three images uploaded to a computer, the user can then measure or estimate the three cardinal facial features. Landmark points on the inner (endocanthi) and outer (exocanthi) eye corners are manually annotated by the clinician on the front portrait image. Palpebral fissure length is calculated as the mean of left and right distances which is weighted using an adjustment factor, subsequently adding 7% to the distance. The weighting constant is representative of the angle of the zygoma (cheek bone) to account for the loss of depth perspective in 2D. The palpebral fissure length measure taken using this method, with the adjustment factor applied, was shown by the authors to have just a 1% margin of error compared to subjects measures with a caliper [23, 24]. However, the use of a 2D photographic assessment of palpebral fissure length have has come under some scrutiny by other researchers and is not always the preferred method by clinicians [6, 25]. This lack

of agreement is primarily due to potential inaccuracies caused by individual variations of the angle of the zygoma, questioning the ability to account for this correction using a constant value. Studies testing the concordance of techniques to record palpebral fissure length have found measurements taken using 2D imaging to be consistently shorter [25, 26] than physical measurement, although it should be noted that a subsequent study has shown 2D palpebral fissure length measurements to be greater, although not significantly different to those taken using a ruler [27].

Standard physical examinations and those undertaken using 2D imaging are reliant on subjective methods for lip and philtrum assessment. Likert scales matched by ancestral origin are used to compare an individual's philtrum smoothness and upper lip by holding a chart up against the subject or the ¾ view using the FPA software. The lip-philtrum guides provide a scoring system from 1–5, ranging from 1 a full/grooved lip/philtrum to 5 a FAS-like thin/smooth lip/philtrum. The consistency and accuracy of this assessment are paramount to a FAS diagnosis, and in most criteria, a misclassified score of just a single rank difference will change diagnosis and potentially the interventions received throughout life. Likewise, for the palpebral fissure length measurement, a deviation of just 1 mm can result in the measure being 1 s.d out of range, potentially to altering diagnostic outcome.

Clinical Recognition of Minor Facial Anomalies

FASD diagnostic criteria provided by Hoyme and colleagues [6] describe the importance and prevalence in FAS of a series of minor facial anomalies, secondary to cardinal FAS features. Primarily, they observe the presence of midfacial hypoplasia (reduced growth of the upper jaw, cheekbones and eye sockets), epicanthal folds (skin fold on the upper eyelid, on the inner part of the eye), a long philtrum, decreased interpupillary distance (distance between the midpoints of the eyes), a flat nasal bridge, prognathism (protrusion of the lower jaw), and anteverted nares (upturned nostrils). With the recognition of these features being mostly reliant on subjective identification, it remains a challenge to accurately and objectively recognize these mostly soft-tissue deformations, to the point that clinical observation by trained dysmorphologist is still prone to error. These "non-cardinal" FASD features do not contribute to any formal diagnosis in the most commonly used diagnostic criteria, other than in the Emory-Fetal Alcohol Center Clinical Criteria, which uses a weighted checklist of 40 items to provide an overall dysmorphology score [7]. This score sets a scale from 0 to 57 to measure the physical effects of alcohol rather than focusing only on cardinal features. An approach such as this could benefit diagnostic outcomes, especially if methods for objective identification on a continuous scale were to be integrated into the clinical workflow. Midfacial hypoplasia, for example, is characterized primarily by a flattened nasal bridge, an upturned nose, long philtrum, and flatness of the midface region. To date, there have been few attempts to objectively identify this discriminative and prevalent feature with visual and imaging-based approaches [28].

Detecting FASD Associated Facial Dysmorphism Using Image Analysis

Face Analysis using 2D imaging suffers limitations of perspective and dimensionality, and analysis of surface shape and finer detail are restricted. Based on the subjectivity and inaccuracies of manual assessment, we assert that an objective and accurate approach would provide an optimal clinical protocol for FASD evaluation. One such strategy is to apply shape analysis to facial images acquired from stereo-photogrammetric cameras. Stereo-photogrammetric cameras digitally combine two or more simultaneously acquired 2D dimensional images and apply an algorithm to produce a 3D representation of surface geometry with perspective information and accurate dimensionality. Surface images obtained from 3D camera systems provide tens of thousands of points accurately representing facial shape with sub-millimeter accuracy, producing morphology data more accurately and in a more convenient form for shape analysis than 2D. Shape analysis of 3D images can take several forms, the most simplistic of which is based on anthropometric measurement derived from landmarks placed on the image surface.

Several studies have used the highly detailed and accurate reconstructions of the human face provided by 3D photography and statistical analysis techniques to address the problem of identifying FAS facial features [28–30]. The earliest of these studies [3] utilized stereo-photogrammetry systems to obtain reliable 3D images of the face to determine clinically relevant anthropometric measurements. Before this investigation, previous studies using photographic methods for distance measures had been performed using the FPA software. Measurements imperative to diagnosis using 2D photography, such as palpebral fissure length, must be synthetically adjusted to account for the points being off the midline perspective. The ability for 3D images to reliably capture points that lie in different planes highlights the inadequacies of single planar 2D photographic techniques.

Obtaining point-to-point distance measures using 3D images has shown to be a reliable method comparable to anthropometric measures attained physically by a trained dysmorphologist [31, 32]. utilized 3D images of 276 subjects (aged 2.8 to 21.2 years), clinically labeled as FAS or control from 4 different ethnic populations (Cape Coloured [37%], Finnish Caucasian [36%], African American [9%] and North American Caucasian [18%]). The hypothesis of this study assumed that a unique combination of anthropometric features would best discriminate FAS and controls in each of the four ethnic populations. To test this, images captured using a commercially available laser scanning system were manually annotated with 20 anthropometric landmarks. Discriminant analysis was performed to classify FAS and controls utilizing an optimal combination of age at evaluation and 16 measurements derived from the landmarks. From the discriminant analysis, the Finish cohort performed with the highest overall classification rate (93%) and utilized nine anthropometric measurements (inner/outer canthal distance, palpebral fissure length, midfacial depth, ear length, nasal and nasal bridge length, and bitragal

width). The discriminant function calculated from the Cape Coloured cohort achieved a classification accuracy of 92% and included five measurements (inner canthi distance, philtrum length, ear length, minimal frontal width and bizygomatic width). For the African American cohort, the classification rate of 79% was achieved using only two measures (palpebral fissure length and philtrum length). The North American Caucasian population achieved the lowest classification rate of 77% using only the inner and outer canthi distances. This study showed that anthropometric measures derived from 3D images could discriminate FAS from controls across a wide age range and multiple ethnicities. It also highlights and explicates ethnic differences in the presentation of FAS, which appear to be particularly discriminating in the Cape Coloured cohort. More sophisticated approaches employing statistical shape analysis techniques were later introduced to characterize the facial anomalies associated with FAS. Landmark-based morphometric analysis techniques, such as those defined in Mutsvangwa et al. [28], demonstrate the ability to detect features present in FAS-affected individuals from 3D facial images. By utilizing a landmark-based discriminant function analysis model, the authors evaluated the mean shape differences between FAS and control groups at two different time points (5 and 12 years), achieving overall classification accuracies of 95% and 80%, respectively. As well as demonstrating the value of 3D morphometric analysis for identifying individuals with FAS, this analysis supports the notion that FAS facial features are more pronounced in younger generations and subsequently diminish with age [20]. Another study of facial morphology of prenatally exposed individuals revealed differences in directional asymmetry [33]. 3D scans of children from a Finnish Caucasian cohort (n = 90) and a South African mixed-ancestry cohort (n = 78), were obtained and manually annotated with 17 anthropometric landmarks. Using geometric morphometrics methods, they analyzed shape data from the landmarks to show the degree of directional asymmetry for each individual. The analysis showed average directional asymmetry in the FAS groups to be significantly higher than those in the control groups, primarily consisting of a leftward shift in midline landmarks. These results show the extent of facial dysmorphology in alcohol-exposed individuals goes beyond what is clinically recognizable and can include subtle differences in asymmetry.

3D surface shape analysis supports more sophisticated evaluation and detection of even subtle face shape differences. One such technique is the utilization of dense surface models [34, 35] which have been used extensively to analyze 3D facial characteristics associated with neurodevelopmental and related human conditions [36–43]. These models have an advantage over landmark-based analysis in their potential to assess shape variation across an entire 3D surface. The dense surface modelling algorithm builds surface models from raw 3D data, initially aligning and warping surfaces guided by a series of manually placed landmarks. This provides a dense correspondence, matching points on different face surfaces to produce a shape based on a principal component analysis (PCA) of variation of point displacement from the mean face. Individual 3D face surfaces are then resynthesized as a weighted linear sum of principal components that account for the shape variation.

Facial Dysmorphism Across the FASD Spectrum

Across the literature, facial analysis in FASD had typically focused on the identification of FAS. Identifying non-syndromic, heavily-exposed (HE) individuals without cardinal features remains a clinical challenge. In our 2013 study [43] we aimed to assess facial dysmorphism across the FASD spectrum using DSM constructed from 3D facial images to better identify those with and without cardinal FAS features. In a population of South African children, 3D images of 192 participants from two longitudinal University of Cape Town (UCT) cohorts recruited from a local mixed-ancestry community where the incidence of FAS and alcohol consumption during pregnancy is one of the highest in the world. Prospective drinking histories of mothers were obtained by using a timeline follow-back interview [44] recorded during pregnancy in antenatal clinics and at 6 weeks postpartum to obtain data spanning all trimesters. The threshold for heavy alcohol consumption during pregnancy was set at a level of >13 standard drinks per week or partook in binges of five or more drinks per occasion. Controls were the offspring of those who reported abstinence from alcohol during pregnancy unless they met FAS criteria. Those with a genetic disorder were excluded from the study.

Multiple expert clinicians undertook dysmorphology assessments to obtain a diagnostic consensus. Neuro-behavioral assessments were undertaken at a 9-year follow-up, which included the fourth edition of the Wechsler Intelligence Scale for Children (WISC IV) [45], and the California Verbal Learning Test–Children's Version (CVLT-C) [46]. The WISC IV tests are designed to assess verbal comprehension and perceptual reasoning and provide an intelligence quotient (IQ) score, while the CVLT-C tests word recall and recognition memory after a short period. 3D images were captured using a commercially available stereophotogrammetric system, and each image was annotated with 24 anatomically reliable landmarks. DSM surface models were built, providing principal components representing 99% of face shape variance. Control-FAS discrimination testing using dense surface model representations of face shape using 20 randomly sampled 90% to 10% training-unseen test subsets, and discrimination accuracy was predicted using receiver operating characteristic curve analysis. Closest mean classification testing provided scores of relative similarity to either a control or FAS means, using the whole face and sub-regions of the face (periorbital (eyes), profile, mid-facial, perioral (mouth), and perinasal (nose)). Whole face classification using closest mean returned an agreement of 0.967, indicating a high probability of correctly classifying a pair of control and FAS faces. Linear discriminant analysis and support vector machines provided more sophisticated methods for classification, with the latter resulting in a near-perfect agreement. Of the facial regions tested, the periorbital and profile regions achieved the greatest accuracy with 0.98 and 0.93, respectively, using closest means classification. When applying this to the PFAS subset, we achieve similar but marginally diminished accuracy.

Given the heterogeneous presentation of the HE subset, binary classification as shown for FAS/PFAS was not an effective tool. A different approach was taken,

which encompasses the variation of dysmorphism by computing the 'facial signature' of each individual. Facial signatures calculate point-to-point displacement differences of an individual face normalized against a background set. In this case, we are calculating signatures against a set of 35 age-ethnicity matched controls. Comparison control means were selected by ordering the dataset by age, selecting 35 contiguously aged subjects, and computing the mean face for comparison. Facial signatures visualize surface displacement using a heat map showing normalized contraction, coincidence, and expansion compared to the age-ethnicity matched control mean. They provide a quantitative representation of surface differences relative to a matched control population and can show the significance of dysmorphia. When applied to the means of each FASD subgroup, we can observe the most prominent features across each subset of alcohol-exposed individuals (Fig. 24.1). Facial signature heatmaps observed similar coloration at varying degrees of severity across each FASD clinical category. For FAS and PFAS, there was an indication of the cardinal features with blue coloration (expansion) on the philtrum and reduction of the eye openings. Interestingly, we also identified shortening of the nose (red), flattening of the nasal bridge and glabella (forehead above and between the eyes), malar region (cheek) flattening (yellow anterior zygomatic arch), and micrognathia and retrognathia (deficient growth of the lower jaw and mandible; red chin). Focusing on the HE category, we observed these non-cardinal features in a significant proportion of the dataset, and when applying facial signature analysis to individual faces, we noted the presence and severity of these features across the dataset.

Facial Signature Graphs

While identifying facial dysmorphism using facial signatures of groups provides an insight into mean differences, recognizing individual dysmorphia plays a more vital role in clinical assessment. We applied facial signature graph analysis to improve our ability to identify HE children who lack the FAS facial phenotype (Fig. 24.2). Facial signature graphs provide an unsupervised learning approach to clustering faces based on their facial signature similarity. To construct, we iterate through every pair of faces within a dataset and compute the face signature difference to define the relative proximity/signature similarity as the Euclidean distance between the corresponding vectors indexed by their facial signatures. The facial signature distance accounts for all 3D surface points and corresponding geometry, providing a measure of morphological differences between two individuals normalized with respect to a set of age-ethnicity matched controls. We computed the facial signature distance between 107 alcohol-exposed (FAS, PFAS, and HE) individuals within the dataset and constructed a graph whose vertices were the face signatures (Fig. 24.2a). Directional edges are constructed from the closest individuals with the lowest distance to connect one vertex to another or the most similar normalized face shape difference to the first. A connection between face A and face B does not necessarily equate to the two being similar but rather represents a relative similarity of

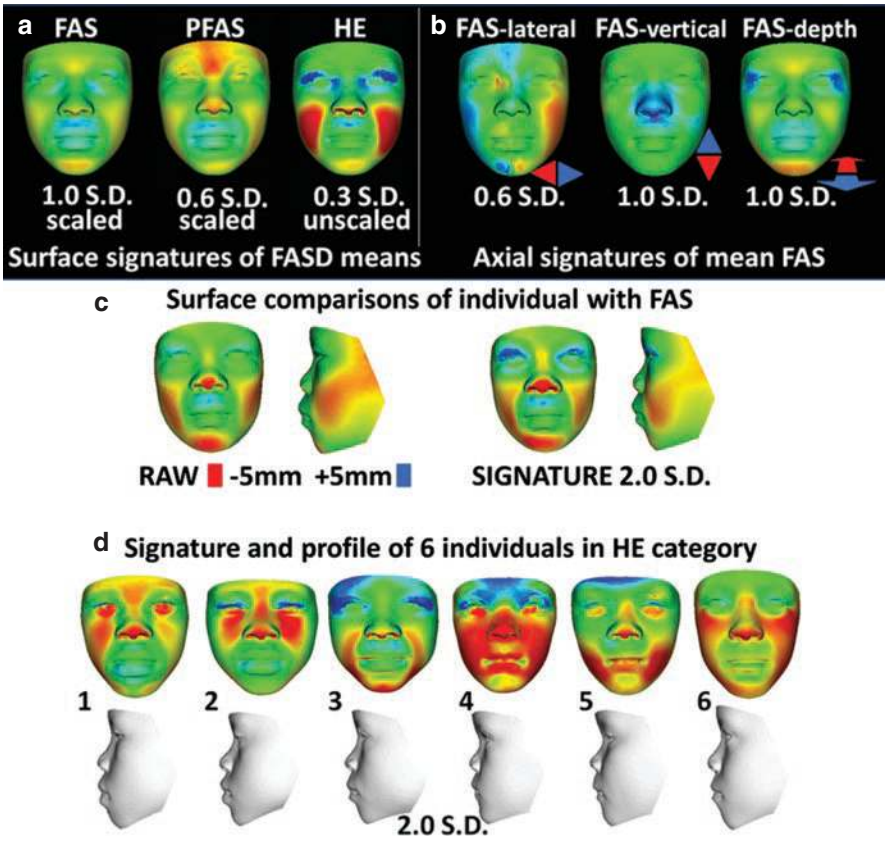

Fig. 24.1 (**a**) Facial signature showing mean differences for each of the clinical categories. Given the significantly reduced growth, FAS and PFAS mean faces were scaled by facial height prior to normalization to reflect shape-only differences. Red-green-blue heat maps reflect contraction-coincidence-expansion along the surface normal, where "±1.0 SD," " ±0.6 SD," and "±0.3 SD," define the upper-lower significance bounds. (**b**) Facial signatures relative to X, Y, and Z axes to depict directional differences in FAS. The lateral axis (left) shows inner canthi differences moving outward—reducing palpebral fissure length, the vertical axis (Y, middle) shows displacement/shortening of the nose relative to the face, and the depth axis (Z, left) indicates midfacial flattening, retrognathia and flattening of the nasal bridge. (**c**) Shows raw differences in mm and the facial signature of an individual with FAS, where red is indicative of mid-face/malar (cheek) flattening, nasal displacement, and retrognathia. This individual also has a smooth philtrum indicated by a blue spot on the philtrum showing an expansion in this region. (**d**) Signatures and profiles of 6 individuals in the HE category who showed affinity to FAS/PFAS in signature analysis. Individuals 1–4 and 6 show red coloring on the mid-face indicating flattening, and individuals 3–6 have red coloring on the chin potentially indicating retrognatia. All individuals have red coloring on the nose indicating reduced nasal length. Extract from Suttie et al. [42]. Reproduced with permission from Journal of Pediatrics, Vol. 131, Page(s) 1, Copyright © 2013 by the AAP

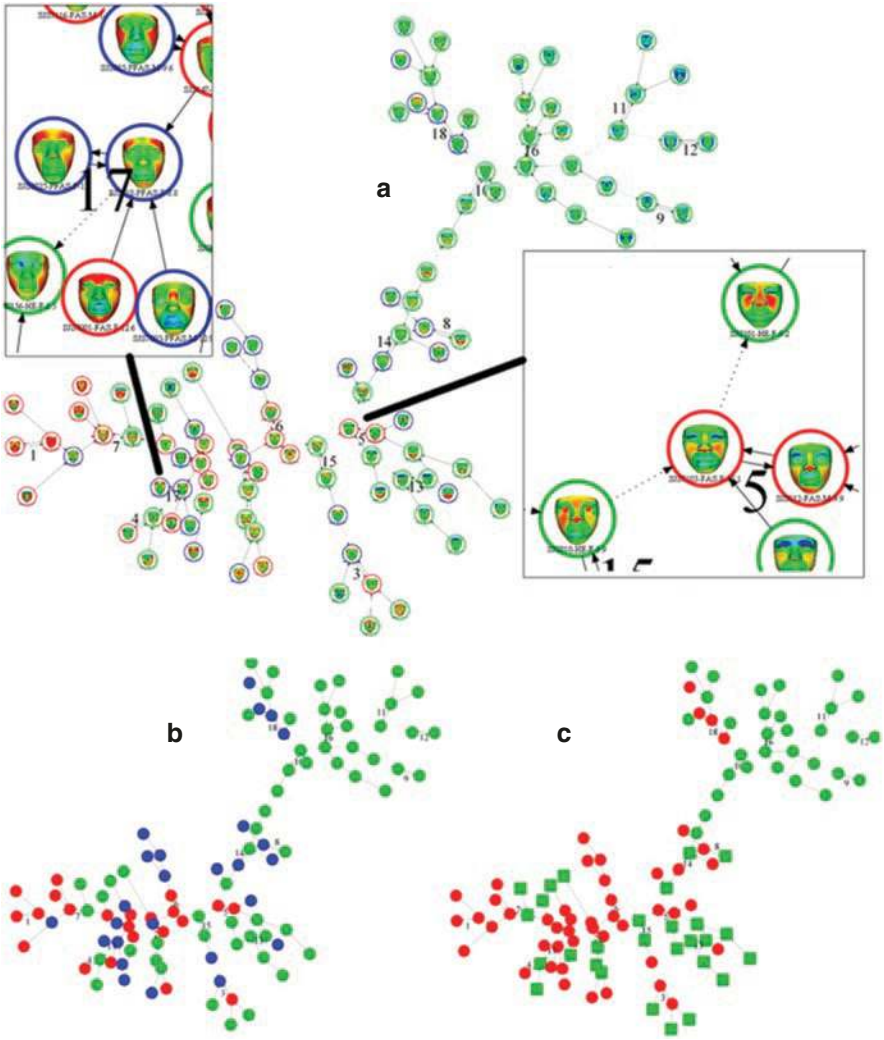

Fig. 24.2 (**a**) Facial signature graphs of 107 alcohol-exposed individuals. This graph is formed of 18 subgraphs from 107 alcohol-exposed individuals (FAS, PFAS & HE) normalized against 35 age-ethnicity matched controls. Left and right boxes show a magnified view of individuals with similar dysmorphism. (**b**) Color-coded signature graph showing HE, PFAS, and FAS represented in green, blue, and red, respectively. (**c**) Alternative node coloring combines FAS and PFAS in red, displaying FAS-like HE subset in green squares and control-like HE subset as green circles. Extract from Suttie et al. [42]. Reproduced with permission from Journal of Pediatrics, Vol. 131, Page(s) 1, Copyright © 2013 by the AAP

normalized shape difference within the dataset. When applying this technique, we observed clustering of the FAS and PFAS as expected (Fig. 24.2b). However, the HE group was separated into two subsets, with nearly half of the group (n = 28) showing a greater affinity to FAS/PFAS signatures (Fig. 24.2c). Upon closer inspection, these individuals exhibited more FAS-like facial features than the remaining HE subset. Examples of six individuals from this subset are shown in Fig. 24.1d, where we observed mid-facial flatness and/or a flat nasal bridge, philtrum smoothness and mid-facial hypoplasia.

No significant differences were found for parity, alcohol exposure, smoke exposure, or maternal age between the HE subset with FAS/PFAS affinity versus the HE subset with more control-like features. However, those with an affinity to the FAS/PFAS signatures scored significantly lower on neurocognitive measures compared to the control-like subset. Particularly affected were the WISC Verbal Comprehension IQ and CVLT-C scores, where the mean for the FAS-like subset closely resembled that of FAS and PFAS scores, while the more control-like subset was also more similar to controls.

The Face: A Window to the Brain

Studies of structural differences of the brain, facial dysmorphism, and neurocognitive impairment in FASD are well characterized in the literature. However, only a handful of studies to date focus on the interactions, relationships, and correlations between each domain [47–49]. DSM based analysis has been previously used for the simultaneous analysis of face and brain in a murine model of in utero ethanol exposure at two time points, gestational days 7 and 8.5, concluding that unique patterns of face-brain dysmorphia correspond with stage-specific exposure [3]. The earlier of these two time points yielded facial traits that resembled an FAS phenotype, while the latter time point characterized more subtle differences such as mid-facial hypoplasia. In this study, stage-specific facial differences were associated with morphological abnormalities of brain components such as the septal region, pituitary, and olfactory bulbs. Mouse studies are advantageous over human studies in that they can provide specific exposure targeting exact dosage and quantity, but it remains important to analyze face-brain correlations in human PAE populations to better understand and identify all possible phenotypic linkages.

Data collected by the Collaborative Initiative of Fetal Alcohol Spectrum Disorders (CIFASD), facilitated analysis of a dataset formed of controls (n = 47), FAS diagnosed (n = 22), and HE (n = 50) of European ancestry with corresponding 3D face, MRI brain and neurocognitive testing data [11]. MRI images were segmented to provide 3D geometry of two critical components of the brain disproportionately affected by alcohol teratogenesis—the corpus callosum and the caudate nucleus. The corpus callosum is a midline white matter tract responsible for interhemispheric connection, and the caudate nucleus is a part of the basal ganglia primarily responsible for executive function. To analyse the morphological covariance

between the face and brain in humans, we developed a technique capable of constructing a combined surface model representation of both components. This combination analysis relies on a method based on active appearance models [50] originally designed to apply a principal component analysis (PCA) to pixel intensity information while accounting for shape variation. In place of pixel intensities, we compute principal component values from separate dense surface models and concatenate them, before undergoing a further PCA on the combined representation. The result is a PCA based model containing both structures represented by a single set of principal components.

Using face and brain models, we were able to replicate control-FAS classification testing, only this time on both the face, brain and their combined representations. Several localized facial regions were tested and showed similar results to those observed in previous studies [42, 51] with results providing near perfect discrimination between FAS and controls. Additionally, using the same set of techniques both the corpus callosum and caudate nucleus yielded a classification accuracy of over 90% (Table 24.1). When testing combined representations of the facial regions combined with the corpus callosum, and caudate nucleus, we observed a consistently improved accuracy compared to analogous testing on face and brain components separately. In particular, results indicated midline facial differences were correlative with midline defects of the brain, as the most significant improvements were made on midline facial combinations with the corpus callosum and; nose (1.00), lip-vermillion (0.90), and philtrum (0.98).

Two previous face-only studies [42, 51] had shown that facial signatures graphs of alcohol-exposed individuals revealed a subset of HE individuals with FAS-like dysmorphism. Reassuringly, this dataset also produced a facial signature graph partition analogous with previous results. Applying this technique to produce a caudate nucleus signature graph also partitioned the HE subgroup into an FAS-like subset with similar brain morphology. From this graph we observed a significant overlap

Table 24.1 Extract from Suttie et al. [11]: separate face and brain control–FAS discrimination rates estimated as the mean area under the ROC curves of 20 cross-validation tests, corresponding to the probability of accurately classifying Pairs of individuals: 1 from control and 1 from FAS

Face	CM	LDA	SVM	Brain	CM	LDA	SVM
Full Face	0.93	0.93	0.95	Corpus Callosum	0.90	0.90	0.93
Profile	0.83	0.88	0.90	Caudate Nucleus	0.88	0.88	0.88
Eyes	0.90	0.93	0.93	Left Caudate Nucleus	0.83	0.85	0.90
Malar	0.95	0.95	0.93	Right Caudate Nucleus	0.83	0.85	0.90
Mandible	0.88	0.88	0.90				
Nose	0.95	0.95	0.98				
Lip Vermilion	0.85	0.85	0.85				
Philtrum	0.83	0.83	0.88				

CM closest mean, *LDA* linear discriminant analysis, *SVM* support vector machines, *ROC* receiver operating characteristic. Full face and facial regions achieved high discrimination rates as did corpus callosum and caudate nucleus. Reproduced with permission from Alcoholism: Clinical and Experimental Research, Vol. 42, Issue(s) 9, Copyright © 2018 by John Wiley and Sons

with the individuals partitioned from the facial signature graph, and more interestingly, when reconstructing the mean facial signature of the FAS-like HE group partitioned by the caudate nucleus we observed a striking similarity with that of the FAS mean. Thereby, essentially reconstructing an FAS-like facial phenotype by individual selection from brain morphology.

The Future of Image Analysis for FASD Diagnosis

In this chapter, we have discussed the importance of pertaining to an accurate, objective, and reliable method for the assessment of individuals with PAE. We have shown how 3D facial analysis can accurately identify those with an FAS diagnosis, and more importantly assist with the identification of those without cardinal features by recognition of subtle, non-cardinal minor facial dysmorphia. Additionally, we observed that the facial dysmorphism in FASD may relate to neurocognitive performance and even provide clues to the underlying brain structures. But, for these works to become clinically useful there are multiple hurdles to overcome.

Firstly, is the complexity and expense of acquisition, in the case of 3D image capture high-resolution commercial photogrammetry systems used in the studies details can often be cumbersome and expensive. However, recent advances in mobile computing-based acquisition of 3D facial analysis may overcome this problem, with Apple Inc. producing an inferred based camera system on the front of standard mobile phones capable of capturing facial geometry with sub-millimeter accuracy. The capture and analysis of brain images would, at current standing, be ruled out for any screening for diagnostic purposes due to the expertise and costs associated with MRI imaging and analysis.

Any screening tool would need to eliminate any subjective assessment prone to error. Thus, it would require reliable automation. Techniques in the above studies all utilise DSMs, to model surface geometry. However, they require the manual annotation of anatomical landmark points to successfully align surfaces and produce linear anthropometric measurements such as palpebral fissure length. Recent advances have shown how this process can be automated using deep learning approaches such as convolutional neural networks in FASD cohorts [52, 53]. These techniques could be utilized to train models on several thousand PAE and control individuals, to automatically and objectively place left and right inner and outer canthi points for palpebral fissure length calculation with a high degree of accuracy.

Additionally, as FASD is a multifaceted diagnosis reliant on multiple domains, any screening tool should be able to account for neurocognitive assessment data. Combination analysis is vital to ensure high sensitivity and specificity, as identifying only the facial features is not sufficient for providing a full insight into a patient's presentation. Finally, as recognized by recent review into the potential for facial screening tools in FASD any tool must and be accessible, compatible with other diagnostic/screening tools, accurate on all skin colors, ethnicities, ages, and sexes, and ensure no discomfort or harm to the patient [54].

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Chapter 25
"From Surviving to Thriving": A Focused Review of Interventions for Fetal Alcohol Spectrum Disorders to Guide a Shift Toward Strengths-Based Intervention Development

C. Kautz-Turnbull, M. Rockhold, and C. L. M. Petrenko

Abstract Fetal alcohol spectrum disorder (FASD) refers to physical and neurodevelopmental symptoms associated with prenatal alcohol exposure. While much is known about deficits in FASD, little research has focused on strengths and quality of life. The "From Surviving to Thriving" model illustrates a perspective shift from deficits-based to strengths-based research and intervention. In order to achieve this, three barriers must be addressed: lack of awareness of FASD, limited access to care, and stigma. This chapter offers a focused review of existing FASD interventions and community programs to illustrate how current work is already confronting these barriers and demonstrating a shift to a strengths-based perspective. It also provides practical guidelines for clinicians to address these barriers in their everyday practice. Future work should build on existing literature to deliberately and systematically design strengths-based interventions to fully support quality of life for people with FASD.

Keywords Fetal alcohol spectrum disorder · Prenatal alcohol exposure · FASD · PAE · Intervention · From surviving to thriving · Strengths · Strengths-based

Fetal alcohol spectrum disorders (FASD) is an umbrella term for a range of physical and neurodevelopmental symptoms associated with prenatal alcohol exposure (PAE). Despite a prevalence estimated at 1–5% [1], FASD continues to be underrecognized and underserved and access to FASD-informed resources is limited [2, 3]. Lack of appropriate, strengths-based, and person-centered supports for

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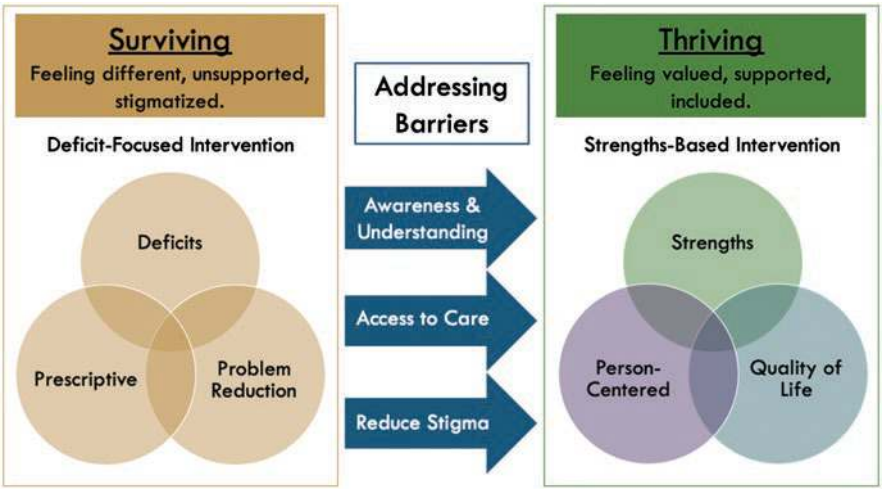

Fig. 25.1 "From Surviving to Thriving", a model for a paradigm shift to a strengths-based perspective in FASD research and intervention [5]

individuals living with FASD may lead to difficulty realizing their full potential for quality of life. Further, without similarly strengths-based caregiver support, families may feel isolated and frustrated, and experience burnout [4].

The "From Surviving to Thriving" model (Fig. 25.1) [5] proposes a shift from a deficit-focused perspective to a strengths-based perspective. Many people with FASD are simply "surviving", or feeling different and stigmatized [6]. They deserve the opportunity to "thrive": to feel valued, supported, and included. Prescriptive or deficit-based interventions, though they may provide some support, often fail to fully meet the needs of individuals and families and may even perpetuate some barriers to care such as stigma [4]. In contrast, strengths-based interventions focus less on problem reduction and more on quality of life; specifically, autonomy, social connectedness, and emotional, physical, and material well-being [7]. This approach works to uncover and recognize assets including abilities and talents, while also accounting for positive family relationships, social supports, interests, hobbies, and goals.

The model also recognizes three barriers preventing a shift from surviving to thriving: lack of awareness and understanding of FASD, lack of access to appropriate, FASD-informed care, and stigma. Continued effort is needed to overcome these barriers and move the field toward a strengths-based perspective, including deliberate and systematic intervention design to support quality of life and thriving for people with FASD. However, existing FASD interventions have already begun addressing these barriers, and provide valuable foundational work and insight in how to move toward person-centered, strengths-based care.

This chapter presents a focused review to illustrate how current FASD intervention work is already demonstrating this shift at the individual, family, and systems levels. Table 25.1 presents a summary of existing intervention approaches to

Table 25.1 Summary of published and in-progress approaches to reduce barriers to a strengths-based perspective in FASD research. Existing intervention work reviewed in this chapter illustrates these approaches across individual, family, and systems levels

	Systems	Family	Individual
Increasing awareness and understanding	• Educational interventions in the education, child welfare, and justice systems	• Parent training, consultations, and skill-building	• Skill-building interventions
	• Consultations with providers through skill-building interventions	• Attachment or relationship-building interventions	• Mindfulness or other therapeutic approaches
Increasing access to care	• Educating providers in diagnosis, assessment, and treatment	• Delivering interventions digitally	• Delivering interventions digitally
	• Improving standards of care within systems	• Connecting families with peer support	• Active outreach for individuals in need of care
	• Reducing resource burden on individuals and families seeking care		• Providing low level of care to broader populations
Reducing stigma	• Policy work	• Family-focused care	• Arts programs
	• Representation		

reducing these barriers reviewed in this chapter. This review is not meant to be exhaustive or evaluative (for systematic reviews including efficacy, see [8, 9]). Additionally, the current chapter does not place a large focus on efficacy of reviewed interventions, both because many intervention and programs have not been formally evaluated and because traditionally outcomes have been deficit-focused. Rather, the current chapter aims to help the reader understand how existing interventions and systems of care are confronting these barriers and provide practical guidelines to continue to guide a shift toward strengths-based work.

Increasing Awareness and Understanding

Physicians, mental health providers, educators, and those in the foster care and justice systems report feeling ill-equipped to recognize, diagnose, and treat FASD [3, 10, 11], contributing to extremely low rates of diagnosis. In fact, an estimated 80% or more of people with FASD are misdiagnosed or undiagnosed [1, 2], delaying or preventing receipt of quality care. Without a diagnosis, parents are left with no explanation for their child's symptoms. They may feel isolated, frustrated, and ineffective, experience blame and judgement by others, and at times even blame themselves [4, 12, 13]. Without understanding and awareness of FASD, providers and parents can misinterpret common symptoms of FASD, like impulsivity, difficulty with self-regulation, processing speed delays, and difficulty with social skills, as

willful disobedience or defiance [13, 14]. Individuals with FASD can internalize these messages, and report feeling like a "bad kid", a problem, and a burden [6, 15, 16]. Thus, increasing understanding and awareness across settings and people is essential to improve quality of life for people with FASD.

Increasing awareness and understanding at the systems level largely consists of trainings for providers working with children with FASD. Clark and colleagues [17] describe POPFASD, an intervention to educate teachers on FASD and support them in developing appropriate and FASD-informed strategies to manage behavior in the classroom [17]. Atkinson [18] goes a step further, educating preservice teachers about FASD and encouraging them to change maladaptive attributions about the behavior of children with FASD [18]. Educational interventions also target Court Appointed Special Advocates (CASA) volunteers, increasing their knowledge, comfort, and confidence in referring and advocating for children with FASD [19]. Another helped justice system providers, such as probation officers and court officials, better understand and reframe behavior related to FASD using documentary-style training videos [20]. Other programs involve training justice system personnel to screen for and diagnose FASD; in some cases, providers help to modify probation or case plans to be more appropriate for an individual with FASD [21].

At the family level, interventions typically help caregivers gain a better understanding of the individual with FASD; support needs of siblings and extended family members have been noted as an area for growth [22]. Parenting a child with FASD often requires different parenting techniques than a typically developing child [12]. Better understanding of FASD can help a caregiver attribute the child's challenges to a neurodevelopmental disability, rather than willful misbehavior, leading to better parenting outcomes including greater feelings of efficacy and an improved parent-child relationship [13]. This theorized process originated in work on the Families Moving Forward (FMF) Program, a behavioral consultation program in which a trained specialist helps the parent to "reframe" the child's behavior, or understand the function and neurodevelopmental nature of the behavior. The parent and provider collaborate to create a behavior plan to better support the child and increase adaptive behavior, including recognizing antecedents and setting events and developing accommodations [13, 23–25]. Other parenting training programs are presented in group-based or workshop formats (e.g., [26–28]), which are less intensive yet maintain the overarching aim of helping parents to better understand FASD, more effectively support their children, and better advocate for services.

Certain skill-building interventions can help people with FASD understand their own profile of strengths and challenges and learn coping strategies that work for them. This type of growth is essential to developing self-confidence, setting goals, and improving in areas of challenge. Some focus on specific skills, like social skills or academic skills. Children's Friendship Training (CFT) [29], based on social learning theory, uses modeling, rehearsal, and performance feedback to help children with FASD improve social skills and adaptive behavior. The Math Interactive Learning Experience (MILE) [30, 31] includes math instruction as well as a comprehensive neuropsychological assessment and recommendations. Others involve a focus on self-regulation. The Alert Program for Self-Regulation (Alert) [32] is a

neurocognitive habilitation program to improve self-regulation and executive functioning in children with FASD. The GoFAR program [33, 34] involves a computer game that teaches the Focus, Act, and Reflect (FAR) metacognitive technique, and works with parent-child dyads to apply this technique to help children with FASD regulate behavior, impulsivity, and attention, and improve adaptive functioning in the home. Though explicit strengths-based assessments are not a large part of these types of interventions, practicing skills such as understanding self-regulation, calming strategies, and social interaction and cooperation can help individuals build their understanding of themselves and how they move about the world.

Integrating parent and child intervention increases awareness and understanding at the family level and helps parents better support child skill building. Some, such as MILE and GoFAR, include training caregivers to help children practice the skills they are learning in everyday life [31, 33, 34]. Interventions like CFT include concurrent parent group sessions, the content of which mirrors child sessions [29]. The Families on Track program [25], combined two evidence-based programs, the FMF Program for parents and the Promoting Alternative Thinking Strategies curriculum (PATHS) [35] for children with FASD, to provide family-based support. Though these types of interventions have been mostly studied with school-aged children, some exist for younger children and adolescents. The Strategies for Enhancing Early Developmental Success Program (SEEDS), a multidimensional school readiness program for children aged 3–5 who were exposed to alcohol prenatally, includes both a child and parent group component as well as combined parent-child time each session. Parents learn how to support skill building as the child learns school readiness skills like transitions and self-regulation [36]. Project Step Up aimed to prevent and reduce substance use among teens with FASD, using FASD-informed strategies to facilitate learning and memory. The parent component empowered parents to help their teens avoid using alcohol [37].

Some individual and family interventions provide an additional consultation to increase awareness of FASD at the systems level. Two notable examples are the FMF Program and MILE [23–25, 30, 31]. Both include a school consultation, during which the provider educates and works with the school team, including teachers and staff, to better support the student with FASD [24, 31]. In the case of MILE, in-service workshops for interested teachers are also incorporated, providing additional strategies for working with students with FASD [31].

Relationship building or attachment-based work is another method of increasing understanding and awareness at the family level. Interventions focusing on the parent-child relationship, like Circle of Security [38] and parent-child interaction therapy [23] aim to increase caregivers' parenting skills, sensitivity and responsiveness to the child with FASD, and self-reflection about their own parenting style. Parents Under Pressure (PUP) is notable as one of the few interventions targeting the parent-child relationship for children older than 7. Including mindfulness and self-regulation coaching, PUP is an individualized, home-based family focused intervention to improve the parent-child relationship, parent emotion regulation, and the child's home environment [39]. Trauma-informed attachment-based interventions, such as Child-Parent Psychotherapy (CPP) [40] are particularly important

in FASD, as research documents high levels of trauma in this population [41, 42]. A 2016 trial is one of the only intervention studies looking at CPP in children with FASD who had experienced trauma. The intervention combined CPP with parent mindfulness training to improve the parent-child relationship and developmental outcomes [43].

Therapeutic approaches to increase awareness and understanding of oneself have been studied in FASD, but larger trials have not yet been conducted in this area. Small pilot studies have shown that mindfulness training is feasible in children [44] and adolescents [45], but conclusions on efficacy of these trainings cannot yet be made due to limited studies and sample sizes. Techniques such as art therapy (see [46] for a review and practice guidelines) may be especially appropriate for people with FASD, as creativity is often a strength in this population [47].

Increase Access to Care

FASD-informed care [48] is a term encompassing best practices in care for people with FASD and their families. This includes providers who understand, accept, and advocate for people with FASD and their families, as well as systemic services making diagnosis and intervention accessible to those who need it, especially traditionally underserved populations such as low-income groups, people of color, and people living in rural areas. Increasing access to care involves both making FASD-specific forms of care more available, but also involves making existing systems of care FASD-informed.

Digital interventions have increased dramatically in recent years and show significant promise in expanding access to care. They are cost-effective, scalable, and widely accessible using a smartphone, to which 85% of the population now has access [49]. Web- and app-based parenting workshops and interventions [27, 50–52] have the capacity to reach many more caregivers than traditional in-person trainings. Computer or video game interventions can provide a more efficient and engaging way to reach individuals, especially children. Examples of these types of interventions studied in individuals with FASD include Cogmed, a computerized working memory intervention [53], and Cognitive Carnival/Caribbean Quest, a video game targeting attention and working memory [54]. Though the vast majority of people have access to the internet, potential adaptations must be made for varying levels of computer literacy, especially for lower-resourced areas in which children or families may not have high levels of exposure to technology. A recently developed cognitive training computer game was customized to lower levels of computer literacy, such as lack of comfort with a keyboard or mouse [55].

Virtual reality (VR) interventions have also increased as the technology becomes more accessible, though VR is not yet as widely available as the internet. Some have used VR to teach children with FASD safety skills [56, 57], taking advantage of the ability to practice interactions and skills in real time, which may improve

generalization. Motor interventions such as STABEL use VR to improve balance, stability, and sensory integration [58, 59].

Increasing access to care at the individual level also includes active outreach for individuals who may be less likely to seek out care. One program focused on outreach for at-risk aboriginal youth suspected of having FASD, and sought to improve a wide range of outcomes including safety, school attendance, sexual health, substance use, and knowledge and use of community resources [60]. The Mind, Body, and Spirit (MBS) program was established in correctional facilities in Canada to develop a screening and diagnostic clinic for FASD, provide support for offenders, and to create a transitional support system. An interdisciplinary FASD diagnostic evaluation identified potential participants, who then completed skill-building around communication and interpersonal skills, regular exercise, and culturally-informed spiritual wellness activities [61].

These types of outreach have the advantage of directly targeting those in need of support. Another method of reaching people who may not be actively seeking care is providing a lower level of care to a large number of people. For example, Wagner and colleagues [62] trained teachers to deliver an adapted version of the Alert Program to an entire classroom, rather than in a clinic setting, in an Aboriginal community with high estimated rates of FASD [62]. Delivering an intervention to a more general population improves the capacity to serve individuals, broadens the scope of the intervention, and lessens stigma around receiving care. It also sidesteps the barrier of limited FASD diagnosis, thereby reaching those in need of extra support including those who may have an undiagnosed FASD.

At the family level, connecting families with peer support is an efficient and effective way to increase access to care for families of children with FASD. Social support has long been known to contribute to a variety of positive outcomes for parents of children with special needs [63, 64]. However, peer-to-peer support provides an added affective benefit, with parents in peer support groups reporting feeling understood and validated, and valuing connection with others who have "been there". This is particularly important for parents of children with FASD, who often report feeling isolated and having no connection with other parents of children with FASD [4]. Some peer support programs provide training and mentorship for caregivers of children with FASD to run their own groups, an aspect especially valued by group participants [26, 65]. Other peer-to-peer approaches target geographical barriers using "hotlines" or app-based interventions. The Family Empowerment Network, based in Wisconsin, uses a telephone-based hotline to connect parents and families to peer support across the country [66]. The FMF Connect app, a mobile health adaptation of the FMF Program, includes a Family Forum, allowing parents to connect with and support each other, seek advice, and share their experiences regardless of geographical location.

Building linkages and advocacy increases access to care by connecting families to existing programs, addressing barriers to care like lack of material resources, respite, and childcare. Programs like the Parent-Child Assistance Program (PCAP) [67, 68], Key Worker program [69], Breaking the Cycle [70], and Coaching Families [71] provide families with information about FASD and assistance in accessing a wide variety

of resources, including parenting skills groups, advocacy services, FASD diagnostic clinics and supports, and substance use counseling. Some, like Breaking the Cycle, also connect families with support for basic needs like clothing and meals [70]. PCAP, a 3-year program to increase access to community resources as well as collaboration and advocacy with case managers and other providers, has also been adapted to parents with FASD to further increase accessibility of care [67, 68].

Other types of programs directly reduce barriers to care related to resources. One program provided increased respite care (i.e., 6 h per week of group-based activities for children without parent attendance) to decrease family stress over a period of 10 months [72]. Many programs, such as the Key Worker program and PCAP are home-based, reducing the need for transportation and childcare. Along these same lines, several therapeutic programs described above can take place in the home (e.g., the FMF Program, PUP, Circle of Security).

Training providers to recognize and diagnose FASD expands access to care within existing systems. The Extension for Community Healthcare Outcomes (ECHO) approach involves tele-mentoring using didactics and case-based learning with an expert "hub" team and community "spoke" sites, enabling efficient and broad dissemination of evidence-based care models [73]. The first trial of the ECHO program for FASD trained primary care providers across the country on FASD diagnosis and care management [74]. In addition to family-focused care, the Family Empowerment Network trains providers on FASD diagnosis, interventions, and planning [66]. One initiative involved social workers, called FASD Success Coaches, who provided support to students with FASD as well as training and case management support to their teachers [65]. The Promising Practices program [75] works within the child welfare system to provide improved care for families; specifically, it provides assessment for suspected cases of FASD and makes training and expert consultation available for both caseworkers and foster parents. Through this program, support plans are developed collaboratively with caseworkers, foster care support workers, and foster parents. Interventions in the justice and school systems discussed above also increase access to care via education and training of providers, especially when delivered using digital means such as videos (e.g., [20]).

Work in the Australian Fitzroy Valley provides an example of wide-ranging FASD-informed systems-based intervention. Titled the Marulu Strategy, the program included expanding FASD screening and diagnosis, building local capacity to serve individuals and families, and identifying and leveraging existing community resources [76, 77]. Not only does this model provide a standard for addressing barriers to care at the systems level, it also shows the potential of community and stakeholder expertise.

Reduction of Stigma

Stigma underlies nearly all barriers to thriving and prevents individuals and families from realizing their full potential for quality of life. A recent literature review [6] lays out the varied types of FASD-related stigma including public stigma,

self-stigma, stigma by association, and structural stigma. Each of these types of stigma are important to consider, as is how they overlap and influence each other; stigmatization toward people with FASD is often intensified given the intersectionality of varying identities impacting the lived experiences of this population [6, 15, 16]. In recent years, movements to explicitly address stigma and discrimination, especially implicit bias, have come to the forefront of discussion both in the FASD field and in society as a whole. Despite this, interventions for stigma reduction are limited. Literature on reducing stigma around mental health disorders suggests that public protest of stigmatizing materials, increasing awareness and education, and contact between majority and minority members will translate to stigma reduction [78]. Further, concrete and purposeful steps must be taken to address the harm that has already occurred as a result of stigma and discrimination, and to continue to make clear a tangible commitment to the value and worth of people with FASD.

Self-stigma, or stigma that is anticipated, expected, and internalized, involves self-blame and shame and leads to people with FASD having lower self-esteem, lacking confidence, and underestimating their own potential [6, 16]. Few interventions actively focus on increasing the self-esteem of people with FASD and little research has been conducted on how self-stigma can be reduced. One potential avenue is arts-based interventions, which have shown positive effects on self-esteem and confidence in children in the child welfare and mental health care systems [79, 80]. An intensive theater training camp in Canada targeted the skills, emotional awareness, and self-esteem of children with FASD aged 9–14, along with skills in acting and performing [81, 82].

Parents, caregivers, siblings, and other family members may experience stigma by association, or social and psychological reactions to an association with a stigmatized person, in this case an individual with FASD. This type of stigma may underlie feelings of isolation and stress experienced by caregivers of children with FASD [4], as well as actual social exclusion. To date no interventions actively address stigma by association. Reid and colleagues [83] propose an intervention framework to place families at the center of interventions and service provision, including recommendations drawn from literature around "excluded families", or families who cannot access mainstream services and resources. The authors provide specific practical recommendations for facilitating family-focused intervention: strengthening the family social economy (both formal supports such as schools and informal social supports such as friends and family), promoting hope, acknowledging family expertise, and providing education and skill building [83].

Structural stigma, or that which is perpetuated in society's institutions and systems, also plays a factor in the lives of those with FASD and is largely unaddressed by intervention work. However, there are multiple efforts being made to reduce structural stigma such as changing policy through the FASD Respect Act in the US and shifting the media portrayal of individuals with FASD through documentary filmmaking. Positive representation for people with FASD may be the most important method of stigma reduction. The Adult Leadership Collaborative of FASD Changemakers represent a group of well-known adults living with FASD who conduct research on well-being in FASD, provide a network of peer-to-peer support,

and advocate for reduction of stigma and greater acceptance. Amplifying the voices of activists and role models with FASD should be one of the highest priorities for the field, and can be accomplished through intervention work, and indeed research in general, at all levels.

Implications for Clinical Practice

Clinical providers can utilize guidelines from interventions reviewed here to reduce these barriers and shift to a strengths-based perspective in day-to-day practice. Clinicians should pursue education about FASD and related conditions and incorporate the knowledge into care, recognizing that many people with FASD will not have access to a diagnosis. Importantly, providers must understand that challenging symptoms stem from a neurodevelopmental disability, and do not indicate willful defiance. A focus on environmental modifications (reducing sensory stimulation, providing visuals or social stories, simplifying language and instructions) should be emphasized. Providers can also help caregivers to understand their child's behaviors from this perspective and validate the stress and isolation they may be feeling as a caregiver of a child with FASD. Clinicians who are knowledgeable about FASD should also educate colleagues and offer support and resources in learning about FASD.

Incorporating a strengths-based perspective into clinical practice is essential. Providers using standardized assessment tools should include a strengths-assessment tool, such as the Values in Action (VIA) Assessment System [84] or the Assessment Scale for Positive Character Traits—Developmental Disabilities [85]. These assessments can provide a profile of character strengths and positive attributes, which offers a more complete picture of an individual than solely deficit-based assessments. Additionally, using a strengths-based framework in clinical care and planning can help a provider to tailor care to the individual's needs and goals. Possible clinical approaches include Buntinx & Schalock's four phase approach [86] or person-centered planning [87, 88].

Providers should also be aware of barriers to accessing care faced by individuals and families. Novel approaches to intervention such as digital interventions can be accessed regardless of geographical location and are often cost-effective, although many are still in initial stages of development and testing. Peer-to-peer support should be emphasized, as should connections to community resources for advocacy, mental health care, respite, and other family needs. When possible, methods of overcoming lack of resources should be used, such as home visitation, collaboration with case managers, and linkages to fulfill basic needs.

Providers also have a role in decreasing stigma around FASD. Understanding the various types of stigma (i.e., self-stigma, stigma by association, structural stigma, and public stigma) is crucial to reducing it. Clinicians must work to build up the self-esteem and confidence of individuals with FASD and validate and support their caregivers. Modifying stigmatizing language (see [89] for a guide) and attending to

the past experiences of individuals with FASD and their families can help them to feel understood and accepted. Finally, providers can work to build hope for their patients by treating them with compassion, empathy, and kindness, recognizing their expertise, strengths, and the ways in which they positively influence the world around them.

Conclusions and Future Directions

The "From Surviving to Thriving" model presents a shift from a focus on problems and deficit-focused intervention to a focus on positives and strengths-based intervention. To make this shift, three barriers must be addressed: lack of awareness and understanding of FASD, lack of access to care, and stigma. The current review illustrates how existing interventions and programs have already begun to address these barriers at the individual, family, and systems levels. Much of this work focuses on increasing awareness and understanding, especially with the goal of helping caregivers and providers "reframe" behavior or understand challenges as part of a neurodevelopmental disability, and not as willful defiance or misbehavior. These types of interventions range from intensive skill-building programs to educational videos but are often effective in increasing knowledge and awareness of FASD [8, 9]. Work focusing on increasing access to FASD-informed care includes both finding novel ways to deliver care, such as technology training teachers or peer mentors, as well as improved methods of reaching underserved populations. Limited formal work is explicitly aimed at reducing stigma, but informal approaches such as grassroots work and positive representations of FASD can reduce stigma and increase hope for people with FASD. Though existing intervention work lays a foundation for a shift to a strengths-based perspective, future intervention work should be person-centered and deliberately designed around strengths and positives.

Perhaps most importantly, future work on effectiveness should no longer be limited to reduction of deficits, but should take steps to understand and address the issues important to individuals living with FASD and those around them. Very little is known about the effectiveness of existing interventions on including quality of life and promoting thriving in people living with FASD. Many of the interventions reviewed in the current chapter are shown to be effective in achieving outcomes such as reduction of problem behavior, improved adaptive or social skills, and reduced stress and burden on caregivers (for systematic reviews including efficacy, see [8, 9]). However, larger and more diverse trials are needed to make conclusions about broader efficacy as well as in subgroups such as racial/ethnic minorities. Future work should continue to rigorously test effectiveness of these interventions, both in the context of randomized controlled trials and in community settings, especially in the context of quality of life and strengths-based outcomes.

In addition to these calls to action for future work, the "From Surviving to Thriving" model presents six directions to continue to promote thriving in FASD (Table 25.2).

Table 25.2 Future directions to shift to a strengths-based perspective in FASD research and intervention

Future directions to promote thriving in FASD [5]
1. Transform how we view FASD
• Reduce stigma and use of stigmatizing language
• Promote a balanced view of people with FASD
2. Study and measure strengths and thriving
• Develop more appropriate and valid measures of strengths
• Conduct research to understand thriving in FASD
3. Utilize strengths-based frameworks for FASD intervention in research and practice
• Promote use of existing strengths-based frameworks in care
• Adapt strengths-based interventions to FASD
4. Translate research knowledge to community and public health settings
• Combine approaches to increase access to care with approaches like community-based participatory research [90]
• Increase education around additional experiences such as comorbid mental health disorders, trauma, school disruption, and justice system involvement
5. Adapt existing models and technologies for FASD
• Adapt and study existing evidence-based mental health treatments for people with FASD
• Establish efficacy of digital interventions and foster implementation in community
6. Develop novel approaches to address gaps in the literature
• Work to reach underserved populations like diverse cultural groups, biological parents, underserved geographical areas, and adolescents and adults with FASD
• Research specific needs of these populations and develop or adapt interventions to address them

FASD intervention research and community programs to provide and improve care have made significant steps in improving the quality of life of people with FASD and their families. However, the evidence base for effectiveness is still in need of growth; larger samples and more rigorous study designs are necessary to understand how intervention programs can be most helpful to people with FASD. Trials in community settings focusing on implementation are particularly important to building a sustainable and effective system of FASD-informed care. Continuing to shift to a strengths-based perspective, guided by the "From Surviving to Thriving" model, will build on this foundation to purposefully and intentionally promote thriving for people with FASD.

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Part V
Structural and Functional Central Nervous System Pathology in Alcohol Addiction

Chapter 26
Structural and Functional Imaging of Alcohol's Effects on the Brain

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Abstract Brain imaging has helped reveal acute and chronic effects of alcohol use, and how these effects relate to clinical outcomes in adults with alcohol use disorder (AUD). The prefrontal cortex and the mesocorticolimbic reward system have shown consistent effects of chronic alcohol use. For example, adults with AUD show reduced dopamine D2 receptor density in the striatum, reduced volume of medial prefrontal cortex (PFC), and decreased white-matter tract integrity. Although most studies only examine individuals at a single point in time, supporting evidence suggests a causal relationship between chronic heavy alcohol use and altered brain structure and function. Individuals with AUD, relative to controls, display greater activation of the cingulate and ventromedial PFC in response to alcohol cues, less activation of the striatum during the anticipation of monetary reward, and less activation of the insula and ventromedial PFC in response to negative emotional cues. Differences in brain anatomy and function can inform clinical practice, as indicated by studies that show that AUD can be identified based on neuroanatomical differences, and that neural activation to alcohol cues may predict which individuals will benefit from treatment.

Keywords Dopamine · Reward · Emotion · Grey matter · White matter · fMRI · PET

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Alcohol Use Disorder (AUD) has social, environmental, and biological origins. Of biological contributors, the brain is paramount. The brain is a complex organ that controls thought, motivation, and behavior, and each of these processes affects likelihood of harmful alcohol use. Yet identifying specific features of brain structure and function associated with AUD has been challenging. Alcohol exerts its effects primarily by altering brain function, but identifying precise changes in brain function that result from acute and chronic alcohol use remains difficult. Alcohol affects multiple aspects of cellular function, albeit mostly in small ways that fail to account fully for its effects. Historically, researchers studying the effects of alcohol on the brain have focused primarily on individuals with alcohol dependence, which is the diagnostic predecessor of AUD. Although some of the literature discussed in this chapter involved studies of individuals diagnosed with "alcohol dependence," "alcoholism," or "alcohol abuse," we use the term AUD to encompass all previous diagnostic terms used in older studies. Research aimed at understanding how AUD affects human brain function has benefited from neuroimaging tools, including magnetic resonance imaging (MRI), positron emission tomography, and single photon emission computed tomography. These tools have helped researchers quantify structural and functional changes associated with AUD.

Effects of Alcohol on Brain Structure

Brain Structures Associated with Cognitive Control. Early evidence that alcohol affected the brain came from post-mortem brain studies. Post-mortem data suggest that alcohol changes brain structure, and certain brain regions may be at greater risk for damage. For example, post-mortem studies have shown significant brain atrophy among individuals with AUD compared to healthy adults, primarily within the prefrontal cortex (PFC) [1]. The PFC has many functions, many of which relate to cognitive control. Brain regions underlying cognitive control include the inferior frontal gyrus (IFG), dorsolateral prefrontal cortex (DLPFC), dorsal anterior cingulate cortex (ACC), and parietal cortex [2] (see Fig. 26.1, which will be a reference point for neuroanatomy throughout this chapter). AUD may involve changes within neural circuitry underlying control over behavior [3]. An imbalance between neural reward and control functions may explain why individuals with AUD seek alcohol instead of other reinforcers and have difficulty reducing alcohol consumption despite experiencing negative consequences associated with drinking [4]. MRI studies have reported lower gray matter in the PFC, IFG [5, 6], dorsal ACC, and parietal cortices [7] among individuals with AUD compared to healthy adults.

A valuable approach to gathering scientific evidence, meta-analysis, combines data from multiple studies to assess the strength of evidence. Numerous meta-analyses show volume loss within control-related brain regions among adults with AUD relative to healthy adults. One meta-analysis of 12 studies representing 433 individuals with AUD and 498 healthy adults revealed that the AUD group demonstrated lower gray matter volume within the insula, DLPFC, ACC, striatum,

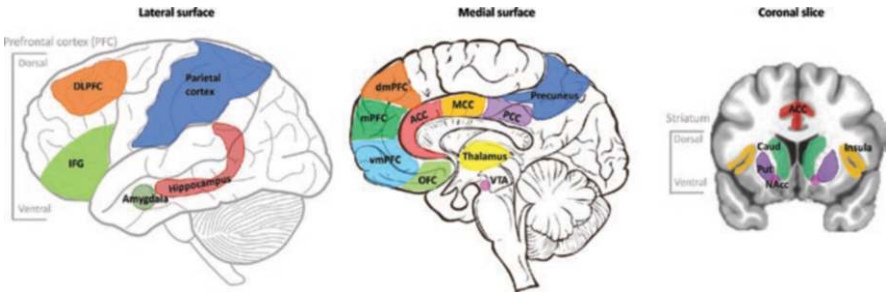

Fig. 26.1 Brain areas referenced in this chapter. DLPFC, dorsolateral PFC; IFG, inferior frontal gyrus; dmPFC, dorsomedial PFC; mPFC, medial PFC; vmPFC, ventromedial PFC; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex; MCC, middle cingulate cortex; PCC, posterior cingulate cortex. Note that the amygdala and hippocampus are located in the medial temporal lobe but are depicted on the lateral surface image. On the coronal slice, the striatum comprises the caudate (Caud), putamen (Put), and nucleus accumbens (NAcc)

thalamus and hippocampus [8]. A larger meta-analysis included 23 studies representing 846 individuals with AUD and 878 healthy adults and found lower gray matter volume in the right cingulate gyrus, insula and middle frontal gyrus for those with AUD [9]. Finally, the largest meta-analysis to date included 27 studies representing 1045 individuals with AUD and 1054 healthy adults and found gray matter reductions in the cingulate gyri, medial frontal gyri, left anterior and right posterior insula and left superior frontal gyrus among individuals with AUD [10]. Volume loss in these regions may contribute to the impaired control over drinking that is commonly seen in AUD [11].

Brain Structures Associated with Reward. In the 1950s, a series of groundbreaking studies was conducted in which rats were implanted with electrodes that allowed them to electrically stimulate specific brain areas by pressing a lever [12, 13]. When an electrode was placed within dopaminergic regions in the midbrain, the rats repeatedly pressed the lever (up to 2000 times per h over 24 h) and ignored other reinforcers such as food. Following this work, researchers identified the mesocorticolimbic dopaminergic circuitry as the primary neural pathway underlying reward-related responses to addictive substances [14]. The mesocorticolimbic pathway includes the ventral tegmental area (VTA), striatum (composed of caudate, putamen, and nucleus accumbens [NAcc]/ventral striatum [VS]), hippocampus, amygdala, and PFC. Other brain regions involved in addiction-related reward-processing include the insula and thalamus [15–17]. Reduced gray matter volumes have been observed in the striatum, hippocampus, amygdala, PFC, insula, and thalamus in AUD individuals relative to healthy adults [5, 18–21]. Volume loss in these regions may contribute to deficits in reward processing, such as overvaluing alcohol reward and undervaluing natural reinforcers [22].

The hippocampus plays a critical role in learning and memory and is implicated in the development of AUD [23]. A study exploring differences in hippocampal volumes between men with AUD and age-matched healthy adults found that the

AUD group showed lower total hippocampal volume [24]. A recent analysis synthesizing published results from 23 studies on hippocampal volume demonstrated that heavy alcohol use is associated with smaller hippocampal volume [25].

Compelling evidence regarding the effects of alcohol on brain volume emerged from several large-scale studies that pooled data from multiple research sites. These studies have leveraged large, carefully selected samples that screened out for psychiatric comorbidities to provide stronger evidence that any differences were due to alcohol use, but not other causes. One such study pooled data from 10 research sites and included 660 people with AUD and 326 healthy adults. They found that, on average, individuals with AUD, relative to healthy adults, had 3–9% lower gray matter volumes in the hippocampus, putamen, pallidum, and thalamus, as well as lower total gray matter [26]. In another study, data were pooled across 23 sites and included 1100 healthy adults and 2140 individuals who were dependent on alcohol, nicotine, cocaine, methamphetamine, or cannabis. This study showed that substance-specific associations with gray matter volume were stronger with alcohol relative to the other substances, and AUD was associated with reduced gray matter volume in the amygdala, hippocampus, NAcc, putamen and thalamus [27]. These studies indicate that AUD is associated with broad gray matter volume loss, especially within reward- and control-related regions, and suggest the potential for specific patterns of regional brain volume loss to serve as a diagnostic marker for AUD.

Effects of Alcohol on Brain Function

Alcohol Cue-Reactivity. Stimuli associated with alcohol consumption, including the sight, smell, and taste of alcohol (i.e., "cues"), have long been known to elicit alcohol craving [28]. With the advent of functional magnetic resonance imaging (fMRI) in the 1990s, alcohol cue-reactivity paradigms were among the first tasks tested in the fMRI environment. In the most common versions of these tasks, the neural signal elicited during blocks of alcohol-related cues, including visual [29], olfactory [30] and gustatory [31] stimuli, is contrasted with the signal elicited during blocks of neutral stimuli that are closely matched on other sensory characteristics. This contrast yields an estimate of differential activation, either throughout the brain or within a specific region of interest. Numerous studies have demonstrated that alcohol cues, relative to neutral cues, elicit activation of a variety of brain areas, particularly those related to reward processing and decision-making.

A seminal meta-analysis [32] analyzed 28 studies of alcohol cue-reactivity, representing 679 individuals with AUD and 174 healthy adults, and concluded that the ventral striatum (VS), ventromedial prefrontal cortex (PFC), posterior cingulate cortex (PCC), and precuneus were most consistently activated by alcohol cues, relative to neutral cues. Of these areas, the PCC and the adjacent precuneus demonstrated greater alcohol cue-elicited activation among individuals with AUD, relative to healthy adults. The VS and ventromedial PFC lie along the mesocorticolimbic dopamine pathway and underlie neural reward processing, while the PCC and

precuneus, situated in the parietal lobe, are associated with risky decision making [33] and subjective valuation of potential rewards [34]. Since that analysis included hypothesized regions of interest as well as whole-brain analyses, a subsequent meta-analysis considered only 17 whole-brain fMRI studies, representing 457 people with AUD and 360 healthy adults, and identified greater alcohol cue-elicited activation among AUD individuals, relative to healthy adults, in the ACC, middle cingulate as well as the ventromedial PFC [35]. Among individuals with AUD, these findings indicate that alcohol-associated cues acquire the ability to hijack brain systems associated with reward and decision-making, but treatment for AUD can change this altered brain function [35].

Studies have also examined whether neural activation to alcohol cues has clinical implications. Among treatment-seeking individuals with AUD scanned prior to treatment initiation, greater cue-elicited activation of several brain areas has been associated with relapse, but the studies have shown inconsistency in the specific regions implicated. For example, a review [36] reported that of seven studies that identified a relationship between cue-elicited activation and relapse, greater dorsomedial PFC activation was associated with relapse in two studies, greater VS activation in two other studies, and several other regions in one study each. In contrast to these varying associations, several randomized controlled trials examined cue-elicited activation of the VS as a predictor of response to naltrexone, a frontline medication for AUD. Naltrexone is an opioid antagonist with greatest affinity for the mu receptor, and one of its mechanisms of action is reducing alcohol craving [37]. Individuals with AUD who drink because they enjoy the rewarding effects of alcohol are more likely to respond to naltrexone [38]. Consistent with these data, naltrexone (50 mg daily), relative to placebo, reduced cue-elicited VS activation in three separate studies [39–41]. Greater VS activation prior to naltrexone initiation was associated with longer time to relapse. Incubation of neural alcohol cue reactivity after withdrawal and its blockade by naltrexone [41, 42], and greater reduction in VS activation during the first 2 weeks of naltrexone treatment was associated with significantly fewer heavy drinking days in the subsequent 14 weeks [40]. These data suggest that alcohol cue-elicited VS activation is a promising predictor of response to AUD medications that block alcohol craving or reward.

Reward Processing. In addition to neural responses to alcohol-related cues, many studies have investigated whether general reward processing differs between individuals with AUD and healthy adults. In one commonly used fMRI reward processing task, participants see a cue that indicates either a potential monetary gain or loss, followed by a target symbol. Participants attempt to respond to the target by pushing a button as quickly as possible and receive feedback after each target indicating their success or failure and their earnings [43–45]. In healthy individuals, anticipation of winning $5 versus $0 results in a large increase in VS activation [45]. A recent meta-analysis of this task that included six studies representing 131 individuals with AUD and 155 healthy adults found that individuals with AUD demonstrated less VS activation when given the chance to win $5 [46]. Additionally, individuals with AUD exhibited increased VS activation when receiving feedback that they had won $5 compared to healthy adults. Differential striatal activation in

individuals with AUD may be the result of difficulty in learning during reward prediction when presented with non-alcohol related cues [46] and this may result in inappropriate decision making that leads to continued alcohol use.

Emotion Processing. Emotion processing and regulation are necessary for recognizing and responding to emotional stimuli [47], and are critical for effective interpersonal functioning [48]. Facial expression recognition tasks aim to assess recognition of emotional facial expressions (e.g., fear, surprise, happiness). In a systematic review of 26 studies of facial expression recognition by individuals with AUD, the majority of studies found that individuals with AUD had deficits in the recognition of disgust and sadness and they needed images with greater intensity of emotional expression for successful recognition of fear and anger [49]. They also took longer to recognize emotions, compared to healthy groups [49]. One study used fMRI to examine neural activation in response to emotional faces by 29 detoxified individuals with AUD and 31 healthy adults [50]. Individuals with AUD were less able to recognize fearful faces compared to healthy adults and demonstrated decreased activation in the orbitofrontal cortex (OFC) and insula, brain regions associated with the identification of emotional stimuli [50]. Individuals with multiple detoxifications showed the greatest decrease in activation in these regions. These findings suggest that individuals with AUD have difficulties with processing emotions and producing appropriate emotionally driven behaviors [50].

Research has also examined response to stressful or threatening situations, such as interpersonal conflict. Individuals with AUD, compared to healthy adults, show decreased activation in the cortico-limbic-striatal regions—associated with identification, processing, and modulation of stressful stimuli—during high-stress situations, but over-activation of these regions during low-stress situations [51]. A study examining problematic drinking, reward, and viewing emotional faces in 759 college students found neural activation patterns were associated with harmful drinking patterns over time. For example, individuals who exhibited low VS activation in response to winning money and high amygdala activation in response to angry faces were more likely to meet criteria for AUD at the time of the neuroimaging and more likely to engage in problematic drinking 3 months later [52].

Another approach to examining stress involves creating an audio-recorded script based on a participant's report of a recent stressful life event, a recent alcohol use-related event, and a neutral-relaxing event [53, 54]. Creating stressful stimuli from the person's lived experience helps ensure that the stimulus is salient and elicits negative feelings. Relative to healthy adults, adults with AUD had increased alcohol craving after hearing the stress- and alcohol-related scripts [54, 55]. Healthy individuals show increased neural activation when hearing the stress- and alcohol-related scripts in the medial and lateral PFC, ACC and PCC, striatum, and anterior insula, indicating recruitment of emotion, stress, and reward processing brain regions [55]. A study of 37 patients with AUD who had been abstinent for 4–8 weeks and 37 healthy adults found that individuals with AUD demonstrated less activation in the medial PFC, ACC and PCC, ventrolateral PFC, and insula, during the stress- and alcohol-related scripts compared to the neutral-relaxing scripts [56]. These regions are involved in cognitive control and the modulation of emotional

behaviors in response to conflict. The individuals with AUD demonstrated difficulties with regulating negative emotion following stressful stimuli [56]. Neural regions associated with emotional processing and modulation in response to stressful stimuli may be impaired in adults with AUD, which may lead to impulsive behavior and loss of control over drinking.

Positron Emission Tomography (PET) Studies of Alcohol Effects

Dopamine Transporters and Receptors. Studies have also used PET to investigate changes in neurotransmitter function that are associated with AUD, including the distribution of receptors and the release of neurotransmitters following a probe. Research has examined the dopamine system more than other systems because of the wider availability of tools to study this system, and because of empirical and theoretical associations between dopamine function and addictive disorders. One tool to study dopamine function is a radiotracer that binds to dopamine transporters at the presynaptic terminals in the striatum. Dopamine transporters remove dopamine from outside the neuron and return it to the cytosol. In periods of early abstinence (less than 3 weeks), individuals with a history of heavy drinking show reduced dopamine transporter availability relative to non-heavy drinkers [57]. Following protracted abstinence, several studies have shown that dopamine transporter availability in adults with a history of heavy drinking resembles that of healthy adults [58–60], suggesting recovery of dopamine transporters over time.

Another key set of tools for examining the dopamine system are tracers that bind to striatal D2/3 receptors on the postsynaptic terminal. Studies that have examined D2/3 receptor availability have found lower levels of these receptors in individuals with AUD relative to healthy groups [59, 61], suggesting suppression of dopamine signaling. Decreased D2/3 receptor availability may extend to regions beyond the striatum, as some studies have investigated other regions and found lower levels in the insula, thalamus, and hippocampus among individuals with AUD relative to healthy adults [62]. Decreased D2/3 receptor availability among individuals with AUD is consistent with the effects of alcohol, which acutely elicits greater dopamine release, relative to non-alcoholic juice, in the VS [63]. Men may release more dopamine after drinking alcohol than women [64]. Elevated VS dopamine release caused by chronic heavy drinking may thus lead to compensatory pruning of D2/D3 receptors.

In addition to reduced D2/3 receptor availability, adults with AUD may also have altered dopamine release. In two studies, adults with AUD showed lower dopamine release following administration of methylphenidate, a stimulant that induces dopamine release, relative to healthy adults [61, 65]. Two studies have examined whether D2/3 receptor levels recover with abstinence from alcohol. One study showed persistent decreases in D2/3 receptors in adults with AUD even after 4 months of abstinence [66]. The other showed evidence of recovery, where the adults who remained

abstinent no longer differed from adults with no history of AUD [62]. More evidence is needed to determine whether D2/3 receptor availability recovers with abstinence.

Other Neurotransmitters. While dopamine is widely associated with addictive behaviors, another key neurotransmitter in alcohol pharmacology is ɣ-aminobutyric acid (GABA). GABA-A receptors are the site of benzodiazepine action, and tolerance to benzodiazepines is associated with cross-tolerance to alcohol, suggesting alcohol also acts at GABA-A receptors. Several tools help study GABA-A receptors in the brain, such as the radiotracer [^{123}I]iomazenil. One study used [^{123}I]iomazenil to show that adults with AUD exhibited lower levels of GABA receptors across the brain, particularly in the PFC, ACC, and occipital cortex [67]. Even when accounting for lower gray matter among adults with AUD, there were still fewer GABA receptors [68]. Another study, with twice as many participants, showed higher GABA-A receptor levels among adults with AUD, relative to healthy adults, during the first week of abstinence, but no difference between these groups after a month of abstinence [69]. Part of the discrepancy may stem from the lack of specificity of [^{123}I]iomazenil, which binds at many receptor types. Another study used [^{11}C]Ro15 4513, which binds specifically to the α5 subunit of the GABA-A receptor. This study showed large reductions in the availability of this receptor throughout the brain in participants with AUD, with the largest differences in the VS [70].

Several other neurotransmitter systems have been examined in relation to chronic alcohol use. For example, a study of 25 adults with AUD and 30 healthy participants showed significantly greater mu-opioid receptor availability across many brain regions, with the largest differences in the striatum [71]. The study also examined delta-opioid receptors and similarly showed greater availability in the adults with AUD, but the differences were smaller in magnitude than the mu receptor ones, and did not reach statistical significance in any regions [71]. Another study showed that greater mu-opioid receptor availability remained after 5 weeks of abstinence [72]. The differences could result from "up-regulation of mu-opioid receptors and/or reduction in endogenous opioid peptides following long-term alcohol consumption, dependence, and/or withdrawal" [71].

Brain Metabolism and AUD. Numerous studies have investigated how AUD affects the brain's metabolism. The first tool that allowed researchers to study metabolism was the radiotracer [^{18}F]fluorodeoxyglucose, in which a radiolabeled fluorine was inserted into a glucose molecule [73], allowing PET scans to track glucose uptake. In the mid-1980s, studies had shown that adults with a history of alcohol misuse, relative to those without such a history, showed lower uptake of [^{18}F]fluorodeoxyglucose, indicating a lower level of glucose metabolism [74]. Levels of glucose metabolism were lower across the whole brain, but the magnitude of differences was greatest in the frontal and parietal cortices [75]. Participants in the study had been abstinent between 4 and 22 days, and shorter durations of abstinence were associated with lower levels of glucose metabolism [76]. This suggests that glucose metabolism deficits may begin to recover following sustained abstinence for several weeks.

A prospective, longitudinal study showed that individuals with AUD had the lowest levels of cerebral glucose metabolism in the first 15 days after the last drink

[76]. Between 16 and 60 days of abstinence, glucose metabolism increased to higher levels, but there was no evidence of additional recovery between 30 and 60 days. Individuals who had a longer history of heavy alcohol use showed lower levels of glucose metabolism, and while they were also older, there was no correlation with age in healthy adults. This suggests that a longer history of heavy alcohol use may cause decreased glucose metabolism. Another study tested the glucose metabolism of healthy adults and adults with AUD under baseline conditions and again 1 day later after administration of alcohol (1 g/kg), a dose targeted to bring participants to 0.1 g/dL blood alcohol concentration, above the legal limit for driving. While alcohol administration reduced glucose metabolism for both groups, the reduction was more pronounced in the group with AUD, and was greater in adults who consumed greater amounts of alcohol [76, 77].

One possible explanation for the decrease in cerebral glucose metabolism is that the energy source for the brain may shift in response to acute and chronic alcohol exposure. One clue for this possibility came from a study of heavy drinkers and light drinkers that found higher acetate levels in the blood in heavy drinkers. In normal circumstances, blood glucose concentrations, at 15 mM [78], are about 100 times greater than acetate concentrations, at 0.17 mM [79], which could explain why the brain would prefer the more widely available energy source. Following alcohol exposure, acetate levels increase by 65% to as much as 0.75 mM [80], and glial cells in the brain have been shown to use acetate as an energy source when levels are higher [81]. A PET study used [$^{1-11}$C]acetate as a tracer and examined a group of occasional social drinkers and a group of heavy drinkers under a placebo condition and an acute alcohol condition. The heavy drinkers showed higher levels of cerebral acetate uptake than the social drinkers across both the placebo and alcohol conditions. Both groups showed a significant increase in acetate uptake during the alcohol administration scan relative to the placebo scan [82]. The largest increases occurred in the cerebellum and the occipital cortex. Glial cells, such as astrocytes, express monocarboxylic acid transporters that can bring acetate into the cytosol, whereas neurons do not, so glial cells may be more apt to use acetate than neurons. The shift to acetate as an energy source may be primarily for resting energy use, as another study examined glucose metabolism both at rest and in response to visual stimulation. The study found that visual stimulation led to increases in glucose metabolism and that alcohol did not reduce the stimulation-induced metabolism [83]. This suggests that alcohol may reduce resting glucose metabolism, but not stimulation-induced increases in glucose metabolism. While the implications of lower glucose metabolism among adults with AUD are unclear, it may affect energy availability or metabolic byproducts.

Recovery of Brain Volume with Sustained Abstinence

Clinically, several critical questions are whether the structural changes associated with alcohol are reversible with abstinence, to what extent alcohol-related changes may be reversed, and how long it takes for abstinence-related brain volume recovery

to occur. In one study, which followed 23 individuals with AUD over 12 months, greater recovery of brain tissue volume was observed in the first month of abstinence than in the following 6–9 months, during which time modest gray matter volume gains continued to occur. The short-term increases in volume were reversed with a return to drinking [84]. In certain brain regions, volume recovery to levels comparable with healthy adults may be possible after extended abstinence. For example, in 85 individuals with AUD who were measured at 1-week, 1-month, and 7-months of abstinence, increases in volume were observed after 1-week, 1-month and 7-months. After 7 months of abstinence, ACC and DLPFC volumes were equivalent to those of healthy individuals [85]. Some volume loss, however, appears to persist even after sustained abstinence. Another study showed that after 7.5 months of abstinence, individuals with AUD had lower parietal, temporal and total cortical gray matter and thalamus volumes than healthy adults, despite showing increases in frontal, parietal, occipital, total cortical gray matter volume, and thalamus volume over the course of the abstinence period [86]. Recovery of brain tissue over the course of abstinence also appears to differ based upon the extent of volume loss an individual has incurred, as another study in an AUD sample found that smaller baseline brain volumes prior to 7 months of abstinence predicted larger rates of brain volume change during the abstinence period [87]. The structural changes that occur in the context of heavy or chronic alcohol consumption may be partially reversible with prolonged abstinence, with the largest improvements occurring in early abstinence [86].

Interaction Between Structural and Functional Neural Changes in AUD

Both brain structure and function may change among individuals with AUD, and there may be an interaction between structural and functional changes. Although most AUD neuroimaging studies have analyzed primarily structural or functional data, multimodal neuroimaging studies examining both have become more common in recent years. Perhaps the earliest of these studies used PET and fMRI in the same participants and found that VS dopamine D2 receptor availability was lower among 11 men with AUD than 13 healthy men, and less D2 availability was correlated with greater cue-elicited ventromedial PFC activation [88]. These data suggested that cue-elicited brain activation is dopaminergically modulated, that VS dopamine is associated with frontal processing of alcohol cues, and that AUD alters this pathway.

A key question regarding the relationship between AUD-associated changes in brain structure and function is whether volumetric deficits among individuals with AUD relative to healthy adults mask, or exaggerate, differences in function. If alcohol neurotoxicity reduces the number of neurons in a brain region, the BOLD response of this region to a functional task may be affected. A study evaluated this possibility by scanning 46 healthy adults and 46 detoxified AUD patients, who were followed for 3 months after scanning, during which 30 relapsed to heavy drinking

and 16 remained abstinent [89]. Relative to healthy adults and adults with AUD who remained abstinent, the AUD patients who relapsed displayed smaller volumes of several cortical areas, including the OFC, medial PFC, and ACC. Individuals who relapsed, relative to those who remained abstinent, demonstrated greater alcohol cue-elicited activation of the medial PFC even after accounting for the atrophy they displayed in this region. The effect was larger than previous reports indicated, suggesting that volumetric differences might have masked an even greater difference in cue-elicited activation of this region, and that structure and function may interact to predict relapse in AUD.

Studies have also examined the relationship between white matter structure and brain function. Assessment of white matter integrity is useful because alcohol damages brain myelin [90]. One study reported that 18 AUD individuals, relative to 17 healthy adults, displayed less correlation of activation between the middle cingulate cortex (MCC) and PCC during an inhibitory control (Stroop) task, but greater correlation between MCC and midbrain activation [91]. Enhanced MCC-midbrain correlation was associated with lower integrity of fiber bundles in the PCC as measured by diffusion tensor imaging (DTI), which assesses white matter integrity. This suggests that structural compromise of major white matter tracts in AUD manifests in differences in neural activation patterns during inhibitory control. Another study employed structural MRI, DTI, and resting-state fMRI to evaluate differences in a circuit between the NAcc (the primary structure within the VS) and OFC, between 39 detoxified AUD patients and 18 healthy adults [92]. The AUD individuals displayed smaller NAcc volumes and reduced integrity of the whiter matter tract between the NAcc and OFC, and these measures were correlated, suggesting that alcohol-associated atrophy of the NAcc, OFC, or both might have led to degradation of their connecting fibers. Although the groups did not differ in the strength of correlation between OFC and NAcc activation, individuals with stronger correlation had greater alcohol craving. This pathway may therefore contribute to motivation to use alcohol.

Brain structure and function have also been examined as metrics to identify adults with vs. without an AUD diagnosis. A recent study used several structural neuroimaging measures, including gray matter and cerebrospinal fluid density and cortical thickness, along with functional measures, including monetary reward task and resting-state fMRI, to develop a classification model to differentiate 119 AUD individuals from 97 healthy adults [93]. The combination of these measures achieved 79.3% accuracy in predicting AUD diagnosis, although this was only modestly better than the single most predictive measure, gray matter density, which achieved 76.6% diagnostic prediction accuracy. A similar study examined 177 adults with a history of weekly binge drinking and 309 adults who did not binge drink, and was able to discriminate the groups using neural and personality measures at 80% accuracy [94]. The best performing fMRI task data achieved 66% accuracy, whereas the worst performing task was no better than chance, and another analysis on the same dataset showed that the fMRI tasks that discriminated between risky drinkers and healthy adults also showed greater test-retest reliability [95]. Thus, structural abnormalities may better distinguish individuals with AUD from healthy adults, perhaps

because many fMRI tasks lack the within-person stability necessary for them to represent a stable, trait-like measure of brain function. Although it has become more frequent, studies that use multiple types of imaging measurements remain relatively rare compared to single-modality studies. However, this approach may help advance understanding of the effects of AUD on the brain.

Conclusions

Chronic heavy drinking among individuals with AUD has consistently been associated with differences in brain structure. Numerous brain regions are impacted by chronic alcohol use, and these generally correspond to brain systems that have been linked to problematic alcohol use, such as frontal control systems and the mesocorticolimbic reward system [2, 5, 96]. Structural alterations, such as reduced D2 receptor density, cortical volume, and white-matter tract integrity, may relate to differences in brain activation elicited by alcohol cues. The direction of these relationships remains unclear but structural deficits may presage functional effects or may represent a consequence of changes in function. Most studies only examine individuals at a single point in time, precluding conclusions about whether alcohol use directly causes these differences, but supporting evidence suggests a causal relationship with chronic heavy alcohol use. There is also evidence that individuals with AUD display greater activation of the cingulate and ventromedial PFC in response to alcohol cues, less activation of the VS during the anticipation of monetary reward, and decreased activation of the insula and ventromedial PFC in response to negative emotional cues. Evidence from PET studies suggests that AUD is associated with reduced glucose metabolism, possibly reflecting a shift to acetate metabolism by glial cells. There is also evidence that GABA receptors are reduced and that mu-opioid receptors are increased. Differences in brain anatomy and function can inform clinical practice, as indicated by studies that show that AUD can be identified based on neuroanatomical differences, and that neural activation to alcohol cues can offer predictive value to determining which individuals will benefit from treatment.

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Chapter 27
Brain Microstructure in Alcohol Addiction: Characterization of Diffusion-Based MRI Biomarkers, Neuropathological Substrates, and Functional Consequences

Silvia De Santis, Mohamed Kotb Selim, and Santiago Canals

Abstract Alcohol consumption is associated with structural alterations in the brain parenchyma. Understanding the longitudinal brain transformations that occur from an alcohol naïve state to alcohol addiction, through cycles of consumption, abstinence and relapse, is crucial to progress towards effective treatments. Magnetic Resonance Imaging, and in particular diffusion-weighted approaches, have been a breakthrough for neuroscience, neurology and psychiatry due to their ability to access different aspects of brain anatomy in a non-invasive and longitudinal way. In the last 20 years, this technique has been instrumental in characterizing brain alterations associated to alcohol consumption.

In this chapter, we will first introduce diffusion-weighted Magnetic Resonance Imaging and its most widely adopted formulation, Diffusion Tensor Imaging, describing its utility to look at brain microstructure and its most used biomarkers, mean diffusivity and fractional anisotropy; we will then focus on the importance of diffusion tensor imaging in alcohol use disorders, describing the most salient aspects of the pathology that can be captured and dissected; next, we will discuss several recent advances in the field that were possible thanks to the use of diffusion tensor imaging. Finally, we will disclose important limitations of the technique that must be taken into account in the interpretation of alcohol-driven alterations, challenging the conventional view that diffusion tensor imaging can discern between "healthy" and "damaged" microstructure under all circumstances.

Keywords Alcohol use disorder · Diffusion-weighted MRI · Mean diffusivity · Fractional anisotropy · Microstructural integrity · Extracellular space

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Introduction

Magnetic Resonance Imaging (MRI), and particularly diffusion weighted approaches, were a breakthrough for neuroscience, neurology and psychiatry due to their ability to access distinct aspects of brain anatomy non-invasively and without the use of ionizing radiation and/or contrast agents. Being non-invasive and translational, diffusion-weighted MRI can be used to monitor brain changes over time *in vivo* in humans and in animal models, giving access to fine microstructural properties of the brain parenchyma, and has proven pivotal to characterize brain changes associated with alcohol consumption.

In this chapter, we will first introduce diffusion-weighted MRI and its most widely adopted formulation, diffusion-tensor imaging (DTI), describing its utility to look at brain microstructure; we will then focus on the importance of DTI in alcohol use disorders, describing the most salient aspects of the pathology that DTI can capture and dissect; next, we will discuss several recent advances in the field that were possible thanks to the use of DTI. Finally, we will disclose important limitations of the technique which need to be taken into account in the interpretation of alcohol-driven DTI alterations, challenging the conventional view that DTI can discern between "healthy" and "damaged" microstructure under all circumstances. Throughout the chapter, we will focus on the so-called uncomplicated alcohol use disorder, i. e., in absence of other degenerative conditions associated with alcohol, like the Wernicke-Korsakoff syndrome.

Measuring Water Diffusivity Non-invasively Through Diffusion-Weighted MRI

At absolute temperatures higher than zero Kelvin, water molecules are in constant motion due to their intrinsic thermal energy, colliding with each other and with the obstacles they find in their path. Due to such collisions, the trajectories followed by the particles are random, so their motion is referred to as "random walk". Within living organisms, soft tissues like the brain contain a high percentage of water, and the diffusion trajectories of the water molecules are influenced by the shape and orientation of the structures in which they are embedded (e.g., axons, cell bodies, glia, . . .). Through the diffusion-weighted MRI sequence, a label is imparted to hydrogen nuclei (by manipulating the phase of the transverse magnetization), and the displacement of the water molecules is characterized by reading this label at a later time; normally, in the milliseconds rage. The larger the signal attenuation recorded between the two time points, the larger the motion that has occurred. In this way, the diffusion of water during the allowed diffusion time can be quantified.

One of the main advantages of diffusion-weighted MRI is the ability to highlight what is known as diffusion *anisotropy*, that is, when molecules exhibit different diffusion properties along different orientations in space. For example, the axonal

bundles that constitute the white matter of the brain present a structure dominated mostly by cylindrical elements, the axons. Since the cell membranes are largely impermeable to water, the movement of water in these structures is less hindered parallel to the fiber bundle than in the perpendicular direction. If one were to measure water diffusion parallel to the fibers, this would appear much faster than perpendicular to them.

To quantify anisotropy, as well as the average water diffusion in the tissue, several indices can be extracted from the diffusion-weighted MRI measurements; the most popular are the *fractional anisotropy*, FA, and the *mean diffusivity*, MD. While MD quantifies the average water displacement in the tissue and is measured in square meters per second (m^2/s), FA is a dimensionless index ranging from 0 (no anisotropy) to 1 (maximum anisotropy). The mathematical framework used to define FA and MD is detailed in Box 27.1.

Box 27.1

The simplest mathematical structure capable of accounting for diffusion anisotropy in a 3D environment is a tensor. In the diffusion-tensor imaging (DTI) framework, water diffusion in each voxel is modeled with a 3 × 3 diffusion tensor $\boldsymbol{D}$.

$$\boldsymbol{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix}$$

$\boldsymbol{D}$ is constituted by 9 components, but only 6 of them are independent, due to the tensor symmetry. For this reason, to measure the diffusion tensor, a minimum of 6 measures along different orientation, plus one measure without diffusion weighting, are needed, although to have a better signal-to-noise ratio it is highly recommended to use more [1]. From the eigen-decomposition of $\boldsymbol{D}$, different scalar indices can be obtained, like the mean diffusivity (MD) and the fractional anisotropy (FA), as defined in the following expressions:

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}.$$

$$FA = \sqrt{\frac{3}{2}} \frac{\sqrt{\left(\lambda_1 - MD\right)^2 + \left(\lambda_2 - MD\right)^2 + \left(\lambda_3 - MD\right)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}.$$

The mean diffusivity is the mean of the three diffusivities measured along the eigenvectors and is interpreted as the average water diffusivity in the measured volume (the voxel in MRI). Fractional anisotropy is proportional to the standard deviation of the diffusivities measured along the eigenvectors divided by their root mean squared; importantly, fractional anisotropy quantifies the

degree of anisotropy of the media. Anisotropic tissues have fractional anisotropy values closer to 1, meaning that the diffusion of water molecules in such environments occurs predominantly in one orientation, and can be visualized as an ellipsoid, while isotropic tissues have fractional anisotropy values closer to 0, meaning that the diffusion is similar in all directions, and can be visualized as a sphere [2]. These scalars are translationally invariant, meaning that they do not depend on the specific orientations along which the diffusion is measured. This makes their use ideal as quantitative biomarkers of brain microstructure.

Among the diffusion-based indices extracted from MRI, fractional anisotropy has probably become the most popular in neurology and psychiatry, with its reduction commonly interpreted as a marker of loss of white matter integrity [3]. However, many factors contribute to determining the value of the resulting fractional anisotropy, such as the amount of myelin, axonal diameter, axonal density and fiber dispersion. Therefore, it is not straightforward to assign a specific tissue configuration or biological state to a measured fractional anisotropy change, as discussed below. Figure 27.1 illustrates a typical diffusion-weighted MRI acquisition and processing pipeline.

It is important to note that the direction of the greatest diffusivity, i.e., the orientation of the principal eigenvector, can be interpreted as an estimate of the fiber orientation in the white matter under the assumption that when a number of neuronal axons are aligned along a common axis, the diffusion of water molecules will be hindered to a greater extent across this axis than along it [7]. By integrating these estimates across the whole brain white matter, it is possible to generate a 3D representation of the major axonal bundles. These are the basis of the technique called *tractography*, which quickly became a powerful tool to look at brain anatomy. Several more advanced non-tensor approaches have been introduced in recent years to extract the fiber orientation distribution; each of these models has its own strengths and pitfalls, nevertheless, a comparative discussion is outside the scope of this chapter.

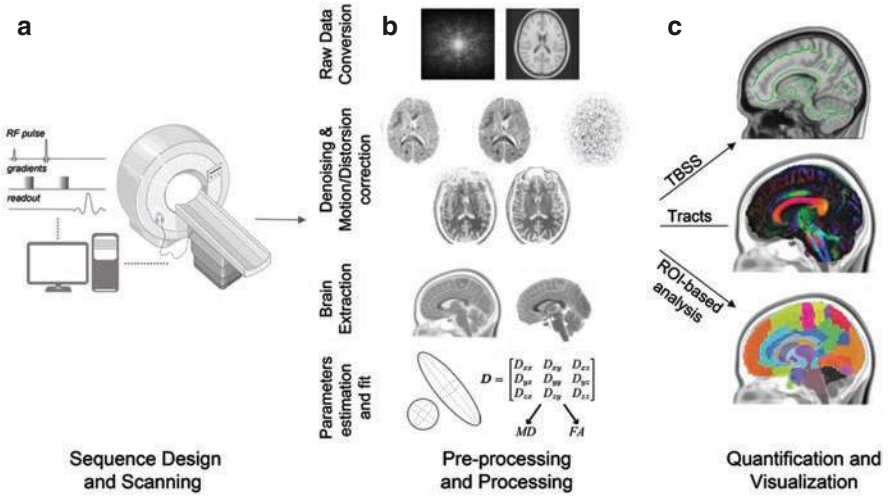

Fig. 27.1 Diffusion-weighted MRI acquisition and processing pipeline. (**a**) Diffusion-weighted MRI experiments normally encompass several acquisition and processing steps. The diffusion-weighted MRI sequence is tuned according to the specific need of the experiment in terms of diffusion weighting (quantified by the *b-value*) which, by defining the time allowed for the labeled water molecule to diffuse and the range of diffusivities on which the sequence focuses, weights the contribution of the different tissue compartments to the diffusion measurement (e.g., diffusion in the intracellular vs. extracellular space). Other important parameters are the number of gradient orientations, which define the directions in which diffusion will be quantified; or the image resolution, which is a compromise between the capabilities of the MRI system to acquire data with good signal-to-noise ratio and the time devoted to acquiring them, and which determines the voxel size. (**b**) After the desired diffusion-weighted MRI sequence is defined and acquired, the preprocessing might involve denoising, motion and distortion corrections, brain extraction and normalization to other contrasts. Then, the desired diffusion model is applied, and the data are visualized, ready for the statistics of choice. (**c**) Statistical analysis normally involves voxel-wise approaches like the widely adopted tract-based spatial statistics [4], or region-of-interest approaches like those based on parcellated brains in standard space [5], or on tractography in single subject space [6]

Neuropathological Substrate and Utility of DTI Biomarkers in Alcohol Use Disorders

Difficulty walking and talking, blurred vision, slowed reactivity: clearly, alcohol affects the brain, but while some of these impairments quickly resolve when alcohol is discontinued, chronic drinking produces the accumulation of deficits that persist into sobriety, generating a disability with great impact on society. As such, alcohol neurotoxicity has been widely investigated, with the goal of achieving a mechanistic understanding of this complex behavioral and medical condition.

The histopathological substrate underlying the well-characterized brain shrinkage in alcohol use disorder (AUD) patients involves changes in both myelination and axonal integrity, as well as region-selective neuronal loss [8]. Dendritic and

synaptic changes have also been well documented in people with alcohol addiction [9]. Importantly, alcohol exposure not only affects neurons, but also the development, morphology, physiology and gene expression in astrocytes, oligodendrocytes and microglia cells [10].

In this context, neuroimaging techniques like DTI-based approaches represent a unique tool to assess alcohol-induced brain alterations, being sensitive to fine details of the brain parenchyma's microstructure. DTI can detect changes in both myelination and axonal integrity in white matter with high sensitivity [11]. While the technique is preferentially used to look at white matter microstructure, there are also numerous applications to grey matter morphology, where the diffusion of water molecules is expected to sense and reflect the complex geometry of a tissue containing neurons, their dendrites and axons with different levels of myelination and orientations, and glial cells of different morphologies [12, 13].

A major feature of DTI is that it affords relatively high resolution compared to other whole brain imaging techniques, going from 2–3 mm in humans, to few hundreds of microns in animals. Furthermore, being non-invasive, the technique allows for longitudinal studies. In the context of alcoholism, this means that the dynamic course of the disease, going through periods of drinking, sobriety, and relapse, can be followed [14].

Another important feature of DTI, shared with the other MRI-based techniques and sometimes underestimated, is the possibility to perform translational studies. DTI can be applied across species, in humans and animal models. This important feature allows scientists to interrogate the causality of alcohol-related brain alterations in well-controlled animal experiments, something that is difficult to achieve in studies with AUD patients where alcohol-induced damage almost inevitably coexists with that produced by tobacco and other drugs of abuse, medication and other comorbid factors. A reverse translational approach, in which alterations found with DTI in patients find an equivalent counterpart in animal models, and the latter are used to investigate the underlying neurobiological mechanism, has proven successful in bringing causality to AUD [15, 16].

Multiple DTI metrics can be combined, and they can also be combined with other imaging and non-imaging measures, using machine learning frameworks. Pioneer work in this field has shown that multi-parametric approaches including DTI have high potential to serve as valuable biomarkers for early diagnosis and response to treatment in AUD, by demonstrating high accuracy and precision in classifying baseline, alcohol drinking and abstinence states, and even the effect of medication with naltrexone in an animal model [17]. Those multimodal studies pointed to water diffusion measures as the more informative feature to differentiate between AUD associated states. Recently, machine learning algorithms using structural MRI data have also been able to predict in adolescents future patterns of alcohol binge-drinking, providing important information of the substrate that may favor the development of alcohol addiction [18].

Regional Specificity of Altered Diffusivity in AUD: Frontal White Matter Vulnerability

By mapping DTI parameters in AUD versus age- and sex-matched controls, a pattern of region-specific vulnerability emerged. In white matter, frontal and dorsal tracts consistently show the greatest abnormalities in people with alcohol addiction relative to controls, while more posterior and ventral bundles were relatively spared [19, 20]. A recent meta-analysis including over 900 subjects identified four significant clusters of convergent microstructural white matter alterations in AUD patients that were assigned to the genu and body of the corpus callosum, anterior and posterior cingulum, fornix, and the right posterior limb of the internal capsule [21]. Genu and fornix seem to be particularly vulnerable tracts in alcohol use disorder, showing alterations after a single episode of binge drinking and suggesting potential for rapid neuroplasticity [22]. Importantly, these same axonal bundles are also preferentially affected in rat models of AUD longitudinally investigated with DTI [15, 20], which in addition to attributing a causal role to alcohol (see above), suggest the existence of some fundamental biological principle, generalizable across species, that determines heterogeneous white matter vulnerability to alcohol. White matter tracts showing alterations in the AUD population compared to controls are shown in Fig. 27.2.

The described microstructural alterations are expected to have functional implications. Abnormalities in the white matter tracts connecting regions of the

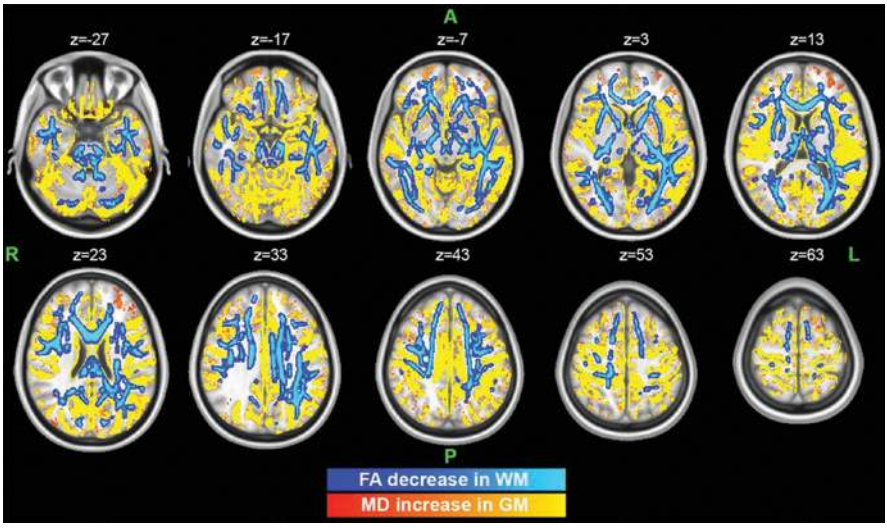

Fig. 27.2 White matter regions of significantly reduced fractional anisotropy (FA) in AUD vs. controls (blue, filled with the tbss_fill routine) according to the cohort in [15], and grey matter regions of significantly increased mean diffusivity (MD) in AUD vs. controls (red-yellow, $p < 0.05$) in the same cohort [23]

mesolimbic pathway, known to play an important role in the rewarding effects of drugs, are associated with higher impulsivity and alterations of the reward network [24]. The fimbria, for instances, is the main pathway connecting the hippocampus and the prefrontal cortex and plays a fundamental role in memory formation and extinction, executive function and emotional processing [25]; thus, the high vulnerability of the fimbria white matter to alcohol drinking might be an important contribution to the observed inability of AUD patients to suppress maladaptive memories, and the display of behavioral inflexibility [20]. More generally, the fact that alcohol consumption causes heterogeneous alterations of white matter tracts could be interpreted in functional terms as the basis for the observed imbalances between fMRI brain networks, some of them showing decreases in functional coupling and others increases, possibly reflecting a new functional equilibrium characteristic of an AUD state (allostasis).

Finally, the patterns of region-specific white matter vulnerability are not limited to AUD; negative associations between alcohol intake and brain microstructure are already apparent in individuals consuming an average of only one to two daily alcohol units, and become stronger as alcohol intake increases, in a region-specific manner, with the fornix and the corpus callosum featuring prominently as areas associated to the largest effect sizes [26].

DTI in Grey Matter: Functional Consequences

While DTI has traditionally been used to assess white matter integrity, it can be also used to look at microstructure in grey matter. The preferred biomarker in grey matter is the mean diffusivity, due to the low degree of anisotropy which characterizes grey matter microstructure. Microstructural information has the potential to complement and, most importantly, anticipate macroscopic alterations like volume and cortical thickness changes, which are normally employed as surrogate markers of neuronal degeneration, but which are believed to pick up alterations only when these are in a relatively advanced state [27].

Higher mean diffusivity was detected in frontal, temporal and parahippocampal grey matter, and in the cerebellum of alcohol dependent patients compared to controls. Low verbal episodic memory performance was associated with higher mean diffusivity but not shrinkage in parahippocampal areas, in frontal cortex and in the left temporal cortex, suggesting that regional microstructural but not macrostructural alteration of the brain parenchyma might be responsible, at least in part, for episodic memory deficits in alcohol dependence [28]. Another study measured increased mean diffusivity in the medial prefrontal cortex of rats exposed to chronic intermittent ethanol vapor, associated to deficits in retrieval and recall of fear memories, and whose neuropathological substrate included demyelination and mitochondrial damage [29].

A recent study comparing water diffusivity in the grey matter of AUD patients and a rat model of chronic voluntary drinking in a two-bottle free-choice paradigm

[23], showed widespread increases in mean diffusivity in both species. Grey matter areas showing alterations in the AUD population compared to controls are shown in Fig. 27.2. The authors demonstrated that 1 month of chronic alcohol drinking (4–6 g/kg/day) is sufficient to trigger the mean diffusivity effect in the rat model and in both, humans and rats, higher mean diffusivity persisted during abstinence. However, as we have mentioned already and will discuss further in the last section, interpreting DTI findings in biological terms is challenging, as all water compartments (i.e., intracellular and extracellular) contribute to determine mean diffusivity. To answer this question, the authors turned into the animal model and, using an invasive technique called iontophoresis, they quantified with high precision the diffusion in the extracellular space of the brain. Together with a small decrease in the total volume fraction, they found a large and significant reduction in the extracellular space tortuosity triggered by alcohol drinking, this is, a reduction in the diffusion barriers. The reduction in tortuosity was sufficient to explain the increase in mean diffusivity measured with DTI and suggested a significant change in the manner in which solutes can diffuse through the extracellular space of the brain.

To study the possible functional impact of the increased mean diffusivity in the grey matter of alcohol-exposed individuals, a mathematical model was used to investigate how the above tortuosity change might influence the diffusion of extrasynaptically released neurotransmitters, such as dopamine [23]. The authors found a marked increase in the spatial reach of the released neurotransmitter, a potentiation of volume neurotransmission, so that a same amount of dopamine will diffuse farther and in greater concentration in the same amount of time [23]. The authors speculated that a synergistic combination of a primarily weak reinforcer like ethanol, which is known to raise dopamine levels (albeit modestly), with progressively enhanced volume neurotransmission due to increased extracellular diffusivity, might comprise a novel mechanism to explain the slow onset but potent addictive effect of alcohol. Further experimental work will be necessary to confirm or refute this hypothesis. The impact of mean diffusivity changes on other important biochemical processes heavily relying on extracellular space diffusivity, like the clearance of metabolic byproducts, might also be interesting to investigate. This result already serves as an example to illustrate the mechanistic insight that can be gained from investigating the neurobiological basis of DTI biomarkers.

Progression of Diffusivity Alterations During Early Abstinence

Longitudinal DTI acquisitions have been used to measure microstructural integrity in the white matter of abstinent AUD patients from 1 to a few years after the last drinking episode [30, 31]. These studies reported the recovery of DTI values towards control levels in long-term abstinent subjects, providing a microstructural substrate for the observed clinical improvement. However, AUD is associated with a chronically relapsing-remitting course over lifetime, and most individuals treated for AUD are known to relapse to hazardous alcohol

consumption within just 6 months of treatment [32]. What happens then with the microstructure of the brain during the most critical early phase of alcohol abstinence? To answer this question, a recent DTI study acquired longitudinal DTI data in AUD patients and rat models thereof at different time intervals from 2 to 6 weeks, during early abstinence [15]. Interestingly, in both species it was shown during this period that, far from recovering, the reduction in fractional anisotropy measured in the white matter tracts progressed, becoming reduced after 3 weeks of abstinence, and even further reduced at 6 vs. 3 weeks of abstinence. The progression during early abstinence suggests the existence of an underlying process that evolves soon after cessation of alcohol consumption, challenging the conventional idea that the microstructural damage starts to repair immediately after discontinuing alcohol drinking.

What might this process be? Answering this question will require further experimental work, but a first clue can perhaps be seen in the change in extracellular space tortuosity found in the grey matter [23]. In that study, it was shown that the reduction in tortuosity explaining the increased water mean diffusivity in the grey matter was associated with a microglial response. Microglial cells retracted their cellular processes and engrossed the cell body acquiring an amoeboid morphology. This change did not affect other glial subtypes, extracellular matrix proteins or neuronal density. The morphological change in microglia explained the decrease in diffusion barriers and the increased mean diffusivity. Indeed, reactivation of microglia independently of alcohol drinking with lipopolysaccharide, or their elimination with the CSF1R inhibitor PLX5622, provided causal evidence to support their effect on diffusion [23]. A role of microglial cells and neuroinflammation in the neurotoxic effects of alcohol has long been proposed [33]. Taken together, these results suggest that the biological process underlying the progression of microstructural alterations during early abstinence could be an inflammatory response, maybe triggered by alcohol withdrawal.

Overall, this result, besides proving that DTI can provide unique information to understand the neuroadaptations occurring during abstinence, puts this critical phase in the spotlight as a central target for therapeutic interventions [16].

Sex Differences in Alcohol Use Disorder

While alcohol neuroimaging investigation has been affected, like many other fields, by the systematic underrepresentation of female subjects, especially in basic research, there is a remarkable convergence of evidence pointing towards a sex effect in AUD, confirmed by DTI studies. In addition, over the last 10 years, rates of alcohol use disorder have increased in women by 84%, while they have increased by 35% in men [34], highlighting even more the importance of including both sexes in AUD studies. The drawback of this necessary change of paradigm is that including an additional factor might further reduce power in neuroimaging studies, many of which have by design small sample size.

Pioneer work showed that AUD women have more DTI features of white matter degradation than men of the same age in several fiber bundles [19]. Also in animal models, female rats have been reported to be more affected in a chronic intermittent ethanol paradigm, as shown by greater reduction of fractional anisotropy in the fornix. This vulnerability was explained by higher initial blood alcohol levels in females [35]. In women but not in men, more frequent binge drinking was associated with lower fractional anisotropy values, a result that was interpreted as evidence of higher vulnerability to alcohol in females [36]. However, a recent study using multimodal brain imaging in a large general population (36,678 generally healthy middle-aged and older adults from the UK Biobank) [26], found consistent associations between daily alcohol units consumed and lower fractional anisotropy values in several white matter tracts, with the strongest effects in the fornix, but no significant or weak association to sex. Therefore, while sex seems to be an important factor to develop brain damage in AUD, the interaction between sex and less severe levels of alcohol drinking will require further clarification. Furthermore, women and men's white matter microstructure is affected differently by age [37], suggesting that longitudinal studies involving larger cohorts than those normally employed in neuroimaging studies might be necessary to characterize the complex interaction between AUD and age in a sex-specific manner.

Finally, the reported difference in DTI features across sexes in AUD are not limited to differences in effect size. Indeed, opposite patters of DTI changes between sexes have been also reported. Fractional anisotropy is systematically reduced in AUD males compared to controls but, interestingly, seems to be increased in some fiber tracts in AUD women [38, 39]. Before going into interpreting these apparent discrepancies, it is important to remember what is being measured with DTI and how it relates to the underlying neurobiological substrate. While the classical view of DTI associates a reduced fractional anisotropy to impaired microstructure (demyelination and axonal damage), as discussed in the next paragraph, other factors are contributing; according to this rationale, it is not possible to univocally associate an increase in fractional anisotropy to an improvement in the quality of microstructure.

DTI Limitations and Caveats in the Interpretation of DTI Biomarkers in AUD

DTI-derived measures are affected by at least two limitations: the lack of specificity to sub-compartments of the tissue, meaning that very different neuroanatomical configurations can result in the same measured values of water diffusion [40], and the limited utility in structures like grey matter lacking macroscopic anisotropy.

Given the lack of specificity of DTI measures, interpreting the underlying neurobiological substrate that is causing the observed change is challenging. As mentioned earlier, postmortem studies consistently report compromised white matter

integrity in AUD that could explain, at least in part, the observed DTI changes. In these patients, the reduction in white matter volume is normally explained as a process of demyelination and axonal loss produced by the regional neuronal loss that can occur especially in the dorsal frontal cortex [41]. Alcohol drinking induces loss of mainly small fibers, myelin irregularity and segmental de/remyelination [42], accompanied by neuroinflammation. Also, the possibility of excessive intracellular and extracellular fluids accumulation has been proposed to explain some DTI changes in AUD vs. age matched controls [43].

However, recent *in silico* data challenged the idea that it is possible to infer the specific microstructural alteration causing the observed pattern of DTI changes. It was shown that a different balance between the restricted, hindered and isotropic water pools in the tissue can explain the DTI alterations found in AUD patients with totally different neurobiological underpinnings, simply depending on the underlying geometry [15]. Importantly, the increase in the proportion of the isotropic water pool, which can be a model for both an accumulation of fluids in the extra-cellular space as well as a glial reaction, results in an increase of fractional anisotropy in areas of white matter with single fibers, and a decrease of fractional anisotropy in areas of crossing fibers. Overall, the observed changes in fractional anisotropy across the different phases of AUD can thus be equally explained by progressive myelin damage, axonal loss, and/or a glial/cellular reaction, for instance, during an ongoing inflammatory process. In addition, the same biological phenomenon can impact with opposite trends in DTI (fractional anisotropy either increasing or decreasing) depending on the affected brain region (areas of predominantly single fiber vs. areas of crossing fibers). Possible neuropathological substrates generating fractional anisotropy alterations are illustrated in Fig. 27.3.

All in all, DTI is non-specific to neurobiological correlates of brain tissue, and needs to be complemented with other approaches to dissect cell-specific patterns of alterations in AUD. For example, diffusion-weighted MRI has been shown to be sensitive to axonal density [44] and diameter in white matter [45]; DTI measures can be complemented with myelin specific sequences like those based on magnetization transfer [46].

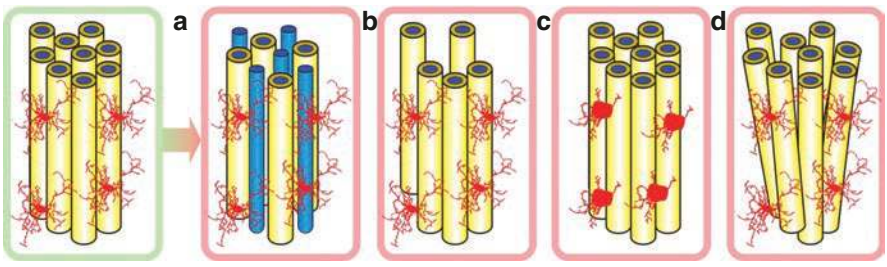

Fig. 27.3 Possible biological substrates driving changes in fractional anisotropy: demyelination (**a**), axonal loss (**b**), glia morphological changes (**c**) and increased fiber dispersion (**d**). Myelin is shown in yellow, axons in blue and microglial cells in red

The other major limitation of DTI is that, given the low anisotropy of grey matter tissue, fractional anisotropy is a poor predictor of microstructural integrity in grey matter. As detailed earlier in the chapter, mean diffusivity has been successfully used to highlight grey matter alterations in AUD; however, it suffers from the same lack of specificity of other DTI parameters. A recent approach based on stereotaxic injections of neurotoxins affecting selectively glia, myelin and neuronal compartments showed that mean diffusivity is highly sensitive to changes, but poorly specific [13], with similar effect sizes in demyelination, inflammation and degeneration. As such, to achieve augmented specificity, it is advisable to choose more advanced, grey-matter specific diffusion-weighted MRI sequences focusing on neurite morphology [47] or inflammation [13]. The drawback here is that, for the time being, such advanced techniques entail longer and more complex data acquisitions.

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Chapter 28
Determinants of Risk Developmental Trajectories for Risky and Harmful Alcohol Use: Lessons from the IMAGEN Consortium

Justin Böhmer, Andreas Heinz, Gunter Schumann, and Henrik Walter

Abstract Adolescence is a key period for the initiation of alcohol drinking. Escalating alcohol use in adolescence, however, increases the risk for developing alcohol-related problems later in life, including alcohol use disorder (AUD). Thus, early identification of risk factors for developmental trajectories of alcohol abuse are crucial for preventing the development of addiction. To this end, the IMAGEN Consortium, a longitudinal neuroimaging-genetic study investigating reinforcement-related behaviors and their role for normal and psychopathological development in adolescence, was established. With more than 2000 adolescents repeatedly assessed in eight European study centers across four successive time points during adolescence and young adulthood, the IMAGEN study constitutes one of the world's largest longitudinal neuroimaging-genetics studies in adolescence. Since its inception, the IMAGEN Consortium has published a number of studies revealing environmental, behavioral, neurobiological and (epi-)genetic determinants of risk developmental trajectories for adolescent alcohol use. In this chapter, we will synthesize findings from these studies by delineating relationships between structural and

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functional brain characteristics, genetic variation, epigenetic modification and alcohol use trajectories in adolescence and summarize the relative contribution of these factors for the prediction of alcohol abuse.

Keywords IMAGEN consortium · Adolescence · Alcohol use · Binge drinking · Imaging genetics · Longitudinal study · Trajectory · Prediction

The IMAGEN Study: Identifying Risk Developmental Trajectories in Adolescence

Adolescence is a key period for alcohol use initiation [1]. Escalating levels of alcohol use across adolescence increase the risk for transitioning to alcohol addiction later in life [2], presumably due to common genetic, family and personal vulnerabilities [3–5]. Thus, identifying risk factors for developmental trajectories of alcohol abuse in adolescence is crucial in alcohol research. In order to understand complex interactions and temporal changes in neurobiological mechanisms and to infer causality, prospective longitudinal population studies are necessary that compare individuals regarding neurobiological, genetic, behavioral and environmental differences, allowing for the identification of vulnerability markers early in the course of the disease before the manifestation of alcohol use disorder (AUD).

To this end, the IMAGEN Consortium was established in 2010—a longitudinal neuroimaging-genetic study focusing on reinforcement-related behaviors and their role for normal and psychopathologic development in adolescence, including alcohol abuse [6]. The IMAGEN Consortium comprises eight European study centers in Germany (Berlin, Dresden, Hamburg, Mannheim), the United Kingdom (London, Nottingham), Ireland (Dublin) and France (Paris). More than 2000 adolescents at the age of 14 years were initially included in the IMAGEN cohort and followed up repeatedly across adolescence and young adulthood at ages 16, 19 and 22 (see Fig. 28.1), making it one of the world's largest longitudinal neuroimaging-genetics study in adolescents. Neuroimaging assessment included structural T1-weighted magnetic resonance imaging (MRI), functional MRI from three behavioral paradigms on reward processing [7], response inhibition [8] and emotional processing [9], resting-state functional MRI, diffusion tensor imaging (DTI) and neurite orientation dispersion and density imaging (NODDI). Moreover, blood samples were drawn in order to perform genome-wide (GWAS) and epigenome-wide association analyses (EWAS) as well as RNA, proteomic and metabolic analyses. Neuropsychological tests together with measures on personality, clinical symptoms (e.g., substance use, cognitive functioning, stress) and environmental factors for disease susceptibility (e.g., family environment, urbanicity,

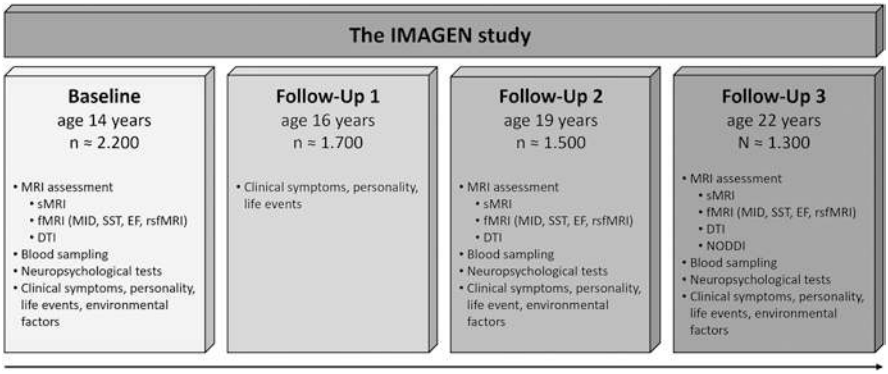

Fig. 28.1 Overview of the IMAGEN study. Within four successive time points from early adolescence to young adulthood, a set of neurobiological, genetic, behavioral and environmental characteristics are repeatedly measured

air pollution) from both adolescent self-report and parental reports completed the study's assessment battery, yielding a well-characterized, phenotypically rich, longitudinal study cohort.

To date, more than 100 papers from the IMAGEN Consortium have been published that helped to elucidate neural and genetic influences on reinforcement-related behaviors, symptoms and disorders (see https://imagen-project.org/publications/ for full list of published IMAGEN papers), including a systematic review on the significant contributions of IMAGEN to the field of imaging genetics [10]. In this chapter, we will describe findings from IMAGEN studies specifically related to alcohol use by delineating the relationship between structural and functional brain characteristics, genetic variation, epigenetic modification and developmental trajectories of alcohol use behavior in adolescence and summarize the relative contribution of these factors for the prediction of alcohol abuse. Please note that "prediction" in that case might refer to different phenomena associated with varying underlying methodologies. In cross-sectional analyses, prediction usually refers to an association or correlation between variables assessed with specific statistical methods. In longitudinal analyses, which is mostly the case in IMAGEN studies presented here, prediction typically refers to statistical approaches to measure associations between a given variable at one time point (e.g., brain structure at age 14) and a subsequent clinical phenotype (e.g., binge drinking at age 16). In some cases, within the context of machine learning applications, prediction also refers to the classification of individuals based on specific features. In order to assess the strength of the identified relationships, we report estimates of explained variance and effect size provided that it is stated in the original studies.

Brain Structural Predictors for Adolescent Alcohol Use

Previous studies demonstrated alcohol-related changes in brain structure across widespread regions of the brain (Chap. 26) [11]. The extent to which these changes reflect neurotoxic effects that arise as a consequence from alcohol exposure or delineate cerebral predispositions that contribute to vulnerability for detrimental alcohol use remains largely unknown. Therefore, several studies within the IMAGEN Consortium leveraged the longitudinal data structure to identify neuroanatomical predictors for developmental trajectories of adolescent alcohol use.

In this regard, Seo et al. [12] analyzed regional gray matter volume and cortical thickness in 14-year-old adolescents and used them as input for a machine learning classification to predict adolescent drinking behavior phenotypes (n = 550 light vs. n = 464 heavy drinkers) at age 19. Specifically, drinking behavior at 19 years was predicted by gray matter volume at the same age, although classification accuracy was only slightly above chance level (max. 58.6%). Heavy drinkers exhibited lower gray matter volume in bilateral anterior cingulate cortex, orbitofrontal cortex, medial prefrontal cortex, thalamus and left anterior insula. However, gray matter volume at age 14 did not predict drinking behavior longitudinally at age 19 [12]. In another study, gray matter volume was used to predict change in alcohol use over the course of adolescence (14–19 years) using latent growth curve modeling [13]. Accordingly, increased gray matter volume in the caudate nucleus and the left cerebellum at early adolescence predicted the increase of alcohol consumption from early to late adolescence. However, there was no association between gray matter volume of either brain region and alcohol use in early adolescence, suggesting that the observed increases in brain volume might be particularly relevant for alcohol use trajectories over the course of adolescence. Along this line, Kühn et al. [14] showed that reduced gyrification in the left orbitofrontal cortex predicts the increase of alcohol use-problems from early to mid-adolescence [14].

Robert et al. [15] specifically investigated the directionality of the association between brain structure and alcohol abuse in adolescence using temporal directionality analyses. Controlling for various confounding factors such as demographic, behavioral and genetic influences, increased frequency of drunkenness was associated with accelerated gray matter atrophy in the posterior temporal cortex, left prefrontal cortex, left anterior insula and the anterior cingulate cortex. Importantly, Causal Bayesian Network (CBN) analysis revealed substantial evidence (73% of the CBNs) for a directionality from gray matter atrophy during adolescence (age 14 to 19) to more frequent states of drunkenness. Moreover, gray matter volume at age 14 was associated with frequency of drunkenness between ages 14 and 19 (β = 0.23, n = 604) but, in turn, drunkenness frequency at age 14 years was not associated with future gray matter development (β = 0.03, n = 726) [15]. Therefore, these findings suggest that the observed reductions in brain volume might already be present prior to harmful alcohol use. Another study from the IMAGEN Consortium draws a similar conclusion. Ottino-González et al. [16] analyzed structural covariance networks (SCN) based on cortical thickness and

compared the properties of these networks between heavy drinking adolescents ($n = 297$) and non-drinking controls ($n = 594$) at ages 14 and 19 using graph theoretic metrics. Heavy drinkers at age 19 exhibit lower network segregation and higher network integration than controls. Importantly, the same SCN pattern was observed in the same adolescents 5 years earlier at age 14, when they had little to no lifetime alcohol exposure, suggesting a pre-existing risk factor for problematic drinking [16]. Similarly, probing alterations in white matter (WM), reduced WM integrity within the upper posterior pons in 14-year-old adolescents without substantial alcohol exposure was associated with heavy drinking at age 16 ($n = 24$) [17].

In sum, findings from these studies challenge the neurotoxicity hypothesis as the only cause of brain structural changes and suggest that findings in widespread brain areas can predict adolescent alcohol use. However, these studies used different brain structural features and distinct phenotypes of adolescent alcohol use and findings were complex, including increases and decreases of specific brain volumes. A recently published study [18] expanded previous analyses by incorporating multiple morphometric brain features from both white and gray matter collected at ages 14, 19 and 22 in a machine learning framework to predict alcohol use at age 22. Moreover, they compared distinct phenotypes of adolescent alcohol use based on different assessment metrics such as frequency, amount or onset of alcohol use and bingeing frequency. In this study, binge drinking behavior was the most predictable phenotype of adolescent alcohol use with accuracies of 73.1% (age 14), 75.5% (age 19) and 78.0% (age 22). The most informative features for predicting alcohol use included increases and decreases of different brain structural indices relating to widely distributed cortical and subcortical regions of the brain, including several white matter tracts of the corpus callosum, internal capsule and brain stem as well as occipital brain regions, the anterior cingulate cortex, middle frontal gyrus, precentral gyrus, hippocampus and parahippocampal gyrus [18]. Based on the relatively high accuracies for predicting future alcohol use already present at ages 14 and 19, this study corroborates the notion that certain brain structural changes at least partly precede alcohol abuse in adolescence. Future research should assess whether such changes are due to shared genetic factors or associated with traumatic or stressful life events [19–22].

Brain Functional Predictors for Adolescent Alcohol Use

As the brain's anatomical architecture scaffolds its functional activation (e.g., [23–25]), a series of behavioral tasks were implemented in the IMAGEN study to probe the relationship between activity in brain functional networks underlying reward processing, inhibitory control, emotional processing and the resting-state of the brain and adolescent alcohol use.

Alcohol use in early adolescence (age 14) was associated with increased activation in reward-related brain areas, including the left orbitofrontal cortex ($\beta = 0.05$, $n = 1778$) [26], ventral striatum (left: $\eta^2_p = 0.022$; right: $\eta^2_p = 0.015$; $n = 393$) [27],

dorsal striatum ($r = 0.09$; $n = 1483$) [28, 29], the interaction between orbitofrontal cortex and ventral striatum ($r^2 = 0.05$, $n = 1080$) [30] as well as blunted activity in frontal brain areas, namely inferior frontal gyrus ($\beta = -0.09$, $n = 1778$) [26], when anticipating reward. Concurrently, when subjects are supposed to choose between a smaller immediate amount of money and a larger delayed amount, adolescents with higher alcohol use exhibited less brain activation in frontal regions, including the dorsolateral prefrontal gyrus ($r = -0.163$, $n = 202$) [31].

Importantly, due to the longitudinal data structure, the IMAGEN study provides insights into the temporal relationship between brain activation and alcohol use trajectories across adolescence. In normal development, neural responses related to reward sensitivity and impulsivity decrease across adolescence while activation in cortical regions associated with behavioral control performance increases [32, 33]. However, impulsivity, defined as premature action without considering consequences [33], increased and the developmentally expected decrease in reward sensitivity was significantly altered in heavy drinking individuals across adolescence [32–34], suggesting disruptive effects of alcohol use on normative development of reward sensitivity and behavioral control. In this regard, rising levels of alcohol use from early to mid-adolescence (14 to 16 years) were predicted by increased ventral striatal activation ($\beta = 0.082$, $n = 1327$) and a blunted ventromedial prefrontal response ($\beta = -0.086$, $n = 1327$) [35] during reward anticipation. Additionally, increases in alcohol use across adolescence were predicted by increased ventral striatal activation ($\beta = 0.079$, $n = 1327$) [35] as well as reduced activity in medial orbitofrontal cortex ($\beta = -0.167$, $n = 304$) [33] and insula ($\beta = -0.057$, $n = 1327$) [35] during reward receipt. Moreover, Qi et al. [36] identified a specific reward-related brain network underlying high novelty seeking in adolescence, including the prefrontal cortex, striatum, amygdala and hippocampus, which longitudinally predicted alcohol use from early to late adolescence at age 19 ($r = 0.263$, $n = 219$) [36].

During resting-state, when subjects are not actively engaged in a specific task, a recent study showed that 19-year-old adolescents with increased risk for alcohol use disorder exhibited reduced resting-state functional connectivity within all seven large-scale brain networks under study [37]. Particular attention has been drawn to resting-state functional connectivity of networks involved in reward-related circuitry. Using data from the Human Connectome Project [38], Cheng et al. [39] demonstrated aberrant resting-state functional connectivity in adult high amount drinkers compared to low amount drinkers between nodes implicated in reward processing, including the medial orbitofrontal cortex, anterior cingulate cortex, parahippocampal cortex, supramarginal gyrus, insula, and superior temporal gyrus. Interestingly, replication analyses in the IMAGEN cohort ($n = 1176$) found similar patterns of functional connectivity for the same connections in non-drinking adolescents at age 14, who reported drinking alcohol at age 19, suggesting that altered resting-state functional connectivity in reward-related circuitry might play a causal role in the development of alcohol abuse [39].

In sum, these IMAGEN studies highlight an increased involvement of reward-related brain networks and decreased activation in fronto-cortical brain areas during reward-related behavioral tasks, and reward-related brain networks were also implicated in resting-state studies as an important antecedent of adolescent alcohol abuse.

Impact of Genetic Variation on the Association Between Brain Structure/Function and Adolescent Alcohol Use

In the past years, significant efforts have been made to identify variation within the genome related to alcohol use (see [40] for a review). Determining genetic loci and their relationship with alcohol use during adolescence is particularly crucial as it expands our understanding of possible molecular mechanisms underlying the development of alcohol abuse. In the following, we will outline associations between genetic variation, adolescent alcohol use and features of brain structure as well as functional brain activation during reward processing, response inhibition and emotional processing identified by the IMAGEN Consortium.

Genetic Variation, Brain Structure and Adolescent Alcohol Use

In an animal model, Mielenz et al. [41] identified the lack of the EF hand domain containing 2 (*EFhd2*) protein coding gene as a genetic determinant of enhanced sensation-seeking and attenuated anxiety sensitivity, both traits associated with reduced behavioral control and increased alcohol consumption [42, 43]. Along this line, *EFhd2* knockout (KO) mice exhibited reduced volume in the prefrontal and sensorimotor cortex, portending to a role of *EFhd2* for the development of fronto-cortical brain regions. Importantly, these findings translated to the IMAGEN sample (n = 1773), yielding a positive association of the single nucleotide polymorphism (SNP) rs112146896 in the *EFhd2*-coding region with lifetime alcohol intake (r = 0.099) and a negative association with anxiety sensitivity (r = −0.067) in 14-year-old adolescents. Moreover, a negative association was found between alcohol consumption and thickness of the superior frontal gyrus in adolescents (r = −0.067). Similarity analysis indicated significantly similar genetic contribution of all SNPs under study to lifetime alcohol drinking and superior frontal cortex thickness. Together, findings from the animal and human study suggest that genetic variation in *EFhd2* plays an important role for maturation of the frontal cortex and its role in intentional behavior and executive control, which might contribute to risky and harmful alcohol consumption [41, 44].

Genetic Variation, Reward-Related Brain Activation and Adolescent Alcohol Use

Dysfunctional reward processing, in particular reward anticipation, has been proposed as an important pathogenetic mechanism implicated in the development of substance use disorders (e.g., [45, 46]). A pivotal brain region involved in the anticipation of reward is the ventral striatum [47]. Elucidating how genetic variation impacts on ventral striatal activity during reward anticipation might help identify genetic risk constellations for alcohol use trajectories in adolescence.

In this regard, Stacey et al. [48] investigated the association of SNP rs26907 of the ras-specific guanine-nucleotide releasing factor 2 (*RASGRF2*) gene, which has previously been linked with alcohol intake [49], with ventral striatal activation during reward anticipation and the development of drinking behavior [48]. In an animal model, they showed that *Rasgrf2* KO mice exhibit reduced ethanol intake and ethanol preference as well as attenuated dopamine release following alcohol intake in the ventral striatum compared to wild type controls. In 14-year-old male adolescents from the IMAGEN cohort (n = 663), who had no or low exposure to alcohol, the *RASGRF2* haplotype containing rs26907 was associated with reduced ventral striatal activation during reward anticipation [48]. These results suggest that *RASGRF2* has an impact on ventral striatal activation through modulations of mesolimbic dopamine activity and may thus influence reward sensitivity, a risk factor for future alcohol use in adolescents (e.g., [50]). In this regard, the same haplotype was associated with more frequent drinking episodes at age 16, substantiating the notion of a genetic constellation that might render individuals at risk for alcohol abuse by affecting the incentive value of rewards [48]. In a follow-up study, Stacey et al. [51] identified a module of functional genes associated with mesolimbic dopamine signaling (M5) that correlated with *Rasgrf2* KO status in mice. Afterwards, they analyzed the human orthologues of M5 genes and tested their association to ventral striatal activity and alcohol use in 14-year-old male adolescents from IMAGEN. One haplotype block consisting of rs1648821 and five other SNPs of the EH-domain containing 4 (*EHD4*) gene exhibited a significant association with right ventral striatum activation during reward anticipation and binge drinking, suggesting the involvement of *EHD4* in alcohol-related reinforcement mechanisms [51].

Several other translational studies within the IMAGEN Consortium identified further genetic contributions to ventral striatal activity. For instance, the gene encoding Ras suppressor 1 (*Rsu1*) modulates ethanol preferences and reduces sensitivity to ethanol-induced sedation in flies [52]. In 14-year-old adolescents (n = 1908), *RSU1* polymorphisms were associated with activation in the ventral striatum (r = 0.020) and increased frequency of lifetime alcohol use (r = 0.020). Moreover, the findings replicated in an independent adult sample, showing an association of *RSU1* with alcohol dependence [52]. In another translational model, Peña-Oliver et al. (2016) identified genetic variants related to impulsive behavior in mice, defined by premature responding in a five-choice serial reaction time task, and evaluated their human homologs with respect to ventral striatal activation and

alcohol use in adolescents. Variation in the kalirin RhoGEF kinase (*KALRN*) gene was associated with increased activation in the right ventral striatum during anticipation of reward (G major allele of the SNP rs6438839) and with increased frequency of binge drinking (A minor allele of SNP rs4634050) in 14-year-old adolescents, suggesting that *KALRN* increases the risk for alcohol abuse [53].

Other studies focused on functional polymorphisms specifically related to dopaminergic signaling. In this regard, Baker et al. [30] analyzed genetic variation of the D1 and D2 dopamine receptor (*DRD1*, *DRD2*) genes and found that variation in the proximally located Ankyrin repeat and kinase domain (*ANKK*) rs11800497 was associated with activity of the lateral orbitofrontal cortex ($\beta = -0.09$). Conversely, *DRD1* rs686 expression modulated medial orbitofrontal cortex activation ($\beta = -0.08$). Moreover, *DRD1* rs686 was indirectly related to alcohol use at age 14 through an interaction between activation of the medial orbitofrontal cortex and the ventral striatum, which in turn predicted alcohol use at age 16 ($\beta = 0.08$). This analysis suggests a molecular pathway, in which *DRD1* rs686 impacts orbitofrontal cortex and ventral striatum signaling and thus renders adolescents susceptible to a rather early onset of alcohol use that may facilitate progression towards excessive alcohol use [30]. Another gene related to dopaminergic neurotransmission and possibly involved in the development of alcohol misuse behavior is the vacuolar protein sorting-associated protein 4a (*VPS4A*). In a GWAS study, the major C allele of SNP rs16958736 of *VPS4A* was linked to decreased striatal activation during reward anticipation ($r = -0.14$, $n = 1403$). On a behavioral level, however, no significant association could be observed for alcohol consumption and activity of the striatal node alone, but only for the link between the striatal node and another node comprising occipital areas V1/V2 [28].

Previous studies also highlighted the role of the brain-derived neurotrophic factor (*BDNF*) for alcohol consumption [54]. Along this line, Nees et al. [29] investigated the impact of genetic variation in *BDNF* that encodes the VAL66MET amino acid substitution (rs6265) on brain activation in different subdivisions of the striatum (putamen, nucleus caudatus, nucleus accumbens) and its predictive value for adolescent alcohol use. Specifically, they compared carriers of Met alleles (Met/Met homozygotes and Val/Met heterozygotes, $n = 167$) with homozygous Val allele carriers (Val/Val, $n = 363$). During reward anticipation, Val/Val genotype carriers exhibited lower activation in all subdivisions of the striatum compared to Met allele carriers. However, decreased activity in the putamen of Met carriers significantly predicted current (age 14) and future levels of alcohol consumption at age 16 (explained variance: 6.8%). This relationship was only apparent for putamen activation and not for activity in other striatal subdivisions [29]. The putamen has been implicated in mechanisms relevant for the transition towards alcohol addiction, such as reinforcement learning and the formation of habitual behaviors [55, 56]. Therefore, genetic variation in *BDNF* VAL66MET might reflect a risk factor for the development of excessive and harmful alcohol use by modulating neural activity that regulates the link between reward anticipation and drug seeking [29, 57].

Genetic Variation, Brain Activation During Response Inhibition and Emotional Processing and Adolescent Alcohol Use

With respect to response inhibition, the β1-containing $GABA_A$ receptor gene (*GABRB1*) has been previously associated with alcohol dependence and substance use disorders [58]. Allelic variation in SNP rs2044081 of *GABRB1* is associated with altered brain responses during regulation of reward-related behaviors such as behavioral inhibition and reward anticipation in 14-year-old adolescents before manifestation of alcohol symptoms, highlighting *GABRB1* as a potential contributor to addictive phenotypes [59]. In another study, the *Arf6* activator *Efa6* was associated with ethanol-induced behaviors like sedation and tolerance development in drosophila, and analysis of the human orthologs of *Arf6* and *Efa6* (*PSD1–4*) in 16-year-old adolescents of the IMAGEN sample found a link between SNP rs13265422 in *PSD3* with increased frequency of binge drinking ($r = 0.06$) and reduced activation in the right inferior frontal cortex during behavioral inhibition ($r = 0.06$) [60]. Thus, *PSD3* possibly affects excessive alcohol drinking behavior by modulating regional activity critical for executive control [60, 61].

In a large GWAS study, Schumann et al. [62] identified an association of the minor A allele of SNP rs197273 of the TRAF family member-associated NF-κB activator (*TANK*) gene with reduced alcohol consumption [62]. Müller et al. [63] set out to determine the functional relevance of *TANK* polymorphism for alcohol drinking in adolescence and found that carriers of the minor A allele of SNP rs197273 showed increased negative affective responses in brain regions associated with interoceptive processing, particularly the insula. Moreover, the minor A allele of rs197273, which results in lower *TANK* expression, was associated with lower alcohol consumption in 14-year-old adolescents. These results also translated to an animal model in which *Tank* KO mice exhibited attenuated ethanol consumption and preference as well as enhanced anxiety-related behaviors relative to wild type controls. Thus, *TANK* polymorphism modulates aversive emotional processing, possibly serving as a protective factor for developing alcohol use behavior [63].

A growing body of evidence also suggests an involvement of the endocannabinoid system (eCB) in addiction and particularly in alcohol use disorder (see [64] for a review). In a recent IMAGEN study [65], two SNPs within the eCB system were significantly associated with increased risk for developing alcohol-related problems (AUDIT score > 7) in adolescents aged 14, 16 and 18 years ($n = 575$), including SNP rs9343525 of the gene coding for the CB1 receptor protein (*CNR1*) and SNP rs507961 of the gene coding for the monoacylglycerol lipase enzyme (*MGLL*). Moreover, an SNP × SNP interaction analysis revealed a robust three-SNP interaction involving rs484061 (*MGLL*), rs7766029 (*CNR1*) and rs4963307 of the gene coding for diaglycerol lipase (*DAGLA*) that predicted AUDIT scores >7 in the same adolescents with 54% accuracy [65]. However, future research needs to investigate the potential role of the identified SNPs for addiction-related brain mechanisms of reward processing, response inhibition and emotional processing.

Epigenetic Mechanisms Affecting Adolescent Alcohol Use

Environmental processes can regulate the functionality and expression of genes without alterations in the DNA sequence, referred to as epigenetic modification [66]. One of the most widely studied forms of epigenetic modification are alterations in DNA methylation. Investigating DNA methylation changes in the brain is a major challenge in psychiatric epigenetic studies due to limited availability of post-mortem brain data. However, recent studies indicated significant overlap between methylation profiles in human brain and blood [67–70]. Therefore, in the IMAGEN cohort, peripheral blood tissue samples were used as surrogates for identifying DNA methylation changes in the brain. So far, four epigenome-wide association studies (EWAS) have been conducted within the IMAGEN Consortium to investigate the link between DNA methylation, environmental factors and alcohol use phenotypes in adolescence.

In the first study, hypermethylation in the locus of the 3′-protein-phosphatase-1G (*PPM1G*) gene has been found to be associated with increased trait impulsiveness and functional activation in the right subthalamic nucleus during response inhibition ($\eta^2_p = 0.013$, $n = 393$) [71], a brain structure responsible for the integration of neural signals also relevant for inhibitory control [72]. Moreover, *PPMG1* hypermethylation was also related to rising daily alcohol consumption levels from early to mid-adolescence ($\eta^2_p = 0.014$, $n = 352$) [71], suggesting that *PPM1G* methylation impacts brain mechanisms necessary for exerting inhibitory control and thus contributes to escalating alcohol use, a risk factor for developing alcohol-related problems later in life [2]. Another EWAS identified the upstream region of the gene that codes for the disks large-associated protein 2 (*DLGAP2*) as a differentially methylated region that has previously been related to the manifestation of alcohol dependence [73]. This finding was supported in 19-year-old adolescents from the IMAGEN sample by showing that decreased methylation levels of *DLGAP2* were significantly associated with increased frequency of drunkenness and reduced functional activation during reward anticipation in widespread regions of the brain, with the largest cluster located in the precuneus. These results suggest that *DLGAP2* hypomethylation is related to both altered reward processing and increased risk for problematic alcohol use in adolescence, and may contribute to the manifestation of alcohol dependence later in life [73].

Adolescence is a key developmental period for brain maturation processes, which render adolescents particularly vulnerable to psychosocial stressors such as relationship problems, peer victimization, family or school problems [74]. Psychosocial stress exposure, in turn, confers vulnerability for problematic alcohol use, e.g., by disrupting reward-related neural circuitry (e.g., [75]). Ruggeri et al. [27] revealed an epigenetic mechanism that helps to elucidate the link between adverse life events, reward processing and alcohol use in adolescence. Specifically, they found that methylation of the nociceptin receptor/opioid receptor like-1 (*NOP*/*OPRL1*) gene mediates the effect of psychosocial stress on binge drinking behavior and neural activity in the ventral striatum during reward anticipation in

14-year-old adolescents (η^2_p = 0.020, n = 393). *OPRL1* hypomethylation may thus result from psychosocial stress exposure and eventually increase reward sensitivity and the propensity of excessive alcohol use [27]. Similarly, increased methylation in the sterile alpha motif/pointed domain containing the ETS transcription factor (*SPDEF*) gene was associated with a greater number of adverse life events (r = 0.082, n = 1287) and with higher levels of lifetime binge drinking (r = 0.099, n = 413), which, in turn, was associated with decreased gray matter volume in the right caudal cuneus [76]. Specifically, among carriers of the minor G-allele of rs2233631, *SPDEF* methylation moderated the association between stressful life events and alcohol abuse, portending to a differential genetic vulnerability in adolescents for developing excessive alcohol use behaviors through environmental stress [76]. However, future research is needed to validate the identified changes in DNA methylation in human brain tissue.

Breaking Down the Relative Impact of Different Predictors for Adolescent Alcohol Abuse

As outlined above, the IMAGEN study has identified a multitude of environmental, behavioral, neurobiological and (epi-)genetic factors implicated in adolescent alcohol use. Understanding the relative contribution of these factors in predicting future alcohol abuse can provide risk profiles, which, in turn, may help to develop targeted interventions that aim at preventing the emergence of alcohol use disorder in individuals at risk.

Predictive models of alcohol use in early adolescence (age 14) found that personality traits explained the largest amount of variance in adolescent alcohol use (16% explained variance), while functional brain activation during reward anticipation and reward-related behavior contributed only marginally with 0.6% and 0.4% explained variance, respectively [77]. Additionally including candidate genetic variants [78] as well as stressful life events, use of other substances and family history of drug use [35] as further predictors yielded comparable prediction performances with 13% and 24% explained variance, respectively. Reward-associated personality traits, including novelty seeking, impulsivity, sensation seeking and extraversion (β = 0.35–0.41) [77, 78] as well as smoking status (β = 0.385) [35] were the most significant predictors for alcohol use in early adolescence. In contrast, the level of future alcohol consumption at age 16 was most strongly influenced by personality factors (β = 0.260) and genetic variation in candidate genes (β = 0.270) [78] as well as alcohol drinking levels at age 14 (β = 0.258) [35]. Importantly, variation in candidate genes was most important for the increase in alcohol use between 14 and 16 years (β = 0.330) [78]. However, these factors have been shown to contribute differentially to the prediction of alcohol use when adolescents are stratified based on family history of substance use ([35], but see also [79]). Together, these results suggest that the initiation of alcohol use in early adolescence

is determined by a different set of predictors, namely stress exposure, reward-related personality traits and concurrent substance use, as compared to the transition to increased levels of alcohol consumption, which is mostly associated with genetic predispositions and previous alcohol use. This finding supports etiological models on substance use in which early patterns of consumption are strongly determined by social and familial environmental factors rather than genetic influences, while later levels of use are more strongly influenced by genetic predispositions, showing that different predictors act dynamically across development [80–82].

Reward-related brain activation, however, was less important for predicting alcohol use as compared to other predictor variables, with standardized regression weights (β) ranging from 0.007 to 0.040 in early adolescence and 0.010 to 0.082 in mid-adolescence [35, 77, 78]. The low predictive value of functional brain activation compared to other factors could be due to the impact of transient, state-dependent components in brain activation [83] while genetic or personality factors reflect more stable trait-like characteristics [84, 85] which might be more useful for predicting pathological psychiatric conditions at an early stage [86]. Moreover, recent studies highlighted deficits in psychometric properties of task fMRI measures, demonstrating low test-retest reliability for a range of widely adopted fMRI tasks comparable to those implemented in IMAGEN that impede their eligibility for biomarker discovery [87, 88].

Using a machine learning approach, Whelan et al. [43] generated multivariate models for the prediction of current ($n = 115$) and future binge drinking ($n = 121$) in 14-year old adolescents of the IMAGEN cohort. Based on more than 40 different variables from different domains, the models correctly predicted 82% of current (age 14) and 66% of future binge drinkers (age 16). While personal life experiences (e.g., romantic history, deviance history, family drug use etc.) were the most important features for the classification of current bingers (β = 0.022–0.244), identifying future binge drinkers primarily relied on personal life events (β = 0.024–0.184), personality factors (β = 0.025–0.086), and characteristics of brain structure and function (β = 0.071–0.225). Regarding brain structure and function, future binge drinkers compared to controls exhibited reduced gray matter volume and increased activation upon reward receipt in the superior frontal gyrus as well as increased gray matter volume and increased activation during failed response inhibition in the precentral gyrus [43]. A study combining resting-state functional connectivity and machine learning expanded these results, showing that changes in functional connectivity in frontal regions significantly contributed to the prediction of binge drinkers [89]. In contrast, candidate genetic variants only contributed to a minor degree to current (β = 0.018–0.065) and future binge drinking (β = 0.028–0.067) [43].

Together, these studies expand our understanding of the relative contribution of different predictors for adolescent alcohol use and reveal important antecedents, which may inform the development of more individualized interventions in the future. Moreover, the studies demonstrated that (1) incorporating multiple features from different domains outperformed the prediction of any single feature and that (2) single predictors relate differently to the initiation of alcohol use than to the

transition to increased alcohol consumption, thereby highlighting the multifactorial nature of harmful alcohol use with distinct factors acting and interacting dynamically across development [80–82].

Conclusion and Outlook

Since its inception in 2010, the IMAGEN Consortium helped to elucidate environmental, neurobiological and genetic factors and mechanisms involved in the formation of reinforcement-related behaviors, symptoms and disorders. From the large body of IMAGEN publications, in this chapter we focused on studies uncovering determinants for alcohol use in adolescence, a key developmental period in which heavy alcohol use can pave the way for transitioning to addiction later in life [2].

Structural differences in widespread cortical and subcortical brain regions as well as increased functional activation of reward-related brain networks and decreased activation in fronto-cortical networks implicated in goal-directed behavior, response inhibition and executive control [44, 90] predicted developmental trajectories of alcohol abuse from early to late adolescence. The IMAGEN study revealed a range of genetic polymorphisms and epigenetic modifications modulating the association between brain structure/function and alcohol use. Research breaking down the relative impact of different predictor classes additionally highlighted the importance of life experiences and environmental factors as well as reward-related personality traits, indicating multiple causal factors for the development of alcohol abuse across adolescence. Together, these findings might facilitate the stratification of individuals based on their individual risk constellation and promote the development of targeted interventions, which could reduce the emergence of AUD later in life. However, as an important limiting remark, it should be noted that effect sizes and proportions of explained variance, though consistent with previous studies in the field of (imaging) genetics [91–93], were relatively small based on conventional thresholds, particularly for brain structural/functional and genetic predictors. Therefore, it is crucial to validate the identified neurobiological mechanisms in specific clinical cohorts and in culture spaces outside Europe. To this end, the IMAGEN Consortium has aligned itself with other neuroimaging-genetics studies across the world [94] to further expand our understanding of determinants contributing to the development of alcohol abuse.

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Chapter 29
Impulsivity and Alcohol Use Disorder

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Abstract Alcohol use disorder (AUD), along with other substance use disorders, can be understood at least partially as disorders of impulsivity, or a persistent deficit in self-regulation in which the individual is increasingly unable to control arising impulses to consume the substance. In turn, understanding impulsivity in relation to AUD can inform our understanding of its aetiology and potentially identify novel treatment targets. A challenge to this ostensibly straightforward perspective is the many ways impulsivity can be measured and the highly variable associations among these measures, revealing a highly multidimensional construct. Recent investigations have begun to delineate the latent structure of impulsivity, and, in this chapter, we review how a conceptual model of three broad domains relates to AUD. These comprise impulsive personality traits (i.e., self-attributions on personality inventories), impulsive choice (i.e., overvaluation of immediate rewards), and impulsive action (i.e., behavioural inhibition). In each case, we review the state of the evidence in relation to AUD, followed by a discussion of these domains as potentially modifiable risk factors. Although intervention research is relatively nascent, clinical interventions to improve self-regulatory capacities are promising and have potential in the context of a precision medicine approach.

Keywords Alcohol · Alcohol use disorder · Addiction · Substance use disorders · Impulsivity · Self-regulation · Personality · Delay discounting · Behavioral inhibition

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Introduction

Despite clinically significant harm, distress, and negative consequences, why do individuals with alcohol use disorder (AUD) keep drinking? This is arguably the central question in alcohol addiction research. One answer is that AUD, along with other substance use disorders (SUDs), can be understood as self-regulatory disorders, in which a person gradually loses their capacity to regulate arising impulses to drink [1]. From this perspective, impulsivity is a cardinal feature of AUD and other health conditions associated with excessive consumption. These include not only other SUDs but behavioral addictions, like gambling or gaming, risky sexual behavior, and even obesity, which typically results from excess food consumption. Accounts of addiction that emphasize self-regulatory capacity over impulses differ from those that emphasize the development of overlearned habitual behavior [2, 3], and are more consistent with theories emphasizing maladaptive goal directed behaviour, which arguably map better to the clinical manifestations of addiction [4]. Given its potential importance, understanding the role of impulsivity in AUD and factors that influence the ability to self-regulate this behaviour may afford aetiological insights into the development and maintenance of the disorder.

Despite this ostensibly straightforward approach, however, research on impulsivity in AUD is made substantially more difficult by foundational questions about the nature and definition of the construct. Impulsivity is often broadly described as a tendency towards rash actions, but despite this seemingly simple definition, numerous measures of impulsivity and related constructs exist and the relations among these measures are highly heterogeneous. Rather than a singular psychological trait, it is increasingly clear that impulsivity is a superordinate construct, one that is multidimensional in nature, comprising multiple operational definitions that are conceptually related to each other but often quantitatively distinct and with differing relations to AUD. Only recently have investigations more clearly delineated latent structural models of impulsivity, quantitatively revealing the multidimensionality of the construct [5–8].

In the context of these definitional and measurement questions, the first goal of the current chapter is to review and evaluate the different definitions and measures that exist and their relation to AUD. This perspective considers impulsivity as an aetiological and maintaining factor in the disorder. The second goal of the chapter is to evaluate the clinical relevance of impulsivity and the extent to which facets of impulsivity may be viable treatment targets using the lens of experimental medicine. The experimental medicine framework [9] proposes the development of treatments that are specifically directed at targeting constructs closely associated to underlying aetiological mechanisms of the disorder. It is similar to a precision medicine approach insofar as it emphasizes careful assessment of specific features of an individual and the development of treatments that address those specific features. Collectively, the purpose of the chapter is to offer the reader a deeper understanding of what impulsivity is, how it relates to AUD, and the extent to which facets of impulsivity may be modifiable risk factors for treatment and prevention.

Impulsivity as a Multidimensional Construct

Early conceptualizations of impulsivity in personality psychology considered it to be a unitary trait (e.g., the Impulsivity subscale of the Eysenck Impulsivity Questionnaire [10]), generally capturing a tendency to respond rashly without foresight. Subsequently, behavioral paradigms measuring self-regulation emerged, moving away from self-attributions and operationalizing self-control based on emitted behavioral outputs, either as choices on decision making tasks or performance in motor tasks. Consequently, the concept became increasingly broad and, based on highly variable relations among measures, understood to be multifaceted [5, 6, 11]. Thus, the term impulsivity can be thought of as a superordinate construct, or a family of constructs that are conceptually related to each in natural language but independent, not different measurements of a common underlying process.

Importantly, the heterogeneity of relations among impulsivity measures is not such that every measure reflects an orthogonal process. Rather, a number of studies suggest that there is a latent structure among the various measures. In other words, there is a middle ground between all measures reflecting the same construct and all being independent, with certain ones overlapping with each other. In a metaphorical extended family of impulsivity measures, some constructs are more closely related than others. One approach to the latent structure of impulsivity is a tripartite model that consists of Impulsive Personality Traits (i.e., self-reported attributions of ability to self-regulate), typically measured using personality questionnaires; Impulsive Choice (i.e., preference for smaller sooner rewards over larger later rewards, often referred to as delay discounting), typically measured using delay discounting tasks; and Impulsive Action, also referred to as "Impulsive Waiting" (i.e., the capacity to inhibit a prepotent motor response), typically measured using Go/NoGo or Stop Signal tasks [6] (Fig. 29.1). While this is not the only proposed latent model of impulsivity [12], it provides a heuristic conceptual framework for understanding the various facets for considering the varying relations to AUD.

Impulsive Personality Traits and AUD

A number of self-report measures purport to measure impulsivity [13, 14]. Among the most common are the UPPS-P Impulsive Behaviour Scale (UPPS; [15], and the Barratt Impulsiveness Scale 11 (BIS-11) [16]. The UPPS includes five subscales representing five traits originally identified via the Five-Factor Model of personality [17]. The traits are Sensation Seeking, Lack of Perseverance, Lack of Premeditation, Negative Urgency, and Positive Urgency. Sensation Seeking refers to the tendency to seek out novel and thrilling experiences; Lack of Perseverance refers to the tendency to have difficulty completing task; Lack of Premeditation refers to a tendency to act rashly without forethought about the consequences of an action; and Positive and Negative Urgency refer to the tendency to act out under conditions of strong

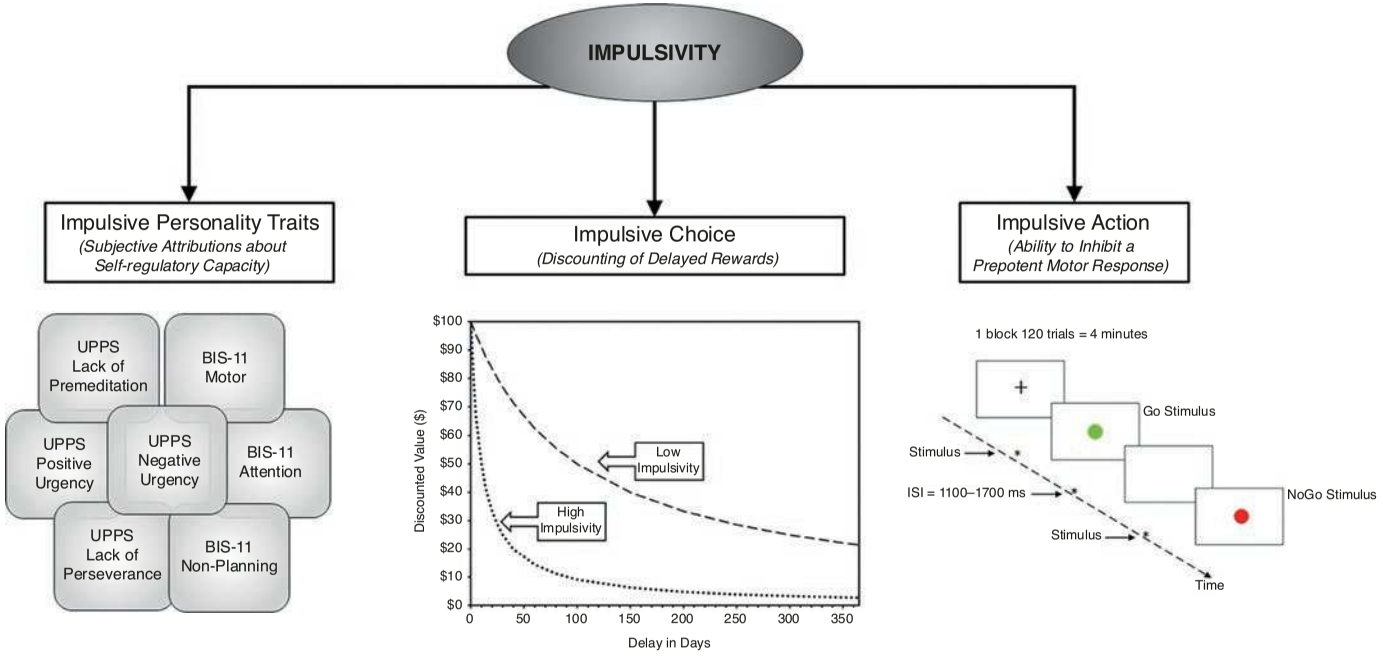

Fig. 29.1 A tripartite model of impulsivity. Impulsive personality traits reflect reports from self-report questionnaires; impulsive choice reflects overvaluation of immediate rewards; and impulsive action reflects ability to suppress an arising prepotent response

positive or negative affect, respectively [15, 18]. The BIS-11 parses impulsive personality differently. It includes six lower-order factors (Attention, Cognitive Instability, Motor, Perseverance, Self-Control, and Cognitive Complexity) that are grouped into three higher-order factors. The first higher order factor is Attentional Impulsivity, which is the ability to focus on the task at hand without racing thoughts and consists of the lower order factors Attention and Cognitive Instability. The second higher-order factor is Motor Impulsivity, which is the tendency to act on the spur of the moment. It consists of the lower order factors Motor and Perseverance. Finally, the third higher-order factor is Non-Planning, which is lack of forethought regarding the future. It consists of the lower order factors Self-control and Cognitive Complexity [16]. Higher order factors on the BIS-11 tend to have more robust psychometric properties than the lower order factors because of the larger number of items. Research using the BIS-11 may also use a total score, but that is not the case for the UPPS.

Cross-sectional research implicates various impulsive personality traits in relation to alcohol use and misuse. Research using the BIS-11 has generally found positive associations between AUD status, severity of alcohol use problems, and BIS score (total and higher-order factors) [19–21]. For example, in a sample of young adults, BIS total score predicted total number of drinks, drinking days, and days intoxicated [20]. Cross-sectional research using UPPS subscales have primarily implicated both measures of urgency as strong predictors of alcohol use problems and AUD, while Sensation Seeking tended to predict drinking frequency [13, 22–24]. Another study found that Sensation Seeking was related to heavy drinking at least partially via younger age of initiation [25]. Two meta-analyses have also converged in finding that negative drinking outcomes (i.e., drinking problems, alcohol dependency, and problematic use) were most strongly and consistently related to urgency traits, with the most severe outcomes (AUD) consistently and strongly related to Negative Urgency, specifically [26, 27]. In other words, those who struggle most with coping with strong negative emotions were most likely to have an AUD. This provides an appealing candidate mechanism for the well-known ability for stress to promote relapse [28]. Regarding level of alcohol consumption, one meta-analysis found positive relation for all UPPS traits, but particularly strong relations with Sensation Seeking and Positive Urgency [26], whereas the other found that quantity was most strongly related to Lack of Perseverance and frequency was equally predicted by all traits [27].

Beyond cross-sectional studies, longitudinal evidence supports bidirectional relationships between impulsive personality traits and alcohol use [29, 30]. For example, in a sample of college students, Positive Urgency predicted higher levels of alcohol use 1 year later, and baseline alcohol use predicted higher levels of Urgency (positive and negative), Sensation Seeking, and Lack of Premeditation a year later [29]. Another longitudinal study following college students from high school to college graduation found evidence for transactional relations between heavy drinking and impulsivity (here, considered unitary) and Sensation Seeking. Sensation Seeking and impulsivity at time 1 predicted increases in drinking across the first 2 years of college, while heavy drinking predicted individual differences in

personality change [30]. In addition to comparatively few longitudinal studies, another potential limitation that has not been addressed is that even though the UPPS or BIS-11 do not ask about alcohol, the statements could be endorsed by participants *specifically* because of behavior in the context of alcohol. For example, the 'acting out' when feeling bad could reflect drinking when feeling bad from the perspective of a participant. This link would make associations potentially spurious.

In sum, research has suggested that traits of impulsivity are differentially related to alcohol use and problematic use. Varying traits seem to be related to consumption variables (i.e., frequency and quantity), with evidence suggesting that Sensation Seeking may be especially important and may also be related to age of alcohol use initiation. With regard to alcohol problems and AUD, the Urgency traits (especially Negative Urgency) have been implicated in both alcohol use and problematic alcohol use. Together, this suggests that impulsive personality traits may differentially influence drinking, whereby drinking quantity and frequency are influenced via thrill or excitement (Sensation Seeking), while drinking problems are associated with emotional regulation, including managing both positive or negative affect (Urgency). Bidirectional relations suggest that impulsive personality traits forecast alcohol use and problems, but also that, conversely, alcohol misuse appears to also give rise to self-regulatory deficits. Although the large majority of studies are cross-sectional and cannot speak to causality, there is clear evidence for an association between impulsive personality traits and multiple features of drinking and AUD.

Impulsive Choice: Delay Discounting

The second facet of impulsivity—impulsive choice—is the preference for smaller immediate rewards over larger, delayed rewards is often referred to as delay discounting [6], that is, how much a reward is *discounted* based on its *delay* in time. Delay discounting in general refers to the behavioural economic principle that rewards progressively lose value based on their temporal delay, and it is conceptually similar to the capacity to delay gratification (i.e., *in vivo* ability to wait for a reward while in its presence). Among the most common measures are the Delay Discounting Task, Monetary Choice Questionnaire, and the Effective Delay-50 [31, 32]. Common to all instruments is the requirement for participants to choose between smaller-sooner rewards or larger-later rewards at varying values and temporal lengths (e.g., $1 now or 10 tomorrow, $20 now or $100 at the end of the week). In extended delay discounting tasks, the point at which a participant equates the smaller-sooner and larger-later rewards, the point of indifference, can be used to generate an empirical discounting curve that can be modelled using nonlinear regression to determine a person's temporal discounting function [33]. Steeper discounting curves represent a higher preference for smaller-sooner rewards, while shallower curves represent a more balanced preference between larger-later and smaller-sooner reward (more value allotted to larger-later reward). Brief tasks permit inferring the temporal discounting function.

Research has consistently demonstrated associations between high delay discounting and alcohol-related indicators, including alcohol use, AUD, and AUD severity [34–43]. In each case, elevations in drinking-related variables (AUD+ samples included) are associated with being likely to choose smaller-sooner rewards over larger-later ones. Studies of subclinical drinkers have found that those who drink more heavily or more frequently exhibit greater discounting [36–38]. For example, one study found that in a sample of adolescent drinkers, the heavier drinkers exhibited steeper discounting than lighter drinkers [36]. Other studies have compared AUD samples to controls, revealing that those with AUD displayed steeper discounting compared to controls [34, 35, 42, 43]. For example, one study examined delay discounting differences among an actively drinking AUD sample, abstinent AUD sample, and control sample. They found that the actively drinking AUD sample discounted future reward most rapidly, followed by the abstinent AUD sample, and finally, the healthy controls, who demonstrated the least discounting [34]. In addition to individual studies, meta-analyses provide further support for steeper discounting in alcohol-related samples. One meta-analysis examined categorical differences (e.g., differences between AUD and controls, or heavy drinkers and light drinkers). That study found a medium effect size (d = .68) in clinical samples and a notably smaller effect size (d = .29) in studies using subclinical samples [44]. Another meta-analysis examined continuous associations between delay discounting and drinking variables (e.g., quantity, frequency, and AUD severity), finding a highly significant, albeit small magnitude association overall, and a larger effect size for AUD severity measures compared to quantity or frequency measures [45]. Notably, both meta-analyses reported minimal evidence of potential publication bias. A small number of studies suggest that excessive delay discounting predates substance use and may be a vulnerability marker [46]. Longitudinal studies suggest that high discounting at one timepoint predicts increases in drinking at later timepoints [47, 48], providing further support for delay discounting as a vulnerability marker. For example, one longitudinal study of high schoolers found that initial delay discounting predicted alcohol involvement 6 months later, but that heavy drinking was not associated with any subsequent changes in delay discounting [47].

Studies have generally converged to suggest steeper discounting of future rewards in AUD or heavy drinking samples, but do these findings generalize to real-world encounters? Discounting relevant to AUD decision-making is cross-commodity in nature: it requires the individual to discount future rewards of one commodity type (e.g., monetary, relational, health, occupational) for immediate reward of another commodity (alcohol). Research has examined delay discounting in non-monetary commodities, and found that although AUD/heavy drinking samples still exhibit steeper discounting than healthy controls in these designs, consumable commodities (e.g., drug and food related rewards) are more steeply discounted compared to monetary rewards across the board, even among healthy controls [40, 49]. However, these paradigms remain single commodity in nature (e.g., alcohol now vs. alcohol later).

Comparatively less work has examined cross-commodity discounting (money-now/alcohol-later and vice versa). Indeed, a review on addiction related

cross-commodity delay discounting found that only three studies included all four possible combinations of discounting (money-now/money-later; other-now/other-later; money-now/other-later; other-now/money-later). The review found that contrary to expectations, the drug-now/money-later condition was not the most steeply discounted condition [50]. One study examined cross-commodity discounting in a crowd-sourced sample of drinkers. They found that those with the highest scores on a measure of AUD had significantly steeper discounting in the alcohol-now/money-later condition compared to those with lower scores [51]. It remains important to note that the existing literature on cross commodity discounting is relatively nascent.

Further, there are many more permutations of discounting paradigms that map onto the decision-making situations relevant to those with an AUD. For example, in contrast to delayed *reward* discounting, significantly fewer studies have investigated delayed discounting of *aversive outcomes* (e.g., choosing between a smaller-sooner and larger-later loss). Some studies have been conducted in rat models [52, 53], while others have looked at hypothetical aversive outcomes in human studies [54, 55]. Current conclusions are limited to the fact that the hyperbolic discounting processes remains consistent across reward/punishment paradigms, suggesting that discounting tasks can be used to analyse discounting of aversive outcomes. To our knowledge, discounting of losses/aversive outcomes remains to be studied in populations with greater levels of choice impulsivity, such as AUD and SUD populations.

Another important consideration from a developmental perspective is that in certain environments, a preference for smaller, immediate reward can actually be an adaptive, skillful response. In unstable or otherwise chaotic environments, resources and rewards may be scarce or unpredictable and there may be little to no value of delayed gratification for future rewards. Instead, the adaptive response is to focus on surviving the here-and-now (e.g., surviving living on the street). A developmental approach would hypothesize that these environmental contexts also help shape delay discounting/substance use. Indeed, studies exhibit associations between variables such as childhood socioeconomic hardship, childhood adversity, abuse/neglect, and increased delay discounting [56–58]. Specifically, some studies have found indirect links between adverse childhood neglect/abuse and substance use via delayed discounting [57, 58].

An emerging literature suggests that delay discounting may be a mechanism by which genetic variation confers risk for addictive behavior. For example, there is evidence that delay discounting is at least moderately heritable: twin studies conducted in rodents and humans suggest that heritability rates of delay discounting is around 30–50% [59]. Studies have also suggested that the heritability of delay discounting increases over time, with one set of studies finding heritability rates of 30% in 12-year-olds, steadily increasing to 51–62% by age 18 [60, 61]. Similarly, steeper delay discounting has been found to be associated with family history of substance use, including prior to any onset of use [62–64]. Although initially promising candidate gene studies have not yielded robust findings over time, larger scale genome-wide association study (GWAS) samples have identified novel variants and revealed significant aggregate molecular genetic heritability [65, 66]. On balance,

the evidence suggests that delay discounting is heritable and may inform understanding the mechanisms of genetic influences on addiction, although the specific variants and pathways remain unclear.

A further nuance is that some evidence suggests elevated delay discounting may not be unique to AUD or even SUDs, but rather a psychological mechanism implicated in externalizing/disinhibitory disorders more broadly, as well as other psychiatric disorders and other health outcomes [67–69]. Indeed, some research suggests that delay discounting may be a transdiagnostic process across numerous psychiatric disorders [70, 71]. Furthermore, in addition to undervaluing delayed rewards, it appears that excessive orientation toward the future can be maladaptive also, reflecting excessive overcontrol. In particular, an interesting line of inquiry has found that excessively shallow discounting (i.e., excessive preference for future reward) is present in individuals with disorders that are associated with excessive self-control, such as anorexia nervosa and obsessive-compulsive personality disorder [72–74].

In sum, the construct of impulsive choice can be operationalized via delay discounting tasks and provides one specific vantage point for understanding impaired self-regulation in AUD (immediate pull to drink at the cost of long-term outcomes). Alcohol studies consistently reveal that heavier-drinking samples and samples with AUD exhibit more impulsive discounting than comparison group. More broadly, there is evidence that maladaptive delay discounting is observed across addictions more generally, along with other externalizing disorders and disorders of excessive self-control. Heritability studies suggest that delay discounting is moderately influenced by genetic variation, but developmental and environmental factors are also robustly implicated, making it a biobehavioral process that is jointly determined by 'nature' and 'nurture.' Limitations to the existing literature are that it primarily comprises cross-sectional associations, with longitudinal and experimental studies needed to determine causation more definitively, and more research is needed on related processes, such as cross-commodity discounting, to provide more ecologically valid conclusions.

Impulsive Action

The second behavioural measure of impulsivity is impulsive action (behavioural/response inhibition, waiting impulsivity), an individual's ability or inability to inhibit a prepotent motor response [75]. Behavioural inhibition is conceptually linked to all behaviours that involve self-regulation, including alcohol and other substance use [76]. Behavioural inhibition is generally measured via laboratory tasks such as the Stop Signal Task, Go-NoGo Task, or the Continuous Performance Task [77–79]. Generally, these measures require participants to quickly respond by pressing a button upon the presentation of certain stimuli ("go" stimulus) but not other stimuli ("stop" stimulus) [75]. Response patterns can either generate an accurate response, or two types of errors: errors of commission (pressing during "stop" stimulus; indicative of impulsivity), or errors of omission (failure to respond to the

"go" stimulus; error of inattention). In general, a number of studies have found that poorer responding on response inhibition tasks (more errors, longer response time) is associated with SUDs and other areas of psychopathology [80, 81].

The empirical literature on alcohol suggests that worse performance on response inhibition tasks is related to increased alcohol consumption, alcohol-related problems, and AUD severity [20, 82–85]. One 4-year longitudinal study found that heavy drinkers performed worse on all behavioural impulsivity measures, and that specifically for response inhibition, difficulty with the stop-signal task was associated with an increased risk for alcohol dependence 4 years later [85]. Some research also suggests that there may be familial links to response inhibition. A cross-sectional study examined adults without a history of alcohol or other substance use disorder, either with or without a family history of AUD. They found that those with a family history had more errors of commission compared to those without a family history of AUD, suggesting that poorer response inhibition is likely associated with family history of AUD [86].

Meta-analyses provide further support for response inhibition impairments in those with AUD and heavy drinking samples [80, 81]. One recent meta-analysis examined behavioural inhibition in SUDs ($k = 97$). With regards to alcohol use, they found that those with alcohol dependence exhibited poorer task performance on the Go-NoGo task (significantly more errors of omission and commission; $g = .35$ and .43, respectively), and exhibited longer stop reaction time on the Stop Signal Task ($g = .40$) compared to controls. They also found that heavy drinkers had more errors of omission (inattention) on the Go-NoGo task when the NoGo condition was rare and poorer stop signal reaction time (gs = .48 and .25, respectively) [81].

Longitudinal studies with adolescents and young adults suggest that poor response inhibition may be a risk factor for later AUD or alcohol related problems [76]. For example, one study of 498 children from 275 families found that poorer response inhibition (longer reaction time) was a significant predictor for the number of alcohol-related problems and illicit drug use, while other measures of executive function were not [76]. Aside from examining task performance, a number of longitudinal neurocognitive studies combine both measures of response inhibition and fMRI methods and have found that blunted activation in prefrontal cortical regions of the brain during response inhibition tasks predicted negative outcomes such as problem substance use, heavy drinking 4 years later, and alcohol dependence symptoms 18 months later [87–90]. Other studies have found greater activation in the cerebellum during failed response inhibition (errors of commission) in those with an AUD family history [91, 92]. Together, results suggest that those at risk for AUD or hazardous drinking also show abnormalities in brain functioning while completing these tasks.

Some studies have examined how inhibition is impacted by acute alcohol consumption. In these studies, participants are administered either ethanol or saline, and subsequently complete behavioural inhibition tasks. Generally, results suggest that alcohol administration impairs behavioural inhibition [93]. For example, one study found that moderate amounts of ethanol in healthy participants resulted in decreased stop reaction times, indicative of impaired behavioural inhibition [94].

Other studies have made efforts to increase ecological validity by including alcohol-relevant cues in the measures or exploring how variables such as craving influence response inhibition [95, 96]. One comparative study of individuals with AUD and healthy controls found that the AUD sample made significantly more errors (both commission and omission), and that errors of commission (impulsivity) were increased when AUD participants had to detect alcohol-related stimuli [96]. Another study found that in a sample of inpatient alcohol-dependent participants, response inhibition was associated with absolute craving in a bar-restaurant setting, whereby those who performed poorer on the task experienced higher levels of craving when exposed to alcohol cues. They also found that trait impulsiveness (measured via BIS-11) was associated with absolute craving and craving increases [95].

In summary, cross-sectional, longitudinal, laboratory, and meta-analytic studies suggest that poor behavioural inhibition is related to AUD. Additionally, some research suggests that there may be familial links in response inhibition in those with a family history of AUD. Another branch of research suggests that behavioural inhibition is further weakened by alcohol intoxication and alcohol-related cues, and that those who perform more poorly on response inhibition tasks also report higher levels of craving in alcohol-relevant situations.

Impulsivity as a Modifiable Intervention Target

Given the evidence that higher levels of impulsivity—regardless of how it is measured—is related to AUD, the next logical question is whether there are treatment implications. Indeed, higher levels of impulsivity facets predict relapse and treatment drop-out [97–100]. These findings suggest that measures of impulsivity may be viable tools to identify those at the highest risk for drop-out or generally poorer treatment outcomes. They also implicate impulsivity as a candidate treatment target: addressing impulsivity in treatment might ameliorate or bolster treatment outcomes. The final section of this chapter will review treatments related to the three facets of impulsivity, specifically, psychosocial interventions and prevention programs for impulsive personality traits, episodic future thinking for delay discounting, and behavioural inhibition training for impulsive action.

Psychosocial Treatments and Impulsive Personality Traits

While there are no current interventions that specifically target impulsive personality traits, some research employing the UPPS model suggests that some traits are modified throughout the course of SUD treatment [101–103]. For example, one study examined impacts of a trauma-informed yoga intervention to treatment-as-usual in a sample of women with SUDs. Results revealed that both conditions experienced improvements in Negative Urgency, and that only those in the yoga condition

exhibited improvement in Premeditation [101]. One review on UPPS traits in psychological treatment for SUDs found limited data on how UPPS factors change during various SUD treatments. The majority of studies examined Negative Urgency, which reduced following a number of treatment modalities (e.g., Dialectical Behaviour Therapy, 12-step groups, Cognitive Behavioural Therapy, Motivational Interviewing). Fewer studies examined changes in Premeditation, and they found no available empirical evidence for changes in Positive Urgency, Sensation Seeking, or Perseverance [102]. One meta-analysis examined the relations between UPPS traits and psychotherapy outcomes for substance use, and found promising evidence of significant decreases in Sensation Seeking and Negative Urgency, although effect sizes were small (gs = −.10 and −.25, respectively). Importantly, these effect sizes were based on a small number of studies (k = 4–8) [103] and were generally within-subjects changes, rather than evidence that the treatment selectively reduced impulsive personality traits. In other words, it is also possible that these traits generally attenuate following reduction of active substance use.

Related, a small number of studies have examined the use of Dialectical Behaviour Therapy (DBT) skills training. DBT skills training focuses on, among other things, emotional regulation with obvious relevance to the urgency measures. Studies have found preliminary support for its use with SUD populations [104]. One systematic review found that studies varied in their implementation and adaptation of the skills, but that generally, DBT skills training was an acceptable and feasible treatment for SUD populations. Further, they found preliminary support for emotion regulation enhancement and substance use reduction. Research has also examined the effectiveness of a prevention program specifically targeting impulsivity (here, defined as a unitary construct), and sensation seeking. One cluster randomized control trial found that high risk students (high scores on impulsivity and sensation seeking measures) who received a personality-tailored intervention exhibited long term effects on drinking rates, binge drinking rates, and problem drinking. They also exhibited benefits in drinking quantity and drinking frequency [105].

At this point, while the research on interventions for impulsive personality is at an early stage, the existing evidence does support applications in prevention and that certain impulsive personality traits (specifically, negative urgency and premeditation) are modified throughout the course of various psychosocial treatments. Further, DBT skills may be relevant in their capacity to bolster emotion regulation, and some preliminary evidence suggests that DBT skills may be helpful for those with SUDs, but direct links have not been made to positive and negative urgency.

Interventions for Delay Discounting: Episodic Future Thinking and Pharmacological Interventions

Given the evidence that AUD is associated with an increased propensity to discount delayed rewards, it is intriguing that some research suggests prospective memory (remembering to do something in the future) is negatively correlated with drinking

severity and dependence [106, 107]. Episodic future thinking, or the ability to self-project and pre-experience a future event, is a skill that can be trained to widen the temporal window that those with AUD consider while making inter-temporal decisions [108]. Some studies have found that episodic future thinking training reduced delay discounting in cigarette smokers [109–111], with one study finding that it also reduced cigarette self-administration [109].

To date, four studies have examined effects of episodic future thinking on delay discounting in drinking and AUD samples, and generally converge to find that episodic future thinking reduces delay discounting. One study examined relations between episodic future thinking, delay discounting, and alcohol decision-making in a samples of college students. During a delay discounting task, participants either imagined events they were looking forward to in the future (episodic future thinking condition) or completed a control imagery task. When participants imagined the personally relevant future event, they performed less impulsively on the delay discounting task relative to the control condition. Further, in the episodic future thinking condition, participants reported less intensity of alcohol demand (reporting they would drink fewer drinks at zero cost) [112]. Another study of participants with AUD found similar results, in which episodic future thinking reduced delay discounting and intensity of alcohol demand [113]. The effects appear to be cumulative, as a study of episodic future thinking in a sample of participants with AUD found reduced delay discounting rates that the effect grew with repeated administrations [114]. Finally, in a sample of treatment-seeking adults in an inpatient alcohol program setting, episodic future thinking reduced delay discounting and generalized to other alcohol-related decision-making measures [115].

A related line of research has investigated whether stimulant medications commonly used to treat attention-deficit-hyperactivity-disorder (ADHD) also improve delay discounting, both because ADHD is often comorbid with AUD [116], and it is also associated with elevated delay discounting [68]. Studies using preclinical rodent models have suggested that D-amphetamine may reduce delay discounting [117–119]. Atomoxetine has also been the focus of some preclinical studies, with results generally finding that the drug reduces delay discounting [120, 121], although discrepancies exist [122]. Finally, other research has revealed that methylphenidate reduced delay discounting in rats, Rhesus monkeys, and in children with ADHD [123–125]. Related, one prospective study actually revealed that youth with ADHD who were prescribed methylphenidate had a lower risk for alcohol and drug use [126], and another study found that methylphenidate did not increase ethanol consumption in rats with ADHD-like qualities [127]. However, delay discounting was not evaluated as a possible mechanism in either case. On the other hand, as most ADHD medications are dopamine agonists, it remains important to consider the possibility that these compounds may precipitate substance use in someone who is in early or sustained remission, akin to the risks of prescribing opioids during a hospital stay to person with a history of opioid use disorder. Indeed, the ability of pharmacologically alike compounds to revive drug-seeking is referred to the reinstatement model of drug relapse [128] and is a widely used preclinical paradigm.

In sum, delay discounting appears to be malleable via both psychological and pharmacological interventions. Episodic future thinking trains participants in the

ability to prospectively consider the future when making decisions in the present. While findings that reveal episodic future thinking reduces delay discounting and substance consumption are promising, it remains important to consider that existing studies examining alcohol related samples are small in number (only four studies to date) and small in sample size (*n*s = 28–50). Furthermore, the evidence that reduced impulsive delay discounting generalizes to substantive reductions in drinking is limited. Acknowledging these considerations, episodic future thinking as a potential intervention for AUD is nonetheless a promising line of inquiry. Regarding pharmacotherapies, there is preclinical evidence that stimulant medications, such as methylphenidate, d-amphetamine, and atomoxetine reduce delay discounting. However, these findings have not been translated into clinical populations and a degree of caution is warranted in doing so because of possible reinstatement effects.

Inhibitory Control Training for Behavioural Inhibition

Numerous studies have examined effects of inhibitory control training on alcohol consumption. In these studies, participants complete modified versions of Stop-Signal or Go-NoGo tasks in which participants learn to inhibit their responses upon presentation of alcohol related cues. Individual laboratory studies have generally found evidence for reduced alcohol consumption immediately following the tasks, but not reductions in implicit alcohol value or general inhibitory control [129–131]. Two meta-analyses also found evidence for appetitive and health behaviour change (including alcohol consumption) in laboratory studies in the short-term [132, 133]. Unfortunately, randomized controlled trials (RCTs) suggests that these interventions are not generalizable outside of the laboratory, and that they do not result in longer-term change in alcohol consumption [134, 135]. For example, an RCT of heavy drinkers compared the effect of two sessions of inhibitory control training to two sessions of an active control condition [134]. For both conditions, one session took place in a laboratory, the other in a naturalistic bar setting. Results demonstrated no differences in alcohol consumption in either setting compared to the control group, and that there were no effects of inhibitory control training on inhibitory control processes more generally, or on alcohol value [134]. Another RCT examined the effect of 4-week, 14-session internet-based inhibitory control training treatment compared to an active control [135]. The participants of this study were 246 heavy drinkers that were motivated to reduce their alcohol consumption. The study did find significant reductions in alcohol consumption, but these effects were non-specific to the inhibitory control training groups, as similar levels of alcohol consumption reductions were also observed in the control condition, suggesting that the training did not help heavy drinkers reduce their alcohol consumption beyond a placebo effect [135]. Together, evidence suggests that while inhibitory control training may reduce alcohol consumption in the short term in laboratory-based studies, these effects do not appear to extend beyond the laboratory and do not appear to result in any long-term alcohol consumption changes. These findings are reminiscent of facilitative intervention training treatments for ADHD, where treatments

focused on repeated training of specific tasks, with the prediction that skills would generalize with repetition, but little evidence that was the case [136]. This is an instance in which the impulsivity marker is clearly implicated in AUD but may not be itself amenable to direct interventions, at least with the strategies employed to date.

Final Conclusions and Summary

Understanding the nature of the inability of an individual with AUD to self-regulate arising impulses to drink is important to both understand the disorder and potentially to develop the next generation of behavioral treatments to address these self-regulatory deficits. At the heart of this issue, impulsivity is a multidimensional construct comprising impulsive personality traits, impulsive choice (delay discounting), and impulsive action (behavioural inhibition). Studies examining each of these domains have repeatedly shown them to be associated with drinking behaviour and AUD. Furthermore, some longitudinal evidence suggests that these self-regulatory deficits predict alcohol misuse, suggesting they are more aetiological than consequences of substance use. Indeed, some evidence suggests they may be bidirectional, with continued alcohol use further exacerbating pre-existing deficits.

Early-stage intervention research reveals that at least some existing interventions may be helpful in improving various facets of impulsivity. For example, arguably the most promising candidate is EFT for delay discounting, where participants focus on salient future events to scaffold out their temporal window of consideration, ameliorating delay discounting in the process. Additionally, some more limited research suggests that Urgency and Premeditation are improved throughout the course of psychosocial treatments for SUDs. Unfortunately, research examining response inhibition training in AUD has shown that gains do not translate past the laboratory setting. More broadly, if efficacious intervention modules can be developed to address discrete forms of impulsivity, precision medicine applications are clear. Carefully characterizing an individual's profile in these different domains would provide a blueprint for specifically matching the patient to the module and putatively to the greatest clinical benefit. Although significant gaps in knowledge remain, investigating the various forms of impulsivity in the context of AUD has clear promise, both for understanding the causes of AUD and addressing those causes as part of evidence-based treatment.

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Chapter 30
Brain-Immune Mechanisms in Alcohol Use Disorder Targeting Neuroimmune Signaling in Alcohol Use Disorder: Opportunities for Translation

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Abstract A growing body of literature implicates the neuroimmune system in the development and maintenance of alcohol use disorder (AUD). This chapter broadly covers the field's progress in characterizing brain-immune mechanisms in AUD from basic to clinical research. We provide an overview of the neuroimmune hypothesis of addiction, cover proposed mechanisms through which alcohol is hypothesized to alter the immune system and increase neuroinflammation, and review evidence from preclinical and clinical studies that demonstrate the relationship between the immune system and AUD.

Given preclinical and clinical evidence to date, there is high enthusiasm for the development of AUD treatment options that target peripheral and neural immune pathways and restore healthy levels of proinflammatory signaling as a way to mitigate drinking behaviors and promote recovery. We review relevant pharmacotherapies and psychotherapies that have been shown to affect the immune system and highlight those that demonstrate particular promise as novel treatments for AUD.

Finally, we conclude by providing recommendations for further development of immune treatments for AUD. We emphasize the importance of target-specific screening methods, such as neurocognition, stress-reactivity, and gut microbiota.

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Further, we suggest that future work should investigate proinflammatory biomarkers, such as C-reactive protein and plasma cytokine levels, as precision medicine approaches that may help identify individuals with AUD who benefit most from immune treatment.

Keywords Alcohol use disorder · Inflammation · Immune system · Neuroinflammation · Heavy alcohol use · Addiction · Medications development · Neuroimmune modulator · Immune therapy

Introduction

The Immune System

Increasing evidence suggests that the immune and neuroimmune systems play a critical role in the development and maintenance of alcohol use disorder (AUD) [1]. The immune system, which is essential for humans' well-being, is comprised of innate and adaptive immune mechanisms and serves as the body's primary defense against pathogens [2]. Upon activation of the innate immune system, proinflammatory responses are prompted by detection of conserved features of microbes, termed pathogen-associated molecular patterns (PAMPs), such as the surface membraned component of most Gram-negative bacteria and endotoxin, lipopolysaccharide (LPS) [3]. Among individuals with AUD, LPS levels are elevated, but may normalize after a period of abstinence [4, 5]. Toll-like receptors (TLRs) are a common family of receptors found on immune cells and can recognize PAMPs and consequently activate transcription factors, including nuclear factor-κB (NF-κB), interferon (IFN) regulatory factors, and cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) [6–8]. TLRs are widely implicated in alcohol-related neuroimmune signaling. Subsequently, activated transcription factors drive the expression of proinflammatory immune proteins, termed cytokines, which are released from immune cells and can either promote or dampen inflammatory processes. Cytokines coordinate inflammatory cell functions and are thought to have a wide-range of effects on physiological and behavioral responses [9]. Cytokine types, including interleukin (IL)-1, IL-2, IL-6, IL-8, and tumor necrosis factor-α (TNF-α), are each shown to have specific mechanisms [10]. In the central nervous system (CNS) specifically, microglia and astrocytes are considered the primary mediators of immune responses, such that they respond to and release immune signals. Neurons interact with microglia and astrocytes and express receptors capable of immune signaling. Further, anti-inflammatory factors, such as brain-derived neurotrophic factor (BDNF) and the cytokine IL-10, are necessary to manage and resolve proinflammatory and immune responses.

Alcohol and the Immune System

There are two proposed mechanisms through which alcohol is hypothesized to alter immune signaling and increase neuroinflammation: (a) indirectly, by initiating production of proinflammatory cytokines in the periphery that subsequently signal to the CNS, e.g., by binding at vagal afferent sites, crossing the blood-brain barrier (BBB) via immune-mediated active transport, or by reaching the CNS through disruption of the BBB [11]; and (b) directly via actions in the brain, wherein alcohol and alcohol-induced neural damage [12] stimulate the release of proinflammatory molecules [13]. Alcohol is thought to promote systemic inflammation by acting on peripheral immune receptors in the gut [10] and by breaking down lymphatic duct lining and endothelial cell junctions, whereby proinflammatory molecules leak into the bloodstream, termed "leaky gut" [14]. These proinflammatory molecules in the periphery subsequently provoke a proinflammatory response within the CNS, termed neuroinflammation. Proinflammatory molecules in the brain are suggested to alter neural circuit functioning and neuronal plasticity [10]. A prolonged or excessive proinflammatory response can negatively impact the individual and, among samples with AUD, is suggested to contribute to compulsive alcohol intake and other AUD symptomatology.

Preclinical Evidence

Preclinical animal models have provided strong support for the neuroimmune hypothesis of AUD. For instance, brain transcriptome studies have found that in adolescent alcohol-preferring rats, binge-like alcohol consumption resulted in upregulation of cAMP signaling system genes in the central amygdala and nucleus accumbens [15]. Chronic alcohol vapor exposure in mice led to upregulation of genes related to inflammatory diseases in the amygdala and nucleus accumbens [15]. Beyond alterations in immune gene expression, voluntary alcohol consumption has been demonstrated to increase cytokine and chemokine levels in both the CNS and the periphery in mice [16] and monkeys [17]. Specifically, in monkeys, alcohol consumption was correlated with hippocampal levels of the chemokine MCP-1 [17]. In mice, chronic alcohol consumption resulted in increases in levels of cytokines (IL-1β, IL-17, and TNF-α) and chemokines (MCP-1, MIP-1α, and CX3CL1) in the striatum and serum in wild-type mice [16], while mice with knockouts in the TLR system (i.e., TLR4, TLR2) were protected from these effects. This provided evidence in support of the importance of the TLR system in alcohol-related neuroinflammation [18]. Specifically, TLR4 is thought to contribute to alcohol-related neuroimmune effects [19]. Blocking TLR4 in glial cells protects against alcohol-induced glial activation, the induction of inflammatory mediators (i.e., proinflammatory cytokines), and apoptosis [19]. Furthermore, in male mice, chronic binge-drinking induced a microglia-driven neuroimmune response in the

prefrontal cortex, which led to abnormal synaptic pruning and ultimately resulted in synapse loss and increases in anxiety-like behavior [20]. Additionally, 24–48 h of alcohol withdrawal, a critical window during which motivation to self-administer is increased, resulted in the upregulation of mRNA proinflammatory expression of innate immune markers (e.g., TNF-α, IL-1β) in rat cortical tissue [21, 22]. These findings indicate that upregulated immune signaling occurring during alcohol withdrawal may contribute to the maintenance of AUD.

Further, preclinical work suggests that neuroinflammation and modulation of immune signaling induced by chronic alcohol use alters alcohol-related behaviors, including heightening motivation for intake, enhancing alcohol-related reward, and contributing to substance-related cognitive impairments and depression-like behavior [19, 23–26]. In rats, artificially induced proinflammatory states via LPS or cytokine administration sensitized alcohol withdrawal-induced anxiety in a dose-dependent manner [24]. Relatedly, microglia depletion prevented escalations in voluntary alcohol intake and decreased anxiety-like behavior in mice [27]. Chronic binge-pattern alcohol consumption among alcohol-preferring rats resulted in an anhedonia phenotype and decreased BDNF effects during withdrawal, whereas a BDNF agonist rescued these effects, indicating that BDNF may modulate the effects of chronic alcohol consumption on depressive symptoms [25]. Several mouse knock-out models have been used to evaluate the role of cytokines and chemokines on alcohol-related behaviors [28–31]. Knocking out or preventing the expression of cytokines, IL-6, IL-Ra, IL1R and TNF1R, resulted in reduced alcohol consumption, thus implicating these cytokines in alcohol drinking behavior (see [10] for detailed review). Moreover, chronic stress may further exacerbate alcohol-associated tissue injury in the gut, liver, and brain, such that chronic corticosterone treatment increases alcohol-induced expression of proinflammatory cytokines, resulting in mucosal barrier dysfunction, endotoxemia, and systemic inflammation [32]. Together, results from preclinical studies spanning molecular and genetic investigations to behavioral studies consistently find that the immune system plays a critical role in the development and maintenance of AUD.

Clinical Evidence

To date, human research in AUD evaluating the presence of enhanced activation of proinflammatory signaling in the CNS has been quite limited. It is remains unclear to what extent acute versus chronic alcohol exposure, quantity and frequency of alcohol intake, and recovery from AUD influence alcohol-related neuroinflammation [33, 34]. Existing work in this area has come primarily from postmortem brain studies [13, 35, 36], positron emission tomography (PET) studies imaging the translator protein (TSPO), and studies measuring levels of proinflammatory makers in cerebrospinal fluid (CSF) [37–39]. For example, an early report on gene expression profiles of postmortem human brains of individuals who had AUD found

that identified genes could be categorized into functional groups relevant to immune response, cell survival and communication, signal transduction, and metabolism, particularly in the frontal cortex [36]. In a study which collected CSF, researchers assessed whether MCP-1 concentrations were elevated among those with AUD versus healthy controls and if these concentrations were correlated with markers of liver damage, as this proinflammatory chemokine is shown to induce alterations in BBB permeability [39, 40]. In line with expectations, among treatment-seeking individuals with AUD, concentrations of MCP-1 were elevated in CSF and associated with peripheral liver markers of inflammation.

Labeling of TSPO, which is localized mainly in the outer mitochondrial membrane and upregulated during neuroinflammation, is utilized in PET imaging for inflammatory conditions, as it is suggested to be a biomarker sensitive to neural damage, inflammation, and reactive gliosis [41]. TSPO is involved in a broad range of biological functions and is typically expressed in reactive microglia and astrocytes. Across investigations of TSPO imaging in psychiatric disorders, differing patterns and wide variabilities in findings have emerged, such as elevated TSPO in major depressive disorder (MDD) and reduced levels in psychosis [42, 43]. For AUD, clinical studies have reported reduced binding of PET TSPO ligands in the brains of individuals with AUD relative to controls, which may be suggestive of reduced levels of activated microglia or downregulation of proinflammatory responses following chronic inflammation from alcohol [33]. In one PET study that enrolled 15 healthy controls and 15 individuals with AUD, results showed that the AUD group had 10% lower TSPO levels than controls; and TSPO levels in the hippocampus were negatively correlated with disorder severity [44]. However, interpretation of these findings is complicated by limitations of this technique, genotype-specific responding, and discrepant results from *in vitro* animal studies [45]. TSPO binding affinity may be influenced by competition of endogenous cholesterol, which binds to TSPO for transport during steroidogenesis [46]. For instance, a recent report examined the impact of *TSPO* polymorphism rs6971 on plasma levels of triglycerides and cholesterol in participants with AUD and controls [47]; researchers showed that only among the group with AUD did *TSPO* rs6971 significantly relate to plasma levels of total cholesterol, triglycerides, and HDL, along with withdrawal severity. In contrast to the imaging findings, a postmortem brain study showed upregulated TSPO mRNA in the amygdala and prefrontal cortex of brains from males who had AUD, in comparison to age-matched controls. This finding provides initial support for the neuroinflammation hypothesis of AUD. In sum, while results from studies on postmortem brains, CSF, and PET imaging are suggestive of altered CNS immune activity in human samples of AUD, much more research in this area using diligent experimental design and careful interpretation is needed to adequately establish the neuroimmune hypothesis of AUD and specific factors influencing immune signaling in the CNS.

Clinical research has also provided evidence that levels of systemic inflammation are elevated in samples of AUD. As discussed above, alcohol is thought to increase intestinal permeability, leading to elevations in circulating LPS and

bacteria, and subsequent activation of immune cells and peripheral inflammation. These signals can reach the CNS through various pathways and contribute to neuroinflammation [48]. Acute alcohol intake appears to modulate peripheral cytokine concentrations, as evidenced by reductions in TNF-α and elevations in IL-6 levels 3 h following an oral alcohol administration challenge in 25 heavy drinking individuals [49]. Regarding chronic alcohol intake, elevated levels of serum LPS were detected at treatment onset among individuals with AUD, but decreased after 3 weeks of detoxification, reaching points comparable to individuals classified as healthy controls [5]. Moreover, other markers of peripheral inflammation partially decreased over this time and both proinflammatory and anti-inflammatory cytokines were correlated with ratings on psychological measures assessing alcohol craving, depression, and anxiety. A meta-analysis pooling data from 17 studies that collected a wide range of peripheral inflammatory markers in samples of AUD, provided evidence for higher cytokine concentrations (e.g., IL-6, TNF-α, IL-8) among individuals with AUD compared to a healthy control group [50]. Results varied greatly depending on the cytokine type examined, and these abnormalities were more prominent during active drinking and acute withdrawal periods compared to those of early or prolonged abstinence. Importantly, translational work has started to improve the field's understanding of how alcohol-stimulated peripheral inflammation alters neuroimmune signaling in human samples of AUD. For instance, one study examining associations among liver function, peripheral inflammation, and brain alterations among both non-cirrhotic patients with AUD and alcohol-preferring rats, demonstrated a strong link among these factors [51]. For example, proinflammatory cytokines and liver fibrosis were correlated with brain macrostructure abnormalities in patients with AUD and microglia activation in the rodent model. In sum, results from clinical studies indicate that the peripheral immune and neuroimmune system are related to AUD symptomatology, but much remains unclear regarding specific mechanisms, causal links, and recovery from AUD in human samples.

Implications for Treatment

Promising Immune Treatments for AUD

Given the preclinical and clinical evidence reviewed above, treatments targeting peripheral and neural immune pathways represent an important direction in the development of novel and more effective treatment options for AUD. In this section, we outline research covering immune pharmacotherapies and psychotherapies with promise to mitigate drinking behaviors and promote recovery from AUD (see Fig. 30.1).

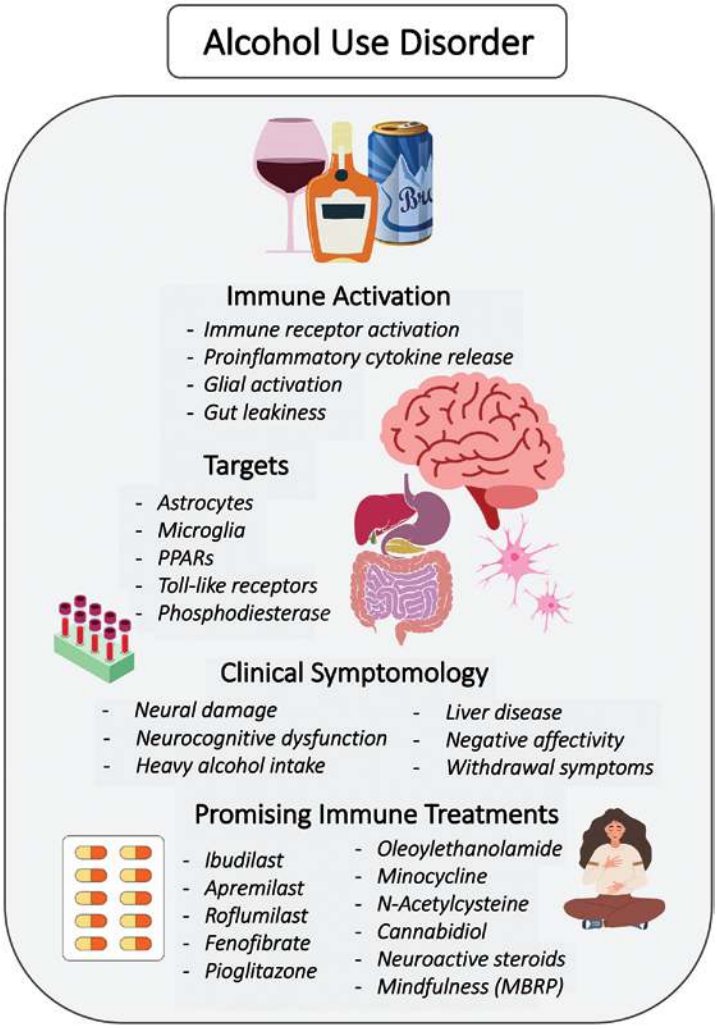

Fig. 30.1 Translational Science of Brain-Immune Mechanisms in Alcohol Use Disorder. Multiple components of the immune system are impacted by heavy alcohol intake, leading to altered immune signaling, dysfunction, and inflammation. In return, these changes are thought to influence and maintain clinical symptoms of alcohol use disorder (AUD). Both pharmacological and psychosocial therapies hypothesized to alter relevant immune targets have shown initial promise for treating the complex, multisystem symptoms of AUD; PPARs, peroxisome proliferator-activated receptors; MBRP, mindfulness-based relapse prevention

Ibudilast

Ibudilast is a selective PDE3A, PDE4, PDE10A, and PDE11A inhibitor [52] and an allosteric macrophage migration inhibitory factor (MIF) inhibitor [53]. Ibudilast has demonstrated initial efficacy in preclinical and clinical studies of AUD. In multiple rodent models of AUD, ibudilast attenuated drinking and relapse, and preferentially reduced drinking in alcohol-dependent, compared to non-dependent mice [54]. In clinical samples, two completed randomized controlled trials investigating the effect of PDE inhibition in humans with AUD have been published. First, a crossover human laboratory trial of ibudilast was conducted to evaluate safety, tolerability, and initial efficacy in a non-treatment seeking sample with AUD. Among 24 individuals enrolled in the trial, ibudilast was safe and well-tolerated, and, compared to placebo, decreased tonic craving for alcohol and improved mood following alcohol cue and stress exposure [55]. Second, results from a 2-week trial of ibudilast in non-treatment-seekers with AUD (n = 52) showed that ibudilast reduced rates of heavy drinking and neural alcohol cue-reactivity in the ventral striatum compared with placebo [56]. Ventral striatal activation to alcohol cues further interacted with ibudilast to predict reductions in drinking [56]. Ibudilast was well-tolerated in the 2-week trial, as there were no significant differences between medication groups in the occurrence of adverse events [56]. Presently, ibudilast (50 mg b.i.d.) is being evaluated in a 12-week randomized clinical trial in treatment-seeking individuals with AUD (NCT03594435). The trial's primary and secondary outcomes are reductions in percent heavy drinking days and examination of peripheral markers of inflammation and depressive symptomology, respectively.

Apremilast

Apremilast is a partial competitive PDE4 inhibitor, which is FDA-approved for the treatment of psoriasis, and shows promise as an AUD pharmacotherapy. In mice, apremilast reduced alcohol intake and preference, but did not alter sucrose preference, indicating its beneficial effects may be alcohol-specific [23]. Apremilast may impact alcohol consumption by increasing the aversive properties of alcohol, including decreasing functional tolerance to alcohol and increasing its sedative effects [23]. Apremilast was recently tested in five animal models of AUD, where it was found to reduce binge-like alcohol intake, motivation for alcohol, and stress-induced alcohol drinking [57]. Moreover, a proof-of-concept, double-blind, human laboratory study of apremilast (90 mg) in non-treatment-seeking individuals with AUD found that apremilast reduced the number of drinks participants consumed each day during the 11-day treatment period relative to placebo [57]. There were no serious adverse events in the proof-of-concept trial but adverse drug effects, including gastrointestinal symptoms were twice as likely in the apremilast group than placebo [57]. A 2-week clinical trial of apremilast (50 mg, b.i.d.) in non-treatment-seeking individuals with AUD (NCT03175549) has been completed, but results have yet to be posted.

Roflumilast

Roflumilast is a second-generation PDE4 inhibitor, with a potentially more favorable side effect profile than first-generation PDE4 inhibitors. Roflumilast is already FDA-approved for treatment of chronic obstructive pulmonary disease. In rodents, roflumilast dose-dependently reduced alcohol intake and preference in two separate mouse models [58]. Roflumilast did not impact sucrose or quinine drinking, indicating that it does not alter preference for natural rewards nor act through aversive mechanisms. However, at the highest dose tested, roflumilast decreased locomotor activity [58]. There have been no translational studies of roflumilast in clinical samples with an AUD, nor are there any ongoing clinical trials of roflumilast.

Fenofibrate

Peroxisome proliferator-activated receptors (PPARs) are transcription factors and members of the nuclear hormone receptor superfamily, whose actions can reduce proinflammatory immune signaling and regulate other physiological and cellular processes. PPAR agonists have been tested for their potential role in addiction processes as well as other CNS diseases. Fenofibrate, a PPARα agonist, shows promise preclinically as a pharmacotherapy for AUD. In both chronic voluntary and limited-access binge drinking mouse models, fenofibrate reduced alcohol intake and preference [59]. In chronic-drinking rats, fenofibrate markedly reduced voluntary alcohol intake [60, 61], but also reduced saccharin intake, indicating that fenofibrate may alter the rewarding effects of both alcohol and non-alcohol rewards. Additionally in rats, fenofibrate treatment had dose-dependent effects on self-administration and reduced both the reinforcing and motivational effects of alcohol [62]. This compound's mechanism of actions may be partially related to its effects on genes involved in energy metabolism, as fenofibrate administration was shown to increase levels of blood acetaldehyde [60], which is an aversive reaction similar to the effects of disulfiram, an FDA-approved medication for AUD. A clinical trial of fenofibrate for AUD was recently completed (NCT02158273) but trial results have yet to be published for the primary outcome of alcohol craving or secondary outcome of drinking reduction. Of note, initial trial reporting indicates that no serious adverse events occurred, suggesting that this medication was safe and well-tolerated.

Pioglitazone

The PPARγ agonist, pioglitazone, has been tested extensively in animal models of AUD. In rats, pioglitazone reduced voluntary drinking, motivation for alcohol as assessed via lever pressing, and reinstatement of alcohol-seeking behavior, but did not prevent cue-induced relapse [63]. Changes in alcohol-drinking behavior were not associated with changes in alcohol metabolism or blood

glucose levels, suggesting that these effects were not metabolic [63]. A follow-up study in rats combined pioglitazone with naltrexone, an FDA-approved pharmacotherapy for AUD, and found larger reductions in alcohol drinking with this combined medication administration [64]. These findings highlight the potential enhanced benefit of combining novel neuroimmune compounds with existing, approved medications to treat AUD. Rat models indicate that pioglitazone may furthermore have anxiolytic and neuroprotective properties as it prevented stress-induced reinstatement of alcohol seeking [65] and protected against binge alcohol-induced neuronal and cognitive damage [66]. In humans, a randomized clinical trial of pioglitazone (NCT01631630) resulted in early termination due concerns over myopathy risk in the active treatment group [67] and another trial was terminated early due to the COVID-19 pandemic (NCT03860753). At present, one human clinical trial of pioglitazone for AUD (45 mg) is ongoing (NCT03864146) and another human laboratory trial, which will examine the effect of pioglitazone (45 mg) on stress-induced relapse and drinking in the natural environment is posted but not yet recruiting (NCT05107765). Promisingly, a recent analysis of data from the Veterans Health Administration found that individuals who were prescribed pioglitazone for diabetes and were also heavy drinkers showed reduced alcohol intake after receiving the pharmacotherapy [68].

Oleoylethanolamide

Oleoylethanolamide (OEA) is a bioactive lipid mediator and member of the acylethanolamide family. OEA is a known satiety factor that has demonstrated strong anti-inflammatory and antioxidant effects, potentially mediated through activation of PPARα with protective actions in both the intestinal tract and CNS [69]. In rat and mouse models, OEA reduced operant alcohol self-administration, prevented cue-induced and withdrawal-induced reinstatement of alcohol-seeking, and reduced withdrawal severity [69]. In rats exposed to intragastric binge alcohol, administration of OEA blocked the expression of TLR4 in the frontal cortex and inhibited alcohol-induced NF-kB proinflammatory cascade, resulting in reduced proinflammatory marker levels, such as IL-1β, TNF-α, and COX-2 [70], providing support for a neuroimmune mechanism of action for OEA. In the same study, OEA pre-treatment provided antidepressant-like effects during acute alcohol withdrawal [70]. A clinical trial for a dietary supplement containing the precursor of OEA in young adult heavy drinkers was completed (NCT01902069) [71]. Treatment with the precursor supplement significantly improved performance on a Go/No-Go task of inhibition, which was correlated with reductions in alcohol intake [71]. However, larger clinical trials enrolling samples with AUD are needed to evaluate this compound's efficacy in regard to drinking outcomes.

Minocycline

Minocycline is a broad-spectrum antibiotic that crosses the BBB. Minocycline is a microglial attenuator, which has been shown to alter neuroimmune and cytokine expression in the brain and periphery [72]. Support for minocycline as a pharmacotherapy for AUD has been mixed. In male and female mice, minocycline modestly reduced voluntary alcohol intake [73]. Minocycline has also been shown to modulate a host of AUD-related behaviors including reductions in alcohol-induced sedation, withdrawal-induced anxiety, alcohol-induced reinstatement, and alcohol-induced cognitive disturbances and neurodegeneration [74–76]. However, minocycline's effects may be non-specific, as it reduced both alcohol and water consumption in mice [77]. In humans, a completed clinical study found that short-term treatment of minocycline was well-tolerated but no beneficial effects on subjective response to alcohol, alcohol craving, nor peripheral proinflammatory markers (e.g., serum cytokine levels) were found among heavy drinkers [78]. At present, a randomized, double-blind placebo-controlled trial of minocycline is underway; this trial aims to evaluate the effect of minocycline (200 mg) on cue-induced alcohol craving, alcohol consumption, neurocognitive impairment, and neuroinflammation—as assessed via multimodal neuroimaging—in non-treatment-seeking individuals with AUD (NCT04210713).

N-acetylcysteine

N-acetylcysteine (NAC), an over-the-counter dietary supplement, is an antioxidant precursor to glutathione that is approved for treatment of acetaminophen poisoning [79]. NAC has been shown to reduce proinflammatory cytokines in rodents and humans. In male rat models of AUD, NAC reduced alcohol-seeking, motivation for alcohol, and abstinence-induced alcohol reacquisition [80], but did not prevent cue-primed reinstatement of alcohol-seeking in a separate study [81]. NAC may protect against chronic alcohol-induced neuroinflammation in the rodent frontal cortex and hippocampus, as treatment with NAC prevented increases in proinflammatory cytokines and decreases in anti-inflammatory cytokines in male rats [82]; however, treatment with NAC did not affect serum cytokine levels [82]. Moreover, the co-administration of NAC and aspirin, which is anti-inflammatory, markedly reduced alcohol intake and relapse binge drinking in alcohol-preferring rats to a greater degree than the administration of either NAC or aspirin alone [83]. In humans, a secondary analysis of a clinical trial of NAC as a pharmacotherapy for cannabis use disorder found that NAC treatment reduced alcohol consumption by 30% relative to placebo [84]. However, a small human laboratory study of NAC in individuals with AUD found no effect of NAC on alcohol self-administration or subjective response to alcohol [85]. Despite this negative result, there is clear enthusiasm for NAC, as several clinical trials of NAC are ongoing and will examine the potential efficacy of the supplement in adolescent and adult samples with AUD (e.g., NCT03238300, NCT03216954, NCT04964843, NCT03707951).

Cannabidiol (CBD)

CBD is a non-psychoactive component of the cannabis plant that has received notable attention as a possible therapeutic for many psychiatric disorders, including AUD. While CBD has diverse biological effects, research supports its anti-inflammatory effects with immune signaling actions seen in the periphery and CNS. Anti-inflammatory targets of CBD include PPARγ, COX-2 enzymes and NF-κB, among others [86]. Numerous preclinical studies have tested whether CBD administration can reduce alcohol intake and related harms (see systematic review [87]), and findings consistently support CBD as a candidate pharmacotherapy for AUD. Animal studies have shown that CBD reduces alcohol administration, decreases motivation for alcohol, reduces relapse-like behavior, and improves withdrawal symptoms in animals exposed to chronic alcohol [88–92]. The majority of this research has yet to examine CBD's influence on the immune system, but one study showed that CBD attenuated alcohol-induced increases in liver enzymes, mRNA expression of cytokines TNF-α and IL-1β, and several chemokines [93]. In humans, CBD has been proven to be safe and well-tolerated in a range of clinical samples [94], although translational challenges exist, such as the low bioavailability of oral CBD in humans and potential contraindications for those with liver impairment [87]. A recent naturalistic study found that administration of a predominantly CBD cannabis strain reduced drinking in cannabis and alcohol co-users [95]. Researchers are currently conducting numerous randomized clinical trials of CBD for AUD across adolescent and adult samples (NCT03252756, NCT05159830, NCT05159830, NCT04873453, NCT05317546), as well as comorbid samples (i.e., AUD and PTSD; NCT03248167).

Neuroactive Steroids

Neurosteroids, or endogenous neuroactive steroids, are thought to be involved in neuroimmune signaling in AUD. Neurosteroids have a range of actions, including modulation of $GABA_AR$-mediated neurotransmission, TLR-dependent signaling [96], and CRF signaling, giving these compounds the potential to target the complex symptomatology of AUD [97, 98]. Two neurosteroids, allopregnanolone and pregnenolone, have been investigated in several preclinical studies and have demonstrated promising results [99]. In male mice, allopregnanolone dose-dependently modulated alcohol intake, with low doses increasing and high doses suppressing alcohol intake [100]. In male rats, allopregnanolone dose-dependently modulated alcohol-reinforced operant responding via lever pressing, such that lower doses increased operant responding whereas higher doses decreased response rates [101], indicating that higher doses of allopregnanolone may alter the reinforcing effects of alcohol. In alcohol preferring rats, pregnenolone reduced alcohol intake and preference; however, chronic treatment did not significantly alter intake or

preference [102], thereby limiting enthusiasm for this compound. There have been limited studies of neuroactive steroids in clinical samples. A human laboratory study of dutasteride, a 5-alpha steroid reductase (5AR) inhibitor that limits the production of dihydrotestosterone and the 5a-reduced neuroactive steroids allopregnanolone, pregnanolone and 3a,5a-androstanediol, found that males with heavy drinking patterns reported fewer days of heavy drinking after pretreatment for dutasteride compared to placebo [103]. Dutasteride pretreatment also reduced the subjective sedative effects of alcohol administration in male heavy drinkers [103]. Finally, several randomized clinical trials of neuroactive steroids for the treatment of AUD are ongoing (NCT03872128 [pregnenolone]; NCT02582905 [citicoline and pregnenolone]; NCT04098302 [dutasteride]; NCT04015869 [allopregnanolone]; NCT05223829 [brexanolone]). These trials include important investigations into sex differences as well as effects on alcohol intake, withdrawal, stress reactivity, and mood symptoms. Future trials will also test the benefit of neurosteroids on treating comorbid conditions, including PTSD and AUD, and bipolar disorder and AUD.

Mindfulness-Based Relapse Prevention

Mindfulness-based relapse prevention (MBRP) is a mind-body therapy designed for individuals with addiction. MBRP is delivered in 2-h group sessions aimed to cultivate increased awareness of present-moment cognitive, emotional, and physical states, especially as they relate to cravings and withdrawal [104]. The anti-inflammatory effects of mind-body therapies have been explored in other psychiatric and neurological conditions, but limited research has been focused specifically on samples of AUD [105]. It is hypothesized that these therapies work to reduce inflammation by impacting downstream stress reactivity pathways, thereby reversing the activation of proinflammatory mechanisms [106]. While there has only been a small number of randomized trials of MBRP conducted in the context of AUD, there is evidence that MBRP may be most effective for individuals with severe AUD or comorbid mood symptomatology [107], which is supported by the wide-body of literature linking depression and inflammation [108]. One trial examined the impact of MBRP on peripheral proinflammatory marker levels in adults with AUD and found that greater time spent practicing mindfulness predicted lower levels of circulating IL-6. This suggests that regular mindfulness practice may reduce peripheral proinflammatory marker levels [105]. One clinical trial of MRBP has recently been completed, although results have yet to be published (NCT02994043). The results of this study will extend this area of research by exploring immunological, epigenetic, and neurobiological changes associated with 8 weeks of MBRP in AUD. Additional trials will seek to further test MBRP efficacy as a treatment for AUD, but do not include explorations of potential anti-inflammatory mechanisms of this treatment (NCT03842670; NCT0214783).

Treatment Development Recommendations

These efforts to elucidate the role of the immune system in the development and maintenance of AUD have contributed to a host of potential novel treatments, both pharmacological and psychosocial [34]. Research establishing immune signaling as a novel treatment target supports interventions that reduce (neuro)inflammation and alter aberrant immune processes as plausible candidates for AUD medications development. Findings in this area of research represent an exciting development and illustrate the desired progression from basic discoveries to tangible improvements in healthcare [10, 13, 56, 57, 73, 103]. Yet, careful consideration of optimal approaches to treatment development is necessary, as medications development for AUD is known to be costly and time-consuming and has produced limited success over the past several decades [109, 110]. The following recommendations are offered to enhance the efficiency of developing neuroimmune modulators for AUD.

To start, novel immune targets and compounds may signify novel biobehavioral mechanisms of action. It is possible that certain established pharmacological mechanisms of action, which are often examined through experimental human laboratory designs to inform decisions about novel medications, may not effectively capture the clinical effects of neuroimmune modulators for addiction. For instance, research has demonstrated that not all effective medications for AUD alter the sedative properties of alcohol or reduce alcohol cue-induced craving, such as was shown in a trial of varenicline [111]. We recommend considering novel mechanisms informed by the application of neuroimmune modulators in psychiatry [112–114] as well as novel hypotheses informed by preclinical and clinical studies of neuroimmune function and behavior in AUD. Therefore, screening methods specific to these compounds' targets, such as neurocognition, stress-reactivity, gut microbiota, or immune biomarkers will help capture these potentially unique mechanisms of change.

Second, establishing the relationship between any novel treatment and drinking outcomes is critical. While elucidating mechanisms of action and moderators of treatment response remains a high priority area, enthusiasm and support for treatment development hinges on the demonstration of a "main effect" of an intervention on drinking outcomes in clinical samples of AUD. Reductions in drinking that translate into improved health and social function are the "meaningful clinical benefit" that regulators require for approval of a new drug application (NDA), or a new indication (NI) for a medicinal product with current marketing approval on another indication. As discussed in detail elsewhere [110], the utility of screening models for medications development for AUD hinges on the relationship between the screening paradigm and the regulatory standard outcome of drinking reduction. To that end, we recommend that screening models be selected to optimize translational potential for clinical trials outcomes, which will ultimately determine the fate of the novel treatment and its approval and uptake in clinical settings.

In addition, as we learn from the application of neuroimmune (dys)function and psychiatric disorders, including AUD, we refine our understanding of clinical

profiles of treatment responders to neuroimmune modulators. The literature on MDD discusses persuasive evidence that immune mechanisms contribute to the pathology of MDD in subpopulations of patients [115]. Individuals with a proinflammatory profile of MDD may have unique clinical features that are resistant to typical treatments, such as elevations in peripheral biomarkers of proinflammatory activity and sickness behavior [108]. It is plausible to hypothesize that certain clinical presentations of AUD may similarly represent a higher contribution of neuroimmune dysfunction. Characterizing the unique clinical profile of individuals with AUD for whom immune dysfunction is most salient has great potential to maximize the benefits of neuroimmune treatments in clinical settings through precision medicine principles. In line with this, the application of insights from basic and clinical research in this area may elucidate treatment-responsive biomarkers. For example, peripheral markers of inflammation, such as C-reactive protein (CRP) are widely used in clinical settings for a range of diseases and inflammatory conditions. This presents an opportunity to leverage well-established resources in clinical care to identify individuals with AUD for whom rescuing healthy neuroimmune function may be most beneficial. For example, individuals with AUD and clinical elevations in markers such as CRP, IL-6, or TNF-α may be fitting candidates for treatment with neuroimmune modulators. Notably, however, these elevations will likely be less pronounced than inflammation caused by certain inflammatory health conditions (e.g., bacterial infection) and will require the use of highly sensitive assays, such as high-sensitivity CRP (hs-CRP). These tests can detect slight increases that still fall within the normal range of standard CRP values, such as is often used to detect one's risk for developing coronary artery disease. Improvements in neuroimmune function (i.e., return of CRP levels to normal range or decreases of CRP within that range) may thus be associated with improvements in alcohol consumption and recovery from AUD. Preliminary work from our laboratory supports the utility of identifying elevations in CRP as an indicator of beneficial response to ibudilast treatment, but much more work on this topic is needed; other biomarkers may prove more sensitive or specific to inflammation in individuals with AUD. In summary, biomarker development is a long-awaited step forward in AUD treatment, and neuroimmune and AUD literature provides several avenues for clinical translation and biomarker development.

Conclusions

This chapter broadly covers the field's progress in characterizing brain-immune mechanisms in AUD from basic to clinical research. Alcohol is believed to alter immune signaling and contribute to neuroinflammation through various pathways, including by initiating systemic production of proinflammatory cytokines and inducing neural damage. While preclinical models provide strong support for the neuroimmune hypothesis of AUD, more work is needed to adequately establish these mechanisms in human clinical samples. For instance, much remains unknown

regarding the relevance of specific immune signaling mechanisms and their causal links to AUD symptomatology and recovery in human samples. In addition, we reviewed translational work seeking to ultimately support individuals' recovery from AUD, testing the biological and clinical plausibility of treatments that target peripheral immune and neuroimmune dysfunction. Despite the alcohol research field's strong focus on medications development over the past several decades, a paucity of new pharmacotherapies has demonstrated robust efficacy or received FDA approval. This highlights the necessity of developing and testing novel treatments for AUD with novel mechanisms, such as the immune system. We covered the limited number of randomized clinical trials of immune treatments for AUD that have been conducted to date but noted numerous ongoing clinical trials that may support the safe and effective clinical application of immune treatments for AUD and perhaps psychiatric disorders more broadly. Finally, recommendations for treatment development are provided, such as attempting to identify individuals who are most treatment-responsive, considering potentially novel mechanisms of actions for compounds, and in turn, assessing these through experimental laboratory screening methods and collection of relevant biomarkers in clinical samples of AUD.

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Chapter 31
Brain Alterations and Cognitive Deficits Induced by Alcohol Use Disorder

Mickael Naassila

Abstract Chronic alcohol use, either in the context of repeated binge drinking behavior or alcohol use disorder, induces brain and cognitive alterations. These brain and cognitive alterations can be reversible and a recovery, at least partial, is seen in general after weeks or months of abstinence. Cognitive deficits are highly frequent in patients with alcohol use disorder and are largely under-diagnosed and under-treated as for example for the Gayet-Wernicke encephalopathy that could be easily treated and or prevented (if suspected) by thiamine treatment. Cognitive deficits have an impact on the treatment and should be well identified and targeted in order to improve care of patients and increase the success rate in maintaining long term abstinence or reduced alcohol intake.

Keywords Binge drinking · Alcohol use disorder · Brain · Alterations · Neurotoxicity · Cognitive deficits · Gayet-Wernicke encephalopathy · Korsakoff syndrome

Effects of Acute Consumption

The ethanol contained in alcoholic beverages is rapidly absorbed from the gastrointestinal tract and the peak blood-alcohol concentration (BAC) is usually reached after 10 to 60 min. Ethanol reaches the brain very easily and since the brain has a high rate of blood flow per gram tissue, it rapidly equilibrates with the concentration of ethanol in the arterial blood. Ethanol depresses brain activity in a dose-dependent manner. At low BAC, about 0.3–0.5 g/L, ethanol has disinhibiting effect associated with a mild euphoria. Between 0.5–1.0 g/L, impairments such as slurred speech, slow reaction time, difficulty in information processing, sedation and ataxia are

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observed. From 2 g/L BAC, emesis and stupor are obvious and at 3 g/L, a coma can occur. Around 4 g/L, respiratory depression and death are possible, even at lower levels, death can occur with asphyxia from inhalation of vomit. Alcohol drinking has a biphasic effect with both positive (pleasure, euphoria) and negative (sedation, ataxia, memory and attention deficits) effects. Alcohol affects brain function from the first drink and GABA type A receptors appear to be particularly sensitive to the effects of alcohol. From the first drink, and an alcohol level of only 3 mM, the activity of GABA type A receptors containing the delta subunit, which are preferentially located on the extra synaptic side, is modified [1].

As other drugs of abuse, acute alcohol intake activates brain reward system, thus inducing pleasant effects [2]. Rewarding effects increase the probability to repeat alcohol drinking also called positive reinforcement. Reward is mediated by dopamine release in the ventral part of striatum, the nucleus accumbens. Dopamine is released by neurons from the ventral tegmental area and the effect of alcohol on dopamine release is mediated by different mechanisms. Alcohol targets directly the dopamine neurons and increases their firing rate. Alcohol also increases dopaminergic neuron activity by their disinhibition through the inhibition of GABAergic neurons either directly or through the release of β-endorphin by opioidergic neurons.

Acute alcohol intake, particularly if consumed rapidly, has also memory impairing effects, the so-called blackouts. Blackouts are episodes of amnesia, during which subjects continue to interact with others during the event but that they later cannot remember [3].

Effects of Binge Drinking on Brain and Cognition

Definition of Binge Drinking

Excessive alcohol intake (e.g., heavy or hazardous drinking) has massive physiological, psychological and cerebral consequences [4]. Binge drinking is a specific consumption pattern that has raised as a major research topic due to its ubiquity and widespread effects [5]. Binge drinking behavior is characterized by excessive (i.e., leading to drunkenness) but episodic alcohol consumption [6, 7]. Recently, an operational definition of binge drinking behavior has been proposed with six specific characteristics including the presence of physiological symptoms related to binge drinking episodes, the presence of psychological symptoms related to binge drinking episodes, the ratio of binge drinking episodes compared to all alcohol drinking occasions, the frequency of binge drinking episodes, the consumption speed, and the alternation between binge drinking episodes and soberness periods [8]. Among adolescent drinkers, alcohol-related blackouts, or acute alcohol-related memory loss, may occur after consuming ≥12 drinks (126 g) per occasion for males and ≥ 7 drinks (70 g) for females [9].

The repetition of such drunkenness episodes results in an alternation between intense alcohol intoxications and abstinence periods, constituting a specific pattern of alcohol intake. Binge drinking behavior is the most prevalent alcohol-related behavior among youth in Western countries [10], 40% of young adults reporting at least one binge drinking episode per month during the last 6 months. Converging data have demonstrated the rapid and long-lasting psychological and cerebral consequences of binge drinking behavior [11]. The specific neurotoxicity of this behavior results from the repetition of intoxication-abstinence cycles, leading to multiple withdrawals that are particularly harmful for the brain. This even led to the "continuum hypothesis" suggesting that binge drinking pattern might constitute the first step towards severe alcohol use disorder (AUD): neurocognitive impairments would initiate the addictive vicious circle by reducing inhibitory abilities and increasing automatic attraction towards alcohol [12]. A recent study has suggested that frequent (more than twice a month) binge drinking behavior (≥5 drinks (50 g ethanol) for male and ≥ 4 (40 g ethanol) for female students) during adolescence (18–25 years) is predictive of AUD at adulthood (25–45 years, adjusted OR = 2.83, 95% CI 1.10 to 7.25) [13]. The increased vulnerability to AUD at adulthood after repeated exposure to binge drinking episodes during adolescence has also been demonstrated in animal models [14].

Effects of Binge Drinking on Memory

During adolescence, binge drinking can be harmful to the brain, as it may interfere with ongoing maturation of its neuronal circuits. Several studies have suggested that binge drinking may have neurotoxic effect through the induction of neuroinflammation that damages both white and grey matters and loss of hippocampal neurogenesis [15]. Animal models have demonstrated that both hippocampal synaptic plasticity and memory impairments induced by binge drinking episodes are prevented by an anti-inflammatory treatment [16]. Compared with social drinkers, binge drinkers display reduced white matter integrity and performance in spatial working memory [17]. Binge drinkers also display altered verbal memory due to low proficiency in encoding, storage and retrieval processes that are impacted by consumption speed and intoxication episodes [18]. In the latter study, binge drinkers displayed the following characteristics (versus social drinkers; data are given as mean ± SD (min–max)): Consumption speed (drinks/hour) 2.83 ± 0.78 (2–5) versus 1.52 ± 0.71 (0.5–3), Episodes of intoxication in previous 6 months 16.65 ± 10.77 (6–50) versus 1.22 ± 1.44 (0–4), Percentage of times drinking to intoxication 56.52 ± 19.68 (10–90) versus 12.61 ± 12.87 (0–50) and Alcohol units per week 17.35 ± 14.91 (3–63) versus 5.14 ± 4.66 (1–20) [18].

Effects of Binge Drinking on Decision Making and Emotion Processing

In addition to learning and memory impairments, binge drinking behavior is also associated with dysfunctions in decision making, executive functioning and in affective processing [6, 19–21]. Previous study suggested that binge drinking would induce brain alterations in amygdala and prefrontal cortex, leading to comparable cognitive and affective impairments than AUD [22]. Difficulties to process emotional contents are associated with behavioral deficits in binge drinkers who display poorer performance for the identification of anger and fear affective bursts and for the recognition of fear and sadness facial expressions [20, 23]. When binge drinkers have to identify affective bursts, results show lower activations in the bilateral superior temporal gyrus together with increased activations of the right middle frontal gyrus [23]. Beyond emotional identification, binge drinkers presented differential brain responses following the implicit processing of emotions and the emotional difficulties in binge drinking might be related to a more automatic/unconscious processing of emotions [21]. Difficulties in emotion processing observed in binge drinkers, as also observed in patients with AUD, may have important implication by underlining emotional processes as a potential target to prevent the appearance of problematic alcohol use [21].

Effects of Binge Drinking on Brain Structure and Functioning

Numerous studies suggested that binge drinking during adolescence is associated with various regions of lower cortical, subcortical, and cerebellar grey-matter volume; however, studies have also suggested greater volumes in other brain regions in control subjects. A follow-up study that investigated gray-matter volumes in adolescent binge drinkers at baseline and during multiple follow-ups found that binge drinkers displayed greater reductions in overall neocortex volume, as well as in frontal, lateral frontal, and temporal cortex volumes [24]. The number of binge drinking episodes in the past year was negatively associated with frontal and parietal cortex thickness and adolescent binge drinkers also displayed thinner total, frontal, temporal, and cingulate cortices than nondrinkers [25]. Longitudinal studies have demonstrated reduction in white-matter volumes both before and following initiation of binge drinking [24, 26]. Binge drinking has been linked with degradations in neural white matter and that compromised white matter at this period of brain development has also been linked with impaired cognitive functioning [17].

A single binge drinking episode may be particularly harmful for the brain and may have long-lasting effects. For example, a prospective study showed a single night of extreme drinking (21st birthday celebration) is enough to immediately alter brain structure, revealing changes to the corpus callosum that remain at least 5 weeks following the drinking episode [27]. A single injection of ethanol in

adolescent baboons, reaching in blood ethanol levels of 0.8 g/L, showed an increase in inflammatory response both acutely and even 7 months after the binge drinking episode [28]. The persistent effect suggested a 'priming' of glial cell function after initial alcohol exposure.

Similarities Between the Effects of Binge Drinking and AUD

As for emotional processing deficits seen in both binge drinkers and patients with AUD, the electroencephalographic (EEG) profile of binge drinkers and alcohol-dependent individuals displays similarities [29]. Young binge drinkers seem to display a similar profile as that of subjects with AUD during resting state and visualization of alcohol-related pictures. The representation of the brain overactivation observed in binge drinkers during some cognitive tasks accompanied by a satisfactory level of performance is presumably related to a neurocompensatory mechanism [29].

Overall, brain and cognitive alterations described by a large number of studies support the *continuum* hypothesis suggesting that binge drinking and AUD may share several common features [29]. To date, the literature does not support a greater vulnerability of females to the effects of binge drinking.

Only very few studies have investigated potential brain recovery after reducing or stopping binge drinking behavior. One study demonstrated that abandoning the binge drinking behavior for 6 years may lead to partial recovery of working memory deficits, particularly perseverations and low working memory span in demanding trials [30]. Another study revealed a recovery in problem solving impairment in young binge drinkers after 4 weeks of abstinence, regarding tests of prospective memory, cognitive switching, inhibition task accuracy, verbal memory, visuospatial construction, and language and achievement [31].

Effects of Chronic Alcohol Intake on Brain

Chronic alcohol intake is known for inducing brain alterations even at moderate levels of intake. For example, a study on a longitudinal cohort of 550 men with AUD, demonstrated that higher alcohol consumption over the 30 year follow-up was associated with increased odds of hippocampal atrophy in a dose dependent fashion [4]. While those consuming over 280 g ethanol a week were at the highest risk compared with abstainers (odds ratio 5.8, 95% confidence interval 1.8 to 18.6; $p \leq 0.001$), even those drinking moderately (112–168 g ethanol/week) had three times the odds of right sided hippocampal atrophy (3.4, 1.4 to 8.1; $p = 0.007$). There was no protective effect of light drinking (8 ≤ 56 g ethanol/week) over abstinence. Higher alcohol use was also associated with differences in corpus callosum microstructure and faster decline in lexical fluency [4].

Patients with AUD display variable brain alterations and variable cognitive deficits. More than 50% to 80% of patients with AUD display cognitive deficits thus suggesting that they are largely under-diagnosed. The most frequent ones are: (i) temporo-spatial orientation (disorientation in time and/or space), (ii) memory (difficulty in retrieving old memories, (iii) difficulty in learning new things), (iv) reasoning (problem solving, mental planning difficulties, mental rigidity, etc.), (v) visual-constructive praxis (difficulty in drawing, structuring space).

Among the risk factors for brain and cognitive alterations in AUD there are the following ones: (i) younger age or older age (>60 years), (ii) women, (iii) hepatic damages, (iv) psychiatric comorbidity, (v) low educational level and (vi) positive history of fetal alcohol syndrome.

Structural magnetic resonance imaging studies of patients with AUD highlighted reduction in the volume of both grey matter and white matter in the cerebral cortex with greater loss in the frontal lobes, the hippocampus, the mamillary bodies, the thalamus and the cerebellar cortex. Studies have also showed thinning of the corpus callosum and the pons, reduced volume of the cerebellar vermis. The different studies that investigated difference between men and women yielded controversial results and so far, the findings suggesting that women would be more vulnerable to alcohol-induced brain damage have not been confirmed.

Alcoholic Dementia

Dementia is a clinical syndrome characterized by a progressive deterioration in cognitive ability and the capacity for independent living and functioning.

Alcoholic dementia could be characterized with the following criteria: (i) multiple cognitive impairments, (ii) decline from previous state, (iii) chronic alcohol use >5 years, 28 to 35 drinks per week, (iv) cognitive deficit persisting 60 days after withdrawal, (v) no vascular factors on brain imaging, (vi) signs of alcohol-induced somatic damage, and (vii) progressive evolution over time without being able to date the onset of the disorder (different from Gayet-Wernicke).

Dementia affects memory, thinking, behavior, and the ability to perform everyday activities, and is a leading cause of disability in older individuals. Although causality has not be established, light to moderate alcohol intake in middle to late adulthood has been associated with a decreased risk of cognitive impairment and dementia; however, heavy alcohol intake has been associated with changes in brain structures, cognitive impairments, and an increased risk of all types of dementia [32]. A recent study suggested that AUD is a major risk factor for onset of all types of dementia, and especially early-onset dementia [33].

About 80% of patients with AUD present cognitive deficits [34] and a standardized cognitive evaluation carried out in detoxified patients in care in the French health system showed that more than half of the patients in an addiction service display cognitive dysfunctions [35]. The most frequent disorders are impairments of executive functions, episodic memory and visuospatial construction abilities. They

are often referred to as moderate because they are about one standard deviation below the mean of control subjects regardless of the neuropsychological domain considered [36]. However, these average results conceal a great heterogeneity of impairments which may be absent in some patients, mild to moderate in others and severe in still others.

There is a lot of evidence to show the effects of AUD on executive functions. The body of research suggests global executive dysfunction, affecting the abilities to rank, strategize, be mentally flexible, inhibit, reason, update and plan [37]. Decision making has also been shown to be impaired in patients with AUD who tend to engage in risky behavior by not considering the future consequences of their actions [38].

Studies on episodic memory in AUD have concluded that there is an alteration in long-term memory learning capacities even though some results show contrary results or reveal performance only one standard deviation below the mean of control subjects [36]. The authors suggested that the apparent contradiction could be explained by (1) the heterogeneity of the tasks used, (2) the intrinsic heterogeneity of the clinical population and (3) the great variability of the clinical characteristics of the AUD patients included in the studies (duration of abstinence, history of alcohol use). Between 2 weeks and 2 months of abstinence, patients with AUD would present episodic memory disorders which would be difficult to detect with classical tests but observable with more elaborate tests [39]. Patients with AUD and recently detoxified display an alteration of all components of episodic memory (encoding, retrieval, contextual memory and autonoetic awareness) but preserved storage abilities [40]. The authors suggested the existence of a real impairment of episodic memory in patients with AUD, not linked to executive dysfunction, and well before the development of a Korsakoff syndrome [40]. Patients with AUD are able to learn new complex informations despite impairments in episodic memory and executive function [41]. Patients with AUD use learning strategies that are cognitively more costly than those used by control subjects [41]. The neuropsychological disorders of patients can also have a deleterious impact on their ability to associate essential information in daily life such as names and faces. One study has showed that the learning deficit of name-face associations in patients with AUD would reflect a more general dysfunction of long-term memory capacities [42]. These difficulties in acquiring new complex cognitions evidenced in patients with AUD following withdrawal require consideration during care. Indeed, a number of patients may be cognitively unable to take full advantage of proposed treatments to promote maintenance of abstinence, thereby increasing the risk of relapse [43]. Patients with cognitive problems could be less attentive during therapeutic workshops, less motivated and more in denial than those with preserved cognitive abilities [43].

Although it is now established that a large proportion of patients with AUD display neuropsychological disorders after withdrawal, the factors explaining the heterogeneity of these disorders remain unknown. Some patients display severe cognitive impairment while others, with the same drinking pattern, present no apparent impairment. Thus, the history of alcohol intake alone does not explain the nature and severity of cognitive dysfunction. The difficulties of patients with AUD

in acquiring new complex information indicate that a lengthening of the treatment (repetition of learning sessions or postponement after a recovery period with abstinence) would be favorable for patients with neuropsychological disorders. Another possibility for adapting the management of patients with cognitive disorders could be the use of cognitive remediation techniques.

Gayet-Wernicke and Korsakoff Syndrome

Definitions and Clinical Characteristics

One of the first clinical descriptions of an amnesic syndrome due to alcohol intoxication was made by Sergei Korsakoff during a conference in Paris in 1889 and the first detailed description was probably published in a paper by Robert Lawson in 1878 [44]. For more details, the reader is also referred to Chap. 72 focusing primarily on the Wernicke-Korsakoff syndrome. The Korsakoff syndrome is the result of a combination of chronic and excessive alcohol consumption and a thiamine (vitamin B1) deficiency. Gayet-Wernicke encephalopathy (GWE) often precedes the severe and long-lasting amnesia characteristic of Korsakoff syndrome, but this syndrome can also have an insidious onset. Patients with Korsakoff syndrome present neuropsychological disorders and equivalent brain damage whether they have Gayet-Wernicke encephalopathy or an insidious onset, suggesting that it is the same pathology regardless of the mode of entry into the disease. Gayet-Wernicke encephalopathy, related to thiamine deficiency, is largely underestimated in patients with AUD [45] who are nevertheless at high risk for this neurological complication [46].

The prevalence reported in the literature is 1% to 2% in the general population and 12% to 14% in the population of subjects with AUD [47]. However, this prevalence would be underestimated because of the difficulty in identifying GWE or Korsakoff syndrome during the patient's lifetime [48].

Clinically, Korsakoff syndrome is classically described as an amnestic syndrome comprising massive anterograde amnesia and retrograde amnesia of varying amplitude. Working memory and executive function disorders are sometimes associated. Confabulations, which are also part of the classic clinical picture, are mostly present early in the disease, during or just after GWE. False recognitions, erroneous identifications of persons or places, are also very frequent at the beginning of the disease but tend to disappear quite quickly, just like confabulations. Finally, anosognosia is frequent in Korsakoff syndrome. It refers to the patient's lack of knowledge of his or her disease or condition. In the DSM-5 classification, Korsakoff syndrome would be coded as "major alcohol-related neurocognitive disorder, confabulatory amnesia". The term "with moderate or severe use disorder" could be added. The DSM-5 also specifies that the term "persistent" should be used when the disorder does not recover with a period of abstinence. The ICD 10 describes this condition as a syndrome dominated by the presence of chronic memory disorders (recent and old events). Short-term memory is usually preserved and recent memory is more

severely disturbed than the memory of old events. Obvious disturbances in the perception of time and the chronology of events, as well as difficulties in learning new knowledge also characterize this syndrome. The syndrome may involve intense fabulation. Other cognitive functions are usually relatively unaffected, and memory problems are disproportionate to other disturbances. In contrast to the DSM, the ICD specifies that it is "Korsakoff's syndrome induced by alcohol or other psychoactive substances or unspecified".

Korsakoff syndrome is primarily a permanent amnesic syndrome. In contrast to patients with AUD, the episodic memory disorders of patients with Korsakoff syndrome are not reversible. In addition to amnesia, patients with Korsakoff syndrome have impairments of working memory and executive functions. Like patients with AUD, they may have impaired intellectual and visuospatial abilities. Impaired working memory and executive functions are not specific to patients with Korsakoff syndrome and therefore do not differentiate between patients with AUD from those with Korsakoff syndrome [49]. The severe episodic memory deficits of patients with Korsakoff syndrome seem to hamper their ability to acquire new complex knowledge, whether semantic or procedural [50]. The executive disorders of patients with Korsakoff syndrome, equivalent to those of patients with AUD, seem to contribute little to their learning difficulties.

Brain Alterations

Regarding structural brain alterations in patients with Korsakoff syndrome, post-mortem studies have generally reported damage to the medial temporal lobe, the mammillary bodies and the anterior or dorsomedial nuclei of the thalamus [44]. Alterations of the white matter of patients with Korsakoff syndrome have been shown post-mortem, particularly at the hippocampal and prefrontal levels. The damage to the white matter could even partly explain the volumetric cerebral alterations in patients with Korsakoff syndrome. Studies using magnetic resonance imaging have confirmed both cortical and subcortical structural alterations in patients with Korsakoff syndrome. *In vivo* imaging studies also reveal a decrease in the volume of the medial temporal lobe and more specifically the hippocampus. The atrophy would also concern the thalamus, the mammillary bodies and the frontal lobes. In 2009, one study showed a graded effect of structural damage ranging from mild to moderate damage in patients with AUD to severe damage in patients with Korsakoff syndrome in the mammillary bodies, hippocampus, thalamus, cerebellum and pons [51]. Significant structural and metabolic damage have been shown in patients with Korsakoff syndrome, particularly in the Papez circuit and the fronto-cerebellar circuit. The dysfunction of these two circuits is consistent with the neuropsychological profile of patients with Korsakoff syndrome, which combines severe amnesia, working memory disorders and ataxia [44].

Postmortem studies in patients with AUD have shown reduced brain weights compared with controls, as well as ventricular dilation [52]. Overall, neuropathological studies indicate atrophy of the frontal and cerebellar cortex. However,

quantification of neuron number only showed neuronal loss in certain regions of the frontal cortex, but not in the hippocampus, mammillary bodies, thalamic nuclei. The alteration in brain volume is thought to be primarily related to white matter impairment, as well as possible associated thiamine deficiency and liver disease [52]. *In vivo* neuroimaging studies have described a reduction in gray matter volume in patients with AUD compared to control subjects particularly in the frontal and more specifically dorsolateral cortex, hippocampus, thalamus, mammillary bodies, caudate nucleus and putamen, and cerebellum. Fluorodeoxyglucose positron emission tomography imaging studies reported a decrease in cerebral glucose consumption in the whole brain [53], in some regions up to 20%. Hypometabolism has been found particularly in the parietal and frontal cortex [53] and in the cingulate cortex. Functional brain imaging studies of brain activity and functional connectivity in patients with AUD have suggested brain reorganization with, for example, recruitment of different brain networks, greater activation of certain regions and recruitment of more regions [54]. Even in the absence of significant macrostructural damage, patients with AUD appear to involve different regions than controls in certain cognitive tasks [54]. Most of studies that investigated potential brain recovery in patients with AUD have investigated the effect of abstinence. Many studies have globally shown a cerebral recovery after abstinence but it is difficult to compare them because of their differences in the duration of the follow-up (from a few weeks to several years) or their definition of abstinence (total abstinence or certain level of consumption). Longitudinal studies have reported an improvement in ventricular dilation in the cortical level and more particularly in the temporal, cingulate and insula as well as in the amygdala, thalamus, hippocampus and cerebellar cortex. Partial recovery from frontal hypometabolism and hypoperfusion has also been reported after abstinence. Among the explanatory mechanisms of this brain recovery, neurogenesis and the involvement of oligodendrocytes that enable myelin repair and remyelination have been proposed. Very interestingly one study demonstrated that brain recovery in the cerebellum (gray and white matter), striatum, cingulate gyrus, corpus callosum and periventricular white matter was inversely related to the amount of alcohol during the 6 months period of abstinence [55]. In this study brain recovery was not only observed in the case of total abstinence, but could be observed in patients who had drastically reduced their consumption (approximately 120 units (1200 g ethanol) in 6 months, i.e., the equivalent of one glass per day on average).

Mechanisms Involved in Brain Alterations

Two main mechanisms have been proposed to explain the neuronal and cognitive alterations induced by chronic alcohol intake. The first one is the direct neurotoxic effect of ethanol by disturbing the excitatory/inhibitory balance (glutamate/GABA balance) and the deficiency in thiamin (B1 vitamin), see Fig. 31.1.

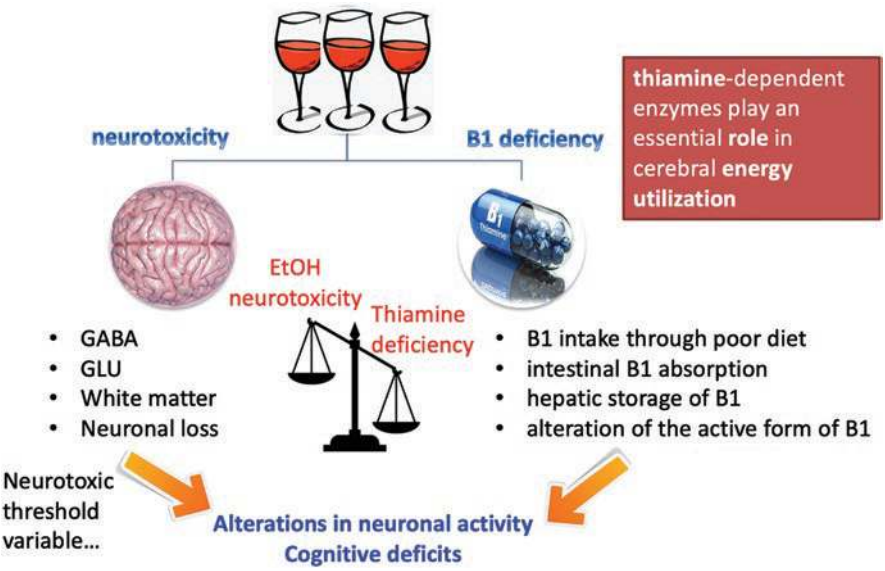

Fig. 31.1 M Naassila. Two main mechanisms have been proposed to explain the neuronal and cognitive alterations induced by chronic alcohol intake. The first one is the direct neurotoxic effect of ethanol by disturbing the excitatory/inhibitory balance (glutamate/GABA balance) and the deficiency in thiamin (B1 vitamin)

Thiamine deficiency is a crucial factor in the etiology of Korsakoff syndrome. Although alcohol abuse is by far the most important context in which thiamine deficiency occurs, there is no convincing evidence for an essential contribution of ethanol neurotoxicity to the development of GWE or to the progression of GWE to Korsakoff syndrome [44].

The frontal cortical damages may be primarily due to the direct toxic effect of ethanol [44] while the damages due to thiamine deficiency may primarily affect the dorsomedial thalamic nuclei, the mammillary bodies, the basal forebrain, the dorsal and median raphe nuclei with floor and walls of the third and fourth ventricles, and the cerebellar vermis [44].

Finally, a continuity (or continuum) hypothesis has been proposed but still a matter of debates [44, 49, 56], see Fig. 31.2. The continuity hypothesis states that cognitive deficits in patients with AUD but with no Korsakoff syndrome and patients with AUD and Korsakoff syndrome lie a long continuum of mild to moderate deficits due to ethanol neurotoxicity [44]. However, this hypothesis does not fit with all clinical situations and has been proposed to become obsolete now [44].

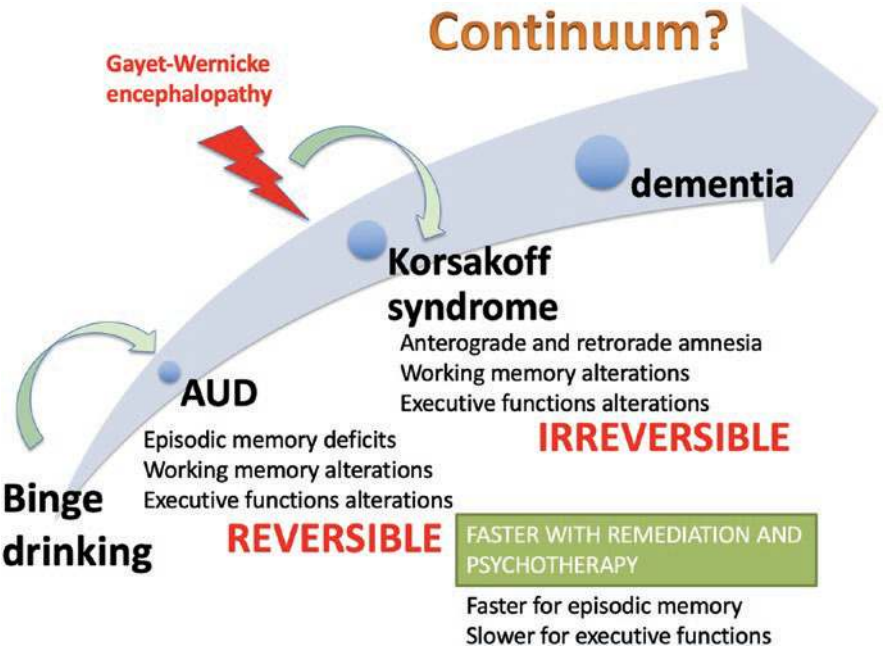

Fig. 31.2 The continuity hypothesis states that cognitive deficits in patients with AUD but with no Korsakoff syndrome and patients with AUD and Korsakoff syndrome lie a long continuum of mild to moderate deficits due to ethanol neurotoxicity. M Naassila

Recovery and Treatments

Some studies have suggested the existence of a subgroup of patients with AUD at risk to develop a Korsakoff syndrome on the basis of clinical signs of GWE, episodic memory performance and volumetric damage to the thalamus [57]. The evolution of the GWE (acute state, life threatening, confusion, incoherence, attentional deficits) has been proposed to be the following: 12% will display minor to no cognitive sequelae, 68% will display the Korsakoff syndrome (chronic state, clear consciousness, coherence) and 20% will die [58].

In patients with established GWE, parenteral thiamine 200–500 mg three times a day should be given for 3–5 days, followed by oral thiamine 250–1000 mg/day [59]. In patients with suspected GWE, parenteral thiamine 250–300 mg should be given two times a day for 3–5 days, followed by oral thiamine 250–300 mg/day [59].

There is currently little or no therapeutic management of patients with Korsakoff syndrome [44]. The priority is to stop drinking and maintain abstinence. No drug treatment is available for these patients once Korsakoff syndrome is established. The best treatment strategy remains prophylaxis with identification of patients with AUD at risk for developing Korsakoff syndrome and treatment with thiamine at the first sign of GWE [60]. While it is clear that thiamine injection improves the neurological signs observed during this acute episode, there is no evidence for an effect

of thiamine on cognitive recovery. Once the severe memory problems of Korsakoff syndrome have set in, neuropsychological treatment can be proposed. The goals of this treatment are to adjust the environment or to compensate for the disorders with external memory support or alternative learning methods.

A large number of studies have shown the possibility of a recovery, at least partial, of the cognitive impairment of patients with AUD with the complete cessation of alcohol intake. These studies have either compared groups of patients with AUD who have been abstinent for different durations (cross-sectional studies), or have followed up a group of patients who have finished their treatment (longitudinal studies). Studies on cognitive changes during a period of abstinence has examined memory, visuospatial or executive function. In general, the studies showed an improved performance with alcohol cessation, sometimes even reaching normalization in long-term abstinent patients [61, 62]. However, some cognitive problems have been described as persistent even after several years of abstinence [61]. Some authors suggest a deterioration of neuropsychological abilities in relapsing patients [62]. The duration of abstinence necessary to normalize cognitive functions is still poorly known, in particular because of the selectivity of cognitive recovery. Indeed, the reversibility of cognitive impairment could be different depending on the cognitive function studied, in relation to the macrostructural and microstructural recovery of the brain substrates involved. Age at alcohol cessation may particularly influence the cognitive recovery abilities of patients with AUD [39, 62]. Older patients with AUD would also have less opportunity for cognitive recovery due to reduced brain plasticity. Cognitive abilities of smoking patients with AUD would also recover less well than non-smokers. Longitudinal studies of patients with AUD have failed to demonstrate whether the presence of cognitive impairment at discharge from withdrawal might be predictive of longer-term treatment outcomes. One study demonstrated that the episodic memory and executive function performance of abstinent patients with AUD was normalized after 6 months of abstinence, whereas in relapsed patients, flexibility problems worsened [63]. These results suggest that when neuropsychological impairments are identified after withdrawal, deferring treatment until after a stay in an alcohol-free environment that promotes spontaneous recovery seems to be an appropriate treatment strategy. The effects of reduced consumption on cognitive recovery remain unknown.

Patients with AUD recently detoxified may have impaired episodic memory, decision-making abilities, executive impairments of metamemory abilities, learning of new complex information and motivation. These impairments hinder the management and limit the ability to maintain abstinence. It is essential to detect cognitive disorders in patients with AUD and to identify those with neurological complications in order to adapt the care and to implement cognitive remediation to promote brain and neuropsychological recovery.

Finally, some pharmacological interventions may be neuroprotective against the toxicity induced by alcohol withdrawal. About 50% of alcohol-dependent patients develop clinically relevant symptoms of withdrawal [64]. Multiple withdrawals may be particularly toxic for the brain of detoxified patients and this situation has been described as the withdrawal kindling model in which the increase in the

number of alcohol withdrawals may be associated with an increase in brain toxicity [65]. As brain damage is more severe after multiple withdrawals and the severity of withdrawal is predictive of relapse, it is essential to prevent the neurotoxicity of alcohol withdrawal [66, 67]. Since chronic alcohol consumption is associated with neuronal changes related to NMDA receptors, this neuroprotection may be particularly important in the treatment of alcohol use disorder [68, 69]. Acamprosate may reduce neuronal hyperexcitability, a phenomenon that occurs during acute withdrawal and prolonged abstinence from alcohol. Acamprosate is thought to act on the glutamatergic system as a partial co-agonist of NMDA receptors and an inhibitor of pre- and post-synaptic metabotropic glutamate receptors type 5 (mGluR5) [70, 71]. In addition to inhibiting the excitatory glutamatergic system, acamprosate is also thought to prevent neuronal hyperexcitability by facilitating the release of GABA, mediated by inhibition of presynaptic GABAB receptors and increased taurine release. Acamprosate could therefore also have neuroprotective effects [72, 73]. The inhibitory effect of acamprosate on glutamate release, and thus on the reduction of neuronal hyper-excitability in alcohol withdrawal, has been demonstrated both in humans [74] and in animals [71, 75]. In cell culture and organotypic brain slice models, acamprosate treatment reduces neuronal death induced by alcohol withdrawal [76–78].

Acamprosate is one of the standard treatments for maintaining abstinence in patients with AUD. The initiation of treatment is very often carried out at the beginning of alcohol withdrawal and no studies has yet investigated the potential benefit of initiating treatment before alcohol withdrawal.

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Part VI
Risk Factors for Alcohol Addiction and Lessons from Basic Science

Chapter 32
The Genetics of Alcohol Use Disorder

Josephin Wagner, Andrew S. Bell, Jeesun Jung, and Falk W. Lohoff

Abstract The genetic factors which underly Alcohol Use Disorder (AUD) risk and progression have long been the subject of intense research. Early familial studies established a genetic component by observing disease risk within families, while later linkage studies were able to locate genomic regions with some influence over disease incidence. Later, candidate gene association studies began to identify single nucleotide polymorphisms (SNPs) and pathways statistically associated with AUD and other drinking phenotype outcomes. The recent development of Genome Wide Association Studies (GWAS), which analyze common SNPs across the genome for association with AUD risk has resulted in robust associations with various SNPs. Newer methods, including methylome scanning techniques employing next neneration sequencing, and Mendelian randomization have enabled more precise estimation of the contribution from individual variants to AUD risk and progression. As study methodology improves, it has become increasingly clear that while AUD is a heterogenous and vastly polygenic disease, with dozens, if not hundreds of genes playing a role in AUD pathogenesis, certain genes are frequently observed to influence disease risk across several populations. Genes affecting the activity of the ethanol metabolic enzymes alcohol dehydrogenase and aldehyde dehydrogenase 2 are among the most common targets of genomic investigation into AUD, and variants in these genes are commonly found to play a role in AUD risk in a variety of populations. However, while decades of research have identified numerous candidate genes which influence AUD incidence between patient populations, no gene or group of genes has been identified which singlehandedly explains AUD risk at the individual level. The large number of genes found to associate with AUD suggest that AUD is an exceptional disease candidate for personalized medicine, and new

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pharmacological treatments should seek to address the genetic pathways identified in recent genomic research. Nonetheless, it is now clear that environmental and genetic factors as well as gene-environment interactions all play a role, and further research into the precise interactions of each factor will inform clinical decision making.

Keywords Alcohol Use Disorder · Alcohol · Addiction · Genomics · Genetics · Genome-Wide Association Study · Personalized medicine

Introduction

Alcohol Use Disorder (AUD) has long been theorized to have a heritable component, due to early observations that AUD and other addictive disorders are common in families [1]. Documentation of the observation that certain family or social groups appear predisposed to addictive disorders appeared as early as in ancient Greece [2]. It was not until recent decades, however, that the heritability of AUD became a topic of serious empirical study. Until recently, heritability in general has been primarily assessed through twin studies and has been limited to simple observations of differences in AUD risk between families. Furthermore, modern research has identified a host of non-genetic considerations that can influence family-specific behavioral predispositions, including environmental and social factors, which complicates the concept of a purely "genetic" explanation for AUD heritability. Further, advances in genetic research methodology such as the Human Genome Project enabled the proliferation of genomic techniques for assessing the contribution of specific genes to certain outcomes, including AUD and AUD-associated mechanistic pathways [3].

Identifying the genetic underpinnings of a disease and how they can be modulated by pharmacological and/or lifestyle means has become a pillar of modern pharmacological and medical research. Since the early days of pre-sequencing genetic study, research methods have been devised to investigate the source of disease heritability, including AUD. An overview of significant advances in genetic investigation which have played a role in AUD pathogenetic research is provided in Fig. 32.1. AUD is a complex disorder with a wide range of genetic factors having been identified in each phase of disease, from risk to enzyme-mediated symptom severity to drug response. Despite this, much of AUD's genetic profile is uncertain, and in particular the search for a single "AUD gene" or group of genes has so far proved fruitless. It is therefore crucial for the advance of clinical treatment to understand in detail the intertwining genetic pathways implicated in AUD and to develop drugs capable of acting on pathways identified by genetic and genomic methods in order to design effective treatment regimens for as many patients as possible [4].

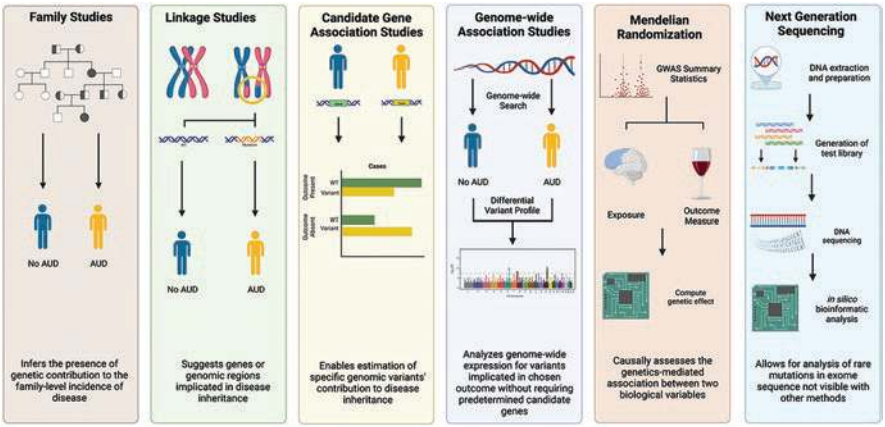

Fig. 32.1 Overview of selected study methods which have been used for genetic research into AUD. Figure created using Biorender.com

Early Familial Association Studies and Twin Studies

Early studies reported that relatives of individuals with AUD exhibited an increased risk for AUD themselves [2]. It was initially unknown to what extent this was the result of genetic or environmental effects. In the 1970's, the potential role of genetics in determining AUD predisposition was first assessed in a number of twin studies. In 1972, it was reported that harmful alcohol-associated phenotypes among offspring were more closely related to AUD diagnosis in a biological parent than an adoptive or foster parent, suggesting that AUD development is at least in part mediated by genetics [5]. In 1981, AUD incidence was studied in 862 Swedish men adopted at an early age in what came to be known as the Stockholm Adoption Study (SAS) [6]. The SAS found that AUD has both genetic and environmental causes, and was one of the first twin studies to identify at least two subgroup of AUD patients, characterized by patterns of parental AUD incidence [6]. The study, which was replicated in 1996, noted a significantly higher rate of "type 2" AUD among adopted sons irrespective of postnatal environment, suggesting that there was a strong genetic component to AUD heritance and helping to usher in the modern era of genetic AUD research [7, 8]. Indeed, later studies found that children of individuals with AUD, even those adopted into other families as infants, demonstrated at least a threefold increase in risk for AUD themselves [9–11].

Later studies of monozygotic and dizygotic twins provided further evidence of strong genetic factors underlying AUD development and prognosis. The use of both mono- and dizygotic twins allows heritability to be estimated while controlling for potential influence of shared environment using structural equation modeling. For instance, Falconer's formula (Eq. 32.1) is used in twin studies to estimate the contribution of genetic and environmental factors to total variation in a particular trait by assuming that environmental contribution in monozygotic and dizygotic twins is

equal. In this way, the obvious confound of shared upbringing environments is addressed, and more valid quantitative assessments can be made.

$$H_b^2 = 2\left(r_m - r_d\right) \tag{32.1}$$

Falconer's formula for heritability, where H_b^2 is the total (broad-sense) heritability for a trait, r_m is the twin correlation for monozygotic twins, and r_d is the twin correlation for dizygotic twins

The Australian OZALC twin study of AUD found a stronger association of alcohol dependence phenotypes between monozygotic twins compared to dizygotic twins and reported a heritability estimate for AUD of 64%, with no between-sex differences reported [12]. More recent twin studies have generally reported heritability ranges between 40% and 70% and have generally not identified significant differences in genetic AUD risk between males and females, despite incomplete overlap of genetic sources of AUD vulnerability between sexes [13–16]. Additionally, underlying symptoms and phenotypes associated with AUD have been independently assessed, with heritability estimates as high as 53% for certain severe phenotypes [17]. While these findings have demonstrated that genetics plays a major role in AUD risk, gene-environment interactions or rare somatic de novo mutations in the genome may contribute to the outstanding portion of heritability not explained by genetics or environmental exposures alone [18].

Linkage Studies

While family, adoption and twin studies provided early evidence for the existence of a genetic component underlying AUD risk, they were unable to identify specific genes or pathways involved in AUD heritability. The early desire to identify key genes or alleles motivated the use of genetic linkage studies in AUD patients. In general, linkage studies identify broad regions of an individual genome associated with a phenotype of interest; thus, linkage studies made it possible to identify genomic regions associated with significantly increased risk of AUD in certain populations. These studies make use of genetic linkage, the phenomenon in which two genetic markers on a single chromosome have a lower probability of recombination during meiosis, and are therefore increasingly inherited together more often than would be expected by chance the closer physical proximity they are located in within the genome [19]. Linkage studies typically use families with a high incidence of disease such that chromosomal regions are likely to exhibit common genetic risk variants. Linkage studies are also generally more effective for studying rare autosomal dominant diseases with relatively high penetrance, as these disorders have a high degree of genetic heritability and shared risk alleles.

Linkage studies on alcohol dependence and AUD have found chromosomal regions associated with AUD incidence in several populations. The Collaborative Study on the Genetics of Alcoholism (COGA) mapped a range of genetic variants

associated with AUD [20, 21]. The COGA reported a region on chromosome 4q broadly associated with AUD risk, a finding which was also reported in a sib-pair study of individuals from a Southwest Native American tribe, which also identified a region on chromosome 11p strongly associated with AUD incidence [21, 22]. Among others, this region contains genes that encode isoforms of alcohol dehydrogenase, an enzyme now known to be intimately linked to alcohol metabolism and AUD risk [23]. Later linkage studies have implicated other chromosomal regions. Specifically, regions encoding both the muscarinic acetylcholine receptor M2 (CHRM2) on chromosome 7 [24] and GABA-A receptors [25], which were also identified in the COGA and Southwest Native American studies, have been identified within primary linkage sites corresponding to AUD. The results of early linkage analyses suggested genomic regions within which to search for specific genes capable of influencing AUD pathogenesis.

Candidate Gene Association Studies and the Identification of Gene Targets

While linkage studies provided a wealth of potential regions observed to be associated with genetic AUD heritability, and suggested gene locations with potential effects on disease risk, they were unable to identify specific genes and alleles underlying these observations. Candidate gene association studies (CGAS) were designed to overcome this limitation. CGAS evaluate the association between a predetermined set of gene loci (i.e., genes associated with a disease outcome of choice) and disease occurrence in a case/control random sample, leveraging the availability of large datasets to make population-level genetic inferences [26]. Fundamentally, this is achieved by comparing allele frequencies between cases and controls, and test for deviation from random. Thus, CGAS were among the first studies to identify specific alleles with influential effects on AUD pathogenesis and have more clearly delineated the genetic pathways reported in earlier linkage studies.

Based on previous linkage study findings, follow-up fine mapping and candidate gene studies have demonstrated associations between AUD and the genes encoding alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*), both crucial enzymes which regulate the metabolism of ethanol in the liver and digestive tract [27]. Ethanol is oxidized to acetaldehyde by ADH, while ALDH catalyzes the subsequent oxidation of acetaldehyde to acetate. Acetaldehyde is a toxic intermediate, and significant buildup of acetaldehyde causes several aversive reactions, including tachycardia, nausea, and flushing syndrome. Disruptions to the normal ethanol metabolic pathway, particularly those that result in the accumulation of acetaldehyde, are associated with increased severity of these aversive effects and therefore lower incidence of AUD [27]. Those disruptions that promote the buildup of acetaldehyde, especially those that increase the rate of oxidation of ethanol or decrease the rate of oxidation of acetaldehyde, therefore act as negative regulators of drinking behavior.

In the liver, many isoforms of the *ADH* gene are present that differentially impact alcohol metabolism. *ADH* isoforms ADH1A, ADH1B, ADH1C, and ADH4–7 have been identified as the primary isoforms involved in hepatic ethanol oxidation. The majority of ethanol oxidation is usually carried out by ADH1B. However, the ADH1B*2 single nucleotide polymorphism (SNP) [rs1229984 (Arg48His)] oxidizes alcohol much faster than the typical ADH1B*1 variant [27]. ADH1B*2 has been demonstrated to have a protective effect on AUD risk, likely due to the aversive effects of an increased rate of formation of acetaldehyde after drinking, and is known to be more common in East Asian populations than in groups of other ancestry [28–31]. ADH1B minor alleles, especially rs1229984, have also been shown to reduce the risk for AUD in population groups of other ancestry, including Native American, European, and African American samples [32, 33].

While ADH1B has been the subject of much research for its marked variation across populations and strong effect on alcohol metabolism, other ADH isoforms, in particular ADH1A and ADH1C, have also been identified as drivers of genetic variation in alcohol consumption behavior and AUD. At lower concentrations, these isoforms contribute to overall ethanol metabolism. Studies have reported that the ADH1C*2 SNP rs698 (Ile350Val) is associated with a decreased rate of alcohol metabolism and impairment of reaction time at lower alcohol concentration [34]. Individuals expressing such variants are prone to more severe adverse effects after drinking, and are consequently at less risk of developing AUD [30, 35]. Studies of individuals from several populations have identified certain variants associated with AUD risk in a geography- or ancestry-dependent manner. For instance, the variant ADH1B*3 was found to have a protective effect against AUD in Native American individuals [36].

CGAS of AUD have yielded a set of genes involved in AUD risk and severity and many endophenotypes associated with AUD. At least 20 genes identified by COGA analyses have been associated with AUD or alcohol phenotypes [37]. While enzyme gene polymorphisms involved in ethanol metabolic pathways are among the most common candidate genes identified in COGA, genetic variants in neurotransmitter pathways have also been reported to be significantly associated with AUD risk [27]. Among others, the glutamate receptor (GIRK1), gamma-aminobutyric acid receptor (GABA-A), D2 dopamine receptor (DRD2), dopamine transporter (SLC6A3), serotonin transporter (SLC6A4), tryptophan hydroxylase 1 (TPH1), catechol-O-methyltransferase (COMT), cholinergic muscarinic receptor (CHRM2), and u-opioid receptor (OPRM1) have each been found in candidate gene studies to associate with AUD incidence [24, 38–46].

CGAS provided initial evidence of specific genes and variants potentially responsible for the heritability patterns observed in family and linkage studies. However, a number of inherent concerns are associated with this methodology. It became increasingly recognized that the risk alleles identified typically conferred small estimated effect sizes, which combined with low statistical power resulted in a high false discovery rate (i.e., nominally significant results that were the result of random variation). The relationship between a risk allele's observed frequency and the magnitude of its effect is summarized in Fig. 32.2. There is also concern regarding the potential for ethnic stratification, i.e., that allele frequencies often differ between cases and controls not because of an association with disease, but because of

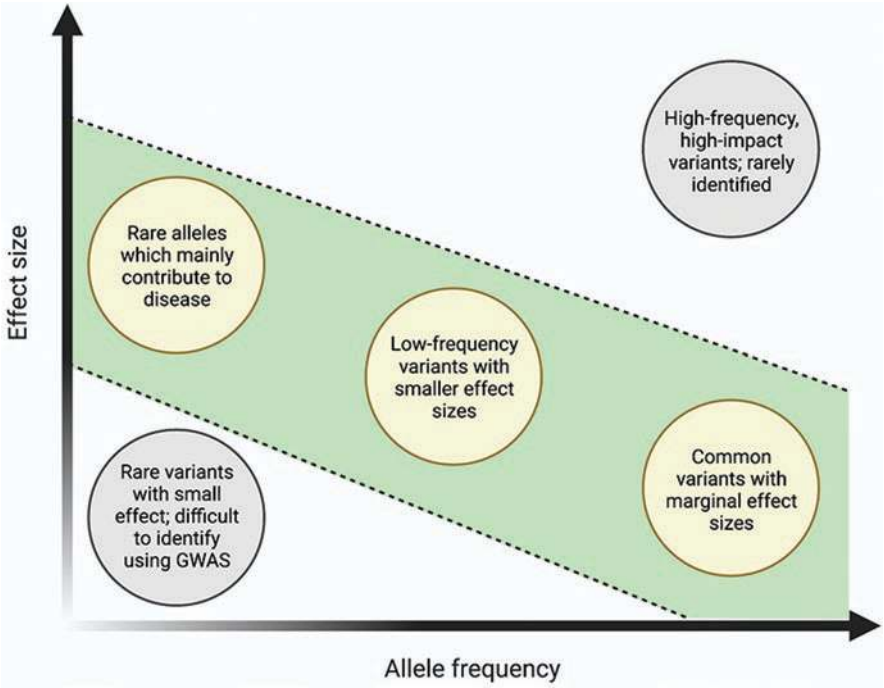

Fig. 32.2 Simplified scheme showing the effect size of a generic risk allele on a disease outcome as a function of the frequency of observation in candiate gene or genome-wide association studies. Adapted from Manolio et al. [47] Figure created using Biorender.com

different ethnic composition of cases and controls. Finally, clinical and genetic heterogeneity between individuals categorized as having "AUD" became increasingly realized. Collectively, these factors have posed a challenge for replication and interpretation of candidate gene studies. The desire to overcome these limitations has led to the development of newer genomic methods for quantifying variant-level contribution to AUD pathogenesis.

Genome-Wide Association Studies

One inherent issue with candidate gene studies that poses a challenge for disease and drug research is the requirement of *a priori* hypotheses. Specifically, candidate gene studies require prior knowledge of the underlying neurobiology of the risk variants assessed in the study. These methods cannot similarly gauge unknown variants or pathways and are thus limited to areas identified by prior studies. In contrast, Genome-Wide Association Studies (GWAS) are able to analyze hundreds of thousands to millions of SNPs across the entire genome to identify differences in genetic variants between case-controlled individuals, without requiring an initial selection of candidate genes. In contrast to the limited scope of a CGAS, a GWAS could

theoretically identify every genetic variant associated with a disease or phenotype, and can evaluate genetic risk variants in complex or polygenetic disorders wherein very small effect sizes would be expected. In practice, this is unlikely, given genomic heterogeneity between patients and the high statistical power required for such findings. GWAS have recently come into focus as a useful tool for assessing the genetic contribution from a number of gene variants to mental illness risk, including that for schizophrenia and bipolar disorder [48–50]. GWAS have also been used in patient samples with AUD.

Among the first GWAS to demonstrate genome-wide significance of individual variants on alcohol dependence identified two intergenic loci on chromosome 2q35, rs7590720 and rs1344694, near the peroxisomal trans-2-enoyl-CoA reductase (*PECR*) gene; PECR catalyzes the reduction of medium chain enoyl-CoAs to saturated acyl-CoAs when triglycerides are mobilized to produce energy [51]. Chronic alcohol use is associated with increased serum triglyceride concentration, and *PECR* expression patterns may thus play a role in alcohol-mediated lipid metabolic disorder [52]. An internal replication study identified 15 SNPs showing association with at least nominal significance (i.e., prior to correction for multiple-testing bias), including rs11640875 in cadherin 13 (*CDH13*), rs1614972 in *ADH1C*, and rs13273672 in the GATA binding protein 4 (*GATA4*) [51]. While this study was the first GWAS to demonstrate associations between GATA4 and AUD, prior studies have found that GATA4 is associated with alcohol dependence and relapse risk, as well as limbic gray matter volume, which is itself predictive of alcohol relapse risk [53, 54]. However, later replication studies failed to confirm this association using data from an independent sample [55].

GWAS have identified many other gene targets displaying complex relationships with AUD incidence. For instance, peroxisome proliferator-activated receptors (PPARs) have been associated with neuroinflammatory processes known to be involved in AUD etiology [56]. Reanalysis of GWAS data identified SNPs in PPARA and PPARG associated with alcohol withdrawal and PPARGC1A associated with alcohol dependence in mouse and human samples, suggesting a potential role for PPAR agonists in pharmacological interventions for alcohol-associated liver disease (ALD), in which PPARs have been known to play a role for some time [57, 58]. In the past decade, GWAS studies of AUD have proliferated, and many genes have been identified in a range of populations [27]. In German and German-descent individuals, GWAS have reported associations between *PECR, CDH13, ADH1C, GATA4, ALDH2, ADH1* [51, 59]. In Korean individuals, C12ORF24, ALDH2, ADH1B, ADH7, have been associated with AUD. [60, 61] Studies of European and European-American individuals have identified, among many others, SNPs in CDH11, CDH13, ANKRD, CYTL1, MARK1, DDX6, KIAA1409, SEMA3E, AUTS2, C15ORF53 [46, 62–66]. GWAS meta analyses of combined European-American and African-American individuals have reported effects of genes in the KEGG pathway, *GABRA2, PKNOX2, ADH1B, ADH1C, EDNRB, TPARP, CYFIP2, THEMIS, PSG11, KIAAA0040, THSD7B, NRD1, PTP4A1, SH3BP5, NR2C2, PLGLB2, NKAIN1-SERINC2, IPO11-HTR1A,* and genes located between *MTIF2* and *CCDC88A* on chromosome 2 [65, 67–73]. Meanwhile,

ALDH2 was associated with AUD in Chinese and Japanese individuals, while *ADH1B* was associated with AUD only in Japanese individuals [74, 75].

While early GWAS studies were limited by small sample sizes and consequently low statistical power, efforts have been made to increase predictive power by analyzing genome-wide SNPs from large datasets, including those from the COGA, the Australian twin-family study of alcohol use disorder (OZALC), the Million Veteran Program, the Psychiatric Genomics Consortium, and the Study of Addiction: Genetics and Environment (SAGE) dataset [27, 76, 77]. Early GWAS of the COGA dataset were unable to identify single SNPs with genome-wide significance but identified a gene cluster on chromosome 11 associated with alcohol dependence [78]. A meta-analysis and replication study of 11,120 SNPs sourced from the COGA, SAGE, and OZALC samples identified three novel loci and replicated previous findings of an association between *PKNOX2* and alcohol dependence [65]. However, later studies and meta-analyses have failed to identify variants with genome-wide significance with data from COGA, OZALC, or SAGE [65, 67, 68, 70, 72, 73].

In general, GWAS investigations of AUD have supported the findings of earlier methodologies, including linkage studies, family studies, and candidate gene studies; SNPs in genes encoding alcohol metabolizing enzymes are among the variants most often found to associate with AUD [32]. In particular, SNPs located on the *ADH* and *ALDH* genes are among the variants with the greatest effect on AUD risk. A GWAS of AUD in German individuals identified the variant rs1789891 in the *ADH* gene cluster, a SNP which was also found to be in linkage disequilibrium with the functional variant *ADH1C* (Arg272Gln) [59]. The *ADH* gene cluster was also identified in a GWAS using a Korean sample, which found multiple nominally significant SNPs in the cluster located on chromosome 4q22-q23; genome-wide significance for rs1442492 and rs10516441 in *ADH7* and rs671 in *ALDH2* were also reported [61]. Other studies of East Asian populations have similarly found that the *ALDH2*2* variant rs671 (Glu504Lys) is associated with a decreased risk of AUD in these individuals [59, 75, 79]. There is also evidence that certain variants or genes may be protective in multiple distinct populations of different ancestry. For instance, GWAS studies have found that the *ADH1B*2* SNP rs1229984 is protective against AUD development in East Asians, associating both with lowered alcohol use and more severe adverse effects associated with drinking; recent genomic studies have also reported an increased risk of alcohol-related cancer among rs1229984 carriers in East Asian populations [80, 81]. Other studies have found that rs1229984 decreases the risk of AUD in European-Americans, while the analogous ADH1B SNP rs1789882 (Arg369Cyc) decreases the risk of AUD in African-Americans [61, 69, 75].

One of the greatest strengths of GWAS has been the ability to quickly and efficiently identify a huge number of SNPs which are associated with disease outcomes, and their proliferation has led to a surge in the understanding of genetic pathways involved in AUD pathogenesis. In the past 5 years alone, dozens of GWAS studies have shed light on new, complex factors involved in AUD risk, a selection of which is summarized in Table 32.1. However, the wide scope and relatively small

Table 32.1 GWAS summary

Author	Year	Outcome measure(s)	Main finding	Sample size (*n*)	Link
Walters et al.	2018	17 neuropsychiatric outcomes	Alcohol depdence associated with psychiatric disease, cigarette/cannabis use, low socioeconomix status, poor behavioral health; differences in ADH1B variant expression between European and African American participants	52,848	https://www.nature.com/articles/s41593-018-0275-1
Mallard et al.	2021	AUD	A novel weighted AUD severity score correlated with psychiatric disorder and not with positive health outcomes previously associated with certain AUDIT criteria; PRS associated with poor mental health only with AUD diagnosis	160,824	https://ajp.psychiatryonline.org/doi/10.1176/appi.ajp.2020.20091390
Kranzler et al.	2019	AUD and related disorders	Large genetic overlaps in genomic expression profiles between PRS for alcohol consumption and AUD; heavy drinking is a key risk factor for AUD but does not itself cause it	274,424	https://www.nature.com/articles/s41467-019-09480-8
Sanchez-Roige et al.	2019	AUD	Loci in *JCAD, SLC39A13, ADH1B, ADH1C, KLB,* and *GCKR* associated with AUD; alcohol consumption and problematic drinking exhibited differential expression	20,328	https://pubmed.ncbi.nlm.nih.gov/30336701/
Marees et al.	2019	Socioeconomic status, substance use disorders, psychiatric disorders, psychological/personality traits	Frequency and quantity of alcohol consumption display generally similar health marker associations but opposite associations with socioeconomic status-related phenotypes	438,308 (consumption) 307,098 (quantity)	https://pubmed.ncbi.nlm.nih.gov/30874500/
Gerlernter et al.	2019	Alcohol dependence	Identified five novel risk loci including *ADH1B* variants in European American and African American invidiauls; *CRHR1* particularly associated	143,965	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6919570/
Gerlerneter et al.	2022	AUD	Maximum habitual alcohol intake closely aligns with other predictive drinking phenotypes; supported a two-factor mdoel in which drinking frequency and problematic drinking act individually to influence outcomes	247,755	https://www.medrxiv.org/content/10.1101/2022.05.02.22274580v1

Liu et al.	2019	N/A	Smoking and alcohol use share a large amount of risk variants, but display opposite asssociations with negative health outcomes; nicotinic, dopaminergic, and glutamatergic systems involved in smoking and AUD	337,334–1,232,091	https://pubmed.ncbi.nlm.nih.gov/30643251/
Kapoor et al.	2021	AUD, drinks per week	rs56030824 on chromosome 11 reduces immune activation and AUD risk; *MAPT* is specifically associated with drinks per week; high overlaps with genes involved in neurodegenerative disorders	739,353	https://www.nature.com/articles/s41467-021-25392-y
Linner et al.	2021	Self-regulative disorders	AUD and other disorders involving self-regulation have a shared genetic underpinning which is independently associated with more than 500 genetic loci	1,373,240	https://www.nature.com/articles/s41593-021-00908-3#MOESM3
Luo et al.	2019	Epigenetic aging	AUD is associated with epigenetic age acceleration; disease severity further accelerates epigenetic aging	532	https://pubmed.ncbi.nlm.nih.gov/31466081/
Lohoff et al.	2021	AUD	Differential methylation in genome regions encoding glucocorticoid signaling and inflammation-related proteins are associated with alcohol use behaviors	625 (AUD) 4798 (replication)	https://pubmed.ncbi.nlm.nih.gov/32398718/
Munn-Chernoff et al.	2021	Substance use and eating disorders	AUD is positively associated with other substance abuse and eating disorders; some of the association is explained by intermediate association with major depressive disorder	2400–537,000	https://pubmed.ncbi.nlm.nih.gov/32064741/
Wetherill et al.	2019	Alcohol or illicit drug dependence (ANYDEP)	Novel loci associated with individual differences in reward-related ventral striatum activity contribute to ANYDEP and alcohol dependence risk	10,218	https://pubmed.ncbi.nlm.nih.gov/31099175/
Zhou et al.	2020	Problematic alcohol use (PAU)	PAU was genetically correlated with substance use and psychiatric traits; genetic heritability of PAU was enriched in brain and in conserved and regulatory genomic regions	435,563	https://pubmed.ncbi.nlm.nih.gov/32451486/
Meyers et al.	2021	AUD	Variants associated with posterior interhemispheric low theta EEG coherence were also associatyed with AUD; interhemispheric theta EEG coherence may be able to proxy AUD-associated neural connectivity disregulation	8810	https://pubmed.ncbi.nlm.nih.gov/32433515/
Sun et al.	2019	Alcohol dependence	Variants in ALDH2 (rs671) and ADH1B (rs1229984) significantly associated with alcohol dependence in a Han Chinese cohort	3381	https://www.nature.com/articles/s41398-019-0586-3

sample size of many GWAS studies in relation to the small genetic effects most commonly identified also poses a fundamental limit on the statistical power of GWAS predictions, and the heritability attributable to collective SNP associations that survive multiple testing correction in GWAS studies is significantly less than heritability estimates from corresponding family studies, a phenomenon known as the "missing heritability" problem [32, 47]. It is known that a significant portion of the genetic contribution to AUD risk is attributable to common SNPs, with one analysis finding that they may represent as much as 33% of total genetic AUD risk and at least 16% and 18% for alcohol dependence and consumption, respectively [82, 83]. To overcome this limitation, newer methods have been designed which make up for the lost power when conducting large GWAS analyses.

Next-Generation Sequencing and Whole Genome Sequencing

While once a prohibitively expensive and time-consuming process, modern technology has enabled rapid and reliable genome sequencing as a means of obtaining research data for genetic and genomic investigations into disease phenotypes. Next-Generation Sequencing (NGS) has significantly reduced the barriers to genome sequencing, enabling a proliferation in genetic and genomic research. Traditional Sanger sequencing methods were originally limited to very small genomes comprised of at most ~5000 bases, and while later improvements and computer programs increased the maximum base sequence size about tenfold, until recently Sanger sequencing required specialized and expensive equipment and infrastructure [84]. It was not until the development of next-generation, or "massively parallel" sequencing methods capable of simultaneously analyzing up to billions of templates simultaneously, that genome sequencing became an accessible research method for use in studies of disease phenotypes such as AUD [85].

NGS methods include targeted deep sequencing, whole-exome sequencing (WES), and whole-genome sequencing (WGS) [86]. Each has contributed significantly to the study of precision genetics. WES systematically scans an individual's entire set of coding DNA regions, or exome, for all known genes. Using high-throughput sequencing methods, WES generates a library of every variant present in an individual exome. While WES analyzes only the exome, comprising about 90 million nucleotides, or 3% of the entire genome, it assesses more than 95% of all exons and as much as 85% of all disease-associated genes [87–89]. Targeted deep sequencing may also be used to provide exceptionally high-resolution genomic description with high statistical power even with smaller sample sizes than low coverage whole genome or exome sequencing [90]. However, deep sequencing requires significantly greater overall sequencing effort [90, 91]. Targeted resequencing of smaller regions containing up to a few dozen genes can be used to accurately detect low-frequency or rare gene variants without drastic increases in resource use [91].

Compared to analytic methods employing existing datasets, including GWAS, and less inclusive sequencing techniques such as WES, WGS is more expensive and

time consuming but is more likely to identify functional uncommon mutations in exome sequences and is particularly useful for identifying rare recessive mutations [92]. Further, whole genome/exome sequencing has successfully identified genetic mutations associated with a variety of psychiatric disorders, including autism spectrum disorders, schizophrenia, depression, bipolar disorder, and others [93]. WGS studies also have the benefit of potentially identifying uncommon variants not previously found using conventional GWAS methods. For example, rare variants of the K2P channel gene *KCNK2* and rare missense and splice-site variants in the pro-inflammatory mediator gene PDE4C were associated with incidence of alcohol-related life events reflecting AUD severity in both Native American and European American individuals [94]. Additionally, a NAF1-FSTL5 intergenic variant and an FSTL5 variant were associated with alcohol-related life events in both samples, while other genes in the serine/threonine protein kinase and interleukin subunit families, and long non-coding RNA sequences, were associated with Native American or European American samples, respectively [94].

NGS methodology holds great promise for identifying novel genes previously inaccessible to researchers, as well as identify currently unknown variants potentially driving ancestry-related and ethnic differences in AUD risk and severity. However, this technology is still new, and studies employing WGS and NGS broadly are rare in the field of addiction research. Future studies identifying further unexplored genomic regions or variants with influence on AUD development in specific patient populations in order to elucidate the impact that WGS may have on research into AUD risk and heritability.

Polygenic Risk Scores

Consistent findings of AUD heritability and a large number specific genes with an identified etiological role suggest that AUD is highly polygenic; while a great number of genes may contribute to patterns of AUD heritability and symptom severity, the specific genetic pathways and variants appear to differ between ethnic and patient populations, and likely also between individuals within those populations. While CGAS and GWAS have been able to identify some variants associated with AUD and alcohol dependence, the missing heritability confound limits the clinical significance of any one of these variants. To overcome this limitation, polygenic risk scores (PRS) were developed to aggregate genetic liability across many variants, each with a nominal level of association, in an effort to establish tools for assessing individual genetic AUD risk. To develop a polygenic risk score, a GWAS is conducted in a discovery sample; using a threshold p-value, a risk profile score equal to the sum of the count of risk alleles for one subject weighted by their effect sizes in the discovery sample, is generated for each subject in a target sample. The score is regressed to evaluate its association with the phenotype of interest and the proportion of variance (R^2) explained by the score [95]. Importantly, however, while the reliance on a strict ($p < \sim 10^{-8}$) threshold p-value may make type I errors rare, it may

also bias results toward type II errors, excluding SNPs with marginally lower significance but which may still contribute to the observed genetic variance.

Polygenic risk scores have both increased the predictive value of SNPs identified in GWAS studies and enabled the discrimination between variant-level sources of interpopulation differences in genetic AUD risk. For instance, a polygenic risk score developed by Lai et al. used 858 variants from 410 genes, each significantly associated with AUD in African American and European American subjects. Compared to the bottom decile, those in the top PRS decile were almost twice as likely to be diagnosed with AUD. [96] Since the first score to be developed specifically for alcohol dependence in 2012 [95], several risk scores have been created for the study of the genetics of AUD and related disorders, each associating significantly with AUD incidence and confirming the polygenetic nature of AUD and other substance use disorders [83, 97–101].

While PRS have had some success in assessing individual-level risk of AUD, most studies have reported very small R^2 values, with several explaining less than 1% of total variance [100–102]. Thus, studies using PRS have allowed for the aggregation of many individual risk factors into a cohesive unit for proxying genetic risk, but have thus far been unable to fully solve the missing heritability problem. Some authors have suggested that with much larger sample sizes, the variance attributed to PRS would rise considerably; however, there are currently no datasets containing alcohol-related sample statistics from which a risk score of this magnitude could be derived [103]. Nonetheless, PRS have played an important role in the study of the genetics of AUD, and while currently their value for predicting AUD is limited, they are useful associative tools for studying the association of AUD with genetic factors and other phenotypes [103, 104].

Mendelian Randomization

Research into the genetic factors that underly both AUD heritability as well as AUD's complex associations with other disease phenotypes, including other substance abuse and psychiatric disorders with potential common neurological pathways and metabolic disorders influences by AUD, such as hyperlipidemia and cardiovascular disease (CVD), has made use of GWAS studies and the variants discovered by earlier candidate gene studies. In particular, Mendelian randomization (MR) has emerged as useful tool for studying these associations. MR is a genetic analytical tool which uses genetic variants as instrumental variables to explore causal relationships between exposures and phenotypic outcomes, using summary statistics taken from large GWAS databases [105]. By using variants strongly associated with instrumental variables in relatively large patient samples, MR can effectively estimate the causal effect of modifying an exposure on an outcome. For example, an MR assessing alcohol consumption may use as an outcome measure a patient's average alcoholic drinks per week. If researchers sought to estimate the effect of subcortical brain structure on average alcohol consumption, either

with an *a* priori directional hypothesis or as a bidirectional study, MR can, and has been used to examine this relationship [106]. A key advantage of MR derives from the fact that germline variants are randomly assorted during meiosis at the beginning of life and are thus not influenced by lifestyle or environmental factors, effectively allowing MR to naturally proxy a randomized controlled trial in order to assess the causal relationships between two biological variables and reducing susceptibility to methodological confounds or reverse causation [107].

In alcohol research, MR has been used both to identify factors exerting causal influence on alcohol as well as those phenotypes for which alcohol use has a causal role. Among others, it has been shown that alcohol consumption levels, global cortical thickness, and educational attainment each display causal associations with AUD pathogenesis [104, 106, 108]. An even greater number of studies have chronicled the causal associations between alcohol misuse and a wide range of metabolic and biological measures. MR studies have found that GWAS measures of chronic alcohol use are associated with hypertension, hyperlipidemia, early-onset Alzheimer's disease, type 2 diabetes, age-related macular degeneration, and shorter telomere length [109–113]. MR has also been used, with mixed results, to explore the complex and often unclear relationship between varying levels of alcohol use and cardiovascular risk [114–116].

Among the advantages of MR as a tool for studying the genetics underlying AUD and alcohol-associated disease is the relative efficiency of MR studies, which use summary statistics sourced from pre-existing GWAS datasets. As a result, MR studies can rapidly estimate the possible causal relationship between AUD, alcohol use, binge drinking, and several other alcohol phenotypes with a vast number of biological outcomes. This has led to a continuing surge in studies connecting alcohol with a new and growing number of behavioral and biological outcome measures. However, MR's reliance on pre-existing data can also limit the scope of investigations. Specifically, outcome measures for which no GWAS summary statistics are available or for which sample sizes are prohibitively small, which limits statistical power, may not be possible to assess using MR and related methods. The growing interest in using MR to identify clinically relevant lifestyle or environmental factors that can influence an individual's predisposition to AUD highlights the need for more recent and larger consortium datasets with alcohol and metabolic phenotype data.

Future Directions in the Genetics of AUD: Working Towards Personalized Medicine

Decades of research have brought to light numerous genetic pathways with complex and varied effects on AUD risk and pathogenesis. As the genetic underpinnings of AUD and other substance use disorders have become more apparent, new opportunities for pharmacological treatment of AUD are emerging. However, despite the proliferation of studies into the genetic factors underlying AUD and potential

druggable genes, to date only three FDA approved medications exist for the treatment of AUD—disulfiram, acamprosate and naltrexone [117]. Importantly, while these medications can reduce drinking for certain patients, as many as 40% to 70% of individuals taking naltrexone or acamprosate fail to exhibit an efficacious drug response [118–120]. While several other drugs are used off-label to treat AUD, and several others are in various stages of clinical development, many of these also display limited effectiveness, and none have been demonstrated to systematically reduce AUD symptoms for wide population groups, with the possible exception of topiramate [121–123]. Until such time as medication becomes available, which is capable of altering drinking behavior in AUD patients, further genomic investigation into AUD, and in particular the distinct neurocognitive and genetic pathways that are responsible for the considerable heterogeneity in patient presentation, may help to elucidate novel treatment mechanisms which are most likely to be effective for specific patients.

As genetic research in the psychiatric sphere has developed, new frameworks for analyzing genetic variation between individuals and populations have emerged as research tools. While GWAS have contributed greatly to the understanding of genetic variants associated with AUD pathology and risk, multi-omics approaches, including epigenome-wide association studies (EWAS), phenome-wide association studies (PheWAS), and proteome-wide association studies (PWAS), among other tools, have been designed to explore all manifestations of genetic influence under a combined framework. The integration of distinct yet interrelated domains and the proliferation of biobanks and large consortium datasets have enabled a growing number of high-power analyses of psychiatric illnesses, including alcohol [104, 106, 124]. As the size and scope of large datasets increases, it will be all the more important to leverage their statistical power to explore these multi-omic relationships to develop more effective medications capable of addressing the needs of specific patients.

Personalized medicine refers to the idea that an individual's specific genomic expression patterns can be used to predict scientifically which specific medications would be most likely to have a beneficial effect and least likely to have any adverse outcomes for an individual patient [4]. Personalized medicine draws on the results of genomic investigations identifying specific variants or expression patterns associated with biological variables such as drug response, reported adverse outcomes, and disease and comorbidity risk. As the volume of research into individual genome-level associations between AUD risk variants and biological outcomes increases, AUD has become a leading candidate for personalized medicine, and a growing number of researchers have proposed applying the concept of personalized medicine to the clinical treatment of AUD patients [117, 125–128]. However, there remains a great need for data from studies with much larger sample sizes and with more diverse outcome measures, including detailed drinking assessments, in order for GWAS and MR studies to more accurately visualize the factors underlying AUD risk and biological consequences.

Personalized medicine offers promise for a new approach to AUD treatment which emphasizes the heterogeneity of the disease and works to adapt selection of

pharmacological interventions to individual-level differences in disease presentation such as to ensure the highest likelihood of success for every patient [117, 128]. In order to account for the considerable heterogeneity among AUD patients, however, it is necessary to fully explore the genomic variation in wide and diverse population groups of AUD patients, and to characterize not only drug interactions, but neuropsychological processes underlying disease severity. Only then will the possibility that a clinician can draw upon an individual's genome to make prescription decisions become reality.

Special Considerations in the Genetics of AUD

Like many psychiatric disorders, AUD is extremely heterogenetic, and there is a large degree of difference in both the reported symptoms and underlying neurobiology between AUD subjects. The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) uses 11 criteria assessing excessive alcohol use, alcohol abuse, and alcohol dependence to diagnose AUD in patients; these criteria, which encompass drinking behavior alone, demonstrate considerable differences even between patients with identical diagnoses [27]. The DSM-V also classified AUD severity along a continuous scale for the first time, removing the less severe "abuse" and more severe "dependence" categories in favor of mild, moderate, and severe diagnoses based on the number of applicable criteria [129]. Additionally, a meta-analysis of MRI studies found that while AUD patients generally exhibited reduced white matter volume, there was also significant heterogeneity between study populations [130].

The heterogeneity of AUD patients is of particular importance for genetic and drug development research, as any genetic pathways responsible for inter-patient variation may also affect certain drugs' efficacy or safety profiles. AUD's complex genetic foundation has been explained by some authors to result from a high degree of both heterogeneity and polygenicity. Heterogeneity assumes that either a single or few genetic variants determine disease risk, and that different alleles can lead to similar clinical presentation in different individuals. Meanwhile, polygenicity assumes that a given phenotype is the simultaneous result of the effect of multiple genetic variants [131]. The evidence suggests that both of these frameworks describe certain aspects of the heritability and neurobiology of AUD and other substance use disorders [132]. Certain endophenotypes, or measurable intermediate characteristics between a disorder and the biological processes underlying the disorder, have helped to distinguish differences in underlying AUD biology; among others, differences in facial flushing syndrome in East Asians facilitated the discovery of ADH2 subtypes, variants of which have become a primary target for genetic research [133, 134]. More recently, neurofunctional domains have become endophenotypes of interest for their potential to identify clinically significant patient groups with shared neurocognitive structures which may be effective targets

for new medication [4]. In any case, identification of new and more strongly associated endophenotypes is likely to provide molecular targets for novel medications, and research in this area is of particular importance for bringing the promise of personalized medicine into the addiction sphere [135].

Also of note is the high degree of comorbidity between AUD and other SUDs and psychiatric illnesses, offering both promise and challenges for genetic risk variant identification and subsequent targeted medication development. That AUD and other SUDs may arise from common predispositional factors has been suggested for decades, and environmental, cognitive, and biological factors have been proposed as potential mechanisms for this association [136, 137]. The relationship between AUD and smoking is particularly well-chronicled; in 2005 it was reported that up to 80% of individuals with DSM-IV alcohol dependence smoke cigarettes, and studies in European populations have reported over a fourfold increase in AUD risk among those meeting criteria for tobacco dependence compared to those who never smoked [138, 139]. Significant comorbidity between AUD and opioid use have been reported, and alcohol is a major contributor to opioid-related deaths [140, 141]. Alcohol and cannabis misuse are also significant, and associated with additive impairments in neurocognitive performance and more frequent use of both drugs [142]. AUD is also highly comorbid with other psychiatric disorders, especially anxiety and depressive disorders, and studies have found that any prior mental health disorder significantly increases the risk of problematic alcohol and drug use [143–145].

That AUD and other psychiatric disorders exhibit such a high degree of comorbidity is an important consideration for drug development. Besides the necessity to ensure patient safety by minimizing drug-drug interactions outside of the disorder for which a drug is prescribed, comorbid disorders are a key consideration for the application of personalized medicine and drug research. Patients with certain comorbid disorders may respond better to certain forms of treatment, and recent and ongoing clinical trials of psychological and pharmacological interventions are attempting to distinguish which drugs work best for patients with certain comorbid disorders [146–150]. Future research should focus on comorbid disorders as a useful grouping method for AUD patients in order to design treatment plans most likely to address individuals' total psychiatric health.

Conclusions

Despite the fact that the genetics of AUD has been a subject of interest for many years, and that a significant (appr. 50%) contribution of genetics to AUD has been established for quite some time, identifying the underlying genetic factors has been challenging. As genomic investigations have grown more complex, it has become clearer that there is no single genetic cause of AUD in any patient population, and that at least dozens of factors, including genetic, environmental, and gene-environment interactions, may work together to ultimately influence genetic AUD

risk. While in recent years genomic studies of AUD have grown more common and concerns regarding small sample sizes and statistical power have been addressed, there is still a need for large, multi-ethnic datasets with data for specific drinking phenotypes and life histories in order to disentangle the complex system of relationships between genetic factors, environmental influences and underlying disease processes, which may have different effects for different patients. Recently developed methods can help address some of the deficiencies in the understanding of AUD as a genetic disorder, but further innovation is needed to devise statistically powerful and clinically useful classification methods and analytical tools that can account for the combined influence of genetic and lifestyle factors. In the meantime, further genetic investigation into AUD is warranted in order to determine which variants that hold promise for future drug development and better understanding of the pathophysiology of individuals with AUD.

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Chapter 33
Alcohol Responses as Phenotypic Markers of AUD Risk: Lessons from Longitudinal Studies

Andrea King

Abstract Alcohol response phenotypes play an important factor in the vulnerability to alcohol use disorder (AUD). The majority of research in this area has employed controlled laboratory "alcohol challenge" studies in cross-sectional designs to determine alcohol response phenotypes. However, longitudinal studies that identify alcohol responses as phenotypic markers of AUD risk are crucial to determine which alcohol response phenotypes best predict problem drinking and severity and maintenance of AUD. The two most comprehensive longitudinal studies of alcohol response and future drinking are the San Diego Prospective study and the Chicago Social Drinking Project. These studies include alcohol and placebo response testing with long-term follow-up of drinking behaviors and AUD symptoms over a decade with exceptionally high retention over a decade of participation. The Chicago study also has included an important alcohol re-examination component to determine if alcohol responses are stable or change over time at different stages of AUD. This chapter reviews the central tenets and theoretical frameworks underlying these two longitudinal studies, summarizes their strengths and weaknesses, and compares their main findings in terms of alcohol's euphoric and rewarding effects relative to its sedating and impairment effects. These hallmark studies represent seminal contributions to the alcohol field and to our understanding of AUD risk and maintenance, as well as protective alcohol response factors in those at low risk. The chapter concludes with a call for consilience and directions to build on these research frameworks in future studies.

Keywords Alcohol use disorder (AUD) · Subjective alcohol response · Stimulation · Reward · Sedation · Risk factor · Family history · Binge drinking · Longitudinal · Follow-up

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Costs and Consequences of Excessive Drinking

Excessive alcohol use remains the third leading preventable cause of death [1] and is a major preventable contributor to disability and mortality worldwide [2]. In the United States alone, the annual economic costs of excessive drinking, including health care, loss of productivity, crime and accidents are estimated at over $249 billion [3] with three-quarters of the costs attributed to binge drinking [4], defined as consuming five or more drinks in an occasion for men, four or more drinks for women [5]. Binge drinking in early adulthood is a strong predictor of the development and risk for future alcohol use disorder (AUD) [6, 7] which is associated with serious health and safety consequences and impairments [8]. Given the global burden of alcohol misuse, identifying factors that increase its susceptibility and persistence is critical for public health [9] and crucial for improving prevention, education and intervention strategies [10].

Excessive Drinking in Young Adulthood and Across the Lifespan

While there are multiple trajectories of drinking behavior over time [11, 12], for many individuals, binge drinking originates during adolescence and emerging adult years [13]. Frequent binge drinking behavior has been described by the term heavy drinking, i.e., five or more binge episodes per month [14] that may become a chronic behavior pattern [14, 15]. Indeed, one-third to one-half of young adult binge drinkers persist with alcohol misuse and heavy drinking during adulthood [16]. The consequences of persistent heavy drinking are high—with an estimated 5.1% of the global burden of disease and injury attributed to alcohol and 283 million adults aged 15 and older meeting criteria for AUD [17]. Thus, there is a need for a better understanding of the resilience, vulnerability and mechanisms of excessive drinking and its co-morbidities across the lifespan. Outside of several known sociodemographic risk factors for the disorder, including male sex, lower education, delay of developmental transitions, family history, and impulsivity [18–23], the biopsychosocial causal factors that influence the persistence or escalation of heavy drinking and AUD are unknown.

Subjective Alcohol Response Research Approaches

An important factor to elucidate increased vulnerability to AUD is characterizing acute subjective alcohol responses, or alcohol response phenotypes, at different stages of the disorder. The majority of research in this area has employed controlled laboratory “alcohol challenge” studies. In these paradigms, alcohol is administered

under controlled conditions to maximize internal validity and reduce external factors (environmental context, etc.) that may confound assessment. Oral or intravenous alcohol is delivered by a variety of methods, including a fixed dose, an extended clamped dose [24], or serial increasing doses of alcohol [25]. Participants are usually medically and psychiatrically healthy young adults who vary on high- and low-risk status for AUD by virtue of binge drinking patterns, family history, or both. Responses are measured both before and after alcohol consumption, i.e., versus placebo in double-blinded studies, and compared between the high- and low-risk groups. The dependent alcohol response variables often include a battery of responses, such as self-report scales, physiological parameters [26, 27], and performance tasks [27–30].

Cross-sectional studies have revealed a myriad of responses to alcohol that differ between high- and low-risk subgroups [28, 29]. Relative to low-risk drinkers, high-risk drinkers have shown lower sensitivity to some of alcohol's acute effects, including subjective intoxication or sedation [31, 32], body sway [30], cognitive measures [33] and stress hormone response [26, 27, 32]. In contrast, high-risk drinkers have exhibited higher sensitivity to alcohol in terms of subjective stimulation [32, 34–36], liking [32] and wanting [32]. Other studies have shown no differences on subjective [37] or objective responses in high- and low-risk individuals [38]. While cross-sectional studies have provided information on alcohol responses as a function of purported risk for the disorder, these designs are not the optimal method to determine how alcohol responses prospectively predict future drinking and AUD propensity. From a clinical neuroscience perspective [39], longitudinal alcohol response studies are crucial to determine alcohol response phenotypes that predict problem drinking and severity of AUD. Despite numerous studies of acute oral or intravenous alcohol effects [40], there have been few programs of research utilizing longitudinal frameworks to examine alcohol response phenotypes related to vulnerability to AUD. It is not surprising that longitudinal investigations of alcohol response are less common than cross-sectional studies, as these designs are time- and labor-intensive, financially costly, and require extensive planning and perseverance to achieve high follow-up rates [41, 42].

The two most comprehensive longitudinal studies of alcohol response to future drinking include: (a) the San Diego Prospective study with participant enrollment in the 1970s to 1980s; and (b) the Chicago Social Drinking Project with participant enrollment in the 2000s to 2010s (and still enrolling). These studies are similar in that they combined well-controlled laboratory alcohol (and placebo) challenge sessions with extensive and high-retention subsequent follow-ups of drinking patterns and addiction symptoms. They differ in their criteria for high- and low-risk groups and measures employed, and also whether alcohol responses were measured at enrollment only or repeated over an extended period of time.

The focus of this chapter is to review the central tenets and theoretical frameworks underlying these two longitudinal studies, summarize their main findings, and discuss their relevance and contributions to the alcohol field. An important caveat to mention before describing these studies is that they were undertaken roughly 20–30 years apart. Over that time, the measurement of alcohol's effects has

changed from a general model of global alcohol intoxicating effects to a more evolved pharmacological and evidence-based structure with at least two main factors, one stimulating and rewarding factor and another sedative and ataxic factor, and these factors are inversely correlated [43]. There may also be a third factor for tension reduction [44] that is being investigated in AUD subgroups [45]. Given these developments, as outlined in the discussion, the Chicago study and newer models have set the stage to evaluate multifaceted effects of alcohol in high- and low-risk drinkers [46].

Finally, the chapter concludes with a call for consilience and future directions to build on this work, as it relates to the role of individual differences in subjective alcohol response and vulnerability and persistence of alcohol misuse that is so costly in terms of personal, health, and societal harms.

Theories of Alcohol Response and Risk for Excessive Drinking

Several theoretical models have been proposed to explain how an individual's response to alcohol may influence hazardous drinking leading to alcohol problems, consequences, and harm [28, 29, 46–50]. These models involve aspects of rewarding and/or aversive responses to alcohol, and include early-age alcohol responses as a risk factor for the future development of alcohol problems. They also include potential adaptations in alcohol responses during the course, chronicity, and increasing severity of AUD in a drinkers' lifetime. In terms of risk for future AUD, a prominent and longstanding theory is the low level response model [51] that purports lower sensitivity to alcohol's effects [51] as a risk factor for AUD. At-risk individuals are described as lacking the interoceptive signs of intoxication and therefore need to consume more alcohol to achieve a "desired effect." However, subsequent studies failed to support low level responses in at-risk persons [52, 53] and animal studies have supported psychomotor stimulant mechanisms of drug reinforcement [54]. Thus, a competing theory was introduced, the differentiator model, that posited that greater pleasurable and excitatory alcohol effects during the ascending limb of the breath alcohol concentration (BrAC), and lower sedative responses during the declining limb, increase risk for future AUD [47]. Further work called for a simplified theory, i.e., the modified differentiator model, as greater stimulatory and rewarding alcohol effects and lower sedative effects at peak BrAC, and not as limb-dependent, were shown to predict future alcohol misuse in at-risk drinkers [55]. As the low-level response and (modified) differentiator models hypothesize about alcohol response phenotypes that increase the risk for developing addiction, it is important to note that there are also theories about adaptive changes in alcohol responses as they relate to the neurocircuitry of the addiction process. These are briefly reviewed in the next section.

Neurobiological Models of Addiction

Addiction has been explained as a "disease of the brain's reward system" [56–59] and alcohol promotes dopamine release in the brain [60] and activates opioid peptides, glutamate, GABA, and other neuromodulators [61, 62]. During the course of increasing severity of addiction, neurobiological theories have proposed adaptive processes that underlie the stages of addiction. The longstanding model of adaptive changes in addiction is tolerance, i.e., diminished response to the same amount of alcohol [63, 64], that has been shown in psychopharmacological and neurobiological pre-clinical studies and is also one of the DSM criteria for AUD [65].

Another model, the allostasis model [66] draws from data in neuroimaging and animal studies and describes a series of stages in addiction. The first stage is described as the binge/intoxication stage, and is marked by positive reinforcement and drinking for pleasurable effects. The next stage is marked by neuroadaptations depleting dopamine function in brain reward circuits and pathological recruitment of stress- and anti-reward systems, resulting in drinking for negative reinforcement to lessen negative affective states and withdrawal. The third stage is termed preoccupation and anticipation and accompanied by executive function deficits.

Finally, another widely-cited theory of neuroadaptive changes in addiction is the incentive sensitization model [67, 68]. This model, based on extensive animal work, posits the addiction process involves independent changes in two neural systems: chronic excessive alcohol drinking sensitizes the neural circuits underlying motivational reward (wanting) but not hedonic reward (liking).

There are individual differences in the neural circuitry of risk for alcohol problems [69], and variation in the progression and intensity of addiction cycles as they relate to disruptions of the underlying neurobiological circuits [70]. However, convergent tests of these theories are critical and are lacking, due to methodological constraints and the difficulties in translational research between animal and human studies, particularly in prospective neuroscience and longitudinal studies. Further compounding the issue is that in early work in human laboratory studies, reliable and valid measures of stimulating and positive rewarding effects were largely absent, meaning that only "one side of the coin" (impairment/sedation) was being measured in high risk individuals [46]. These are important factors to consider in this review of the two largest longitudinal studies of alcohol response summarized in the next section. Figure 33.1 shows the models of addiction tested and supported by these studies.

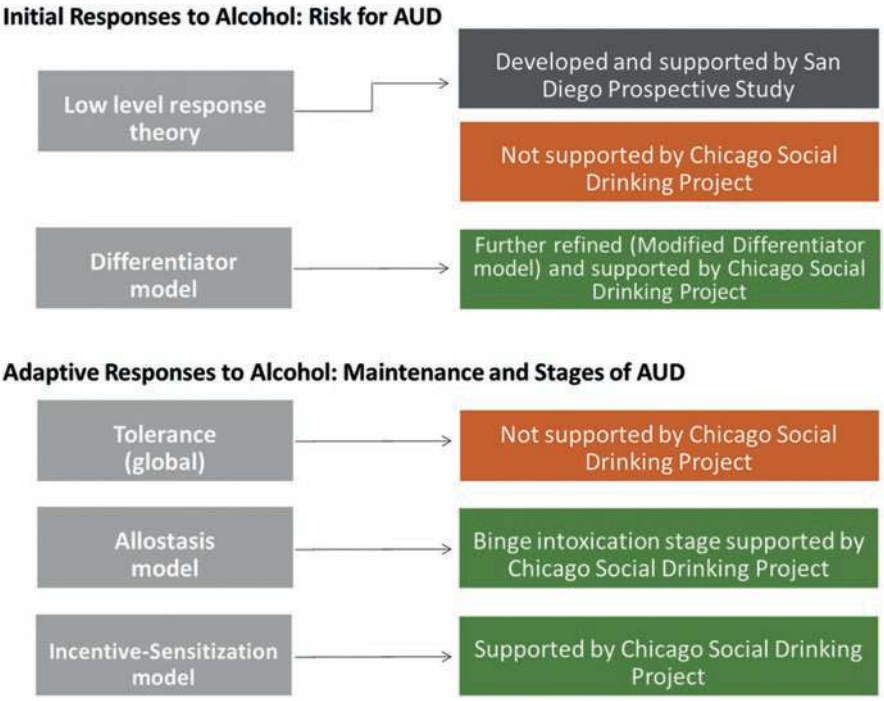

Fig. 33.1 Models of addiction

Longitudinal Approaches to the Study Alcohol Responses in At-Risk Individuals

As stated earlier, two longitudinal studies have set the benchmark for examining acute responses to alcohol in at-risk individuals. They represent the gold standard of well-controlled, quality research with moderate-to-large sample sizes, well-characterized participant subgroups, and high longitudinal retention rates of 98–99%. The first of these two studies, the San Diego Prospective Study, was founded on prior work [71] and originated in 1978 with laboratory alcohol and placebo challenge among a reported 453 participants. Approximately one decade later, the participants were contacted for in-person follow-up for symptoms of alcohol abuse and dependence and other biometric data. This study is remarkable in that the follow-up was conducted before the digital age, so that methods used to contact participants consisted of mail, telephone, and/or publicly available records [72].

The second of these longitudinal studies, the Chicago Social Drinking Project [50], originated in 2004, with laboratory alcohol and placebo challenge in 397 participants (and counting). After the laboratory sessions, the first cohort of 190 participants completed quarterly follow-ups for 2 years and near-annual follow-ups for a decade thereafter. They were also invited to return to the laboratory to engage in

re-examination laboratory sessions 5- and 10-years after their initial testing. As over 40% of participants had relocated, transportation and travel allowances were provided, as needed. Two additional cohorts of 207 participants (described in more detail later) were added to the study starting in 2009 and 2016, respectively. These cohorts underwent similar laboratory testing and repeated follow-up assessments of drinking behaviors and AUD symptoms.

The Chicago Social Drinking Project is a significant next-generation study extending the precedent set forth by the San Diego Prospective study. It capitalized on technology advances in the intervening years to employ more frequent follow-ups and the use of email, text messages, and internet surveys not possible at the time of the San Diego study [41]. Notably, the Chicago study is the only investigation to date to re-examine drinkers over an extended period of time in order to elucidate the dynamic changes and/or stability in alcohol responses. The next section provides a more detailed review of each of these high-impact longitudinal studies examining alcohol responses and their associations to future drinking and AUD symptoms over time. Both the San Diego and Chicago studies reported outstanding 98–99% follow-up rates. The methodology and mindset to achieve such high retention has been outlined by both groups [41, 72].

San Diego Prospective Study

Over the course of a decade (1978–1988), the San Diego Prospective Study reported enrolling 453 non-alcohol dependent male drinkers between ages 18 to 25 years. These men were recruited from surveys given to students and staff at a state university, and selected for participation based on their self-reported family history of alcoholism. They were deemed high risk if they reported a positive paternal family history of DSM-III (or DSM-III-R) alcohol dependence, and low risk if they had no known family history of alcohol abuse or dependence [49, 73]. Investigators matched individuals in each group on sociodemographic and drinking characteristics. Laboratory sessions were double-blinded, and began at 7:00a after participants had an overnight fast for at least 10 h. After baseline measures, alcohol was consumed over 10 min as a 20%-by-volume room temperature beverage at doses approximating 0.6 and 0.87 g/kg alcohol (or placebo).

A series of publications reported on outcomes in subgroups of the total sample (Ns ranging from 40–68 subjects). The initial findings showed a less intense response to alcohol in individuals with positive versus negative family history of alcoholism, on measures of subjective intoxication, static ataxia, and stress hormone markers including cortisol [27, 30, 73, 74]. Measures of stimulation and reward were not obtained, and many of the responses were reported during post-consumption intervals corresponding to the peak BrAC or descending limb [74]. The men were re-contacted approximately 8–12 years later for face-to-face follow-up interviews, with a very high 99% retention rate reported. Findings showed that, regardless of family history status, those with lesser alcohol responses on subjective

intoxication or body sway (ataxia) had a several-fold higher risk of meeting criteria for alcohol abuse and dependence approximately a decade later [51]. This was confirmed in a subsequent analysis of the highest and lowest 15% of individuals on level of response [49]. Subsequent path modeling in a subset of participants showed that low response to alcohol on the same measures predicted alcohol abuse and dependence symptoms at a 20-year follow-up wave [75]. The genetic contributors to alcohol sensitivity in this study may be related to GABA, glutamate, opioid, dopamine, serotonin and the cholinergic system, but more work is needed before conclusions can be drawn [76].

In sum, the San Diego Prospective Study was the first of its kind to examine the response to alcohol on some measures with long-term follow-up to examine risk for AUD. This study demonstrated that low acute alcohol response phenotypes on measures of ataxia or subjective responses favoring sedative effects prospectively predicted future diagnoses of alcohol abuse or dependence in men who did not meet those criteria at enrollment. The results also demonstrated that there may be other predictive factors for future problem drinking outside family history for the disorder. Indeed, the majority of the children of people with AUD do not develop AUD themselves [77], and heritability accounts for about 50% of the variance in the risk for developing AUD [78]. So while family history is a highly heritable phenotype for risk for AUD, acute alcohol responses may be just as important—or even more important—as a marker of risk.

While the San Diego Prospective Study had strengths with a well-controlled laboratory paradigm, subjective and objective measures, and highly successful follow-up rates to circumvent problems associated with loss to follow-up [41], it also had some limitations. First, as collection of data took many years to accrue, publications have different sample sizes with most papers presenting data from less than half of the full sample. Second, as the work is decades old, instruments were limited on their psychometric properties to measure the many effects of alcohol. The main subjective instrument came from research on subjective effects of lithium [79], and items favored sedative and sluggish effects over pleasurable effects. The few positive effects included in the scale have low comprehension and association to alcohol's effects (i.e., "high", etc.) [80]. Third, the sample described in many reports from this study was homogeneous on sex (all male), race (all Caucasian) and religious affiliation (non-Jewish) potentially limiting generalizability to the larger population of people with AUD, including women [81] and non-White racial and ethnic subgroups [82]. More inclusive studies with alcohol-specific response measures would overcome these obstacles, as discussed in the next section.

Chicago Social Drinking Project

The Chicago Social Drinking Project has thus far enrolled 397 young adult drinkers aged 21 to 35 years, in three successive cohorts from 2004 to 2019. The study included individual, random-order 0.8 g/kg alcohol or placebo beverage

administration (consumed over 15 min with 16%-by-volume alcohol) in separate individual sessions. The sessions were conducted during afternoon timeframes and after a calorie-controlled snack to minimize nausea and aversive effects, as opposed to the overnight fast and early morning timeframes in the San Diego study. Participants were recruited from the community by flyers, media advertisements and word-of-mouth referrals. As early-age binge drinking is an important risk factor for vulnerability to AUD [83, 84], high-risk individuals were defined as non-alcohol dependent persons reporting weekly binge drinking (i.e., five or more standard drinks per occasion for men, four for women) [14, 85] with 10 or more drinks per week for at least the last 2 years. The control group was light drinkers who consumed less than six drinks per week with rare binge drinking occasions. The overall sample was diverse with inclusion of both sexes (44% women) and broad representation in terms of race (26% reported as: Black, Asian, Native American, or More than One Race), and ethnicity (13% Hispanic) to reflect the heterogeneity of persons with excessive drinking behaviors [82].

The original cohort of 104 young adult binge/heavy drinkers and 86 light drinker controls was enrolled between 2004 and 2006 and underwent alcohol and placebo laboratory testing and follow-up interviews on their drinking and AUD symptoms at near-annual intervals. Those eligible for repeat alcohol challenge testing (96% of the sample) were invited back for two re-examination laboratory testing sessions at two intervals corresponding to 5 and 10 years after initial participation with 88% and 91% participation rates, respectively.

A second cohort of 104 young adult heavy social drinkers was enrolled between 2009, and 2011, and a third cohort of 103 young adult AUD drinkers between 2016 and 2019. These cohorts underwent similar alcohol and placebo challenge testing and follow-ups. The second cohort was recruited to increase the sample size of at-risk drinkers for a replication sample, and the third cohort was included to test adaptive responses to alcohol in individuals at the higher end of the drinking severity continuum. The drinking patterns of this AUD cohort differed from those in prior heavy drinker cohorts, i.e., 41.9 versus 20.9 drinks per week, respectively, and on all other quantity-frequency and alcohol consequence measures.

Thus, a total of 397 young adult drinkers comprised three cohorts in the Chicago Social Drinking Project. They have ranged across the spectrum of light, heavy, and AUD drinkers with an overall mean age of 26 years at enrollment. Family history of alcoholism was obtained as part of background measures but was not a selection criterion. Of note, more than one-quarter of the sample had indeterminate family history (adoption, lack of knowledge of family members' drinking, etc.), and including family history as a covariate in analyses did not affect the results. To provide a complete biphasic alcohol response profile, primary measures were assessed before beverage consumption and repeated at several intervals after consumption to capture rising, peak, and declining BrAC. In order to decrease alcohol expectancy, participants did not know the actual study purpose or the class of substance in their beverage until debriefing [86]. In a series of papers, differences in alcohol responses between heavy and light drinkers was demonstrated on a variety of measures, including subjective response, psychomotor performance, eye movements, and

cortisol [26, 32, 38, 87, 88]. Subjective responses to alcohol were ascertained by reliable and valid scales sensitive to alcohol's effects: the Biphasic Alcohol Effects Scale for stimulation and sedation [80, 89] and the Drug Effects Questionnaire for liking and wanting [90].

Relative to low-risk drinkers, heavy drinkers exhibited heightened sensitivity to alcohol's pleasurable effects (stimulation, liking and wanting) and lower sensitivity to subjective sedation [32]. Heavy drinkers also had lower cortisol response than light drinkers and self-perceived they were less impaired from alcohol [32, 38, 91] despite having similar performance impairment [38], with a few exceptions [87]. This alcohol response phenotype of heavy drinkers in the original cohort was reproducible in the second cohort of heavy drinkers [92].

Over the course of quarterly follow-ups, with 99.1% retention in the first 2 years after the sessions, in heavy drinkers, heightened alcohol stimulation, liking, and wanting as well as low sedation predicted binge drinking exacerbations, which in turn predicted the likelihood of meeting AUD criteria as participants were entering their late 20's [32]. Light drinkers continued with low-risk drinking patterns. At the 6-year follow-up interval, 98% of all possible follow-ups were successfully completed. The findings in heavy drinkers provided a critical extension of the aforementioned predictive models: greater stimulation and reward sensitivity, in addition to lower sedation, predicted future AUD symptoms and drinking exacerbations 6 years later. This was observed during a developmental epoch where alcohol misuse largely deviates from age-related norms [55]. The positive subjective effects of alcohol at peak BrAC were more predictive of future drinking and AUD symptoms than effects during either rising or declining limbs, providing further support for the modified differentiator model [55]. Trajectory analyses showed that about one-third of high-risk drinkers moderated their drinking with few AUD symptoms, but more than half continued with weekly binge drinking and met symptoms consistent with mild or moderate AUD. About 10% progressed to severe AUD.

In the re-examination testing of alcohol responses at the 5–6 year interval, those with the highest AUD symptom counts exhibited pronounced alcohol stimulation, wanting and liking, and lower sedation, compared with individuals with intermediate or few AUD symptoms. Light drinkers continued to show a largely "protective profile", with persistently low sensitivity to alcohol stimulation and reward, together with high alcohol sedation and stress hormone responses [42].

A 10 years of follow-up, re-examination of alcohol and placebo responses were conducted when most participants were in their fourth decade of life [93]. At this juncture, 21% met criteria for past-year AUD (three times higher than national norms). Those who had the highest alcohol stimulation, liking and wanting at the initial challenge were most likely to have developed AUD a decade later [93]. Further, re-examination testing showed that alcohol-induced stimulation and wanting increased in intensity among those who developed AUD versus those who did not [93]. The same pattern of results was observed in trajectory analyses in the growth of AUD symptoms: the highest AUD symptom trajectory subgroup showed an increased magnitude of alcohol stimulation and wanting over time, with persistently high levels of alcohol liking. Unlike the prior follow-up intervals, sedation

was no longer an inverse predictor of drinking exacerbations or AUD symptoms and physiological responses to alcohol also were not predictive.

In sum, over the decade of re-examination waves, there was a potentiation of stimulation and motivational salience (wanting) such that these responses were magnified over time in those who developed AUD versus those who did not. Hedonic effects of alcohol hedonic (liking) were sustained at a higher level in those with AUD than those without AUD. These findings in those with or without AUD at the end of the decade ("the destination") were corroborated by examining the sample on AUD symptom trajectories (the "journey"), as individuals in highest trajectory subgroup of AUD symptoms, relative to those with low or no AUD symptom progression, initially had the highest stimulating and rewarding alcohol responses that were either magnified or sustained at 10-year re-examination.

Finally, a subsequent third cohort of 103 young adult AUD drinkers in the Chicago Social Drinking Project was enrolled to examine a sizeable sample of drinkers at the highest level of the drinking continuum, i.e., AUD, in order to provide a comprehensive test of adaptive alcohol response models of addiction. The methods of random-order alcohol (0.8 g/kg; high dose) and placebo laboratory sessions were similar to that in prior cohorts. A subset of this cohort also had an additional randomized session with 1.2 g/kg alcohol (very high dose) to more closely match the excessive drinking behaviors characteristic of AUD drinkers. This dose was feasible with few adverse effects [94].

Results showed that both the high and very high doses of alcohol produced marked biphasic effects in AUD drinkers, with rising limb to peak BrAC increases in stimulation, liking and wanting as well as heart rate increases, and declining limb increases in sedation [95]. Thus, instead of developing tolerance and lack of sensitivity to alcohol's desirable effects, AUD drinkers exhibited high alcohol sensitivity, with stimulation and reward of a magnitude comparable or even higher than previously observed in heavy drinkers. These findings show that AUD drinkers haven't "lost that positive feeling" [95] or developed tolerance to alcohol's pleasurable effects. To the contrary, AUD drinkers exhibited heightened pleasurable effects of alcohol that align with the reward-sensitive binge intoxication stage of addiction in the allostasis model, and not with thef purported reward-deficit second stage of allostasis. Currently, participants are undergoing annual follow-ups for four years to examine their future drinking and potential onsets and offsets of AUD. Retention rates are in the 98–99% range over first few years and results will address whether alcohol responses have prognostic significance in the progression or regression of AUD.

Overall, the findings of the extensive longitudinal Chicago Social Drinking Project challenge the conventional notion of a "low responses to alcohol" as the driving force in the progression of AUD. Rather, there is a prolonged and pronounced sensitivity to stimulation and reward consistent with what is described in the first stage of the allostasis model. Results also support the incentive-sensitization model, as motivational salience (wanting) became sensitized over time while hedonic reward, i.e., liking, remained elevated but did not significantly increase in magnitude over time. As such, while there are multiple pathways to development of

a disorder as complex as AUD, the maintenance and potentiation of stimulatory and rewarding alcohol effects appear to play an important role in the continuation and progression of alcohol addiction. In contrast, an attenuated or absent sedative response becomes less predictive of future drinking as follow-up waves proceeded.

Conclusions: Alcohol Response Phenotype as Contributors and Markers of Addiction Process

The two longitudinal studies reviewed herein have advanced our understanding of the quality and magnitude of alcohol responses as related to excessive drinking and risk for future AUD through young and middle adulthood. Notably, both the San Diego and Chicago studies examine alcohol response phenotypes and their associations to the risk for excessive drinking. These programs have expanded scientific questions and testing of neurobiological theories of addiction by enrolling additional cohorts of at-risk drinkers, examining the progeny of participants and genetic contributions [96] and/or employing newer methods and measures for precise ascertainment of a myriad of alcohol responses. Beyond laboratory assessments, both groups have developed and tested self-report retrospective scales to ascertain alcohol responses [97, 98] that correlate with laboratory-derived measures [99]. In terms of other advancements in recent years, the Chicago study has employed examination of real-time drinking and alcohol effects in the natural environment with high-resolution ecological momentary assessment (HR-EMA) using participant's own smartphones and alcohol biosensors [99, 100].

While laboratory alcohol challenge continues to be the gold standard for translational research in ascertaining acute alcohol response, refinement of complementary methods may circumvent the limitations of labor-intensive and costly individual laboratory sessions. Retrospective scales or technology-based HR-EMA methods enable data collection on a larger scale, and overcome external validity concerns within the controlled laboratory environment. The COVID-19 pandemic has necessitated more urgency in developing real-time methods and may mark an inflection point in the use of alternative methods to examine alcohol responses.

Another major issue in the field is overcoming the apparent discrepancies between the findings of the two research programs presented herein. The earlier work of the San Diego Prospective study in the 1970s and 1980s set the stage for the widely-adopted low-level response and other low-sensitivity models of risk for AUD [51, 101]. However, the findings of the Chicago Social Drinking Project from the 2000s to 2020s, and other cross-sectional work, demonstrate that less intensive alcohol effects are not ubiquitous in at-risk individuals and highly sensitivity to alcohol's pleasurable effects are the most predictive of future drinking problems and AUD [32, 93, 95].

To resolve the apparent discrepancy on whether lower or higher sensitivity to alcohol concurs heightened vulnerability to addictive disorders, qualitative and

quantitative reviews have suggested that there may be more than one phenotypic pattern of response to alcohol in high-risk individuals, depending on whether risk is conferred by family history of the disorder or heavy drinking patterns [28, 29]. Others have argued for a paradigm shift to change the zeitgeist of global alcohol response characterization, i.e., low alcohol sensitivity or low level response [51], to a more accurate, specific and descriptive framework, i.e., low level response to alcohol sedation or low sensitivity to alcohol-induced ataxia. In addition, many have called for terms to describe alcohol's effects in lieu of a newer and more specific characterization framework stressing the use of measures with strong psychometric properties to detect alcohol's effects, and specifying the dose of alcohol, interval on the breath alcohol curve, and the risk group being studied [28, 50], i.e., low response to alcohol sedation after peak BrAC, or heightened sensitivity to alcohol stimulation during the rising BrAC limb in binge drinkers. These proposed more specific characterizations would be akin to animal models that identify particular drug effects tested Such models in rodents include measures of the sedating effects of alcohol by the loss of righting reflex or rotarod test, and measures of positive reinforcing and drug preference effects by the two-bottle choice, self-administration or conditioned place preference paradigms [102]. The advantage of human research is that instead of making inferences about internal mood state or behavioral responses, as in animal research, the use of subjective measures with good psychometric properties allow direct self-report measurement of how one feels at certain intervals before and after alcohol consumption. Going forward, best practices for human models should use more specific terminology and frameworks, as mentioned earlier, as well as avoid paradigm choices lacking external validity and potentially confounding measurement, such as morning sessions, overnight fasting, or participant isolation.

An overall understanding of the findings of the San Diego and Chicago studies may come down to the inverse relationship between alcohol stimulation and sedation [43] that may necessitate a paradigm shift [46, 50]. This becomes clear when scales that measure pleasurable alcohol effects are included in laboratory protocols [80, 89, 90]. Contrary to theories purporting low levels of response to alcohol [51] and reward insensitivity as drivers of risk and maintenance of AUD [56, 57], an increased focus on heightened sensitivity to alcohol stimulation, euphoria, and reward may is warranted. In contrast to what has been postulated, positive subjective effects of alcohol persist and, in some cases, sensitize as individuals develop AUD, at least in young and early-middle aged adulthood [93]. As mentioned earlier, adopting more precise phrasing beyond level of response or low sensitivity for more well-defined concepts will enable the field to move forward with consilience on methodological considerations and strengthen the impact of the findings.

A third important issue is to better understand the importance of longitudinal studies for translational neurobiology. While studies with long-term follow-up and re-examination phases are challenging in terms of coordination and organization to yield high retention and continued participation rates, this type of research in humans allows direct testing of the main theories regarding the role of alcohol responses on heightened vulnerability and maintenance of AUD. This knowledge is

necessary to adequately test the translational significance of animal models of addiction to humans [103] and recent results from the Chicago study indicate that the excitatory effects of alcohol remain heightened (liking) or are potentiated (stimulation, wanting) as drinking exacerbates and AUD symptoms increase over time. At the same time, the acute sedative effects of alcohol decrease in problem drinkers. Genetic factors underlying alcohol response phenotype have been examined in both studies and shown a range of possible variants related to acute effects of alcohol [76] or as moderators of the predictive relationship of alcohol liking and wanting to future AUD as shown with a dopamine signaling variant related to reward in the Chicago study [96].

In sum, longitudinal research can be integrated with state-of-the-art laboratory methods and the lessons learned from the two most prominent of these investigations can uniquely help future research. First, the San Diego and Chicago studies described in this chapter showed exceptional attention to detail and investment in their paradigms to yield outstanding high-retention cohorts to advances in our understanding of the development of alcohol addiction and translation of animal research to the human condition. Continued longitudinal examination and re-examination of alcohol response phenotypes will foster better understanding of the development, maintenance and treatment of AUD. Second, improved methods in translational laboratory and longitudinal investigations and measurement of effects across the spectrum of positive- and negative-reinforcing effects is paramount. Because it is more recent, the Chicago Social Drinking Project is has methodological advantages over the San Diego study, but the latter study contributed greatly to long-term outcomes and pathway models, integrating genetic heritability and psychosocial risk factors related to alcohol response phenotype. Expanding participant samples to individuals in middle and older ages would provide a lifespan perspective on whether alcohol responses may stabilize or change over time during aging. This would be an important future direction, and this testing is underway in a fourth cohort in the Chicago study.

In conclusion, the ultimate goal for longitudinal investigations based on those described in this chapter will be to offer new empirical insights into the etiology and maintenance of harmful drinking and AUD. This can be undertaken by bridging neuroscience and basic preclinical studies to the human realm for laboratory and longitudinal hybrid studies that lead to innovative prevention and targeted intervention strategies [104]. Yet the combination of rigorous and well-controlled laboratory and longitudinal methodologies is not without practical challenges and methodological considerations for a long course of data collection. Expansion of the two longitudinal programs of research presented in detail in this chapter—by independent scientific teams and new study samples—will enable a better understanding of sensitivity and insensitivity to specific biphasic alcohol responses in at-risk individuals and protective alcohol response factors in low-risk drinkers. New avenues and novel methods of re-examination testing, convergences of animal and human methodology to the highest extent possible [102], and a lifespan approach will enable better targeted and empirically-based strategies for prevention, early intervention and treatment.

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Chapter 34
Early Life Adversity as a Risk Factor for Alcohol Use Disorder

Melanie Schwandt

Abstract Childhood adversity and maltreatment, encompassing early life stressful events ranging from parental illness or divorce to more severe cases of physical and sexual abuse, represents a significant risk factor for adult mental health outcomes include alcohol use disorder (AUD). Childhood adversity and maltreatment is surprisingly prevalent in the general population worldwide, with even greater prevalence in individuals with AUD. These stressful experiences are associated with developmental alterations in key biological systems that contribute to risk for AUD, including the neuroendocrine and corticolimbic systems, the inflammatory response, and epigenetic modifications. These alterations can become embedded, resulting in long-term adverse consequences on health and behavior. Childhood maltreatment in particular has been linked to an earlier age of onset for alcohol consumption, greater likelihood of adolescent binge drinking, and increased probabilities of drinking to cope and impaired control over drinking in young adulthood and beyond, all of which contribute to vulnerability for AUD. Furthermore, individuals with a history of childhood maltreatment who ultimately seek treatment for AUD often present a greater challenge for health practitioners. A better understanding of the role of childhood adversity and maltreatment in the etiology of AUD can assist in meeting that challenge.

Keywords Abuse · Neglect · Maltreatment · Trauma · Alcohol · Stress

Introduction

Risk factors for the development of alcohol use disorder (AUD) are traditionally classified into one of two categories: genetic and environmental. The genetic risk factors for AUD are covered here in a separate chapter. Environmental risk factors

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for AUD are diverse in scope and stem from a wide range of contexts, including the individual social network (parents, family, peers), the community level (work, school, social norms, religion, alcohol availability), and the societal level (policies and laws, taxation) [1, 2]. The impact of these risk factors also varies across individuals, communities, populations, and geographic regions. For the individual, it also varies across the life course.

A prominent theme in epidemiological research is that environmental exposures occurring during sensitive periods of development can exert significant and long-lasting effects on health and well-being. Adversity and maltreatment during childhood, especially in the context of family and the home environment, has been linked to a vast array of negative consequences for physical and behavioral health during both childhood and adulthood. These consequences include somatic health conditions such as cardiovascular disease, respiratory disease, stroke, diabetes, cancer, autoimmune disorders, and pain disorders [3–7], and nearly all forms of psychopathology, including alcohol and other substance use disorders [8–11]. A recent review and meta-analysis of the consequences of childhood adversity on health outcomes reported estimated annual costs of $581 billion in Europe and $748 billion in the US [4], underscoring the fact that childhood adversity exerts a significant impact on public health.

Problematic alcohol use also poses a significant burden to public health, with an estimated annual cost of $249 billion in the U.S. (www.cdc.gov/alcohol), an estimated economic cost of 2.6% of the gross domestic product worldwide, and an estimated 5.1% of the global burden of disease [12, 13]. This chapter examines how childhood adversity and maltreatment pose a significant environmental risk factor for alcohol use disorder. Following a review of definitions and prevalence estimates for adversity and maltreatment, the biological and behavioral pathways that ostensibly link these early life experiences to alcohol use disorder are discussed. Figure 34.1 presents a conceptual illustration of the main points and pathways that will be covered in this discussion.

Data on individuals seeking treatment for AUD paint a compelling picture: greater than 50% of patients report being exposed to at least one type of childhood maltreatment (e.g., abuse or neglect), and a history of maltreatment is associated with earlier onset of AUD, greater severity of addiction, increased risk for psychological comorbidity, and poorer treatment outcomes [14–19]. Paradoxically, a history of maltreatment has been associated with both an increased probability of accessing treatment, and greater perceived barriers to treatment, e.g., believing that treatment would not be helpful [20]. Increased awareness and understanding of the role of childhood adversity and maltreatment in the etiology of AUD is crucial not only for efforts at early prevention or intervention, but also for the development of current and future treatment approaches.

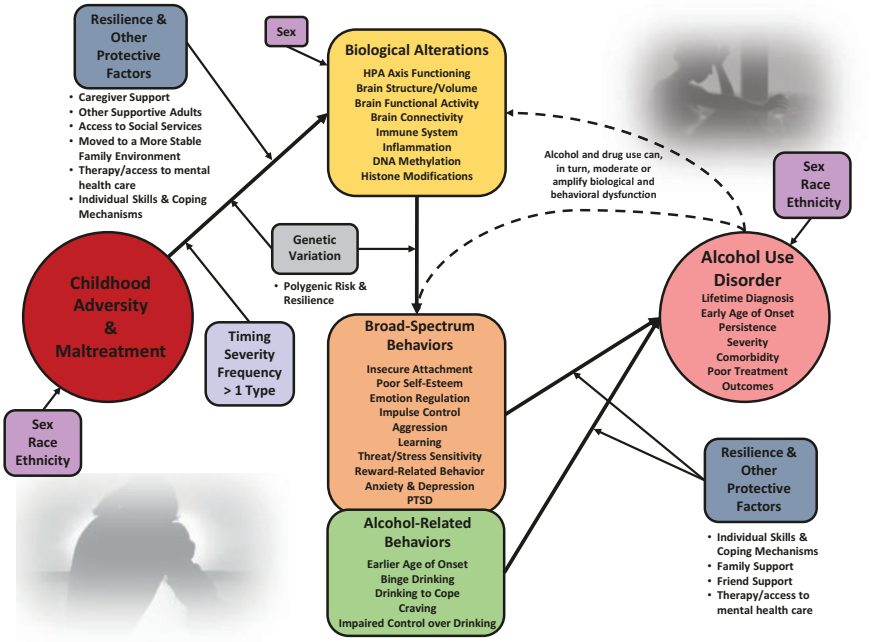

Fig. 34.1 Illustration of the potential pathways from early life adversity to alcohol use disorder. Exposure to childhood adversity and maltreatment can lead to alterations in multiple biological systems, some of which may become embedded. These biological alterations can have long-term consequences on behavior, including alcohol-related behaviors that are known to increase risk for developing alcohol use disorder. At multiple points along these pathways, effects may be moderated by the timing and severity of exposure, genetic variation, sex, race, and various factors associated with resilience. The dashed arrows indicate how alcohol misuse can, in turn, result in further biological and behavioral dysfunction

Definitions: Adversity, Maltreatment, and Trauma

A variety of terms are used in the literature to describe negative childhood experiences, including "early life stress", "childhood adversity", "adverse childhood experiences" (ACE), "childhood maltreatment", and "childhood trauma". Conventionally, these terms all refer to experiences occurring up until 18 years of age. While these terms are often used interchangeably, in truth they represent different concepts with varying degrees of overlap. Early life stress, childhood adversity, and ACE are broad terms that cover a wide range of experiences, from common events such as divorce or death of a family member to more extreme events such as witnessing domestic violence, being subject to abuse or neglect, and experiencing natural disasters, warfare, or terrorism. These experiences may happen directly to the child, or may affect the child together with others around them. Childhood maltreatment is directed exclusively toward the child, and occurs when a parent or other person responsible for the child commits acts that result in actual, potential, or

threat of harm to the child [21]. Maltreatment covers behaviors such as physical, sexual, and verbal or emotional abuse, neglect (failure to meet the physical and emotional needs of a child), abandonment, and sexual exploitation [22]. Childhood trauma is a more complex concept to define. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) defines trauma as exposure to actual or threatened death, serious injury, or sexual violence [23]. Yet this definition implicitly excludes emotional or psychological abuse, which can actually be quite harmful, especially for children, despite the lack of physical injury. Alternative definitions of trauma focus less on the type of event and more on the response to that event. For example, the Substance Abuse and Mental Health Services Administration (SAMSHA), which oversees the National Child Traumatic Stress Initiative (NCTSI) in the United States (US), defines trauma using the three "E's"—an *event* (or series of *events*) that is *experienced* by an individual as emotionally or physically threatening and that has lasting adverse *effects* on the individual [24].

Childhood maltreatment and childhood trauma show a high degree of overlap in the literature, as studies using these terms typically focus on abuse and neglect. Childhood abuse and neglect are traumatic for many if not most children, thus it is common for these experiences to be labeled as childhood trauma. This is reflected in the Childhood Trauma Questionnaire (CTQ), a well-validated and widely-used assessment developed by Bernstein and colleagues [25], which measures presence and severity of five types of trauma: physical abuse, sexual abuse, emotional abuse, emotional neglect, and physical neglect. Physical abuse is defined as risking or inflicting physical injury on a child, usually by a parent or adult caregiver, through bodily contact or other means (e.g., tying up a child to restrict movement, forcing exercise to the point of exhaustion). Sexual abuse is defined as sexual contact between a child and an adult or older person, or other non-contact interactions such as voyeurism and exploitation, that are used for the sexual stimulation of the perpetrator [25, 26]. Emotional abuse is defined as verbal attacks on a child's sense of worth or well-being, or other acts or demeaning behavior directed toward a child by an adult or older person, that cause emotional and/or psychological damage to the child [25–27]. Emotional neglect is defined as the failure of caretakers to meet a child's basic emotional and psychological needs (e.g., attention, love, nurturance, and support), while physical neglect is defined as the failure to provide for a child's basic physical needs (e.g., food, shelter, clothing, medical care) [25, 26].

For AUD, the most notable effects tend to be linked to childhood maltreatment/trauma experiences such as abuse and neglect, and thus the primary focus of this review will be on those type of exposures. However, the impact of other forms of early life stress on alcohol use and misuse are still relevant, especially if there is a history of exposure to multiple types of stressful events.

Prevalence of Childhood Adversity and Maltreatment

Prevalence in the General Population

According to the World Health Organization (WHO) it is estimated that globally, up to one billion children aged 2–17 years have experienced physical, sexual, or emotional abuse or neglect in the past year [28]. Past year prevalence estimates based on a 2016 systematic review of reports covering 96 countries indicate that at least 44% of children in developed countries, and 59% of children in developing countries, experience physical, emotional, or sexual abuse or witness domestic or community violence [29]. In the United States, it is estimated that 1 in 7 (14.4%) children experienced abuse or neglect in the past year [30], while lifetime prevalence estimates in North America based on meta-analysis are 18.2% for sexual abuse, 18.1% for physical abuse, 23.9% for emotional abuse, and 30.1% for neglect (either physical or emotional) [31]. Higher prevalence of childhood maltreatment has been reported in certain populations including Native Americans [32], African Americans, and Hispanic Americans [33, 34]. In Europe, lifetime prevalence estimates based on meta-analysis are 13.2% for sexual abuse, 12.2–22.9% for physical abuse, 21.7–29.1% for emotional abuse, and 27.0% for neglect, and in Asia the estimates are 16.3% for sexual abuse, 13.9% for physical abuse, 33.4% for emotional abuse, and 47.2% for neglect [31, 35]. When considering the broader experience of childhood adverse events, rates are notably higher: from 46–61% among U.S. youth under 10 years of age, up to 77–99% in several countries assessed for childhood adversity by the WHO [36].

Prevalence in Individuals with AUD

Prevalence rates for child maltreatment are in general higher among individuals with AUD, especially those who seek treatment, compared to the general population. That said, precise prevalence rates in AUD populations vary somewhat, due to factors such as method of assessment, sample size, and sample composition. Several studies using the CTQ have reported prevalence of the five common types of abuse and neglect based on a cut-off point of "moderate-to-severe" [37]. These rates range from 11.0–24.0% for sexual abuse, 18.0–31.1% for physical abuse, 21.4–32% for emotional abuse, 20.4–39.7% for emotional neglect, and 19.9–28.2% for physical neglect [14, 16, 38]. Using a "low-to-severe" cut-off, however, reported rates in an AUD sample are somewhat higher: 38.9% for sexual abuse, 21.1% for physical

abuse, 47.5% for emotional abuse, 57.1% for emotional neglect, and 27.5% for physical neglect [15]. Data from the large National Epidemiologic Survey on Alcohol and Related Conditions (NESARC), which measured child maltreatment with questions based on the CTQ, indicate prevalence rates among all adults with AUD as 12.1% for sexual abuse, 22.9% for physical abuse, 11.2% for emotional abuse, 10.2% for emotional neglect, and 29.6% for physical neglect [39]. Among emerging adults (18–25 years of age) with AUD, however, rates are substantially higher: 40.2% for sexual abuse, 47.0% for physical abuse, 52.9% for emotional abuse, and 48.0% for physical neglect (emotional neglect was not reported) [20].

In addition to prevalence, severity scores for each of the five abuse and neglect measures of the CTQ are also significantly higher in individuals with AUD compared to those without [15]. Moreover, individuals undergoing inpatient treatment for AUD report greater severity of emotional abuse and physical neglect compared to individuals with AUD that do not seek treatment [40].

Characteristics of Exposure

The impact of childhood adversity and maltreatment on an individual, both immediately and in the long term, is influenced by several key characteristics of exposure. These include the timing of exposure, the severity and/or frequency of exposure, and the accumulation of different types of exposures. Timing, or the age at which a child is exposed to maltreatment, can be a determinant of developmental process and behaviors that are most likely to be affected. For many outcomes, exposure to maltreatment in the first few years of life has the greatest impact; however, exposure at later ages (e.g., puberty) can also have long-lasting effects [41]. "Sensitive periods", or windows of time during which vulnerability to maltreatment-related changes is relatively high, have been identified for both brain development [6, 42] and hypothalamic-pituitary-adrenal (HPA) axis functioning [43], systems that have long been implicated in the etiology of AUD. Severity of exposure is also important and can reflect specific aspects of the maltreatment, such as the likelihood to cause serious injury, or even just the frequency of occurrence. Severe exposure to a single abuse type, most often sexual abuse, has been associated with increased risk for substance use disorders and having more than one psychiatric disorder [44]. Likewise, frequent or chronic exposure to maltreatment or adverse events is associated with greater vulnerability for a range of mental health and substance abuse problems [45]. For problematic drinking and AUD, experiencing two or more events confers greater risk compared to experiencing only a single event [46, 47], and exposure to four or more events is associated with a 7.2-fold increase in risk for developing an AUD [45]. Somewhat related is the concept of cumulative exposure to more than one type of maltreatment, otherwise known as "poly-victimization". Exposure to multiple types of maltreatment is a stronger predictor of psychopathology than exposure to a single type, even if chronic or severe [36, 48, 49]. Individuals with AUD report exposure to two or more types of childhood abuse or neglect at a much higher rate compared to those without AUD [15]. However, findings on

whether cumulative maltreatment exposure increases risk specifically for AUD, above and beyond exposure to a single type, are inconsistent [20, 50].

Biological Alterations Associated with Childhood Adversity and Maltreatment

A fundamental question is how exposures that occur during childhood can have such persistent and long-lasting effects on an individual. The answer lies in extensive evidence obtained from both animal and human studies that childhood maltreatment impacts the biological development of the individual, including changes in the brain, body, and even gene expression. These changes can ultimately become "biologically embedded", i.e., progress from transient responses to long-term, stable alterations that influence health and well-being throughout the life course [51]. A summary of some of the more well-documented changes is provided below. Much of what we know about early maltreatment and biological development stems from research using animal models, where experimental manipulations such as maternal separation, variation in maternal care, and social isolation have been used as proxies for childhood maltreatment. However, the validity of such models for the human experience of childhood maltreatment is debated. Consequently, the review below focuses on findings that have been corroborated in human studies as well as in animals.

Hypothalamic-Pituitary-Adrenal (HPA) Axis

The HPA axis is the primary biological system regulating the neuroendocrine response to stress. Childhood maltreatment results in chronic activation of the HPA-axis, which can lead to altered regulation of this axis throughout the life span. The expression of this dysregulation is inconsistent, however, both in childhood and later in adulthood. In studies of both animals and humans, maltreatment has been linked to both higher and lower basal and diurnal cortisol levels, and to both increased and attenuated cortisol responses to stressful stimuli. Contradictory findings for ACTH have also been reported [43]. There is some converging evidence that childhood maltreatment results in an initial hyperactivity of the HPA axis, followed by attenuation of response in adolescence and into adulthood [52–54]. However, several factors are known to moderate effects of maltreatment on the HPA-axis, including but not limited to sex, genetic make-up, and the timing and type of exposure [55]. Altered functioning of the HPA-axis has long been implicated in problematic alcohol use, with both augmented and blunted cortisol responses reported as possible precursors to AUD. Cortisol also interacts with other brain mechanisms, such reward, learning, and memory, all of which are also implicated in the etiology of AUD [56]. Consequently, the HPA axis is a prime suspect for being one of the pathways by which childhood maltreatment increases risk for AUD.

Brain Structure and Function

Another pathway is through changes in brain structure and function. Human brain development takes place over a long period of time, from before birth and into early adulthood, although the vast majority of volumetric growth occurs in the first 2 years of life. Exposure to stress during sensitive periods of development, and the ensuing release of glucocorticoids and neurotransmitters, affects neurogenesis, synaptic pruning, and myelination processes [57]. Childhood maltreatment has been associated with decreased gray matter volume in various brain regions, most notably the hippocampus, amygdala, anterior cingulate, and prefrontal cortex, regions that make up the corticolimbic system involved in emotion and cognition, as well as control of the HPA axis [51, 58]. Interestingly, these reductions are often seen in adults but not in children—for example, studies suggest an initial increase in volume of the amygdala during childhood that may precede a decrease in volume later in adulthood [57]. Attenuated development or reduced volume has also been reported for the caudate nuclei, the insula, and the cerebellum [59–61]. White matter changes have also been documented, the foremost being reduced size and integrity of the corpus callosum, a crucial white matter tract that connects and allows neural transmission between the two hemispheres [43, 62]. It is important to note that maltreatment-related effects on brain structure may be further augmented by heavy alcohol consumption in adolescence, which has also been shown to impair brain development processes during this stage of life [63].

With regards to brain function, two consistent findings in those exposed to childhood maltreatment are a diminished response of the striatum (nucleus accumbens, caudate, and putamen) to anticipated or actual reward, and hyper-reactivity of the amygdala to threat and negative emotional stimuli [51, 57, 58]. Relatedly, studies show changes in functional connectivity following maltreatment in both reward circuitry and stress/emotion circuitry, including increased connectivity of the salience network to both the insula and the amygdala, and reduced connectivity between the amygdala and cortical regions [64]. The latter is likely to contribute to heightened amygdala responses through diminished top-down control [62, 65]. Of note, all of these regions are considered part of "addiction circuitry", encompassing the processes of stress, emotion, reward, executive function, and motivation [66]. This reinforces the potential for alterations in brain structure and function to serve as a potential link between childhood maltreatment and risk for AUD.

Immune System and Inflammation

Similar to the brain, development of the immune system is continuous throughout childhood and is sensitive to environmental factors. Childhood maltreatment has been linked to elevated levels of C-reactive protein (CRP), a global marker of

inflammation, and to proinflammatory cytokines such as Interleukin-6 (IL-6), Interleukin 1-beta (IL-1β), and Tumour Necrosis Factor (TNF-α) [67–69]. While many of these findings stem from studies of adults with a history of childhood maltreatment, prospective studies have also reported increases in inflammatory makers in children recently exposed to maltreatment [70, 71]. Overall, there is stronger evidence for effects of abuse on inflammation, compared to neglect [68]. Chronic inflammation can result in immunosuppression, and links between childhood maltreatment and low-level immunosuppression have also been identified [51]. Chronic activation of the immune system in childhood may also affect brain development and functioning of biological stress systems [69] which, as described above, are notable pathways linking childhood maltreatment exposure to risk for AUD. Of note, alcohol consumption also triggers neuroimmune activation and inflammation. Furthermore, both posttraumatic stress disorder (PTSD) and depression share a high comorbidity with AUD, and it has been postulated that immune/inflammatory mechanisms play a significant role in risk for comorbidity of these disorders [72].

Epigenetics

Epigenetics changes comprise several chemical modifications to the DNA and DNA-associated proteins that can alter gene expression in a persistent manner, but do not modify the DNA sequence. Modifications include DNA methylation, histone modifications, and alterations in the level of non-coding microRNAs (miRNAs), processes that occur throughout the lifespan but are also susceptible to environmental factors [73]. These processes are some of the primary drivers of the biological embedding of early life experiences [51]. Evidence from both animal and human studies identify a number of genes that show changes in methylation levels associated with early maltreatment, including the glucocorticoid receptor (NR3C1), FK506-binding protein (FKBP5), arginine-vasopressin (AVP), brain derived neurotrophic factor (BDNF), oxytocin receptor (OXTR), serotonin transporter and synthesis (SLC6A4 and TPH2), dopamine receptor (D2), monoamine oxidase A (MAOA), and the glutamate receptor (NMDA), with the caveat that most human studies have been limited to analyses of blood and saliva rather than brain tissue [73–75]. Histone modifications and miRNA dysregulation associated with childhood maltreatment have likewise been reported for some of these genes in both animals and humans. Many of these genes are involved in stress-system regulation, either directly or through other processes such as reward, fear-conditioning, and cognitive function, all of which are implicated in addiction. However, identifying DNA methylation patterns as precursors for risk for AUD is problematic, given that alcohol consumption itself is known to cause methylation changes [76, 77]. Childhood maltreatment-associated changes in plasma levels of miRNAs, which can target many different genes, have also been identified in pathways for neurodevelopment and inflammation [78].

Behavioral Effects Associated with Childhood Adversity and Maltreatment

Childhood maltreatment has been associated with a broad range of behavioral problems and challenges across the life span. During childhood, these include insecure attachment, poor self-esteem, difficulty controlling emotions, poor impulse control, learning difficulties, aggressive behaviors, internalizing behaviors, and PTSD [7, 79]. These early disruptions can persist into adolescence and young adulthood, manifesting as interpersonal problems, self-regulation/emotion regulation difficulties, anxiety and mood disorders, executive function difficulties, and impulsivity. A history of childhood maltreatment has also been associated with poor sleep quality, alterations in reward-related behavior, and a heightened threat sensitivity [80–86]. Many of these broad-spectrum behaviors contribute in varying degrees to a general vulnerability for addictive disorders, including alcohol use disorder [87, 88]. In addition to the above, childhood maltreatment has been directly associated with behaviors specific to problematic alcohol use and the development of AUD, as detailed below.

Alcohol-Specific Behaviors

Alcohol use disorder is explicitly tied to alcohol consumption behaviors. The earlier these behaviors begin, the longer they persist, and the context or motives for continuing these behaviors are all important factors predicting risk for AUD. As a result, adolescence and young adulthood are crucial periods of risk, especially for individuals who have experienced childhood maltreatment. Both an earlier age of onset for alcohol use and adolescent binge drinking are associated with exposure to childhood adversity and maltreatment. Studies of large representative samples in the U.S. have shown that exposure to specific types of adversity including emotional, physical, and sexual abuse, emotional and physical neglect, and parental discord/divorce, is associated with increased odds of alcohol use initiation by or before ages 13–14. In addition, exposure to more than one type of adversity is associated with even greater odds of early initiation, as high as 3.6-fold among those experiencing four or more types [47, 89, 90]. Similar studies have also shown that childhood abuse and neglect are significant risk factors for adolescent binge drinking (consuming five or more drinks in a row at least two to three times in the past year), as well as a sharper increase in rates of binge drinking across adolescence and persistence into young adulthood [91, 92].

Among the motives for consuming alcohol, “drinking to cope” refers to consuming alcohol as a means to alleviate stress, anxiety, and other negative emotions. Drinking to cope is a strong predictor of long-term drinking behavior and AUD [93] and can emerge as early as adolescence, particularly among those exposed to

maltreatment and thus are more likely to experience frequent emotional distress [89]. In both adolescents and young adults, drinking to cope has been shown to mediate the effects of childhood maltreatment and alcohol use problems [94, 95]. In adults, drinking to reduce negative affect is often described as "self-medication", and while self-medication may alleviate distress in the short-term, in the long-term it often results in increased severity of symptoms and problematic alcohol use [96].

Other behaviors linked to AUD that may be impacted by childhood maltreatment include craving, impaired control over drinking, and "high-intensity" binge drinking. For craving, the effect of childhood maltreatment is often indirect, i.e., acute stress in adulthood results in increased craving for alcohol among individuals with a history of childhood trauma, more so than in those without such history [97, 98]. Impaired control is defined as the inability to stop drinking after beginning to consume alcohol, despite one's intentions, and like craving is one of the key criteria for AUD. Studies have indicated both direct and indirect links between childhood maltreatment and impaired control, with the indirect effects primarily mediated by PTSD in young adults [99, 100]. High-intensity binge drinking is defined as drinking two or more times the standard threshold for binge drinking. In adults, a history of family dysfunction and substance abuse, as well as childhood maltreatment, is associated with greater odds of high-intensity binge drinking [101].

Alcohol Use Disorder

Quite a few studies have linked childhood adversity and maltreatment to increased risk for AUD later in life. As mentioned previously, experiencing more than one type of adversity during childhood is associated with increased risk for lifetime AUD, with four or more events conferring even more severe risk [45, 46]. With respect to maltreatment, data from NESARC indicate odds ratios (ORs) for lifetime AUD ranging from 1.1 to 1.8 for the five types of maltreatment measured by the CTQ [39, 102, 103], with the higher estimates attached to the abuse subtypes. Physical and emotional abuse have also been associated with increased odds of seeking treatment for AUD among emerging adults [20]. Increased risk for a diagnosis of AUD is only part of the story, however. Childhood adversity and especially maltreatment is associated with an accelerated transition through the stages leading to the disorder (from age of first opportunity to use, to age of first drink, to age of regular alcohol use, and to age on onset for AUD) [104, 105], an earlier onset of AUD, especially in women [105, 106], persistence of AUD for at least 3 years [107], greater severity of AUD [15], and increased rates of psychiatric comorbidity [14]. Furthermore, a history of childhood maltreatment, particularly abuse, has been linked to poorer outcomes in those seeking treatment for AUD. These include increased probability of relapse, a shorter amount of time to relapse, and a decrease in global functioning [17, 18, 108].

Moderating Factors

Several important moderating factors can influence vulnerability to childhood adversity and maltreatment and the long-term effects of early stress exposure. These include intrinsic factors such as sex, race, and genetic make-up, as well as extrinsic factors such as early intervention, and family and social support. Notably, not all individuals exposed to childhood adversity and maltreatment experience lasting negative consequences, including AUD. While early adverse experiences are prevalent, so are individuals who successfully cope with or manage to overcome them. Indeed, moderating factors play a role in resilience as well vulnerability.

Sex and Race

Sex differences in exposure to childhood maltreatment have been reported, with the most consistent findings being higher rates of childhood sexual abuse among women [31, 109, 110]. Furthermore, sex differences in key biological systems known to be impacted by childhood maltreatment (e.g., HPA axis, corticolimbic system) have been documented [111]. However, findings on sex differences in the *consequences* of childhood maltreatment for behavioral health have been inconsistent. In terms of general psychopathology, some studies have suggested that women are more vulnerable to the effects of childhood maltreatment, while other studies suggest it is men that are more vulnerable [112]. Prevalence rates for AUD are consistently higher in men compared to women, and yet while some studies have found a stronger association between childhood maltreatment and problematic alcohol use in women compared to men [113, 114], more recent studies have found little to no sex differences in the association of childhood maltreatment history with either problematic alcohol use or AUD in adults [102, 103, 109]. There is evidence to suggest that the pathways from childhood maltreatment to AUD may differ between the sexes, however, despite no overt differences in diagnosis outcome. For example, the magnitude of effects of sexual and physical abuse on early onset of substance use and past year alcohol problems may differ between the sexes [115, 116]. All things considered, the relationship between sex, childhood maltreatment, and risk for AUD is complicated, as variables such as maltreatment type and severity, development stage, genetics and biological factors, and even cultural norms can play a role. Nonetheless, awareness of potential sex differences remains important when considering the mechanisms underlying a diagnosis of AUD [112].

Prevalence rates for childhood maltreatment tend to be higher among minority populations such as Native Americans, African Americans, and Hispanics, and it is argued that the true burden of childhood maltreatment and trauma in these groups is routinely underestimated [36]. Interestingly, there are some studies that suggest African Americans may be less vulnerable to the subsequent development of psychopathology compared to White Americans [117–119]. However, long-term trends

indicate that past year prevalence of AUD has been increasing among African Americans and Hispanics at a higher rate than in White Americans [120]. Whether this increase is associated to any degree with childhood maltreatment rates is undetermined.

Genetics

Just as there is a genetic risk for AUD, genetic make-up can serve as both a risk factor for, and a protective factor against, the effects of childhood maltreatment on biological functioning and behavior. Studies of gene-environment interaction (i.e., individual genetic variation influences the response to environmental exposures) in the context on childhood maltreatment exposure have for the most part focused on stress-related genes. Candidate gene approaches have identified several specific genes of interest that interact with childhood maltreatment, including FKBP5, SLC6A4, MAOA, and corticotropin-releasing hormone receptor 1 (CRHR1) [9, 121, 122]. However, the effect of individual genes can be quite small and are not always replicated. Accordingly, new approaches using polygenic risk scores (PRS), or weighted sums of risk alleles across the whole genome, could offer a more comprehensive view of genetic risk and resilience in the face of childhood maltreatment. PRS for AUD have been identified and found to predict AUD independent of family history [123]. However, whether PRS for AUD interact with childhood maltreatment is still unknown, therefore more research is needed in this area.

Resilience and Other Protective Factors

The fact that many individuals who experience childhood maltreatment do not develop psychopathology or substance use problems indicates an important role for resilience in the etiology of AUD. Resilience is generally defined as the ability to adapt or cope with significant adversity, trauma, tragedy, or stress, and is considered a multidimensional construct. Sources of resilience can be intrinsic to the individual (i.e., genetics, neurobiology, personality, individual coping mechanisms) or extrinsic protective factors existing at the individual, familial, and community levels. Examples of the latter include social support from a caregiver, family and/or friends, community involvement, access to social services and mental health care, and educational support [36, 124, 125]. Resiliency and protective factors can have an impact across the time course from childhood maltreatment to AUD, but particularly during the early stages immediately following exposure, and in the later stages where an individual is liable to transition from low-risk alcohol use to AUD. Studies have shown that indicators of resilience such as personality, emotional control, and a supportive home and social environment are associated with decreased risk for AUD

[126, 127]. These findings have important implications for intervention and treatment approaches, as discussed below.

Methodological Challenges

One of the biggest challenges in assessing the impact of childhood maltreatment on lifetime outcomes is the fact that many studies rely on retrospective self-reporting of childhood experiences, which is highly subject to recall bias. Prospective measurement, typically involving family reports made at the time of maltreatment, or use of official records (public and/or police reports), circumvents the problem of recall bias and yet is not without downsides. Family reports may not always be reliable as events often occur in private and are not reported, and official reports often only capture the more severe cases of maltreatment, while missing most forms of emotional abuse. As a result, prospective reporting can actually underestimate the actual prevalence of childhood maltreatment [110]. Studies of agreement between retrospective and prospective measures of childhood maltreatment generally result in poor concordance between measures, although concordance is somewhat higher if retrospective measurement is interview-based rather than questionnaire-based [128]. Another significant challenge in assessing childhood maltreatment as a risk factor for AUD specifically is the necessity to separate that risk from familial factors, both genetic and environmental. AUD is highly heritable [129], and a parent or parents with AUD may be more likely to abuse or neglect children [130]; both of these are confounding factors when investigating the association between history of childhood maltreatment and AUD. A handful of retrospective studies have suggested an effect of childhood maltreatment independent of these factors [131, 132], however, prospective studies offer greater reliability in teasing out the unique effects of maltreatment and familial factors. One such study looking at substance use disorders overall indicated that childhood maltreatment is still a substantial risk factor even when controlling for familial confounding [11].

Implications for Prevention and Treatment

Ideally, childhood adversity and maltreatment is addressed early on through intervention and prevention, such as removing the child to a safe environment. Unfortunately this is not always possible, and in the context of AUD, which develops primarily in adulthood, the prospect of early intervention has already passed. Individuals with AUD and a history of childhood adversity and maltreatment can present an extra challenge for treatment providers, due to greater severity of addiction and increased comorbidity with anxiety, depression, and PTSD. Consequently, screening of adult patients for a history of early adversity and trauma is strongly

recommended as standard practice [51, 110]. Obtaining this information is key to assessing individual treatment needs, as integrative treatments that simultaeously address both trauma and substance use can be implemented. There is a variety of treatment modalities that are utilized for individuals with AUD, and while a review of these is beyind the scope of this chapter, some methods have been specifically put forward as beneficial to those with a history of childhood maltreatment. These include complementary mental health care for comorbid psychiatric disorders (including medications), cognitive behavioral therapy (CBT), mindfulness, and more recently, eye movement desensitization and reprocessing (EMDR) [43, 133]. In addition, resilience-oriented approaches that involve individual, family, and community-based factors linked to resilience, and strength-based approaches that increase an individual's sense of purpose and encourage prosocial acts, may be particularly beneficial as they can attenuate stress and increase well-being [36]. These and other interventions, while unlikely to reverse the effects of maltreatment and trauma during development, may help provide compensatory mechanisms to minimize it's effects, with the added benefit of contributing to treatment and recovery outcomes for alcohol use disorder.

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Chapter 35
Animals Models Used to Study Alcohol Use Disorder

Asmae Lguensat, Andrea Coppola, and Eric Augier

Abstract For ethical and technical reasons, research in humans has some limitations and requires the support of animal models. Numerous animal models have been developed over the years to study alcohol consumption and model alcohol-related behaviors in several species, including non-human primates, rodents and more recently zebrafish, fruit flies and C. elegans. In this chapter, we provide an overview of the most commonly used animal models of alcohol use disorder (AUD) and discuss their pros and cons. We classify animal models of AUD into two main categories, operant and non-operant paradigms, which covers behavioral procedures developed to model several aspects of human addiction, including primary alcohol reinforcement, physical dependence, loss of control over alcohol intake, progressive choice of alcohol over healthy rewards and relapse. Finally, we will conclude and discuss about other important aspects of human addiction, including interindividual differences, sex differences and social factors, that need to be incorporated into preclinical models of AUD to improve their translational value.

Keywords Alcohol use disorder · Animals · Preclinical models · Behavior · Reward · Motivation · Choice

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Introduction

Animal Models in the Context of AUD: Definition and Generalities

An animal model refers to a non-human animal which is used in research to investigate biological processes or disorders in humans, including alcohol use disorder (AUD). Numerous animal models have been developed over the years to study alcohol consumption and model AUD-related behaviors in rodents [1–4], non-human primates (for review, see [5]) and more recently in zebrafish [6], fruit flies [7] and C. elegans [8]. Despite the utility of such models in investigating AUD on a molecular and a circuitry level, concerns have recently emerged regarding the limited translation of findings obtained in these models to humans [9]. Nevertheless, the recent failure in clinical trials of promising mechanisms identified and validated in animal models of AUD does not necessarily invalidate animal models, or render them useless [10, 11].

Historical Background

The use of animals to study and understand basic physiology and vital functions began back in ancient times [12]. The rationale behind such use is that humans and animals have comparable physiological processes and thus animals can be used to study and better understand human physiology [12]. Animal research has contributed to the fundamental understanding of human physiology and disease and to the development of vaccines and pharmacotherapies [13]. When it comes to understanding addiction, the use of animals to achieve that aim only started in the twentieth century, given the common belief at the time that the intense desire for drugs, a core feature of addiction, was uniquely human and thus addiction could not be modeled in animals [14]. Moreover, the few animal studies of drugs of abuse at the time (morphine studies: [15, 16]) largely focused on how the body responds to drugs, while overlooking their behavioral effects [17]. The first records of behavioral drug effects in dogs showed an increased desire to get injected with the drug and stopping to resist being injected when they become addicted [17–19]. However, the only observable manifestation provided by these studies that was taken as measure of addiction was the submissiveness of the animal to injections, and other measurable manifestations were lacking. It was only in the late 1930s that Sidney Spragg [20] demonstrated, for the first time, that animals were able to voluntarily work for a dose of drug. He developed a paradigm in which chimpanzees could choose between morphine and a natural reward, a banana or an orange. The animals exclusively chose morphine when they were under withdrawal of the opioid drug, but almost exclusively chose the fruit in other experimental conditions. This confirmed that

drugs of abuse could also exert reinforcing properties in animals, and that animals could display addiction-like symptoms. This established that using animal models in addiction research was possible.

Paradigms to Model AUD in Animals

In this chapter, we classify animal models of AUD into two main categories, operant and non-operant paradigms. Operant conditioning (also known as instrumental or Skinnerian conditioning) requires training in which performing a certain task, such as pressing a lever, is rewarded by the delivery of the drug. Animals learn to work for a reward (positive reinforcement) but to avoid punishment (negative conditioning). Non-operant models refer to approaches that rely on spontaneously emitted behavior, such voluntary drinking from a bottle available in homecage.

Non-operant Animal Models of AUD

These procedures are very commonly used in the context of alcohol research. They include, for example, free choice drinking paradigm [3], conditioned place preference [21] and intermittent exposure to alcohol vapor [22, 23].

Free Choice Drinking Paradigm

The standard free choice drinking paradigm, also known as two-bottle choice (2 BC) typically involves individually housing the animals and giving them free access to two bottles containing either water or a solution of alcohol. Variation of this protocol have also been reported, in which animals can drink from three or more bottles [24] (one water bottle and the others containing varying concentrations of alcohol) either continuously (24 h, several consecutive days) or intermittently (one day on and one day off) [25]. Several factors, including the alcohol concentration, the schedule of alcohol availability, and the number of available bottles can influence alcohol consumption [11]. For example, intermittent access to alcohol (repeated cycles of free access followed by a withdrawal period) induces escalation of alcohol intake in rodents [25, 26], therefore mimicking an aspect of human addiction, which is the progressive transition from controlled to excessive consumption. Other advantages of this paradigm are that the procedure is simple and straightforward, making it highly reproducible among laboratories. Alcohol consumption is voluntary, and animals initiate drinking without the need of saccharin fading procedure. Finally, FDA-approved drugs, such as naltrexone, significantly reduce

voluntary alcohol drinking in these models [27, 28], providing predictive validity to the model. However, free-choice drinking models have several limitations, such as the fact that animals are frequently single housed. Social isolation is a well-known stressor in rodents and therefore could be a confounding factor when studying addiction-like behaviors [29]. A key challenge for these models is also the low amount of effort required to obtain alcohol, which makes is difficult to assess the level of motivation, and the relatively low blood alcohol concentrations (BACs) typically achieved, which limit conclusions whether the alcohol is consumed for its pharmacodynamic effects of the central nervous system, or other reasons, such as calory content or taste. Approaches have been developed to address these limitations, in which free choice drinking can result in higher blood alcohol levels compared to operant self-administration [30, 31], but even when these are used, the levels remain below those seen with vapor exposure models, and withdrawal symptoms are not observed during water days [31].

Moreover, using the 2 BC model does not permit measuring any observed behavioral effects of alcohol withdrawal, nor modeling motivation and compulsive seeking of alcohol [26], suggesting that the two-bottle choice could be useful in modeling the consummatory side of alcohol addiction but does not capture other behavioral aspects of it.

Chronic Intermittent Exposure to Alcohol

Under most conditions, laboratory animals will not voluntarily consume sufficient amounts of alcohol to induce tolerance and withdrawal, processes typically seen as human AUD develops. To address this limitation, chronic intermittent exposure (CIE) to alcohol vapor is an increasingly common procedure used in preclinical alcohol addiction research. It consists of exposing rats to alcohol vapor in standard housing cages connected to a vapor inhalation chamber [22, 32]. Commonly, rodents are exposed to daily alcohol vapor inhalation (14–16 h per day) during several weeks to months [33]. The main advantage of this procedure is that the experimenter can manipulate and control the induction of physical dependence in exposed animals, by varying multiple parameters such as the dose, the duration, and the pattern of exposure. As a result, animals reach pharmacologically relevant BACs, and exhibit robust signs of physical dependence, which can be measured using behavioral indicators such as withdrawal–related behaviors [34]. Although this model is effective in inducing physical dependence, it lacks face validity given that the route of self-administration differs from the oral route use by humans. Another common criticism of CIE is that it is experimenter-imposed (i.e., non-contingent), and that the forced exposure lacks the motivational aspects of human addiction. To address this, CIE is often used with the objective of inducing neurobiological processes of relevance for a AUD, and then coupled with other procedures, such as the two-bottle choice [32] and operant self-administration [35] to determine the motivational and behavioral consequences of these processes. It has been reported that alcohol vapor

exposure leads to escalated alcohol consumption, compulsive-like drinking (i.e., continued use despite negative consequences) and other physical and motivational symptoms reminiscent of AUD (for detailed reviews, see [33, 35]. Moreover, this combined approach offers a good predictive validity since many tested drugs have been shown to be effective in reducing addiction-like symptoms in animals (Naltrexone: [36]; Baclofen: [37]; Prazocin: [38]) Finally, recent attempts have been made to couple the CIE with operant self-administration [39].

Conditioned Place Preference

Perhaps the first demonstration of conditioned place preference in animals was provided by Olds and Milner in their seminal study, in which they demonstrated the existence of "reward centers" in the brain [40]. They found that rats that voluntary pressed for electrical stimulation in the septal area returned and spent time in the compartment in which they had received these rewarding stimulations. The conditioned place preference (CPP) was later extensively used to study the motivational effects of drugs of abuse in laboratory animals (for review, see [41]), with the first study investigating the reinforcing effects of morphine [42]. This kind of conditioning involves associating the substance, which in this model serves as an unconditioned stimulus, with the environmental context in which the substance effects are experienced, and which thus becomes a conditioned stimulus. If the animal spends more time in the side associated with drug injections, it can be concluded that this substance has a rewarding effect.

Several substances, including alcohol, can induce a robust conditioned place preference, although the strength of this phenomenon varies strongly with the species used, as well as the dose and the time point in relation to drug administration (see below) [43]. Although the dose, the time of injections and the frequency of administration of the substance are controlled and imposed by the experimenter, this model has been used extensively to study the predisposition of animals to develop addiction-like symptoms and the impact of stopping the administration of a given drug (withdrawal). This model is therefore useful to understand drug-seeking behavior [21] which is considered a key parameter in the development and maintenance of addiction.

Place preference models are widely used across a wide ranges of species, including C elegans, fruit flies, rodents, primates and humans [44], indicating that this procedure and its findings translate between species. More specifically, in the case of alcohol, CPP studies in rodents [45–47] and humans [48, 49] pointed out the development of a preference for a given context following the pairing between the rewarding properties of alcohol and that context, showing that interpreting CPP in rodents as drug reward is validated by human research. The main criticism of this procedure is its lack of face validity, given that alcohol is injected by the experimenter and not voluntarily consumed by animals. Moreover, this paradigm only assesses the rewarding effect of alcohol and/or its ability to reduce negative emotional states as well as seeking of the substance

after withdrawal and after reinstatement [50, 51]. It does not model other addiction-like behaviors, such as motivation to drink alcohol or compulsive seeking despite negative consequences. CPP is also very sensitive to several experimental parameters, such as the dose and the duration of the pairing between alcohol and the context. In fact, CPP is inversely proportional to the duration of the context-alcohol pairing, with greater preference obtained with shorter exposure period [52] and with lower doses (1–2 g/kg [53]).

To conclude, although CPP is a simple and short procedure that is helpful in measuring the addictive potential of alcohol in several species, it remains sensitive to several procedural and experimental design related variations, and therefore requires more standardization.

Operant Animal Models of AUD

Operant Alcohol Self-Administration

In addition to models of voluntary oral ingestion and forced passive administration described above, alcohol research relies on operant self-administration models to investigate the reinforcing properties of the drug.

Operant self-administration in the alcohol research field traces back to early 1970s, when researchers were able to show that both non-human primates and rodents could be trained to self-administrate alcohol by performing simple tasks, such as pressing a lever [54, 55]. Even though early studies used this model to induce physical dependence and to study pharmacological effects of the drug, Woods and colleagues [56] already reported the importance of operant models to study the reinforcing properties of the drug, modelling aspects of drug-seeking in the human condition. In a subsequent study, Winger & Woods [57] also reported how acquisition and maintenance of alcohol self-administration was influenced by different schedules of reinforcement.

Operant models have been useful to investigate, among others, the role of stress in alcohol consumption and escalation of alcohol self-administration [58–60]; several studies also used operant models to report dependence-induced escalation of alcohol intake [32, 61–64]. FDA-approved medications, such as naltrexone [65], naloxone [65, 66], acamprosate [67] have also been shown to reduce alcohol consumption and alcohol seeking in rodents, assessed with operant models. Moreover, operant models provide a good potential to investigate more complex aspects of human addiction. Indeed, by modifying the environmental conditions under which operant responding occurs, schedules of reinforcement or availability of the drug, self-administration procedures have been used to model motivation for alcohol, loss of control over alcohol intake, aversion-resistance (“compulsivity”), choice of alcohol over a natural reward, and relapse.

Different Routes of Operant Self-Administration

Early attempts at using operant models mostly used intravenous administration procedures [for review, see [68]]. The main advantage of this route of administration is that it allows to investigate the reinforcing properties of the drug while bypassing the potential confounds from orosensory properties of alcohol (i.e., taste, smell) and individual variations in its absorption, since alcohol is directly infused into the bloodstream [68]. For the same reason, other non-oral routes of administration have been used, such as intragastric self-infusion [69–71] and intracerebral self-infusion [72]. With these procedures, researchers aimed to induce voluntary self-infusion of alcohol doses that could lead to intoxication levels comparable to humans [68]. However, despite some positive results, especially in non-human primates [73, 74], others failed to show intoxication with intravenous self-administration, especially in rats [75, 76]. Further criticism of the non-oral routes of self-administration is sustained by its poor face validity since humans mainly consume alcohol orally.

For this reason, development of reliable models for oral operant self-administration was needed. Early attempts were characterized by the use of several manipulations prior to the training, such as water/food deprivation [77, 78] and sweetening the alcohol solution [79], or for using secondary conditioning procedures [80, 81]. The reasoning behind these approaches was that animals, especially rodents, were not thought to initiate and maintain stable alcohol oral self-administration because of its aversive taste [82]. However, recent efforts have shown that rats can be trained to orally self-administer alcohol without the use of any water/food deprivation or sweetener fading [83–85]. This is crucial to assess the role of alcohol as a reinforcer, while eliminating the confounding effects of its caloric and sensory properties.

Models of Motivation for Obtaining the Drug

Maintenance of operant responding at increasing costs, such as increasing ratio requirements or with more stringent schedules of reinforcement, is considered as a measure of the motivational effects of alcohol. The most frequent strategy is to use progressive ratio (PR) schedules of reinforcement, in which the ratio (i.e., number of responses) requirement to obtain a single unit of alcohol reward increases progressively within a single session [86, 87]. The last completed ratio requirement is defined as “breaking point” or breakpoint and can represent the maximum “price” the animal is willing to spend to reach the reward. Breakpoint is a quite reliable parameter, as it is consistent over days under baseline conditions [88]. It can also be evaluated by increasing fixed ratio requirements of self-administration day by day, between single sessions, as has been done in previous studies with other drugs of abuse [89, 90]. This strategy is preferable to the previous one especially for psychostimulants, since it rules out the confounding effects of cumulative infusions in within-session progressive ratio schedules [91]. However, these procedures require

extensive training and multiple days of testing. To our knowledge, there are no studies reporting between-session breakpoint assessments in alcohol research.

Other protocols that rely on operant responding to model motivation for obtaining the drug include the extinction paradigm and second-order schedules of reinforcement [92]. In the extinction paradigm, the persistence of the animals to perform a task even when it is not reinforced with the drug anymore can be used as a parameter for the motivation for the drug. Eventually, all animals would extinguish the operant behavior, a condition that is then necessary to test propensity to relapse in the reinstatement model (see paragraph below).

Second-order schedules of reinforcement are schedules in which completion of a schedule contingency serves as a unitary response that is reinforced following a fixed schedule of the primary reinforcement [93]. In most cases, upon completion of the n-th response on a fixed ratio, a drug-associated cue (tone or light) is briefly presented, and the first completion of this schedule after a fixed interval elapses is reinforced with the drug and longer presentation of the drug-associated cue [94]. Second-order schedules have the advantage of maintaining high operant responding even when drug presentation is limited to small amounts. This allows researchers to better evaluate the role of drug-seeking and motivation to take the drug, without the potential confound of cumulative infusion/ingestion present in conventional progressive ratio schedules. These schedules are also able to capture complex behavioral sequences of drug-seeking that are similar to humans [95, 96], and are sensitive to increasing doses of the drug [97]. However, second-order schedules seem to be less stable over time than primary reinforcement schedules, and their complexity limited their extensive use to non-human primate models [98]. However, studies of second-order schedules in rodents have also been reported [99, 100].

Animal Models of Loss of Control Over Alcohol Intake and Aversion-Resistant Drinking

Loss of control over alcohol intake is one of the hallmarks of AUD. Operant animal models attempt to model uncontrolled alcohol-seeking by inducing escalation of alcohol intake in experimental settings. Contrary to intravenous psychostimulants and opioid drugs, for which long-term access to the drug (6 h minimum) during daily sessions produces a robust escalation of responding [101], no such operant model exists for alcohol. Escalated alcohol intake has been shown using persistent drug access for several weeks [102, 103] or by intermittent alcohol access [104] in non-operant settings, as described above. Dependence-induced [63] and stress-induced escalation of alcohol [60] have also been reported using operant self-administration models.

Loss of control over alcohol intake has been widely studied in relation to other aspects of AUDs. Perhaps most important among these is compulsive-like drinking, i.e., use of the drug despite adverse consequences [105]. This is typically operationalized as aversion-resistance, i.e., the insensitivity of experimental animals to aversive stimuli associated with alcohol reinforcement. In these approaches, alcohol can be associated with the bitter taste of quinine [63, 102, 106], with contingent

punishment such as a foot shock [106–108], by the presentation of foot shock-associated cues (conditioned suppression [109]), or by contingent exposure to lithium chloride with passive infusion of alcohol [110, 111]. The use of quinine as aversive stimulus has been quite popular in the alcohol field for its face validity [112]. Indeed, alcohol dependence in humans leads to increasing acceptability of bad tasting cheap liquor, or even non-beverage alcohol such as mouthwash or eau de cologne [113], a condition mimicked by taste aversion-resistance induced by quinine in laboratory animals. The use of foot shock, instead, is sometimes preferred to quinine for its flexibility, since it allows aversive stimulation to be presented at different schedules (i.e., probabilistic punishment) or even exploited to test conditioned suppression to shock-associated cues [109]. Moreover, intensity of aversion can be more easily titrated by adjusting duration and amplitude of the punishment [105]. However, its face validity is poor since electric shock is never experienced by humans in relation to their alcohol consumption. Devaluation of alcohol by postingestive injection of lithium chloride has been postulated as a model to specifically devalue the psychoactive pharmacological effects of the drug, rather than its taste or its associated seeking responses. Suppressed responding by this devaluation strategy showed that alcohol seeking is also reinforcing for the drug's pharmacological effects and not only for its taste or caloric value [110].

Aversion-resistance models have been used to investigate possible interindividual differences in the vulnerability to develop addiction-like behavior in animals [106] (see Conclusion below). Indeed, at specific intensities of aversion, different subpopulations of sensitive and resistance animals were identified, both for quinine-aversion [103] and for foot-shock punishment [114]. Despite its promising results, criticism about these being proper models for compulsive drinking in humans still exist [105]. Mainly, it is hard to say that resistance to aversive stimuli associated with drug-seeking reflects loss of control over intake, especially because animals had no other alternatives than taking the drug in these experimental settings [115].

Finally, it is important to state that loss of control models have been also studied in terms of inflexibility of behavioral responding, or the inability to adapt seeking behavior in response to changing environmental conditions. A big part of the literature reports that inflexibility might be also a consequence of transition from goal-directed seeking behavior to habit formation [see [116] for review]. Modeling this type of transition in operant settings has been intensively used by protocols of outcome devaluation, such as pre-feeding or satiety [117] or by contingency degradation [118], assessed in operant settings. However, contributions and role of habit formation in addiction phenotype, such as compulsive drinking is still debated [119, 120] and it is out of the scope of this review.

Choice Models Between Alcohol and Healthy Rewards

Alcohol addiction in humans occurs typically—but not always—in complex environments, in which access is available to possible alternative, healthy, rewards, and can promote voluntary abstinence. The development of drug addiction is

characterized by a shift in decision making, in which drugs become increasingly chosen over these healthy rewards. The fact that the availability of alternative non-drug rewards has been so far largely overlooked in animal studies of drug addiction and alcohol therefore appears as a potential limitation [68, 115]. In fact, in his landmark monograph [20], Sidney Spragg already showed the importance of providing alternative rewards to drug. In this study, morphine-dependent chimpanzees only preferred morphine infusions over a palatable food (a fruit) when tested under morphine withdrawal. However, in baseline conditions, they almost always favored the sweet taste of the banana or orange over a morphine injection. Following this seminal work, two main strategies have been employed: concurrent choice schedules of reinforcement, where animals have simultaneous access to two alternative rewards contingent to different levers present in the operant chambers; and discrete-choice schedules where instead access to one reward mutually excludes the other.

Concurrent choice schedules built on basic findings from early work by Myers [121] which assessed alcohol preference over water under operant conditions. Following this, extensive work from Marylin Carroll and colleagues [122, 123] showed that rats stopped cocaine self-administration when concurrently presented with availability of non-drug rewards, such as sweetened water solutions. Concurrent choice protocols have also been studied in relation to behavioral economics of AUD, reporting that uncontrollable use in patients is reflected by inelastic demand for the drug [124]. Inelastic demand of alcohol has been reported in previous studies in rodents [125, 126] and reflects the insensitivity to decrease seeking responses for the drug at increasing ´´prices´´ (i.e., schedule requirements) when palatable non-drug alternative reward, such as food, is available. Even though this model's good face validity and simple methodology make it advantageous for addressing choice behavior, it does not provide a realistic proxy of individual preference for the drug over non-drug rewards, since the two options are both available at the same time.

Paradigms in which choices are mutually exclusive in a series of discrete choice trials have been more widely used. Using this type of approach, early studies reported choice of cocaine over food in rhesus monkeys [127, 128]. Later adaptations of the complex discrete-choice paradigms used in non-human primate research were successful in replicating similar findings in laboratory rats, which broadly increased the utility of these models [129]. These studies reported that rats shifted their choice preference from cocaine to saccharin rewards when these were presented as alternatives in discrete-choice paradigms [130]. However, a subpopulation of animals retained the preference for cocaine [131]. We recently extended these findings to alcohol [106], and reported that in accordance with other drugs of abuse, only a significant minority of outbred Wistar rats will keep working for alcohol when a sweet alternative is available (about 15% of animals). This subpopulation of alcohol preferring rats also show increased motivation for alcohol, assessed in a progressive ratio procedure, and aversion-resistant drinking, assessed both with quinine-adulteration and foot shock-punished protocols, compared to saccharin-preferring individuals [106]. These findings indicate that discrete-choice paradigms

could be useful for investigating factors of individual vulnerability to AUD, also given that proportion of alcohol-preferring rats closely reflects the epidemiological data in humans [106, 132]. Exclusive choice models have also been used to investigate the role of other non-drug alternatives such as social rewards to induce voluntary abstinence of drugs of abuse [133]. We and another lab have recently applied it to alcohol [134, 135]. Quite unexpectedly, we found that outbred Wistar rats almost exclusively responded for alcohol when offered the opportunity to access the social reward as an alternative, independently of the nature of the social partner (cagemate vs. novel rat), the length of social interaction, housing conditions (group housed vs. short isolation before the operant session or chronic isolation) or sex. The reason for this discrepancy with models of choice between alcohol and sweet rewards remains unclear and more studies are needed.

Animal Models of Craving and Relapse

A key challenge of clinical addiction treatment is to prevent relapse after patients achieve abstinence.

Since its introduction in a seminal study [136], reinstatement of drug seeking following extinction has been widely used to model relapse in animals, and to investigate the underlying neural mechanisms [137]. To reinstate alcohol-seeking, it is first necessary to initiate robust and stable levels of alcohol self-administration. Once operant responding for alcohol is acquired, the reinstatement procedures start with an extinction phase, in which the operant response that previously led to an alcohol delivery no longer has a programmed consequence. Following extinction training, responses on the alcohol-associated lever decrease to low levels, or stop. Reinstatement of responding for alcohol under extinction conditions (i.e., in the absence of the reinforcer) can then be induced by triggers that parallel those promoting relapse in patients, with discrete cues and stress being most robust for alcohol. The rate of operant responding (i.e., reinstatement) on the lever previously associated with alcohol delivery is taken as a measure of the animal's urge to obtain alcohol, a model of craving in patients (see our recent review [138] for a detailed overview of preclinical models of alcohol relapse).

Despite its advantages in modelling multiple aspects of human disease, conventional operant self-administration models are not sensitive to interindividual differences. Meanwhile, in humans, not all individuals that drink alcohol develop addiction. In animal models that rely on instrumental responding, almost all the animals always learn the contingency between the drug and the specific requirement. However, by modulating the context and conditions at which drug is delivered, operant procedures have served as a tool to study effects of vulnerability and resilience to the development of alcohol addiction.

Concluding Remarks and Future Directions

Although many advances and improvements have been made during the years to improve the translational value of animal models of AUD, several other important aspects of human alcohol addiction need to be incorporated to these models.

Individual Differences, Genetic Heterogeneity, and the Use of Inbred vs. Outbred Rodent Lines

Animal lines selectively bred for high alcohol preference (e.g., Sardinian rats: [139]; alcohol-preferring P rats [140, 141] or AA (alko, alcohol) rats [142, 143]) or strains displaying contrasting emotional and cognitive responses (e.g., high/low anxiety: [144]) have been used to elucidate the implication of specific genes or behavioral traits in vulnerability or resilience to AUD [11]. Individual differences that are observed to occur spontaneously within populations of outbred lines such as Wistar rats have also been extensively used in AUD research (e.g., [106]). The use of lines selectively bred for alcohol preference may be useful for studying and identifying genetic factors that predispose to alcohol [145], but the generalization of findings from these studies, and their translational value could be limited given that these models do not reproduce the heterogeneity of human genetics and responses. Furthermore, in a phenomenon that is often overlooked, selective breeding over many generations results in random allelic fixation throughout the genome (see e.g., [146]). As a result, allelic variation at a large number of loci will appear to be associated with the alcohol-related phenotype for which the line was bred, despite a lack of any functional contribution from those loci. Thus, studies in these lines require the inclusion of interventional experiments to demonstrate a causal contribution from identified alleles. Meanwhile, the use of outbred rats has shown that only a minority of individuals exposed to alcohol develop addiction-like symptoms [106, 108, 114], replicating human findings, and suggesting both face validity and utility for discovery of molecular mechanisms. Thus, selectively bred lines and spontaneous individual variation in genetically heterogenous populations represent complementary approaches.

Incorporating Interindividual Differences

Capturing interindividual differences has been a challenge for animal models of AUD. This comes from the fact that most animals trained with standard self-administration procedures end up acquiring the response for alcohol [84]. Here, an important distinction has to be made between models of “non-addictive substance use” that assess rewarding properties of drugs, on one hand, and models of

addiction-related behaviors on the other. Models such as CPP and operant self-administration have been long considered as models of addiction but in fact they are now more classified as models of substance reward, instrumentalization, and non-addictive substance use [9]. This leads to the necessity of shifting the focus into developing models that take into account more than the consummatory or preference side of addiction and thus capturing interindividual differences in AUD. One of the attempts to establish models capturing interindividual differences was in the context of cocaine addiction with the three criteria model [147], modelling several of the diagnostic criteria for substance use disorders. Another model was the choice model [130] that managed to demonstrate that the majority of rats are resilient to develop cocaine addiction-like behaviors, given that they prefer saccharin over cocaine. In the context of AUD, Jadhav et al. [108] and Augier et al. [106] have extended the three criteria model and the choice model, respectively, to study individual vulnerability to alcohol. They were able to show that only a minority of rats show a combination of addiction-like behaviors such as high motivation for alcohol, resistance to footshock and higher addiction scores. This gives more validity to these models shedding light on the heterogeneity of responses within the exposed populations and calls for a systematic use of them in preclinical AUD studies.

AUD Animal Models and Sex Differences

The inclusion of female subjects in animal models of AUD has largely been neglected until recently. The classical justification of this is the fact that AUD is more prevalent in men [148] and therefore it is appropriate to focus research on male subjects. However, the prevalence of AUD in women has dramatically risen in the recent years compared to men (women, 84% increase; men, 35% increase [149]). Some evidence indicates that females may be affected differently by alcohol: they, for example, may initiate the use earlier, progress to addiction quicker [150] and seek treatment earlier than men [151]. However, this is still controversial, and a recent systemic review didn't find evidence in both clinical and animal studies to support the notion that women are more vulnerable to psychostimulant and opioid craving and relapse [152]. More research is needed to understand whether this is also the case for alcohol.

Altogether, this suggests that females represent a population that has different and very understudied addiction-related characteristics [35] and highlights the importance of a systematic inclusion of female subjects in all preclinical addiction investigations.

The Inclusion of Social Factors into AUD Models

The evidence that social factors are important in drug addiction comes from human studies, showing for instance that proximal factors (the immediate presence of peers) are contributing to the onset of substance use (e.g., [153]). As opposed to mice, rats are highly social, and thus suitable for studying social factors in addiction [11]. To date, the majority of studies examining the impact of social factors on alcohol addiction in animals (alcohol self-administration and seeking behaviors) have studied the influence of distal social factors, such as social stress [154], early life social stress [155] and isolation [156]. However, such models give limited information regarding the impact of the immediate presence of peers given, that the social factors are absent at the moment of drug exposure. This points out the necessity of having models integrating social factors during behavioral testing. There were fewer attempts in integrating proximal social factors into AUD models compared with animal models of other drugs (morphine: [157]; methamphetamine: [133]; heroin: [158] and cocaine: [159, 160]). A previous report indicated that the presence of a peer promotes drinking in a two-bottle choice setting [161]. Two laboratories, including ours, have recently employed a choice model in which animals are given the option of choosing between alcohol and a brief interaction with a peer [134, 135] and examined the impact of the concomitant availability of a social reward on alcohol self-administration. The results point out that social reinforcement provided by the brief interaction with a peer is weak compared to alcohol reward, which differs from stimulants and opioids [133, 158]. More efforts are therefore needed in order to elucidate the mechanism by which alcohol acts as powerful reinforcer surpassing social reward.

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Chapter 36
Alcohol Use Disorder: Stress, Negative Reinforcement, and Negative Urgency

Leandro F. Vendruscolo, George F. Koob, and Eric P. Zorrilla

Abstract Alcohol use disorder is a chronically relapsing disorder that involves aspects of compulsivity in alcohol seeking and taking, difficulty limiting alcohol intake, and the emergence of negative emotional states, such as dysphoria, anxiety, irritability (e.g., hyperkatifeia), in the absence of alcohol. Alcohol addiction encompasses a three-stage cycle that intensifies with continued alcohol use: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. These stages engage neuroadaptations in brain circuits that involve the basal ganglia (incentive salience), extended amygdala (reward deficit/stress surfeit), and prefrontal cortex (executive dysfunction). Here, we discuss key neuroadaptations in stress systems in alcohol addiction. These neuroadaptations contribute to negative emotional states and negative urgency that are hypothesized to powerfully drive alcohol drinking and seeking and promote relapse. Changes in stress systems, combined with the disruption of prefrontal cortex function that leads to cognitive deficits, impairments in inhibitory control, and poor decision making, contribute to the chronic relapsing nature of alcohol addiction.

Keywords Addiction · Reward · Glucocorticoids · Mineralocorticoids · Steroids · Hypothalamic-pituitary-adrenal axis · Extended amygdala

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Introduction

Alcohol use disorder (AUD), also termed alcohol addiction, is a medical condition that is characterized by an impaired ability to stop or control alcohol use despite adverse social, occupational, or health consequences. Currently, three medications—disulfiram, acamprosate, and naltrexone—are approved by the United States Food and Drug Administration (FDA) for the treatment of AUD [1]. However, although these medications are effective, they are underutilized. Like the treatment of mental illness in general, more individually targeted, practical, affordable, and acceptable treatments would alleviate suffering and increase wellbeing.

In individuals who are not diagnosed with AUD, the consumption of alcohol typically causes pleasurable effects (e.g., euphoria). These pleasant effects increase the probability of drinking, which is defined as positive reinforcement. However, AUD involves aspects of compulsive alcohol seeking and drinking, difficulty limiting alcohol intake, and the emergence of a negative emotional state, such as dysphoria, anxiety, and irritability (hyperkatifeia), during alcohol abstinence. These negative emotional states can persist long into alcohol abstinence and are hypothesized to help perpetuate compulsive alcohol drinking and seeking via negative reinforcement. Negative reinforcement is defined as the process by which the removal of an aversive state, such as somatic and motivational signs of withdrawal, increases the probability of a response (e.g., alcohol is consumed to alleviate anxiety, pain, and dysphoria). Negative reinforcement is conceptually distinct from punishment, which involves the presentation of an aversive stimulus contingent to a behavior.

Alcohol addiction encompasses a three-stage cycle [2]: binge/intoxication (heavy alcohol drinking), withdrawal/negative affect (hyperkatifeia), and preoccupation/anticipation (craving; Fig. 36.1). These stages are interconnected and intensify with continued alcohol misuse. They involve neuroadaptations in numerous brain circuits that include the basal ganglia (incentive salience), extended amygdala (reward deficit/stress surfeit), and prefrontal cortex (PFC; executive dysfunction). Here, the extended amygdala is defined as a supra-structure that includes the central nucleus of the amygdala (CeA), the bed nucleus of the stria terminalis (BNST), the sublenticular substantia innominata, and a transition zone in the medial part of the nucleus accumbens (e.g., shell) [4].

The multidimensional nature of AUD involves a complex interaction between alterations in brain reward and stress systems and executive function that engage multiple neurocircuits and neurotransmitter systems [5, 6]. Much work has focused on incentive salience and repetitive behavior (often described as habits) that are associated with the alcohol binge/intoxication stage. However, repeated cycles of alcohol intoxication and withdrawal produce neuroadaptations in reward and stress systems and cognitive function that in turn drive alcohol drinking. Thus, stress systems have an important effect on driving alcohol misuse and precipitating craving and relapse in later phases of AUD [2].

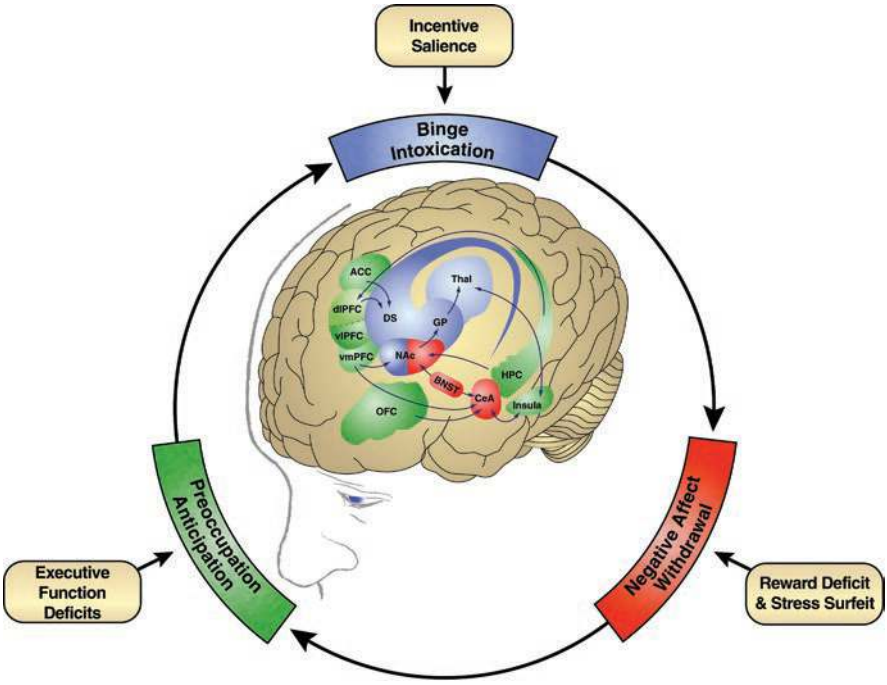

Fig. 36.1 Conceptual framework for the neurobiological basis of addiction. In the *binge/intoxication* stage, reinforcing effects of drugs may engage neurocircuits of the basal ganglia (blue structures). Reward neurotransmitter activation and associative mechanisms engage the nucleus accumbens shell and core, and then stimulus-response habits engage the dorsal striatum. Two major neurotransmitters that mediate the rewarding effects of addictive drugs are dopamine and opioid peptides. In the *withdrawal/negative affect* stage, the negative emotional state of withdrawal may engage activation of the extended amygdala (red structures). The extended amygdala is composed of several basal forebrain structures, including the bed nucleus of the stria terminalis, central nucleus of the amygdala, and possibly a transition zone in the medial portion (or shell) of the nucleus accumbens. Major neurotransmitters in the extended amygdala that are hypothesized to function in negative reinforcement are corticotropin-releasing factor, norepinephrine, and dynorphin. There are major projections from the extended amygdala to the hypothalamus and brainstem. The *preoccupation/anticipation* (craving) stage involves neurocircuitry of the cortex and allocortex (green structures). The processing of conditioned reinforcement involves the basolateral amygdala, and the processing of contextual information involves the hippocampus. Executive control depends on the prefrontal cortex and includes the representation of contingencies, the representation of outcomes, and their value and subjective states (i.e., craving and, presumably, feelings) that are associated with drugs. The subjective effects, termed "drug craving" in humans, involve activation of the orbital and anterior cingulate cortices and temporal lobe, including the amygdala. A major neurotransmitter that is involved in the craving stage is glutamate that is localized in pathways from frontal regions and the basolateral amygdala that project to the ventral striatum. ACC, anterior cingulate cortex; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; DS, dorsal striatum; dlPFC, dorsolateral prefrontal cortex; GP, globus pallidus; HPC, hippocampus; NAC, nucleus accumbens; OFC, orbitofrontal cortex; Thal, thalamus; vlPFC, ventrolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex. (Modified from [3])

The biological mechanisms of alcohol addiction can be investigated using animal models. These models provide critical information about the etiology and pathophysiology of AUD and will continue to guide the discovery and development of new treatments for AUD. The use of experimental animals in preclinical alcohol research gives the experimenter control over several factors, such as genetic background, the environment, and exposure to alcohol, which are all difficult to control in humans. Although no animal model fully recapitulates AUD in humans, experimental animals exhibit behaviors that have predictive validity in the domains of alcohol drinking that persists despite negative consequences, motivational withdrawal, hyperkatifeia, and hyperalgesia [7–9].

Here, using rodent models as a framework, we argue that repeated, intense cycles of alcohol intoxication and withdrawal elicit neuroadaptations first at the level of the hypothalamic-pituitary-adrenal (HPA) axis, a primary neuroendocrine stress system, and subsequently in extrahypothalamic brain regions (i.e., basal ganglia, extended amygdala, and PFC). These physiological and neural adaptations lead to hypofunctional brain reward systems (reward tolerance, hypohedonia), stress surfeit (anxiety, irritability, and pain), and executive dysfunction (cognitive deficits, poor decision making, and poor judgment) that are associated with AUD. These neuroadaptations are hypothesized to maintain alcohol misuse via negative reinforcement and contribute to relapse risk even long into protracted abstinence.

Neurocircuitry Perspective of Allostatic Stress System Changes in Addiction

Our hypothesis is that as motivational dependence on alcohol develops, reward systems are compromised, and brain stress systems are recruited in the extended amygdala. We further hypothesize that these brain stress neurotransmitters that are known to be activated during the development of excessive drug taking comprise a between-system opponent process, and this activation is manifest when the drug is removed, producing such negative emotional symptoms as anxiety, depression, irritability, and pain (both emotional [hyperkatifeia] and physical) that are associated with acute and protracted abstinence. Between-system neuroadaptations can also impact within-system neuroadaptations to further exacerbate negative emotional states by suppressing reward function, which was originally hypothesized for dynorphin by Carlezon et al. [10]. The activation of cyclic adenosine monophosphate response element binding protein (CREB) by excessive dopamine and opioid peptide receptor activation in the nucleus accumbens is hypothesized to trigger the induction of dynorphin to feedback to suppress dopamine release. Thus, we argue that anti-reward circuits are recruited as between-system neuroadaptations [11] during the development of addiction and produce aversive or stress-like states via the direct activation of stress systems (e.g., corticotropin-releasing factor [CRF] in the

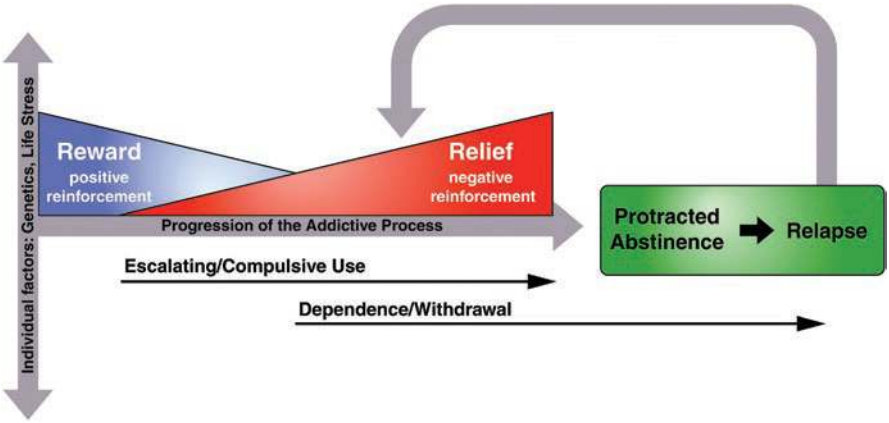

Fig. 36.2 Conceptual framework of sources of reinforcement in addiction. Positive reinforcement, in which the drug typically engenders positive hedonic effects, is defined as an increase in the probability of responding that is produced by the presentation of a drug. Positive reinforcement is associated with the early stages of addiction as part of the binge/intoxication stage but persists throughout the addiction cycle. Negative reinforcement is defined as an increase in the probability of responding for a drug to relieve hyperkatifeia or stress, in which drug withdrawal during the withdrawal/negative affect stage of the addiction cycle typically engenders hyperkatifeia and stress. Both sources of reinforcement can co-exist and be perpetuated by protracted abstinence and cue-, drug-, and stress-induced reinstatement in the preoccupation/anticipation stage of the addiction cycle. (Figure modified from an original diagram from Dr. Loren Parsons)

extended amygdala) and the indirect activation of a hypohedonic state by suppressing dopamine.

The long-lasting neuroadaptations in stress and reward systems that lead to motivational symptoms during acute withdrawal and protracted abstinence may provide the basis by which drug priming, drug cues, and acute stressors acquire additional power to elicit drug-seeking behavior. Notably, multiple alcohol detoxifications and relapses can further enhance withdrawal that is increasingly less responsive to treatment [12]. This "kindling" effect may be reduced by the blockade of brain stress systems, suggesting a key role for brain stress systems in this phenomenon [13] (Fig. 36.2).

Dysregulation of the Hypothalamic-Pituitary-Adrenal Axis in Alcohol Addiction

The endocrinologist Hans Selye conceptualized stress and HPA axis function as adaptive responses to environmental challenges, termed "general adaptation syndrome" [14]. The HPA axis is a primary neuroendocrine system that is engaged in response to environmental stimuli [15, 16]. Knowledge of such an HPA axis, with glucocorticoids as an endpoint, encouraged the search for hypothalamic releasing

factors (i.e., CRF) [17] that control adrenocorticotropic hormone (ACTH), which in turn drives glucocorticoid release from the adrenal gland [18]. Stress stimulates the paraventricular nucleus (PVN) of the hypothalamus to release CRF that acts in the anterior pituitary to release ACTH into the bloodstream, which then activates melanocortin receptor 2 (an ACTH receptor) in the cortex of the adrenal glands. This causes the rapid production and release of glucocorticoids into the blood circulation. Glucocorticoids have physiological effects on many tissues and alter behavior. In situations of high or chronic stress, negative feedback mechanisms along the HPA axis, including the PVN, pituitary, and hippocampus, prevent further glucocorticoid release.

In the brain, glucocorticoids bind to two types of receptors: mineralocorticoid receptors (type I) and glucocorticoid receptors (type II). Mineralocorticoid receptors have high affinity for glucocorticoids, whereas glucocorticoid receptors have lower affinity for glucocorticoids. Thus, at normal circulating levels of glucocorticoids, the occupancy of mineralocorticoid receptors is already substantial, and high circulating glucocorticoid levels are necessary to activate glucocorticoid receptors. Activation of the HPA axis is adaptive and critical for survival. However, intense and sustained HPA axis activation may lead to long-lasting detrimental neuroadaptations that contribute to the development of mental disorders [15, 16].

Like stress, acute exposure to alcohol activates the HPA axis [19]. In nondependent rats, adrenalectomy decreased alcohol drinking, which was restored by corticosterone replacement [20]. Glucocorticoid receptor antagonism blocked alcohol-induced conditioned place preference (a measure of reward) in mice [15, 21]. These findings suggest that glucocorticoids contribute to alcohol's acute rewarding/reinforcing effects in a nondependent state. By extrapolation, glucocorticoids contribute to incentive salience [22]. The effects of glucocorticoids on alcohol's motivational effects may involve the release of dopamine in the mesolimbic system [23]. Extracellular dopamine levels decreased in the nucleus accumbens shell [24] in rats that were subjected to adrenalectomy to suppress endogenous glucocorticoids. Thus, glucocorticoids may contribute to reward/incentive salience function in response to alcohol in the binge/intoxication stage.

Excessive activation of the HPA axis by repeated alcohol exposure and withdrawal leads to the dysregulation of HPA axis activity [25–28]. Activation of the HPA axis by alcohol is blunted (i.e., neuroendocrine tolerance) in alcohol addiction [27, 29] (Fig. 36.3). An injection of alcohol increased blood ACTH and corticosterone levels in nondependent rats but much less so in alcohol-dependent rats [27]. This blunted HPA axis response to alcohol may contribute to the decrease in alcohol-induced rewarding effects in alcohol addiction. Dysregulation of the HPA axis in alcohol addiction is associated with alcohol craving and relapse [28]. The opioid receptor antagonist naltrexone, which activates the HPA axis, has anti-craving effects [33]. Activation of the HPA axis may be a consequence of the naltrexone-induced blockade of tonic inhibitory effects of endogenous opioids [29] on the PVN and other modulatory stress-related regions (e.g., locus coeruleus). However, naltrexone can also cause a direct aversive/stressful response in rats and humans that

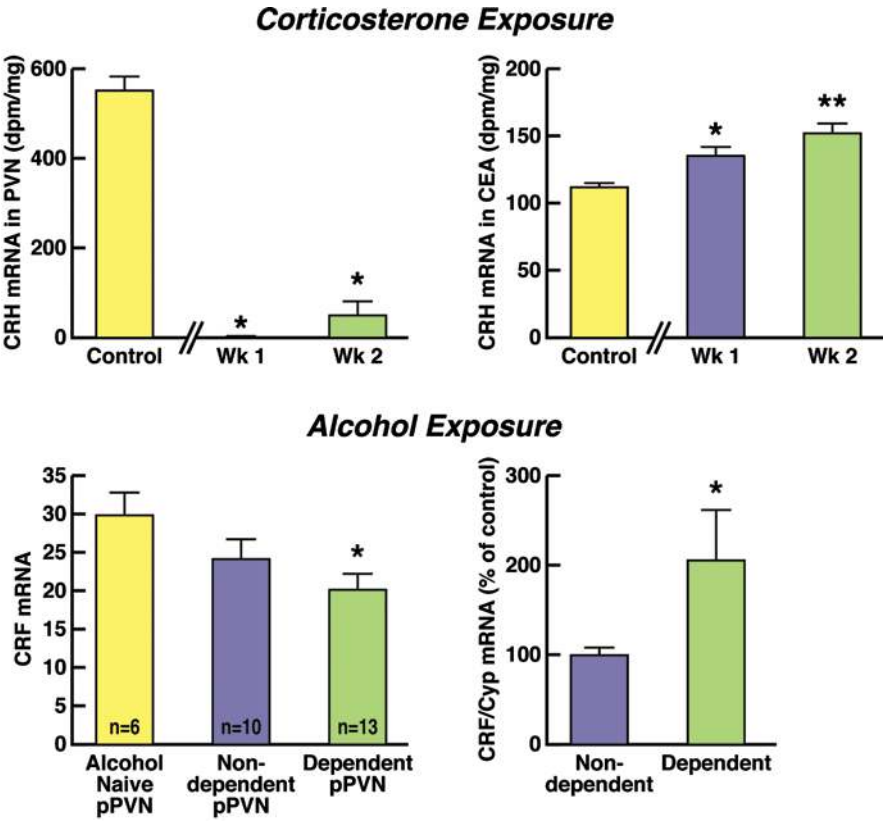

Fig. 36.3 Corticosterone blunts hypothalamic-pituitary-adrenal axis and sensitizes the central nucleus of the amygdala. (Top left) Corticotropin-releasing hormone (CRH; the nomenclature for the CRF gene) mRNA hybridization levels in the paraventricular nucleus induced by corticosterone pellet (200 mg) implantation. Control rats ($n = 12$) were obtained from the pool of rats that were euthanized at the same time points as the experimental group ($n = 7$ for each time point). The data are expressed as the mean + SEM. $*p < 0.001$, *vs*. control (Taken with permission from [30]). (Top right) CRH mRNA hybridization levels in the central nucleus of the amygdala induced by corticosterone pellet implantation over 2 weeks. Control rats ($n = 12$) were obtained from the pool of rats that were euthanized at the same time points as the experimental groups ($n = 7$ for each time point). The data are expressed as the mean + SEM. $*p < 0.01$, $**p < 0.001$, *vs*. control (Taken with permission from [30]). (Bottom left) CRF mRNA significantly decreased in the pPVN in dependent animals compared with alcohol-naive controls ($*p = 0.01$) but not compared with nondependent animals. The groups did not differ in CRF mRNA levels in the magnocellular division of the PVN (mPVN; data not shown). The data are expressed as the mean ± SEM. (Taken with permission from [31]). (Bottom right) In alcohol-dependent rats ($n = 8$), levels of CRF mRNA, normalized to cyclophilin A, significantly increased in CeA punches ($*p < 0.05$) compared with naive controls ($n = 11$), measured by quantitative real-time polymerase chain reaction. (Taken with permission from [32])

consume high levels of alcohol [34]. Furthermore, alcohol-associated cues stimulate glucocorticoid release in humans with a history of AUD who are abstinent from drinking [35] and produce craving, suggesting that glucocorticoids contribute to conditioned responses [36] to promote relapse.

The blunting of HPA axis responses in alcohol addiction may be attributable to CRF downregulation in the PVN [27, 30, 37]. Concomitantly with the downregulation of CRF in the PVN, there is a paradoxical upregulation of CRF levels in extrahypothalamic brain regions (see Fig. 36.3), such as the CeA and BNST, following chronic exposure to either glucocorticoids or alcohol [30, 32, 38]. This bidirectional regulation of CRF has been hypothesized to depend on the interaction between glucocorticoid receptors and various steroid-related co-regulators [39]. Functionally, these neuroadaptations in the extended amygdala may mediate hypohedonia and stress sensitization in alcohol addiction.

Role for CRF and Vasopressin in Alcohol Addiction

The dysregulation of brain CRF and vasopressin systems is hypothesized to underlie the enhanced anxiety-like behavior and enhanced alcohol self-administration that are associated with alcohol withdrawal and protracted abstinence. The pharmacological blockade of CRF_1 receptors or vasopressin (a co-regulator of the HPA axis that potentiates CRF's effects) V_{1b} receptors reduced alcohol drinking, especially in dependent rodents [32, 38, 40–47]. In mice [48] and high-intake nondependent rats [49], CRF receptor antagonists reduced binge-like drinking and stress-induced alcohol drinking. Electrophysiological studies of the CeA showed that the effects of alcohol on the presynaptic activation of γ-aminobutyric acid (GABA) interneurons were enhanced in dependent rats. These effects were abolished by CRF_1 receptor antagonists [32]. Moreover, a CRF_1, but not CRF_2, receptor antagonist normalized the long-term potentiation/intrinsic excitability of BNST neurons in rats during protracted abstinence [50]. There are prominent CRF projections from the CeA to the BNST. The optogenetic inactivation of CRF neurons in the CeA that project to the BNST during alcohol withdrawal reduced dependence-induced alcohol intake [51]. The blockade of CRF_1 receptors (systemic or intra-CeA) also decreased anxiety- and depression-like behavior [52–55] and attenuated hyperalgesia (i.e., an exacerbated tactile response) in alcohol-dependent rats [56] (Fig. 36.4). Anxiety and pain are both hypothesized to contribute to alcohol drinking [59, 60] in the withdrawal/negative affect stage.

Two double-blind, randomized, placebo-controlled, human laboratory studies with treatment-seeking subjects with AUD failed to support the efficacy of CRF_1 receptor antagonists in reducing craving for alcohol [61, 62]. However, based on these studies that have several limitations, one cannot conclude that CRF_1 receptor antagonists, or other drugs that target the CRF system, are ineffective for the treatment of AUD. Several reasons may explain the apparent lack of efficacy of CRF_1 receptor antagonists in humans. These include inadequate pharmacodynamic

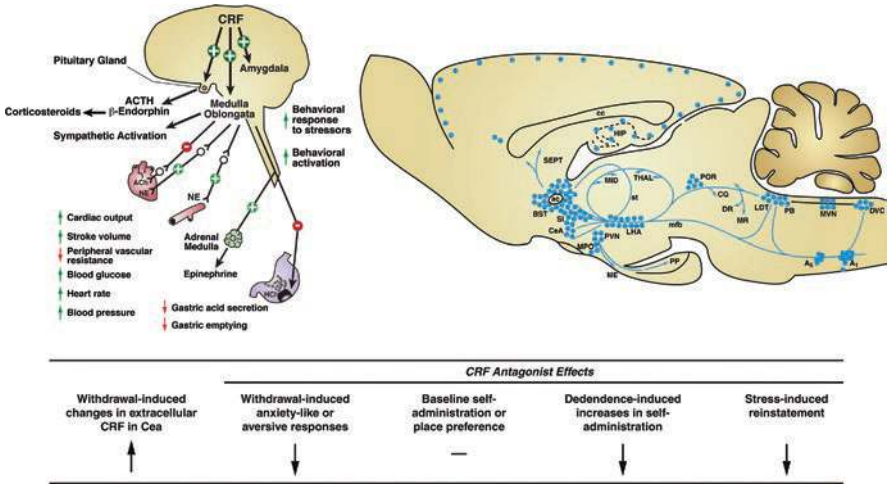

Fig. 36.4 Interactions between CRF and stress systems. (Left) Diagram illustrating the multiple actions of CRF in mediating stress responses in the body. CRF drives the hypothalamic-pituitary adrenal axis by acting to release adrenocorticotropic hormone (ACTH) in the portal system of the pituitary. CRF activates the sympathetic system by actions in the brainstem and mediates arousal and behavioral responses to stressors by actions in the amygdala, other basal forebrain regions, and ventral midbrain, such as the ventral tegmental area. Ach, acetylcholine; NE, norepinephrine (Taken with permission from [57]). (Right) Localizations and projections of brain stress systems. Corticotropin-releasing factor. The major CRF-stained cell groups (dots) and fiber systems in the rat brain. Most of the immunoreactive cells and fibers appear to be associated with systems that regulate the output of the pituitary and the autonomic nervous system and with cortical interneurons. Most of the longer central fibers course either ventrally through the medial forebrain bundle and its caudal extension in the reticular formation or dorsally through a periventricular system in the thalamus and brainstem central gray. The direction of fibers in these systems is unclear because they appear to interconnect regions that contain CRF-stained cell bodies. Three adjacent CRF-stained cell groups—laterodorsal tegmental nucleus, locus coeruleus, and parabrachial nucleus—lie in the dorsal pons. Uncertain are which of these cell groups contributes to each of the pathways shown and which of them receives inputs from the same pathways. ac, anterior commissure; BST, bed nucleus of the stria terminalis; cc, corpus callosum; CeA, central nucleus of the amygdala; CG, central gray; DR, dorsal raphe; DVC, dorsal vagal complex; HIP, hippocampus; LDT, laterodorsal tegmental nucleus; LHA, lateral hypothalamic area; ME, median eminence; mfb, medial forebrain bundle; MID THAL, midline thalamic nuclei; MPO, medial preoptic area; MR, median raphe; MVN, medial vestibular nucleus; PB, parabrachial nucleus; POR, perioculomotor nucleus; PP, peripeduncular nucleus; PVN, paraventricular nucleus; SEPT, septal region; SI, substantia innominata; st, stria terminalis. (Modified from [58])

profiles (e.g., fast receptor off-rates, inability to block ligand-independent CRF_1 signaling or other CRF system elements [CRF-BP, CRF_2]), plasticity/sensitization in mechanisms downstream of the CRF_1 receptor, heterogeneous or incomplete experimental populations (one study only involved women; subgroups defined for genetic or endophenotypic vulnerability, such as hyperkatifeia, might respond), and the temporal challenge of experimentally intervening in dynamic, motivational withdrawal symptoms [63]. Another important consideration is the brain site of

action of receptor blockade. The doses of antagonists that were used in these clinical studies were possibly not sufficiently high for complete CRF_1 receptor blockade in all relevant regions of the forebrain, even if some target engagement was demonstrated. As discussed elsewhere [64], high doses could produce such side effects as Addison's disease-like symptoms by the complete blockade of CRF_1 receptors in the anterior pituitary (outside the blood-brain barrier). Additional well-designed studies with novel anti-CRF compounds or, perhaps even better, the indirect modulation of CRF activity, such as with glucocorticoid receptor antagonists and steroid receptor coregulators [39, 65], may facilitate the discovery of drugs with clinical efficacy (see below).

A recent double-blind, placebo-controlled, multisite clinical trial investigated the role of vasopressin, which is known to modulate HPA axis activity and potentiate CRF's effects, in treating AUD. Vasopressin V_{1B} receptor antagonism increased the percentage of days abstinent compared with placebo [66].

In summary, like stress (e.g., life adversities), repeated alcohol exposure is hypothesized to lead to a delayed hypofunctional HPA axis and decreased reward/incentive-salience system and brain (extended amygdala) stress sensitization in vulnerable individuals. These adaptations may contribute to negative emotional states that are hypothesized to drive alcohol drinking and seeking via negative reinforcement (i.e., "self-medication"). Similar neuroadaptations may underlie the vulnerability to reward- and stress-related mental disorders, such as anxiety, depression, and pain, which are highly comorbid with AUD.

Increased Glucocorticoid Receptor Activity in Alcohol Addiction

Alcohol addiction is associated with glucocorticoid-dependent plasticity in brain reward and stress regions. Acute alcohol withdrawal (i.e., 6–24 h after the end of alcohol exposure) was accompanied by the downregulation of glucocorticoid receptor mRNA in the PFC, nucleus accumbens, and BNST, results that were interpreted as a compensatory effect of receptor overactivation in alcohol withdrawal [67]. Consistent with this hypothesis, glucocorticoid receptor signaling in the CeA, measured by levels of phosphorylated glucocorticoid receptors, increased in dependent rats compared with nondependent rats [68]. Notably, neurophysiology and regulation of the HPA axis and glucocorticoids are complex. Plasma levels of glucocorticoids do not necessarily reflect levels in the brain. Little et al. [69] reported that corticosterone levels increased in several brain regions in alcohol-dependent mice compared with nondependent mice, whereas both dependent and nondependent mice had similar blood corticosterone levels. These findings again suggest the greater activation of glucocorticoid receptors in the brain during acute alcohol withdrawal. Higher corticosterone levels in the brain in animals that are made dependent on alcohol may involve the activity of 11β-hydroxysteroid dehydrogenase 1, which colocalizes with glucocorticoid receptors and converts inactive glucocorticoids

(e.g., cortisone and 11β-dehydrocorticosterone) into active glucocorticoids (e.g., cortisol and corticosterone). The inhibition of 11β-hydroxysteroid dehydrogenase 1 with carbenoxolone reduced escalated alcohol drinking in both rats and mice [70].

The functional role of glucocorticoid receptors in alcohol drinking during acute withdrawal was evaluated using both acute and chronic glucocorticoid receptor antagonism. Chronic mifepristone (also called RU-38486 or RU-486) administration blocked the escalation of alcohol drinking in alcohol-dependent rats at doses that did not affect drinking in nondependent rats [67] (Fig. 36.5). Acute systemic or intra-CeA treatment with mifepristone or the more selective glucocorticoid receptor antagonist CORT113176 decreased escalated alcohol drinking in dependent rats but not in nondependent rats [68] and decreased binge-like alcohol drinking but not low drinking levels in mice [71]. Mice that exhibited high alcohol drinking, compared with mice that exhibited low drinking, exhibited alterations of the expression of several genes that are related to the glucocorticoid system in the nucleus accumbens [72]. The glucocorticoid receptor modulators CORT118335, CORT122928, and CORT125134 reduced alcohol self-administration in alcohol-dependent and nondependent rats, whereas CORT108297 had no effect on alcohol drinking in either

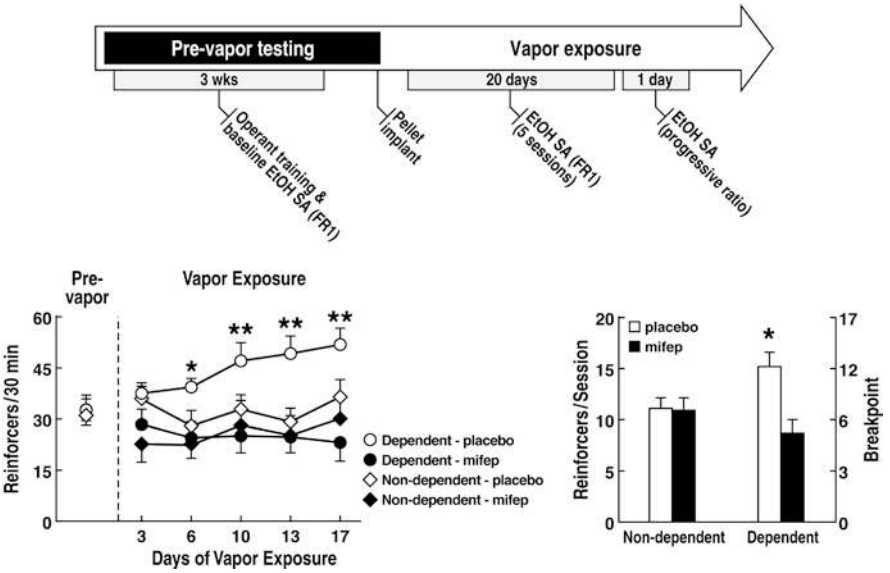

Fig. 36.5 Chronic glucocorticoid receptor blockade by mifepristone prevented the escalation of alcohol intake and motivation for alcohol in vapor-exposed animals. (**a**) Timeline of the experiment. Dependent and nondependent rats were implanted with pellets for the chronic release of mifepristone (150 mg for 21 days) or placebo before exposure to alcohol vapor. Mifepristone-treated vapor-exposed rats did not exhibit an escalation of alcohol intake (**b**) or an increase in progressive-ratio responding (**c**) compared with placebo-treated vapor-exposed rats. Mifepristone did not influence alcohol intake in nondependent rats. The data are expressed as the mean ± SEM. $^{*}p < 0.05$, difference from mifepristone-treated vapor exposed rats; $^{+}p < 0.05$, difference from placebo-treated nondependent rats. n = 9–10/group. (Taken with permission from [67])

group [73]. Acute systemic treatment with mifepristone reduced heavy alcohol drinking in rhesus macaques but did not block alcohol-induced relapse-like behavior in early abstinence [74].

In contrast to dependent rodents, mifepristone did not affect alcohol drinking in nondependent male rodents [20, 67, 68, 75–78] or baboons [79]. However, mifepristone reduced alcohol consumption in female rats [76] and the stress-induced reinstatement of alcohol seeking in male rats [78]. The intra-CeA (but not intrabasolateral amygdala) administration of mifepristone also suppressed the stress-induced reinstatement of alcohol seeking in nondependent rats [78]. Mifepristone prevented the increase in alcohol drinking in mice [80] and decreased alcohol drinking and seeking in rodents under stress conditions [80, 81]. Additionally, a systemic mifepristone injection reduced alcohol self-administration in nondependent male and female Wistar rats, but mifepristone had less of an effect in Marchigian-Sardinian alcohol-preferring rats. CORT113176 decreased alcohol self-administration in male and female Wistar rats and in female Marchigian-Sardinian alcohol-preferring rats [82]. Mifepristone did not affect anxiety-like behavior in Marchigian-Sardinian alcohol-preferring rats [83]. Altogether, these findings suggest that glucocorticoid receptor antagonism reduces alcohol drinking and seeking under conditions of binge-like drinking, stress, and dependence.

During protracted alcohol abstinence (i.e., several weeks of abstinence from alcohol), glucocorticoid receptor mRNA expression levels were upregulated in the nucleus accumbens, BNST, and CeA in dependent rats compared with nondependent rats [67]. These findings suggest that HPA axis activity may be at least temporarily reduced during protracted abstinence [26, 84] and indicate that the expression of glucocorticoid receptors is dynamically regulated in alcohol-dependent and post-dependent states. These neuroadaptations may contribute to long-lasting symptoms of anxiety, craving, and irritability that persist into protracted abstinence.

During protracted abstinence, the gene that encodes glucocorticoid receptors, *Nr3c1*, was identified as a master regulator of gene expression in multiple brain regions, including the medial PFC, nucleus accumbens, CeA, and ventral tegmental area, in rats that were made dependent on alcohol [77]. The chronic systemic administration of mifepristone and acute administration of mifepristone in the nucleus accumbens and ventral tegmental area decreased the escalation of alcohol drinking in rats with a history of alcohol dependence but not in nondependent rats [67, 77]. Furthermore, rats that were chronically exposed to alcohol drinking and dependence exhibited robust cue/context-induced reinstatement of alcohol seeking during protracted abstinence, an effect that correlated with higher glucocorticoid receptor activity in the medial PFC [85]. These results suggest that the dysregulation of glucocorticoid receptor function may be a mechanism by which negative emotional states persist into protracted abstinence in the preoccupation/anticipation stage.

In electrophysiological studies of neurons in the CeA, mifepristone reduced the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) without affecting postsynaptic measures, suggesting a decrease in GABA release, with the largest effect in dependent rats compared with nondependent rats. The glucocorticoid receptor modulator CORT118335 did not significantly alter GABA transmission in naive

rats but decreased sIPSC frequency in dependent rats. Mifepristone decreased amplitudes of evoked inhibitory postsynaptic potentials only in dependent rats and during protracted withdrawal. These findings suggest that the efficacy of mifepristone and CORT118335 increases in rats that are made dependent on alcohol [86].

Glucocorticoid receptors and glucocorticoids are implicated in other alcohol-related behaviors. During alcohol withdrawal, circulating glucocorticoid levels positively correlated with the severity of cognitive deficits in individuals with AUD [87]. In rodents, glucocorticoids contributed to alcohol withdrawal-induced brain neurotoxicity via the activation of glucocorticoid receptors [49, 88–90]. Treatment with mifepristone during acute withdrawal attenuated memory deficits in mice during protracted alcohol abstinence [88]. The dentate gyrus of the hippocampus exhibits robust neurogenesis and is affected in animals that are made dependent on alcohol [91]. Mifepristone exerted a neuroprotective effect in this brain region in rats that were exposed to binge-like alcohol [49]. Mifepristone also attenuated motor cross-sensitization between stress and alcohol in mice [92] and reduced somatic signs of alcohol withdrawal [88, 90], indicating that the decrease in glucocorticoid receptor function via receptor antagonism normalized several signs of enhanced stress and alcohol addiction-like behaviors.

The positive results of the role of glucocorticoid receptors in animal models of AUD encouraged studies in humans. The effect of mifepristone on alcohol drinking and seeking was tested in a double-blind, randomized, placebo-controlled human laboratory study that included 56 non-treatment-seeking individuals who were diagnosed with AUD [68]. Mifepristone treatment (600 mg daily, orally, for 1 week) lowered craving for alcohol compared with placebo treatment. Craving was precipitated by the presentation of cues that were associated with alcohol-containing beverages in the laboratory. Mifepristone also reduced self-reported alcohol drinking during pharmacological treatment and at least for 1 week after treatment cessation compared with placebo. Mifepristone caused few adverse effects and appeared to improve liver function.

The methylation of the NR3C1 exon variant 1H increased and glucocorticoid receptor mRNA and protein levels decreased in the PFC in individuals with AUD compared with controls. Additionally, the expression of other stress-related genes, such as *CRF*, *POMC*, and *FKBP5*, was altered in the PFC in individuals with AUD compared with controls [93].

Alcohol-Induced Sensitization of Glucocorticoid Receptor Systems

The glucocorticoid receptor is a transcription factor that belongs to the nuclear receptor superfamily. When activated by glucocorticoids, glucocorticoid receptors translocate from the cytoplasm to the nucleus. The process of intracellular glucocorticoid receptor trafficking is regulated by a host of chaperones. In the nucleus, glucocorticoid receptors bind directly to glucocorticoid response elements in the DNA

or indirectly by tethering to other transcription factors to cause the activation or repression of gene expression [94]. Chronic alcohol exposure and withdrawal downregulate CRF in the PVN (i.e., HPA axis tolerance that contributes to reward deficits). CRF upregulation in extrahypothalamic regions (e.g., extended amygdala) contributes to stress sensitization. Thus, an intriguing question is how glucocorticoid receptor activation causes opposite effects in the regulation of CRF expression in two distinct brain regions. A potential mechanism is that the valence of glucocorticoid receptor actions on gene transcription largely depends on co-regulators, and the expression of these regulators is site-specific.

Steroid receptor coactivator 1 (SRC-1) has been implicated in glucocorticoid receptor-mediated CRF transcription, with two isoforms (SRC-1a and SRC-1e) playing opposite roles in CRF transcription. SRC-1a, which represses CRF transcription, is abundantly expressed in the PVN, whereas SRC-1e, which is more highly expressed in the CeA [95], has been shown to lack repressive function [96]. Antisense oligonucleotides, which are synthetic single-stranded strings of nucleic acids that interfere with gene expression by binding to RNA, were used to regulate SRC-1 splice variants *in vivo* [96]. An antisense oligonucleotide infusion in the CeA that favored the expression of SCR-1a over SRC-1e repressed CRF expression and decreased anxiety/fear-like behavior in mice [96]. The effect of manipulating SRC-1 splice variants in alcohol drinking remains to be determined, but a decrease in opioid addiction-like behaviors has been reported [65]. This is an exciting new therapeutic possibility, given that antisense oligonucleotides have already been successfully used in humans for the treatment of neurodegenerative disorders. Furthermore, the indirect and opposite actions of glucocorticoid receptors in different brain regions represent a potential novel mechanism of CRF regulation that may have advantages compared with direct CRF_1 receptor blockade.

Potential Role of Mineralocorticoids in Alcohol Addiction

Much less is known about the role of mineralocorticoids in alcohol addiction [97]. Both glucocorticoids (centrally and peripherally) and aldosterone (mainly peripherally) bind to mineralocorticoid receptors. Aldosterone is a mineralocorticoid steroid hormone that is produced in the zona glomerulosa of the adrenal gland. Aldosterone controls blood pressure and electrolyte levels through the mineralocorticoid receptor, which is encoded by the *NR3C2* gene. However, mineralocorticoid receptors are also expressed in the brain, including the CeA, hippocampus, and PFC. In the brain, mineralocorticoid receptors are preferentially activated by glucocorticoids. The expression of mineralocorticoid receptor mRNA in the CeA but not PFC negatively correlated with anxiety-like behavior and aversion-resistant alcohol drinking in dependent rats but not in nondependent rats [59]. Similarly, alcohol drinking negatively correlated with mineralocorticoid receptor

expression in the CeA but not PFC in long-term drinking rhesus macaques [59]. Both alcohol-drinking rhesus macaques and humans with AUD exhibited higher plasma aldosterone levels compared with controls, and aldosterone levels correlated with craving, anxiety, and the number of drinks consumed by humans with AUD [59].

Systemic or intracerebroventricular administration of the nonselective mineralocorticoid receptor antagonist spironolactone and the more selective mineralocorticoid receptor antagonist RU28318 did not reduce alcohol drinking in male rats [20, 75] or mice [80] in a continuous (24 h) two-bottle (water vs. alcohol) choice model or in a limited (1 h) two-bottle choice model following fluid restriction [81]. However, 7 days of oral spironolactone treatment decreased alcohol drinking (and blood pressure) in high drinking but not low drinking male rats that were given continuous two-bottle choice access [98]. In nondependent male and female rats, the systemic administration of spironolactone reduced operant alcohol self-administration [99]. In nondependent male and female mice, spironolactone dose-dependently reduced binge-like alcohol drinking [100]. In male and female alcohol-dependent and nondependent rats, spironolactone dose-dependently reduced operant alcohol self-administration [100]. Intra-CeA infusion of the selective mineralocorticoid receptor antagonist eplerenone and mineralocorticoid receptor knockdown in the CeA transiently reduced alcohol intake in nondependent rats [101]. In humans, two pharmacoepidemiological studies that used high-dimensional propensity score matching found that spironolactone dispensation was associated with a decrease in alcohol drinking [100, 102]. These findings suggest that mineralocorticoid receptors may be implicated in alcohol reinforcement and that spironolactone may be further studied as a potential pharmacotherapy for AUD.

Brain Stress Systems: Beyond Glucocorticoids and Corticotropin-Releasing Factor

The central role of glucocorticoids and CRF in the brain in behavioral responses to alcohol and stressors does not exclude the role of other brain stress systems. There is strong evidence for norepinephrine, dynorphin, hypocretin (also called orexin), substance P, and neuroimmune modulators in the higher alcohol intake that is associated with alcohol addiction-like behaviors in animal models (for review, see [5, 103]; Fig. 36.6). Thus, the activation of a pro-stress, pro-negative emotional state system is multidetermined and comprises the neurochemical bases of hedonic opponent processes. However, one may hypothesize that there is a multidetermined anti-stress buffer neurocircuitry that may help return the organism to homeostasis. The vulnerability to AUD may also involve hypoactive anti-stress circuitry. Indeed, neuropeptide Y, endocannabinoids, and possibly nociceptin may contribute to such circuitry [5, 103].

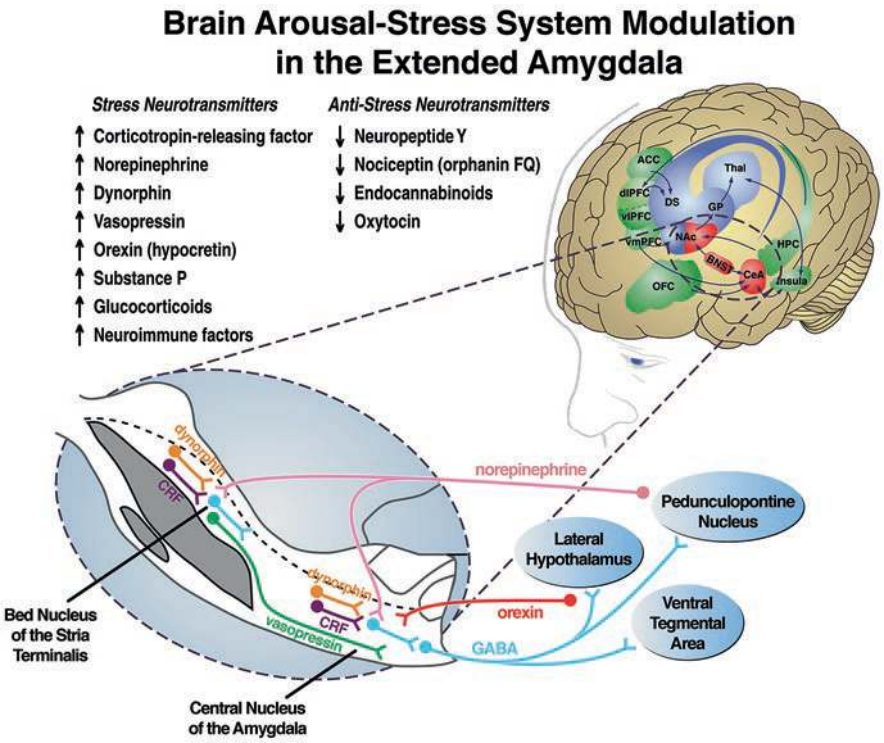

Fig. 36.6 Targets for hyperkatifeia in the withdrawal/negative affect stage (Modified from [104]). Shown is a representation of limbic cortical and associated striatal circuitry, with tentative localization of functions involved in drug addiction. Key structures include the medial prefrontal cortex (mPFC), which also includes the anterior cingulate (AC), orbitofrontal cortex (OFC), hippocampus (Hippo), ventral striatum (VS), nucleus accumbens, dorsal striatum (DS), globus pallidus (GP), amygdala (AMYG), and thalamus (Thal). The zoomed section shows the extended amygdala and its afferent and major efferent connections and modulation via brain arousal-stress systems. Note that dynorphin may activate CRF neurons or be activated by CRF neurons, that norepinephrine and CRF are hypothesized to be involved in a feed-forward circuit, and that vasopressin for the central nucleus of the amygdala is hypothesized to derive from the bed nucleus of the stria terminalis. Neuropeptide Y and nociceptin are not depicted in this figure but may act either via modulation of the CRF system or independently, directly on the output of the central nucleus of the amygdala. (Taken with permission from [105])

Construct and Measurement of Negative Urgency

The construct of negative urgency (i.e., the disposition to act rashly under extreme distress) [106] may further link opponent-process affective dysregulation to greater use in the stress surfeit model of AUD. Negative urgency involves impairments in inhibitory control while under stress [106]. In humans, negative urgency is measured via the validated Negative Urgency subscale of the Urgency, Premeditation (lack of), Perseverance (lack of), Sensation Seeking, Positive Urgency, Impulsive

Behavior Scale (UPSS-P) [106–108]. The Negative Urgency subscale includes negative reinforcement-oriented items, such as "When I feel bad, I will often do things I later regret in order to make myself feel better now." Accordingly, individuals who are high in negative urgency are high in negative reinforcement efficacy and motive [109–111], initiate substance use during stressful life situations [112], and are predisposed to use alcohol to reduce negative affective symptoms of abstinence [113].

A second set of Negative Urgency items measures impairments in inhibitory control during distress or craving, such as (*i*) "I have trouble resisting my craving" and (*ii*) "It often makes matters worse when I act without thinking when I am upset." Accordingly, individuals who are high in negative urgency show impairments in executive function performance in neuropsychological tests [114]. Twin studies showed that negative urgency predicted externalizing psychopathology, including substance use disorders. In these studies, impairments in executive function showed phenotypic correlations with impulsivity and had genetic and environmental bases [115]. A third set of negative urgency items suggests impairments in the negative-outcome-feedback control of behavior, a compulsivity-like construct [113] (e.g., "Sometimes when I feel bad, I can't seem to stop what I am doing even though it is making me feel worse").

Negative Urgency and Alcohol Use

Negative urgency often co-occurs with alcohol misuse [116] and may be an endophenotype of AUD [106, 116]. Negative urgency is a risk factor for the initiation and exacerbation of alcohol use in young people during stress or depression, exemplified in studies of school- and college-age transitions [112, 117–122]. Meta-analyses have found that among UPPS-P impulsivity dimensions, negative urgency is the strongest predictor of problematic alcohol use, especially during late adolescence [123, 124]. Each point increase in negative urgency predicts a fourfold higher rate of alcohol use problems [125], such as driving under the influence [126, 127].

Several additional findings support a role for negative urgency in promoting the negative reinforcement use of alcohol. For example, daytime anxiety in individuals with dependence symptoms predicted subsequent alcohol intoxication only in individuals who were high in negative urgency [128]. In undergraduates with a history of self-harm, negative urgency was associated with greater affective lability, impairments in self-control, problematic alcohol use, and eating problems [129]. In community-dwelling adults, negative urgency predicted greater mood changes, alcohol craving, alcohol seeking, and blood alcohol levels in response to negative mood induction [116]. In college students, path analysis found that the relationship between negative urgency and alcohol drinking was mediated by alcohol outcome expectancies and affect enhancement motives [111], consistent with the hypothesis that individuals who are high in negative urgency drink alcohol to enhance their positive affect and relieve their emotional distress.

Several findings also suggest a differential role for negative urgency relative to other aspects of impulsivity. Urgency (more than sensation seeking, the lack of premeditation, and the lack of perseverance) mediated the relationship between alcohol addiction symptoms and adult symptoms of attention-deficit/hyperactivity disorder [130], linking it to comorbid AUD and attention-deficit/hyperactivity disorder. In Australian young adults, only negative urgency and the lack of premeditation were unique predictors of binge drinking when UPSS-P impulsivity dimensions were regressed simultaneously. Similarly, only negative urgency and positive urgency were unique predictors of alcohol-related problems [131]. In the Rockland Study of community-dwelling adults, negative urgency, not positive urgency, was a unique mediator of relationships between depressive symptoms and problematic alcohol use [132].

Several findings similarly point to a key role for negative urgency in AUD. A study of 454 participants found that negative urgency was 50% higher in participants with AUD than those without AUD and loaded strongly on an impaired inhibitory control factor and also, less so, on a negative emotionality factor [133]. In a structural magnetic resonance imaging study of 33 patients with AUD compared with 32 healthy controls, negative urgency was significantly greater in AUD patients in association with increased anxiety [134]. In a study of 793 patients with AUD by the National Institute on Alcohol Abuse and Alcoholism Clinical Neurogenetics team, negative urgency, more than other UPPS-P dimensions, was related to a greater severity of alcohol addiction symptoms. This relationship was seen across physical ("hangovers, shakes, vomiting"), perceptual ("seen, heard, or felt things not there"), and neurobiological ("passed out, stumbling drunk") domains [135]. Consistent with the reviewed recent findings, an earlier meta-analysis of 2381 individuals found that negative urgency had the strongest relationship to alcohol addiction among all UPPS-P impulsivity dimensions (effect size r = 0.38) [123].

Sex, Age, and Negative Urgency

Negative urgency is a relevant construct in both men and women [136]. Although no consistent sex differences in negative urgency have been reported at the population level [136], negative urgency might still play differential roles between sexes for specific phenotypes [137]. Perhaps accordingly, levels of negative urgency were higher in women with AUD than in men with AUD [135]. This finding is especially salient because clinical and population studies also indicate an increase in the comorbid prevalence and symptom severity of many anxiety and depressive disorders in women with AUD compared with men [138–142]. Altogether, the results potentially suggest a greater role for negative urgency in the pathophysiology of AUD in women.

Levels of negative urgency vary across the lifespan. They increase across puberty [143, 144], and these increases may predict higher drinking frequency and binge eating [145, 146]. Conversely, urgency levels inversely correlated with age in

adulthood, decreasing in older and elderly adults ($r = -0.17$); these findings might account for the "aging out" of drinking for some individuals [147].

Neurocircuitry Implicated in Negative Urgency and Addiction

Initial neuroimaging data [132] support the hypothesis that negative urgency involves impairments in "top-down" cortical control over both basal ganglia and extended amygdala function, leading to a loss of control over pathological habits [148–153] and greater attention to, incentive salience of, or cognitive resource interference from emotion-evoking stimuli.

As summarized in Fig. 36.7, alterations of the structure, function, or connectivity of orbitofrontal cortex (OFC) and ventromedial PFC (vmPFC) projections to the basal ganglia and extended amygdala have been proposed to underlie negative urgency [106, 136, 154]. Accordingly, urgency was associated with the amplitude of resting-state low-frequency fluctuations in the lateral OFC and vmPFC in healthy volunteers [155]. In social drinkers, negative urgency predicted greater activation of the vmPFC in response to an alcohol odor cue and mediated the relationship of vmPFC activation with alcohol craving and problematic drinking [156]. Negative urgency also predicted greater OFC and amygdala activation in response to negative visual stimuli in direct relation to higher levels of risky behavior [157]. In tasks that require inhibition, such as Go/No-Go and gambling tasks, negative urgency predicted differential activation in other cortical regions that are associated with self-regulation and decision making, including the dorsolateral and ventrolateral PFC, anterior insula, and cingulate [155, 158–161]. Greater insula activation predicted real-world substance use in subjects who were high in negative urgency [158]. Finally, negative urgency correlated directly with larger amygdala and thalamus volumes bilaterally in patients with AUD relative to healthy controls [134].

Neurochemically, negative urgency may reflect deficient 5-hydroxytryptamine (5-HT) and dopamine activity in the OFC and vmPFC [106, 162], leading to the disinhibition of basal ganglia- and extended amygdala-subserved impulses. Thus, a composite polygenic 5-HT risk score predicted alcohol use problems via greater negative urgency rather than other aspects of impulsivity [125, 163]. Variants of the *GABRA2* gene, which encodes the $GABA_{A\alpha 2}$ receptor subunit and influences dorsolateral PFC GABA levels, were associated with alcohol use problems via urgency [164] and alterations of insula activation responses [165]. Future work should study the role of glucocorticoid receptors, CRF, hypocretin, dynorphin, and other molecular systems that were reviewed in the stress surfeit model as underlying individual differences in, or chronic alcohol use-induced adaptations in, negative urgency circuitry.

Although traditionally viewed as a stable, trait-like construct that potentiates responses to stress and distress, negative urgency itself is subject to change. Situational factors can increase urgency, potentially by impairing inhibition from

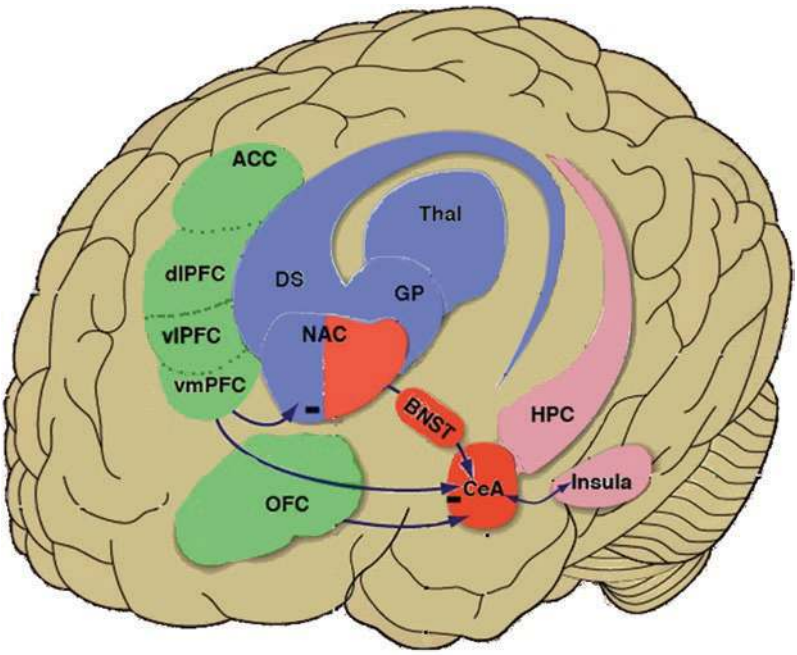

Fig. 36.7 Negative urgency circuitry in the neurobiology of alcohol use disorder. Simplified relationships are shown between higher-order cortical regions (green regions: orbitofrontal cortex and prefrontal cortex, including anterior cingulate cortex, dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, and ventromedial prefrontal cortex) that normally exert top-down regulation over the extended amygdala. Red regions: central nucleus of the amygdala, bed nucleus of the stria terminalis, a portion of nucleus accumbens shell, and basal ganglia. Blue regions: nucleus accumbens core, dorsal striatum, and globus pallidus. Under conditions of stress and extreme negative affect, individuals who are high in negative urgency are hypothesized to exhibit impairments in the efficacy of higher-order inhibitory control from such regions as the orbitofrontal cortex and ventromedial prefrontal cortex over the basal ganglia and extended amygdala, the latter leading to an increase in alcohol use and seeking behaviors that are motivated by negative reinforcement. Negative urgency is also hypothesized to reflect alterations of cortico-amygdalar and cortico-striatal modulation by the insular cortex (representing interoceptive state and context) and other prefrontal cortical regions, including the prelimbic and anterior cingulate cortices. Chronic alcohol use is hypothesized to increase negative urgency progressively by further disrupting top-down control of the OFC and vmPFC over the extended amygdala, thereby increasing the vulnerability to engage in negative reinforcement drinking under conditions of stress or hyperkateifia. Note that regions boundaries are heuristic and not anatomically precise. ACC, anterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; vlPFC, ventrolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex; OFC, orbitofrontal cortex; CeA, central nucleus of the amygdala; BNST, bed nucleus of the stria terminalis; HPC, hippocampus; NAC, nucleus accumbens; GP, globus pallidus; Thal, thalamus; DS, dorsal striatum. (Modified with permission from [104])

the OFC/vmPFC to the amygdala [166]. More enduringly, childhood abuse persistently increased amygdala activation and reduced prefrontal cortical control over amygdala responses [167]. Conversely, effective psychological interventions can reduce negative urgency [168, 169].

Relevant to alcohol use, greater activation in response to alcohol and drug cues occurs within the hypothesized negative urgency network across the course of the addiction process (i.e., basal ganglia, amygdala, OFC, cingulate cortex, vmPFC, dorsolateral PFC, and anterior insula) and may reflect use-associated plasticity [158, 170–174]. In contrast, in nondependent subjects, neither alcohol images nor negative urgency was associated with activity of the lateral PFC [158]. Relevant to the stress surfeit model, alcohol cue-induced activation in negative urgency circuitry is hypothesized to involve not only reward processing, as is often interpreted (e.g., [175–177]), but also an increase in aversive stress processing [103].

As further possible evidence of alcohol use-related changes in negative urgency circuits, opponent-process decreases in striatal dopamine D_2 receptor availability in individuals with AUD correlated with lower glucose metabolism in frontal cortical regions that subserved inhibitory control, such as the dorsolateral and anterior cingulate cortices [178]. These relationships were not seen in non-AUD controls. Similarly, negative urgency correlated directly with larger amygdala and thalamus volume in patients with AUD but not in healthy controls [134].

Animal Model of Negative Urgency?

Some attempts have been made to back-translate the negative urgency concept to animal models. Many of these have utilized unexpected reward omission as a probe, based on findings that human subjects who were high in negative urgency exhibited greater behavioral responding and self-reported frustration following reward omission in a monetary-based task compared with subjects who were lower in negative urgency [179]. Accordingly, unexpected reward omission increased subsequent operant responding for intravenous amphetamine and sucrose pellets in rats [179] at levels that were greater than after the contingent delivery of an expected reward [180]. In this animal model, higher V_{max} uptake values for the dopamine transporter in the nucleus accumbens and serotonin transporter in the OFC correlated positively with responding following omission. The results suggest that rats that exhibited higher responding after reward omission (i.e., a putative index of negative urgency) had greater dopamine transporter function in the nucleus accumbens and serotonin transporter function in the OFC [180].

Similarly, Cifani and colleagues showed that the unexpected nonreward of being placed in a context where a previously available, preferred food could be seen and smelled but no longer eaten elicited behavioral and neuroendocrine signs of stress and subsequently greater binge eating of palatable food [181, 182]. The increase in responding was accompanied by CRF activation in the extended amygdala and reduced by local CRF receptor antagonist pretreatment [183, 184]. The results highlight the animal modeling potential of reward omission and frustrative nonreward, which resemble translationally relevant “frustrative” alcohol and drug cues [185]. Remaining to be shown is whether unexpected alcohol reward omission can “drive forward” alcohol use impulsively, meaning (*i*) rapidly, (*ii*) prioritizing immediate

vs. later outcomes (e.g., in a delayed-discounting task framework) [186], and (*iii*) in a risky fashion without behavioral consideration to negative outcomes [187, 188].

From a "negative emotional side" perspective, changes in negative urgency circuits may represent a stress-related mechanism through which impulsivity and deficits in inhibitory control gain prominence in the transition to negative reinforcement-based drinking, away from positive reinforcement and reward motive use. Analogously, Belin and colleagues showed that high impulsivity in the 5-choice serial reaction time task predicted the development of compulsive-like cocaine self-administration and compulsive-like adjunctive behavior in a manner that was mitigated by the norepinephrine transporter inhibitor atomoxetine [149, 189, 190]. Negative urgency impulsivity may similarly ultimately potentiate the development of compulsive alcohol use and relapse in the stress surfeit model of AUD.

The collective results suggest how negative urgency contributes to alcohol use-related behavior in the opponent-process, stress surfeit disorder framework. From a bottom-up perspective, negative urgency may potentiate the experience of self-medicating and urge to self-medicate negative emotions that result during the withdrawal/negative affect stage, thereby increasing negative reinforcement. From a top-down perspective, impairments in executive control and outcome feedback that are associated with negative urgency may decrease the ability to resist urges to pursue alcohol use in the preoccupation/anticipation stage.

Summary

The complex nature of AUD involves multiple neurotransmitter systems that mediate stress sensitization, dysphoria, and hypohedonia (hyperkatifeia) and mediate executive dysfunction (reward, pro-stress and anti-stress neurotransmitter systems). Here, we focused on the role of the HPA axis and extrahypothalamic stress systems in AUD, largely via results from animal models of AUD. Repeated episodes of alcohol intoxication promote neuroadaptations that underlie incentive salience, reward hypofunction, stress sensitization, and impairments in executive function. These neuroadaptations contribute to the development of negative emotional states (i.e., hyperkatifeia: anxiety, dysphoria, pain, irritability) and negative urgency that are hypothesized to drive alcohol drinking and seeking via negative reinforcement in moderate to severe AUD. The HPA axis is necessary for alcohol reinforcement during the initial phase of alcohol use in the binge/intoxication stage of addiction that involves the basal ganglia. In the transition to alcohol addiction, the HPA axis becomes blunted (neuroendocrine tolerance), and extrahypothalamic stress systems (e.g., extended amygdala) become concomitantly sensitized. These changes are accompanied by the disruption of PFC function, which compromises executive function (i.e., inhibitory deficits and poor decision making) that may contribute to craving and relapse in the preoccupation/anticipation stage.

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Part VII
Alcohol-Related Liver Disease: Diagnosis

Chapter 37
Laboratory Parameters in Heavy Drinkers

Sebastian Mueller

Abstract Alcohol-related liver disease (ALD) is the most common liver disease in the Western world and for many reasons underestimated and underdiagnosed. Its early diagnosis is essential since it helps to identify patients at an increased genetic risk for disease progression and to initiate screening programs to prevent life-threatening complications such as bleeding from varices, spontaneous bacterial peritonitis, or hepatocellular cancer (HCC). The prediction and early diagnosis of ALD is still insufficiently solved although routine laboratory parameters in combination with abdominal ultrasound, liver elastography and clinical observation can establish the diagnosis and disease stage with high accuracy. This chapter focuses on the typical laboratory findings in chronic alcohol consumers and presents novel data from a large cohort of more than 1000 heavy drinkers. Moreover, the dependence of laboratory data on fibrosis stage are demonstrated and discussed in detail. Typical laboratory profile of ALD patients encompasses elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), GGT, ferritin and mean corpuscular volume (MCV) of red blood cells (RBC). Already in medium fibrosis stages, levels of transferrin, albumin and RBC count can be decreased. While transaminases and ferritin typically resolve rapidly within 1 week or at least after 4 weeks of abstaining from alcohol, normalization of RBC and MCV can take many months.

Keywords Alcoholic hepatitis · ALD · Liver transaminases · Serum marker · Steatosis

Abbreviations

ALT	Alanine aminotransferase
ALD	Alcohol-related liver disease
AP	Alkaline phosphatase

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ASH	Alcoholic steatohepatitis
AST	Aspartate aminotransferase
AUROC	Area under reciever operating characteristic
CAP	Controlled attenuation parameter
CDT	carbohydrate-deficient transferrin
CK	Cytokeratin (e.g. CK18 and CK19)
CRP	C-reactive protein
EtG	ethyl glucuronide
EtS	ethyl sulfate
GGT	Gamma-glutamyl transferase
GOT/AST	Glutamic oxaloacetic transaminase, see AST
GPT/ALT	Glutamate-pyruvate transaminase, see ALT
HCC	Hepatocellular cancer
LDH	Lactate dehydrogenase
LS	Liver stiffness
MCV	Mean corpuscular volume
PEth	phosphatidylethanol
RBC	Red blood cell
SWE	Shear wave elastography
TE	Transient elastography

Introduction

Alcohol-related liver disease (ALD) is the most common liver disease in the Western world and for many reasons underestimated and underdiagnosed. Its early diagnosis is essential since it helps to identify patients at an increased genetic risk for disease progression and to initiate screening programs to prevent life-threatening complications such as bleeding from varices, spontaneous bacterial peritonitis or hepatocellular cancer (HCC). The prediction and early diagnosis of ALD is still insufficiently solved although routine laboratory parameters in combination with abdominal ultrasound, liver elastography and clinical observation can establish the diagnosis and disease stage with high accuracy. This chapter focuses on the typical laboratory findings in chronic alcohol consumers and presents novel data from a large cohort of >1000 heavy drinkers. Moreover, the dependence of laboratory data on fibrosis are demonstrated and discussed in detail. Typical laboratory profile of ALD patients encompasses elevated levels of AST, ALT, GGT, ferritin and mean corpuscular volume (MCV) of red blood cells (RBC). Already in medium fibrosis stages, levels of transferrin, albumin and RBC count can be decreased. While transaminases and ferritin typically resolve rapidly within 1 week or at least after 4 weeks of abstaining from alcohol, normalization of RBC requires longer and normalization of MCV can take many months.

General Diagnostic Aspects of ALD

The diagnosis of ALD is further complicated by three major challenges: (1) underreporting by patients (2) lack of good biomarkers for alcohol consumption and (3) a rather diverse clinical presentation. These are the reasons why ALD is routinely underestimated both by physicians and health statistics [1, 2]. The diagnosis of ALD relies on a combination of clinical, laboratory, elastographic and imaging findings. For several reasons, despite their high frequency, ALD is often overlooked in daily clinical practice. Although diagnosis requires some experience, the combination of laboratory parameters and experienced clinical observation normally allows an almost certain diagnosis. This chapter will rather focus on the typical laboratory parameters. What complicates the interpretation of laboratory parameters in ALD and contributes to the confusion is the fact that almost all **laboratory markers depend on fibrosis stage**. Since elastography is increasingly available and should actually be available in the near future even in peripheric medical centers and for general practitioners, the changes of laboratory with increasing fibrosis stages are discussed in more detail. To better illustrate the diagnostic findings, data from the Heidelberg cohort of heavy drinkers will be shown in some tables. Table 37.1 shows patient characteristics of important laboratory parameters of this cohort. More details are shown in Tables B.1 and B.4. More details about fibrosis screening are separately discussed in Chaps. 40 and 42 in this book part.

The early and exact diagnosis of ALD and namely the manifestation of fibrosis/cirrhosis are important since:

1. patients receive an explanation for their symptoms and complaints.
2. prognostic information can be obtained.
3. the diagnosis ALD can be separated from other comorbidities (e.g., viral hepatitis) or disease modifying factors (e.g., obesity, drugs).
4. a targeted search for potential complications such as varices or HCC can be started and surveillance intervals, e.g., for HCC can be defined.
5. by understanding of disease mechanisms, the patient gets the opportunity to control disease progression through change of life style, avoidance of alcohol and reducing other potentially harmful factors such as obesity.

Liver diseases are in general hardly to detect and commonly show no or unspecific symptoms. Even end-stage liver cirrhosis remains undetected in routine laboratory testing or ultrasound screening in ca. 40% (see Table 37.2) [3]. For instance, if one considers bilirubin, INR and signs of cirrhosis by abdominal ultrasound as easily accessible parameters that are widely available, Table 37.1 demonstrates that, despite advanced fibrosis, these parameters can be normal in 43% of a large cohort of heavy drinkers. Here, elastography really fills the gap. Although ALD follows the typical sequence of chronic liver diseases including alcoholic fatty liver, steatohepatitis, fibrosis and eventually cirrhosis, the early recognition of severe steatohepatitis and alcoholic cirrhosis is most important since it will save lifes, prevent complications and initiate follow up programs (Fig. 37.1). Most critical and life-threatening

Table 37.1 Typical routine blood tests in ALD in low fibrosis stages (F0-2) and advanced fibrosis stages (F3-4)

			F0–2		F3–4	
Parameter	Units	Normal	Mean	Path. (%)*	Mean	Path. (%)*
Blood count						
Erythrocytes	/pL	4.5–5.9	4.8	*47.5*	3.9	*76.5*
Hemoglobin	g/dL	12–16	14.5	*6.1*	12.8	*33.0*
MCV	fL	80–96	92.3	23.6	96.2	50.9
Liver parameters						
AST	U/L	<50	91.3	52.2	99.1	69.6
ALT	U/L	<50	71.3	44.6	50.7	37.0
GGT	U/L	<60	301.8	68.0	648.2	87.3
AP	U/L	40–130	91.7	10.9	154.4	49.9
Bilirubin (total)	mg/dL	<1.2	0.7	11.1	3.3	50.3
M30	U/L	<200	453.4	57.4	1000.8	83.3
M65	U/L	<400	820.6	55.2	1871.4	84.5
INR		0.85–1.15	0.9	3.2	1.2	46.7
Routine laboratory						
CRP	mg/L	<5	4.2	15.6	12.9	48.1
CRP > 30	mg/L	<5	4.2	2.1	12.9	13.3
Glucose	mg/dL	60–100	108.4	49.4	119.8	64.3
Albumin	g/dL	3.4–5.4	4.5	*1.8*	3.9	*24.3*
Triglycerides	mg/dL	<150	209.4	43.1	163.7	35.2
Cholesterol	mg/dL	<200	225.4	65.9	187.3	39.4
HDL Cholesterol	mg/dL	>40	79.0	*9.7*	50.0	*45.0*
LDL Cholesterol	mg/dL	<160	118.7	15.9	101.1	12.5
Potassium	mM	3.5–4.6	4.1	*12.5*	3.9	*19.9*
Sodium	mM	136–145	138.3	*18.4*	135.8	*35.2*
Hemolysis parameter						
LDH	U/L	<250	223.6	26.3	260.5	41.8
Haptoglobin	g/L	0.3–2.0	1.5	*2.9*	1.2	*15.2*
CD163	ng/mL	<800	1118.0	63.0	2218.8	94.4
Iron-related parameters						
Ferritin>400	ng/mL	50–150/400	567.0	41.7	674.4	50.3
Ferritin>1000	ng/mL	50–150/400	567.0	17.2	674.4	25.6
Serum iron	ug/dL	95–158	129.2	25.6	117.9	25.1
Transferrin	g/dL	2.0–3.6	2.5	*2.3*	2.0	*44.8*
Transferrin saturation	%	16–45	40.7	31.1	48.3	44.5
Alcohol marker						
Serum alcohol level	g/L	<0.1	1.0	51.7	0.9	47.5
EtG	ng/mL	0	1191.6	85.5	1436.9	79.2
EtS	ng/mL	0	446.6	75.4	553.4	70.8
PEth	ng/mL	0	1733.2	100.0	1586.4	100.0

Table 37.2 Diagnosis of cirrhosis (confirmed by histology and elastography) is understestimated by conventional clinical parameters (ultrasound, laboratory). As an example, only ca. 43% of patients with manifest F3–4 cirrhosis are diagnosed by a combination of bilirubin, INR and ultrasound signs of liver cirrhosis (highlighted in bold)

Parameter	Pathologic	F0–2 Elevated (%)	F3–4 Normal (%)	Mean F0–2	Mean F3–4
Bilirubin, INR, Platelets, Spleen size, Sign of liver cirrhosis	>1	27.7	22.6	0.28	0.77
Bilirubin, INR, Sign of liver cirrhosis	>1	10.0	**43.5**	0.10	0.57
Bilirubin	>1.3 mg/dL	7.6	58.3	0.08	0.42
INR	>1.27	1.2	74.8	0.01	0.25
Platelets	<150/nL	18.5	49.6	0.18	0.50
Spleen size	>11.5 cm	6.4	70.4	0.06	0.30
Signs of liver cirrhosis	>0	1.6	59.1	0.02	0.41

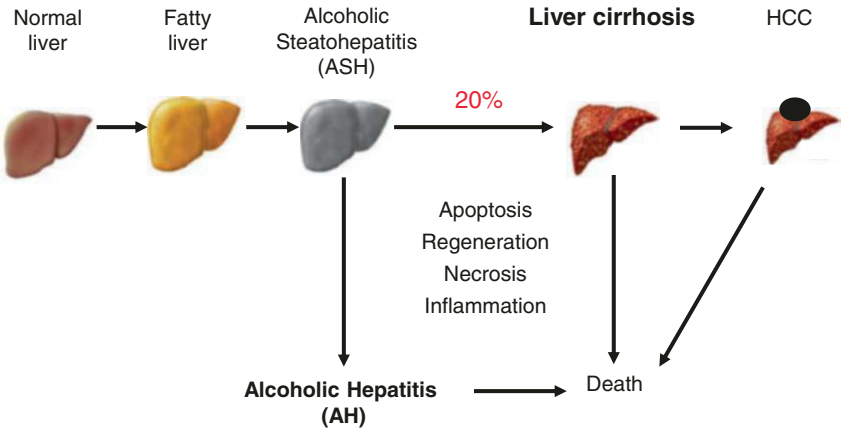

Fig. 37.1 Natural course of ALD and major end points

end points are **decompensated alcoholic liver cirrhosis** and the rare and clinically defined **alcoholic hepatitis (AH)**. Fibrosis screening and AH will be separately discussed in respective book chapters.

The slow progression of ALD towards liver cirrhosis can be undetectable and unnoticed for many years. For these reasons, patients who are sensitive to alcohol-mediated liver damage but diagnosed too late may have an unfavourable outcome. These patients are listed late for transplantation and are at high risk of dying from complications while waiting for a transplant. Of course, modern imaging techniques are absolutely essential for HCC screening and are continuously improved. Liver biopsy still remains an important option for some patients to confirm the relative contribution of alcoholic liver damage in relation to other potential causes. The reader is referred to the book chapters on liver histology (Chap. 38) and AH (Chaps. 64–68).

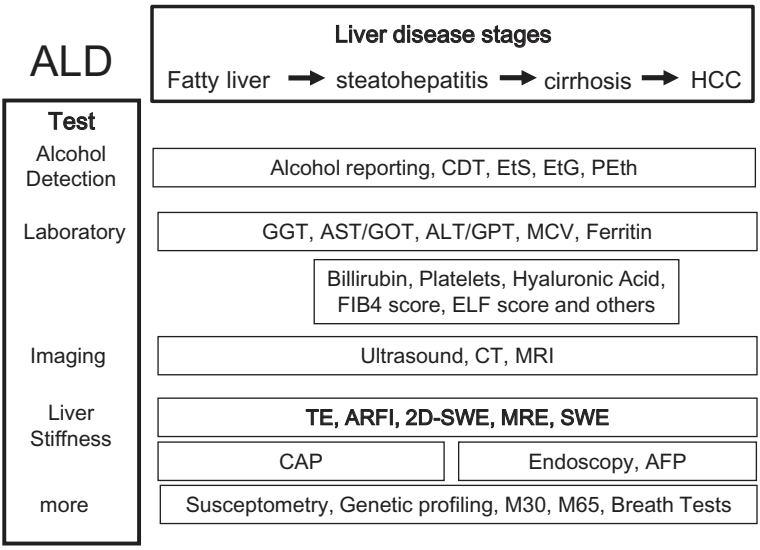

Fig. 37.2 General non-invasive approaches for patients with suspected ALD. The various tests will help to establish alcohol as cause and the progression stage of the disease

Clinical Approach to ALD

The diagnosis of ALD has first to establish the consumption of alcohol as cause of the liver disease. Then, the different clinical stages of ALD should be ascertained such as fatty liver, alcoholic steatohepatitis, fibrosis and eventually cirrhosis (Figs. 37.1 and 37.2) [1]. Reported alcohol consumption of more than 20–30 g alcohol per day for females/males is a prerequisite although genetic background or other comorbidities play also an important role. Except direct measurement of alcohol in serum as sign of acute alcohol intake within the last 20 h, no single laboratory marker definitively monitors chronic alcohol consumption. Alcohol biomarkers such as ethylglucuronide (EtG), ethylsulfate (EtS) and phosphatidylethanol (PEth) are highly specific. EtG levels in the urine (up to 3 days) and, more widely, carbohydrate deficient transferrin (CDT) are used frequently to detect previous alcohol consumption (4–21 days). However, CDT has only a moderate sensitivity of 60% at a daily alcohol consumption of more than 50 g. A rather new and longer tracking of alcohol consumption is provided by determination of EtG in the hair. Especially PEth, although covering a rather long period of alcohol consumption of the last 3 weeks, has distinct degradation kinetics that varies strongly inter-individually. For instance, novel data show that PEth levels, typically produced within the red blood cell, are strongly affected by red blood cell degradation [4]. Moreover, patients with cirrhosis have delayed elimination kinetics of alcohol and, hence, blood alcohol concentration and levels of alcohol biomarkers can be elevated (see Table 37.1) [4]. More information of biomarkers is also provided in the Chap. 40. The diagnosis of ALD sometimes requires a more extended clinical experienced view on the patient

symptoms. Thus, alcohol consequences may manifest first in the brain (see Chap. 72), in the peripheral nerves (polyneuropathy) or as alcoholic cardiomyopathy (Chaps. 70–71) to name only a few. Likewise, rib fractures are common on X ray images and may be even more diagnostic for ALD than an elevated GGT. Some other clinical symptoms more specific for ALD include parotid enlargement, Dupuytren's contracture, and especially signs associated with feminization.

Histology and ALD

Detailed features of liver histology in ALD are described in Chap. 38. Histological characteristics of heavy drinkers are shown in Appendix Table B.9. It should be pointed out, however, that a single histological slide does not allow the diagnosis of ALD, alcoholic hepatitis nor non-alcoholic fatty liver disease (NAFLD) nor does it allow to discriminate between them. Moreover, it is long known that typical hallmarks of ALD such as Mallory bodies (now MDBs) do also occur in other liver diseases (Wilson's disease, NAFLD). Especially in the light of liver elastography, biopsies should not any longer be performed to screen for liver fibrosis stage as it has a quite high sampling error of up to 30% [5–9]. Further, it is invasive with mild (pain and small bleedings in 6%) or severe (0.1%) complications and rarely fatal perforations and bleedings. The most important role of biopsy remains to dissect and identify comorbidities in addition to ALD.

Alcohol-Related Hepatic Steatosis

As shown in Fig. 37.1, the majority (>90%) of moderate and heavy drinkers have hepatic steatosis, but only 15% progress to fibrosis. Therefore, it remains actually an open question to what extent steatosis is indeed a necessary prerequisite for liver fibrosis or even hepatitis (see also Chap. 49). Already in the mid nineteenth century, Theodor Ferichs showed (before actually being able to obtain photographs of microscopic images) that feeding dogs transiently a high fat diet induces a reversible fatty liver [10]. However, as steatosis may be one of the early signs of ALD and may be present in the absence of inflammation or fibrosis, it may be the only sign to show that "at least the liver responds to alcohol intake". Therefore, screening for steatosis may add to the diagnosis and may be the only parameter that can be followed up if other parameters are normal. Table 37.3 shows actual correlation between histology, MRI, controlled attenuation parameters (CAP) and ultrasound. Figure 37.3 shows comparison for all non-invasive techniques with histology. Abdominal ultrasound is quite robust in identifying steatosis [11]. But as seen in Fig. 37.3, it is not linear, especially at higher degrees of steatosis. Recent ultrasound-based techniques such as CAP are promising. They are reproducible and quantitative with an AUROC up to 90% for fatty liver [12]. In clinical

Table 37.3 Spearman correlation between different methods to assess hepatic steatosis from the Heidelberg cohort (n = 41)

	Histological degree of steatosis	Hepatic fat fraction (MRI)	CAP	Steatosis (US)
Histological degree of steatosis	–	0.836***	0.604***	0.358*
Hepatic fat fraction (MRI)	0.836***	–	0.577***	0.304**
CAP	0.604***	0.577***	–	0.408**
Steatosis (US)	0.358*	0.403**	0.408**	–

MRI magnetic resonance imaging, *CAP* controlled attenuation parameter, *US* ultrasound
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

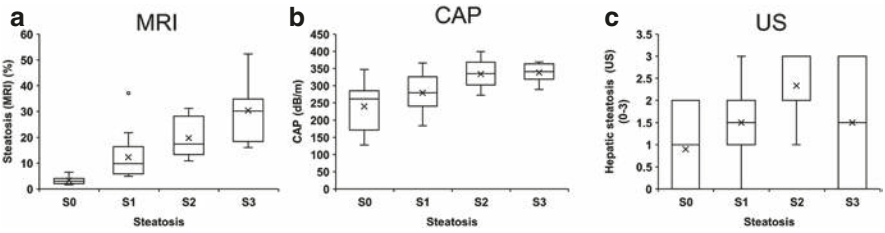

Fig. 37.3 Boxplots with medians to assessing histological steatosis with MRI, CAP and ultrasound. Data are based from an ongoing study in Heidelberg (n = 42). MRI is highly linear, while both ultrasound-based techniques show a saturation and higher steatosis degrees

practice, ultrasonography may be proposed in heavy drinkers as a screening procedure for steatosis [13]. US has the advantage of being already available everywhere. CAP is excellent in ruling in any steatosis and as a quantitative parameter it allows real follow-up studies. Even 1 week of alcohol detoxification, for instance, decreased reliably hepatic steatosis by 30 dB/m [14]. The limitation is that cut-offs between S1–3 are quite narrow and in the light of the importance of steatosis mentioned above, US remains useful for daily practice. In addition, as shown in Fig. 37.3, it becomes "saturated" at higher steatosis degrees. MRI, as also shown in Table 37.3, is the most accurate and correlates best with histology. It is highly quantitative and its only limitations are costs and availability. However, in the future, it may be used more frequently [15, 16]. Table 37.4 shows typical US parameters in patients with ALD and different fibrosis stages.

Introduction to Routine Laboratory Markers in Alcohol Consumers

Alcohol consumers show typical changes of routine laboratory markers. They are dependent on genetic background, disease and fibrosis stage and show a great variability. As already mentioned earlier, an ultrasound and additional liver

Table 37.4 Typical parameters of abdominal ultrasound and liver elastography in 1260 patients with ALD

Parameter	Units	Normal	F0–2		F3–4	
			Mean	Path. (%)*	Mean	Path. (%)*
Ultrasound parameter/Liver elastography						
Liver size	cm	<16	15.7	40.1	16.8	52.8
Hepatic steatosis (US)	0–3	0	1.8	90.4	2.1	93.2
Spleen size	cm	<11.5	9.5	8.7	11.6	47.6
Ascites	0–1	0	0.0	0.3	0.3	31.6
Signs of cirrhosis (US)	0–1	0	0.0	2.2	0.6	57.3
Liver stiffness	kPa	<6	6.4	38.7	43.2	100.0
CAP	dB/m	<240	286.6	79.1	293.1	75.0

US ultrasound, * either increased or decreased according to normal cutoff

elastography are the best prior to laboratory evaluation. Major and typical changes affect the blood count (erythrocytes, MCV, platelets, leukocytes), liver parameters (AST, ALT, GGT, AP, bilirubin), iron-related parameters (Ferritin, Transferrin). Many other routine parameters (Uric acid, creatinine, LDH) or more specific markers (M30, M65) may also be useful to evaluate the disease stage. With some experience, ALD stage can be quite well diagnosed based on routine laboratory and ultrasound. The combination of these routine blood tests further increases the diagnostic accuracy for ALD with a sensitivity and specificity >90% for GGT, MCV, IgA, CDT, and AST/ALT ratio.

Blood Count

As shown in Table 37.1, RBC count decreases with fibrosis stage in ALD. Although full criteria of anemia are only reached in ca. 12% of cirrhotics, a significant decrease of the RBC mass of ca. 20% can be observed quite frequently and the hemoglobin is about 1–2 g/L lower as compared to the mean of a normal population. The underlying mechanisms are discussed in more details in Chaps. 57 and 58. A typical feature of the blood count is an elevated MCV. In the large Heidelberg cohort (see Table 37.1), almost 25% of non-cirrhotic drinkers have an elevated MCV, in cirrhotics more than 50%. Platelet count is also affected by alcohol-related bone marrow toxicity and, at later stages, by portal hypertension and splenomegaly. After alcohol detoxification, RBC count, hemoglobin and MCV even worsen in the first week due to aggravated ineffective erythropoiesis for not yet clear reasons (see Chap. 57 on ALD and iron). While RBCs normalize typically after 4 weeks of abstinence, MCV may take much longer, even many months or even years. However, in a series of 15 patients from Heidelberg, blood count completely normalized even in patients with manifest cirrhosis after an abstinence of 5 years. As can be seen in Table B.29, B12 levels are typically increased in ALD patients with macrocytosis

while folic acid levels are still in the normal range. There are first indications that these patients may still have a relative deficit of folic acid and the elevated B12 levels seems to be due to compensatory adaptations. The exact mechanisms have not been clarified yet.

Gamma Glutamyl Transferase (GGT)

GGT has an important function for replenishing the glutathione pool in cholangiocytes (see also Figs. A.49 and A.50). GGT is one of the best markers of ALD with a combined sensitivity and specificity of >70%. Elevation of GGT is not due to liver damage but induction of enzymatic activity. GGT loses its alcohol specificity in more advanced stages. GGT activity can be also caused by other conditions namely cholestatic liver disease, cardiac insufficiency, drugs and many more. Figure 37.4a shows mean GGT levels dependent on fibrosis stage in comparison to a large population with portal liver disease (hepatitis C infection). Data are taken from a large multicenter cohort with biopsy proven fibrosis stages [17]. It is clearly visible that

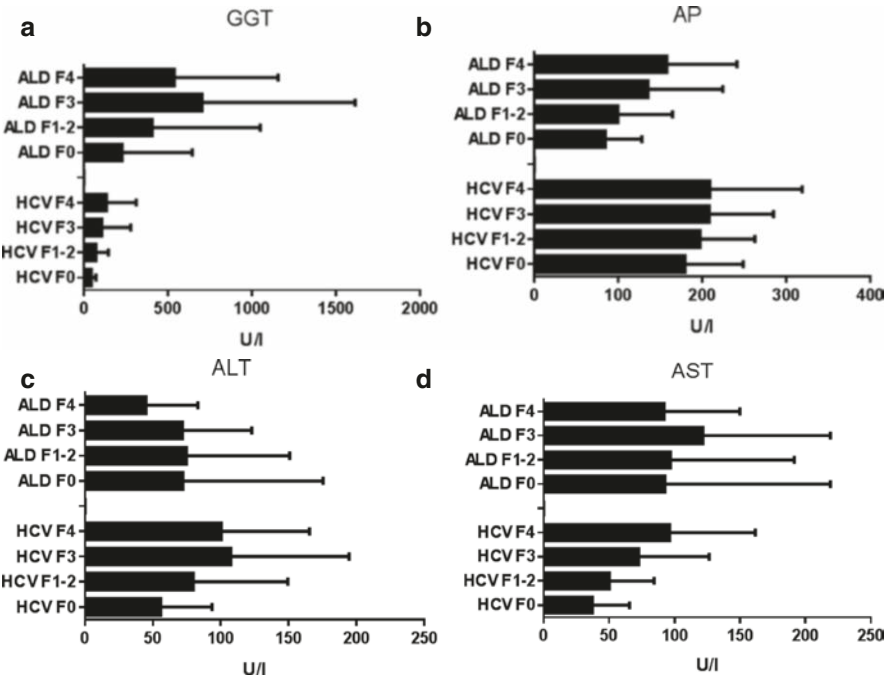

Fig. 37.4 Mean laboratory values for ALD and HCV for respective fibrosis stages (biopsy proven). For (**a**) GGT, (**b**) AP, (**c**) AST (**d**) ALT. Data are from a multicenter study based on 1391 biopsy-proven HCV and 677 ALD samples [17]

GGT levels are higher in ALD patients for all fibrosis stages as compared to HCV. According to Table 37.1, however, about 30% of heavy drinkers without advanced liver disease have no elevated GGT. In contrast, in advanced stages, only ca. 10% have a normal GGT.

Alkaline Phosphatase (AP)

AP's biochemical reaction is shown in Fig. A.49. Its exact role in ALD is not completely clear. As shown in Fig. 37.4b, as compared to GGT, it gradually increases in ALD with fibrosis stage. This may be also the reason why AP has a highly prognostic values with regard to long-term mortality (see Chap. 7). However, levels are still lower as compared to portal HCV and this may one reason why, in daily practice, with limited information, AP has not gained too much attention. It should be noted, however, that a continued increase of AP, even in patients with complete abstinence, is a prognostically unfavorable development.

Liver Transaminases (AST/GOT and ALT/GPT)

AST is typically elevated to a level of two to six times the upper limits of patients with ALD or patients with alcoholic hepatitis (AH) while AST levels of more than 300 IU/L are rarely seen. In about 70% of patients, the AST/ALT ratio is higher than two. This development is especially seen in cirrhotics. As is discussed in more detail in Chap. 41, however, AST level remain elevated throughout all fibrosis stages, in contrast to ALT. Figure 37.4c, d shows AST and ALT for each fibrosis stage both for ALD and HCV [17]. In contrast, in patients with viral hepatitis, AST continuously increase with fibrosis stage. As is discussed in detail in Chap. 41, AST in alcohol consumers is most likely not derived from the liver but rather from red blood cells. The existence of AST in RBCs has already been described early on and seems to have been neglected over the years [18].

Bilirubin

Bilirubin elevation in drinkers is typically an indication of cirrhosis, alcoholic hepatitis or ACLF. The difficulties to discriminate clearly between these entities is discussed in the respective book chapters and still difficult to comprehend. Typically, more than 90% of the total bilirubin in ALD is conjugated bilirubin. It is often ignored that indirect bilirubin is also elevated and an expression of the hemolytic anemia as discussed in Chaps. 57 and 58. Bilirubin has an important prognostic value and is part of many prognostic scores whether for cirrhosis or AH (see Appendix Tables A.9 and A.11).

Iron Related Parameters

Alcohol also causes typical iron changes that are still not very much appreciated and that depend on fibrosis stage. As is discussed in more detail in Chap. 57 on ALD and iron, serum ferritin is typically elevated in drinkers. It is higher than 400 ng/mL in non-cirrhotics in ca. 40% and in cirrhotics in more than 50%. Levels higher than 1000 ng/mL, a typically screening cut-off value for hereditary iron overload disease, is also frequently seen in drinkers, in cirrhotics in up to 25% (see Table 37.1). In contrast to hereditary iron overload diseases, however, ferritin rapidly decreases with a half time of 2 days. Significant decreases can already be seen after 1 week of abstaining from alcohol and ferritin usually completely normalizes after 4 weeks. Ferritin seems to be elevated due to masked hemolytic anemia and ineffective erythropoiesis. As mentioned above, B12 levels can be even elevated, and folic acid levels are normal in most patients. Another typical configuration about iron in ALD patients are significantly decreased transferrin levels and elevated transferrin saturation. This makes it rather difficult per se to differentiate between hereditary iron overload and ALD. Haptoglobin levels are also typically difficult to interpret since they may be elevated in inflammation, reduced in fibrosis and levels also depend on the degree of hemolysis.

Markers of Inflammation

As can be seen in Table 37.1, in this large population of heavy drinkers, levels of inflammation markers are rather moderate. For instance, mean CRP levels are in the normal range with 4.2 mg/L (normal <5 mg/L) for patients without advanced liver fibrosis (<F3). Only ca. 15% have pathologically elevated CRP. In patients with F3–4 fibrosis stage, mean CRP levels are higher with 12.9 mg/dL, but these levels are still moderate and cannot be compared to levels of patients with viral or bacterial infections that can easily reach levels of 50 or higher than 100 mg/dL. Independent of fibrosis stage, mean leukocyte count was always within the normal range and only elevated in ca. 15% (see Tables B.1 and B.4). Of note, levels of important cytokines such as TNFalpha, IL6 or TGFbeta are typically in the upper normal range or just slightly elevated [19]. Only IL8 levels were significantly increased with ca. 120 pg/mL as compared to median levels of healthy donors (below 20 pg/mL). Cytokines were only slightly higher in cirrhotics [19] and decreased after alcohol detoxification. Taken together, markers of inflammation are typically only slightly elevated in large cohorts of heavy drinkers.

Prognosis and New Biomarkers in ALD

Among the many potentially useful markers to be used in ALD in the future, cytokeratins have emerged as markers of hepatocyte damage. Mallory bodies, the hallmark of alcoholic hepatitis, contain cytokeratin-18 and cytokeratin-19 [20]. Serum

levels of CK-18 and CK-19 are increased in patients with AH compared to fatty liver or controls [20]. In a recent study, Bissionette and colleagues reported higher levels of total and microvesicle-bound M65 and M30, circulating fragments of cytokeratin-18 in the circulation in biopsy-proven AH [21]. A cutoff of 2000 U/L for M65 has a positive predictive value of 91% and a cutoff of 642 U/L had a negative predictive value of 88% [21]. It should be noted that CK-18 fragments are markers of hepatocyte apoptosis, thus, are not specific for AH [22]. Furthermore, high levels of CK-18 were reported in non-alcoholic hepatitis and toxin-related steatohepatitis [23, 24]. Of note, M30 levels (caspase3-cleaved Cytokeratin-18) is induced in response to alcohol detoxification [25]. These data suggest that alcohol suppresses apoptosis which is unchained after alcohol withdrawal.

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Chapter 38
Histology of Alcohol-Related Liver Disease

Stephan Sygulla and Carolin Lackner

Abstract This chapter describes the typical histologic features of compensated and decompensated alcohol-related liver disease (ALD). Connotations are provided to put pathogenesis and morphologic alterations into context. In addition, an ALD-specific and prognostically relevant semiquantitative scoring system for grading and staging is presented that has been recently developed by the SALVE Histopathology Group.

Keywords Alcohol-related liver disease (ALD) · Steatohepatitis due to ALD · Histology · Fibrosis due to ALD · Pathogenesis · Semiquantitative grading and staging · Ballooning

Histological Types and Natural History of Alcohol-Related Liver Disease

Alcohol-related liver disease (ALD) [1] covers a spectrum of liver diseases ranging from alcohol-related steatosis to steatohepatitis (ASH) and fibrosis/cirrhosis due to alcohol consumption. These types of ALD are defined by histology. The natural course of the disease has not been described in detail. However, a summary of data from the literature indicates that approximately 90–100% of people who consume more than 40 g of alcohol per day over a longer time (many months to years) will develop fatty change of hepatocytes (steatosis due to alcohol). A minority of these individuals, between 10–35% progress to ASH which is an inflammatory condition associated with liver injury and fibrogenesis. Of the individuals with ASH 8–20% develop cirrhosis which provides the basis for progression to hepatocellular carcinoma in another 2% of patients [2].

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Histology of ALD: General Remarks

The histologic key features of ALD comprise four types of lesions which in pre-cirrhotic stages of the disease are typically present in centrilobular regions of the hepatic lobules and which either occur as single features or in any combination in individual patients. These features include steatosis of macrovesicular and eventually microvesicular type(s) (see below), lobular inflammation, a certain form of hepatocellular injury termed hepatocellular ballooning (Fig. 38.1) which can be combined with necrosis, and fibrosis [3].

The centrilobular distribution of liver lesions is promoted by the zonation of the hepatic lobules. Pericentral hepatocytes exhibit increased expression of lipogenesis- and reduced expression of fatty acid ß-oxidation genes as compared to periportal hepatocytes thus contributing to the fatty change (i.e., macrovesicular steatosis) in centrilobular hepatocytes. In addition, cytochrome P450 genes such as Cyp2 E1, one of the major ethanol-metabolizing enzymes is increased in centrilobular hepatocytes as compared to the periportal ones. The alcohol-mediated oxidative stress in centrilobular hepatocytes presumably plays an important role promoting hepatocellular injury leading to the disintegration of the intermediate filament cytoskeleton and impaired mechanical stability of hepatocytes eventually resulting in the ballooning change, hepatocellular necrosis, and inflammation [2].

Other characteristic findings include visible bile pigment in hepatocytes (hepatocellular cholestasis), canalicules (canalicular cholestasis) and ductules (ductular cholestasis) as well as perivenular fibrosis, sclerosing hyaline necrosis and fibro-obliteration of hepatic veins [4].

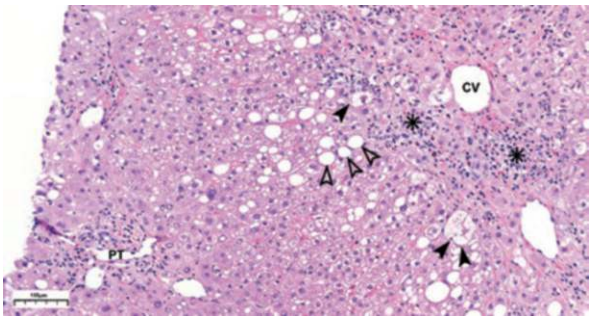

Fig. 38.1 The key features of ASH are accentuated in central and intermediate portions of hepatic lobules. Hepatocytes with macrovesicular fat and ballooned hepatocytes are marked with open and black arrow heads, respectively. The inflammatory infiltrates are indicated by asterisks (H&E; PT, portal tract; CV, central vein)

Alcohol-Related Steatosis

Macrovesicular Steatosis

Macrovesicular steatosis is the most frequent type of fatty change in ALD. Alcohol-associated enhanced lipolysis and uptake of free fatty acids into the liver, increased hepatocellular lipogenesis and reduced lipolysis as well as decreased secretion of lipids via very low density lipoproteins are among the main mechanisms leading to the accumulation of lipid, mainly triglycerides, phospholipids and cholesterol ester deposited in membrane-coated vesicles in the cytoplasm of hepatocytes. Lipid vesicles are dynamic structures involved in the distribution and metabolism of lipids [5–7]. After periods of abstinence, alcohol-related steatosis is fully reversible [8]. Macrovascular steatosis is considered a benign condition with low potential for disease progression. However, in 10% of people alcohol-related steatosis may progress to cirrhosis also in the absence of ASH [9].

In the early stages, small lipid vesicles are typically located around the nucleus of centrilobular hepatocytes. Macrovesicular forms of steatosis are thought to result from confluence of smaller lipid vesicles and comprise most of the hepatocellular cytoplasm. They lead to dislocation of the organelles and nucleus to the periphery of the cell [10, 11]. In settings of ongoing alcohol consumption also hepatocytes of the intermediate and eventually periportal portions of the hepatic lobules are affected by steatosis (Fig. 38.2). Isolated hepatocellular steatosis is a rare finding. Frequently, a mild inflammatory reaction is present. A local inflammatory response consisting of Kupffer cells and few mononuclear cells termed microgranulomas may result from the rupture of lipid-laden hepatocytes (Fig. 38.3). However, there may also be larger lesions termed lipogranulomas with extrahepatocellular lipid droplets surrounded by Kupffer cells, lymphocytes and few eosinophils or neutrophils.

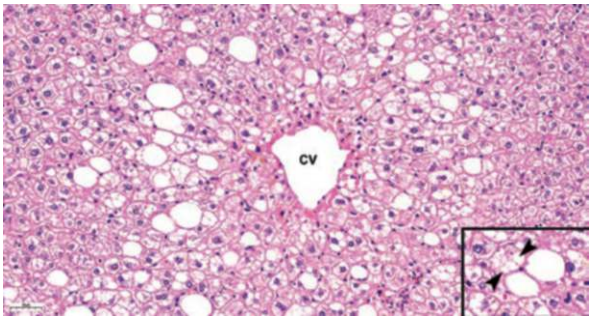

Fig. 38.2 Alcohol-related macrovesicular steatosis: Hepatocytes in intermediate and central portions of the hepatic lobules contain large lipid vesicles taking up most of the hepatocellular cytoplasm and dislocating the nucleus to the periphery of the cell (inset). The large macrovesicles may result from confluence of several smaller ones (marked by arrow head in inset) (H&E; CV, central vein)

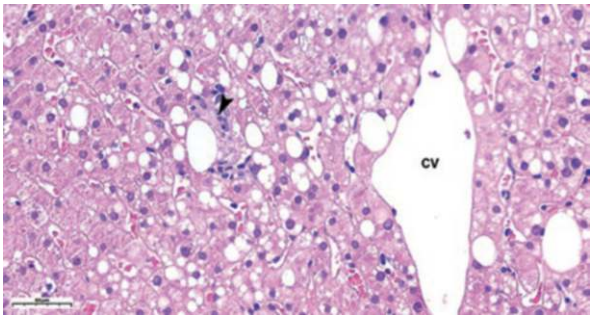

Fig. 38.3 Macrovesicular steatosis and lobular inflammation. Small microgranuloma consisting of Kupffer cells and few lymphocytes is present adjacent to a hepatocyte with large lipid vesicle (arrow head) (H&E; CV, central vein)

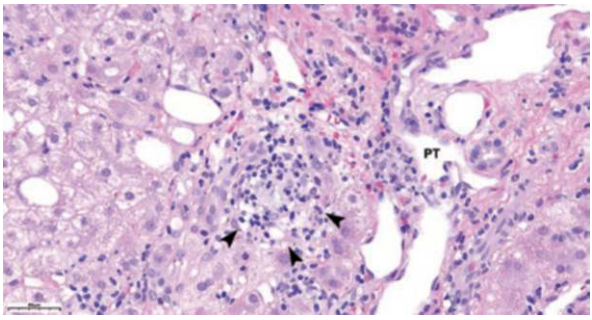

Fig. 38.4 Lipogranuloma adjacent to portal tract consisting of fat vacuoles surrounded by histiocytes and lymphocytes (arrow heads) (H&E; PT, portal tract)

Lipogranulomas can also occur in portal tracts and may be surrounded by collagen fibers (Fig. 38.4) [11, 12].

Sudden death of unknown cause affecting mostly middle-aged females has been described as a rare complication of heavy drinking associated with massive hepatomegaly, macrovesicular steatosis and bilirubinostasis (visible bile pigment) [13].

Microvesicular Steatosis

Microvesicular steatosis is defined as minute lipid droplets only visible at high magnification on H&E histology filling and extending the cytoplasm of hepatocytes. The nucleus is often pycnotic and located in central position within the hepatocytes (Fig. 38.5). It is an infrequent type of steatosis as compared to macrovesicular steatosis which is present in most cases of ALD. Macrovesicular and microvesicular steatosis can also coexist as so-called mixed type steatosis which seems to be associated with higher risk of disease progression [9]. An extensive form of microvesicular steatosis affecting large portions of the parenchyma is termed alcoholic foamy degeneration (AFD) [14] and is believed to represent a type of acute alcohol-related toxicity in chronic drinkers [15]. On histology, AFD is typically not associated with lobular inflammation or fibrosis. Megamitochondria (Fig. 38.6) are

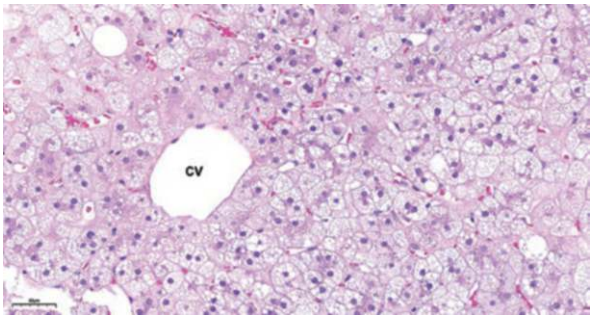

Fig. 38.5 Microvesicular steatosis. Hepatocytes adjacent to central vein are filled and expanded by minute lipid droplets. The pycnotic appearing nucleus is situated in central position in the cytoplasm. Few hepatocytes with macrovesicular fat are present. Lobular inflammation is absent (H&E; CV, central vein)

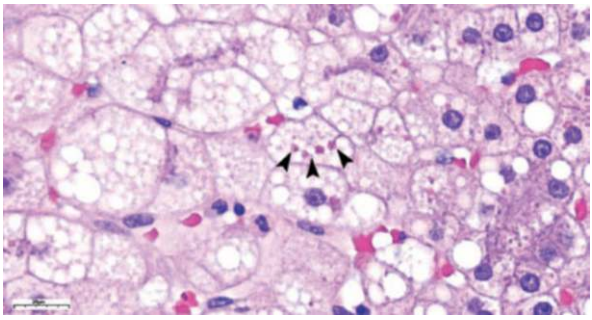

Fig. 38.6 Megamitochondria are seen as globular-shaped eosinophilic cytoplasmic inclusions (arrow heads) in a case of alcoholic foamy degeneration (H&E)

frequently, and bile pigment is occasionally found in the cytoplasm of affected hepatocytes.

In a recent study, Spahr and colleagues investigated patients with AFD in comparison to patients with ASH [16]. In patients with AFD, a pronounced downregulation of genes associated with inflammation, fibrogenesis and detoxification was found and may contribute to mechanisms underlying the non-inflammatory and fibrogenic nature of this condition as suggested by the histological pattern. In addition, upregulation of genes associated with lipid metabolism and mitochondrial function including among others mitochondrial glycerol-3-phosphate acyltransferase (GPAM) was found. GPAM catalyzes the first committed step in triglyceride and phospholipid biosynthesis. Increased expression of GPAM in the mouse model leads to massive accumulation of triglycerides in hepatocytes and to impaired ß-oxidation of fatty acids [16]. Non-esterified fatty acids such as amphiphilic compounds may be involved in the formation of an emulsifying rim around a core of triglycerides thus linking the overexpression of GPAM to the morphological feature of microvesicular steatosis seen on histology [17, 18].

The clinical course differs among individuals. A spectrum of conditions ranging from an asymptomatic condition to a clinical syndrome resembling alcoholic hepatitis [16]. Rare cases of microvesicular steatosis and liver failure have been described and AFD seems to be reversible after alcohol withdrawal [14, 15].

Steatohepatitis Due to ALD (ASH)

The correlation between the clinical syndrome of alcoholic hepatitis (AH) based on anamnestic and biochemical parameters only and ASH defined by histology is not optimal. It has been estimated that approximately 25% of patients with AH do not have ASH on histology [19]. The exact incidence of ASH in people with ALD is uncertain [20–23]. Most clinical studies to date are based on clinical diagnosis of ALD and/or AH without histological analysis. In a large cohort of patients with alcohol abuse, 44% of patients with cirrhosis and 12% of those without cirrhosis had ASH on histology [24].

Most cases of ASH are defined by three key features including macrovesicular steatosis, hepatocellular ballooning, and lobular inflammation [4, 25, 26]. After periods of abstinence or in severe ASH or cirrhosis, macrovesicular steatosis can be very limited. Therefore, the minimum requirement for the histological diagnosis of ASH is based on the presence of hepatocellular ballooning and lobular infiltration by neutrophils [27].

Hepatocellular ballooning is an ill-defined morphological term. It designates a presumably degenerative hepatocellular change characterized by a significantly enlarged pale staining (also referred to as cytoplasmic clarification) and rounded cytoplasm on H&E histology (usually >2× normal sized hepatocytes). Ballooned hepatocytes may contain garland shaped hyaline eosinophilic cytoplasmic inclusions termed Mallory Denk bodies (MDB) (Fig. 38.7) [28]. Compared to normal sized hepatocytes ballooning is also associated with loss of cytoplasmic staining with antibodies against the intermediate filament (IF) components Keratin (K) 8 and 18 and marking with antibodies against sonic hedgehog (see below) [29]. Ballooning can be very severe in ALD. Typically, the ballooned hepatocytes harbour very large MDB (Fig. 38.8).

The mechanisms underlying the ballooning change are not known in detail. Based on the results from in vitro experiments and mouse models it can be concluded that alcohol-related oxidative stress is involved in the upregulation of K8 and

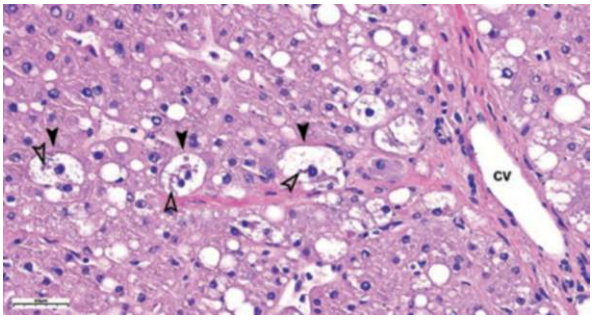

Fig. 38.7 Ballooned hepatocytes with cytoplasmic enlargement, rounding and clarification (examples are marked by black arrow heads) and Mallory Denk bodies present as eosinophilic garland shaped hyaline inclusions (open arrow heads) (H&E; CV, central vein)

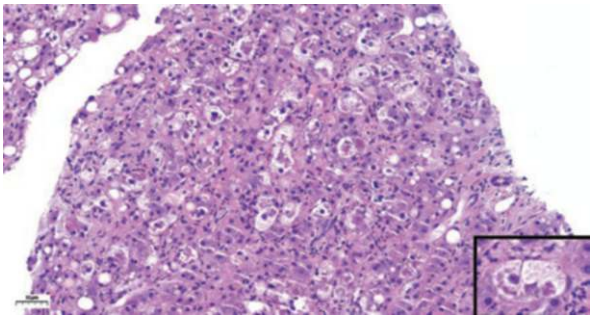

Fig. 38.8 Parenchymal area with massive ballooning. The ballooned hepatocytes contain large Mallory Denk bodies present as eosinophilic garland shaped hyaline inclusions (inset) and are surrounded by neutrophils (H&E)

to a lesser extent K18 as well as hyperphosphorylation, transamidation and crosslinking of these proteins. Furthermore, the keratins become partially degraded and ubiquitinated. These modifications promote disintegration of the IF detectable as loss of cytoplasmic K8/18 staining on immunohistochemistry (Fig. 38.9). The decreased mechanical stability of hepatocytes presumably contributes to the ballooned phenotype. In addition, p62, which is a stress- inducible ubiquitin binding protein is overexpressed. It binds the modified keratin species and targets them for degradation by the proteasome or autophagosome. However, oxidative stress also causes an accumulation of other misfolded proteins. Large amounts of modified proteins overwhelm proteasomal and autophagic capacities resulting in the aggregation of crosslinked and ubiquitinated K8 and 18, p62 and ubiquitin and the formation of MDBs [28, 30, 31]. The role of impaired proteasomal and autophagic degradation for the generation of MDBs was recently also confirmed in mouse models and humans [32, 33]. Hepatocellular ballooning is not specific for ASH. It can also be a feature of other chronic liver diseases associated with chronic oxidative stress like non-alcoholic steatohepatitis (NASH), conditions of chronic cholestasis, copper storage disorders, apha-1-antitrypsin deficiency, and ischemia-reperfusion injury [34]. Loss or regularly structured keratin in ballooned hepatocytes may render them prone to apoptosis [35].

Lobular inflammation by neutrophils is one of the key components of ASH. In some cases, neutrophils surrounding hepatocytes that contain MDBs can be found, a feature referred to as satellitosis (Fig. 38.10). In the early literature it was postulated that MDBs have leukotactic properties. Recently this hypothesis was supported by results from a study by Liu and colleagues using a MDB-inducing cell model. They showed that compounds which can induce MDB in vitro also produce IkappaB alpha loss. Furthermore, IkappaB alpha is sequestered into proteinaceous aggregates mostly consisting of p62, which is also a component of MDBs (see above). Because of the sequestration of IkappaB, NFkappaB signalling is activated, and inflammation is enhanced. The authors also identified another 10 IkappaB alpha- interacting and -aggregating proteins using four different proteomic approaches [36]. The presence of protein aggregates such as MDBs in ASH may therefore be regarded as indicators of oxidative stress-associated propensity of

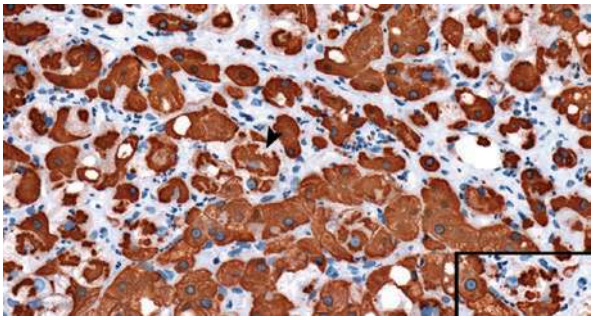

Fig. 38.9 Ballooned hepatocytes characterized by a loss of cytoplasmic staining by antibodies against keratin (K) 8 and K18. Mallory Denk bodies contain the aggregated forms of K8 and 18 as well as other proteins and are therefore stained with the respective antibodies (arrow head) (immunohistochemical stain with antibodies against K8/K18)

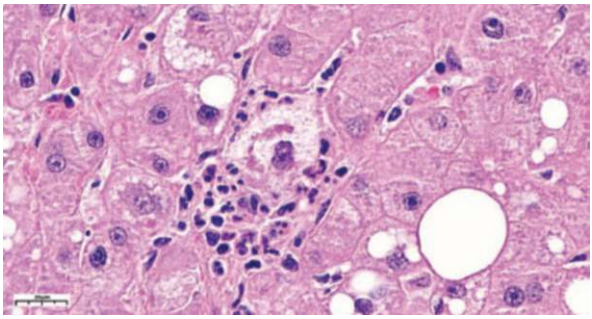

Fig. 38.10 Satellitosis. Ballooned hepatocyte with Mallory Denk body surrounded by neutrophils (H&E)

protein aggregation and a proinflammatory setting promoted by activated NFkappa B signalling.

In decompensated stages of ALD, characterized by bilirubin levels >3 mg/dL and/or signs of liver decompensation (new onset jaundice, ascites, hepatic encephalopathy, portal hypertensive gastrointestinal bleeding), cholestasis can be also a feature in the pre-cirrhotic setting. Visible bile pigment is seen in the hepatocellular cytoplasm (hepatocellular cholestasis), canalicules (canalicular cholestasis) (Fig. 38.11a) and ductules (Fig. 38.11b) (ductular cholestasis) of the so called ductular reaction (see below). However, it is a very rare feature in compensated clinical stages of ALD. Canalicular and/or ductular cholestasis are associated with sepsis [21, 23, 37, 38], unfavourable prognosis and higher short-term mortality rates [27]. Ductular cholestasis has also been identified as an independent predictor of treatment response to corticosteroids in patients with severe AH. The pathomechanism(s) underlying cholestasis are not well described. Some of the factors responsible could include ballooning-associated obstruction of bile radicles [39], impaired bile formation and secretion via the canalicular transport systems [40] and reduced bile flow. Some of these pathways could be implicated in the sepsis-associated ductular cholestasis [41, 42].

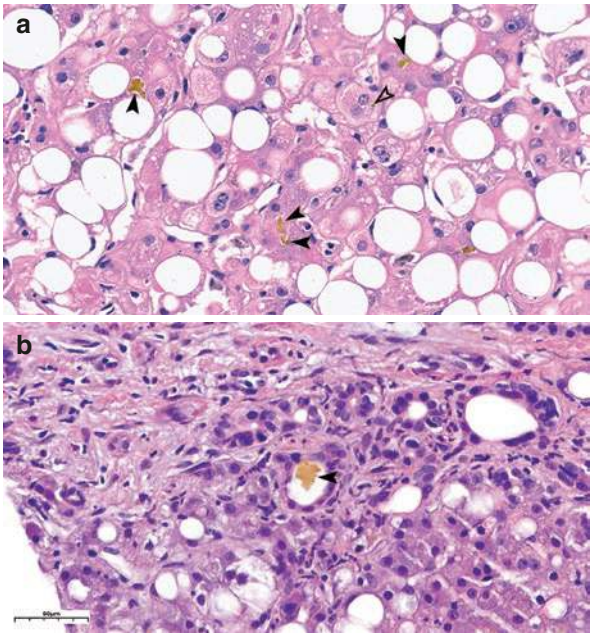

Fig. 38.11 Hepatocellular, canalicular and ductular choleastasis. (**a**) Bile pigment is present in hepatocytes (open arrow head) and canalicules (black arrow head) in a case with the clinical syndrome of alcoholic hepatitis (AH) and severe steatohepatitis due to alcohol-related liver disease (ASH) (H&E). (**b**) Ductular cholestasis with bile pigment in the lumen of a ductule at the mesenchymal-parenchymal interface in a case with AH and sepsis. The ductules are surrounded by some neutrophils (H&E)

Canalicular cholestasis is also a feature of Zieve-syndrome which is characterized by jaundice, hyperlipidemia and hemolytic anemia in a background of cirrhosis and steatosis in patients with ALD [43].

Other Histologic Changes in ALD

Megamitochondria

Mitochondria play a central role in the pathogenesis of fatty liver diseases as a source of reactive oxygen species triggering proinflammatory and fibrogenesis signals via the activation of Kupffer and stellate cells. In ALD mitochondria are damaged by oxidative stress due to induction of cytochrome P450 2E1 and hydroxyl radicals from the Fenton reaction [44].

Normal sized mitochondria are too small to be detected on light microscopy. In ALD they are often enlarged and appear as pink or bright red globular or needle shaped structures in the cytoplasm of hepatocytes in H&E (Fig. 38.6) and chromotrope-aniline-blue (CAB) stained sections, respectively. On electron microscopy, mitochondrial enlargement is accompanied by a reduced number of cristae, intramitochondrial cristalline inclusions and multilamellated membranes [4]. More recently a reduced number of mitochondria of stunted shape have also been described in patients with severe AH.

Megamitochondria appear to be an adaptive phenomenon. Their decreased capacity of oxygen consumption and phosphorylation may be associated with decreased production of reactive oxygen species (ROS) in hepatocytes with beneficial consequences for survival [37].

Megamitochondria are not specific for ALD. They can also be found in other liver diseases like diabetes mellitus or Wilson disease.

Other Adaptive Hepatocellular Changes

Eosinophilic homogenization of the cytoplasm of the liver cells morphologically resembling ground glass inclusions of hepatitis B virus infection are considered a reactive hepatocellular change in ALD caused by an increased smooth endoplasmic reticulum [45, 46]. Another change is so called mitochondriosis due to a proliferation of mitochondria giving rise to an eosinophilic granular cytoplasm (Fig. 38.12) [46]. Adaptive changes are more frequent in cirrhotic than compared to pre-cirrhotic stages of ALD.

Iron Storage

Iron storage in hepatocytes (parenchymal siderosis) as well as in sinusoidal lining and Kupffer cells (mesenchymal siderosis) is a common finding in patients with ALD (Fig. 38.13) [4, 25, 47, 48]. In some cases, particularly in the cirrhosis stage, the extent of parenchymal siderosis can be comparable to siderosis in hereditary hemochromatosis. Iron storage in ALD is due to an increased uptake of iron from the intestine resulting from decreased production of hepcidin in hepatocytes. However, hepcidin expression is upregulated due to alcohol-related ER stress and

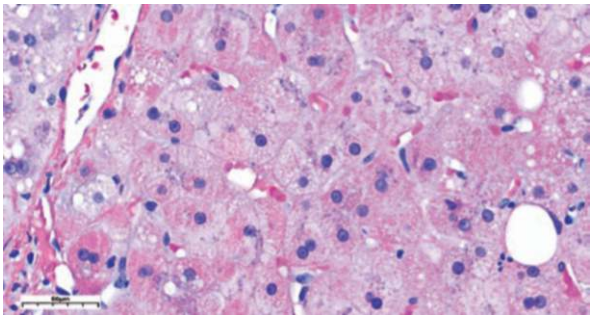

Fig. 38.12 Eosinophilic and granulated cytoplasm of hepatocytes in mitochondriosis is presumed to be due to a proliferation of mitochondria (H&E)

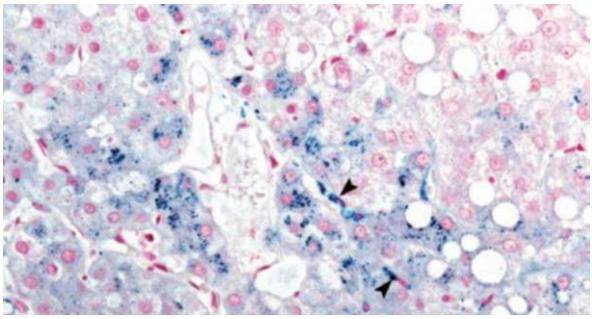

Fig. 38.13 Mixed type siderosis in a case with steatosis due to alcohol-related liver disease. Iron deposits are stained blue in the hepatocellular cytoplasm as well as in Kupffer and sinusoidal endothelial cells (arrow heads) in the Prussian blue stain

inflammation promoting the retention of iron in Kupffer cells. The extent of iron storage may be determined by the ratio of hepcidin suppression and induction in hepatocytes and Kupffer cells, respectively [49]. In addition, comorbidities, genetic factors, fibrosis stage and inflammation may also influence the extent of iron storage [49]. Free iron promotes liver injury and inflammation via oxidative stress and increased production of TNF-alpha [50–52]. In patients with ALD parenchymal siderosis has been shown to correlate with fibrosis stage [53], the development of HCC [54], and prognosis [55].

Ductular Reaction

Ductular reaction (DR) is a frequent histopathological feature in ALD and other chronic liver disease associated with parenchymal loss or mechanical obstruction of bile flow [4, 25, 45, 56, 57]. The ductules consist of epithelial cells with cholangiocellular differentiation set in a loose inflamed fibrous stroma at the mesenchymal parenchymal interface (Fig. 38.14). DR may arise from stem cell proliferation or ductular metaplasia of periportal hepatocytes. The ductules are capable of differentiation into mature cholangio- or hepatocytes and are presumed to contribute to restoration of hepatocellular mass. Transcriptomic analysis of DR revealed a proinflammatory profile with expression of CXC and C-C motif chemokine ligand chemokines [58] as well as fibrogenic factors which activate stromal myofibroblasts, promote the recruitment of neutrophils and the development of periportal fibrosis [59].

DR may also result from mechanical obstruction of bile flow, e.g., related to fibrosis of the papilla of Vater in cases with alcohol-related chronic pancreatitis.

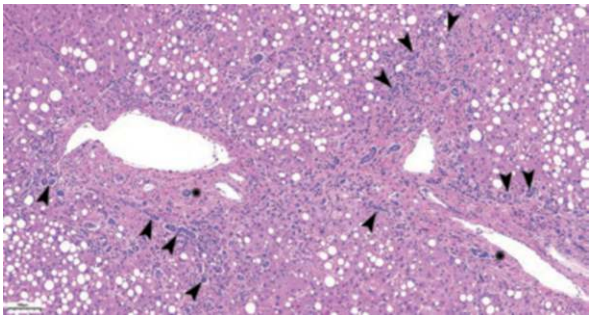

Fig. 38.14 Ductular reaction in a case with severe fibrosis due to alcohol-related liver disease. The interlobular bile ducts of the original portal tracts (marked by asterisks)are located adjacent to the A. hepatica and portal vein branches whereas the ductular structures of ductular reaction are present at the mesenchymal-parenchymal interface (examples marked by arrow heads) (H&E)

Fibrosis and Cirrhosis Due to ALD

Alcohol or acetaldehyde-associated liver injury promotes ballooning degeneration of centrilobular hepatocytes. Ballooned hepatocytes release hedgehog ligands and, in a paracrine fashion, induce hedgehog-responsive genes in hepatic stellate cells (HSC) residing in the space of Disse`. Activated HSCs are involved in the production of extracellular matrix and collagen deposits along sinusoids and around ballooned hepatocytes, giving rise to a particular type of fibrosis typically seen in fatty liver diseases like non-alcoholic fatty liver disease (NAFLD) as well as ALD. According to the characteristic pattern of collagen deposits, the term pericellular fibrosis (PCF) has been coined (Fig. 38.15). Profibrogenic hedgehog ligands can also be released from dying hepatocytes, hepatic progenitor cells and a few other cell types of the injured liver. Furthermore, HSC can also be activated by growth factors secreted by Kupffer cells which are activated by damage-associated molecular patterns (DAMPs) and ROS from dying hepatocytes as well as pathogen-associated molecular patterns (PAMPs) and lipopolysaccharides (LPS) resulting from ethanol-associated gut injury (so called leaky gut). In addition, fibrogenesis is propelled by components of the extracellular matrix (ECM), like collagen-1 and integrin promoting HSC survival which is also enhanced by alcohol-mediated inhibition of natural killer cell (NK)-associated removal of HSC. Activated HSC produce tissue inhibitors of metalloproteinases (TIMPs) and thus inhibit the fibrolytic activity of matrix metalloproteinases (MMPs). Maturation of fibrous tissue is accompanied by loss of hepatocytes from areas with fibrosis, the formation of condensed collagen and accumulation of elastic fibers as well as clusterin resulting in dense, scar-like fibrous septa that resemble septa in chronic viral hepatitis (reviewed in [60]).

Zonation of the hepatic lobules may also play an important role in the shaping of central-based fibrosis patterns. HSC just like hepatocytes differ with respect the expression of zonation-specific protein markers. In a mouse model of centrilobular

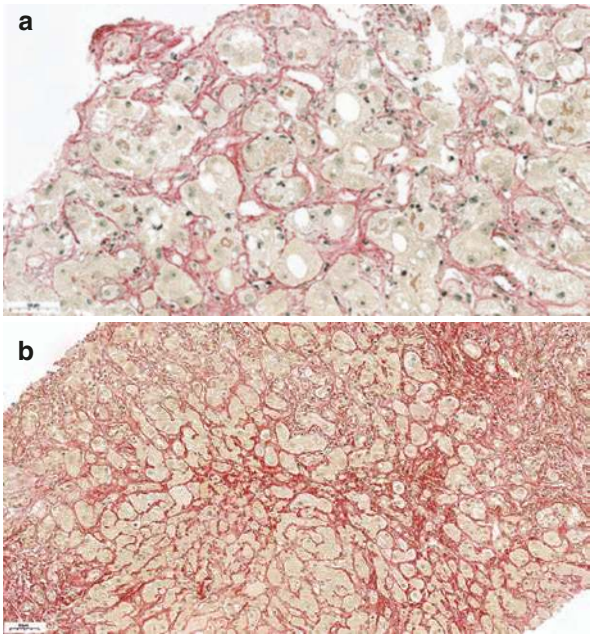

Fig. 38.15 Pericellular fibrosis. (**a**) Single or small groups of hepatocytes are surrounded by collagen fibers giving rise to a chicken wire pattern of fibrosis (sirius red). (**b**) Severe pericellular fibrosis is dissecting the parenchyma (sirius red)

fibrotic injury the central stellate cell type contributed most to the injury-related collagen production whereas the periportal stellate cells are less involved [61].

Compared to normal liver (Fig. 38.16a), in ALD, fibrosis is restricted to perivenular areas and around centrilobular hepatocytes in the early stages of the disease (Fig. 38.16b). In settings of ongoing alcohol abuse, PCF can extend to the intermediate portions of the hepatic lobules. In some cases, the entire lobules are dissected by PCF and/or septa consisting of PCF with admixed hepatocytes (septal PCF) forming bridges between central veins and the portal tracts (bridging fibrosis) (Fig. 38.16e, f). This pattern is often associated with the fibro-obliteration of central veins [27]. Fibro-obliterated veins are typical for ALD and have not been described in NAFLD. A particularly severe form of centrilobular fibrosis is termed sclerosing hyaline necrosis. It designates severe perivenular fibrosis in the setting of lobular inflammation and hepatocellular ballooning [62]. Although central-based fibrosis is the common fibrosis type in ALD, portal-based fibrosis has also been described in a minority of cases [63] (Fig. 38.16c). Portal-pased fibrosis may occur together with central-based PCF (Fig. 38.16d). Ongoing liver injury finally leads to the destruction of the lobular architecture and the formation of parenchymal nodules surrounded by fibrous septa characterizing the cirrhosis stage (Fig. 38.17a–f). In cases of actively drinking patients, PCF may be very severe, dissecting the parenchymal nodules and causing an indistinct nodular outline (Fig. 38.17b, d, f). In such cases the term parenchymal nodularity may be used. Again, this pattern of fibrosis is also typical for ALD and has not been described in NAFLD [27].

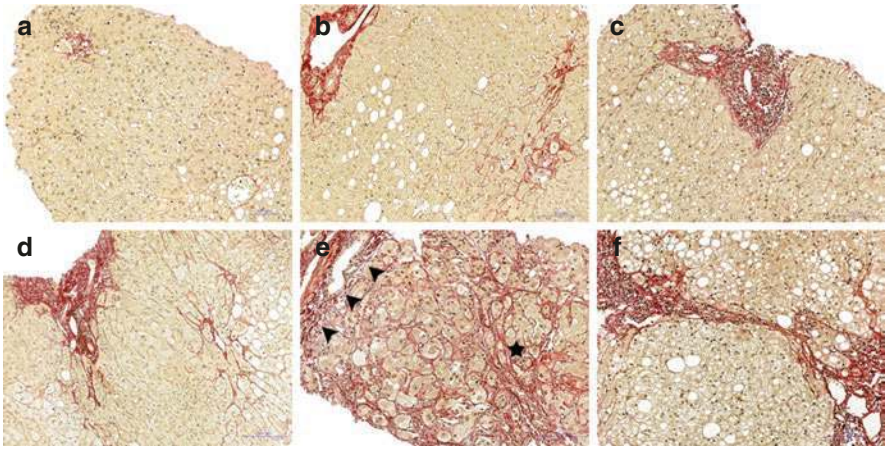

Fig. 38.16 Fibrosis progression in precirrhotic stages of alcohol-related liver disease. (**a**) Normal lobular architecture. Fibrous tissue is restricted to portal tracts (SFS 0). (**b**) Fibrogensis starts in centrilobular areas as pericellular fibrosis withe deposits of collagen fibers around single or groups of hepatocytes (SFS 1P) (**c**) In some cases there is a predominantely periportal fibrosis type (SFS 1). (**d**) With progressive disease both, cetrilobular and periportal fibrosis may be seen (SFS 2) and (**e**) can progress to severe pericellular fibrosis dissecting the lobules or bands of pericellular fibrosis connecting portal tracts and central veins (i.e. bridging fibrosis) (SFS 3P). This fibrosis stage is often associated with obliteration of central veins, a pattern not decribed in NAFLD. (**f**) Bridging fibrosis (SFS 3) ca also present in the form of dense septa resembling septa in chronic viral hepatitis

Even in the cirrhosis stage, there can be disease progression with parenchymal loss and increase of fibrous septa giving rise to different stages of cirrhosis severity (see below) (Fig. 38.17a–f). Notably, most patients with decompensated ALD are in the cirrhotic stage at the time of diagnosis. Substages of cirrhosis severity have been shown to be of prognostic relevance [64, 65] in all clinical settings of ALD including compensated and decompensated ALD as well as patients with the clinical diagnosis of AH and histological ASH [27, 66].

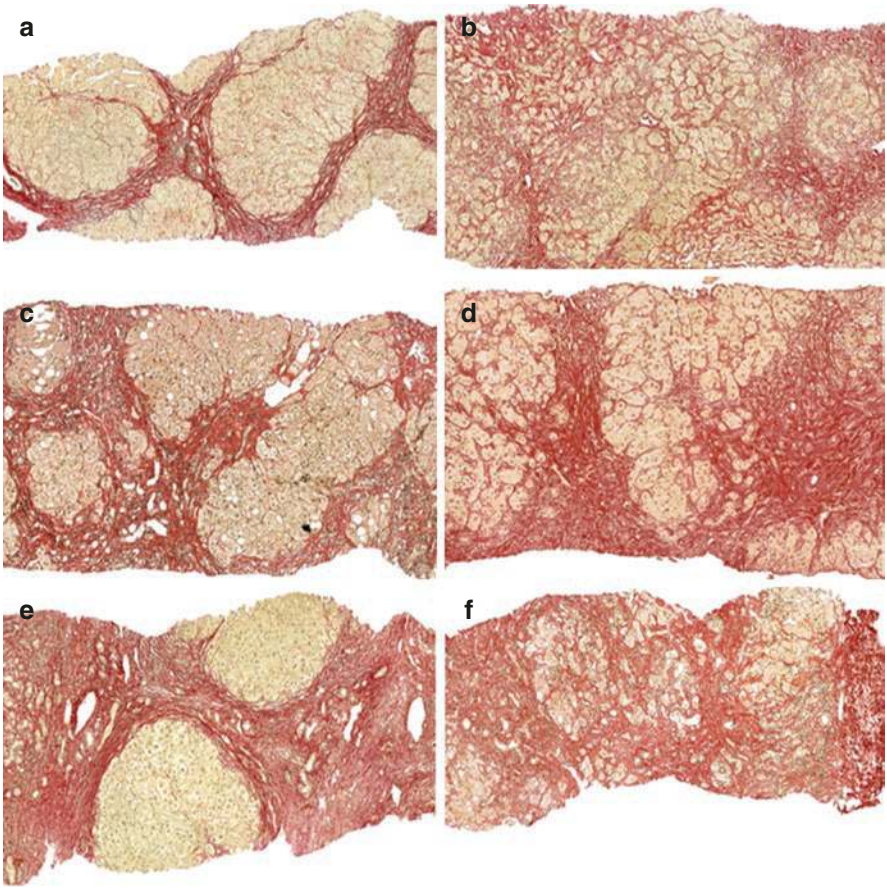

Fig. 38.17 Fibrosis progression in cirrhostic stages of alcohol-related liver disease. (**a**, **c**, **e**) Progresssive disease leads to the destruction of the lobular architecture and the formation of parenchymal nodules surrounded by fibrous septa. The progression of fibrosis leads to thin (SFS 4A) to broad (SFS 4B) and very broad septa (SFS 4C). In all stages of cirrhosis (**b**, **d**, **f**) severe pericellular fibrosis (comprising more than 50% of the parenchyma)

Histologic Grading and Staging of ALD

Histologic methods for the semiquantitative assessment of disease activity (grade) and non-structural collagen, i.e., fibrosis (stage) have been developed and validated for many chronic liver diseases including chronic hepatitis, biliary disease and NAFLD and are widely used in clinical practice and clinical trials for the standardized histological assessment and prognostication. Recently an ALD-specific histological grading and staging system was developed by an international group of pathologists [27], members of the Consortium for the Study of Alcohol-related Liver Disease in Europe (SALVE). The design of this histological method was based on characteristic and prognostic histologic parameters of ALD.

Semiquantitative Assessment of Disease Activity (SALVE Grading)

The histological scoring system for the assessment of grade was defined using numerical scores representing the extent of macrovesicular steatosis, hepatocellular ballooning, MDB, lobular neutrophils as well as canalicular and ductular cholestasis (Table 38.1). Macrovesicular steatosis is assessed on a three-step scale according to the percentage of parenchymal involvement ranging from less than 5% of hepatocytes with macrovesicular steatosis to severe steatosis with more than 66% of parenchymal involvement. Activity is defined as the sum of semiquantitative scores for ballooning or MDBs depending which of these features is more pronounced to avoid overrating of hepatocellular damage, and scores for lobular infiltration by neutrophils, both assessed on a two-step scale. Canalicular and ductular cholestasis is scored as absent or present. SALVE grade is then described by itemization of the scores for steatosis (range 0–3), activity (range 0–4) and canalicular as well as ductular cholestasis (range 0–1, respectively).

Table 38.1 SALVE grading [27]

Steatosis (S) grade: Macrovesicular steatosis[a]; % parenchymal involvement
Score 0: <5%
Score 1: 5–33%
Score 2: 34–66%
Score 3: >66%
Activity (A) grade: Sum of scores for hepatocellular injury (Ballooning [B] *or* Mallory-Denk bodies [MDB][b]) and lobular inflammation
Score 0: None-rare
Score 1: Few[c]
Score 2: Many[d]
Lobular neutrophils (LN)
Score 0: None-rare
Score 1: Few[c]
Score 2: Many[d] and/or satellitosis[e]
Cholestasis type
Canalicular cholestasis (CC)
Score 0: None
Score 1: Present
Ductular cholestasis (DC)
Score 0: None
Score 1: Present
SALVE grade is described by itemization of each of the component scores:
S 0–3**, A (B/MDB** 0–2 **+ LN** 0–2**), CC** 0–1**, DC** 0–1

[a]Lipid vacuoles in the cytoplasm of hepatocytes larger than the hepatocellular nucleus
[b]If scores for ballooning and Mallory-Denk bodies are unequal the higher score is applied
[c]Feature is appreciated after a reasonable search and is present in few microscopic fields
[d]Feature is frequent and easy to find without searching and present in many microscopic fields
[e]Neutrophils surrounding ballooned hepatocytes

Semiquantitative Assessment of Non-structural Collagen (SALVE Staging)

SALVE staging comprises seven main fibrosis stages (SALVE fibrosis stages, SFS) ranging from no fibrosis, SFS 0 to severe cirrhosis, SFS 4C (Table 38.2). SFS 1 can have two different patterns of periportal or centrilobular PCF. In SFS 2 periportal and centrilobular fibrosis is present. Bridging fibrosis (SFS3) is characterized by complete septa that can consist dense fibrous septa devoid of hepatocytes resembling septa in chronic viral hepatitis or consist of PCF (septal PCF) connecting central veins and portal tracts.

SALVE cirrhosis stages are defined by destruction of the lobular architecture and the occurrence of parenchymal nodules surrounded by fibrous septa by the diameter of fibrous septa in relation to the diameter of the smallest distinct parenchymal nodule present in a biopsy. In SFS 4A the diameter of the septa measured at the minimal distance between two parenchymal nodules is less than 50% of the diameter of the smallest parenchymal nodule, whereas broad septa of SFS 4B are wider than 50% and very broad septa of SFS 4C are wider than the complete diameter of the smallest nodule. In all cirrhosis stages, variable amounts of PCF can be seen.

Table 38.2 SALVE fibrosis stage (SFS) [27]

Stage	Fibrosis	Definition
SFS 0	No	No fibrosis
SFS 1	Mild	**1A**: Portal & periportal fibrosis
		***1P**: PCF[a] in zone 3 ± zone 2*[b]
SFS 2	Moderate	**2**: PCF in zone 3 ± zone 2 and periportal fibrosis
SFS 3	Bridging	**3A**: ≥1 complete dense septum[c]
		***3P**: >50% of the parenchyma with PCF up to zone 1, no distinct nodules*
SFS 4	Cirrhosis	**4A**: ≥1 distinct parenchymal nodule[d], most septa are thin[e], 1 broad septum[f] allowed
	Thin septa	***4AP**: >50% of parenchyma with severe PCF[g] and indistinct parenchymal nodules[h]; thin dense septa allowed*
	Severe PCF	
	Broad septa	**4B**: Distinct parenchymal nodules, >1 broad septum; 1 very broad septum[i] allowed
		***4BP**: 4B and >50% of parenchyma with severe PCF*
	Very broad septa	**4C**: Distinct parenchymal nodules, >1 very broad septum[i]
		***4CP:** 4C and >50% of parenchyma with severe PCF*

[a]Pericellular fibrosis: Collagen fibers surrounding single or small groups of hepatocytes
[b]*P stages: optional, for use in research settings*
[c]Dense septum: Septum consisting mainly of collagen fibers and eventually very occasional hepatocytes
[d]Distinct nodule: Parenchymal nodule without portal-central relations surrounded by dense septa
[e]Thin septum: Dense septum, <25% of diameter of smallest distinct nodule
[f]Broad septum: Dense septum, >25% and ≤50% of the diameter of smallest distinct nodule
[g]Severe PCF: PCF visible at 4× magnification
[h]Indistinct nodule: Parenchymal area of indistinct nodular shape dissected by severe PCF
[i]Very broad septum: Dense septum >50% of the diameter smallest distinct nodule

The Diagnostic and Prognostic Relevance of PCF in ALD

PCF is devoid of elastic fibers typically present in dense septa. In mouse models it has been shown that dense septa with elastic fibers are more resistant to degradation than septa without elastic fibers [67, 68]. Therefore, it can be speculated that PCF is an immature type of fibrosis prone to degradation in settings of disease regression and thus may be useful to monitor early antifibrotic effects of therapeutic interventions. Furthermore, because severe PCF affects large proportions of the parenchyma it may be correlated with higher liver stiffness than compared to only septal fibrosis in a particular stage and could affect the interpretation of non-invasive fibrosis tests such as elastography. Finally, severe PCF also characterizes two distinct ALD-specific fibrosis patterns which are part of the spectrum of bridging fibrosis and cirrhosis with thin septa. Bridging fibrosis can be present as panlobular or septal PCF associated with fibro-obliterative lesions of central veins (see above) and destruction of the lobular architecture mainly due to PCF can be seen in early cirrhosis. These fibrosis patterns have not been described in NAFLD and may therefore also be of diagnostic relevance. Data from a recent study also indicate that severe PCF in cases with bridging fibrosis and cirrhosis was associated with significantly worse survival at 5-years compared to cases without PCF [27]. Because of these reasons an ALD-specific staging system should provide the possibility to also record severe PCF.

Expanded SALVE Fibrosis Staging with Emphasis on PCF

The SALVE staging system has also been adapted and expanded by the so called P-stages to represent severe forms of PCF. These stages are shown in the grey insets in Table 38.2. The P-stages are optional and may be used in study settings which require a more detailed description of fibrosis. Stage 1P is for cases with centrilobular fibrosis only, stage 3P is characterized by predominantly panlobular or septal forms PCF with bridging. In the cirrhotic stages 4AP, BP and CP severe PCF is seen in more than 50% of the parenchyma.

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Chapter 39
Non-invasive Fibrosis Assessment in Alcohol-Related Liver Disease

Maria Silva and Emmanuel A. Tsochatzis

Abstract Non-invasive identification of advanced stages of fibrosis is of crucial importance in ALD in order to define preventive actions against the occurrence of liver related events. Several non-invasive tests have been tested in ALD populations with high diagnostic yield. Serum biomarkers are valuable tools, mostly to exclude severe fibrosis in low cirrhosis prevalence populations. In case of positive results, the sequential use of a liver elastography to measure liver stiffness (LS) is recommended, provided that alcohol consumption and biochemical evidence of inflammation are excluded or minimized. In high prevalence scenarios, elastographic techniques are preferable. Transient elastography is the most validated LS measurement method, despite the increasing evidence for other SWE techniques with important practical advantages. Disease-specific threshold values for advanced fibrosis/cirrhosis have been proposed, with dual cut-offs providing optimal sensibility and specificity. In terms of prognosis assessment, both biomarkers and LS methods have displayed value, however, sufficient evidence for disease-specific recommendations is lacking.

Keywords Fibroscan · ELF · FIB4 · Cirrhosis · Prognosis · HCC

Introduction

In chronic liver disease, fibrosis develops as a response to repeated liver injury with excess extracellular collagen deposition [1]. Cirrhosis, the most extreme stage of fibrosis, is characterized by abnormal liver architecture with fibrous tissue circumscribing parenchymal nodules and hepatic vasculature alterations [2]. Alcohol consumption is a dominant cause of cirrhosis, with an increasing mortality and morbidity burden worldwide [3]. Alcohol-related liver disease (ALD) patients have

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higher risk of cirrhosis complications and death at an early age in comparison with another aetiologies [4]. The risk of cirrhosis development increases in a cumulative alcohol dose-dependent manner [5], which highlights the importance of early diagnosis and prompt life-style interventions.

The standard method for liver fibrosis assessment is liver biopsy. However, it displays several limitations including potential non-representability of the specimen [6], reproducibility difficulties among pathologists [1], limited availability and a small risk of serious complications [7]. These issues have led to the development of non-invasive tests (NITs) for the assessment of liver fibrosis. The term "cirrhosis" implies a pathological diagnosis with the above-mentioned limitations. For that reason, and considering severe fibrosis and cirrhosis as part for a continuous spectrum, the concept of compensated advanced chronic liver disease (cACLD) has been introduced at the Baveno VI conference, as a stage of disease with significant risk of cirrhosis complications [8]. The Baveno VII workshop reinforced the importance of identifying patients with cACLD with NITs and, among those, the ones with clinically significant portal hypertension (CSPH), defined as hepatic venous pressure gradient (HVPG) ≥10 mmHg [9].

NITs are divided into two main categories (Table 39.1): serum biomarkers, that directly or indirectly are associated with liver fibrosis, and liver stiffness (LS) measurement tools [10]. Most NITs were developed in chronic viral hepatitis cohorts

Table 39.1 Advantages, limitations and specificities of non-invasive tests on alcohol-related liver disease

	Serum biomarkers		Liver stiffness measurement tools		
	Direct/ patented	Indirect/ non-patented	Transient elastography	pSWE/2D-SWE	MRE
Advantages	High reproducibility; High applicability; Prognostic value		High accuracy for diagnosis (advance fibrosis/cirrhosis)		Uses regular MRI machine
			Prognostic value (best validated for TE)		Entire liver examination
	Accuracy (> non-patented)	High availability	Most validated technique	Uses regular echography device	Low failure rate
		Low cost	Good reproducibility	Selectable area of interest	
			Fast learning curve	Low failure rate	
			Well defined quality criteria	Higher accuracy in milder fibrosis stages (2D-SWE)[a]	

Table 39.1 (continued)

	Serum biomarkers		Liver stiffness measurement tools		
	Direct/ patented	Indirect/ non-patented	Transient elastography	pSWE/2D-SWE	MRE
Limitations	Non-liver-specific		False positives (inflammation, congestion, extra-hepatic cholestasis, food and alcohol intake)		MRI machine required
	Inferior accuracy compared to TE				Time-consuming
	False positives (extra-hepatic inflammatory conditions)[b]	False positives (inflammation)[c]	Dedicated device required	Less validated than TE	High cost
	Low availability	Lower accuracy	Highest failure rate (obesity, ascites, inexperience)	Compatible device required	Not applicable if iron overload, non-compatible prothesis and claustrophobia
	High cost			Not well stablished quality criteria	Uncertain prognostic value
Specificities on ALD	Lower accuracy for milder fibrosis stages		High accuracy for diagnosis (advance fibrosis/cirrhosis)		High accuracy for diagnosis
	Uncertain value in CSPH		Prognostic value (liver related events > mortality)[b]		Lack of experience
	Accuracy detecting severe fibrosis (best: ELF® and FibroTest®)	Accurate ruling out severe fibrosis in low prevalence populations (best: Forns index and FIB-4)	Higher false positive risk (steatohepatitis/alcohol intake)		Uncertain prognostic value
	Prognostic value (ELF® and FibroTest®)[a]	Prognostic value[a]	Uncertain value in CSPH		

pSWE point shear wave elastography, *2D-SWE* 2D-shear wave elastography, *MRE* magnetic resonance elastography, *TE* transient elastography, *CSPH* clinically significant portal hypertension, *ELF* enhanced liver fibrosis score

[a]Further validation needed

[b]False positives for FibroTest® in Gilbert syndrome and hemolysis and for FibroMeter® in acute hepatitis

[c]False positive for FIB-4 and NFS if age > 65 years

[10]. However, there is increasing evidence regarding their application on ALD [11]. In this chapter, we review the available NITs for liver fibrosis assessment in ALD, focusing on their diagnostic and prognostic applicability.

Considerations on Accuracy and Limitations of Non-invasive Tests

An ideal Non-Invasive Test (NIT) should be cheaper than LB, accessible, reproducible, and reliable in providing prognostic information [11]. Frequent NITs limitations include lack of accuracy in identifying early fibrosis stages, discriminating between adjacent stages of fibrosis and diagnosing CSPH [10].

The performance of NITs is usually evaluated through an area under the receiver operator characteristic curve (AUROC) taking liver biopsy as the reference. However, as previously stated, liver biopsy is not a perfect standard, meaning that an AUROC >0.90 may not be achieved even by a perfect NIT [12]. The AUROC varies according to the prevalence of fibrosis and the distribution of each fibrosis stage [13]. Thus, the choice of NITs must take into account the clinical scenario. Most NITs were developed in secondary/tertiary settings where advanced fibrosis prevalence may reach >50% in ALD [14], contrasting with <10% in risk patients from the general population [15]. In addition, the AUROC does not inform about the clinical impact of false negatives and false positives [16]. This was addressed by Majumdar et al. who integrated decision tree and curve models to define the minimum acceptable accuracy of NITs compared to liver biopsy in terms of 2-year mortality, in function of cirrhosis prevalence [17]. The authors reported minimal sensitivity and specificity of 89% and 88%, 94% and 85%, and 94% and 87% in populations with 5%, 20% and 50% cirrhosis prevalence, respectively.

Serum Biomarkers of Liver Fibrosis

A wide variety of biomarkers have been proposed for the assessment of liver fibrosis [10]. FibroTest® [18] was the first algorithm combining direct fibrosis markers, followed by four additional patented tests (FibroMeter® [19], FibroSpectII® [20], Enhanced Liver Fibrosis score® (ELF) [21] and HepaScore® [22]). Non-patented methods use algorithms that combine indirect routinely available markers. Serum biomarkers have the practical advantages of reproducibility, applicability and, for non-patented scores, availability at low cost. However, they are not liver-specific and may include parameters that are predominantly altered in advanced disease stages, that vary in other conditions and/or in healthy individuals [23]. Most biomarkers were validated in chronic hepatitis C (HCV) patients in secondary/tertiary settings [10]. However, there are increasing data on their use in other populations.

A large systematic review addressed the diagnostic performance of biomarkers in ALD concluding that they could identify severe fibrosis/cirrhosis but not milder stages of fibrosis [24]. The most common single marker was hyaluronic acid (n = 1360) which presented consistent high yield in excluding severe fibrosis/cirrhosis, despite of a wide range of cut-off values and of reported inferiority when compared to panel markers [25]. The patented panels FibroTest®, FibroMeter®, HepaScore® and ELF® showed excellent accuracy in detecting cirrhosis with AUROCs >0.9, while one non-patented test (PGA: prothrombin time, gamma-glutamyl transpeptidase and apoliprotein AI score [26]) showed similar diagnostic yield to patented tests in an 103-patient cohort [25]. Importantly, due to heterogeneity, the definition of pooled cut-off values was not possible in this review. A more recent 289-participant study evaluating eight biomarkers, in both primary and secondary facilities, concluded that ELF® and FibroTest® were excellent markers of advanced fibrosis, with similar diagnostic yield as LS measurements in the intention-to-diagnose analysis [15]. Cut-offs of 10.5 for ELF® and 0.58 for FibroTest® had 94% and 90% negative predictive value (NPV) to rule out advanced fibrosis in all patients, which rose to 98% and 97% in primary care patients, respectively. Among indirect markers, Forns index [27] was the best identifying advanced fibrosis, with a NPV of 91% at a threshold of 6.8, that increased to 97% in primary care settings, suggesting that it might be useful in sequential NITs strategies (see below). FIB-4 [28] was a close second. In a high-cirrhosis prevalence cohort of 193 participants, however, these scores did not perform well and were outperformed by PGAA (PGA and α-2-macroglobulin score) [29] that came close to FibroTest® accuracy [30].

Biomarkers may also be valuable in defining CSPH and prognosis in ALD. In a cohort of 219 cirrhotic ALD patients, biomarkers were outperformed by LS measurements and LS–spleen diameter to platelet ratio score (LSPS) in defining CSPH [31]. However, the non-patented biomarkers FIB-4 [32] and Lok index [33] were the only NITs independently associated with mortality in this analysis. Regarding patented tests, a 462-participant study with 4-year median follow-up showed that ELF® outperformed FibroTest® and other biomarkers in prognostic value, being only inferior to elastography [34]. ELF® and FibroTest® could independently group patients according to rates of liver-related events during follow-up, with minimal risk for ELF® <9.8 (5%) and FibroTest® <0.31 (8%) and maximal for >10.5 (53%) and >0.58 (55%), respectively. Forns index, NFS [35] and FIB-4 had good prognostic accuracies, however, they were not able to define risk groups. The prognostic value of FibroTest® had been previously addressed in a 218-patient cohort where it independently predicted 10-year mortality, performing better than other biomarkers and similarly to histological defined fibrosis stages [36].

In general, biomarkers are reliable tools in ALD, mainly for the exclusion of severe fibrosis/cirrhosis. Patented scores haver higher diagnostic accuracy and are recommended to rule-out advanced fibrosis when TE is not available, with FIB-4 as an alternative [11]. Serum markers appear to have promising prognostic value, however further validation is necessary to advice their use in that regard.

LS Measurement Tools

Transient Elastography

Transient elastography (TE) is the most stablished LS assessment tool. More details are provided in Chap. 42. Briefly, it is based on the propagation of low-frequency shear waves through the liver translated in 1-dimention ultrasounds [37], with higher propagation velocities in stiffer tissues. Measurements are expressed in kilopascal (kPa). TE is reproducible, rapidly obtained and regarded as a fast learning curve technique [38]. However, reliable measurements are not always attainable, mainly due to patient obesity and/or operator inexperience, but also to narrow intercostal spaces [37]. Large series have reported rates of failed/technical unreliable results up to 19% [39]. In addition, TE results are influenced by inflammation [40, 41], hepatic congestion [42], extra-hepatic cholestasis [43] and steatosis [44], as well as by food and alcohol intake [45]. TE has shown better results ruling out cirrhosis than making a positive diagnosis or identifying milder stages of fibrosis [11]. It has been applied to multiple causes of chronic liver disease and disease-specific LS cut-offs for staging fibrosis have been proposed, with generally higher thresholds for ALD [46]. Regardless of the aetiology, according to the Baveno VI consensus, LS between 10 and 15 kPa is suggestive of cACLD and LS >15 kPa is highly suggestive [8].

There are several aspects to take into account when applying TE to ALD. In the presence of steatohepatitis and alcohol consumption, TE may significantly overestimate fibrosis stages. During alcohol detoxification, decreases in transaminases, particularly aspartate aminotransferase (AST), correlate with lower LS values [47–49]. In a cohort of 101 participants with alcohol abuse, the exclusion of patients with AST >100 IU/L increased TE specificity for cirrhosis from 80% to 90% [47]. Interestingly, significant decreases in LS after alcohol withdrawal, occur even if baseline AST is <100 IU/L [48]. In another cohort of 677 patients, LS exponentially increased with AST baseline levels and 16% of patients showed decrease in LS after 5 days of alcohol withdrawal [49]. Surprisingly, with normal AST, LS cirrhosis thresholds in ALD were quite similar to HCV, suggesting that LS may be comparable among aetiologies in the absence of inflammation. In the presence of AST elevation though LS increased much more in ALD. Importantly, LS only increased in 30% of patients with AST >100 IU/L which may help explain the wide range of cirrhosis cut-offs previously reported in ALD, from 11.5 [47] to 25.6 kPa [50].

In ALD, the most robust evidence favors TE use in identifying advanced fibrosis in secondary/tertiary settings, with AUROCs consistently ≥0.9 [11, 15, 46]. A 834-participant meta-analysis concluded that TE is valuable in ruling out significant fibrosis, severe fibrosis and cirrhosis (cut-off: 7.0–7.8 kPa, sensitivity 94%, specificity 89%; 9.5 kPa, 92%, 70%; 12.5 kPa, 95%, 71%, respectively), in high cirrhosis prevalence scenarios [51]. This cut-off values were the most frequently reported by individual studies, since pooled cut-offs could not be defined. A more recent 1026-patient meta-analysis obtained pooled LS thresholds for each fibrosis stages,

reporting 12.1 and 18.6 kPa for severe fibrosis and cirrhosis, respectively [46]. Increased AST and bilirubin values, as well as histological features of alcoholic hepatitis, were associated with increments in LS. If AST >75 IU/L, LS was twice as high and if bilirubin >16 μmol/L, it was three times higher than if both were normal. The reported cut-off values for cirrhosis ranged from 12.1 kPa, if AST <38.7 IU/L and bilirubin <9 μmol/L, to 25.9 kPa, if AST >75 IU/L and bilirubin >16 μmol/L. Inflammation-adjusted cut-off values had been previously proposed regarding AST but not bilirubin [49].

The LS thresholds for cACLD recommended by Baveno VI were challenged by a >5000-patient multi-aetiology meta-analysis with 946 ALD participants that proposes dual cut-offs of <8 kPa to exclude cALCD (93% sensitivity) and >12 kPa for its diagnosis (92% specificity) [52]. Following these results, recently published EASL guidelines propose a general dual cut-off of 8–10 kPa to exclude and 12–15 kPa to diagnose advanced fibrosis in the absence of inflammation [11].

Regarding CSPH, TE performed better than biomarkers in a study on 88 compensated alcoholic cirrhosis patients, with similar accuracy to LSPS [31]. Importantly, none of the NITs was reliable in predicting high-risk varices and LS/LSPS were not independently associated with mortality, being outperformed by Lok index and FIB-4. Nevertheless, in an analysis of 462 compensated ALD patients, TE had de highest accuracy among NITs in predicting liver-related events, with a 28-fold increased risk for LS >15 kPa, when compared with <10 kPa [34]. Notably, 21% of patients with LS between 10 and 15 kPa developed liver-related events, which might reflect a faster disease progression in ALD. The results regarding mortality were less accurate. Despite being the most validated NIT for the diagnosis of severe fibrosis/cirrhosis, further validation is needed to advice TE use for prognostic definition in ALD.

Shear Wave Elastography

Other LS measurement techniques include point shear wave elastography (pSWE) [53], also known as acoustic radiation force impulse imaging (ARFI), and 2D-shear wave elastography (2D-SWE) [54]. They are implementable on commercially available ultrasonography devices and enable the selection of the region of interest under B-mode echography visualization. The measurements are expressed in m/s or kPa. Like TE, these techniques are influenced by food intake [55] and inflammation [56]. However, they perform better in the presence of ascites and obesity and have reported failure rates of around 2–4%, significantly lower than TE [57–60].

The accuracy of pSWE/ARFI and 2D-SWE was addressed in large meta-analyses with 3951 and 1134 participants, respectively, concluding that both had good to excellent yield in detecting advanced fibrosis and cirrhosis [59, 61]. The comparison between pSWE/ARFI and TE performances has also been systematically analyzed, in a 1163-patients study, that did not report significant differences [57]. A prospective 349-participant study comparing 2D-SWE, pSWE/ARFI and TE

concluded that 2D-SWE was superior to TE in identifying severe fibrosis but not cirrhosis, in which all three methods were similar [62]. There was a tendency towards 2D-SWE being better predicting milder stages of fibrosis, a finding that had been previously reported [63]. In the majority of these studies, ALD was not frequently represented nor separately analyzed.

Regarding specifically ALD, a 99-participant study found pSWE/ARFI to be accurate in identifying severe fibrosis/cirrhosis in the presence of normal alanine aminotransferase (ALT), with a threshold of 1.41 m/s for cirrhosis [64]. This cut-off rose to 1.65 m/s when ALT was elevated, with much lower diagnostic yield. These results are in line with a 82-patient study on alcoholic detoxification that reports a cirrhosis cut-off of 1.94 m/s [65]. The fact that these patients had continuous alcohol consumption and elevated baseline AST may explain the higher cirrhosis threshold values. A more recent cohort of 251 participants reported higher diagnostic yields with cut-offs for severe fibrosis and cirrhosis of 1.47 and 1.66 m/s, respectively [66]. Contrary to previous evidence, pSWE/ARFI had excellent accuracy in detecting significant fibrosis. The authors included 138 patients with histologically diagnosed alcoholic steatohepatitis (ASH), in whom LS was higher, with a 2.52 m/s cut-off for cirrhosis with ASH. However, there was an important overlap between patients with cirrhosis without ASH and patients with ASH without cirrhosis, which highlights the limitations of SWE in identifying ASH when cirrhosis is not previously confirmed. Nevertheless, in known cirrhotic patients, SWE might help obviate liver biopsy as well as unnecessary corticosteroid treatment.

There are few studies evaluating 2D-SWE accuracy on ALD patients. A prospective study of 199 participants with alcohol abuse history concluded that 2D-SWE and TE had equally high accuracy in detecting severe fibrosis and cirrhosis, with high NPV in both secondary and primary care settings [60]. The authors propose dual thresholds with optimal cut-off values of <12.1 kPa to exclude and >27.3 kPa to rule in cirrhosis. Regarding CSPH diagnosis by elastography, a 328-participant meta-analysis, with more than half ALD patients, could not report reliable LS cut-offs, probably because the majority of patient had previously experienced cirrhosis decompensation [67]. The authors concluded, nevertheless, that a 2D-SWE LS cut-off of <14 kPa should be further studied in cACLD. Subgroup analysis of ALD and comparing abstinent versus non-abstinent patients did not reveal significant differences.

Despite suboptimal results in CSPH, 2D-SWE has revealed prognostic value in ALD. In a cohort of 462 patients with 4-year median follow-up, 2D-SWE predicted liver-related events with excellent accuracy, similar to TE [34]. In a cohort of 1827 chronic liver disease patients (including 414 with ALD), a 2D-SWE LS cut-off ≥20 kPa combined with model for end stage liver disease (MELD) score ≥10 was associated with 36.9% 2-year mortality and 61.8% 2-year decompensation rate, contrasting with rates of 1.1% and 3.5%, respectively, if none of the two criteria was fulfilled [68]. This model displayed similar results for cACLD, for patients with previous decompensations and for specific aetiologies, including ALD.

Overall, pSWE/ARFI and 2D-SWE show similar diagnostic and prognostic value as TE, with the above-mentioned practical advantages. Technical guidelines have been published, standardizing both techniques and recommending their use in viral hepatitis [69]. Further validation is needed to advice their practice in ALD.

Magnetic Resonance Elastography

Magnetic resonance elastography (MRE) uses a modified MR phase to image the propagation characteristics of shear waves, translating the results in kPa [70]. Contrary to other methods, MRE has the ability to examine the entire liver and is not limited by obesity and ascites. However, it is a time-consuming expensive method not routinely available in clinical practice. In a study with 90 ALD patients, MRE showed excellent accuracy in detecting severe fibrosis and cirrhosis with 3.31 and 4.0 kPa cut-offs, respectively, using TE as the reference [71]. AST values did not influence these results, however, since biopsy was not preformed, the influence of inflammation is uncertain. No further disease-specific studies were conducted.

Combined and Sequential Non-invasive Tests Strategies

Given the higher applicability and availability of biomarkers and the general higher diagnostic accuracy of LS measurement methods, it has been postulated that strategies combining these groups of NITs could be beneficial. A previously mention analysis regarding the minimum acceptable accuracy of NITs concluded that sequential NITs performed better than isolated ones, regardless of cirrhosis prevalence [17].

In low prevalence scenarios, like patients with harmful use of alcohol in primary care, the evidence aligns towards the use of a serum biomarker to rule out advanced fibrosis, with further testing needed to make a positive diagnosis. This strategy increases diagnostic yield and reduces unnecessary referrals to liver specialists [11]. Sequential NITs also proved to be cost-effective in ALD when severe fibrosis/cirrhosis prevalence is taken into account [72]. Sequential strategies have been proposed with high accuracy for ALD in low cirrhosis prevalence populations, including: ELF® followed by a LS measurement if ELF® >10.5 [72]; Forns index, ELF® if Forns >4.1 [15] and LS if ELF® >10.5 [15, 72]. In contrast, in secondary/tertiary settings with high cirrhosis prevalence, the sequential use of biomarkers followed by LS measurement is not superior to direct referral for a LS method and might result in increased false positive risks and higher health costs [15, 25, 30].

There is lack of evidence concerning the prognostic value of combining NITs. Combinations of MELD with LS measurements and with biomarkers have been proposed with interesting results in liver-events prediction, but inconsistent ones regarding mortality [31, 68].

Conclusions

Non-invasive identification of advanced stages of fibrosis is of crucial importance in ALD in order to define preventive actions against the occurrence of liver related events. Several NITs have been tested in ALD populations with high diagnostic yield. Serum biomarkers are valuable tools, mostly to exclude severe fibrosis in low cirrhosis prevalence populations. In case of positive results, the sequential use of a LS method is recommended, provided that alcohol consumption and biochemical evidence of inflammation are excluded or minimized. In high prevalence scenarios, LS techniques are preferable. TE is the most validated LS method, despite the increasing evidence for other SWE techniques with important practical advantages. Disease-specific threshold values for advanced fibrosis/cirrhosis have been proposed, with dual cut-offs providing optimal sensibility and specificity. In terms of prognosis assessment, both biomarkers and LS methods have displayed value, however, sufficient evidence for disease-specific recommendations is lacking.

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Chapter 40
Noninvasive Biomarker Screening and Alcohol-Related Liver Disease in the General Population

Ellen Lyngbeck Jensen and Maja Thiele

Abstract Alcohol-related liver disease is present in 4–9% of the population and occurs after years of excessive drinking. Liver cirrhosis develops after decades of heavy drinking, but the accumulation of liver fibrosis which accumulates into cirrhosis is asymptomatic and not detectable by either clinical acumen or the routine liver blood tests available to primary care. Consequently, 75% of patients are diagnosed when symptoms and decompensation occur. At this time, prognosis is poor, and the effect of alcohol rehabilitation diminished. Consequently, there is a need for case-finding efforts in primary care or the population. This could be in the form of population-based screening programs, or opportunistic testing when patients visit their general physician for other reasons. There are clear advantages of early disease detection of alcohol-related liver disease, most notably the opportunity to deliver effective interventions to treat harmful use of alcohol or alcohol dependence, thereby improving health and quality of life. Yet, there are also possible negative consequences of screening, for example in the form of overdiagnoses and anxiety/worry induced by fear of disease. Unfortunately, we still lack evidence for the benefits and harms of screening for alcohol-related liver disease in the population. In line with this, the biomarkers commonly used for screening for fibrosis in low prevalence populations such as primary care lack diagnostic accuracy. In the future, more accurate biomarkers such as patented blood-based tests or elastography may be available to screening programs, but this would require reduced costs, improved knowledge for their use by general practitioners, and wider availability.

Keywords Alcohol associated liver disease · Alcohol-related liver disease Cirrhosis · Fibroscan · Screening · Non-invasive tests · FIB-4 · AUD · Alcoholism

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The Burden of Alcohol-Related Liver Disease

In a global context, excessive use of alcohol has become an increasing problem, with 1.3 billion of the adult world population consuming levels of alcohol that poses a possible risk to their health (above the non-drinker equivalent) [1]. Europe has the highest alcohol consumption in the world [2]. More information is available in part I of the book. The liver is the most common organ to be affected by alcohol-related harm, with 335,000 annual deaths from alcohol-related cirrhosis, corresponding to one in eight of all deaths attributed to alcohol [3]. Depending on the amount and duration of alcohol consumption, 5–15% will develop severe liver fibrosis or cirrhosis, making alcohol-related liver disease (ALD) the most frequent cause of severe liver disease in Europe [4, 5].

ALD in the population includes steatosis, steatohepatitis, progressive liver fibrosis, and cirrhosis [6]. All the types of ALD suffer from increased all-cause, liver-related and cancer mortality [7, 8]. Patients with cirrhosis experience the highest rates of liver related complications and mortality, but even in patients with moderate fibrosis (Kleiner fibrosis stage F2, where F0 is no fibrosis and F4 is cirrhosis), one study found a 20% the rate of liver related events within 4 years [9]. Further, patients with ALD and concomitant metabolic comorbidity in the form of obesity, insulin resistance and dyslipidaemia exhibit two to three times the risk of progressive fibrosis and development of decompensation as those without [10, 11].

The age-standardised prevalence of alcohol dependence is 1.3% globally, ranging from <1% in most of Asia and 1.9% in North America, up to 4.2% in Eastern Europe [11]. Beyond dependence, far more are excessive drinkers: 4–9% of Europeans report weekly drinking above the recommended limits [2, 12–14]. Despite the large burden of disease and the availability of effective treatments in the form of behavioural and pharmaceutical alcohol rehabilitation, most cases of ALD are diagnosed at a late stage, for 75% of patients when decompensation occurs [15–17]. When ALD is discovered this late, the survival benefit of alcohol abstinence is substantially attenuated, quality of life is severely impaired, and only 12% of patients will still be employed or under eduction, compared to 59% 10 years before the diagnosis [18, 19].

Consequently, there is an urgent need to detect asymptomatic ALD patients with presence of moderate or severe fibrosis before transition to decompensated cirrhosis has occurred. Accurate case finding of these patients, for example through screening or referral programs, will allow for timely promotion of abstinence, treatment of comorbid risk factors, socioeconomic interventions, and monitoring for progression, which, in the end, may improve survival [20].

Arguments for Screening in the General Population

One of the major problems with current referral pathways for ALD is that they rely on the general practitioner's (GP) clinical acumen in combination with routine liver blood tests [21]. These methods are characterised by poor sensitivity and specificity

for detecting fibrosis and compensated cirrhosis [22]. As a result, less than one-third of referrals from primary to secondary care are on time, while 54% are futile referrals having no or mild fibrosis, and 17% are referred too late, with decompensated cirrhosis [23]. This pattern of wasteful use of health care resources, high rate of overdiagnoses, and high rate of missed diagnoses continue despite ample opportunities for case finding among patients with an alcohol use disorder. For example, 5% of patients with any contact to the healthcare system with problems related to alcohol, from intoxication to dependence, develops cirrhosis within 11–12 years [24]. Beyond hospital care, it is highly likely that patients at risk of progressive ALD even more frequently visit their GP. A UK survey found that men who died from alcohol-related causes, mostly cirrhosis, had seen their GP on average 24 times in their lifetime, 5 times for women. One in five had no record of ever having been advised to abstain from alcohol [25]. This emphasizes the need for (a) improved detection of alcohol use disorder in primary care and subsequent use of validated alcohol-rehabilitation treatments; (b) systematic detection of those with severe fibrosis, or at highest risk of progressing to liver-related outcomes, for people with a harmful use of alcohol in general population or in primary care practices, followed by referral to secondary care.

Some evidence suggests that unselected, broad population-level screening for liver fibrosis may not be practical or accurate due to many false positives and false negatives when using the currently available tests. An example is FIB-4, which is cheap, but has a low discriminative accuracy for detecting significant fibrosis (fibrosis-4 algorithm, consisting of age, platelet count, AST and ALT) [26]. However, more accurate tests such as transient elastography may be cost-effective both in the general population and in patients at risk of ALD [27, 28].

The main advantage of case finding on a population level is timely referral to alcohol rehabilitation. Today, less than 10% of people who are eligible for alcohol rehabilitation treatment receives such treatment [29]. Participation in an opportunistic screening program may lead to higher disease awareness among both patients and physician, potentially increasing delivery of brief interventions or referral to alcohol treatment. There are also some indications that the screening result in itself may be used as biofeedback and increase motivation for and adherence to alcohol treatment [30]. A UK pilot study showed that a higher proportion of screened patients with evidence of liver fibrosis by a simple algorithm (the Southhamptom traffic light score) had decreased AUDIT during follow up than the group with a negative screening test [31]. Of interest, the group with a negative screening test also decreased alcohol intake and AUDIT score. Improved delivery of effective alcohol interventions has a particular advantage for patients with severe ALD, where alcohol rehabilitation rapidly improves survival [32, 33].

Pitfalls of Screening for Alcohol-Related Liver Disease in the population

When considering implementing a screening program, the negative consequences of screening need to be investigated [34]. Negative consequences of screening include overdiagnoses, unnecessary invasive diagnostic procedures, and psychological stress or anxiety for participants, the latter especially in case of a positive test. Those aspects are not yet determined for ALD screening in the population.

Another pressing issue is the question of effects. All studies on screening programs so far have case finding of advanced fibrosis or compensated cirrhosis as their primary outcome [12, 35]. None have shown improved survival or reduced liver-related morbidity in the screened group, compared to a non-screened group. These hard outcomes are best evaluated in a randomized controlled trial, as known from cancer screening [36]. This has not been done yet. Unfortunately, progression time from severe fibrosis to clinical events or death is longer than for premalignant lesions or small cancers, why a randomized screening trial would likely need at least 5 years of follow up to show any effect on clinical outcomes. Similarly, in contrast to cancer, it is difficult to establish what the screening target should be, as a form of "premalignant" condition: advanced fibrosis? Significant fibrosis? Compensated cirrhosis? For this to be evaluated, we need more detailed information on the natural history of ALD in an unselected background population, rate of competing risks in the form of non-liver related death, and progression rates from each fibrosis stage according to age, gender, level of drinking and comorbidities.

From a health economic perspective, a screening program can be very costly, and the costs and benefits of screening must therefore be thoroughly examined. The few health economic studies conducted so far suggest that screening for ALD will be highly cost-effective [27, 28, 37].

Furthermore, unselected screening or opportunistic screening for ALD will likely lead to more health inequality, as it is known that people of low socioeconomic status more often opt out of screening [38]. As ALD already suffer from substantial health inequality, a screening program may not find those at highest need for investigations and treatment [18]. A further worry regarding adherence to screening is the misconceptions regarding the ability and availability of interventions to 'do something' about ALD, not just among physicians, but also among ALD patients [39]. Stigma towards alcohol and ALD likely contributes to the pessimistic expectations to interventions [40, 41].

Finally, screening programs depend on the availability of non-invasive tests with very high negative predictive values (NPV) to ensure low false negative rates, but without losing diagnostic ability to rule in, to avoid a high number of false positives. In a low prevalence population with for example 10% cases, flipping a coin will have a NPV of 90%. Consequently, tests with NPV's of 90% should not be celebrated, as high NPV is an overestimation of a test's discriminative ability. Rather, the NPV should be appraised in the context of the disease prevalence. Opposite to NPV, the positive predictive value (PPV) will be low in low prevalence populations. If disease prevalence is 10%, the PPV of a test with a 95% sensitivity and specificity

will still only be 68%. Consequently, a confirmatory diagnostic test should be planned in all screening positive cases.

Who to Screen for Alcohol-Related Liver Disease?

As in other screening programs, considerations about the target group should be discussed. In at-risk populations with a self-reported history of long-term excessive drinking, the prevalence of ALD ranges between 2% and 5%, but there are big differences depending on drinking history, age, gender, and metabolic or genetic risk. Selecting participants based on such predictors increases pre-test risk of disease, thereby increasing PPV of a screening test.

For drinking history, a cut-off for average alcohol intake of 30 g/day for a minimum of 1–5 years will likely suffice as decision threshold for conducting a non-invasive liver screening test [42, 43]. Women are more susceptible to alcohol-induced liver damage, but there are more heavy drinkers among men, so a selection based on gender is likely not effective. Most patients with ALD cirrhosis are diagnosed at age 50–60 years. Consequently, screening could be initiated at age 30–40 years. In line with this, a Swedish population study found that excess drinking in adolescent adults aged 18–20 years investigated at conscription to military service predicted later occurrence of cirrhosis [44]. The cumulative incidence of cirrhosis in high-risk drinkers departed from controls 5 years after inclusion, but only after 15 years had 1% developed cirrhosis. As mentioned above, concomitant metabolic and genetic comorbidity also increases the risk of advanced fibrosis in ALD patients and could be considered as means to increase disease prevalence prior to testing [10].

An opportunistic screening program where patients with a history of harmful drinking from age 30 or 40 are offered non-invasive testing for liver fibrosis when they visit their general practice would fit in the primary health care system. However, such set up require accurate, effective non-invasive tools to either detect significant or advanced fibrosis, or risk stratify according to prognosis for liver-related events.

Non-invasive Biomarkers for Screening in the Population

Liver biopsy is the gold standard for staging fibrosis in ALD [21]. However, this method is not suited for a screening program because it is invasive, require costly specialist healthcare resources, is time consuming, and far from point of care. Fortunately, several non-invasive methods to assess liver fibrosis have been validated for use in ALD [21, 45]. These are based on either a physical methodology with liver stiffness measurements (LSM) by elastography, or a biological methodology using blood-based biomarkers alone or combined in algorithms [46]. The commonly used non-invasive biomarkers have different advantages and disadvantages for use in primary care or the population, most notable differences in availability, cost, and existence of cut-offs which are validated for ALD (Table 40.1).

Table 40.1 Promising biomarkers for risk stratification and fibrosis staging in early alcohol-related liver disease

Test	Description	Proposed cut-off	Costs	Availability in primary care	Disadvantages
Blood-based tests: Algorithms from routine liver blood tests					
FIB-4	Simple algorithm from age, AST, ALT, platelet count	<1.30 to rule out and ≥2.67 to rule in advanced fibrosis.	$	Good; can be calculated automatically in electronic laboratory systems.	Many false positives. Not accurate for diagnosis. False negatives if age < 35, false positives if age > 65.
APRI	Simple algorithm from AST and platelet count	<0.5 to rule out and ≥1.50 to rule in advanced fibrosis.	$	Good; can be calculated automatically.	Less accurate as FIB-4 in head-to-head comparisons, especially in ALD.
Forn's index	Regression algorithm from age, GGT, cholesterol and platelet count	<4.2 to rule out and ≥6.9 to rule in advanced fibrosis.	$	Good; can be calculated automatically.	Few studies in ALD and in low prevalence populations. Cut-offs not well-validated
NAFLD fibrosis score	Regression algorithm from age, BMI, presence of diabetes, AST, ALT, albumin and platelet count	<−1.455 to rule out and ≥0.676 to rule in advanced fibrosis.	$	Good	Targets NAFLD, not ALD. Need for dedicated calculators to include BMI and diabetes.
Blood-based tests: biomarkers which directly reflect fibrosis					
FibroTest	Patented algorithm of age, gender, bilirubin, GGT, a2-macroglobulin, apolipoprotein A1, and haptoglobin	<0.48 to rule out and ≥0.58 to rule in advanced fibrosis.	$$	Moderate; used in France and marketed in US as FibroSure (company Biopredictive)	Few studies in ALD. One study in low prevalence population. Cut-offs not well-validated
FibroMeter	Patented algorithm of age, body weight, glucose, AST, ALT, ferritin and platelet count	0.254< to rule out and 0.585≥ to rule in advanced fibrosis [47]	$$	Moderate, commercially available (company Echosens)	No studies for screening in low prevalence populations. Cut-offs from tertiary healthcare.

Table 40.1 (continued)

Test	Description	Proposed cut-off	Costs	Availability in primary care	Disadvantages
HepaScore	Algorithm of age, gender, bilirubin, GGT, a2-macroglobulin, and HA	No widely agreed cut-offs	$$	Used in Australia	No studies for screening in low prevalence populations
ELF test	Patented algorithm of PIIINP, HA, TIMP1	No accepted cut-off to rule out; ≥9.8–10.5 to rule in advanced fibrosis.	$$$	Moderate, used in UK and authorized by FDA for US (company Siemens Healthcare)	No studies for screening in low prevalence populations
ProC3	Propeptide of type 3 collagen	No widely agreed cut-offs	$$$	Low, only recently made available on a Roche Cobas (company Nordic Bioscience)	Few studies in ALD. No studies for screening in low prevalence populations
Elastography methods					
pSWE	Also known as Acoustic radiation force impulse, ARFI. Software addition to regular ultrasound.	No widely agreed cut-offs due to many manufacturers, but <1.3–1.7 m/s to rule out and ≥1.7–2.1 m/s to rule in.	$$$	Moderate, available in most medium- and high-end ultrasound equipment	Few studies in ALD and in low prevalence populations. Cut-offs not well-validated
TE	Transient elastography (Fibroscan, Echosens)	<8 kPa to rule out, and ≥15 kPa to rule in advanced fibrosis	$$$$	Moderate, available in some nurse-led FibroScan clinics	False positives in case of liver inflammation (disadvantage of all elastography techniques)
2D-SWE	Software addition to some high-end ultraound equipment	No widely agreed cut-offs to rule in and rule out	$$$$	Low	Few studies in ALD. No studies for screening in low prevalence populations. Cut-offs not well-validated

(continued)

Table 40.1 (continued)

Test	Description	Proposed cut-off	Costs	Availability in primary care	Disadvantages
MRE	Magnetic resonance	No widely agreed cut-offs to rule in and rule out	$$$$$	Low	Few studies in ALD. No studies for screening in low prevalence populations. Cut-offs not well-validated

Based on publications: [45, 46, 48, 49]
2D-SWE 2-dimensional shear wave elastography, *ALT* alanine transaminase, *APRI* AST-platelet ratio index, *AST* aspartate transaminase, *ELF* enhanced liver fibrosis test, *GGT* gamma-glutamyl transpeptidase, *HA* hyaluronic acid, *PIIINP* propeptide of collagen type 3 N-terminal, *pSWE* point shear-wave elastography, *TE* transient elastography, *TIMP1* tissue inhibitor om metalloproteinase-1

Transient elastography (TE) has emerged as the referral standard for non-invasive markers due to its validation for ALD in several diagnostic studies [9, 45, 50, 51]. More details are provided in the respective chapter within this book part. TE has also been investigated for describing fibrosis prevalence in large, low-prevalence populations from mostly Europe, but also North America and Asia [27, 35, 52–56]. These studies often use 8 kPa as the screening cut-off, because, with a sensitivity of 93%, it rules out advanced fibrosis with very high accuracy [57]. The advantages of TE consist of the technique being ultrasound-based which makes it fully non-invasive, and point-of-care, providing the answer immediately. The disadvantage of TE is that the equipment is expensive, both for purchase costs and for an annual calibration fee. However, the FibroScan equipment for TE requires less experience and not dedicated ultrasound knowledge is necessary as compared to 2D-SWE. Finally, elastography may suffer from larger variability than the blood-based tests, especially for patients with elevated LSM [52, 58].

The current recommendation by European guidelines is to initiate case finding in people at risk of liver disease by a history of excess drinking [21]. Next, usage of a cheap algorithm calculated from routinely available liver blood tests, preferably the FIB-4, which seems to perform best among the const-moderate, non-patented blood tests. In case of a FIB-4 above 1.30, the physician may consider referring the patient to LSM by TE. However, this approach was recently criticised, since one-third of patients with FIB-4 $\geq$ 1.30 have liver stiffness below 8 kPa, leading to over-referrals, and FIB-4 was below 1.30 in up to half of patients with highly elevated LSM indicative of severe fibrosis, leading to false negatives [26]. Consequently, a future approach may be a more expensive, but more accurate blood test to select patients

from primary care to referral for LSM [59]. An alternative future approach is to use one of the cheap algorithms first, for example FIB-4, followed by a confirmatory more expensive blood test in case of elevated FIB-4, and only refer those where both blood tests are elevated for LSM [60].

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Chapter 41
Evidence for Red Blood Cell-Derived Aspartate Aminotransferase in Heavy Drinkers

Sebastian Mueller

Abstract Serum transaminase aspartate transaminases (AST/GOT) and alanine transaminase (ALT/GPT) are frequently elevated in alcohol-related liver disease (ALD) and show a typical profile in heavy drinkers with AST being higher as ALT. So far, this has been attributed to the hepatic mitochondrial isoform of AST. Based on recent novel findings in humans and animals, this chapter, however, demonstrates that AST is rather derived from hemolyzed red blood cells (RBC). First, prospective mortality data in heavy drinkers identify hemolytic anemia as novel and most important long-term predictor of survival. Second, in difference to patients with viral hepatitis, AST levels are elevated constantly in ALD throughout all fibrosis stages. Of note, AST-derived from human RBCs not only matches basal serum AST levels in healthy volunteers but also their daily RBC turnover due to RBC recycling. In addition, and third, in a mouse model of mild hemolysis using phenylhydrazine, AST levels are significantly and transiently elevated and follow the kinetics of hematocrit and LDH. Almost no changes of ALT are observed in this model. Finally, during alcohol detoxification of heavy drinkers, AST levels resolve much faster than ALT and correspond to other hemolytic markers. In conclusion, these data suggest that elevated AST and de Ritis ratio in drinkers are likely due to hemolysis and enhanced RBC turnover. These observations should be further confirmed in in vitro erythrophagocytosis studies. The potential ingestions of senescent RBCs by hepatocytes (efferocytosis) also requires further attention as well as the possible contribution of RBC-AST to hepatocyte transamination.

Keywords AST · ALT · Liver transaminases · Red blood cell · Hemolysis · De Ritis ratio

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Introduction to Serum Transaminases

Elevation of transaminases are commonly seen in patients with alcohol-related liver disease (ALD) [1]. Two major transaminases are known: Aspartate aminotransferase (AST), formerly called glutamic oxaloacetic transaminase (GOT, EC 2.6.1.1) and alanine aminotransferase (ALT), formerly known as glutamic-pyruvic transaminase (GPT, EC 2.6.1.2). Both names are still widely used throughout the world. Transaminases or aminotransferases are enzymes that catalyze a transamination reaction between an amino acid and an α-keto acid (Fig. A.48). In the following, the biochemistry of transaminases and their medical use for diagnosis is briefly described. Serum transaminases are elevated in alcohol-related liver disease (ALD) and show a typical profile in heavy drinkers with AST being higher as ALT. So far, this has been attributed to the hepatic mitochondrial isoform of AST.

Data presented in this chapter suggest that elevated AST in drinkers is likely due to hemolysis and enhanced RBC turnover which has important clinical and pathophysiological implications.

Biochemistry of Transaminases

AST and ALT were discovered in 1954 [2]. The transaminases are important for the production of various amino acids. Determination of transaminases in the blood is important for diagnosing many diseases. Transaminases require the coenzyme pyridoxal phosphate (PLP, Vitamin B6), which is converted into pyridoxamine in the first half-reaction (see also Fig. A.48). In this process, the cofactor shuttles between PLP and the pyridoxamine phosphate (PMP) form [3]. Enzyme-bound pyridoxamine in turn reacts with pyruvate, oxaloacetate, or alpha-ketoglutarate, giving alanine, aspartic acid, or glutamic acid, respectively. Many transamination reactions occur in tissues, catalyzed by transaminases specific for a particular amino/keto acid pair. The amino group transfer catalyzed by this enzyme is crucial in both amino acid degradation and biosynthesis. In amino acid degradation, following the conversion of α-ketoglutarate to glutamate, glutamate subsequently undergoes oxidative deamination to form ammonium ions, which are excreted as urea. In the reverse reaction, aspartate may be synthesized from oxaloacetate, which is a key intermediate in the citric acid cycle. Animals metabolize proteins to amino acids, at the expense of muscle tissue, when blood sugar is low. The preference of liver transaminases for oxaloacetate or alpha-ketoglutarate plays a key role in guiding nitrogen from amino acid metabolism to aspartate and glutamate for conversion to urea for excretion of nitrogen. In a similar manner, in muscles, the use of pyruvate for transamination gives alanine, which is carried by the bloodstream to the liver (the overall reaction being termed **glucose-alanine cycle**). Here other transaminases regenerate pyruvate, which provides a valuable precursor for gluconeogenesis. This alanine cycle is analogous to the **Cori cycle**, which allows anaerobic metabolism by muscles [4].

Transaminases in ALD and De Ritis Ratio

The **AST/ALT ratio** or **De Ritis ratio** is the ratio between the concentrations of AST to ALT in the blood of humans or animals [5]. It is useful in medical diagnosis for elevated transaminases to differentiate between causes of liver damage, or hepatotoxicity [6]. An AST to ALT ratio of 2:1 or greater is suggestive of alcohol-related liver disease, particularly in the setting of an elevated gamma-glutamyl transferase [7]. The AST to ALT ratio can also occasionally be elevated in patients with nonalcoholic steatohepatitis (NASH). In addition, patients with Wilson's disease or cirrhosis due to viral hepatitis may have an AST that is greater than the ALT, though the ratio typically is not greater than two. AST may be also elevated due to cardiac ischemia or muscle inflammation. For example, muscle inflammation due to dermatomyositis may cause AST > ALT. It is important to note that, besides clinical information, additional laboratory parameters such as creatine kinase or troponins will help to further dissect the origin of the transaminases. Intense exercise is also able to increase ALT to 50–200 U/L, and AST to 100–1000 U/L [8]. Most causes of liver cell injury are associated with a greater increase in ALT than AST.

Tissue Distribution of AST/GOT

Two isoenzymes of AST are present in a wide variety of eukaryotes. In humans: AST1/GOT1/cAST, the **cytosolic** isoenzyme derived mainly from red blood cells and heart. Already in the first report [2], the high abundance of AST in RBCs had been described. AST2/GOT2/mAST, the **mitochondrial** isoenzyme, is present predominantly in liver. These isoenzymes are thought to have evolved from a common ancestral AST via gene duplication, and they share a sequence homology of approximately 45% [9]. ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is considered to be a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma. The half-life of total AST in the circulation approximates 17 h and, on average, 87 h for mitochondrial AST. Aminotransferase clearance is carried out within the liver by sinusoidal cells [10].

Transaminases in Patients with ALD: Is AST Rather RBC Derived?

In contrast to common believe, AST in alcohol consumers seems to be most likely not derived from the liver but rather from red blood cells. The existence of AST in RBCs had already been described in the first publication and seems to have been

neglected over the years [2]. In most text books, this is not mentioned anymore and AST is usually related to the mitochondrial isoenzyme form. However, it has been always somehow contradictory, why, in the context of hepatotoxicity, AST should be higher based on mitochondrial localization. If mitochondria are damaged the hepatocyte will also die and, eventually be taking up through efferocytosis by neighbouring cells. Even most medical laboratories do not provide anymore specific information as to which isoform is actually measured in patient sera.

Differences between AST and ALT can be seen in the large Heidelberg cohort of heavy drinkers depending on fibrosis stage (see Table B.5). AST elevation is seen in ca. 60% of all patients, more in patients with advanced fibrosis stages (69% vs. 52%). In contrast, ALT is elevated in F0-2 in 42%, and in 37% in patients with F3-4. In line with these observations, the de Ritis ratio increases in this cohort from 1.3 for patients with F0-2 fibrosis to almost 2 for F3-4 fibrosis. Notably, AST has almost no prognostic value in heavy drinkers (see Table B.10 and Chap. 7).

In contrast to ALT, AST rather remains stably elevated throughout all fibrosis stages (Fig. A.79 and Chap. 37). Figure 41.1 shows AST and ALT for each fibrosis stage both for ALD and hepatitis C (HCV) as an example of portal liver disease [11]. As can be seen, in contrast to portal HCV, patients who consume alcohol show an almost constant elevation of AST throughout all fibrosis stages. Only in fibrosis stage 3 (F3), which is a rather transient period and normally a small fraction in study cohorts, it is elevated. In contrast, in patients with viral hepatitis, AST continuously increases with fibrosis stage. Thus, Fig. 41.1 demonstrates important differences between HCV and ALD. While AST is almost constantly elevated in heavy drinkers throughout all fibrosis stages, it continuously increases in HCV. Since the liver mass and function is considered to decrease at higher fibrosis stages, the rather constant levels of AST even in cirrhosis stage F4 may indirectly suggest that AST is not directly derived from hepatocytes.

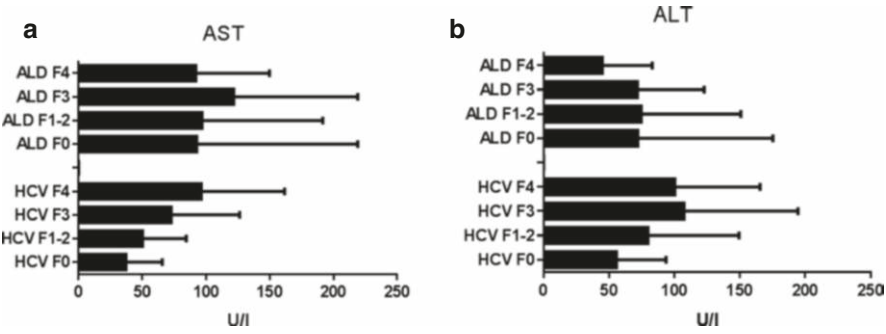

Fig. 41.1 Levels of (**a**) AST and (**b**) ALT in patients with alcohol-related liver disease (ALD) and viral hepatitis C (HCV) depending on fibrosis stage. Note, that AST levels remain rather constant throughout all fibrosis stages in ALD while they continuously increase in HCV. Data are from a multicenter study based on 1391 biopsy-proven HCV and 677 ALD samples [11]

Direct Assessment of AST in Red Blood Cells

The existence of AST in RBCs has already been described in first publications of AST and seems to have been neglected over the years [2]. In a next series of experiments, RBCs from healthy human donors were washed in 0.9% iso-osmotic saline solution three times, then lysed in water, and both AST and ALT activities were measured at the medical laboratory of the University of Heidelberg. As can be seen in Fig. 41.2, at a hematocrit of 1% which corresponds to the daily RBC turnover during physiological RBC recycling, AST levels were 50 U/L, at 5% hematocrit, 200 U/L were reached. In contrast, ALT levels were almost negligible. These data demonstrate that RBC contain significant amounts of AST. It is also interesting to note that AST from an amount of RBCs that are daily recycled due to physiological senescence, already corresponds to basal AST levels in the serum of healthy volunteers.

Tables B.27 and B.28 show Spearman correlations with AST from the 1200 patients of the Heidelberg prospective cohort of heavy drinkers. Only significant correlations are shown in descending order (according to the absolute correlation coefficient r). Accordingly, there are also strong indications that AST is mainly derived from RBCs. AST is highly correlated with hemolysis markers (LDH, indirect bilirubin, CD163 and Haptoglobin). On the other side, it is also clearly associated with liver damage such as liver stiffness (LS), ALT, M65 and M30, histological ballooning and markers of iron overload. These data support the notion discussed in Chap. 57 on iron and ALD, that RBC turnover by erythrophagocytosis and efferocytosis lead to liver damage under conditions of ethanol consumption.

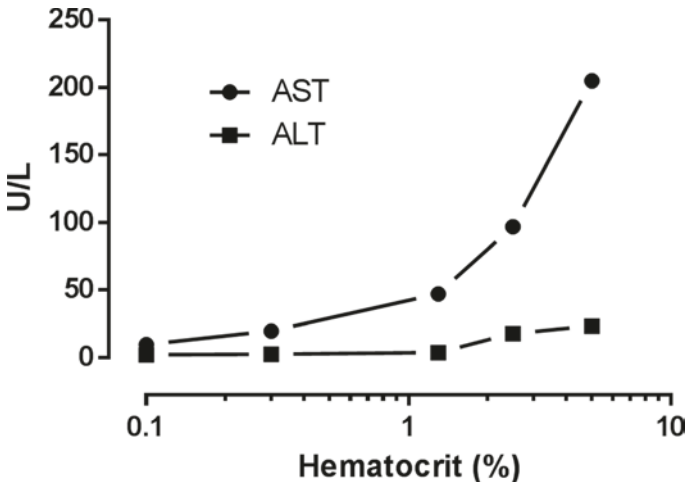

Fig. 41.2 Levels of AST and ALT in washed human red blood cells from healthy volunteer. Representative example. Note that RBCs contain significant AST while ALT is negligible. At a hematocrit of 1% which corresponds to physiological daily RBC turnover, AST levels are in the range of normal basal levels. This suggests that even normal AST levels are mostly derived from RBCs

Transaminase Levels in a Murine Model of Hemolysis

We next studied transaminase levels in mice exposed for 48 h to one injection of 60 mg of the hemolytic agent phenyl hydrazine (PHZ) per kg mouse weight. As can be seen in Fig. 41.3a, hematocrit fell from 48% to 35% in this mild hemolysis model while LDH transiently increased to 1300 U/L (Fig. 41.3b) peaking at ca. 12 h. In line with this (Fig. 41.3c), AST levels increased to almost 600 U/L while almost no change of ALT was observed. Figure 41.3d shows that the heme-binding protein haptoglobin was strongly reduced in response to hemolysis. Of note, in this mice model of hemolysis, mRNA of heme degrading hemoxygenase 1 (HO1) was primarily elevated in the spleen, and so was spleen stiffness. Only a marginal elevation of hepatic HO1 was observed. These data demonstrate that non-toxic hemolysis in mice translates primarily into AST elevation and almost no change in ALT. It should be noted that ethanol exposure of both mice and rats result in higher AST levels and even basal AST levels are already higher as compared to ALT in these animals (not shown).

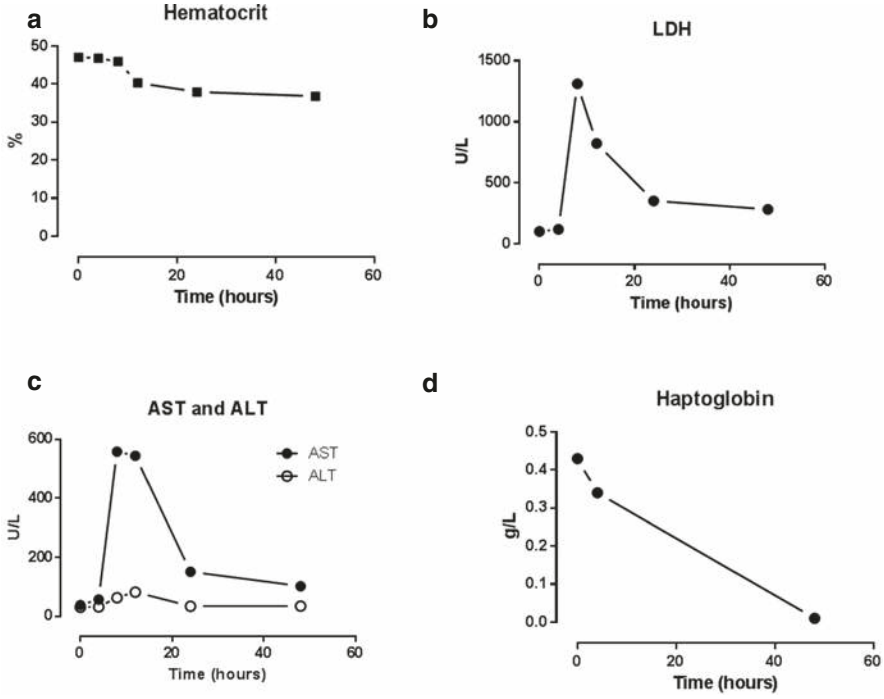

Fig. 41.3 Time course of (**a**) hematocrit (**b**) LDH (**c**) levels of AST and ALT and, (**d**) Haptoglobin in an experimental mouse model of mild hemolysis. Hemolysis was induced using 60 mg per kg phenylhydrazine. Serum was taken at 0, 4, 12, 24 and 48 h. Note that only AST levels increase drastically and transiently during hemolysis, going along with hematocrit, LDH and haptoglobin, but not ALT

Resolution Kinetics After Alcohol Detoxification: Correlation of AST with Hemolysis Parameters

We also analyzed in 19 heavy drinkers the kinetic of AST and various other blood parameters after 1 week of alcohol detoxification. More details about patient characteristics are described in Table B.1. Laboratory parameters were measured in each patient on day 1, 2, 7 and 28. As discussed in more detail in the chapter on bone marrow toxicity, hemoglobin (Fig. 41.4a) slightly decreased after detoxification but recovered after 28 days. During this time, serum LDH and ferritin deceased continuously (Fig. 41.4b, c). A significant difference was seen between AST and ALT. AST fell much faster, already significantly between day 1 and 2, while ALT showed a slower decreased during the whole time period. AST kinetics also corresponded better to kinetics of hemoglobin and LDH that already showed stabilization after 1 week. In contrast, ALT continued to significantly improved in the final weeks of detoxification. We finally, as shown in in Fig. 41.5 and Table B.24, analyzed the association of the erythrophagocytosis marker CD163 with various other laboratory markers. As can be seen in this table, CD163 is highly correlated with indirect bilirubin and shows a twice as high correlation with AST as compared to ALT.

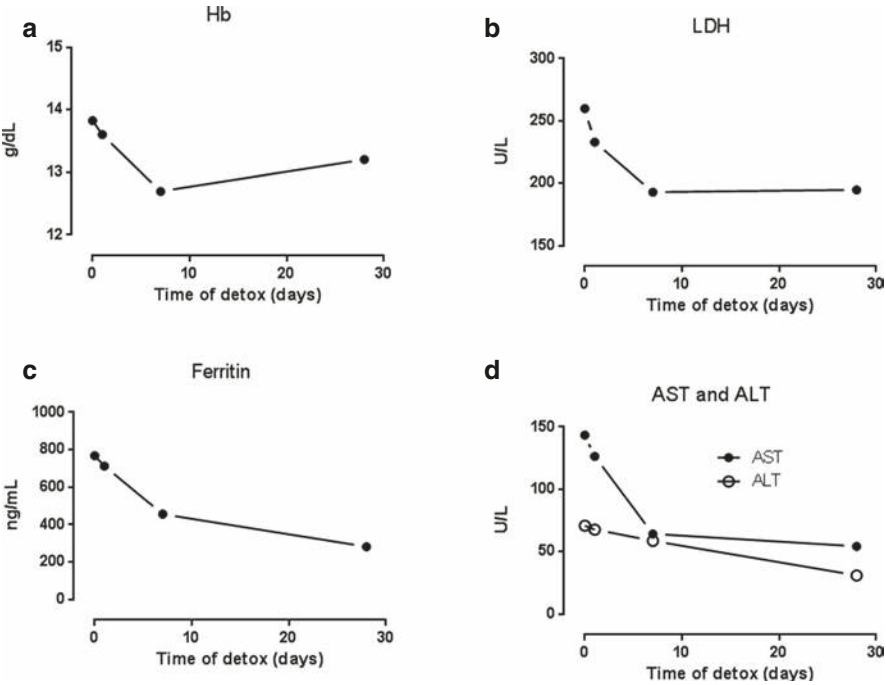

Fig. 41.4 Kinetics of (**a**) hemoglobin (Hb), (**b**) LDH, (**c**) ferritin, (**d**) AST and ALT during alcohol detoxification. Heavy drinkers were withdrawn from alcohol and laboratory parameters were obtained at day 1, 2, 7 and 28 after detoxification. Note that AST levels match the time course of other parameters while ALT shows a very different kinetics. Lab tests are from 19 heavy drinkers

positive Spearman Rho	CD163 r	p	negative Spearman Rho	CD163 r	p
Bile acids (µmol/L)	0.757	3.4E-07	APO A1 after detox (mg/dL)	**-0.772**	5.9E-07
Liver stiffness (kPa)	0.670	2.5E-33	APO A1 (mg/dL)	**-0.639**	1.6E-13
Reticulocytes after detox (°/°°)	0.647	8.3E-02	Albumin (g/dL)	-0.497	3.4E-12
Bilirubin indirect (mg/dL)	0.626	2.5E-07	Transferrin (g/L)	-0.455	3.8E-11
Maddrey	0.580	7.9E-23	Hemoglobin (g/dL)	-0.254	5.6E-05
Bilirubin total (mg/dL)	0.562	8.2E-22	Hemopexin (mg/mL)	-0.236	4.0E-02
M30 (U/L)	0.547	1.8E-20	Serum iron (ug/dL)	-0.067	3.0E-01
AST/GOT (U/L)	0.533	1.5E-19			
Reticulocytes (°/°°)	0.451	1.2E-01			
ERFE (ng/mL)	0.436	1.0E-04			
MCV (fL)	0.345	9.1E-08			
CRP (mg/L)	0.323	2.3E-07			
Ferritin (ng/mL)	0.289	4.2E-06			
GPT (U/L)	0.255	5.1E-05			

Fig. 41.5 Correlation analysis (Spearman Rho) of the hemolysis marker CD163 with other laboratory parameters. Data are from 304 heavy drinkers. Left panel shows positive correlations, right shows negative correlations. Parameters are sorted according to P value in descending order. Note that CD163 is correlated highly with hemolysis markers (e.g., indirect bilirubin) and AST. The association with ALT is much weaker. The relation between hemolysis and liver damage, namely under ethanol exposure, needs to be studied further

Conclusions and Future Directions

As is discussed and shown in this chapter, RBCs have been described already initially as important source of serum AST. In addition, recent prospective mortality data in heavy drinkers identify hemolytic anemia as important long-term predictor of survival (Chap. 7). Several human and animal data are presented here that suggest that AST is also mainly derived from RBCs in heavy drinkers. Thus, as compared to patients with viral hepatitis, AST levels are elevated constantly throughout all fibrosis stages in patients with ALD. Of note, the AST-derived from 1% washed human RBCs correspond to basal AST levels in healthy volunteers thus matching daily RBC turnover due to RBC recycling. In addition, in a mouse model of mild hemolysis caused by phenyl hydrazine, AST levels are significantly elevated matching the kinetics of hematocrit and LDH while almost no changes of ALT are observed. Finally, during alcohol detoxification of heavy drinkers, AST levels also match the course of hematocrit and LDH, in contrast to ALT. In conclusion, these data suggest that elevated AST and De Ritis ratio in drinkers is likely due to hemolysis and enhanced RBC turnover rather that of mitochondrial origin from hepatocytes. To further confirm these observations, direct specific assessment of erythrocyte AST would be helpful. Moreover, the course of AST should be studied during erythrophagocytosis. It can also not be ruled out that some erythrocyte AST is taken up by hepatocytes directly during so-called efferocytosis (see also Chap. 57). It also remains to be solved how enhanced RBC turnover translates into liver damage during ethanol consumption. Finally, like LDH during hemolysis, it remains to be studied whether AST serves additional purposes after the release into the serum.

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Chapter 42
Fibrosis Screening of Alcohol-Related Liver Disease Based on Elastography

Sebastian Mueller and Ioan Sporea

Abstract In alcohol-related liver disease (ALD), transient elastography (TE) to measure liver stiffness (LS) has been demonstrated to have an excellent performance to detect advanced fibrosis and cirrhosis. It correlates well with histological fibrosis stages with a $r > 0.7$ and AUROCs for F3 and F4 fibrosis are typically higher than 0.9. Point and 2D- shear wave elastography (SWE) combine elastography with conventional ultrasound imaging and is also increasingly used in clinical practice for the assessment of LS in ALD. However, the number of published studies is still limited. The initial confusion and controversial debates about varying elastographic cut-off values for fibrosis stages F0–4 in ALD patients is mainly due to inflammation as important confounder of elevated LS. Several studies could show that resolution of liver inflammation due to alcohol withdrawal leads to ca. 20% decrease of LS, within 1 week of alcohol detoxification. Long-term abstinence of more than 5 years seems to further improve LS suggesting partial reversibility of fibrosis. So far, levels of liver transaminases (especially levels of GOT/AST) are providing the best estimate whether LS overestimates histological fibrosis stages due to inflammation. For these reasons, a more accurate fibrosis assessment is obtained either by 1–4 week of alcohol withdrawal and re-measurement of LS or so-called AST-adapted cut-off values that allow for instant fibrosis staging. Moreover, first long-term follow-up data indicate that LS seems to be the best univariate predictor of long-term survival in heavy drinkers. Taken together, liver elastography has drastically improved the screening, early diagnosis and follow-up of fibrosis in patients with ALD. It is expected that the future broader availability of elastographic methods to ALD patients will not only significantly improve the management of the disease, but also help us to finally obtain true prevalence data of this popular, but still underestimated

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disease. There are also preliminary indications that the non-invasive and interactive setting of LS assessment is supporting alcohol withdrawal.

Keywords Alcohol-related liver disease · Fibrosis · Steatosis · Non-invasive tests · Shear wave elastography · Transient Elastography · pSWE · 2D-SWE · Biomarker · Liver stiffness · GOT · AST · Transaminase levels · Inflammation · Lobular inflammation · Pericellular fibrosis · Ultrasound

Introduction

In alcohol-related liver disease (ALD), transient elastography (TE) to measure liver stiffness (LS) has been demonstrated to have an excellent performance to detect advanced fibrosis and cirrhosis. It correlates well with histological fibrosis stages with a $r > 0.7$ and AUROCs for F3 and F4 fibrosis are typically higher than 0.9. Point and 2D-shear wave elastography (SWE) combine elastography with conventional ultrasound imaging and is also increasingly used in clinical practice for the assessment of LS in ALD. However, the number of published studies is still limited. The initial confusion and controversial debates about varying elastographic cut-off values for fibrosis stages F0–4 in ALD patients is mainly due to inflammation as important confounder of elevated LS. Several studies could show that resolution of liver inflammation due to alcohol withdrawal leads to ca. 20% decrease of LS, within 1 week of alcohol detoxification. Long-term abstinence of more than 5 years seems to further improve LS suggesting partial reversibility of fibrosis. So far, levels of liver transaminases (especially levels of GOT/AST) are providing the best estimate whether LS overestimates histological fibrosis stages due to inflammation. For these reasons, a more accurate fibrosis assessment is obtained either by 1–4 week of alcohol withdrawal and re-measurement of LS or so-called AST-adapted cut-off values that allow for instant fibrosis staging. Moreover, first long-term follow-up data indicate that LS seems to be the best univariate predictor of long-term survival in heavy drinkers. Taken together, liver elastography has drastically improved the screening, early diagnosis and follow-up of fibrosis in patients with ALD. It is expected that the future broader availability of elastographic methods to ALD patients will not only significantly improve the management of the disease, but also help us to finally obtain true prevalence data of this popular, but still underestimated disease. There are also preliminary indications that the non-invasive and interactive setting of LS assessment is supporting alcohol withdrawal. Additional schemes and data on elastography/liver stiffness can be found in Figs. A.80, A.81, A.82, A.83, A.84, A.85, A.89, A.90, A.92, and A.93.

Specific Diagnostic Challenges in Patients with ALD

ALD is the most frequent cause of severe liver disease in Europe and according to WHO, more than 40% of the liver deaths are attributed to alcohol consumption [1]. In addition, the number of liver transplantation for patients with ALD-related cirrhosis has increased over the past two decades, both in Europe and in the USA [2, 3]. Despite this high burden of ALD, it is unfortunate that most patients with ALD are diagnosed at the decompensation stage, normally presenting with ascites or jaundice. Moreover, a large proportion of newly diagnosed cirrhosis had recent consultations in primary care or emergency units [4], without any intervention. Alcohol-related liver disease includes a wide spectrum of lesions ranging from steatosis to steatohepatitis, progressive liver fibrosis, cirrhosis and its complications [1]. Although steatosis is almost constant in heavy drinkers, it is estimated that only 10–20% will eventually develop cirrhosis [5]. Since the presence of advanced fibrosis or cirrhosis in compensated patients is the main predictor of long-term survival, it is of clinical importance to diagnose those patients with advanced fibrosis before the decompensated stage, in order to promote abstinence and improve survival [6]. Liver diseases are in general hardly to detect and commonly show no or only mild symptoms. Even end-stage liver cirrhosis remains undetected in routine laboratory testing or ultrasound screening in ca. 40% [7]. The diagnosis of ALD is further complicated by three major challenges: (1) underreporting by patients (2) lack of good biomarkers for alcohol consumption and (3) a rather diverse clinical presentation. These are the reasons why ALD is routinely underestimated, both by physicians and health statistics [8, 9]. Therefore, the diagnosis of ALD normally must rely on a combination of clinical, laboratory, elastographic and imaging findings (Fig. 42.1), where ***liver elastography*** has gained and important novel role for early screening and follow-up [10]. For other non-invasive, e.g., serum tests see other chapters in this book part.

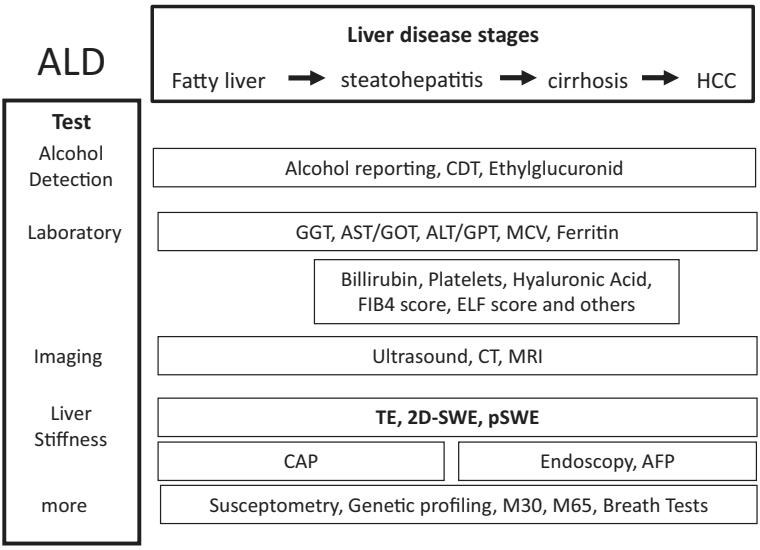

Fig. 42.1 Role of liver elastography within the context of assessing patients with ALD in different disease stages. Note that various clinical, laboratory and imaging tools are used to assess and diagnose ALD patients. Ultrasound-based liver elastography whether 1D-SWE such as TE or 2D- or pSWE elastographic techniques (highlighted in bold) have revolutionized the screening and management of the disease and its complications. There are also indications that elastography is a useful feedback tool for many patients in order to abstain from drinking or cut-down drinking levels

Brief Introduction to Liver Elastography

Ultrasound-based elastographic methods, namely Transient Elastography (TE), have been first introduced in 2003 [10]. Elastography has rapidly gained importance as it allowed within minutes and in a non-invasive manner the accurate screening for liver cirrhosis. Elastography has been so successful as it directly assesses one of the early and most sensitive consequences of liver cirrhosis, an increased stiffness of the liver. Due to the non-invasive nature of elastographic techniques, replicative measurements are possible allowing follow-up studies. First larger cohort studies in patients with ALD were published as of 2008 [11].

In this chapter, we are focusing on quantitative shear wave elastographies (SWE) that should not be mismatched with the qualitative strain elastography (SE) [10, 12, 13].

Transient Elastography was first introduced in 2003 [14] and, consequently, most published data on liver elastography have been based on TE [10]. TE can be also considered a 1D-SWE in comparison to 2D-SWE methods that are integrated or work together with conventional ultrasound imaging devices. Although TE does not allow a simultaneous ultrasound imaging of the liver, it is still the easiest to use system and the most standardized. Typical TE probes and the screen with an actual

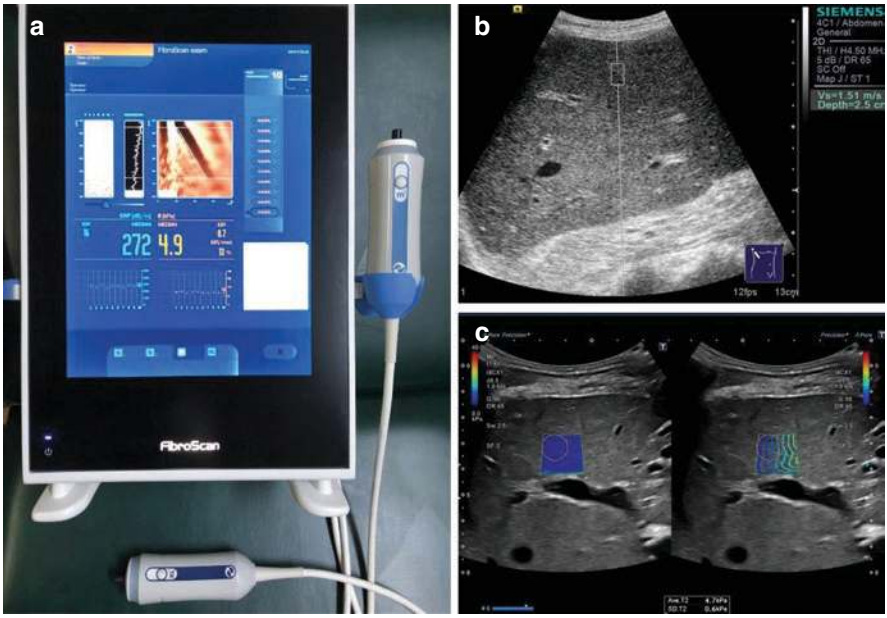

Fig. 42.2 Examples of 1D and 2D ultrasound-based liver elastographies to assess liver fibrosis. (**a**) TE (FibroScan, Echosense) was the first elastographic technic to assess liver stiffness. It does not require dedicated ultrasound knowledge and measurements are highly standardized and interobserver independent. Various probes are available (here shown M and XL probes) and a hepatic steatosis parameter called CAP (controlled attenuation parameter) are also run on the platform. Examples of two 2D-SWE platforms (**b**) pSWE (VTQ from Siemens) and (**c**) 2D-SWE with propagation map from Canon. These platforms have the advantage of being integrated in a conventional ultrasound imaging device but dedicated ultrasound knowledge is required. In addition, elastography can be measured in other tissues but standardization is still a challenge

measurement is shown in Fig. 42.2a. Briefly, in TE, a 50 Hz probe induces a shear wave within the liver whose propagation is quantified with a co-integrated 3.5 MHz ultrasound probe. The liver stiffness is directly and automatically calculated from the shear wave speed. In cases of measuring artifacts or low quality measurements, TE usually will not deliver any LS data. Together with the early well standardized conditions for LS measurements, these criteria have been responsible for the rather low inter- and intraobserver variability with TE. Recently, a liver fat-assessing parameter has been introduced on the TE platform which is called CAP (Controlled Attenuation Parameter). In contrast, 2D-SWE methods have been later introduced to the market and a provide a much larger freedom to the examiner. On the other side, this increased freedom requires experienced ultrasound knowledge and increases the risk of errors. In addition, elastographic platforms have drastically increased on the marked while standardization is significantly lacking behind which has created some confusion with regard to comparability between various techniques. 2D-SWE platforms now also provided fatty liver-assessing parameters. Two examples are shown in Fig. 42.2b, c. For more details, the reader may be referred to

recent publications or guidelines [10, 12]. Since most studies on ALD have been performed with TE, this chapter focuses mainly on this elastographic technique and uses TE-based cut-off values. An update of the first 2D-SWE data will be provided at the end of the chapter.

Elastography in Comparison to Other Alternative Methods to Assess Fibrosis

Although ALD follows the typical sequence of chronic liver diseases including alcoholic fatty liver, steatohepatitis, fibrosis and eventually cirrhosis (Fig. 42.3), the early recognition of severe steatohepatitis and alcoholic cirrhosis is most important since it will save lives, prevent complications and initiate follow-up programs [7]. Most important clinical end points are alcoholic liver cirrhosis and the rare and clinically defined severe alcoholic hepatitis (AH). AH should not be mismatched with the commonly and histologically detectable steatohepatitis (ASH) (Fig. 42.2). Since AH is very rare, typically presents clinically with pronounced jaundice (see also part X) and there are still no early predictors, screening for liver problems in heavy drinkers should primarily focus on the screening for fibrosis [7]. Although liver biopsy is still essential in some patients with liver diseases in establishing the definite diagnosis or in ruling out additional or other causes of the disease, elastographic techniques are the novel gold standard in assessing liver fibrosis. First, liver biopsy is invasive and can cause significant complications in up to 7% [15]. Complications can encompass mild (pain and small bleedings in 6%) or severe

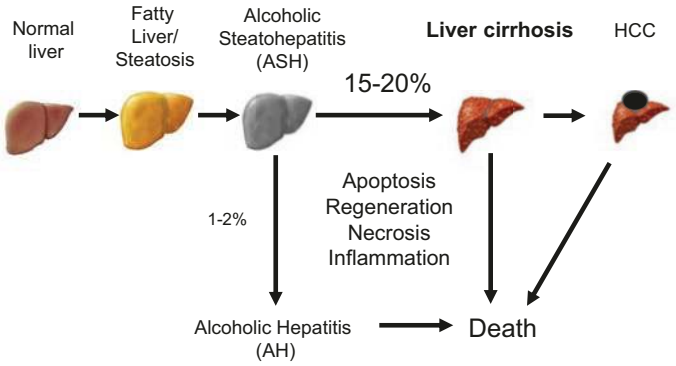

Fig. 42.3 Natural course of alcohol-related liver disease and importance to early detect liver. About 15–20% of heavy drinkers (>80 g alcohol per day) will develop cirrhosis over a period of 15–20 years. Most drinkers will eventually die from cirrhosis or its complications while only very few (<2%) will die due to the rare alcoholic hepatitis (AH). In the compensated state, liver cirrhosis will remain undetected for many years although patients are already at high risk to die from complications of cirrhosis such as bleeding, infections or primary liver cancer (HCC). Consequently, the early detection of those who are going to progress to cirrhosis is essential in order to early screen them for complications, to enroll them in screening programs, and, of course, to motivate them to abstain from alcohol

(0.1%) complications and rarely fatal perforations and bleedings [16, 17]. Moreover, due to the small needle, with regard to fibrosis assessment, the sampling error of a liver biopsy is larger and can reach up to 30% [18–22]. In addition, and the context of ALD, heavy drinkers are typically less likely to see doctors and to undergo invasive diagnostic procedures. With respect to fibrosis assessment, all imaging techniques must rely on so called sure morphological signs of cirrhosis, such as nodular aspects of the liver or recanalization of the umbilical vein, while splenomegaly or ascites are not specific. These imaging signs are only available in about half of ALD patients, with manifest cirrhosis [7]. Serum markers have been long thought to allow for easy fibrosis screening [7, 23]. In ALD, however, a previous study clearly showed superiority of TE with regard to various serum markers [11]. Moreover, this was achieved without sophisticated algorithms. Although this will not be the discussed in detail here, serum markers have important advantages (see also other chapters in this book part), when, e.g., looking for affordable screening tools to be applied worldwide, especially in third world countries.

Fibrosis Screening Using Elastography and Role of Inflammation

Early direct comparison with serum fibrosis markers showed a better performance of TE in patients with ALD [11] and AUROCs are typically >0.9 to detect F4 cirrhosis. The initial major biopsy-proven studies on LS in patients with ALD are listed in Table 42.1. Although an excellent performance could be shown in all

Table 42.1 List of initial biopsy proven studies using transient elastography (TE) to assess fibrosis stage

Reference	Number of patients (n)	Correlation	AUROC F4	Cut-off F4
Nahon et al. [24]	174	0.70, $p < 0.0001$	0.87	22.6
Nguyen-Khac et al. [11]	103	0.72, $p < 0.014$	0.92	19.5
Kim et al. [25]	45		0.97	25.8
Boursier et al. [26]	217	0.87, $p < 0.02$	0.91	17.3
Mueller et al. [27]	101	0.72, $p < 0.001$	0.92	11.5[a]
Janssens et al. [28]	49		0.86	21.1
Fernandez et al. [29]	15		0.93	18.0
Thiele et al. [30]	199		0.94	16.9
Voican et al. [31]	217	0.73, $p < 0.0001$	0.93	20.8

AUROCs and Cut-off values are shown for F4 fibrosis stage (cirrhosis). Note the large difference of cut-off values between different study cohorts. The differences are mainly due to the degree of inflammation and inflammation-associated LS elevation. Consequently, cut-off values should be corrected for inflammation to avoid overestimation of fibrosis stage. This can be done by alcohol-withdrawal and re-measurement of LS after resolution of inflammation or by using co called AST-adapted cut-off values. For more details see text

[a] Patients with AST > 100 U/L were excluded

Table 42.2 Changes of liver stiffness in response to alcohol withdrawal or relapse

Reference	Mean alcohol consumed per day	Patient number/ intervention	LS decrease before and after abstinence	Mean LS change (%)	Time of abstinence/ relapse
Mueller et al. [32]	190 g/day	50/detoxification	Mean LS 20.1–16.5 kPa	17% decrease	5.3 days
Gelsi et al. [34]		23/abstinence relapse		21.7% decrease	7 days
				20% decrease	9 weeks
				32% increase	9 weeks
Trabut et al. [33]	150 g/day	137/abstinence	Median LS 7.2–6.1 kPa		7 days
Mueller et al. [36]	98 g/day	Reduction of 42 g/day total alcohol consumption under Selincro	Mean LS 10.5–8.7 kPa	17%	8 weeks
			Median LS 6.0–5.4 kPa		
Mueller [37]	181 g/day	23/abstinence	Mean LS 20.5–10.5 kPa	48%	5.7 years
Mueller et al. [37]	194 g/day	23/continued drinking	Mean LS 14.8–18.1 kPa	22% increase	5.3 years

Note that absolute and relative LS decreases of 20 kPa or 80% can be observed including complete normalization. LS decrease also depends on initial LS and duration of abstinence. Note that long-term abstaining from alcohol seems to further improve liver stiffness even after 2 years of abstinence potentially suggesting fibrosis reversal in these patients

studies, cut-off values for F3 and F4 fibrosis differed quite significantly between the studies, ranging from 11.5–25.8 kPa. These differences initially caused some confusion, but it was rapidly learnt in an alcohol withdrawal study in 2010, that the cut-off values were strongly dependent on the degree of inflammation in a single patient or the total study cohort [32]. Thus, within 1 week of alcohol withdrawal, LS rapidly decreased independent of fibrosis stage [32]. This study could also show that LS decrease after alcohol withdrawal was inflammation-related and could be best estimated using initial transaminase levels [27]. The importance of inflammation for LS elevation in patients with ALD has been later confirmed by other studies including a meta-analysis [33–35].

Table 42.2 summarizes the data on alcohol withdrawal/relapse and LS. Typically, alcohol withdrawal in heavy drinkers (>80 g alcohol per day) leads to ca. 20% decrease of LS within 1 week of alcohol detoxification [38]. This algorithm has direct implication for fibrosis stages which change in ca. 27% after alcohol withdrawal. Even a 2 months reduction of alcohol consumption by 40% significantly

reduced LS by 17% [36]. In this study, LS decreased significantly in 62 patients (45.3%), and there was a reduction in the estimated stage of fibrosis in 32 (23.3%). In contrast, an increase of LS was observed in 11.7% [33]. The proportion of patients with a significant decrease of LS after alcohol withdrawal increased from 41.7% to 66.7% with the duration of abstinence, from 1 to 9 weeks [34]. There are preliminary observations that long-term abstinence is even more beneficial as LS decreased by 50% if abstained from alcohol for 5 years [39].

Figure 42.4a shows the now widely used algorithm to interpret LS in patients with ALD. When using 1D-SWE such as TE, a concomitant ultrasound is recommended to rule out liver congestion, obstructive cholestasis or nodular masses of the liver. Using 2D-SWE techniques, one platform allows both imaging analysis and elastography using one platform. In addition, as discussed here, the actual AST/GOT levels should be available taken within hours of the elastography. If AST levels are normal, cut-off values from Fig. 42.4a can be directly used to assess fibrosis stage with high accuracy. In case of elevated AST levels, patients should withdraw from alcohol for at least 1–2 weeks, and LS should be re-measured to avoid an overestimation of fibrosis stage due to inflammation.

To avoid the hassle of remeasuring ALD patients after alcohol withdrawal, so called AST-adapted cut-off values can be used [40]. Using the graph shown in Fig. 42.4b, fibrosis stage can be directly read using the measured LS and AST value. AST is indeed the best routine laboratory parameter which allows to estimated LS-elevation due to inflammation and AST performs best both in metabolic but also viral liver disease [40] although different graphs should be used for viral hepatitis. It is still not completely clear why AST has this special impact on LS and not, e.g., ALT? It remains to be addressed in future studies whether this may also be related to extrahepatic conditions, as AST also occurs in muscle cells and erythrocytes. The role of AST was also confirmed in a recent meta-analysis [35]. In this study, also additional levels of bilirubin were found to be useful although we have not been able to confirm this in our Heidelberg cohort. It remains to be confirmed whether bilirubin levels really add to the overall performance of LS, since ALD patients develop jaundice at end stage cirrhosis, where LS normally is higher than 30 kPa and the status of cirrhosis remains undoubted. In contrast, the so called clinical alcoholic hepatitis may develop high levels of bilirubin, in the absence of drastic LS elevation. The correction for AST levels is certainly relevant in daily clinical practice as, in ALD, AST levels are typically higher as compared to ALT and in ca. 70% of patients the AST/ALT ratio is higher than two [41]. On the other side, AST levels higher than 300 IU/L are rarely detected. In cirrhotic stages, transaminases may normalize, while AST levels may be continuously increased despite the absence of alcohol consumption [40].

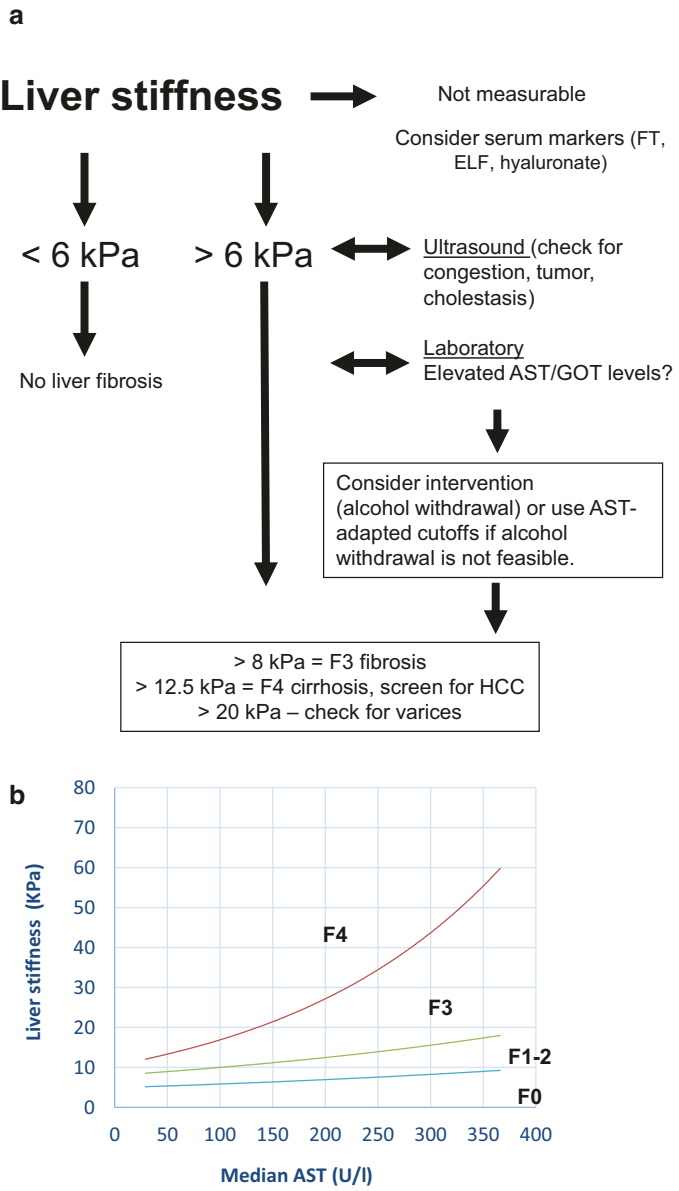

Fig. 42.4 Algorithm to assess fibrosis stage using elastography in patients with excessive alcohol consumption. (**a**) LS is measured and onside ultrasound is performed in order to exclude other potential confounders of elevated LS such as tumors, liver congestion or cholestasis. In addition, actual AST/GOT levels are required to estimate the role of inflammation. If AST levels are highly elevated, patients should abstain from alcohol for 1–2 weeks to allow resolution of inflammation. LS should then be measured in order to accurately apply the indicated cut-off values in the absence of inflammation. (**b**) In addition, so called GOT/AST-adapted cut-off values can be applied for ALD patients without the necessity to abstain from alcohol. Based on the actual LS and AST measurement, the fibrosis stage can be directly read from this graph. Note that other graphs should be used for, e.g., viral liver disease (see also [40])

ARFI Technology for the Evaluation of ALD Patients

ARFI (Acoustic Radiation Forces Impulse) technology is used for the evaluation of liver stiffness in point and 2D-SWE. The advantage of this technology is that this system is implemented inside an ultrasound machine. Consequently, standard ultrasound evaluation of the liver can be performed at the same time of ultrasound-based elastography. The probe produces the ultrasound beam that induce the lateral displacement of the tissue that is used for stiffness measurement. Depending on the platform, results are expressed in kPa and m/s. The ARFI technology is implemented in many ultrasound systems (Siemens, General Electric, Canon, Philips, Samsung, Mindray, Fujifilm, Esaote and others) and published papers about the value of the method in the assessment of liver stiffness are numerous. What are advantages of the ARFI systems? Obviously, it is space saving as no additional ultrasound machine is required, e.g., next to the TE device. As with TE, the measuring time of ARFI devices is less than 5 min. Usually, 5–10 measurements are performed and the criterium of IQR/median < 30 is used for valid measurements. pSWE and 2D-SWE can be performed in patients with ascites as compared to TE. Although ARFI technology is implemented in the high-prize machines of most companies, increasingly middle class ultrasound machines also provide elastographic modules (e.g., Philips Affiniti, Siemens Juniper or General Electric P10) and the technology may be both, pSWE or 2D-SWE.

Once dedicated ultrasound knowledge is available, the ARFI is straightforward and very simple. First, the liver is displayed by ultrasound and important additional information can be rapidly acquired such as tumor masses, signs of cirrhosis, ascites, biliary tree dilatation etc. To measre LS with an ARFI device, a box (region of interest—ROI) of ca. 5–10 mm is placed in an area at least 1–2 cm below the liver capsule (that must be avoided) and free of any larger vessels. The patient is asked to briefly stop the breath in a neutral position and then the button is push and one measurement is obtained. Usually 10 measurements are performed, although some studies point out that 5 measurements may be sufficient for valid LS measurement [42]. Like TE, the pSWE methods are simple and reproducible. For 2D-SWE some experience in field of ultrasound is necessary [43] to obtain the best liver image through the intercostal approach. A box of ca. 2–3 cm is placed at least 1–2 cm bellow the liver capsule avoiding larger vessels. The patient is then invited to stop breathing in neutral position and some measurements are recorded. After this, a region of interest (ROI) of ca. 1 cm is placed in every frame, in the area of the most homogenous image to obtain the final LS values. The advantage of 2D-SWE method is that it is colour-coded and numeric method and for many systems, a quality control of the acquisition exist. Unfortunately, due to a lack of standardization, machine-specific cut-off values are required for each different systems. Some years ago, the SRU (Society of Radiologist in Ultrasound) proposed the “Rule of 4” for different degrees of fibrosis, that are now widely used [44]. In this classification, which has been mostly based without ALD patients, but on patients with viral hepatitis or NAFLD, the following cut-off values can be used:

Table 42.3 Published cut-off values in pSWE and 2D-SWE studies in patients with ALD

Reference	Number of patients (*n*)	Elastography technique	Cut-off values for different fibrosis stage		
			F2	F3	F4
Kiani et al. [45]	69	pSWE	>1.63 m/s	>1.84 m/s	>1.94 m/s
Zhang et al. [46]	112	pSWE	>1.27 m/s	>1.40 m/s	>1.65 m/s
Cho et al. [47]	251	pSWE	>1.46 m/s	>1.47 m/s	>1.66 m/s
Thiele et al. [30]	199	2D-SWE	>10.2 kPa	–	>16.4 kPa

Note that platforms are not standardized and different units are provided. For pSWE, values are typically expressed in m/s

(A) <5 kPa means: normal liver
(B) 5–9 kPa: rule out cACLD (compensated advanced chronic liver disease)
(C) 9–13 kPa: suggestive for cACLD
(D) >13 kPa: rule in cACLD
(E) >17 kPa: suggestive for clinical significant portal hypertension (CSPH).

For ALD, the number of published papers with ARFI technology are rather limited and only one study has been published based on 2D-SWE. These studies are shown in Table 42.3. Point SWE are typically provided in m/s, but can converted into the Young's modulus using a simple formula [10]. 2D-SWE are normally given in kPa like TE and do also correlate well with TE [10].

Practical Example of Fibrosis Assessment by Elastography

After suspicion of ALD either by patients reporting, clinical or laboratory signs, 1D or 2D-SWE is performed directly after the abdominal ultrasound and routine blood tests. A **minimum time of 5–10 min in horizontal position** should be allowed for stabilization of hemodynamics and LS. We normally do the elastography right after the abdominal ultrasound, so the patient has already rested in horizontal position a couple of minutes. During the **abdominal ultrasound**, liver size, antero-posterior diameter of caudate lobe (normal <35 mm), liver morphology, abnormalities such as congestion, cholestasis, morphological signs of cirrhosis, spleen size, the presence of ascites and the diameter of the inferior vena-cava are assessed. In case of TE, LS is measured first with the M probe or in cases of M probe failure due to obesity [48] with the XL probe [48, 49]. If LS is elevated and patients have **AST > 100 U/mL**, alcohol withdrawal for at least 2 weeks (optimal 4 weeks) is recommended, followed by a second LS measurement. In patients with LS > 30 kPa, the diagnosis of cirrhosis is settled despite steatohepatitis as measured by elevated transaminase levels. At these levels, the development of ascites is very likely. This approach will allow definitive non-invasive assessment of fibrosis stage in ca. 95% of patients.

Compared to conventional routine ultrasound, TE identifies almost twice as many patients with advanced fibrosis/cirrhosis (Mueller S, unpublished) and has a smaller sample error as compared to histology (3–5% vs. 20–50%). In a recent French elastography screening study on more than 1000 apparently healthy people older than 45 years, 7.5% had a pathologically increased liver stiffness >8 kPa, with 36% of them eventually being due to ALD [50]. Therefore, it is anticipated that these novel non-invasive screening tools will improve the early recognition and follow up of patients with ALD, the most common and unfortunately too often underestimated liver disease.

Long-Term Liver Stiffness Follow-Up in Patients with ALD

LS measurement allows to monitor drinking activity and ALD progression since LS encompasses the sum of all pathological features from inflammation, ballooning to fibrosis. LS improved shortly after alcohol withdrawal in more than 80% [32]. As shown in Table 42.2, first unpublished preliminary data indicate that LS continues to decrease after further abstaining from alcohol up to 5 years. Thus, in 23 heavy drinkers who were followed-up for 5.5 years, LS decreased by almost 50%. Preliminary unpublished mortality data from 10 year survey in heavy drinkers also shows that LS seems to be the best univariate predictor of death in heavy drinkers [37]. More details are described in Chap. 7. Accordingly, LS predicts mortality independently from bilirubin and INR. A LS >12.5 is associated with 64% survival after 5 years [51].

Additional Information from Spleen Stiffness Measurements in ALD Patients

In addition to liver stiffness (LS), spleen stiffness (SS) is now widely used as novel non-invasive parameter to screen for portal hypertension [52, 53]. Moreover, SS has been demonstrated to outscore liver stiffness or platelet count in **predicting complications of portal hypertension**, such as the presence of **esophageal varices** and the risk of variceal bleeding [54–60]. SS can be easily measured with 2D-SWE or pSWE and also Transient Elastography can well be explored to assess SS [52, 61]. Limitations are: small normal sized spleens and obesity. In addition, since SS is typically higher as LS, the upper detection limit of the Fibroscan device at 75 kPa is more rapidly reached, which has presently resulted in commercially available technically modified dedicated spleen examinations [62].

However, the impact of disease etiology namely the localization of inflammation (portal vs. lobular) has only recently been appreciated. We recently conducted a study that analyzed and compared SS and LS in patients with portal (HCV) and lobular (ALD) chronic liver disease [63]. Our findings clearly showed that SS is

significantly higher in patients with hepatitis C (HCV) as compared to alcohol-related liver disease (ALD) (42.0 vs. 32.6 kPa), $p < 0.0001$), despite a lower mean LS in HCV. Consequently, the **SS to LS ratio** was significantly higher in HCV (3.8 vs. 1.72) through all fibrosis stages. Notably, spleen length linearly increased with SS and the relation between SS and SL was identical in HCV and ALD. In contrast, livers were much larger in ALD at comparable LS. In a prognostic cohort, **ALD patients** had higher LS values, predominantly presented for jaundice and liver failure was the major cause of death [63]. In contrast, spleens were larger in **HCV** with variceal bleeding was the major cause of decompensation. Thus, combined LS and SS measurements provide additional information about disease etiology and disease-specific complications. Figure 42.5 shows SS (in kPa) and spleen size (in cm) both for HCV and ALD in cohorts matched for LS (19 kPa). Cohorts were also matched for age and gender. Although no significant differences were observed with regard to LS, Fig. 42.5 demonstrates that spleens/SS are significantly larger in HCV. Since mean SS/LS ratio did not overlap between ALD and HCV, the SS/LS ratio may be also a useful tool to better emphasize the role of alcohol consumption in HCV patients.

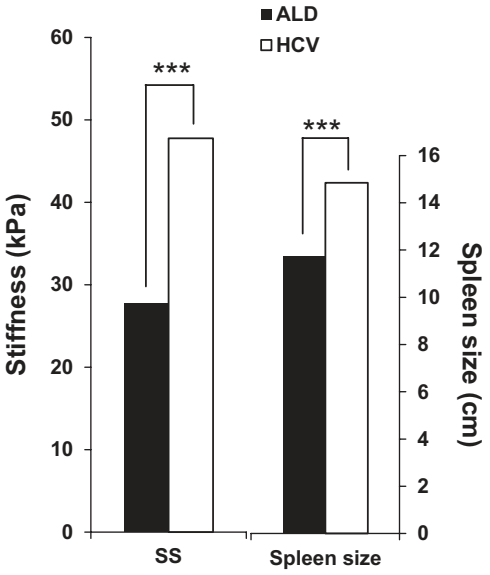

Fig. 42.5 Spleen stiffness (SS) and spleen length are not only highly correlated with portal hypertension but also depend on disease etiology. As shown here, spleen stiffness and spleen length is higher in patients with hepatitis C virus infection (HCV) has compared to patients with ALD. Consequently, the SS/LS ratio can be used to confirm the disease etiology or to assess the relative importance of one disease in patients that present with co-morbidities. More needs to be learnt in future studies about these novel insights

Shear Wave Attenuation Parameters to Assess Liver Steatosis on Elastography Platforms

Controlled Attenuation Parameter (CAP) is a novel tool to non-invasively assess liver steatosis, which measures ultrasound attenuation when travelling through fatty liver tissue, compared to normal liver [64]. It is run on the 1D-TE (Fibroscan) platform (see also Fig. 42.2a). In an individual data meta-analysis, CAP technology was shown to diagnose moderate and severe steatosis with diagnostic accuracies between 0.85 and 0.90 in 2735 patients with mixed liver disease etiologies (mainly viral hepatitis and NAFLD) [65]. In a recent European multicenter prospective study including 562 ALD patients who underwent CAP, regular US and liver biopsy [66], CAP diagnosed mild, moderate and severe steatosis with AUC of 0.77, 0.78 and 0.82, respectively. A CAP value above 290 dB/m ruled in any steatosis with 88% specificity. Moreover, CAP was shown to be superior to regular US to diagnose steatosis in ALD patients. CAP technology appears therefore as an interesting tool to diagnose steatosis and is performed concomitentely with TE, using the FibroScan device: the procedure is non-invasive, non-ionizing, easy to perform and provides immediate results. In addition, CAP can be performed simultaneously to liver stiffness measurement, making possible the simultaneous evaluation of both fibrosis and steatosis [67]. However, diagnostic accuracy appears to be poorer at low steatosis stages and seems lower in ALD compared to other liver disease etiologies. Moreover, optimal cut-offs to rule in, rule out and stage steatosis are varying in the different studies performed. One reason of this variation of CAP within multicenter studies seems to be the rapid response of CAP to drinking dynamics [66]. In case of sequential measurements of CAP in the same cohort, much better accuracy has been observed. It is therefore assumed that specific challenges of ALD study design has also contributed to the rather poor performance of CAP in ALD as compared to NAFLD.

On the other hands, in the last years, **QUS (quantitative ultrasound measurement of steatosis)** have been also implemented on other ultrasound/2D-SWE/pSWE platforms. Examples are the ATI module (Attenuation Image from Canon), or the UGAP module (Ultrasound Guided Attenuation Parameter from General Electric), TAI or TSI (from Samsung) or UDFF (Ultrasound derived Fat Fraction from Siemens). According to the increasing publications, the AUROC of these QUS can reach 0.90–0.95 with some systems, in comparison with liver biopsy [68].

Conclusion

At present, the market of elastographic platforms is continuously expanding and changing. Moreover, there is a certain need for standardization and formal use of units and training that can even confuse experts. Even cut-off values are still controversially used and most likely, some basic training will be required in the near future

in order to help to standardize the "elastographic science" that goes beyond interpretation of "radiological images".

While, already today, liver elastography outscores any other non-invasive fibrosis marker and has significant advantages in comparison to the former gold standard liver histology, a certain knowledge of **confounding factors** that elevate liver stiffness is required.

A **normal liver stiffness of <6 kPa rules out liver fibrosis** since even artifacts are always causing LS elevation but no LS decrease. In ALD patients, liver inflammation is the most important confounder of LS elevation and this is best done by having an actual AST/GOT level available. In cases of highly elevated transaminase levels, elevated liver stiffness is very likely a result of inflammation and either AST-adapted cut-off values should be applied or liver stiffness should be re-measured after an abstaining period of at least 1–2 weeks. If done so, cut-off values of 8 and 12.5 kPa can used to assess for F3 and F4 cirrhosis [10]. A cut-off value of >20 kPa is indicative for significant portal hypertension and patients should be screen for esophageal varices and primary liver cancer (HCC). Future discussion will show whether the "rule of four" (4, 8, 12, 16 kPa for ARFI methods) or the "rule of five" (5, 10, 15, 20 kPa for TE) are more easily applicable in clinical activity. The EFSUMB, WFUMB and EASL Guidelines recommend mainly this methods to rule out liver cirrhosis or advanced fibrosis [69]. Why confounding factors of elevated liver stiffness apply to all elastographic techniques whether 1D or 2D-SWE, there are certain differences to be considered. In 1D-TE, and extra-ultrasound needs to be organized, while 2D-SWE platforms already contain an conventional ultrasound device which facilitates detection of confounders, additional stiffness measurements, e.g., in the spleen. These latter platforms are ideal for hepatologists in order to perform both an abdomincal ultrasound and a Point of Care Elastography (POCE). With the new ultrasound system with the module of elastography (and QUS) inside, after a clinical examination and a standard ultrasound examination, an ultrasound based elastography can be performed (pSWE or 2D-SWE). Both methods can typically be performed successfully in more than 90%. We also have to keep in mind that health care systems are differently organized in different countries. While in some, general practitioners and internists are doing abdomincal ultrasound and are certainly and ideally qualified for performing elastography, ultrasound is restricted in other countries to radiologists. ALD patients will certainly benefit from a future great availably of elastography, due to competition on the market and there may be many niches for stand alone 1D-elastographic platforms and high end 2D-SWE module. Finally, elastography can be explored in a simple "**screening mode**" in search for any elevated LS and the more sophisticated "**expert mode**" that take account of all potential confounders as described above.

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Part VIII
Medical Aspects of Alcohol Consumption and Alcohol-Related Liver Disease

Chapter 43
Portal Hypertension in ALD

Benedikt Silvester Hofer and Thomas Reiberger

Abstract Alcohol-related liver disease (ALD) is among the leading causes of liver-related mortality worldwide. Patients who progress to alcohol-related cirrhosis are at risk for developing portal hypertension (PH), a key pathophysiological driver of disease progression and hepatic decompensation. PH following chronic alcohol consumption develops as a consequence of chronic changes to the hepatic architecture and functional disturbances within intra- and extrahepatic vascular beds. Additionally, acute alcohol intake aggravates PH by increasing intrahepatic resistance as well as portal venous and portosystemic collateral blood flow, thus further increasing the risk of hepatic decompensation. The diagnosis of PH and clinically significant portal hypertension (CSPH) should be based on non-invasive tests, including liver stiffness measurements, or on the invasive gold-standard hepatic venous pressure gradient (HVPG) measurement. As the risk of experiencing PH-related complications is directly related to the severity of PH, any reduction in HVPG results in a risk reduction. Clinically, non-selective betablockers are the therapeutic mainstay for ALD patients suffering from CSPH, as they not only reduce the risk of hepatic decompensation, but also improve survival. Additionally, alcohol abstinence has been shown to significantly reduce HVPG and decrease the risk for PH-related complications in patients with alcohol-related cirrhosis.

Keywords Portal hypertension · Portal pressure · Pathophysiology · Hepatic venous pressure gradient · Non-invasive tests · Advanced chronic liver disease Hepatic decompensation · Non-selective betablockers · Alcohol abstinence

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Abbreviations

acetyl-CoA	Acetyl-coenzyme A
ACLD	Advanced chronic liver disease
ALD	Alcohol-related liver disease
AMP	Adenosine monophosphate
CSPH	Clinically significant portal hypertension
eNOS	Endothelial nitric oxide synthase
FHVP	Free hepatic vein pressure
HSC	Hepatic stellate cell
Hsp90	Heat shock protein 90
HVPG	Hepatic venous pressure gradient
LSEC	Liver sinusoidal endothelial cell
MAP	Mean arterial pressure
NO	Nitric oxide
NSBB	Non-selective betablocker
PH	Portal hypertension
vWF	von-Willebrand factor
WHVP	Wedged hepatic vein pressure

Introduction

Alcohol-related liver disease (ALD) encompasses a spectrum of distinct disease stages, ranging from simple steatosis, to steatohepatitis, fibrosis and eventually alcohol-related cirrhosis [1]. Patients who progress to advanced fibrotic stages of ALD are subsequently at risk for developing portal hypertension (PH), which represents the primary pathophysiological driver of disease progression and hepatic decompensation [2, 3].

Hepatic Haemodynamics and Pathophysiology of Portal Hypertension in ALD

PH is a syndrome defined by an increased pressure within the portal vein, which collects blood from the splanchnic area. The pathogenesis of increased pressure levels in the portal venous system and subsequent PH can be described using a modification of Ohm's law, which defines pressure as the product of the resistance to flow and the flow rate. In the case of cirrhosis, functional and structural pathological changes that affect the hepatic (sinusoidal) microcirculation lead to an increase in resistance, while vasodilatory changes in the splanchnic vascular bed increase the amount of blood inflow into the portal vein [4]. While the overall pathophysiology of increased portal pressure is similar between different aetiologies of

cirrhosis, the haemodynamic profile of PH in alcohol-related cirrhosis demonstrates numerous distinct features. Furthermore, multiple components of the hepatic haemodynamic system are significantly modified by acute alcohol intake. These distinct haemodynamic characteristics of PH in ALD will be covered by this chapter.

Intrahepatic Resistance

The increase in intrahepatic resistance in cirrhosis arises as a consequence of both structural and functional changes, which decrease the calibre of hepatic sinusoids and subsequently hinder portal blood flow.

Intrahepatic Structural Changes

Structural changes originate from cirrhosis-associated alterations of the hepatic architecture, including the formation of regenerative nodules and fibrotic septa [4]. In addition to these macroscopic changes, the intrahepatic resistance is further increased by sinusoidal disturbances, which seem to be particularly pronounced in ALD and are caused by a complex interplay of multiple factors [5].

One key aspect that contributes to distorted blood flow on a sinusoidal level is hepatomegaly, which is caused by hepatocyte hypertrophy and ballooning [6–10]. Importantly, while hepatomegaly can already be observed in early stages of ALD, it is also found in advanced stages of alcohol-related cirrhosis [6]. Mechanistically, hepatomegaly following chronic ethanol consumption is the result of an intracellular accumulation of proteins and lipids, as well as increased levels of intracellular water [8, 11, 12]. In the setting of acute alcohol intake, hepatocyte size may be further increased via a mechanism which is thought to be mediated by the intracellular oxidation of ethanol to acetaldehyde and a subsequent activation of Na-K-2Cl cotransporters [8]. This hypothesis is underlined by the fact that hepatocyte swelling following ethanol exposure can be significantly reduced by inhibiting either alcohol dehydrogenase or the Na-K-2Cl cotransporter [8]. Importantly, this ethanol-induced cell swelling may further inhibit hepatocyte proteolysis and the secretion of proteins and thus further increase hepatocyte volume [8, 9, 13, 14].

The effects of cell swelling and sinusoidal compression on portal pressure levels have been investigated in a pig model of acute PH following intragastric ethanol administration. In this study, the authors observed a marked increase in portal pressure levels upon administration of ethanol, which was paralleled by significant hepatocyte swelling and a subsequent restriction of hepatic sinusoids, as analysed by electron thin-section phase-contrast microscopy [10].

In humans, a number of studies have observed enlarged hepatocytes in histological analyses of liver tissue from ALD patients and linked this phenomenon to increased portal pressure levels [5, 7, 15]. In order to provide pathophysiological

insights regarding this increase in portal pressure, one study demonstrated that the enlarged surface of hepatocytes was directly linked to a significant decrease in relative sinusoidal area [5]. Furthermore, this study revealed that the decreased relative sinusoidal area was significantly more pronounced in patients with ALD compared to patients with non-alcohol-related aetiologies of liver disease [5]. Importantly, in those ALD patients who demonstrated a significant sinusoidal disturbance, the reduction in sinusoidal area was directly correlated to increased portal pressure levels [5]. However, as patients did not abstain from alcohol in this study, the direct (acute) effects of ethanol on hepatocyte volume might have played a significant role.

The presence of a more pronounced sinusoidal disturbance in alcohol-related cirrhosis compared to non-alcohol-related aetiologies of liver disease has been further investigated by assessing hepatic haemodynamics and liver weight in patients with either alcohol-related or viral cirrhosis immediately before and after liver transplantation [16]. As this study was performed in a transplant setting, ALD patients were abstinent for at least 6 months [16]. Liver weight was significantly higher in ALD compared to viral liver disease, regardless of disease severity, thus underlining the increase in hepatocyte volume and sinusoidal resistance in ALD, even in abstinent patients [16]. Furthermore, by using Doppler ultrasound, a significantly reduced portal perfusion rate per gram of liver tissue could be detected in patients with alcohol-related cirrhosis compared to viral cirrhosis, despite a comparable degree of liver fibrosis [16]. The hypothesis of decreased portal perfusion in ALD patients is further supported by liver perfusion assessments using xenon computed tomography, which demonstrated a significantly decreased portal venous tissue blood flow in Child-Pugh A patients with alcohol-related cirrhosis compared to hepatitis C virus-associated cirrhosis [17].

In addition to enlarged hepatocytes, the intrahepatic resistance to blood flow is further increased by significant changes in and around the perisinusoidal space of Disse. In a healthy liver, hepatic sinusoids and the perisinusoidal space of Disse form a complex network within the liver parenchyma and represent the main site of interaction between cellular and liquid blood components and the liver parenchyma [18]. This interaction is facilitated by liver sinusoidal endothelial cells (LSECs), which line the hepatic sinusoids [18]. Importantly, LSECs lack a continuous basal lamina and thus form a netlike structure [18]. In cirrhosis, alterations in the sinusoidal microstructure are caused by the accumulation of collagen in the space of Disse [15, 19] and the formation of a continuous LSEC basal membrane, a process termed "sinusoidal capillarisation" [20]. Hepatic stellate cells (HSCs), which can be found within the space of Disse, are the primary cell type responsible for these perisinusoidal alterations [21]. Upon activation, which can occur due to multiple factors, including transforming growth factor β, as well as various cytokines and chemokines, HSCs transform into fibrogenic myofibroblasts and produce extracellular matrix proteins such as collagen, which subsequently accumulate inside the space of Disse [21]. While this pathomechanism is present in all aetiologies of cirrhosis, studies have shown that metabolites of ethanol, including acetaldehyde and acetaldehyde by-products, directly activate HSCs [22, 23]. Furthermore, the severity of

fibrotic changes, as assessed by serum markers of fibrogenesis including type IV collagen and laminin, has been linked to the amount of ethanol intake in ALD patients [24].

Overall, these alterations not only significantly impair the interaction between hepatocytes and hepatic sinusoids [25], but also increase pressure levels [15]. More information on hepatic fibrogenesis and cirrhosis formation in ALD can be found in a separate chapter within this book.

Intrahepatic Functional Changes

Functional changes that impair the intrahepatic circulation via vasoconstriction are a consequence of HSC activation, LSEC dysfunction and an imbalance between the release of vasodilators and vasoconstrictors [4].

While the activation of HSCs and the subsequent transdifferentiation into myofibroblasts that produce extracellular matrix (i.e., scar tissue) form the key structural resistance factor to portal blood flow, myofibroblasts also contribute to the functional intrahepatic alterations occurring in cirrhosis [4, 21]. These functional changes are facilitated by the fact that HSCs/myofibroblasts line the abluminal sinusoidal wall inside the space of Disse. Thus, a contraction of activated HSCs decreases the sinusoidal lumen and increases the intrahepatic resistance to blood flow [4].

In addition to HSC activation, endothelial dysfunction is another key aspect in the pathogenesis of PH. In healthy liver tissue, LSECs are crucial for maintaining homeostasis and controlling the hepatic vascular tone via the production of vasodilatory nitric oxide (NO) by the endothelial NO synthase (eNOS) [4]. In cirrhosis, however, LSECs become dysfunctional, resulting in a significantly impaired NO production [4]. Simultaneously to the decreased production of vasodilators, there is also an overproduction of vasoconstrictors, including adrenergic stimulants, thromboxane and endothelin, which induce HSC contraction and thus further increase the intrahepatic vascular tone [4]. While LSEC dysfunction is crucial for the pathogenesis of PH regardless of liver disease aetiology, novel evidence suggests that specific causes of cirrhosis may have additional modes of action in terms of modifying eNOS activity [26]. More specifically, it has recently been demonstrated that ethanol intake induces LSEC dysfunction through an interaction with heat shock protein 90 (Hsp90) [26]. Physiologically, Hsp90 is able to induce eNOS activation and, in turn, the production of NO. [27] However, increased levels of acetyl-coenzyme A (acetyl-CoA), which arise from the metabolization of ethanol by LSECs, can lead to the acetylation of Hsp90 [26]. This process decreases the ability of Hsp90 to activate eNOS and thus, the acetylation of Hsp90 decreases eNOS-related NO production [26]. Hence, ethanol directly impacts hepatic vasoconstriction via the induction of LSEC dysfunction.

Splanchnic Vasodilation

Splanchnic vasodilation increases hepatic blood flow and thus the pressure in the portal venous system [4]. The pathophysiology of vasodilation in the splanchnic region in patients with PH is based on the activation of multiple vasoactive systems and the production of vasoactive molecules. One such vasoactive molecule is NO. [28] While reduced NO production within the liver parenchyma induces vasoconstriction, NO production is increased within the splanchnic region [28]. The increased production of NO is primarily a result of the increased shear stress within the splanchnic system due to PH and the translocation of bacteria from the gut into the splanchnic blood stream [28, 29]. As a consequence of these vasodilatory changes, blood pools within the splanchnic region, eventually leading to central hypovolemia [30]. Central hypovolemia and the accompanying decrease in mean arterial pressure (MAP) induce neurohumoral response mechanisms, including the renin-angiotensin-aldosterone system and the sympathetic nervous system [30–32]. Since these response mechanisms aim to increase MAP, they cause an increase in cardiac output and lead to the retention of sodium and water [30]. Overall, these alterations are summed up by the term "peripheral arterial vasodilation hypothesis", which establishes systemic and splanchnic vasodilation as a key factor in the progression of advanced chronic liver disease (ACLD) [30]. Potential differences in the severity of systemic haemodynamic changes in distinct aetiologies of cirrhosis were assessed in a small study by Momiyama et al., which demonstrated a more pronounced decrease in the systemic vascular resistance in alcohol-related cirrhosis compared to hepatitis C virus-associated cirrhosis [33].

While the clinical relevance of these aetiology-specific differences remains insufficiently explored, the impact of acute alcohol consumption on systemic haemodynamics and the ensuing increase in portal pressure is undisputed and has been the topic of numerous previous studies. More than 40 years ago, Villeneuve et al. described the increase in hepatic blood flow in dogs following an acute intragastric or intravenous administration of ethanol [34]. Importantly, haemodynamic assessments in this study were performed without prior anaesthesia and thus, the blunting effects of anaesthetics on the vasodilatory response to ethanol was avoided [34, 35]. These findings were subsequently further investigated in rat studies, which detected a progressive increase in portal pressure following the infusion of increasing amounts of ethanol [36], as well as a dose dependent increase in portal venous blood flow [35].

In subsequent years, the impact of acute ethanol administration on portal venous and splanchnic haemodynamics could also be confirmed in human patients with chronic alcohol consumption, with [37] and without liver cirrhosis [38]. In a study performed in patients without underlying liver cirrhosis and without PH, the intravenous administration of ethanol led to a significant increase in portal pressure, portal blood flow and hepatic vascular resistance [38]. Similar findings were obtained in patients with underlying alcohol-related cirrhosis, who demonstrated a significant increase in hepatic venous pressure gradient (HVPG) and azygos blood

flow following oral ethanol administration [37]. Importantly, both the increase in portal pressure and the increase in azygos blood flow indicate that ethanol consumption might increase the risk of suffering an oesophageal variceal bleeding event [37].

The pathophysiology of increased portal blood flow following ethanol ingestion is based on the metabolization of ethanol and the subsequent production of vasoactive mediators, but not ethanol itself. This is highlighted by the fact that inhibiting alcohol dehydrogenase, the primary enzyme in the degradation of ethanol, using 4-methylpyrazole leads to a suppression of the ethanol-induced increase in portal blood flow [39]. Furthermore, blocking the conversion of acetaldehyde to acetate also suppressed the increase in portal blood flow in response to ethanol [40]. These findings suggest that acetate might be a key metabolite in conveying the vasoactive effects of ethanol. This is in line with the observation that the intravenous administration of acetate causes a dose-dependent rise in portal blood flow [41]. Nevertheless, studies have also indicated that the acetate-induced vasodilation is still dependent on the metabolization of acetate to acetyl-CoA, which is paralleled by the production of adenosine monophosphate (AMP) from adenosine triphosphate [41, 42]. AMP is subsequently further metabolised to adenosine via the enzyme 5′ nucleotidase [42]. The hypothesis that the vasoactive effects of acetate are caused by the subsequent production of adenosine is reinforced by two key observations. Firstly, adenosine is a potent vasodilator [43] with an ability to increase portal blood flow [44]. Secondly, the acetate-induced increase in portal blood flow can be inhibited by administering an adenosine receptor blocker [41]. Of note, hypoxia occurring during the metabolization of ethanol might additionally propagate the increase in adenosine levels [45, 46].

Hepatofugal Blood Flow and Formation of Porto-Systemic Shunts

Hepatofugal blood flow, i.e., a reversal of blood flow within the portal vein, is a phenomenon that has been observed in previous studies using Doppler ultrasound and has been linked to a worse prognosis and the presence of porto-systemic shunts [47–49]. Potential differences in distinct aetiologies were assessed by Hirata and colleagues [49]. The authors observed a high prevalence of hepatofugal blood flow in patients with alcohol-related cirrhosis, while this phenomenon was not observed in patients with viral cirrhosis [49]. A potential explanation for the increased prevalence of hepatofugal blood flow in ALD patients might be found in the increased prevalence of porto-systemic shunts [49]. Porto-systemic collaterals develop as a response to increased pressure levels in the portal vein and shunt blood away from the portal system and into the systemic circulation [49]. As demonstrated by Simón-Talero et al., the prevalence of collaterals increases as portal pressure rises and hepatic function worsens [50]. Importantly, a significantly higher prevalence of

large shunts and paraumbilical collaterals was found in patients with alcohol-related cirrhosis compared to non-alcohol-related aetiologies of cirrhosis [50]. This finding is in accordance with the previously described higher prevalence of paraumbilical vein patency in alcohol-related cirrhosis when compared to viral cirrhosis [16, 51].

Diagnosis of Portal Hypertension in ALD

Invasive Assessment of Portal Hypertension

The invasive measurement of HVPG is the current gold standard method to assess the severity of PH and diagnose clinically significant portal hypertension (CSPH) [52]. HVPG is the most precise surrogate parameter of PH available and has been shown to enable risk stratification, help monitor the treatment response to portal pressure lowering medication and facilitate personalised therapeutic decision-making [3, 52–55]. The current approach to measuring HVPG is based on the insertion of a balloon-tipped catheter into a large hepatic vein and is performed according to a standardised protocol [56]. Specifically, the hepatic blood flow is inhibited by inflating the attached balloon [57], thus forming a static blood column between the hepatic sinusoids and the catheter tip. Therefore, the wedged hepatic vein pressure (WHVP) corresponds to the sinusoidal pressure level. Following the assessment of the wedged pressure, the occlusion is reversed and the free hepatic vein pressure (FHVP) can be measured. Subsequently, the HVPG is calculated by subtracting the mean FHVP from the mean WHVP. While HVPG measurements are considered the gold standard regardless of aetiology, the ability of HVPG to mirror the severity of PH might vary in distinct disease entities [58]. This is highlighted by data on the agreement of the directly measured portal pressure with the WHVP [59, 60]. Of all cirrhosis aetiologies, the correlation between portal pressure and WHVP is the highest in alcohol-related cirrhosis [59]. In patients with non-alcohol-related cirrhosis on the other hand, the correlation performs significantly worse and portal pressure, as assessed by WHVP, may be underestimated [59, 60]. A possible explanation for this discrepancy is the pre-sinusoidal component of PH found in patients with non-alcohol-related cirrhosis [60], as shown for cirrhosis due to cholestatic liver disease [61] or non-alcoholic steatohepatitis [62].

Non-invasive Tests of Portal Hypertension

Due to the invasiveness and the required expertise, HVPG measurements are currently limited to specialised centres and thus, non-invasive tests for the assessment of PH are required. According to the Baveno VII guidelines, the presence of CSPH in patients without previous hepatic decompensation can be assessed using liver

elastography and platelet count [52]. More specifically, liver stiffness values ≤15 kPa, combined with a platelet count ≥150 G/L can be used to rule out CSPH with a negative predictive value and sensitivity of more than 90% [52]. Stiffness values ≥25 kPa on the other hand can be used to rule in CSPH [52]. In patients who do not fall into any of these two categories, the ANTICIPATE model can be employed to assess the likelihood of CSPH [63]. Importantly, non-invasive tools can not only be used to diagnose CSPH, but also to evaluate the presence of fibrotic changes in patients with chronic alcohol intake—a topic which is discussed in depth in a different chapter of this book. More details on PH and measurements of liver stiffness and additional spleen stiffness and important differences between ALD and other liver disease aetiologies are discussed in Chap. 42.

Prognostic Impact of Portal Hypertension in ALD

Regardless of liver disease aetiology, PH only occurs after advanced disease and/or cirrhosis has developed. Thus, the presence of PH itself already indicates a more advanced disease stage that, by definition, is associated with a worse prognosis than non-advanced stages [52]. However, with the concept of using non-invasive liver stiffness measurements to stage liver disease, rather than conducting invasive liver biopsies, the clinical strategy to risk-stratify patients has also changed [64]. A key factor in improving risk stratification in patients with compensated ACLD. following the diagnosis of ACLD via a confirmed liver stiffness measurement of ≥15 kPa, is to assess the presence of CSPH (i.e., HVPG ≥10 mmHg) [52]. CSPH is the main driver of hepatic decompensation [3]. Importantly, liver stiffness measurements can not only be used to detect compensated ACLD in ALD patients, but also to non-invasively classify their risk of suffering from CSPH [63, 65] and to assess PH severity [64]. Nevertheless, the invasive assessment of HVPG still represents the gold-standard for diagnosing CSPH in ALD [56], as it is able to accurately assess the severity of PH in ALD cirrhosis [66] and is thus an important tool for clinical risk stratification [67].

Traditionally, screening for CSPH was mostly conducted by screening for varices using gastroscopy, with the presence and size of varices being associated with prognosis in patients with ALD cirrhosis [68, 69]. However, as the therapeutic focus in patients with (ALD) cirrhosis has shifted from only treating PH once varices have developed (i.e., primary prophylaxis) to treating CSPH (i.e., as soon as HVPG surpasses 10 mmHg regardless of the presence of varices), endoscopic screening for varices should be replaced by other means of diagnosing CSPH [52, 70]. As mentioned above, while HVPG remains the diagnostic gold standard for CSPH, non-invasive surrogates of PH severity are also of prognostic relevance. In this regard, we want to highlight the importance of the liver stiffness-to-platelet ratio score [71], the von-Willebrand factor (vWF) antigen [72] and the vWF-to-platelet count ratio (VITRO) [73, 74] to predict PH-related complications in alcohol-related cirrhosis.

Ultimately, all of the abovementioned studies, which assessed HVPG and non-invasive surrogates of PH, have confirmed that the presence of CSPH, as well as the severity of PH beyond CSPH, are key prognostic factors in patients with alcohol-related cirrhosis.

Treatment of Portal Hypertension in ALD

Despite ongoing research efforts, the current pharmacological treatment options for PH in patients with alcohol-related cirrhosis are limited to non-selective betablockers (NSBBs) [4, 75, 76]. Even though the administration of NSBBs in patients with CSPH has been shown to effectively reduce the incidence of hepatic decompensation, decrease systemic inflammation and improve prognosis, they do not cure the underlying pathophysiological alterations present in cirrhosis [70, 76, 77].

Thus, a particular focus in the treatment of PH in ALD patients should be set on curing the underlying aetiology via sustained alcohol abstinence. The clinical impact of maintaining abstinence from alcohol has been assessed in numerous studies and has been shown to improve the prognosis regardless of disease severity and across all stages of PH [78–82].

Furthermore, persistent abstinence has been shown to significantly decrease the Child-Pugh score, portal pressure and oesophageal variceal size in patients with alcohol-related cirrhosis [67, 83]. Since NSBB therapy was not administered within the context of these studies, their findings were not confounded by a potential difference in treatment adherence between patients with active alcohol intake and abstinence [67]. Importantly, resuming alcohol consumption has been shown to be associated with a renewed increase in PH [83].

Overall, these findings highlight the importance of remaining abstinent on the disease course in patients with alcohol-related cirrhosis and indicate the potential for a regression of PH that warrants further investigation.

Conclusions

Patients with ALD and ongoing pathological alcohol intake are at a high risk of ultimately progressing to cirrhosis, developing CSPH, and subsequently suffering from CSPH-related complications, including variceal bleeding, ascites and hepatic encephalopathy. The diagnosis of CSPH in these patients should no longer rely on screening for the presence of varices by gastroscopy, but rather be non-invasively assessed via liver stiffness measurements and other non-invasive surrogates, or evaluated invasively through HVPG measurements. Patients are at risk of experiencing PH-related complications as soon as CSPH develops and this risk is directly related to the severity of PH, i.e., the level of HVPG. Thus, any reduction in PH severity,

achieved through treatments that decrease HVPG, will result in a risk reduction for PH-related complications. Clinically, NSBBs should be used in patients with alcohol-related cirrhosis as soon as CSPH develops, since NSBBs not only reduce the risk of hepatic decompensation, but also improve survival. In addition to NSBB therapy, alcohol abstinence is a key factor in reducing HVPG and decreasing the risk of CSPH-related complications in patients with alcohol-related cirrhosis. Nevertheless, more effective treatment options for CSPH in patients with alcohol-related cirrhosis are urgently needed in order to improve their prognosis independently from strategies used to treat alcohol use disorders.

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Chapter 44
The Impact of Alcohol Consumption on Inflammatory Bowel Disease

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Abstract Controversial effects of alcohol have been described for patients with inflammatory bowel diseases (IBD) which mainly consists of Crohn's disease (CD) and ulcerative colitis (UC). Besides direct toxic or immunomodulatory effects of alcohol, non-alcoholic ingredients may also show protective effects or modulate relapse induction and exacerbation of symptoms. After a comprehensive literature search, 179 articles were retrieved from Pubmed with the searching terms UC, CD, IBD, and alcohol. Although interpretation remains difficult, studies suggest that chronic heavy drinkers have a higher incidence of abdominal pain, risk of relapse and of colon cancer, while non-smoking moderate drinkers seem to be protected against UC. Moderate alcohol consumption is also safe in inactive IBD patients. The preliminary reports with potentially beneficial effects of non-alcoholic agents in low-to moderate wine consumers warrant further confirmative studies.

Keywords Ulcerative colitis · Crohn's disease · Relapse · Alcohol · Alcohol consumption

Introduction

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine consisting mainly of Crohn's disease (CD) and ulcerative colitis (UC). IBD is a complex disease which arises as a result of the interaction of environmental and genetic factors. Although a wide range of treatment modalities have been established ranging from probiotics to surgery, immunosuppressive therapy can be considered the workhorse. Despite scientific efforts over many decades, the pathophysiology of IBD is still not completely understood. This chapter is not addressing the pathology, diagnosis or treatment of IBD but rather the impact of

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alcohol consumption on the pathogenesis and progression of the disease including important severe complications such as cancer and malnutrition [1]. Altogether, 179 articles were identified reviewed in literature search for the years 1991–2022 in Pubmed using the following terms: UC, CD, IBD, alcohol. Full articles or abstracts in English, German and Italian (systematic reviews, original articles, observational studies, multicenter studies, randomized controlled trials, experimental studies on humans and animals, metaanalysis) were included. Excluded were those articles which did not contain precise data about exact effect on IBD nor the type or ingested quantity of alcohol.

Interactions of Alcohol with IBD Pathology

Alcohol consumption is known to increase the transfer of microbiota from gut to blood ultimately being able to cause endotoxemia [2]. The effects on pro-inflammatory cytokine production seems to depend on the level of drinking [3]; while heavy intake inhibit their production, moderate and chronic drinking stimulates them [3]. Growth factors are also inhibited *in vivo* by heavy chronic alcohol abuse. Alcohol also negatively affects the hypothalamic-pituitary-adrenal axis and the cell-mediated and humoral immunity. For instance, hyperproduction of pro-inflammatory factors (IL1, IL6, TNF-α) has been described in response to chronic alcohol consumption due to increased activity of leucocytes and Kupffer cells [3].

Overall, there was a protective effect of alcohol consumption on three autoimmune diseases mediated by Th1 (IBD, rheumatoid arthritis, multiple sclerosis), but excessive drinking did not lead to decrease of risk of autoimmune diseases [4]. No risk between the alcohol consumption and risk of UC (OR 0.85)/CD (OR 0.95) was observed in two meta-analysis by Nie [5] and Yang [6]. Controversial data have been reported with regard to IBD development after 10 years of heavy alcohol consumption. An increased risk has been reported from Taiwan [7], while no elevated risk was seen in a European study with 262,451 participants [8].

Light drinkers (less than 3 days/week with 0.5–1 drinks per day and corresponding to 1–19 g alcohol per day) seem to be protected against UC (see Table 44.1), if they do not smoke [15]. This protection cannot be seen in smokers [27]. Magee et al. described the lack of association between UC activity and intake of spirits (with no content of sulphites) [26]. Casey et al. proved in a cohort study on 237,835 people that moderate (>1–4/week) intake of beer was associated with low risk of CD, while consumption of >4 portions liquor/week led to increased risk of UC [18].

A genetic predisposition of alcohol consumption and IBD risk has been described, independently of polygenic risk score and in the absence of substance use. SULT1A2 is involved in alcohol metabolism and responsible for regulating alcohol consumption. Gene variants of SULT1A2 were negatively associated with CD risk in children (with no alcohol intake); Chr 16 locus was identified as being connected to

Table 44.1 Summary of alcohol-modulated effects in IBD patients

Study	Year	Consumption	Alcohol dose (wine)	Effect on IBD
Tabasco [9]	2011	low	≤3 days/week, 0.5–1 portions alcohol/day, 1–19 g alcohol/day	prebiotic
Dolara [10]	2005			
Biasi [11]	2014			antimicrobial
Singh [12]	2010			antioxidant, antiinflammatory
Cheah [13]	2013			
Giovanelli [14]	2000			reduces colorectal cancer risk
Jiang [15]	2007			protective in non-smokers (UC)
Cho [16]	2018			higher bone mineral density vs. nondrinkers/big drinkers
Swanson [17]	2011	moderate	1–3 glasses/day; 30 g alcohol/day (men), 15 g/day (women)	safe (inactive IBD), increased risk of flares
Casey [18]	2021		beer (>1–4/week) >4 liquor/week	decreased risk of CD increased risk of UC
Stermer [19]	2002	high	238 g/day	increased colorectal cancer risk
Siegmund [20]	2003			
Swanson [21]	2010		sustained	increased risk of flares (UC)
Jiang [15]	2007		sulfite-containing drinks	increased risk of flares (UC)
Berg [22]	2008			higher risk of hip fracture
Hsu [7]	2016		>10 years (Taiwan)	increased IBD risk
Bergmann [8]	2017		>10 years (Europe)	no IBD risk
Niccum [23]	2021		wine	increased risk of microscopic colitis
Chiba [24]	2000	not mentioned	chronic	complications (malnutrition, diarrhea)
Piovenzani Ramos [25]	2021		gin	increased risk of flares (*Clostridium, Bacteroides)*
Jiang [4]	2021		not excessive amount	protective
Magee [26]	2005		spirits	no association with UC activity

predisposition to alcohol drinking and IBD risk [28]. Ramos et al. identified 240 IBD susceptibility loci in a study including 60,000 people [29]. The anti-inflammatory diet proposed in IBD patients should not contain alcohol [30].

Effect of Alcohol in IBD Relapse

Sustained consumption of high doses of alcohol (238 g/day) leads to increased rate (OR 2.71) of relapses in IBD (Table 44.1) compared to spirits [15, 21] especially due to the sulphite content (component of red and white wine, bitter, beer), but also

a high risk of colorectal cancer [19, 20]. Regarding the complications of chronic alcohol intake in IBD, diarrhoea and malnutrition would be the most important, due to improper absorption of vitamins, folate and thiamine [24]. Alcohol represents a modifiable risk factor of relapse, through formation of crypt micro abscesses, mucosal ulceration, colonic epithelial damage and sulphite-rich (wine, beer) beverages [8]. Although consumption of moderate quantities of red wine (1–3 glasses/day, corresponding to 30 g alcohol/day in men and 15 g/day in women) proved an increased risk of flares in IBD [17], this amount is considered safe in inactive IBD patients, without exposing them to relapse (Table 44.1).

Consumption of alcohol increases the relapse-risk by 2.7 times [31]. Alcohol is the most avoided dietary factor in IBD patients (in 22% patients) [32] and especially women were more likely to avoid alcohol [33]. Among adults with IBD from USA, Xu et al. described a percentage of 57.8% of current drinkers in 2015 (vs. 65.1% among people without IBD) and 16.3% remained all life abstainers (vs. 20.8% in non-IBD people) [34]. Mechanisms that lead to IBD relapse after alcohol intake are complex and include increased permeability, endotoxemia, increased proinflammatory cytokines [25].

Interaction of IBD Treatment with Ethanol

In the context of IBD medication (methotrexate, biologics, 5-aminosalycylates), several interactions with alcohol are of concern, e.g., due to induction or competition with the cytochrome P450 system or adverse events (disulfiram-like reactions) in combination with antibiotics (metronidazole, cephalosporin) [25, 35]. Decreased efficacy of 5-ASS, low levels of cyclosporine (salvage treatment in severe UC) and progression of liver fibrosis after methotrexate/azathioprine have been reported in association with concomitant alcohol abuse [36, 37].

Non-alcoholic Content of Alcoholic Beverages

Beneficial effects of wine have been mainly attributed to non-alcoholic ingredients including antioxidant properties of a wide variety of phenolics, also called polyphenols. Wine phenolics may prevent or delay the progression of intestinal diseases. They act both as free radical scavengers but also modulate specifically inflammation-related genes involved in cellular redox signalling. In addition, the importance of wine polyphenols has recently been stressed for their ability to act as prebiotics and antimicrobial agents. Although questionable with regard to the cancerogenic effect of alcohol in large human studies, low levels of wine have been experimentally shown to reduce inflammatory infiltrates in piglets, rats and mice [12–14].

Conclusions

Chronic heavy drinkers have higher incidence of abdominal pain, risk of relapse and of colon cancer, while non-smoking light drinkers are protected against UC. Moderate alcohol consumption is safe in inactive IBD patients. The preliminary reports with potentially beneficial effects of non-alcoholic agents in low-to moderate wine consumers on reducing the histopathological, endoscopic and immunohistochemical scores in IBD warrant further confirmative studies.

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Chapter 45
Coagulation Disorders in Patients with Alcohol-Related Liver Cirrhosis

Liana Gheorghe and Speranta Iacob

Abstract In patients with liver cirrhosis, the hemostatic cascade is rebalanced at a low levels and its equilibrium is extremely fragile. It can be easily destabilized by various triggers (e.g., infection, alcohol, uremia, anemia, medications etc.) toward either an anticoagulant or procoagulant phenotype. Conventional coagulation tests do not fully reflect the abnormalities of hemostasis and do not accurately predict the risk of bleeding. Commercially available global coagulation tests (viscoelastic tests), including thromboelastography, rotational thromboelastometry and sonorheometry analyze all components of hemostasis including the dynamics of clot formation, clot strength, and clot stability and represent a promising point-of-care tool for assessing bleeding risk and guiding therapy in these patients. In the setting of bleeding, blood product transfusions should be used judiciously because they increase portal pressure and carry a risk of transfusion-associated circulatory overload, transfusion-related acute lung injury, infection transmission, alloimmunization, and/or transfusion reactions. The following transfusion thresholds may optimize clot formation: hematocrit ≥25%, platelet count >50,000/mmc and fibrinogen >120 mg/dL. In the setting of thrombotic complications, systemic heparin infusion is recommended for symptomatic deep vein thrombosis, thromboembolism and portal or mesenteric vein thrombosis. Therapy with low-molecular-weight heparin, vitamin K antagonists, and direct-acting oral anticoagulants improve portal vein re-permeabilization versus observation alone.

Keywords Alcoholic cirrhosis · Procoagulant · Anticoagulant · Bleeding Thrombosis · Platelet · Viscoelastic methods · Clotting · Therapy · Transfusion

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Introduction

The liver plays a pivotal role in hemostasis by synthesizing all coagulation factors and their natural anticoagulants, except for von Willebrand factor (vWF), as well as several fibrinolytic proteins. As a consequence, liver cirrhosis is commonly associated with profound alterations of coagulation by simultaneous reduction in plasma levels of both procoagulant and anticoagulant factors. Coagulation abnormalities in liver cirrhosis pose important clinical consequences representing a key variable of most prognostic scores in liver cirrhosis [1].

Pathophysiology of Coagulation Abnormalities in Patients with Alcoholic Cirrhosis

Liver cirrhosis, irrespective of etiology, is characterized by a state of *re-balanced low-level hemostatic equilibrium* due to the concordant reduction in pro- and anticoagulant components. This balance is extremely fragile and can be easily destabilized by various triggers and superimposed conditions (e.g., infection, alcohol, uremia, anemia, medication, variceal bleeding, decompensated liver cirrhosis, invasive procedures, or inadequate hemotherapy) toward either a procoagulant or anticoagulant phenotype [2, 3]. The resulting clinical state is bleeding or thrombosis according to the predominant complex hemostatic mechanisms [4].

Several well characterized mechanisms are involved in the rebalanced hemostasis that occurs in advanced chronic liver disease [5]: hepatic synthetic dysfunction resulting in decreased levels of liver-derived coagulation, anticoagulation, and fibrinolytic factors; the combination of decreased synthesis of thrombopoietin (TPO) and splenomegaly leading to thrombocytopenia and platelet dysfunction; increasing synthesis of endothelial-derived hemostatic proteins; low-grade activation of the hemostatic system resulting in consumption of hemostatic proteins [6]; posttranslational changes of hemostatic proteins such as fibrinogen associated with functional alteration [7]. Alcohol itself impairs directly or indirectly hemostasis inhibiting platelet aggregation and reducing key components such as vWF, factor VII and fibrinogen levels.

Coagulation disorders may occur in all three phases of hemostasis—primary hemostasis, coagulation (clot formation) and fibrinolysis. Alcoholic cirrhotic patients specifically present multiple alterations of *primary hemostasis* characterized by quantitative (thrombocytopenia attributed to impaired production by decrease in thrombopoietin, splenic sequestration, accelerated turn-over, myelosuppression, and ethanol toxicity) and qualitative (platelet dysfunction resulting in hypo-aggregability) platelet defects, counterbalanced by high vWF and disintegrin and metalloproteinase with thrombospondin type 1 motif 13 (ADAMTS 13) levels [4, 8]. Similarly, in the *coagulation phase,* the low level of liver-derived procoagulant factors, such as fibrinogen and factors II, V, VII, IX, XI, XII, causing

Hemostasis phase	Antihemostatic changes (favoring bleeding)	Prohemostatic changes (favoring thrombosis)
Primary hemostasis	- Thrombocytopenia (splenic sequestration decreased TPO, myelosuppression, ethanol toxicity) - PLT dysfunction (hypo-aggregability)	- High vWF levels - Low ADAMTS 13 levels
Coagulation phase	- Low liver-derived procoagulant factors II, V, VII, IX, XI, XII - Low fibrinogen, Dysfibrinogenemia	- High factor VIII - Low liver-derived anticoagulant proteins S, C, antithrombin III
Fibrinolysis phase	- Low antiplasmin - Low TAFI - High tPA	- Low plasminogen - High PAI1

Fig. 45.1 Hemostasis is rebalanced at unstable low levels in patients with liver cirrhosis

prolongation of the prothrombin time are counterbalanced by a parallel decline in liver-derived anticoagulant proteins C, S, antithrombin III and high factor VIII, while during the *fibrinolysis phase,* to maintain a normal fibrinolytic balance, increase in tissue plasminogen activator (t-PA) is compensated by increase in plasminogen activator inhibitor type-1 (PAI-1) concentrations [2–4] (Fig. 45.1).

Coagulopathy Assessment in Cirrhosis

Simultaneous changes of procoagulant and anticoagulant pathways in patients with cirrhosis result in complex hemostatic changes that are not adequately captured by standard coagulation tests for identifying and monitoring coagulopathy such as platelet count, activated partial thromboplastin time (aPTT), prothrombin time (PT) and international normalized ratio (INR). All currently available laboratory tests of hemostasis have significant limitations in patients with liver cirrhosis and do not reliably predict the risk of bleeding. They do not fully reflect the complex derangement in hemostasis in advanced chronic liver disease since they usually measure procoagulant factors (I, II, V, VII, and X) and do not reflect the reduction in liver-derived anticoagulant factors or the complex interactions between cells and coagulation factors in whole blood [9].

The INR is calculated from the PT ratio (patient PT/control PT) adjusted for the international sensitivity index (ISI). Largely used in clinical practice, the INR was developed to standardize PT reporting for patients on stable anticoagulation with vitamin K antagonists (VKA) and it is not validated for patients with liver disease [4, 9]. Patients with cirrhosis have a more complex and less predictable coagulation profile than patients on VKA with similar INR values. Moreover, there is substantial interlaboratory variability of the INR in patients with cirrhosis across laboratories, especially at high INR, largely due to differences in the thromboplastin used. To

overcome this problematic setting, an alternative INR system has been proposed for patients with cirrhosis (ISI_{liver}) using plasma samples from patients with liver disease for calibration [10]. However, implementation of the INR_{liver} presents logistical challenges that prevent its routine use; for example, a requirement for the manufacturer to provide 2 ISI values (ISI_{VKA} and ISI_{liver}) for each thromboplastin translates into extra-costs and may raise confusion among the laboratory personel [4].

Alternative tools reflecting the interaction between procoagulants, natural anticoagulants, platelets, and the fibrinolytic system (global tests of hemostasis) have been investigated as potentially more reliable for assessing global coagulation in patients with cirrhosis. Global tests of hemostasis, such as thrombin generation or whole-blood viscoelastic tests better capture the general hemostatic status of a patient with cirrhosis but have not been clinically validated.

The thrombin generation test (TGT) dynamically measures the total amount of thrombin generation during *in vitro* coagulation. TGT reflects the balance between pro- and anticoagulant factors in advanced chronic liver disease (ACLD), but remains primarily a research tool, not ready to be translated into a viable clinical instrument and not validated for predicting bleeding risk in patients with ACLD [11, 12].

Recently, whole-blood viscoelastic methods of assessing the clotting mechanism have been showed to be of clinical value in patients with liver cirrhosis. The commercially available viscoelastic methods are: (1) Thromboelastography (TEG, Hemonetics Corporation, Braintree, MA) (2) rotational Thrombelastometry (ROTEM, TEM International GmbH, Munich, Germany) and (3) Sonoclot analysis (SCA; Sienco Inc., Morrison, CO) [13]. These methods are global tests of hemostasis analyzing from a single sample of blood all phases of hemostasis, including the dynamics of clot formation (balance of procoagulant and anti-coagulant factors), clot maturation and strength (platelet and fibrinogen), and clot stability and lysis (fibrinolysis and FXIII) [4, 13].

The principle of the assay consists of (1) *measuring the force* exerted on a small metal rod suspended in whole blood during clot formation while either the cuvette ("resistance to motion") (TEG, ROTEM) or the rod is rotated (ROTEM) or (2) *measuring ultrasound density* during clot formation (sono-rheometry; SONOCLOT). A small amount of whole blood (less than 5 mL) at body temperature (37 °C) is placed in an oscillating cup. A pin suspended from a torsion wire couple with the blood as fibrin form, and the result is increased wire tension as detected by an electromagnetic transducer. The resulting electrical signal is converted to the TEG trace, which can be displayed in real time on a computer monitor allowing the analysis of the TEG curve and variables. Key elements of the whole blood TEG include the reaction time (R time), which reflects the quantity of available factors; clot formation time (K); alpha angle (α) reflecting the rate of clot formation and indirectly indicating fibrinogen levels; maximum amplitude (MA), which is an indicator of platelet activity; and finally, a measure of clot lysis [13, 14] (Fig. 45.2).

Global viscoelastic tests (TEG, ROTEM, and Sonoclot) provide a more physiological assessment of coagulation and should be considered a practical guidance for

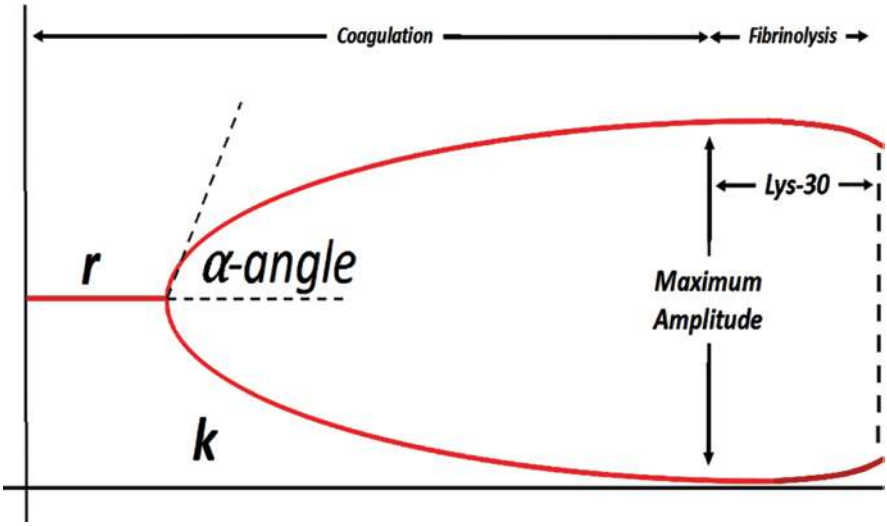

Fig. 45.2 Thromboelastogram curve analysis to assess the bleeding risk in patients with advanced alcohol-related liver disease. (Adapted from Stravitz [13] and Premkumar [14])

blood transfusion requirements in patients undergoing liver transplantation and other major surgeries. TEG is now widely used in cirrhotic patients during surgery and liver transplantation to guide transfusion, coagulation factor replacement, and antifibrinolytic therapy. Only limited data also suggest that TEG more accurately reflects the balance of hemostasis in patients with chronic liver disease outside of surgery [15]. A major limitation in the interpretation of these tests is that they assess *in vitro* hemostasis and cannot express the entirely *in vivo* hemostatic milieu engaging endothelium, tissue factors, portal pressure and flow.

Common Clinical Scenarios of Coagulopathy in Alcoholic Cirrhosis

The unstable *re-balanced* hemostatic state in the setting of liver cirrhosis and portal hypertension could be easily destabilized and tipped toward either a bleeding or a thrombotic phenotype by various triggers. The clinician is facing with two common clinical scenarios (1) *bleeding, related to portal hypertension, spontaneous or procedure-related,* and (2) *thrombosis, either splanchnic or systemic*. Patients with cirrhosis and liver failure are often concurrently coagulopathic, hypercoagulable and hyperfibrinolytic. The resulting clinical state (bleeding or thrombosis) is depends on which hemostatic mechanisms predominates. Moreover, the simultaneously hypercoagulable and hypocoagulable features in an individual patient may

contribute to a complex scenario consisting of concomitant bleeding at one site and thrombosis to another (eg. acute portal vein thrombosis followed by variceal variceal bleeding) [9].

Hypocoagulable/Bleeding Complications in Alcoholic Cirrhosis

Clinical Scenarios

Although considered the dominant clinical problem in cirrhosis, bleeding phenotype is less frequent than generally assumed and may be encountered in several clinical scenarios. Bleeding complications in cirrhosis are infrequently related to abnormal hemostasis. Most clinically significant bleeding episodes are due to increased portal pressure rather than to the deranged hemostasis.

Portal Hypertension-Related Bleeding

In patients with cirrhosis, bleeding is usually the consequence of portal hypertension and primarily related to hemodynamic and mechanical factors, with less relevance of hemostatic mechanisms. Portal hypertension-related bleeding events consist of acute variceal (esophageal, gastric, or ectopic) and non-variceal (portal-hypertensive gastropathy, enteropathy or colopathy) gastrointestinal bleeding. Approximately 50% of cirrhotic patients develop gastroesophageal varices and one-third of them will experience variceal bleeding with an in-hospital mortality rate of approximately 15% per episode [8].

Bleeding Induced by Hemostatic Failure

Bleeding events instigated by hemostatic failure usually occur spontaneously. Spontaneous mucosal or puncture wound bleeding (hematomas, oozing from oropharyngeal or genital mucosa), results from premature clot disolution and hyperfibrinolysis and it is named in the setting of advanced liver disease *accelerated intravascular coagulation and fibrinolysis—AICF*. Although similar to consumptive coagulopathy of disseminated intravascular coagulation (DIC), it can can be

distinguished by the high FVIII level [16]. Apart of these intrinsic alterations, alcohol and drugs (aspirin, AINS, antibiotics, cardiovascular, anti-depression, lipid-lowering drugs) have an important contribution to *acquired platelet dysfunction* in our (overmedicated) society, aggravating hemorrhagic diathesis.

Procedure-Related Bleeding Episodes

Various invasive liver and nonliver specific procedures may be necessary in patients with liver cirrhosis. Procedure-related bleeding events in cirrhosis result from the combination between hemostatic failure, portal hypertension and mechanical factors such as incision, puncture or vascular trauma (vessel rupture/puncture). Accurately determining procedural bleeding risk is a complex process and requires collaboration between specialists to determine the level of bleeding risk before procedures, as well as the periprocedural hemostasis management.

Prediction of the Risk of Bleeding

A procedure-related risk stratification in *low-risk* and *high-risk* procedures (Table 45.1) is accepted based on expert opinion and the overall bleeding risk reported in the literature so far, where low-risk procedures have been defined as procedures associated with significant bleeding in less than 1.5% of cases, that can be easily controlled, while high-risk procedures have a risk of major bleeding higher than 1.5%, can be difficult to control or may lead to catastrophic consequences, even in small amounts (eg, central nervous system bleeding) [17–19]. In clinical practice, it is admitted that several low-risk procedures do not routinely require coagulation assessment in patients with cirrhosis before their performance, including diagnostic and therapeutic paracentesis, thoracentesis, upper endoscopy to screening for and banding esophageal varices, and diagnostic colonoscopy [9]. Assessment of bleeding risk for an individual procedure in patients with cirrhosis is a complex process influenced by numerous interdependent factors grouped into *procedural and technical factors* (such as technique and operator skills and experience), *factors related to liver disease* (severity of liver dysfunction, low fibrinogen levels and advanced portal hypertension are independent risk factors for procedure-related bleeding) and *systemic factors* (comorbidities such as chronic kidney disease, medication such as antiplatelet therapy and anticoagulation which should be carefully considered in the periprocedural period).

Table 45.1 Classification of procedure-related risk of bleeding in patients with cirrhosis

	Low risk procedures	High-risk procedures
Percutaneous	Paracentesis	Biliary interventions: percutaneous biliary drainage
	Thoracentesis	Liver and non-liver intra-abdominal solid organ biopsy
	Drainage catheter exchange	Tumor ablation
		Intrathoracic organ biopsy
		Central nervous system procedures
		Intra-articular injections
Vascular	Central venous catheter line placement and removal	Transjugular intrahepatic porto-systemic shunt (TIPS)
	Diagnostic coronary angiography and right heart catheterization	Transjugular liver biopsy
	HVPG measurement	Locoregional therapies for hepatocellular carcinoma: transhepatic arterial chemo or radio-embolization, radiofrequency or microwave ablation
		Lumbar puncture
		Therapeutic coronary angiography
Endoscopic	Diagnostic upper gastrointestinal endoscopy	Endoscopic polypectomy
	Routine band ligation	Endoscopic stricture dilatation and endoscopic mucosal resection/dissection
	Enteroscopy and colonoscopy (including mucosal biopsy)	Balloon-assisted enteroscopy
	Endoscopic retrograde cholangiopancreatography without sphincterotomy	Endoscopic retrograde cholangiopancreatography with sphincterotomy
	Capsule endoscopy	Percutaneous endoscopic gastrostomy placement
	Endoscopic ultrasound without fine-needle aspiration	Endoscopic ultrasound with fine-needle aspiration
	Transesophageal ecocardiography	Cystgastrostomy
	Diagnostic bronchoscopy without biopsy	Diagnostic bronchoscopy with biopsy or therapeutic bronchoscopy
Others	Skin biopsy	
	Dental non-extraction procedures	Dental extraction

TIPS transjugular intrahepatic porto-systemic shunt
Adapted from Northrup [5], Davidson [17] and Patel [18]

Therapeutic Interventions to Prevent or Control Bleeding

Blood product transfusion can be lifesaving, but the risks and complications of this practice are often underestimated. Transfusion reactions, exacerbation of portal hypertension due to transfusion-associated circulatory volume expansion, bacterial and viral infection transmission, transfusion-related acute lung injury, potential hypercoagulable complications (eg, portal vein thrombosis), as well as long-term

immunologic complications (eg, development of HLA antibodies impacting subsequent transplantation or impairing the ability to receive further transfusions) may alter patient's prognosis by increasing intensive care unit stay, hospitalization, and mortality.

Therefore, it is mandatory to reconsider the practice patterns of blood product transfusion, incorporating agents that have lower volume and risk as well as standardizing the process. A standardized protocol of utilization of blood products and pro-coagulants should be implemented and followed in every unit. The recent AGA Clinical Practice Expert Review on Coagulation in Cirrhosis [1] summarized the current recommendations for active bleeding and bleeding prophylaxis management. Despite the shortcomings of standard coagulation tests, measuring platelet count and fibrinogen level still represent the standard of care assessment of coagulation for all patients with cirrhosis before a procedure.

Hemostatic Strategy and Targets: Utilization of Procoagulant Factors Including Blood Products

In both active portal hypertension-related or spontaneous bleeding, as well as for the prophylaxis of bleeding associated with high-risk procedures, the recommended targets of hemostatic strategy consist of maintaining hematocrit ≥25%, platelet count >50,000/mm^3, and fibrinogen >120 mg/dL in order to optimize clot formation in patients with advanced liver disease [1, 5].

Platelet Transfusion and Oral Thrombopoietin Agonists

Studies have shown that a target platelet count of 56,000/mm^3 suffices to control variceal bleeding because of intact platelet dependent thrombin generation in cirrhosis [11, 15]. There is a consensus that the platelet count should be maintained above 50,000/mm^3 during acute bleeding, the level shown to ensure adequate thrombin generation in vitro.

Given the low-risk of bleeding of many common procedures, potential risks of platelet transfusion, lack of evidence that elevating the platelet count reduces bleeding risk, and ability to use effective interventions, including transfusion and hemostasis if bleeding occurs, it is reasonable to perform low-risk procedures without prophylactically correcting the platelet count [1]. Platelet transfusion derived from single or multiple donors (250 mL of platelet-rich plasma/unit) is expected to increase the platelet count by 5000–10,000/mm^3 [1]. The increment in platelets is often poor in patients with hypersplenism, active bleeding and/or coexistent infection [15]. Oral thrombopoietin agonists (Avatrombopag, Lusutrombopag) are a good alternative to platelet transfusion, but require time (10 to 20 days) to elevate platelet levels and are indicated in patients scheduled to undergo a procedure in elective setting [20, 21].

Red Blood Cell Transfusions

Raising the hematocrit above 25% by red blood transfusion (total volume 250 mL/unit) may improve the margination of platelet and hemostasis and is recommended especially in the setting of renal dysfunction and severe anemia [1].

Cryoprecipitate

Low fibrinogen levels (<100 mg/dL) are associated with spontaneous and procedure-related bleeding in patients with cirrhosis [22]. Recently, fibrinogen levels have emerged as potentially useful tool to couple with platelet count as a measure of bleeding risk, with target levels 120–150 mg/dL. This level is best achieved with cryoprecipitate, a low-volume product having vWF, fibrinogen and fibronectin that does not need cross-matching. Cryoprecipitate is administered on a weight-base dose (1 U of cryoprecipitate per 10 kg body for a volume of 10–20 mL/U). The average dose is 5–10 U resulting from 50–200 mL cryoprecipitate. The increase in plasma fibrinogen from 1 U of cryoprecipitate per 10 kg body weight will be approximately 50 mg/dL.

Fresh Frozen Plasma

Standard doses of fresh frozen plasma (FFP) (approximately 250 mL/U dosed at 10 mL/kg) are not recommended to correct any coagulation factor deficiency [9]. FFP transfusion before procedures is associated with risks and no proven benefits. Increased infused volume of FFP predisposes to transfusion-associated circulatory overload and transfusion-related acute lung injury (TRALI) and determine a substantial increase in portal pressure directly proportional to the volume transfused [23]. For every 100 mL rapid expansion of blood volume, portal pressure increases by 1 mmHg, potentially leading to portal collateral-related bleeding [24].

Concentrated Vitamin K–Dependent Clotting Factors

Prothrombin complex concentrates (PCCs) are plasma-derived products that contain vitamin K-dependent coagulation factors (II, IX, X, VII) and anticoagulant proteins (protein C and protein S). PPCs are available as three-factor (containing factors II, IX, X, very low concentration of FVII and little/no protein C and S) and four-factor (II, IX, X, with the addition of FVII, protein C and S) products. PCC show advantages over FFP including delivery of a smaller volume with a 25-fold higher

concentration of coagulation factors and more rapid correction of hemostatic parameters. In the absence of evidence confirming benefit, the routine use of PCC is not recommended for bleeding complications and may increase the risk of thrombotic events in patients with liver cirrhosis.

Recombinant Factor VIIa (rFVIIa)

Although rFVIIa normalizes a prolonged PT/INR, there is no evidence that it reduces bleeding. Several randomized studies found the use of rFVIIa in addition to standard pharmacologic therapy and endoscopy [25, 26], as well as a Cochrane systematic meta-analysis [27] found that rFVIIa did not reduce mortality in patients with liver disease and upper GI bleeding concluding that there is insufficient evidence to support the use of rFVIIa in this setting.

Antifibrinolytic Agents

Diagnostic laboratory tests for hyperfibrinolysis are not available in clinical practice, and current viscoelastic testing is not sensitive for moderate or mild hyperfibrinolysis. Therefore, the diagnosis of hyperfibrinolytic postprocedural bleeding is made clinically; typical manifestations include continuous venous oozing from skin puncture sites and persistent mucosal or submucosal bleeding. Epsilon-aminocaproic acid (EACA) is a lysine analogue that prevents plasminogen and tissue plasminogen activator (tPA) fibrin binding. Administered for a short period of time either by oral (3 g times per day) or intravenous route (4–5 g in 250 mL by infusion over 1 h, followed by 1 g/h in 50 mL of saline, continued for 8 h or until bleeding is controlled) [1], it has shown benefit in controlling fibrinolytic bleeding without major toxicity. Tranexamic acid is recommended at a dose of 1 g IV every 6 h. Antifibrinolytic agents are commonly used as rescue therapy in major bleeding events that occur after procedures [5].

Vitamin K

Vitamin K will take more than 12 h to correct the hemostatic defect in vitamin K–deficient patients and typically has only a minor impact on the prothrombin time. It can be administered either orally as a 10-mg tablet or 10-mg IV (iv administration carries risk of anaphylaxis), the latter should be mandatory in patients with impaired bile flow, e.g., at bilirubin levels higher than 2 mg/dL. It can be effective when patients have history of prolonged antibiotic therapy, poor nutrition, or severe malabsorption [1].

Hypercoagulable/Thrombotic Complications in Alcohol-Related Cirrhosis

Hypercoagulation in liver disease results from the complex interplay of three pathophysiologic factors: low velocity of portal flow, endothelial dysfunction, and decreased synthesis of the naturally occurring anticoagulant proteins. Therefore, thromboembolic events occur with an increased incidence among patients with cirrhosis, irrespective of etiology, despite standard coagulation tests revealing a prolonged PT/INR [28].

Three main clinical scenarios consisting of thrombotic complications may be encountered: (1) venous thromboembolism (VTE) including peripheral deep vein thrombosis (DVT) and pulmonary embolism (PE), (2) portal vein thrombosis (PVT), and (3) thrombin activation and occlusion of small intrahepatic vessels.

Deep Vein Thrombosis and Pulmonary Embolism

Incidence and prevalence of DVT and PE among patients with cirrhosis have been estimated in several case-control, retrospective and cross-sectional studies reporting an incidence varying from 0.5% to 8.2% [29–33]. A systematic review and meta-analysis of literature evaluating the risk VTE associated with cirrhosis, showed a significantly increased risk in cirrhotic patients as compared with non-cirrhotic controls with a cumulative odds ratio for all venous tromboembolic events of 1.7 for patients with cirrhosis [34]. These results were confirmed when specifically considering the risk of DVT (odds ratio 2.038) and the risk of PE (odds ratio 1.655). Despite this risk, prophylactic anticoagulation for VTE in hospitalized cirrhotics is significantly lower than in non-cirrhotic patients [35], although current evidence supports the use of routine DVT prophylaxis in cirrhotic patients in the absence of evident contraindications [36].

Patients with cirrhosis and VTE may share the same risk factors as other non-cirrhotic patients with thrombotic complications such as venous stasis, infection, congestive heart failure, acute respiratory disease and immobilization. Surgery is one of the major risk factors for VTE in cirrhotic patients [31]. Among the liver-related risk factors, hepatocellular carcinoma (HCC) has been shown to correlate with a higher incidence of thrombotic complications. Although liver disease etiology has not been demonstrated as a risk factor for VTE [37], patients with cholestatic liver disease (including patients with alcohol-related liver disease) show enhanced thrombin generation and platelet adhesion as compared to non-cholestatic etiologies as a result of chronic exposure to high levels of bilirubin. Moreover, patients with non-alcoholic fatty liver disease and metabolic syndrome have been associated with a greater risk of atherosclerosis and endothelial dysfunction contributing to the prothrombotic state [38].

Portal Vein Thrombosis

PVT is the most common and challenging thrombotic event occurring in cirrhotic patients being increasingly recognized due to improvement of diagnostic imaging methods and better awareness amongst clinicians. Incidence of PVT ranges from 5% at 1 year to 40% at 10 years [39]. The relative risk of developing PVT in cirrhosis is sevenfold higher as compared to the risk observed in the general population; it increases with the degree of liver failure and in the presence of HCC, being as low as 1% in patients with compensated disease and rising to 8–25% in patients with end-stage liver disease awaiting liver transplantation and to 40% in patients with HCC [40]. The multicenter prospective study PRO-LIVER (PVT Relevance On Liver Cirrhosis: Italian Venous Thrombotic Events Registry), including 753 Caucasian cirrhotic patients, reported a prevalence of US-documented PVT of 17% and an annual incidence rate of 6.05% [41].

Low portal vein flow velocity, malignancy, prior VTE, thrombophilia, intra-abdominal infection, and history of recent interventions increase the risk for PVT; emerging data suggest that non-alcoholic etiology may be an independent risk factor for thrombotic events, including PVT [42].

PVT classification and terminology are standardized for clinical and research purposes using combined criteria including time course (acute or chronic—if less or more than 6 months), extension (intra- or extrahepatic splenic or mesenteric veins), degree of occlusion (minimal, partial, complete or cavernoma formation), and time change (stable, progressive, regressive) [39].

Most cirrhotic patients are diagnosed with PVT incidentally, during routine ultrasound (US), computed tomography or magnetic resonance imaging evaluation, while some other cases may present with abdominal pain, fever or hepatic decompensation (ascites, encephalopathy, jaundice). The first-line technique for PVT detection is Doppler US (sensitivity about 90% for complete PVT and about 50% for partial PVT) and high operator-dependence. Contrast-enhanced imaging techniques have comparable sensitivity for PVT diagnosis and are used for a better characterization of PVT (extension, degree of occlusion, cavernoma formation).

The most common evolution of acute PVT is spontaneous resolution or disease stability, which have been described in 45–70% of cases [43]. Progression has been reported especially in extensive thrombosis, in one-third of patients at 2 years follow-up.

PVT is associated with a negative impact on the outcome of liver cirrhosis due to the further increase in portal hypertension which may lead to potentially life-threatening bleeding events and worsening of cirrhosis and decompensation caused by decreased hepatic perfusion [44]. Moreover, it definitely increases the technical complexity of liver transplant surgery and may negatively impact transplant outcomes [45]. Improvement in medical and surgical strategies over the last decade overcomes surgical difficulties and, as a consequence, PVT is no longer considered an absolute contraindication for liver transplantation.

An ideal strategy for the treatment of PVT in patients with cirrhosis should have reasonable success rate in reestablishing physiologic flow in the portal system, minimal risk of therapy-induced complications related to the therapy, and be simple and convenient to administer and monitor. The two currently available options include (1) long term anticoagulation therapy and (2) transjugular intrahepatic portosystemic shunt (TIPS) with mechanical or intraprocedural thrombolysis in selected cases.

Anticoagulation is an attractive therapeutic option of PVT since it is widely available, does not require specific procedural interventions, can be started and stopped promptly as needed and has low rates of complications. Pharmacologic agents include vitamin K antagonists (VKAs), low molecular weight heparins (LMWHs), and more recently, the direct acting oral anticoagulants (DOACs) inhibiting factor X or thrombin [46].

Old anticoagulant agents, VKAs and LMWHs, are widely used and appear to be equally effective in cirrhotic patients with PVT. VKAs (warfarin, acenocoumarin) are oral agents that require close monitoring using INR, have a narrow therapeutic window (a target INR of 2.0–3.0), not validated in cirrhotic patients, and are perceived as associated with an increased risk of bleeding in the setting of cirrhosis. Anticoagulation with LMWHs has been extensively studied in cirrhotic population and appears to be safe and effective. Once the risk of bleeding from esophageal varices is controlled by using beta-blockers or band ligation, the rate of bleeding complications in cirrhotic patients with PVT on therapeutic anticoagulation seems to be similar to the non-cirrhotic population. Of note, a significantly lower rate of variceal bleeding in anticoagulated patients was reported in some studies compared to untreated patients [47]. The main limitation of using LMWHs for PVT consists of repeated subcutaneous injections making this therapy poorly acceptable for a long period and causing a high rate of non-adherence; in addition, their efficacy may be compromised by the low plasma level of antithrombin in patients with cirrhosis.

DOACs that inhibit thrombin (dabigatran) or activated factor X (rivaroxaban, apixaban, edoxaban) recently emerged as potential alternatives to VKAs and LMWHs in patients with PVT, because they are oral drugs that do not require laboratory monitoring for dosage [48]. However, they are currently prescribed *off-label* in this setting because patients with cirrhosis were excluded from clinical trials comparing DOACs versus VKAs and LMWHs for prophylaxis or treatment of VTE or atrial fibrillation [49–51].

There are limited data on the pharmacodynamics of DOACs in cirrhosis, rising significant concerns of bleeding and other adverse events. Clinical experience with DOACs in patients with cirrhosis remains sparse and limited to highly select cohorts with well-compensated cirrhosis. Overall, DOACs appear to have a similar safety profile in patients with compensated cirrhosis compared to patients without cirrhosis, and their use is expanding in all indications for anticoagulation, including PVT [48]. Moreover, the availability of direct reversal agents for DOACs (idarucizumab for dabigatran and andexanet alfa for all the others) may attenuate the concerns of bleeding.

Anticoagulation induce reduction in thrombus volume as early as 2 weeks on therapy. The recommended duration of anticoagulant therapy resulting from the

meta-analysis by Loffredo et al. [47] is 6 to 12 months and is associated with 71% of partial and 53% of complete recanalization after 6 months of therapy. Portal vein recanalization (PVR) (thrombolysis) followed by TIPS should be considered in patients with chronic PVT and recurrent bleeding and/or refractory ascites not manageable medically or endoscopically. TIPS insertion was successful in more than 90% of the patients with partial or complete occlusive thrombi with similar rates of complications and mortality compared with patients without PVT [52, 53]. Key recommendations for anticoagulant therapy, but also for controlling bleeding in patients with cirrhosis are shown in Table 45.2 [1, 4].

Table 45.2 Key recommendations for thrombotic complications and bleeding in cirrhosis

Thrombotic complication	Specific management	Comments
Acute setting: acute portal or peripheral vein thrombosis or extension of prior thrombosis (especially if symptomatic)	Systemic anticoagulation with therapeutic dose of I.V. heparin or LMWH	High INR does not indicate an auto-anticoagulant state, and patients will still require anticoagulation
	In acute PVT and superior mesenteric vein thrombosis, catheter-mediated thrombolysis may be indicated	Heparin-induced thrombocytopenia and appearance of new-onset clinically evident bleeding may be challenging
Chronic PVT or history PVT or mesenteric thrombosis	Anticoagulation is indicated in Yerdel grades ≥2, especially in patients who are listed for liver transplantation	Patients with portal cavernoma are less likely to benefit
	Systemic anticoagulation with therapeutic LMWH is indicated	Asymtomatic incidentally discovered PVT does not invariably require anticoagulation
	Oral anticoagulants recommended include VKAs (problematic due to uncertainty of targeting therapeutic INR) and DOACs (safe and effective in Child-Pugh class A and early class B cirrhosis)	Conflicting data are available on primary prophylaxis of PVT to prevent decompensation
DVT therapy or prophylaxis in hospitalized patients if indicated (venous stasis, history of DVT and PE, immobilization, surgery etc.)	In the absence of bleeding, routine DVT prophylaxis is recommended	Variceal eradication is necessary
	Prophylaxis with LMWH should be offered	Anticoagulation is considered for at least 6 months or lifelong in cases of multiple episodes
	Therapeutic anticoagulation should be with oral anticoagulants	

(continued)

Table 45.2 (continued)

Thrombotic complication	Specific management	Comments
Bleeding complication	*Specific management*	*Comments*
Portal hypertension-related bleeding	Timely endoscopic management (variceal banding/sclerotherapy, glue injection etc.)	• Maintain Hb ~ 7 g/dL in all patients and 8 g/dL in cardiac patients (hematocrit >25%)
	Minimize volume expansion during resuscitation	• platelet transfusion for >50,000/mm^3
	Discuss role of emergency TIPS	• Fibrinogen level > 100–120–140 mg/dL
Wound/mucosal oozing suspicious for accelerated intravascular coagulation or hyper-fibrinolysis	Control infection (active infections release endothelial derived heparinoids which can have anticoagulant effect)	• platelet ≥50,000/mm^3, fibrinogen ≥ 120 mg/dL
	Optimize renal function (uremia-associated platelet dysfunction	• Consider anti-fibrinolytic agents (aminocaproic and tranexamic acid)
		• Role of prothrombin complex concentrates not yet defined
Invasive procedures prophylaxis	Risk-benefit ratio and local expertise should be considered	• High-risk: PLT > 50–60,000/mm^3
		• Very high-risk: PLT > 100,000/mm^3

LMWH low molecular weight heparins, *INR* international normalized ratio, *PVT* portal vein thrombosis, *VKAs* vitamin K antagonists, *DOACs* direct acting oral anticoagulants, *DVT* deep vein thrombosis, *PE* pulmonary embolism, *Hb* hemoglobin, *platelet* platelet, *TIPS* transjugular intrahepatic portosystemic shunt

Adapted from O'Leary [1] and Premkumar [4]; Official Learning Resource of AASLD, accessed May 2021

The Role of Hypercoagulability in Hepatic Fibrogenesis and Decompensation: Thrombin Activation and Occlusion of Small Intrahepatic Vessels

Local thrombin generation in portal and hepatic branches triggered by adjacent necroinflammation results in intrahepatic microvascular thrombosis which in turn causes ischemia-induced tissue injury (known as parenchymal extinction). This pathogenic mechanism plays an important role in cellular death and liver atrophy, activates stellate cells, and promotes fibrogenesis emerging as an important mediator of fibrogenesis. Anticoagulation prophylaxis in this setting may be beneficial on disease progression and parenchymal extinction/atrophy [54].

The detrimental effects of hypercoagulability were further documented in a prospective trial of enoxaparin therapy for patients without PVT [55], which demonstrated that enoxaparin treatment lowered the incidence of PVT, decreased the incidence of decompensation, and improved overall survival.

Conclusions

The hemostatic cascade is rebalanced at low levels and its equilibrium is extremely fragile in patients with cirrhosis. It can be easily destabilized by various triggers (e.g., infection, alcohol, uremia, anemia, medications etc.) toward either an anticoagulant or procoagulant phenotype. These complex hemostatic changes are not adequately captured by standard coagulation tests for identifying and monitoring coagulopathy. Further studies of global assessment of coagulation, such as TEG and ROTEM, are needed to determinate appropriate cut-offs for therapeutic interventions. Blood products transfusions should be used judiciously because they increase portal pressure and carry a risk of transfusion-associated circulatory overload, transfusion-related acute lung injury, infection transmission, alloimmunization, and/or transfusion reactions. The following transfusion thresholds for management of active bleeding or high-risk procedures may optimize clot formation in advanced liver disease: hematocrit ≥25%, platelet count >50,000/mmc and fibrinogen >120 mg/dL. In the setting of thrombotic complications, systemic heparin infusion is recommended for symptomatic DVT thromboembolism and portal and mesenteric vein thrombosis. Prophylaxis therapeutic strategy consists of LMWHs, VKAs, and DOACs according to the specific setting. Therapeutic intervention for incidental portal and mesenteric vein thrombosis should be based on careful clinical judgement and estimation of impact on transplantation surgical complexity versus risks of bleeding.

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Chapter 46
Hepato-Renal Syndrome in Patients with Alcohol-Related Liver Disease

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Abstract Hepatorenal syndrome (HRS) is defined as impaired renal function in the context of both acute or chronic liver failure. HRS is considered one of the most dreaded complications in chronic liver diseases. In the context of alcohol consumption, HRS can occur in the acute setting of alcoholic hepatitis (AH) or, in most cases, in alcohol-related liver cirrhosis. It can drastically worsen their prognosis especially for severe AH. This book chapter discusses the new classification of HRS, its diagnosis and underlying pathophysiology with regard to alcohol exposure, and, finally, the therapeutic management ranging from general measures, application of vasoactive drugs, hemodialysis, transjugular intrahepatic stent shunt (TIPS) till Molecular Adsorbent Recirculating System (MARS), and liver transplantation. HRS is considered a frequent complication that arises in cirrhotics and AH causing death in more than 90% of the patients in the first 3 months after the onset of the symptoms, in the absence of liver transplantation.

Keywords Hepatorenal syndrome · Alcoholic hepatitis · Terlipressin · Pathophysiology · Diagnosis · Management · Albumin

Introduction

Hepatorenal syndrome (HRS) is a dreaded complication which develops in patients with liver cirrhosis and has a negative impact on the survival rates. The early recognition of HRS and prompt initiation of specific treatment is mandatory because it may change the prognosis in any cirrhotic patient. The epidemiology of HRS

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differs worldwide, but in the United States, HRS affects between 9000 and 35,000 patients annually. It is considered that approximately 20% of the hospitalized patients with cirrhosis are diagnosed with HRS [1].

Definition of HRS

HRS is defined as the impairment of renal function occurring in the context of both acute liver failure, alcohol or non-alcohol related, and chronic liver failure such as liver cirrhosis [2, 3]. The pathophysiology of kidney failure in HRS is not very well defined, but the most studied mechanism which could induce the malfunction of kidneys in liver diseases is represented by renal vasoconstriction caused by an important decline in effective circulating volume in a setting of systemic and splanchnic vasodilatation, which consequently will lead to a decrease in kidney perfusion [4, 5]. Patients with HRS typically have decreased arterial pressure combined with an elevated cardiac output. The release of circulating vasodilators from the endothelium is one of the leading causes for arterial vasodilatation, and consequent activation of endogenous vasoconstrictor systems resulting in a reduced glomerular filtration rate [6]. The definition of HRS has changed over the years, traditionally including two types. HRS type-1 is characterized by a doubling of the serum creatinine with values higher than 2.5 mg/dL within 2 weeks. HRS type-2 is defined as a slower increase in serum creatinine higher than 1.5 mg/dL [7] (see also Table 46.1). In order to establish the diagnosis of HRS, other causes of kidney injury have to be excluded such as the absence of cardiovascular shock or nephrotoxic drugs. Second, no improvement should be observable after diuretics withdrawal or volume expansion with albumin for 2 days. Third, kidney morphology should be normal in ultrasound, and, finally, no hematuria defined by more than 50 erythrocytes per high power field in urine and absence of proteinuria >500 mg/24 h should be confirmed.

An important change regarding HRS definition emerged with the acceptance of the new definition of Acute Kidney Injury (AKI). The definition of AKI was proposed by the Hepatology community and the International Club of Ascites [8]. The definition of HRS now relies first and foremost on the definition of AKI. The new concept of AKI was introduced because the old definition of HRS had several shortcomings which contributed to delays in establishing the diagnosis, or even misdiagnosis. In the last few years, the Kidney Disease Improving Global Outcome (KDIGO) criteria were modified in relation to AKI. These changes were necessary since serum creatinine-based criteria turned out to be important for estimating mortality rates in patients with liver cirrhosis and deciding early treatment initiation [8]. KDIGO criteria helped to understand HRS and mortality risk stratification according to AKI stage and to speed up the time for diagnosis [9, 10]. After the consensus of the ICA, HRS is now specified by the presence of AKI. Consequently, conventional HRS type-1 is now referred to as HRS-AKI being defined by changes in serum creatinine and/or urine output along to the criteria shown in Fig. 46.1. To

Table 46.1 The evolution of diagnosis criteria for hepatorenal syndrome

	1996 ICA	2007 ICA	2012 KDIGO/2015 ICA	2022 ICA and KDIGO accepted criteria's
Criteria for HRS diagnosis	– Serum creatinine >1.5 mg/dL or 24-h creatinine clearance <40 mL/min. – Absence of shock, nephrotoxic drugs, GI fluid loss, renal fluid loss – No improvement after diuretic withdraws and plasma expansion volume – No US-signs of renal disease – Proteinuria <500 mg/dL	– Type 1 HRS defined by rapidly progressive renal impairment – Doubling sCr at a value >2.5 mg/dL in less than 2 weeks – Type 2 HRS defined by moderate renal failure with sCr between 1.5 and 2.5 mg/dL – Slowly progressive course	– Criteria remove the absolute creatinine value of at least 1.5 mg/dL as a requirement. – Use of acute kidney injury (AKI) criteria. – The importance of having a baseline sCr for diagnosis. Baseline - a prior sCr within 3 months	– Cirrhosis and ascites – Diagnosis of AKI according to ICA- AKI criteria – Absence of shock – No response to diuretic withdrawal and plasma volume expansion – No use of nephrotoxic drugs – No macroscopic signs of structural kidney injury

AKI acute kidney injury, *AKI* acute kidney injury, *GI* gastrointestinal, *HRS* hepatorenal syndrome, *ICA* international club of ascites, *KDIGO* kidney disease improving global outcome, *sCr* serum creatinine, *US* ultrasound

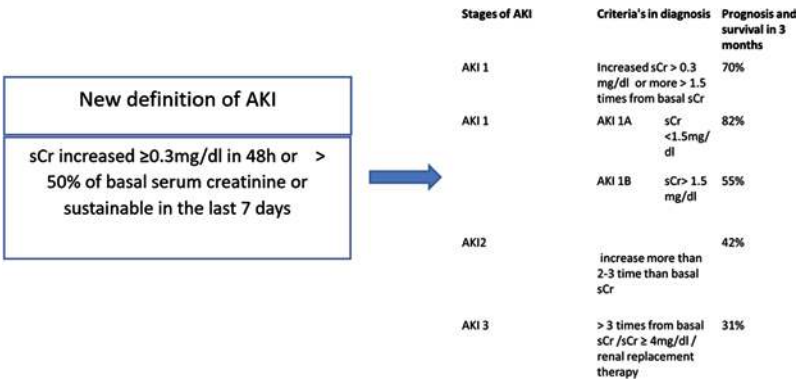

Fig. 46.1 Diagnosis criteria for AKI. Substages of AKI based on serum creatinine. *Created with* BioRender.com. Abbreviations: *AKI* acute kidney injury, *sCr* serum creatinine

improve early treatment efficiency, the new classification removes the cut-off values for serum creatinine of ≥1.5 mg/dL and suggests treatment even in cases of small increases. HRS type-2 is now defined as HRS without AKI and manifest chronic kidney disease [8].

Pathophysiology of HRS in ALD

Alcohol-related liver disease (ALD) is an umbrella term that includes several disease stages ranging from simple steatosis to steatohepatitis, acute alcoholic hepatitis, and liver cirrhosis. Severe alcoholic hepatitis (AH) develops in a minority of patients (typically <2%) with heavy alcohol consumption and is characterized by the onset of jaundice and signs of liver inflammation. Severe AH has a high mortality rate, with approximately 40% patients dying within the first 6 months after hospital admission [11]. Several factors contribute to the high mortality such as sepsis, variceal bleeding, liver failure, and HRS, respectively. Although HRS as long been considered as a specific complication restricted to cirrhotic patients with ascites, it is nowadays considered a frequent complication that also arises patients with severe AH, complicates the course of the disease, and, in the absence of liver transplantation, causes death in more than 90% of the patients in the first 3 months after the onset of the symptoms [12]. Similarly, in patients with liver cirrhosis, HRS is a dreaded complication with a median survival of about one-month [13].

As already mentioned above, HRS is defined as a renal dysfunction which arises in patients with chronic liver diseases – especially in advanced cirrhosis, or in acute liver failure. There are several mechanisms that have been proposed to cause HRS, the most accepted includes splanchnic vasodilation due to portal hypertension which predisposes to peripheral and subsequently renal vasoconstriction leading to a decrease in glomerular filtration rate (Fig. 46.2) [14]. Although this mechanism is the hallmark of HRS, there is evidence for several other contributors, such as systemic inflammation, cirrhotic cardiomyopathy, and adrenal insufficiency.

The Uncompensated Hyperdynamic Circulation

Due to liver inflammation, increased vascular resistance and portal hypertension, several vasodilatory mediators are released into the splanchnic and later systemic circulation which finally lead to arterial vasodilatation. The most known mediators that can induce significant vasodilatation are nitric oxide, carbon monoxide, prostacyclin and endogenous cannabinoids [15, 16]. The splanchnic vasodilation will cause a decrease of effective circulating volume, a decrease of systemic arterial pressure and, ultimately, an activation of systemic vasoconstrictor pathways such as the renin-angiotensin-aldosterone system (RAAS). The sympathetic nervous system acts by releasing angiotensin II, norepinephrine, and antidiuretic hormonein order to increase the effective circulating volume. These mechanisms results in Na^+ retention, water retention through reduced water excretion and renal vasoconstriction which subsequently determines a decreased renal perfusion. In the early stages of liver cirrhosis, the kidneys are able to maintain an adequate blood flow and thus a normal glomerular filtration rate, but with the progression of the disease the glomerular pressure is impaired and leads to renal disfunction [17].

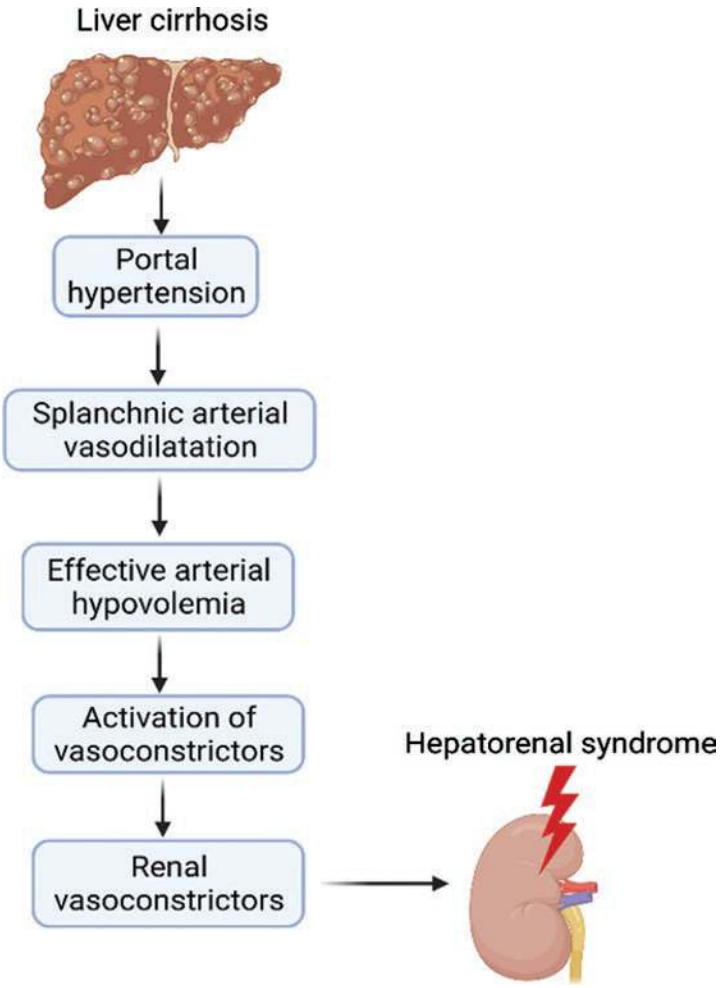

Fig. 46.2 The mechanism of hepatorenal syndrome due to portal hypertension

Systemic Inflammatory Response Syndrome

The systemic inflammatory response syndrome occurring in liver cirrhosis encompasses two types of patterns, the pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). PAMPs are bacterial-released products which result during bacterial translocation whereas DAMPs are cellular products originating from damaged hepatocytes. PAMPs and DAMPs will produce an inflammatory state and release of cytokines by activating toll-like receptors. These events will eventually lead to a systemic inflammatory response which stimulates the arterial production of vasodilators, resulting in a decreased systemic vascular resistance (Fig. 46.3). Data from current literature show that, in patients with

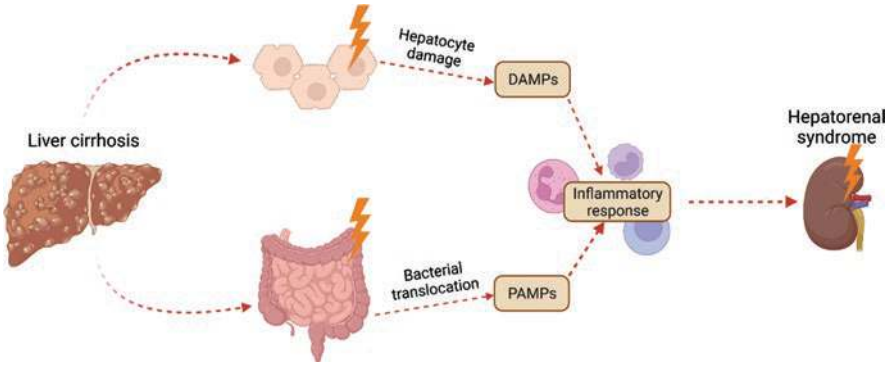

Fig. 46.3 The role of PAMPs and DAMPs in inducing hepatorenal syndrome (*created with* BioRender.com)

liver cirrhosis and impairment of renal function, there is an overexpression of toll-like receptor 4 (TLR4), which has been linked to a prolonged exposure to PAMPs [18]. Furthermore, it has been demonstrated that renal ischemia due to reduced blood flow in HRS induces an increase of TLR4 [19]. There are other possible mechanisms related to innate immune system that can promote HRS, but there is no scientific evidence yet to sustain these theories.

Hepato-Adrenal Syndrome

In about 50% of the patients with decompensated liver cirrhosis and ascites, there is a relative adrenal dysfunction which can be a risk factor for HRS. This category of patients has low blood pressure values, important circulating levels of renin and noradrenaline, and, secondary to the unavailability of cortisol substrates and the disturbance of the hypothalamus pituitary axis in response to cytokines and PAMPs. Patients with decompensated liver cirrhosis and developing HRS have high risks of sepsis and short-term mortality [20].

Bile Cast Nephropathy

Bile cast nephropathy (BCN), known under several terms such as cholemic nephropathy, biliary nephrosis and jaundice-related nephropathy, is frequently found in patients with liver cirrhosis and other cholestatic liver diseases. It seems that BCN affects most patients with high bilirubin levels and HRS and has a negative impact on therapy response and prognosis [21]. Morphologically, it is represented by the

formation of bile acid casts which cause tubular obstruction and toxicity, as well as an altered kidney perfusion. The tubular obstruction has been demonstrated by several histopathologic studies through the presence of intratubular bile acid casts in up to 75% of HRS cases [21]. Furthermore, experimental studies have demonstrated that hyperbilirubinemia can cause "jaundice heart" due to chronotropic and inotropic effects which lead to renal hypoperfusion, corticomedullary junction ischemia and tubular injury, all related to HRS [22]. The diagnosis of BCN can be suggested by elevated urinary bilirubin and urobilinogen levels. Serum bilirubin levels of more than 10 mg/dL are usually associated with a poor response to vasoconstrictors and high mortality rates in HRS [21]. Therefore, targeting bile acids by agents such as ursodeoxycholic acid or norursodeoxycholic acid are thought to improve the response to treatment in patients with HRS and BCN.

Intra-Abdominal Hypertension (IAH)

An important role of a high intra-abdominal pressure (above 12 mmHg) in the pathophysiology of AKI has also been demonstrated. Furthermore, in patients with refractory ascites, IAH seems to be an underestimated cause of HRS. A recent study demonstrated that reduction of intra-abdominal pressure by large volume paracentesis (LVP) short-term improved creatinine levels in patients with HRS [23]. However, it is important to carefully monitor the diameter of the lower caval vein in order to prevent post-paracentesis circulatory dysfunction by applying albumin and plasma expanders.

Direct Alcohol Effects on Kidneys

Although the mechanisms resulting in alcohol-related kidney injury are yet to be established, the role of alcohol in inducing renal dysfunction is well known. Several studies have demonstrated harmful effects of chronic alcohol consumption on kidneys termed "alcoholic kidney injury" [24]. The pathophysiologic mechanisms that have been linked to kidney damage are caused both directly by the toxicity of alcohol and by the increased amounts of the metabolites including acetaldehyde, or the release of NADH and free radicals from damaged cells. Reactive oxygen species (ROS) are thought to trigger alcohol-related tissue injury. Recent data show that alcohol stimulates mitochondrial protein hyperacetylation in the kidney, which may interfere with the function of some mitochondrial proteins involved in alcohol metabolism or defense against oxidative stress which can significantly contribute to alcohol-induced mitochondrial dysfunction in the renal tissue [25].

Predictors of HRS in ALD

In HRS, there are several predictors that could guide the management of these patients. The major risk factors for HRS are low blood sodium, a high level of plasma renin activity, liver size, and the degree of ascites [26, 27]. There are very few cases of unprecipitated HRS, usually it develops in the setting of infections or LVP without standard albumin administration. Data from current literature shows that HRS occurs in more than one quarter of patients with spontaneous bacterial peritonitis (SBP) and has a strong negative impact on survival rates [28]. The prevention of HRS associated to infections could be achieved by administrating albumin in combination with specific antibiotics in patients with SBP or other types of infections, which can significantly improve overall mortality. Furthermore, it has been demonstrated that albumin infusion in patients with infections has a positive impact on effective circulatory volume which is favors an adequate renal function. Other advantages of albumin are represented by the ability to inactivate endotoxins and by the potential immunomodulatory effects [29].

Diagnosis of HRS

As mentioned above, the new diagnostic criteria of HRS allow an earlier diagnosis and specific treatment initiation (Table 46.1, Fig. 46.1). Accordingly, the specific treatment for HRS should be started immediately after the documentation of a lack of response to fluid challenge, without the need to wait for a doubling of creatinine levels. Furthermore, it is important to know that most patients with HRS stage 1A will respond to fluid challenge because it is frequently caused by hypovolemia. In patients with HRS stage 1B, only half will have a favorable response to repletion therapy and therefore current guidelines state that vasoconstrictors should be used only in this category of patients.

The exclusion of structural kidney injury is very important and is stipulated as a diagnostic criterion. Prerenal kidney failure azotemia is the most common cause of AKI in liver cirrhosis, and it develops as a complication to diuretics and LVP without albumin infusion or gastrointestinal bleeding. Thus, for establishing the diagnosis of HRS, the following should be confirmed: the absence of shock or nephrotoxic drugs, no improvement after diuretics withdrawal or volume expansion with albumin for 2 days, normal kidney ultrasound, and the absence hematuria of proteinuria.

Management of HRS

Despite the progresses and standardization of the pharmacological therapy in the last three decades, HRS is considered as one of the cirrhosis complications with the poorest outcome. The immediate goal of treatment in cirrhotic patients with HRS is reversal of the acute kidney injury. Ideally, therapy should include a short-term

improvement of the liver function which most often not possible [30–32]. Numerous confounding factors are known to precipitate HRS. Their prevention can often be achieved by prompt action and close monitoring. HRS regularly develops in patients with systemic bacterial infection (especially, SBP) and/or severe alcoholic hepatitis.

In addition, HRS prophylaxis is an extremely important element and identification, and treatment of precipitating factors is mandatory, given that the prevalence of unprecipitated AKI is low (1.8%) [10]. The following recommendations are mandatory for any patient with liver cirrhosis and ascites: In cases of SBP, a combination of albumin (1.5 g albumin/kg body weight on the first day and 1 g albumin/kg body weight on day 3, up to a total amount of 100–150 g/24 h) with antibiotic therapy has been shown to reduce the incidence of HRS and mortality in patients with PBS [14, 17, 33]. AKI-HRS appears in more than 30% of patients with SBP and is associated with worse outcomes [34]. Patients who benefit particularly from the administration of albumin are those with bilirubin over 4 mg/dL and serum creatinine over 1 mg/dL. Administration of norfloxacin 400 mg/day to patients with proteins in ascites <15 g/L, with bilirubin>3 mg/dL, Child-Pugh score > 10, serum sodium <130 mEq/L and/or serum creatinine>1.2 mg/dL reduces the risk of HRS and improves survival [17, 35]. Regarding therapeutic paracentesis, any paracentesis over 5 L requires an efficient administration of albumin with 8–10 g of albumin for each eliminated liter of ascites [14, 17]. Maintaining the hemodynamic balance, renal perfusion, prophylactic antibiotherapy are mandatory measures in cirrhotic patients with upper GI bleeding, since these measures have been shown to reduce the frequency of infectious complications, HRS, reducing both mortality and length of hospitalization. Generaly, nephrotoxic medication should be avoided in cirrhotic patients despite NSAIDs are required. These drugs should be stopped and replaced by therapeutic classes that do not affect the kidney function. In patients undergoing investigation with iodinated contrast agents, renal injury prophylaxis is recommended. The risk of impaired renal function occurs 72 h after administration of the contrast agent, which is why, after this time, clinical monitoring of serum creatinine is mandatory.

General Measures

Patients with HRS require:

- Monitoring of BP, HR, fluid intake, diuresis, weight.
- Discontinuation of diuretics (spironolactone or other potassium-sparing diuretics are contraindicated due to the risk of hyperkalemia; furosemide may be useful in some patients with low diuresis);
- Central/peripheral venous supply (at least two venous lines).
- The initial assessment includes blood counts, coagulation tests, liver tests, serum albumin, electrolytes, urea, serum creatinine (for calculating the Child Pugh and MELD scores, assessment of renal function and hydroelectrolyte imbalances).
- Exploratory paracentesis is mandatory (albumin, nuclear polymorphism, bacteriology);

– Urine examination - urinary electrolytes, osmolarity, sediment, cultures.
– Excessive fluid administration should be avoided to reduce the risk of dilution hyponatremia and acute pulmonary edema.

Subsequently, every 48 h, urea, creatinine, serum ionogram will be monitored. Patients with ALD need more careful supervision and monitoring because at hospitalization/during hospitalization they may present/develop evolving neuropsychiatric manifestations related to alcohol consumption: delirium tremens, withdrawal syndrome, exacerbations of neuropsychiatric manifestations, etc. Any of these manifestations are often associated with hemodynamic and hydro electrolytic changes that must be closely monitored and psychiatric/neurological consultation is mandatory for each patient.

Specific Treatment of HRS

Therapeutic options for patients with HRS include pharmacological therapy (administration of vasoconstrictor and albumin), hepatic dialysis, transjugular porto-systemic shunt (TIPS) and liver transplantation. Pharmacological therapy, although without a well-defined pathogenic basis, often remains the only therapeutic option for patients who are not candidates for liver transplantation.

Systemic vasoconstrictors are the only therapeutic class with a therapeutic effect demonstrated in randomized studies in HRS. There are three classes of systemic vasoconstrictors: (a) vasopressin analogues (ornipressin, terlipressin) that induce smooth muscle vasoconstriction and decrease portal pressure (b) somatostatin analogues (octreotide) that may inhibit the release of systemic vasodilators [36], and (c) alpha-adrenergic agonists - midodrine and norepinephrine (NE) that act to constrict the smooth muscle and increase systemic vascular resistance. Terlipressin, a vasopressin analog with greater affinity for the vasopressin V1 receptor (V1R:V2R 2-6:1) [37] is the most studied vasoconstrictor in the treatment of HRS and the most utilized vasoconstrictor in Europe, Asia, and Latin America; it is not approved by the Food and Drug Administration (FDA) in North America [37]. The combination of octreotide, midodrine and albumin improves renal function in patients with HRS type 1, without ischemic side effects. These three vasoconstrictors should be mixed with human albumin infusion given at a dose of 20–40 g/day [38].

Clinical experience, efficacy and superior safety profile have made terlipressin the first therapeutic option in patients with HRS type 1. Standard treatment in HRS is terlipressin and albumin, with a minimum duration of 3 days and a maximum duration of 14 days. In single administration, the efficacy of terlipressin in HRS remission is inferior to the combined administration of terlipressin and albumin, suggesting that albumin is a mandatory component of the regimen. The mechanism of action of albumin is not only the effect of plasma expansion but is associated with

a vasoconstrictor effect in the peripheral arterial circulation. The combination of terlipressin with albumin has maximum efficacy, causing improvement in renal function in 65–70% of cases of type 1 HRS.

Terlipressin can be administered as an intravenous bolus (i.v.) at a dose of 0.5–1 mg every 4–6 h or as a continuous infusion; continuous administration is preferred because, although the efficacy is similar, the side effects are less than bolus administration. Treatment is initiated with terlipressin 2 mg/day in continuous infusion, plus albumin 20–40 g/day; terlipressin dose is increased by 2 mg/day if creatinine does not decrease by more than 25% of baseline within 3 days, and may be increased to a dose of 12 mg terlipressin/day (patients who do not respond to 12 mg/day, do not respond to additional dose increases). In patients responding to treatment, it should be continued until serum creatinine drops below 1.5 mg/dL. The only contraindication to terlipressin is ischemic coronary heart disease and during treatment it is recommended to carefully monitor for possible side effects: arrhythmias, mesenteric ischemia; their appearance requires dose modification or even discontinuation of administration, adequate hydration. Vasoconstrictors that can be used in the treatment of HRS are presented in Table 46.2.

After discontinuation of treatment, HRS recurs in a small number of cases (less than 15%) compared to non-specific patients (volume correction with saline or albumin, dopamine, octreotide). Resumption of treatment in case of recurrence has the same effectiveness, and ischemic complications do not exceed 5%.

Transjugular Porto-Systemic Shunt

Although there are data showing that TIPS can be effective in normalizing renal function (creatinine ≤1.5 mg/dL) in a substantial proportion of patients with HRS, its use in practice is usually limited by the fact that HRS usually occurs in patients with severe hepatic impairment (which contraindicates TIPS). The indication is reserved for patients without hepatic encephalopathy, with bilirubin <15 mg/dL and Child Pugh score <12.

Table 46.2 Vasoconstrictor drugs indicated in the treatment of HRS

Drug	Administration
Terlipressin	Continuous iv infusion - 2 mg/day, with the dose increasing by 2 mg/day every 2 days if the creatinine does not decrease by more than 25% of the initial value; maximum dose - 12 mg terlipressin/day
Norepinephrine	Continuous iv infusion with 0.5–3.0 mg/h; the patient should be supervised in an intensive care unit, with careful monitoring of BP and heart rate
Midodrin and Octreotid	7.5–12.5 mg/day midodrin p.o. plus 100–200 μg octreodide, administered subcutaneously every 8 h (the effectiveness of this combination is quite limited, being used in countries where terlipressin is not available)

Renal and Hepatic Replacement Therapy

Hemodialysis is indicated in patients with HRS who are on the waiting list for liver transplantation, in whom renal function is nonresponsive to medical treatment. MARS (Molecular Adsorbent Recirculating System) is indicated in patients without a vasoconstrictor response and with severe liver dysfunction on the transplant list, but the results are contradictory. Liver transplantation is the ideal, curative treatment for patients with end-stage liver cirrhosis, and therefore of those with HRS. In practice, however, performing the liver transplant for HRS is extremely rare, as most patients with HRS type 1 die before the transplant can be performed and in those with HRS type 2, although they manage to survive the transplant, poor control of refractory ascites may contraindicate transplantation. Patients transplanted for HRS have a higher proportion of postoperative complications, increased length of hospitalization in intensive care and significantly higher short-term mortality. In the long term, survival is about 60% at 3 years, compared to 70–80% in transplant patients without HRS. In patients with type 2 HRS, several remarks should be made: treatment with vasoconstrictors is less effective than in type 1 HRS due to the high recurrence rate (although the initial resolution can be obtained in almost 80% of cases, recurrence is the rule in almost all patients); TIPS appears to be an effective therapeutic alternative for the management of HRS type 2 and refractory ascites.

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Chapter 47
Acute Liver Failure Due to Alcohol Intoxication—Therapeutic Options

Carmen Fierbinteanu-Braticevici and Vlad-Teodor Enciu

Abstract Acute liver failure is a relatively common complication of patients with alcoholic hepatitis (AH) and it is characterized by severe liver cell dysfunction. AH can be seen as Acute-on-chronic liver failure (ACLF), as most patients have preexisting liver damage. ACLF is characterized by acute deterioration, multiple organ failure, evidence of systemic and hepatic inflammation and a high risk of mortality. Patients with AH benefit from therapies directed towards reducing liver injury and suppressing inflammation. Traditional, established approaches focus on nutritional support while some patients benefit from corticosteroids. Other therapeutic strategies warrant further confirmation in larger controlled trials. For instance, the addition of intravenous N-acetyl cysteine (NAC) to prednisolone may improve the survival of patients with severe AH. Several studies have demonstrated the efficacy of Granulocyte–Colony Stimulating Factor in mobilizing stem cells from bone marrow to liver, with improvement of clinical, biochemical, and histological profile in patients with liver failure. Some pilot studies suggest that healthy donor fecal microbiota transplantation has a beneficial effect on the outcome of patients with ACLF. In selected cases, the time to liver transplantation may be bridged with liver-assist devices (artificial livers). Liver transplantation has also been shown to improve survival in these patients but acute alcohol intake usually prevent organ allocation.

Keywords Alcohol · Acute liver failure · Therapeutic options · ACLF · Alcoholic hepatitis

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Introduction

For a very long time, alcohol consumption has been an accustomed practice in many cultures. Due to increased availability and globalization, alcohol abuse has become a worldwide health concern. Alcohol-related liver diseases (ALD) includes a wide range of hepatic disorders, from hepatic steatosis to episodic steatohepatitis, alcoholic hepatitis, progressive liver fibrosis and lastly alcoholic cirrhosis.

Acute liver failure (ALF) is a syndrome characterized by an acute deterioration of liver function on an otherwise healthy liver, causing different degrees of hepatic encephalopathy and elevated prothrombin time/international normalized ratio (INR), with onset in under 26 weeks [1]. This definition originates from the studies and observations of drug related liver toxicity [2]. Untreated, ALF has a high mortality and, consequently, early recognition and prompt management are crucial. Considering the severity of the disease, it should be treated in an intense care unit with the possibility for liver transplantation [3].

Based on the debut of **hepatic encephalopathy (HE)** and the onset of jaundice, ALF can be subcategorized in three forms: **hyperacute** (<7 days), **acute** (7–21 days) or **subacute** (>21 days and <26 weeks). Patients with hyperacute presentations usually develop cerebral edema, while patients with subacute forms have renal failure or portal hypertension. These subcategories have different prognoses, reflected more by the underlying cause and not the duration of the disease. For instance, patients with hyperacute liver failure usually have a better long-term prognosis, related to the fact that these patients often have acetaminophen intoxication or ischemic hepatitis, which have a better outcome once the disorder has been recognized. On the other hand, subacute liver failure, secondary to Wilsons disease, comprises a poor long-term prognosis [4].

Recently, **acute-on-chronic liver failure (ACLF)** has been recognized as a distinct disorder, especially in ALD (see also Chap. 67). ACLF involves an acute decompensation of an underlying chronic liver disease, association with other organ failures, and has specific pathogenic mechanism, evolution, and treatment [5]. Regardless of being diagnosed in under 26 weeks, ALF due to alcohol consumption is considered in cases of **severe alcoholic hepatitis (sAH)** even though most patients have a pattern of chronic drinking, with different degrees of subclinical hepatic injury [6]. Particularly, the term ALF due to alcohol intoxication accounts for a minority of cases and should not be used in this context since alcohol intoxication refers more to neurologic or cardiac complications. Nevertheless, since the underlying pathomechanisms are still not completely understood, further studies are needed regarding the terminology. In this chapter we will present the management of ALF due to alcohol consumption as an integrated subgroup of ACLF and sAH. Related chapters on alcoholic hepatitis can be found in Part X (Chaps. 64, 65, 66, 67 and 68).

Assessing Disease Severity

Early assessment of patients with ALF and sAH is vital. Identifying patients who are unlikely to respond to standard therapy and may require liver transplantation is based on severity scores that are poorly standardized. Many patients develop multiple organ failures with rapid deterioration while waiting on the transplant list, hence a readily identification of transplant candidates should be achieved as soon as possible. Clinical and paraclinical features that indicate a poor prognosis include presence of hepatic encephalopathy, extrahepatic organ failure, coagulopathy, and high bilirubin levels. Those have been combined in the classic prognostic and severity scores for alcoholic hepatitis (AH) such as Maddrey's discriminant function, model for end-stage liver disease (MELD), Glasgow alcoholic hepatitis score, ABIC score and Lille score [7–9]. A Maddrey score >32 indicates a severe form of AH, poor prognosis, important hepatic and extrahepatic inflammation, with high risk of organ failure [9]. While the MELD score can be used for severity assessment, it is normally used for liver transplant list stratification [10].

ALF in critically-ill patients can be assessed using scoring systems such as sequential organ failure assessment (SOFA) score and other scores that are used in intensive care units [11]. This score has been also the basis for establishing the CLIF-OF score in the CANONIC study and the definition of ACLF [12]. The clinical prediction scores in ACLF, particularly CLIF-OF, is showing superiority in assessing severity and predicting outcome in this disease category. CLIF-OF includes organ assessment: liver function (bilirubin levels), kidney function (creatinine levels), brain function (hepatic encephalopathy), coagulation (INR), circulatory system (mean arterial pressure), and respiratory function (PaO_2/FiO_2) [12–14]. The CLIF-OF score also helps to determine short and long-term mortality in patients with ACLF, based on the number of organ failure(s) [12].

Diagnosis of Acute Liver Failure

ALF is a rare syndrome with highly specific characteristics consisting of acute modifications of liver blood tests in an individual without an underlying chronic liver disease [4]. While ALF is a characteristic of sAH and ACLF, they are recognized as acute decompensations of chronic liver diseases with particularly higher morbidity and mortality due to concomitant multiple organ failure(s) [14]. **ALF includes the following criteria**:

1. Increased hepatic enzymes,
2. Presence of hepatic encephalopathy and
3. Prolonged prothrombin time (PT) or International Normalized Ratio (INR, higher than 1.5) [15].

Although, INR > 1.5 is still accepted for defining ALF, there is a debate that higher cut-off values should be used or only PT since INR is not standardized and it is used for monitoring Warfarin therapy [16]. The timing of evaluation and diagnosis is crucial as early treatment initiation may improve the patient's outcome. Moreover, a timely evaluation stratifies patients that may benefit from liver transplantation [17].

Key Histologic Features in AH

Details are provided in the histology chapter in this book but are briefly recapitulated within the context of this chapter. Liver biopsy is sometimes needed to advance the diagnosis. In ALF it is a risky maneuver due to the presence of coagulopathy and bleeding complications, especially in the acute episode of liver failure [18]. Moreover, the histologic features are not specific as they can also be found in patients with nonalcoholic steatohepatitis. Histologic finding in AH include: steatosis, hepatocellular ballooning, megamitochondria, lobular inflammation with neutrophilic PMN cells, canalicular and/or ductal cholestasis, Mallory-Denk bodies, fibrosis disposed pericellular, perisinusoidal and perivenular [19, 20]. From these, the presence of neutrophils is more often seen in AH. Mallory-Denk bodies (previously called Mallory bodies) are eosinophilic intracellular protein deposits, which are cytokeratins, a normal component of the hepatocyte cytoskeleton. Mallory-Denk bodies do not appear to play a role in the pathogenesis of the hepatic injury, they are not specific, and can also be found in other liver pathologies [21, 22].

A histological score has been proposed for the severity stratification of AH which includes stage of fibrosis, extent of bilirubinostasis, grade PMN infiltration and presence or absence of megamitochondria. The extent of fibrosis and bilirubinostasis is directly linked to the severity of AH. On the other hand, high PMN infiltration and megamitochondria have been seen in mild cases [19].

Therapeutic Options

General Management

Patients with ALF should be admitted in centers with liver transplantation availability. More than half of alcohol-induced ALF do not recover with standard therapy and require liver transplantation [23]. Patients benefit from early transfers to a liver transplantation center since later transports can become difficult due to clinical deterioration, aggravation of coagulopathy and increased intracranial pressure [24]. Patients are usually managed in intensive care units (ICU) but can also be admitted on a general medical ward provided they have grade I encephalopathy and frequent

neurologic checks. Neurologic stimulation should be minimized, patient's room should be quiet, without audible monitors and dimly lit in order to prevent increased intracranial pressure [4].

Laboratory testing is used to follow the patients' evolution and to monitor for complications. Hepatic enzymes should be tested daily. More frequent lab tests include (up to three times a day) coagulation parameters, complete hemogram, ionogram and arterial blood gas [1]. Glycemia is monitored every 6 h. It is highly recommended that ammonia levels are tested daily, as high values are associated with cerebral herniation [25]. Plasma supplementation should only be used in cases of severe coagulopathy since it can prevent the usage of prognostically important prothrombin/INR monitoring. Increasing bilirubin and INR levels indicate worsening of liver failure. Amelioration of transaminase levels (ALT/AST) should be interpreted with caution since this can indicate either recovery or worsening of ALF as a result of loss of hepatocyte mass [1].

Hemodynamic homeostasis is perturbed in patents with ALF due to low systemic vascular resistance. Glucose levels and intravascular volume are decreased as a result of low oral intake and extravasation of fluid in the interstitial space [26, 27]. Consequently, patients may be either hypotensive or hypoglycemic and fluids should be renewed accordingly using IV saline and glucose fluids. However, fluid supplementation should be managed carefully, as overhydration may worsen cerebral edema [27].

Patients who do not respond to fluid supplementation alone require vasopressor support. Norepinephrine is the preferred agent as it facilitates peripheral organ perfusion with lesser cardiac impact and better preservation of splanchnic blood flow than other vasopressors. The goal is to maintain a mean arterial pressure of 70-75 mmHg. Vasopressin can be added to obtain as it potentiates the effect of norepinephrine [28].

Nutrition is essential in the management of ALF. Early initiation prevents catabolic status and decreases risk of stress ulcers in critically-ill patients. As a result, protein restriction is not recommended and a daily intake of 50–60 g of protein is required to compensate protein catabolism. Oral and enteral nutrition is preferred since this maintains the intestinal barrier and prevents bacterial translocation. Nasogastric tube placement should be done with care as it can increase intracranial pressure through gag reflex, so it should only be performed in intubated and sedated patients [29, 30].

The risk of hemorrhage should be assessed as patients with ALF can develop severe coagulopathy due to decreased coagulation factors synthetization. Conventional indices of coagulation (INR) have been shown to be not accurate in determining bleeding risk in patients with liver diseases. For these reasons, additional tests may be required for patients who require invasive procedures or who develop bleeding, like thromboelastrography or thromboelastrometry such as rotational thromboelastometry (ROTEM), which, unfortunately, are not widely used [3]. Prophylactic administration of fresh frozen plasma has been shown to not

improve survival in a small, randomized trial and can cause fluid overload or interference with liver function assessment [31, 32]. As the gastrointestinal tract is the major site of bleeding in these patients, prophylaxis of stress ulcers should be taken into consideration using proton pump inhibitor or blockers of histamine-2 receptors.

Infection Management and Prevention

Patients with ALF have a high risk of infection and sepsis so close monitoring and prompt actions are necessary. The most common sites of infection are (in descending order of their prevalence) infections of the respiratory tract, the urinary tract and the circulatory system [33]. Symptoms are usually diminished, fever or sputum production may be absent, and the only indication of an infection may be the appearance/worsening of encephalopathy or kidney function. AASLD and EASL guidelines suggest that all patients with ALF, including those without signs of infection undergo routine urine, sputum, blood cultures and chest radiographs to detect a possible infection [4, 27]. This approach is still controversially discussed among specialists in the light of the limited data regarding the prognostic importance of bacterial cultures and imaging surveillance. Moreover, positive cultures, in the absence of infectious signs, may be due to bacterial or fungal colonization or contamination [3].

Patients with ALF and concomitant ascites should undergo diagnostic paracentesis [3]. Antibiotic prophylaxis have shown mixed results. In a randomized trial with 59 patients with ALF who were not infected, antibiotic administration reduced the rate of infection, but did not improve overall survival rate [34]. In a retrospective study on 1151 patients with ALF, antibiotic prophylaxis did not reduce neither bloodstream infection (sepsis) nor short-term mortality [35]. In presence, it is general consensus to give antibiotic therapy in case of any sign of infection and the threshold for initiating antifungal treatment is kept low since many of these patients are at high risk due to prolonged hospitalization, parenteral nutrition and glucocorticoid treatment. If antibiotic therapy is indicated, broad spectrum antibiotics are used, avoiding hepatotoxic and nephrotoxic antibiotics, particularly aminoglycosides. Piperacillin/tazobactam or a fluoroquinolone could be used without the need of culture surveillance [4, 27].

Organ Failure Targeted Therapy

Severe forms of alcohol-induced ALF involve multiple organ failure due to a systemic inflammatory response. Cardiovascular failure should be treated with vasoactive agents preferentially with Norepinephrine. Respiratory failure should be managed with standard sedation and invasive or non-invasive ventilation techniques.

Neurological manifestation due to intracranial hypertension should be treated using mannitol or hypertonic saline solutions. Renal failure often requires extracorporeal renal replacement therapy to correct electrolyte and metabolic imbalances [4].

Treatment for ALF as a Complication of Severe AH

Corticosteroids (CS) remain the first line therapy for severe forms of alcoholic hepatitis (Maddrey>32). For more details, chapters in the AH part of this book are recommended. The response rate is calculated using the Lille model which includes a series of static parameters and bilirubin as a dynamic parameter at the start of treatment and at day 7. A Lille score >0.45 after 7 days predicts failure of corticosteroid treatment and other therapeutic options should be considered. It is interesting to note that the response rate seems to be linked to the degree of ACLF. In other words, the higher the degree of ACLF is the lower will be the response rate [36]. This observation highlights the importance to carefully screening for other organ failure(s) prior to corticosteroid treatment.

Pentoxifylline alone or in combination with CS has been shown to not improve short term survival at day 28, nor does it improve the survival of CS non-responders [37]. Granulocyte colony stimulation factor (G-CSF) has been thought to promote liver regeneration through mobilizations of bone marrow stem cells and proliferation of hepatic progenitor cells [38]. However, as discussed in a recent systematic review, while G-CSF improved survival in steroid non-responders and reduced short-term mortality in patients with ACLF, this could not be confirmed in a prospective multicenter phase 2 study. Here, G-CSF, at a dose of 5 μg/kg per day for 5 days and then every third day until day 26, failed to improve the CLIF-OF score, the MELD score or the incidence of infections [39].

Another important question is centered around intestinal microbiota. Alcohol consumers are knows to have dysbiosis of the intestinal microbiome which contributes to the development of liver injury and maintains liver inflammation [40]. In a pilot study, 1 week of fecal transplantation was effective and improved survival in patients with sAH-ACLF at 1.5 years follow-up [41]. Many patients with alcohol-induced ALF, especially those with ACLF, do not respond to standard therapy. Since liver transplantation is not readily available, new therapeutic options have been studied. Artificial liver support systems are extracorporeal nonbiological dialysis machines are designed to remove albumin-bound toxins (bile acids, bilirubin) and circulating cytokines, thus limiting systemic inflammation. There are five artificial liver support systems: molecular adsorbent recirculating system (MARS), single-pass albumin dialysis (SPAD), fractioned plasma separation, adsorption and dialysis (FPSA)- Prometheus System, selective plasma filtration therapy and hemodiafiltration. While these dialysis systems improve survival in non-alcohol induced ALF, all studies have shown that they do not improve survival patients with sAH and ACLF. However, they transiently reduce pathological levels of liver

biomarkers and are occasionally used for bridging to curative liver transplantation [42–44]. High volume plasma exchange has shown some benefit in patients who are treated early and will not undergo liver transplantation [45].

Liver Transplantation

Patients with sAH usually fail to respond to most therapeutic options including CS, hence liver transplantation remains the only viable treatment option [46, 47]. All causes of ALF are given the highest priority on the transplant list, except ALD (United Network for Organ Sharing [UNOS] status 1). The transplant list stratification is still made based on MELD-Na score which assesses liver and renal function. The newer concept of ACLF highlights the importance of multiple organ failure and increased infection risk in this syndrome. Thus, the CLIF-OF scoring system offers an additional means to assess wait-list mortality and futility of transplantation [48]. Due to the ethical dilemma arising from a high proportion of relapse among ALD patients and the limitation in predicting alcohol relapse, most countries abide by the 6-month abstinence rule [49]. Moreover, these patients must adhere to a rehabilitation and abstinence program. In addition, social support is required maintain sobriety. On the other hand, most of them are critically-ill and would not survive without liver transplantation. For these reasons, and as is discussed in the AH part of thi book, liver transplantation is also performed in alcohol-induced ALF or sAH in highly selected patients with good short and long-term survival rates [50–52].

While continuation of alcohol abuse is associated with post-transplant morbidity and mortality, the prediction of alcohol relapse lacks data and may be similar to patients who follow the six-month abstinence period [52]. In addition, while inflammatory and coagulation disturbances associated with AH could increase perioperative complications, long-term morbidity is more tied to recurrent alcohol abuse rather than complications of transplantation [53, 54]. Overall, there are increasing efforts directed towards establishing liver transplantation protocols in order to facilitate graft allocation in sAH non-responders. Ultimately, patients with sAH who do not respond to conventional treatment, have more than four organ failures and do not meet the criteria for liver transplantation and they are considered for palliative care [55].

Alcohol Withdrawal Treatment

Alcohol withdrawal is only briefly mentioned here, as it is discussed in more detail in other chapters of this book. Alcohol abstinence outweighs any other type of targeted therapeutic approach in patients who have ALD. This usually comes along with a high probability of alcohol withdrawal syndrome, particularly in acute phases. Minimally symptomatic or asymptomatic patients can be prophylactically

treated with oral benzodiazepines, while IV administration is reserved for severe cases with delirium tremens and seizures. Attention is required while administering iv benzodiazepines in patients with ALF as it can worsen pulmonary function and develop acute respiratory distress syndrome. Symptom-triggered approached is favored over front-loading therapy [36].

Conclusions

Excessive alcohol consumption is a world-wide healthcare problem with enormous socio-economic and clinical consequences. ALF consists of an acute deterioration of liver function on a previously healthy liver. Alcohol consumption induces subclinical hepatic steatosis, hence, ALF in drinkers can be seen as deterioration of a chronic disease. The accepted terms are sAH or ACLF that cause ALF and have specific pathomechanisms, prognosis and treatment. Risk stratification and severity is poorly defined due to lack of specific prognostic biomarkers and a yet insufficiently understood underlying disease mechanisms, further complicating the therapeutic management. Despite many previous studies with different agents, corticosteroids are momentarily the only approved option for a limited number of patients. Liver transplantation remains difficult to access in an era of organ-donor shortage and stigma towards a disease that is considered self-inflicted in many countries. While the general management strategies for ALF can also be applied to sAH and ACLF, treatment of associated organ failures and infections are of an additional high priority.

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Chapter 48
Management of Acute Alcohol Withdrawal

V. Enatescu, R. Kalinovic, A. Pascariu, and G. Vlad

Abstract Alcohol use disorders (AUD) are the most prevalent psychoactive substance use disorders worldwide, caused by several biological, psychological, and socio-cultural factors.

Alcohol withdrawal syndrome (AWS) is a frequent complication of those who met the criteria for alcohol dependence. Its intensity may vary from symptoms such as insomnia, tremors, sweating, and tachycardia up to more severe complications such as delirium or seizures, implying 5–15% mortality.

Currently, there are two main directions in the pharmacological management of AWS. First, symptom-triggered therapy is when treatment is provided if the symptoms are severe but not if the symptoms are mild. In this latter case, simple continuous monitoring without medication is sufficient. Therefore, the unnecessary use of benzodiazepines or phenobarbital is avoided. Secondly, in those being delirious or having a prior history of Delirium Tremens (DT) or seizures, a fixed-dose regimen of some type needs to be administered.

On the other hand, the pharmacological treatment of AWS should cover three symptomatic domains: neuropsychiatric symptoms, autonomic symptoms, and motor disturbances. Considering the complexity of AWS, treatment should be individualized according to the physiological and pathological particularities of each case.

Finally, the mainstay of alcohol withdrawal management is early adequate treatment.

Keywords Alcohol withdrawal · Psychopharmacology · Complications · Risk factors · Treatment resistance

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Introduction

Alcohol is the most widely used psychoactive substance worldwide and across the different cultures dating back to 3150 BC in Ancient Egypt [1]. As societies have emancipated, the differences in alcohol consumption have diminished in regard to gender, age of onset, geographical region, and even socio-cultural affiliation [2].

In line with WHO data, in 2016, 2.3 billion (42%) people aged 15 or over were current drinkers meaning at least one use of alcohol in the past 12 months. Based on traditional and cultural considerations, in some low-income and middle-income countries, this data should be cautiously interpreted, as a significant quantity of alcohol is produced in a domestic setting so that official statistics overlook it [3]. On the other hand, due to the high level of stigma associated with Alcohol Use Disorder (AUD), at least a part of patients are often diagnosed with other psychiatric or medical conditions [4]. As a result, AUD could become either a secondary diagnostic or, in some cases, even a hidden or masked condition.

According to a systematic analysis for the Global Burden of Disease Study 2016 that has considered diseases attributable to alcohol and drug use in 195 countries between 1990 and 2016 revealed that globally, in 2016, 99.2 million disability-adjusted life-years (DALYs) (95% UI 88.3–111.2) and 4.2% of all DALYs (3.7–4.6) were attributable to alcohol use. The authors concluded that alcohol significantly contributes to the global disease burden [5].

Alcohol withdrawal syndrome (AWS) is a life-threatening condition that occurs in up to 50% of patients who are affected by AUD when they cease or diminish their alcohol intake [6]. Undoubtedly, AWS needs to be promptly detected and treated, especially in critically ill patients admitted to an intensive care unit (ICU) or referred to the emergency room (ER) when other underlying medical conditions may significantly increase the risk of death [7]. Generally, based on the literature data, AWS implies a 5–15% mortality [8].

Finally, AWS is a severe condition that can evolve to delirium tremens (DT), a state of altered consciousness that often requires higher doses of medications and sometimes physical restraints, making clinical practitioners confront ethical issues [9].

Neurobiological Basis of Alcohol Withdrawal Syndrome

Harmful and chronic alcohol drinking alters the brain's structure and neurotransmitter systems. The main two neurotransmitters that play a key role in AWS are glutamic acid or glutamate (Glu) and gamma-aminobutyric acid (GABA). Glu has an excitatory effect on the central nervous system (CNS), whereas GABA has an opposite inhibitory neurobiological effect. Glutamate acts on both metabotropic and ionotropic receptors; of the latter, N methyl aspartate (NMDA) plays a major role in several neurodegenerative and psychiatric conditions. Glu stimulates all neurotransmitters including catecholamines (dopamine and norepinephrine) contributing to

the increased autonomic sympathetic symptoms of AWS. In a few cases, dopaminergic hyperactivity may result in hallucinatory experiences during DT. Normally, the two neurotransmitters are in a steady balance, GABA being synthesized from glutamate via glutamic acid decarboxylase (GAD) [10, 11]. From a psychoactive standpoint, alcohol stimulates GABA neurotransmission to the detriment of Glu which will lead to the upregulation of NMDA glutamate receptors and glutamatergic hyperactivity if abruptly cessation or significant reduction of alcohol intake happens. The AWS symptoms result from the imbalance in brain receptors between GABA and NMDA that develop on the discontinuation of chronic and harmful alcohol consumption [12].

On the other hand, repeated withdrawal episodes lead to the so-called "kindling," in which neuronal hyperexcitability causes an increased severity of AWS over time that may be complicated by seizures and DT [13].

Symptomatic Polymorphism of Alcohol Withdrawal Syndrome

The clinical picture of AWS is characterized by an intricate combination of signs and symptoms with varying degrees of severity influenced by the time elapsed from the moment of alcohol intake cessation/reduction, the quantity, frequency and duration of the dependence syndrome, number and severity of previous AWSs, patient's age, comorbidities, and concurrent medication or drug use [14–17].

Given the polymorphism of AWS clinical presentation, two approaches can be considered in understanding the symptomatic complexity of this syndrome, a categorial one, respectively a quantitative one.

The categorial approach aims to identify and arrange symptoms into clusters and attribute them, as much as possible into nosologic entities, in order to encompass the effects this pathology exerts on different physiological systems. According to this, the AWS symptoms can be classified in autonomic, motor, neuropsychiatric and related to underlying medical conditions, as detailed in Fig. 48.1 [8, 14–16].

The quantitative approach regards symptomatology as a continuum, with the purpose of quantifying AWS severity. The literature classifies the natural evolution of this syndrome into four main degrees which develop and partially overlap over the course of the first few days [14, 16] as follows:

Mild AWS: is characterized by the presence of autonomic hyperactivity symptoms (see Fig. 48.1), insomnia, anxiety, irritability and general malaise [14–16].

AWS hallucinosis: in this stage patients experience transient auditory (voices, sometimes persecutory), visual (zooscopies) and tactile (formication) hallucinations that develop on a clear field of consciousness, which can increase anxiety to the point of psychomotor agitation [14–16].

AWS seizures: generalized tonico-clonic seizures occur in some patients, and are usually self-limited but in some cases, especially if untreated, they can evolve into DT [15, 16].

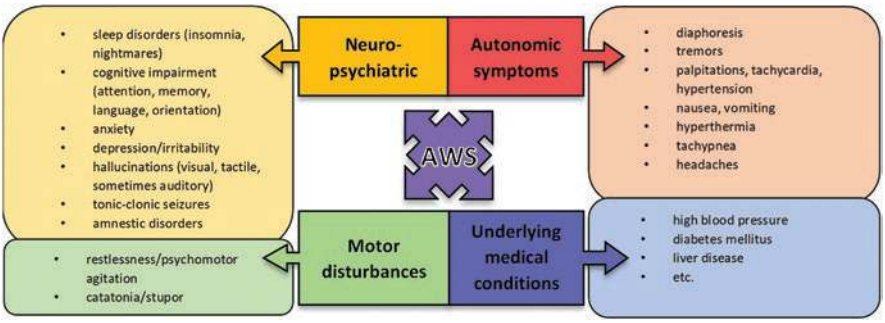

Fig. 48.1 The categorial approach of AWS symptoms

Delirium Tremens: is the most severe form of AWS, manifested by abrupt fluctuations in consciousness, a significant worsening of the aforementioned symptoms (especially the autonomic ones), confusion, disorientation and hallucinations or delusions [14–17].

While the natural evolution of AWS tends to follow this spectrum, clinicians should keep in mind that for some patients the duration and severity of these stages can vary according to the presence or absence of efficient treatment [8, 15, 16, 18].

Measures of the Severity of Alcohol Withdrawal Syndrome

Regarding the assessment of the severity of AWS, the most relevant tool is the revised Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar) scale, which is required for decision-making in AWS management. It must be administered by a trained caregiver, and consists of 10 items, all of which are scored from 0 to 7, except for the orientation category, scored from 0 to 4. The summation of the scores yields an aggregate value that correlates to the severity of alcohol withdrawal, with ranges of scores designed to prompt specific management decisions such as the administration of benzodiazepines. The CIWA-Ar should be used to adjust dosage, detect complications, and identify patients who require more intensive therapy [16].

Risk Factors Associated with the Neuropsychiatric Complications of Alcohol Withdrawal Syndrome

A number of general risk factors are known to predispose patients to developing complications during AWS, such as: advanced age (>65), heavy and regular ethanol use for long periods of time, medical and surgical comorbidities (traumatic brain

injury in particular), marked autonomic symptoms at presentation (especially elevated blood pressure, tachycardia), history of multiple withdrawal episodes (kindling) [16], a positive history for DT or seizures during a withdrawal episode (DT having a stronger predictive value), seizures during the current AWS episode, physiological dependence on drugs that act as GABAergic agents (benzodiazepines, barbiturates) [8, 15, 17].

There are also several individual risk factors: concomitant use of additional addictive substances, active signs and symptoms suggesting at least a moderate severity of psychiatric comorbidity (Dual Diagnosis), concomitant presence of AWS signs and symptoms and a positive blood alcohol concentration (BAC) at presentation [17].

The predictive value of the general and individual risk factors is additive, consequently the more risk factors are identifiable in a patient, the more chances that DT will develop [17].

Thrombocytopenia, low albumin, elevated blood urea nitrogen (BUN) and aspartate aminotransferase (AST) levels could also play a part in identifying patients at risk for developing DT [8, 19].

Although not a direct complication of AWS but rather of thiamine (vitamin B1) deficiency, Wernicke-Korsakoff Syndrome (WKS) often manifests during a withdrawal episode, therefore clinicians should assess if the patient requires thiamine replacement. Besides alcohol dependence, other risk factors include malnutrition and medical conditions which either reduce thiamine absorption or enhance its excretion/consumption [20, 21].

Treatment of Alcohol Withdrawal Syndrome

Objectives

The goals of AWS treatment are to provide symptomatic relief, normalize vital signs, control psychomotor agitation, decrease the risk of seizures, monitoring complications, detection of complications and underlying comorbidities and halt progression to severe withdrawal and prevent death [22–24].

Basic Principles of Acute Alcohol Withdrawal Treatment

It is recommended to administer medication before significant withdrawal symptoms emerge. GABA agonists are the medicines of choice. Delaying the start of treatment can result in withdrawal symptoms that may become difficult to control. Nevertheless, it should be kept in mind that the use of benzodiazepine sedation while the patient is still intoxicated can lead to respiratory depression and death.

For patients with signs of acute alcohol withdrawal on admission, an initial dose of 30 mg chlordiazepoxide is recommended (ideally 6–8 h after the last drink). The patient should then be assessed hourly [25].

Pharmaceutical Compounds and Supplements Used in the Treatment of AWS

Benzodiazepines

Benzodiazepines are the "gold standard" for moderate to severe forms of AWS, given their effectiveness in reducing both withdrawal symptoms and the risk of developing seizures/DT. Benzodiazepines bind allosterically at GABA receptors and increase GABA activity, increase inhibition, and therefore alleviate withdrawal signs and symptoms. The activity of benzodiazepines appears to require some native GABA for benzodiazepines to be effective. Because these drugs have anticonvulsant activity, they are useful in active seizing withdrawal patients. Benzodiazepines have also been shown to reduce mortality associated with AWS. Long-acting benzodiazepines, such as chlordiazepoxide and diazepam provide more protection against delirium and seizures. Short-acting benzodiazepines with renal metabolism (oxazepam, lorazepam) have a better safety profile in the elderly or in those with impaired liver function [16, 26]. The most frequently used BZDs in alcohol withdrawal treatment/prevention are diazepam, lorazepam and chlordiazepoxide.

Administration algorithm: Benzodiazepines are available in different forms (orally, intramuscularly or intravenously) [6]. Oral formulations are preferred in most outpatient settings. Following IV administration, patients should be switched to oral dosing as soon as possible, based on their clinical response. Intramuscular administration should be avoided due to absorption variability [6, 27].

Symptom-triggered regimen: patients are continuously monitored using a structured assessment scale and are given medication only when symptoms exceed a severity threshold. The CIWA-Ar score may also determine the frequency of reevaluation and subsequent dosing until the patient is no longer in weaning and detoxification is complete [27].

Fixed-schedule regimen: fixed-dose administration at predefined intervals (based on AWS severity) according to a schedule. Doses are usually lowered gradually over a few days.

Front-loading regimen: it is recommended for patients at high risk of severe AWS and medical or psychiatric comorbidities. Early control of symptoms with a lower total dose and a lower rate of seizures is achieved by the front-loading therapy method. Starting doses of benzodiazepines should be: diazepam 5–10 mg IV, (lorazepam 2–4 mg IV in patients with severe liver disease) or chlordiazepoxide 25–100 mg orally (oxazepam 10–30 mg orally in patients with severe liver disease) [26]. Loading doses of benzodiazepines should be 5–20 mg of diazepam every

5–10 min, or 2–4 mg of lorazepam every 10–15 min (lorazepam requires a longer time between doses to avoid dose stacking and sudden deep sedation) [22].

Barbiturates

The action of ethanol is replaced by the activity of benzodiazepines, barbiturates and propofol which act on the physiological cause of AWS, thus creating inhibition in an overexcited system. Barbiturates also provide an inhibitory effect caused by the activation of the GABA receptor like benzodiazepines, by a slightly different mechanism at the receptor, which works in the absence of any native GABA receptor. The most used barbiturate is phenobarbital due to its onset and duration of action. The appropriate dose is 65–260 mg every 15–30 min until symptoms are controlled [22].

Thiamine and Folic Acid

The levels of thiamine and folic acid deserve special attention, as long-term malnutrition is common in alcoholic patients. Chronic folic acid deficiencies can lead to the development of megaloblastic or macrocytic anemias, which is why supplementation is recommended [3, 6].

The administration of oral thiamine 100 mg is initiated at the same time as the administration of intravenous therapy and is recommended for a period of 3–6 months in case of abstinence or indefinitely if alcohol consumption is continuous [25].

Anticonvulsants

Antiepileptics such as phenytoin, valproic acid and levetiracetam, do not treat or effectively prevent alcoholic seizures, therefore they are considered adjuvant drugs. Carbamazepine has been shown to have a particular benefit in the treatment of AWS, although it is not available in IV form [22].

While carbamazepine, gabapentin, and valproic acid may be useful in the outpatient management of mild alcohol withdrawal, there is no evidence that this class of drugs can be used effectively in patients with severe AWS [27].

Antipsychotics

When agitation, delirium and hallucinations are not controlled with benzodiazepines, antipsychotics are used as adjunctive therapy. First generation antipsychotics, especially haloperidol, have often been used in patients with psychotic symptoms.

The recommended dose of haloperidol is 2–5 mg intravenously every 0.5–2 h, with a maximum dose of 0.5 mg/kg/24 h. The main disadvantage is that haloperidol is a dopaminergic antagonist and has no effect on GABAergic or glutamatergic systems. It should only be given to patients who have already received benzodiazepine treatment. Intravenous administration of haloperidol may cause QTc prolongation, torsades de pointes, and death. Although a small number of studies have been performed on atypical antipsychotics, it appears that they may have some benefit in reducing withdrawal symptoms, Risperidone and Quetiapine have been shown to be most effective [15].

Alpha-2-Agonists

Alpha-agonists and beta-agonists are used primarily to treat AWS somatic symptoms such as tachycardia or hypertension, but they do not treat the cause [22].

Dexmedetomidine is used to decrease sympathetic overdrive by decreasing norepinephrine release. Some of the more common adverse effects are bradycardia and hypotension, but these have no additive effect to the usual treatment with benzodiazepines, propofol or opioids. Dexmedetomidine is superior to clonidine due to its rapid onset of action and shorter half-life. Studies show that an infusion rate of 0.7 1 g/kg/h is sufficient for most patients, but standardization of dosage for AWS has not been established. It produces light sedation while keeping the patient easily aroused, this helps with routine assessments. Because dexmedetomidine does not prevent withdrawal seizures or DT, they are recommended as adjunctive therapy only if the autonomic symptoms cannot be controlled by benzodiazepines [15].

Clonidine can be used instead of dexmedetomidine but it produces more sedation therefore it is only used as adjunctive therapy in AWS for reducing autonomic sympathetic symptoms [7].

Beta-Blockers (Beta-Adrenergic Antagonists)

Beta-blockers (atenolol) should not be used to prevent or treat AWS, but they are useful for tremors, hypertension and tachycardia, the downside of choosing beta-blockers is that they could mask AWS symptoms and should only be used when the patient is already under treatment with BZDs and presents persistent hypertension or tachycardia [16].

Rehydration and Electrolytes Rebalance

Most patients have hydro-electrolyte imbalances and need to be evaluated and corrected as necessary. Magnesium has received special attention for its potential role in the treatment of AWS since it is an NMDA antagonist. Studies show that there is

a correlation between symptomatic hypomagnesemia and the severity of DTs. Magnesium alone has no beneficial effect on AWS [22].

Sodium oxybate/sodium salt of c-hydroxybutyric acid (GHB) is a natural fatty acid structurally similar to GABA. Alcohol withdrawal symptoms are thought to be suppressed by indirect activation of GABA receptors by GHB. Increased dopamine release in the CNS has been described as an alcohol 'mimicking' effect of GHB [15].

Management of Poorly Controlled AWS

Benzodiazepine-resistant alcohol withdrawal (RAW) is a notion that gained attention in the recent years, even though there is no clear consensus among clinicians regarding the threshold that defines it, the most accepted view defines it as AWS non-responsive to the administration of 40 mg Diazepam (or another benzodiazepine equivalent) in the first 2 h, most often accompanied by seizures and tachycardia [28–31].

Literature suggests that treatment with other agents such as phenobarbital, propofol, dexmedetomidine and ketamine might provide better clinical outcomes, but only as adjunctive therapy to benzodiazepines [31–33].

Phenobarbital is the most commonly used but it has a narrow therapeutic index and requires close monitoring in intensive care settings [33].

Propofol is also considered a viable option, but it is recommended mainly for intubated patients, and it might prolong the need for clinical care [30, 33].

Dexmedetomidine and ketamine yielded promising results in this field but more studies are needed to confirm their reliability for this group of patients [33].

In patients with resistant delirium other etiologies should be screened for [25, 34]. Besides anamnesis, data from caregivers and additional biological and neuroimaging investigations might be needed for identifying a secondary cause or potential medical complications [34]. The importance of a thorough investigation is confirmed by the fact that patients with RAW are more likely to have a psychiatric history, thrombocytopenia, lower potassium and chloride levels and higher values for AST and alanine aminotransferase (ALT) [35]. A cooccurring withdrawal from GABAergic agents such as Gabapentin and a history of repeated AWSs (kindling) might be the cause for the lack of response to benzodiazepines through complex alterations of the GABA receptors therefore considering an alternative strategy is advised [27, 36, 37].

Current literature suggests that for diazepam-resistant delirium or agitation lorazepam and po/im haloperidol should be considered as rescue medication. Clinicians should be careful of contraindications such as CNS depression, coma, long QT syndrome, etc. [25, 34, 36, 38].

Treatment of Alcohol Withdrawal syndrome's Neuropsychiatric Complications

Alcohol-Induced Amnestic Disorders (Wernicke's Encephalopathy and Korsakoff's Syndrome WKS)

The combination of Wernicke's Encephalopathy (WE) and Korsakoff's Syndromes (KS) is a neurological disorder caused by thiamine (vitamin B1) deficiency mostly found in patients with AUD because alcohol is poor in nutrients and high in calories, also patients with AUD tend to have a poor diet, vomiting, diarrhea and liver problems that can lead to improper absorption and storage of thiamine [39]. It can also occur in other diseases that are not associated with alcohol consumption. WE is an acute syndrome characterized by three main clinical symptoms: mental status changes (confusion), ataxia and eye abnormalities (diplopia, nystagmus, ophthalmoplegia and rarely ptosis). Symptoms develop over a few days or weeks and are often reversible [40]. While WE is an acute condition, KS is a chronic condition that can be preceded by WE and that can evolve to dementia, it is characterized by permanent or serious amnesia along with confabulation [41]. Treatment involves stopping alcohol consumption, rehydration and replacing thiamine. Given that oral absorption is compromised in alcoholic patients, it is preferred to administer thiamine IV or IM. IM preparations have a lower incidence of development of anaphylactic reactions. Diagnosed patients should be given parenteral thiamine 200–500 mg three times a day for 3–5 days, followed by oral administration of 250–1000 mg/day. In patients with suspected WE, parenteral thiamine 250–300 mg two times a day for 3–5 days, then oral thiamine 250–300 mg/day [42, 43]. When glucose infusion is required, thiamine is given before or at the same time as glucose to prevent precipitation of WE, because thiamine as a coenzyme that plays a fundamental role in glucose metabolism.

Alcoholic Hallucinosis

Alcoholic hallucinosis can occur both during acute intoxication or during withdrawal [44]. The onset is usually 48 hours after the last alcohol consumption and can last for days [45]. Therapeutic measures involve abstinence and, in some cases, high potent neuroleptic drugs (haloperidol) [46]. Valproate has been shown to be effective and well tolerated [47]. In the case of alcohol abstinence, the prognosis is good, but in 10–20% of cases, alcohol hallucinosis may persist for a long time.

Alcoholic Withdrawal Induced Seizures

Prevention of seizures is an important goal in the treatment of acute AWS syndrome. Alcohol withdrawal seizures usually occur within 6–48 h after cessation of ethanol consumption and are usually generalized tonico-clonic seizures, partial

seizures can also occur [48]. Literature indicates that seizures are more severe in elderly patients or drug users. In cases of recurrent or prolonged seizures, the aetiologies should be investigated. In addition to blood tests, it is recommended to perform electroencephalography (EEG), cranial computed tomography (CT) and in some cases lumbar puncture [48]. Alcohol sudden cessation destabilizes balance between excitatory (glutaminergic) and inhibitory (GABA) neurotransmitters in the brain which decreases the convulsive threshold. Other possible causes of seizures in AWS are hypoglycemia, electrolyte imbalances, blunt head trauma or associated diseases. Benzodiazepines are the drug of choice in treating and preventing seizures in AWS [49]. In some cases it has been shown that anticonvulsants (carbamazepine, valproate) may be effective [50].

Delirium Tremens

Delirium Tremens is a medical emergency and the most severe complication of AWS that significantly increases the morbidity and mortality of patients [51]. DT appears after a period of heavy drinking followed by acute reduction or cessation of alcohol, typically begins about 3 days after the last drink and lasts from 1 to 8 days or more, in some cases the disorder may occur during an episode of heavy consumption [6]. One of the first treatment goals for patients with DT is to control agitation and decrease the risk of injury and death. Non-pharmacological interventions involve a quiet and protective environment, frequent monitoring of vital signs, acid-base rebalancing and liver enzymes, ensuring adequate hydration, and establishing of an intravenous line. In case of extreme agitation, mechanical restrains may be temporarily applied, for as short as possible and under medical supervision, respecting the applicable laws of each country regarding mechanical restraint [6]. This measure is applied to protect the patient and the people around him. Benzodiazepines used to prevent more severe withdrawal symptoms and to control psychomotor agitation and insomnia. Intravenous administration is preferred, BDZs with long half-time (e.g. diazepam, chlordiazepoxide) have the advantage of self-tapering and constant drug serum levels. For patients with hepatic impairment, short half-life benzodiazepines with no active metabolites are preferred [52]. Suggested treatment of DT consists of controlling agitation, promoting sleep and raising the seizure threshold. Benzodiazepines are used in a high enough dosage to achieve sedation or even light sleep, but still have the patient in an arousable state. The recommended starting dosage depends on the severity of the symptoms. Controlling the symptoms must be done on day 1 with a high enough dose of either lorazepam or diazepam. The recommended dosage of diazepam is 10–20 mg intravenously (IV) or orally every 1–4 h, as needed while the recommended dosage of lorazepam is 8 mg IV, intramuscular (IM) or orally every 15 min, as needed. If the patient received 16 mg of lorazepam and the delirium is still severe, a bolus of 8 mg lorazepam IV is recommended, after that a dose of 10–30 mg/h can be maintained. If the patient presents uncontrolled agitation or hallucinations it is recommended to administer an adjunctive antipsychotic such as haloperidol 0.5–5 mg IV or IM every 30–60 min as

needed, without exceeding 20 mg. Thiamine should be administered to prevent WE, 500 mg IV over the course of 30 min once or twice daily for 3 days. IV glucose and fluids with electrolytes are used for rehydration and to restore the normal levels of electrolytes. In the case of refractory DT, propofol should be given in an intensive-care unit if the clinician has experience with treating DT [6]. It should be noted that clinicians need to differentiate early between DT and WE, as it is a neurological emergency with vital risk.

Treatment of Alcohol Withdrawal Syndrome in Particular Patients

Liver Impairment/Disease

Alcohol use disorder is a common cause of advanced liver disease, and some pharmacological agents are likely to be hepatocytotoxic. Several direct and indirect biomarkers can help clinicians detect and quantify alcohol consumption. Since they can be modified in several pathological processes, they have indicative value when correlated with other clinical data, anamnestic and in some cases heteroanamnestic data (Table 48.1).

Pharmacological treatment for AWS is limited in patients with liver disease based on the underlying illness as well as drug interaction, metabolism, bioavailability, and excretion route [53]. Due to their different metabolic pathways, oxidative metabolism and conjugation, the choice of benzodiazepine is made depending on the degree of liver damage. Patients with alcoholic liver cirrhosis have reduced BZD clearance that undergoes oxidative hepatic metabolism. Diazepam undergoes oxidation in the liver and is therefore prone to impairing the metabolic rate in this group of patients while the clearance of lorazepam is assumed to be unchanged [43]. Therefore, in those with moderate liver damage and those who require close monitoring halving the dose of diazepam/chlordiazepoxide or using lorazepam is recommended. Considering potential side-effects of benzodiazepines in patients with advanced liver disease (risk of sedation and drug accumulation), GABAergic non-benzodiazepine drugs such as baclofen are useful in managing withdrawal symptoms, based on their safety hepatic profile [54].

Elderly Patients

In the elderly, clinicians need to be especially careful because these patients often face multiple comorbidities: cardiovascular, renal or liver impairment, dehydration, infections and hydro-electrolyte imbalances. It is essential to evaluate and identify these conditions. In the elderly benzodiazepines should be administered with caution, because these patients may have liver disease or kidney disease, which increase

Table 48.1 Biomarkers for alcohol use and abuse [52]

Biomarker	Clinical significance
Blood alcohol concentration	Useful in detecting acute alcohol intoxication, it shows limited utility in evaluating alcohol abstinence
γ-Glutamyltransferase	The daily consumption of ethanol for several weeks increases the serum γ-glutamyltransferase level, this is an early indicator of liver disease
The mean corpuscular volume (MCV)	Chronic alcohol abuse increases the size of red blood cells. MCV levels normalize, in general, after 3–4 months after alcohol abstinence.
Carbohydrate-deficient transferrin (CDT)	Heavy drinkers have higher levels of the CDT version than non-drinkers
n-acetyl-β-hexosaminidase (Beta-hex)	Found to be increased in heavy drinkers, subsides to normal levels after 7–10 days of abstinence
Fatty acid ethyl esters (FAEE)	Used for distinguishing social drinkers from heavy or alcoholic drinkers, has also been used as a postmortem marker of alcohol consumption
Aspartate Transaminsase (AST)/ alanine transaminase ratio > 2	A ratio of 2:1 or greater is suggestive for alcohol-related liver disease
Uric acid	Indicator of alcoholism through two mechanisms, dehydration and purine intake
Albumin	Marker of liver function and useful for quantifying the severity of chronic liver disease: a decrease in serum albumin is a sign of a poor prognosis
Prothrombin time test	Sensitive indicator in both acute and chronic liver disease

the risk of drug accumulation and sedation, with subsequent risk of falls and fractures, respiratory depression and cognitive impairment. BZD with short half-life (lorazepam, oxazepam) are preferred, and sometimes it is necessary to lower the doses or to increase drug dosing intervals [55].

Pregnant Patients

The first step in approaching women at childbearing age involves performing a pregnancy test. The diagnosis of AWS in pregnant women is difficult to establish, given that certain symptoms are present in pregnancy but also in AWS. Certain items on the CIWA-Ar scale that assess withdrawal symptoms (nausea, headache or seizures) may overlap with symptoms of pregnancy or preeclampsia. There have been case-control studies suggesting that benzodiazepines administered during pregnancy may cause foetal malformations [56]. Most studies disprove this claim. Careful foetal monitoring is recommended in case of administration of BZD. The use of benzodiazepines in the last trimester of pregnancy has been associated with an increased risk of floppy infant syndrome and benzodiazepine withdrawal syndrome [57].

Conclusions

AWS is a prevalent condition in people with long-term alcohol consumption and is often underdiagnosed and undertreated, with short and long-term consequences. Psychometric assessment (AUDIT test) should become a routine practice not only in psychiatric units but in all medical specialties. The global burden of disease (GBD) is very high in the case of AUD and especially AWS. Therefore, evaluation or screening at the level of primary care would substantially contribute to the prophylaxis of complications caused by AUD, which would significantly reduce the costs associated with GBD. The therapeutic management should be established according to biomolecular markers, rating scales and clinical data about the patient's psychiatric and somatic comorbidities, especially age and the presence of pregnancy. The treatment setting is chosen based on the severity of AWS and life-threatening medical comorbidities (outpatient, inpatient units or even ICU). Treatment is individualized respecting the guidelines and therapeutic protocols. Given the pathophysiology of AWS, the most recommended medications are GABAergic agonists, of which BZD represent the first line of treatment, due to their efficacy and safety profile. While long half-life BZDs are preferred for young patients or in those without medical comorbidities, shorter half-life BZDs are the drugs of choice for elderly patients and for those with severe medical comorbidities because they have little hepatic metabolization and no active metabolites so there is no risk of accumulation. Other medications used to manage AWS are antiepileptics, a-blockers, beta-blockers, barbiturates and new drugs like dexmedetomidine and levetiracetam. Thiamine supplementation is widely recommended. In conclusion, AWS is a widespread neuropsychiatric condition that clinicians should be aware of as it often requires an interdisciplinary approach, timely diagnosis and prompt treatment.

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Part IX
Molecular Mechanisms of Alcohol-Related Liver Disease

Chapter 49
Pathophysiology of Ethanol and Unexplained Observations

Sebastian Mueller

Abstract The mechanisms underlying alcohol-related liver disease (ALD) are still poorly understood despite decades of scientific efforts. Ethanol interacts with all structural levels in organisms making the number of interactions difficult to handle. This chapter identifies six larger topics that require more attention to resolve overlooked clinical, partly controversial observations. Briefly, these topics include (a) the role of fat and intermediary metabolism, (b) competition of ethanol metabolites with enzymes, (c) protein retention and endocytosis as adaptive response, (d) enhanced heme and red blood cell (RBC) turnover, in association with reactive oxygen species, erythrophagocytosis and efferocytosis (e) their role for cell death, ferroptosis and regeneration, and (f) biomechanic aspects such as sinusoidal pressure, hepatic arterialization and mechano-signaling. For instance, many enzymes involved in ethanol metabolism such as acetaldehyde dehydrogenases and the P450 systems have other essential substrates. Such a competitive inhibition has been shown to cause teratogenic malformations in early embryonic stage by specifically interacting with retinol aldehyde dehydrogenase 2, thus modulating retinoic acid levels, an important growth factor. Ethanol also interferes with the metabolism of amino acids, steroids, lipids and so-called specialized pro-resolving mediators. Novel prospective survival data clearly link hemolytic anemia and ineffective erythropoiesis to mortality in heavy drinkers. Preliminary data also suggest that hepatocytes can directly engage in RBC turnover (efferocytosis), involve mitochondria, which may open up novel insights with regard to heme metabolism. More targeted strategies will help to dissect the underlying mechanisms of ALD, namely its astonishing histological similarity to non-alcoholic fatty liver disease.

Keywords Alcohol-related liver disease · NAFLD · CYP2E1 · Liver disease · Liver pathophysiology · Mechanic stress · Shear stress · Hemolysis · Nrf2 · AST · Efferocytosis · Erythrophagocytosis

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Introduction

Although ethanol is a relatively simple molecule it undergoes multiple interactions at all structural and organizational levels when exposed to the human organism. As shown in Fig. 49.1, this renders potential interactions rather complex and is one of the reasons that limit our deeper understanding of alcohol-mediated disease mechanisms. The figure also visualizes that many effects of alcohol require the total integrity of a living organism. Although being justifiable to study ethanol-mediated interactions in a solution of isolated proteins, organelles or in cultured cells, the limitations of such approaches should always be kept in mind.

Especially the 60 and 70ies of the last centuries saw an enormous progress in the understanding of alcohol-related diseases. Ethanol as disease causing factor was established. The important role of the liver in the elimination and metabolism of ethanol and its definite role in causing cirrhosis could be demonstrated. However, a couple of years later, in 1995, Peter Scheuer noted in a Foreword to the book entitled "Alcoholic liver diseases" edited by Pauline Hall that he did not understand why "there is an apparent latent period, supported by examination of serial liver biopsies, between the onset of heavy drinking and the development of steatohepatitis". He further asked "why alcohol-related steatohepatitis is so very similar morphologically to non-alcoholic steatohepatitis (NAFLD)? [1].

Today, almost 30 years later, these statements still hold true. In fact, we have noted almost a stagnation over the last two decades. The reasons are manifold. They may also lay in the complexity of ethanol interactions shown in Fig. 49.1. They may also be related to a general change of societies, some stigmata, with which alcohol remains to be associated, the structure how biomedical science is organized and funded etc. It becomes also clear that novel technological achievements, as fascinating as they are such as OMICS will not spare us from synthesizing the vast amount of data into a meaningful story. What is also missing is the classical, successful strategy in biomedical sciences, the tight interaction between clinical observation and basic research at the organ and cellular level. Alcohol-related diseases are primarily human diseases and, despite many other important interactions of ethanol in the living world such as yeast and bacteria, it should not lose its contact to the diseased human body.

Especially ALD offers more unanswered questions, in addition to the above-mentioned stunning similarity between diabetes- and overweight-induced NAFLD. Almost no progress has been made in diagnosing and treating the often-fatal alcoholic hepatitis (see also part X of the book). Modest benefits are seen in only a fraction of patients with steroids. Although the microbiome has gained great attention, simple antibiotic treatment seems not to halt ALD. Moreover, the pathophysiology of ALD is often explained with quite complex and methodologically challenging topics such as reactive oxygen species that require deep physical,

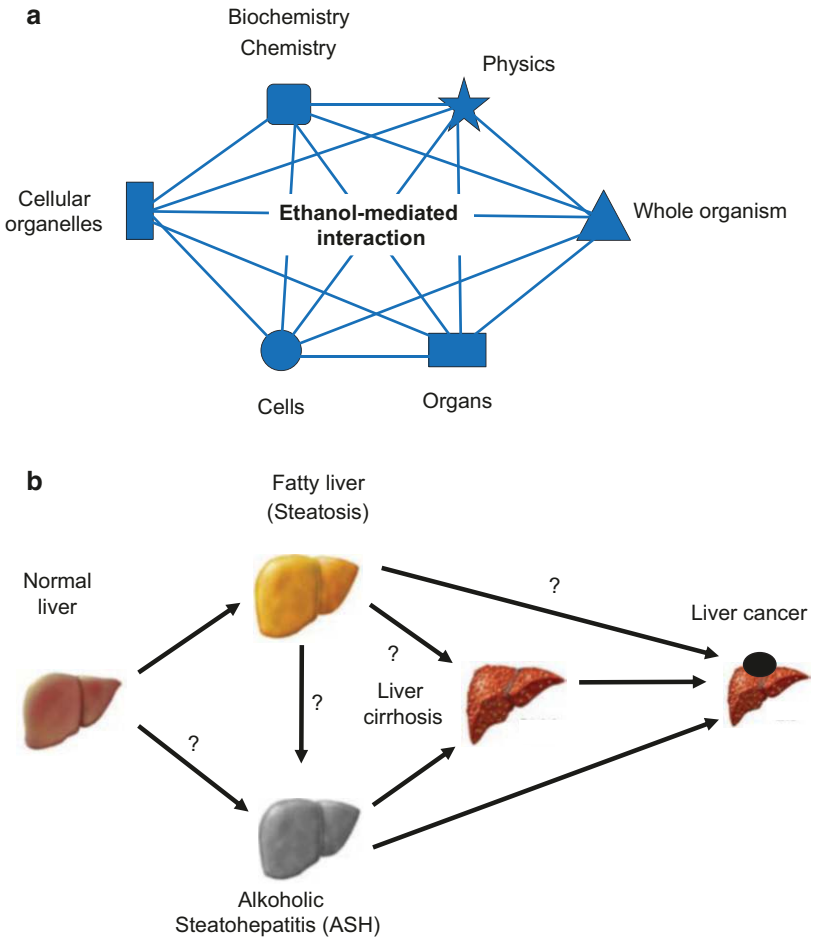

Fig. 49.1 Enhanced intermediary metabolism provides a possible common link between alcohol-related liver disease (ALD) and non-alcoholic fatty liver disease NAFLD. (**a**) Alcohol (ethanol) shows multiple interactions at various organizational levels in humans which complicates the understanding of alcohol-related diseases such as ALD. (**b**) Under ethanol exposure, the transition from normal to steatosis and steatohepatitis to cirrhosis and cancer is established. However, it is still debated whether steatohepatitis can be induced in normal liver or whether steatosis can directly progress to inflammation, cirrhosis and cancer. Moreover, it is still not clear whether steatosis is a bystander or a mandatory step towards the progression of ALD. (**c**) Progression of NAFLD and ALD share many confounding factors. Factors in black are essential for the progression of either NAFLD or ALD while factors marked in blue represent important modulating factors. An enhanced intermediary metabolism with a shift towards reduced NADH, enhanced lipogenesis and elevated glucose could be the joint link of both diseases, potentially better called **metabolic liver disease (MLD)**

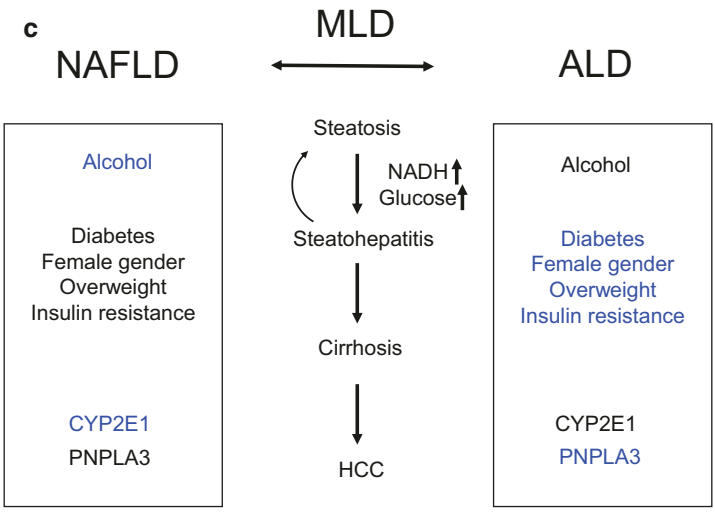

Fig. 49.1 (continued)

chemical and biochemical knowledge. On the other side, enzyme systems such as the p450 system localized in the endoplasmic reticulum are attributed to mediate major disease mechanisms but both knockout animals or pharmacological blockage show less convincing effects. And again, meanwhile, we are used to typical laboratory changes of heavy drinkers although we have no real explanation. What is alkaline phosphatase really doing? Why is GGT frequently elevated in patients with ALD? Where are transaminases originating from? Why are levels of AST always higher than levels of ALT? How about the changes of typical iron-related parameters such as ferritin that are even proposed by guidelines to search for the much rarer hereditary iron overload diseases? In other words: What are the real mediators of ALD?

There can be no doubt that **acetaldehyde** is one of the most important toxic intermediates of ethanol toxicity. As described in another chapter, gene variants that cause a homeostatic elevation of acetaldehyde cause clinical symptoms such as flushing syndrome or hangover. The worse combination is a rapid ADH combined with an almost inactive ALDH. It is also interesting to appreciate that slow ADH metabolizers are more prone to develop alcohol dependence. It seems that, to gain the joy of ethanol, the organism has to cope with the obligatory burden of its oxidation products, especially acetaldehyde. This chapter will not claim to be complete, but it aims at describing in a few rather short paragraphs some basic principles of ethanol-related diseases mechanisms. It also aims at describing some newer clinical and experimental observations in order to continue our journey in better understanding the pathology of alcohol.

Hepatic Steatosis-Bystander or Mandatory Disease Stage?

Steatosis is considered one of the hallmarks of ALD and, indeed, as shown in Fig. A.7 and Table B.7, it is present in more than 90% of heavy drinkers. However, exactly this number has generated a continued debate on whether steatosis is indeed a mandatory precursor of liver cirrhosis or a bystander or even a physiological way of interim storage of fat. If only 20% of heavy drinkers progress to liver cirrhosis, the majority of patients with fatty liver will obviously not undergo any disease progression. As shown in the prospective data in Tables B.21, B.22 in the Appendix B, no significant association can be found between steatosis and death in heavy drinkers. In addition, although discussed controversially, there have been observations of "protective" fat accumulation [2]. Already Theodor Ferichs described in the mid-nineteenth century, that feeding with a high fat diet could induce reversible hepatic steatosis in dogs, and liver fat is typically used by hibernating animals during winter periods to store fat without known negative consequences [3]. As shown in Fig. 49.1b, the transition of the liver to steatosis and of inflammation to cirrhosis are established beyond any doubt. It is, however, surprising to see that the transitions from fat to inflammation and the role of fat for fibrogenesis are still not clearly understood. Based on studies on patients with non-alcoholic fatty liver disease (NAFLD), it is even increasingly discussed whether steatosis can also directly contribute to cancer development.

In summary, liver fat and the role of fatty acids for disease progression is still poorly understood. It is hoped that the identification of the new role of enhanced red blood cell (RBC) turnover in ALD which is presented within this book (chapters on mortality, iron and bone marrow), will provide novel impulses. This "masked hemolysis" is highly associated with steatosis and RBCs uptake by macrophages but also hepatocytes (see Figs. A.33, A.34, A.35, A.36). It is often forgotten that RBC cholesterol primarily accounts for serum cholesterol levels and is in tight homeostasis with it. Moreover, the role of poly unsaturated fatty acids (PUFAs), especially the arachidonic acid metabolism, can give rise both to pro-inflammatory and anti-inflammatory pathways that are still poorly understood. Almost no data are available on these signaling pathways in the context of ethanol metabolism (see also Figs. A.53, A.54, A.55, and A.56). First preliminary data on lipidomics and mortality in heavy drinkers is presented in Table B.10.

Is the Intermediary Metabolism the Link Between ALD and NAFLD?

Why can alcohol-related liver disease be replicated by diabetes and obesity? One of the major cause-specific deaths is liver related mortality with alcohol-related liver disease as one of the famous hallmarks of alcohol consumption. Already in 1995 it was discussed "why alcohol-related steatohepatitis is so very similar morphologically

to non-alcoholic steatohepatitis (NAFLD)? [1] with no clear answers today, almost 30 years later.

Although the oxidation intermediated acetaldehyde is beyond any doubt crucial to explain alcohol-related disease mechanisms, it cannot be the major link to obesity and diabetes.

Rather, both alcohol, diabetes and overweight are characterized by an excess of energy. As can be seen from the original data (see Tables B.4, B.5, B.6, B.7, B.8, B.9) of the Heidelberg cohort followed-up over 15 years, heavy drinkers, despite having access to normal nutrition, cover about 50% of their energy supply by ethanol. In this cohort, during a mean daily consumption of ca. 180-gram alcohol, a mean blood alcohol concentration of 1 °/°° (1 g/L) was reached, corresponding to a mean elimination of ca. 7.5 g alcohol per hour.

Although ethanol, comparable to sugars, only contains the three elements carbon, oxygen and hydrogen, it is neither chemically nor biochemically a carbohydrate and human metabolism is strikingly different. As mentioned above, ethanol is an energy supplier containing almost the double energy as compared to glucose (7 versus 4 kcal per gram). Similar to other physiological energy suppliers such as fatty acids or sugars, oxidation of ethanol leads to NADH which can be further used for mitochondrial respiration. However, in contrast to glucose and fructose, **ethanol metabolisms rather prevents gluconeogenesis** simply due to a balance shift towards reduced NADH (see also Fig. A.38). This shifts the lactate/pyruvate ratio towards lactate and, consequently, away from gluconeogenesis. Through the malate aspartate cycle, reduced NADH is also shifted across the mitochondrial membrane and used for ATP production [4]. First data suggest that the same holds true for other organelles such as peroxisomes [5]. Ethanol also provokes a fast and efficient depletion of glycogen stores (see Fig. A.47). Of note, however, heavy drinkers are characterized by elevated serum glucose levels (see Tables B.2, B.6) which further increase at later fibrosis stages. Due to the enhanced hemoglobin and red blood cell turnover, the diabetic marker HbA1C is often underestimated in heavy drinkers and the diagnosis of diabetes remains obscure (see also Chap. 57).

Glucose is central to energy consumption. Carbohydrates, lipids, and proteins can all ultimately break down into glucose, which then serves as the primary metabolic fuel of mammals and the universal fuel of the fetus. It serves as the major precursor for the synthesis of different carbohydrates like glycogen, ribose, and deoxyribose, galactose, glycolipids, glycoproteins, and proteoglycans [6]. Unlike glucose, which is directly metabolized widely in the body, fructose is almost entirely metabolized in the liver in humans, where it is directed toward replenishment of liver glycogen and triglyceride synthesis [7]. Ca. 40% of fructose is converted in liver to glucose, and about 25% is converted to lactate and ca. 20% is converted to glycogen [8]. Glucose and lactate are then used normally as energy to fuel cells all over the body [9].

Thus, it seems that although not being a carbohydrate, ethanol shares with sugars the immediate energy supply within the intermediary metabolism (see also Fig. 49.1c). They also share to some extent the lack of a negative feedback loop. As mentioned in the Chap. 50, human metabolism can neither escape an excess of glucose/fructose or ethanol and will metabolize it under excess conditions. In this scenario, fatty acid accumulation is the bodies only option to rapidly eliminate carbohydrates or ethanol and store the excess energy through lipogenesis in adipose tissue. Hence, in this context, **fatty liver** may rather be a bystander and a metabolic consequence to quickly remove the excess of energy but not the primary cause of steatohepatitis. Consequently, it is the **uncontrolled excess of rapidly-available energy that may link NAFLD with ALD**. Figure 49.1c shows the common progression factors of NAFLD and ALD, where steatohepatitis results from enhanced intermediary metabolism, most likely due to mitochondrial damage, while steatosis is both a results of the mitochondrial damage (decreased fat elimination) and enhanced lipogenesis in order to rapidly eliminate the energy excess in "safe adipocyte stores".

Since lipogenesis, in this context, would be more a solution than a problem, fatty liver may not be the actual disease hallmark but rather the mitochondrial damage and inflammation due to excess energy supply and mitochondrial damage. The development of mitochondrial damage will later impair the mitochondrial ß-oxidation and, subsequently, further increase steatosis due to decreased fat elimination. More research on carbohydrate metabolism and its relation to ethanol and its hormonal control is needed. Ultimately, with regard to the terminology debate, **metabolic (dysfunction) associated fatty liver disease (MAFLD)** [10] may not be optimal for paving a future path of better understanding the underlying mechanism. As shown in Fig. 49.1c, a potential more optimal alternative could be then broader term **"Metabolic Liver Disease" (MLD)** which would only encompass patients with signs of liver damage and fibrosis while fatty liver would be an important diagnostic feature but not necessarily part of the pathology. Finally, the intermediary metabolism would also provide a novel bridge to **interlink energy and ethanol metabolism to addiction**, whether it is food addiction or alcohol dependence. Here, more detailed studied are needed in the future.

Competition of Ethanol with Important Ethanol-Metabolizing Enzymes

It is important to note that all ethanol-metabolizing enzymes including ADH, ALDH or CYPs are highly evolutionary conserved despite nutritional intake of alcohol. As mentioned in the Chap. 50, this is because gastrointestinal fermentation causes significant intestinal production of ethanol, and, more importantly, these enzymes are involved in the oxidation of other alcohols and substrates such as retinols. The

most impressive example, in my opinion, has been observed in experimental frog embryo models to study Fetal Alcohol Spectrum Disorder (FASD) [11]. For more details, reading of part IV is recommended. In this work, first evidence was provided of a competitive inhibition of **retinoic acid (simplified for all-trans-retinoic acid, abbreviated RA**) biosynthesis under conditions of ethanol metabolism. RA synthesis required the enzymatic activity of RALDH2 (ALDH1A2), the main retinaldehyde dehydrogenase expressed at that stage. As can be seen in Tables A.39, A.40 and A.41 in the Appendix, RALDH2 (ALDH1A2) is also an important acetaldehyde dehydrogenase. Under physiological conditions, in the embryo, RALDH2/ALDH1A2 converts retinal to RA. RALDH2/ALDH1A2 thus provides a molecular link between growth and ethanol metabolism with both substrates, retinal/retinaldehyde and acetaldehyde, competing for the enyzme.

Normally, RA is a metabolite of vitamin A1 (all-trans-retinol) that mediates the functions of vitamin A1 required for growth and development. RA is required in chordate animals, which includes all higher animals from fish to humans. During early embryonic development, RA generated in a specific region of the embryo helps determine to position along the embryonic anterior/posterior axis by serving as an intercellular signaling molecule that guides development of the posterior portion of the embryo [12]. It acts through Hox genes, which ultimately control anterior/posterior patterning in early developmental stages [13].

In the embryo, RA production by RALDH2 (ALDH1A2), the main retinaldehyde dehydrogenase expressed at that stage, is inhibited by ethanol exposure. Pharmacological inhibition of the embryonic alcohol dehydrogenase activity prevents the oxidation of ethanol to acetaldehyde that in turn functions as a RALDH2 inhibitor. Acetaldehyde-mediated reduction of RA can be rescued by RALDH2 or retinaldehyde supplementation [11]. Enzymatic kinetic analysis of human RALDH2 shows a preference for acetaldehyde as a substrate over retinaldehyde. RA production by RALDH2 is efficiently inhibited by acetaldehyde but not by ethanol itself. Thus, these studies elegantly demonstrated that acetaldehyde is the teratogenic derivative of ethanol responsible for the reduction in RA signaling and induction of the developmental malformations characteristic of FASD. It is assumed that this competitive mechanism will affect tissues requiring RA signaling when exposed to ethanol throughout life.

The study [11] also showed that ethanol was only harmful in a specific vulnerable time window, where teratogenic malformations could be observed. The conclusion may even be extended to carcinogenesis and much more work is needed to better understand ethanol interactions with cellular growth by competitive enzyme inhibition. Table 49.1 lists a few ADHs, ALDHs and CYPs with at least some of their known physiological substrates. It becomes rapidly evident that ethanol or, more likely, acetaldehyde can compete with these pathways and cause highly specific interferences.

Finally, with regard to microsomal ethanol oxidation by CYP2E1, it should be noted that other CYPs also metabolize ethanol to a similar or lower extent. There are

Table 49.1 Potential competition of ethanol with physiological substrates of selected ethanol metabolizing enzymes. The competitive inhibition of these enzymes provides a mechanistic rationale for ethanol-mediated disease mechanisms such as the teratogenic effects during embryonic phases involving ALDH1A1-3 (RLDH2). A more complete list is shown in Appendix Figs. A.39, A.40, A.41 and A.42

Enzyme	Substrates or conversion
ADH	
ADH1A	Metabolizes a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. Major role in ethanol catabolism
ADH5	Glutathione-dependent formaldehyde dehydrogenase, has virtually no activity for ethanol oxidation, but exhibits high activity for oxidation of long-chain primary alcohols and for oxidation of S-hydroxymethyl-glutathione, important for the elimination of formaldehyde
ALDH	
ALDH1A1	Converts retinaldehyde to retinoic acid
ALDH1A3	Converts 10-formyltetrahydrofolate to tetrahydrofolate
ALDH1L1	Converts 10-formyltetrahydrofolate to tetrahydrofolate
ALDH1L2	Converts aldehydes from lipid peroxidation to their corresponding carboxylic acids
ALDH6A1	Converts malonate and methylmalonate semialdehydes to acetyl- and propionyl-CoA
ALDH8A1	Converts 2-aminomuconate semialdehyde to 2-aminomuconic acid
ALDH9A1	Oxidizes gamma-aminobutyraldehyde and other amino aldehydes
ALDH18A1	Reduction of glutamate to delta1-pyrroline-5-carboxylate
CYP	
CYP2E1	
	ω-1 hydroxylation of fatty acids
Monooxygenase activity	Metabolizes arachidonic acid to 19-hydroxyeicosatetraenoic acid (19-HETE)
Epoxygenase activity	Metabolizes docosahexaenoic acid to epoxides
CYP1A2	Metabolizes polyunsaturated fatty acids into signaling molecules
Monoxygenase activity	Arachidonic acid to 19-hydroxyeicosatetraenoic acid (19-HETE)
Epoxygenase activity	Metabolizes docosahexaenoic acid to epoxides, primarily 19R,20S-epoxyeicosapentaenoic acid and 19S,20R-epoxyeicosapentaenoic acid isomers (termed 19,20-EDP) and similarly metabolizes eicosapentaenoic acid to epoxides, primarily 17R,18S-eicosatetraenic acid and 17S,18R-eicosatetraenic acid isomers (termed 17,18-EEQ)

still many open questions. The literature focuses on hepatic CYP2E1, but its expression has been noted in macrophages, adipose tissue, intestine. Moreover, it remains unclear why CYP2E1 is typically expressed in the pericentral region (see Fig. 49.2c) and why its expression decreases or is almost abolished in patients with manifest cirrhosis (see mRNA data from ALD patients in Appendix B Table B.13.

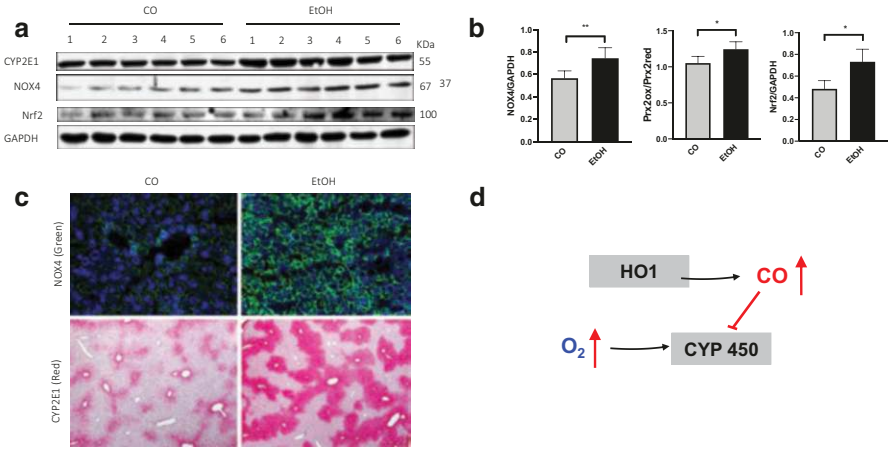

Fig. 49.2 Novel role of redox-sensitive factors to produce reactive oxygen species such as hydrogen peroxide by NADPH-dependent oxidase 4 (NOX4). Mice were exposed for 4 weeks to ethanol. (**a**) Protein expression of CYP2E1, NOX4 and Nrf2. (**b**) Densitometric data. (**c**) Immunostaining of CYP2E1 and NOX4 prior and after 4 weeks of ethanol in mouse liver. (**d**) Possible inhibition of heme-containing CYP2E1 by HO1-released carbon monoxide (CO). Both enzymes are located in the endoplasmic reticulum. CO may also inhibit other heme enzymes such as peroxisomal catalase and mitochondrial cytochromes. This inhibition would finally cause elevation of intracellular oxygen which could be more efficiently processed by NOX4 to yield hydrogen peroxide. More details are provided in the Appendix

Role of Enhanced RBC Turnover, Iron, and Reactive Oxygen Species

The recent novel finding that enhanced red blood cell (RBC) degradation and increased erythropoiesis drive long-term mortality in heavy drinkers (see Chaps. 7, 57 and 58 on mortality, iron and bone marrow) [14] provides a better understanding of the role of hepatic iron accumulation but also reactive oxygen species. Systemic hormonal and cellular control of iron accumulation has long been considered the major reason for pathological iron deposition in livers of drinkers [15]. However, the new insight of enhanced RBC turnover and the importance of hepatocytes in guiding heme degradation and being even able to perform efferocytosis of RBCs (see chapter on "Hepatic iron overload and ALD" and preliminary data in Figs. A.34, A.35, A.36) strongly underline that iron overload is a direct consequence of this heme turnover.

These findings have multiple implications for long known pathological aspects in ALD such as the generation of reactive oxygen species (ROS) or the changes in the methionine, glutathione, vitamin B6 and B12 metabolism. They are especially interesting with regard to the evolutionary role of mitochondria as ancient bacteria (**endosymbiosis theory**). It is quite striking to see that important steps of the heme synthesis only occurs in mitochondria while heme degradation through HO1 is

performed in the ER (see also Fig. A.74). Moreover, first electron micrograph images from Figs. A.33, A.34, A.35, A.36 suggest that RBCs may not only be uptaken directly by hepatocytes (**efferocytosis**) (also shown in Fig. 49.7) but may later fuse with mitochondria. More studies are needed but these impulses are highly fascinating and go beyond the role of ethanol in liver injury but touch fundamental physiological cellular processes. They are especially interesting with the evolutionary conserved **tight link between iron and bacteria**, the high demand of bacterial growth for iron, the high content of iron in mitochondria, the still important role of iron for bacterial infections in humans (see e.g. transferrin versus lactoferrin), and the still unclear role of **megamitochondria** in ALD.

Reactive oxygen species (ROS) have been established to be involved in ALD, however, ROS methodology is challenging and can be easily misinterpreted [16–22] . Important antioxidative systems such as glutathione (GSH) are decreased under exposure to ethanol and oxidative fingerprints such as lipid peroxidation products are increased. All animal cells are capable of synthesizing GSH, and GSH synthesis in the liver has been shown to be essential as Glutamate-cysteine ligase (GCLC) knockout mice die within a month of birth due to the absence of hepatic GSH synthesis [23]. Unfortunately, it tells little about the real underlying molecular mechanisms. Often, unphysiological high ROS concentrations (e.g. hydrogen peroxide) are explored in experimental models while the use of appropriate low and steady state levels show opposite findings [18]. For instance, the redox-sensitive systemic iron masterswitch hepcidin is downregulated at toxic ROS levels [24] while non-toxic H_2O_2 level upregulate [25]. Moreover, under physiological hypoxia levels of 5%, hepcidin is further upregulated [19]. For details see also Figs. A.70, A.71, A.72. There is also the misconception about "physiological oxygen" levels. While human require appropriate oxygen levels in the air of about 21%, single cells are exposed to much less oxygen tension [21, 22, 26].

It has also been less appreciated that either between cells or between organelles within a single cell, there may be important and specific interactions with regard to ROS and oxygen. As an example, Fig. 49.2a shows typical moderate upregulation of CYP2E1 in a 4-week mice model exposed to ethanol in drinking water. As can be seen, NAPDH-dependent oxidase **NOX4** that is readily expressed in hepatocytes, is induced in line with the redox-sensitive transcription factor **Nrf2 (Nuclear factor erythroid 2-related factor 2, or Nuclear factor erythroid-derived 2-like 2)** [27–29]. Figure 49.2b shows the typical pericentral induction of CYP2E1 upon ethanol exposure while NOX4 shows a clear membrane associated, basolateral expression. Figure 49.2d schematically demonstrates that enhanced heme turnover, as is observed in heavy drinkers (see chapters on mortality and iron), will generate **carbon monoxide through HO1 action**, which should block efficiently the heme enzyme CYP2E1. Both enzymes, HO1 and CYP2E1, are localized in the same compartment (endoplasmic reticulum, ER). Figure A.76 goes one step further. If carbon monoxide is released from the ER, it may not only block the mitochondrial respiratory chain but also peroxisomal catalase. In both cases, this will result in less oxygen consumption, increased cellular oxygen levels which then could serve as substrate for NOX4 and lead to elevated hydrogen peroxide levels. The strong

expression of NOX4 rather suggests that oxidase-released H_2O_2 may significantly contribute to elevated ROS in ALD. It is also interesting to note that the redox-sensitive transcription factor Nrf2 is elevated upon ethanol exposure (Fig. 49.2a). **Nrf2** controls many other important target genes such as HO1, Glutamate-cysteine ligase (GCLC), Glutathione S transferase (GST), UDP glucuronosyltransferase (UGT) and Multidrug resistance-associated proteins (MRPs) that are all involved in heme degradation and elimination through the bile. Figure A.75 shows the regulatory network with **KEAP1** including the feedback loop with KEAP1. More detailed studies on this topic will be highly fascinating and hopefully shed more light on the fact that, while NOX1 and 4 are upregulated by ethanol, macrophage NOX2 is downregulated. As shown recently, NOX enzymes induce different target genes and cytokines depending on macrophage or hepatocyte localization [19, 30]. NOX1 and 4 upregulation but NOX repression has also been confirmed in liver specimen from human heavy drinkers (not shown).

Enhanced RBC turnover is also interesting with regard to **vitamin disturbances in ALD. Thiamine (Vitamin B1)** and its thiamine phosphate derivatives are involved in many cellular processes. The best-characterized form is thiamine pyrophosphate (TPP), a coenzyme in the catabolism of sugars and amino acids. **Thiamine deficiency** causes Wernicke Korsakoff syndrome and is more pronounced in patients with ALD (see respective chapter in this book).

Manufactured folic acid, which is converted into **folate** in humans, is required to make DNA and RNA and metabolize amino acids necessary for cell division. Although not completely clear, it is quite intriguing that ethanol metabolism in liver and RBCs (where **folic acid** is stored) causes increased folate utilization and RBC turnover. As can be seen from heavy drinkers (see Chap. 58 on "Bone marrow" and original data in Table B.29), folate decreases in patients with macrocytic anemia. Consequently, a relative folate deficiency may exist due to enhanced RBC turnover. It is also interesting to see, that, most likely in a compensatory manner, **vitamin B12** is elevated in heavy drinkers with elevated MCV. As B12 is controlled by secretion of the gastric intrinsic factor, this could suggest that ethanol specifically blocks certain pathways that require folate and B12 (betaine, methionine). In this context, biochemical pathways of transsulfuration and methionine metabolism are of highest interest (see Figs. A.50 and A.51 and Chap. 55 on methionine metabolism). In conclusion, the role of vitamins, namely the B series, deserve special attention in the light of the important prognostic role of hemolytic anemia (see Chaps 7, 57 and 58 on mortality, iron and bone marrow).

Regeneration, Cell Division and Cell Death

Cell division and regeneration is another important cellular function that is strongly affected by ethanol. On the other side, regeneration is important for cancer development, especially, if cells divide rapidly in a toxic environment that exposes the DNA to genotoxic intermediates such as acetaldehyde. Liver regeneration is also required to recover from alcoholic liver injury and it is long know that a

continuous exposure to alcohol is more detrimental than same quantities that are provided intervals allow phases of recovery. Alcohol-related fatty liver demonstrate increased rates of hepatocyte death, the latter providing a regenerative stimulus. However, unlike mature hepatocytes in healthy adult livers, most surviving mature hepatocytes in alcoholic fatty livers cannot replicate. Therefore, less mature cells (progenitors) must differentiate to replace dead hepatocytes. Little is known about the general mechanisms that modulate the **differentiation of liver progenitors** in adults requiring a better molecular and cellular clarification [31].

In this context, data from patients undergoing alcohol detoxification on growth factors but also on apoptosis are quite elucidating. As shown in Fig. 49.3a, **important growth factors (HGF, hepatocyte growth factor, EGF, epithelial growth factor, and IGF, insulin-dependent growth factor)** are all significantly increased after alcohol detoxification within the first 7 days. IFG even continues to increase thereafter. Figure 49.3b demonstrates that serum erythropoietin (EPO) but also apoptosis as measured by serum M30 levels is transiently upregulated. Induction of liver apoptosis after withdrawal from alcohol had been reported previously [32]. These data, in humans, readily demonstrate that ethanol primarily blocks regeneration and cell division, most likely due to the genotoxic environment. However, under another vital stimulus, such as severe anemia or functional loss of hepatic tissue, the DNA protecting mechanisms that block regeneration may be counteracted by survival signals to maintain a critical cells mass.

This can be studied e.g. by phlebotomy or hepatectomy both of which remove vital tissue mass and, consequently, induced tissue proliferation despite the presence of toxic ethanol metabolism. As ethanol causes both ineffective erythropoiesis (see chapters on iron and bone marrow) and liver damage (see Chap. 38 on histology), these changes can be regarded as "virtual phlebotomy or hepatectomy). As shown in Fig. 49.4, this will result in enhanced RBC turnover, enhanced heme degradation and processing of toxic heme degradation end products by the liver with final excretion of bilirubin. Hence, an ambivalent situation ("signaling conflict") is created that

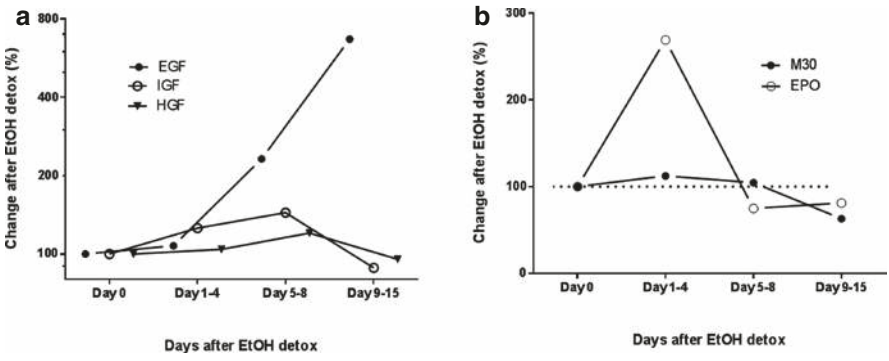

Fig. 49.3 Elevation of important (**a**) growth factors (EGF, IGF, HGF) and (**b**) erythropoietin (EPO) and apoptosis marker M30 after alcohol detoxification. These data demonstrate that ethanol is a strong blocker of growth factors and apoptosis

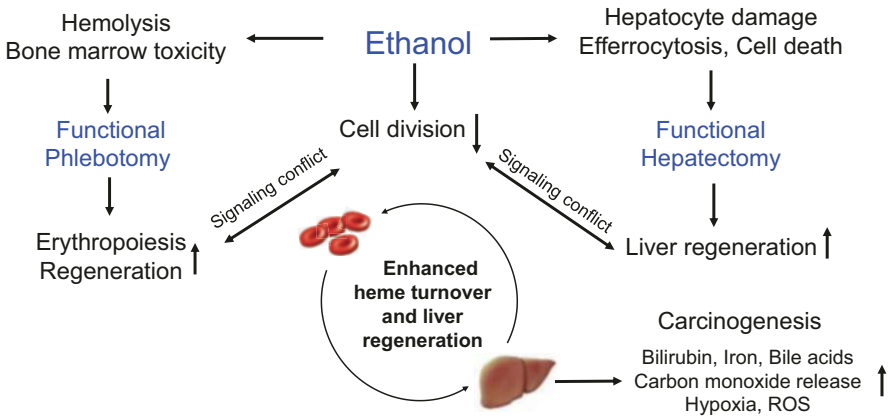

Fig. 49.4 Ethanol provides ambivalent signals to the regeneration machinery and cell division. It is blocking cell division due to toxic ethanol metabolism but stimulates regenerative signals through functional loss of tissues such as hemolysis or liver damage

forces tissues to regenerate and process toxic end products in a genotoxic environment.

Mice and human data suggest that the RBC cycle is modulated by alcohol in a complex manner. As hemolytic anemia is a major driver of mortality in heavy drinkers (see mortality and iron Chaps. 7 and 57) and due to compensatory mechanisms at various levels, it takes several years to develop macrocytosis and liver damage. The transient deterioration of hemolytic anemia after alcohol detoxication demonstrates that ethanol is not alone responsible for the vicious cycle but other cofactors may also contribute. **For instance, accumulation of toxic iron in hepatocytes, macrophages, and erythroblasts could contribute to this mechanism**. Further studies are needed to better understand the exact kinetic response. It is a sign of hope that continued abstaining from alcohol, even after years, can both improve the hematological parameters (MCV, RBC count) and liver parameters (liver stiffness). Preliminary data after 5 years of continued abstaining from alcohol are shown in Figs. A.89 and A.90.

The **mechanisms of cell death regulation** in ALD remain also to be clarified. As is shown in Fig. 49.3, after alcohol detoxification, liver apoptosis increases, so do liver regeneration markers [32]. However, various types of cell death such as **pyroptosis, necroptosis, apoptosis and ferroptosis** (see Fig. A.58) have been identified. So far, it remains unclear whether these death pathways are distinct forms or overlapping events of a general and fundamental death cascade. Moreover, for each classified death type, many open questions remain. It is interesting that reactive and toxic molecules such as ROS (apoptosis) and iron (**ferroptosis**) have been implicated [33–35]. Preliminary data in the Heidelberg cohort of heavy drinkers indicate that markers of ferroptosis such as GPX4 and ACLF are elevated (see Figs. A.3 and A.12). It is intriguing to speculate that ferroptosis is connected to heme turnover,

most likely through efferocytosis of RBCs (see also electron micrographs in Figs. A.34, A.35, and A.36. An interaction of Nrf2 (see above) and ferroptosis has also been discussed [29].

Ethanol, Endocytosis, and Protein Retainment

Alcohol consumption causes hepatomegaly, both in rodents and humans, associated with enlargement of the hepatocytes. Largely overseen today, already Lieber and Baraona showed clearly, almost 50 years ago, that enlarged livers during heavy alcohol consumption are not only due to retention of lipids (steatosis) but also proteins (see Fig. 49.5) [36, 37]. Protein deposition also contributes to a similar extent to hepatomegaly as observed in chronic alcohol consumption. In control rats, lipids represented 15.7% of dry weight and proteins 55.2%. After alcohol consumption, hepatic dry weight increased by ca. 31%, protein by 31% and fat by 83%. Although increasing organelle proteins (mitochondria and microsomes) do contribute to the total increase, the **major fractions of proteins is deposited in the cytosol, including export proteins such as albumin and transferrin** [38]. They also showed that synthesis of liver protein and **proalbumin** were enhanced by chronic ethanol feeding, but this was not associated with a corresponding rise in serum albumin output. There was a significant retention of liver albumin and transferrin with delayed appearance in the serum of ethanol-fed rats. This indicated

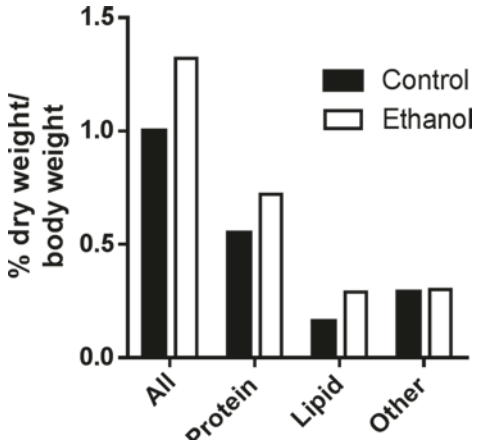

Fig. 49.5 In humans and animals, ethanol not only increases hepatic fat content but also causes protein accumulation. The bars show amount of protein, lipid and other content in control rats and rats treated with alcohol. The underlying mechanisms have been poorly studied but seem to be rather due to an adaptive response in order to e.g. prevent iron uptake in the case of transferrin than a toxic blockage. In support of this hypothesis, some specific proteins are released to the serum compartment such as alpha-macroglobulin. Modified from [36]

that, regardless of the changes in synthesis, the export of protein from the liver into the plasma was impaired. This alteration in export was associated with a decreased amount of polymerized tubulin in the liver of ethanol-treated animals. Thus, both enhanced protein synthesis and defective export contribute to the ethanol-induced accumulation of liver protein, and the decrease in **liver microtubules** represents a possible site for impairment of protein export [38]. **Triglycerides and cholesterol** also decrease after alcohol exposure [39, 40].

As is shown in chapter and Appendix (e.g. Fig. A.57), many important and liver synthesized carrier proteins (albumin, transferrin, haptoglobin, Apo A1) are decreased in serum of heavy drinkers with progressing liver disease. This is not the case for total protein.

It remains to be answered whether protein accumulation is due to ethanol-mediated damage to the protein elimination machinery, microtubule apparatus, cytoskeleton and membranes associated vesicle generation used for endo-, trans- and exocytosis or, whether this could be due to an adaptive response. As is discussed in detail in Chap. 57 on "ALD and iron", due to hemolysis and ineffective erythropoiesis, serum iron typically increases, in line with transferrin saturation and ferritin. As can be seen in the Kaplan Meier plots (Figs. A.86, A.87) from the ongoing survival study in heavy drinkers, **transferrin** is one of the most suppressed serum proteins. This appears logical since the liver is overwhelmed with iron from the erythrophagocytosed/efferocytosed RBCs and it could downregulate transferrin to prevent further iron delivery. More work is needed here that also includes the relation to ferroptosis.

Is Albumin Synthesis Really Impaired in Patients with Liver Cirrhosis?

The strongest argument against a mere toxic or damage-related decrease of serum proteins such as albumin comes from clinical studies of a continued removal of albumin in patients with liver cirrhosis. Thus an **implanted ascites pump** (alfapump®) [41] has been recently introduced for the treatment of therapy-refractory ascites that continuously pumps ascites from the peritoneal cavity into the urinary bladder. The study recruited initially 40 patients of whom the majority (43%) had ALD. In average, the pump removed 1 L ascites per day. As ascites contains significant amounts of albumin (at least 20 g albumin/L ascites), after 6 months, the pump had removed 180 l ascites corresponding at least to ca. 3.6 kg albumin. However, albumin levels were only reduced from 31.9 to 27.2 g/L (by 14.7% or from 0.5 to 0.4 mM)) while, at the same time, bilirubin levels decreased by 20.7% [41]. In other words, while the serum albumin amount decreased by 5 g/L, more than 3 kg had been removed and, consequently, had been replaced by the cirrhotic liver. Even if ascitic concentrations of albumin would be some grams less as compared to the serum, these clinical data clearly suggest that the cirrhotic liver replaces

albumin if it is removed by other means. It also suggests that there could be a signal of the "old albumin" to not further release/secret fresh albumin to the circulation.

One obvious reason of decreased albumin could be that hepatocytes are loaded enough with molecules carried by albumin such as bilirubin. It is often forgotten that albumin not only serves to maintain oncotic pressure but has many other functions. It not only binds thyroid and steroid hormones, vitamin D, fatty acids, but above all, **unconjugated bilirubin**. The amount of bilirubin released from heme degradation can be estimated from novel data of enhanced RBC turnover in heavy drinkers. Each hemoglobin molecule is made up of four heme groups surrounding a globin group, forming a tetrahedral structure. During normal daily erythrophagocytosis, ca. 1% of RBC are recycled which corresponds to 25 ml RBC volume, 8 g or 0.12 mmol hemoglobin. As four heme groups are contained by each hemoglobin, this results in ca. ca. 0.5 mmol or 250 mg bilirubin released every day. Since bilirubin is bound to albumin at a 1:1 ratio [42], and assuming a single path removal of bilirubin by albumin, ca. 0.5 mmol albumin or ca. 30 g albumin total serum albumin would be required. This corresponds to about 20% of the total serum albumin if a total serum volume of 3 L is assumed. In ALD patients with enhanced RBC turnover, a much higher bilirubin release can be estimated potentially by a factor of 3–4 times higher the normal value. These calculations underline how important albumin is for bilirubin removal and that a **blocked albumin uptake by hepatocytes could be seen as a likely adaptive response** to prevent further bilirubin uptake and toxic heme degradation.

Unconjugated bilirubin is typically taken up by the **Organic anion transporting polypeptide called OATP1B3** which is most strongly expressed in pericentral regions of the hepatic lobule. In addition to endogenous substrates (examples include bile salts, thyroid hormones, conjugated steroid hormones, prostaglandins), OATP1B3 transports unconjugated bilirubin from the blood to the liver [43]. See also Fig. A.60. However, how albumin is entering hepatocytes, still remains an open discussion [44]. In an average human, about 13.3 g albumin are lost per day. An **putative albumin-uptake receptor** has been described (Albondin) that allows transcytosis through endothelial cells. Transcytosis of albumin through endothelial cells has been shown to only last 15 s. The strong inhibition of albumin release by colchicine clearly suggests that microtubules are required for albumin excretion [36, 38, 45]. The putative albumin receptor may play an important role in the bidirectional transfer of many classes of endogenous and exogenous substances between albumin and cells [46]. In addition, **lysosomes** also contain a considerable amount of serum albumin and it has been shown in the regenerating liver that partial hepatectomy activates endocytosis and facilitates delivery of endocytosed serum albumin to lysosomes, where albumin is digested to yield amino acids for possible use in protein synthesis during liver regeneration [47].

In conclusion, the long known protein and albumin retention in hepatocytes during alcohol exposures could rather be an adaptive response to prevent further uptake of toxic excretion products such as iron and bilirubin from heme degradation and enhanced RBC turnover. An additional direct efferocytosis of red blood cells by hepatocytes (see Fig. 49.7) would be an additional more efficient strategy

to remove RBC content. Additional electron micrographs both from livers of ALD patients and rodent models of hemolysis are shown in Figs. A.34, A.35, and A.36. These figures suggest that **RBCs could be directly taken up by hepatocytes, and potentially fuse with mitochondria**. In summary, enhanced RBC turnover in ALD, the specific role of mitochondria in heme synthesis, iron and energy metabolism, provide novel highly interesting areas of basic research not only to understand the pathophysiology of alcohol, but physiological routes of iron trafficking between cells, RBCs and organelles.

Biomechanic Aspects for ALD

Finally, studies on ALD and liver pathology should consider recent developments obtained by liver stiffness measurements [48]. The Sinusoidal Pressure Hypothesis (SPH) has been introduced which identifies an elevation of **sinusoidal pressure** (SP) as cause of fibrosis and liver cirrhosis [49–51]. SPH has been a novel concept to better explain macroscopic changes during liver cirrhosis development and the so-called point of no return, at which fibrosis progression becomes irreversible. Normally pressure changes in the context of cirrhosis are associated with portal hypertension which is a consequence of cirrhosis. According to the SPH, however, an elevated SP is the major upstream event that initiates fibrosis (initiation). In healthy liver, liver stiffness (LS) corresponds to SP and increases in response to many pro-fibrogenic stimuli such as inflammation, cholestasis or liver congestion. SPH postulates that extracellular matrix is produced in response to elevated SP to withstand the underlying pressure. Both duration (>4 weeks) and degree (>10–12 mmHg or 10–12 kPa) of SP/LS elevation are critical. While fibrosis can still reverse if the underlying cause of SP elevation is eliminated, the increased matrix deposition causes an increasing blood supply through the hepatic artery. This elevated hepatic arterial flow and the final arterialization of the liver permanently causes pathologically high pressures. A vicious cycle is initiated with further matrix deposition and increased arterial pressure. Thus, **arterialization** defines the **so-called 'point of no return' with irreversible fibrosis progression**. At the cellular level, SP is the actual driving force for the production of collagen by stretching of perisinusoidal cells, pressure-related increase in tissue stiffness and stretch forces transduced via cellular and intercellular bio-mechanic signaling. SPH is able to explain the macroscopic changes of the cirrhotic liver (trajectory forces), the uniform fibrotic response to various etiologies and the point of no return in advanced stages despite elimination of the cause. According to SPH, future treatment options should be targeted at lowering the sinusoidal pressure.

The hepatic artery is directly connected to the sinusoidal bed via arteriole inlets and provides about 20% of blood in a normal healthy liver. The stiffer the liver becomes due to inflammation or fibrosis the more pressure is required to maintain sufficient blood flow. Although the elevation of portal pressure (portal hypertension

>12 mmHg) can partly maintain some portal flow it will hardly reach values higher than 30 mmHg. Under these conditions, the hepatic artery will be the only vessel with sufficiently high pressure to maintain hepatic blood supply. Elevation of hepatic arterial flow and subsequent arterialization is mainly driven by the HABR [52] and hypoxia signaling [53]. SPH postulates that this arterialization defines the so-called **'point of no return'.** It provides a pressure-based rationale to explain the self-perpetuation of fibrosis progression and the uniform, etiology-independent progression of fibrosis. Arterialization of the fibrotic liver ultimately leads to a sustained exposure of the low-pressure organ liver (typically <6 mmHg) to higher pressures. In ca. 7% of patients with cirrhosis, extreme flow changes can be observed such as complete reversal of the portal flow (so called hepatofugal portal flow) [54]. At the end, the arterialized liver (high oxygen, high pressure) together with massive matrix deposition will cause self-inflicted ischemia (see Fig. 49.6a). The combination of these events stimulates the **formation of regenerative nodules** finally causing the typical nodular aspect of cirrhotic livers. High pressure in combination with cell

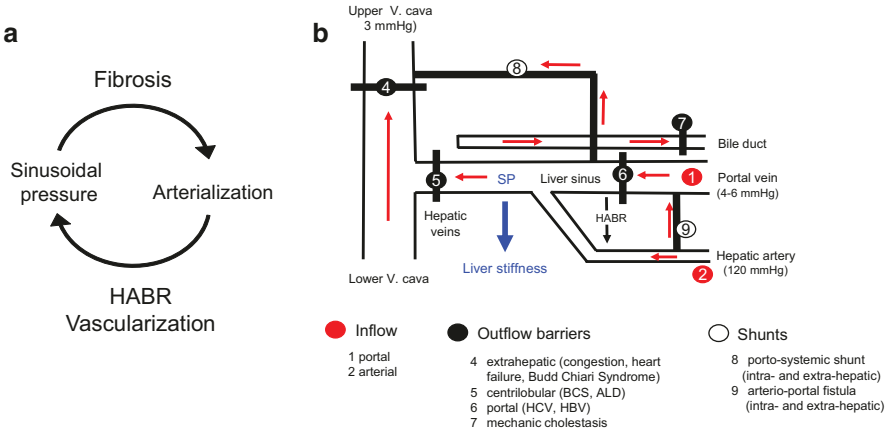

Fig. 49.6 (**a**) Vicious cycle of pressure elevation, matrix generation and arterialization according to the sinusoidal pressure hypothesis [49, 50]. The arterial response is mainly driven by hypoxia signaling and metabolic demand. The hepatic arterial buffer response (HABR) is the first and most rapid step in increasing arterial blood flow in response to decrease portal flow according to the adenosine wash out theory [52]. Later, other vascularization signals establish and secure arterial blood supply. (**b**) Simplified scheme of the hepatic vascular architecture and conditions that result in elevated sinusoidal pressure (SP) and liver stiffness (LS). A normal liver is supplied with blood from the hepatic artery (25%) with arterial pressure (AP) and the portal vein (75%) with the portal pressure (PP). Hepatic blood then leads into the hepatic veins with a central venous pressure (CVP). The hepatic venous pressure gradient (HVPG) determines the flow through the sinusoidal bed. In contrast, the SP is determined by the outflow/inflow ratio and ultimately increases LS. According to the hepatic arterial buffer response (HABR), a reduced portal flow causes compensatory arterial blood supply in a liver-autonomous unidirectional fashion [52]. Arterialization of the liver may have further consequences such as enhanced mechanic shear stress to red blood cells and enhanced RBC uptake by macrophages and hepatocytes in the pericentral region with reduced sinusoidal blood flow. Modified from Mueller S. World J Gastroenterol. 2016;22 (48):10482–10,501

death and enhanced regeneration ultimately provides an ideal environment of genetic instability and formation of cancer (HCC).

The concept of pressure further facilitates to understand typical differences between e.g. portal viral hepatitis (HCV) and lobular disease such as ALD. Figure 49.6b shows a simplified scheme of the vascular and biliary architecture of the liver to better illustrate the role of the various inflow, outflow and shunt factors on sinusoidal pressure. In general, the liver is a low-pressure organ. Pressure in the portal vein is ca. 5 mmHg, while blood leaves the liver through the veins and is ca. 2 mmHg in the caval vein (CVP) [55–57]. Close to the right atrium, this pressure can even reach negative values. Despite this low hepatic venous pressure gradient (HVPG) of ca. 3–6 mmHg, the liver is supplied with ca. 25% of the total cardiac output [57]. According to Ohm's law of streaming fluids it also demonstrates the very low vascular resistance of the healthy liver that easily adapts to flow changes e.g. from the splanchnic side [52]. The sinusoidal pressure (SP) is determined by static and dynamic components (see also Fig. 49.3). The **static part** of the SP is determined by the intravasal pressure and the elastic properties of the vessel walls and also exists in the absence of a functioning blood circulation. Osmotic, oncotic pressure as well as gravitational forces related to the body positioning further contribute to this component. In contrast, the **dynamic component** is represented by the kinetic energy of the blood flow and becomes only relevant under conditions of an operating blood circulation. The flow resistance of the liver, blood viscosity and the blood flow rate all affect this dynamic component. The flow resistance however, will be modulated by many conditions including cellular swelling or infiltration of inflammatory cells. Importantly, the localization of inflammation will increase the vascular resistance locally either in the portal or central areas. It explains why both a rapid increase of arterial [58] or portal [59] inflow or outflow barriers within the venous outflow tract (congestion) [60], bile ducts (mechanic cholestasis) [61] or the sinusoidal bed [62] are able to increase LS. Taken together, the introduction of pressure into the pathology of fibrosis allows various novel insights to understand fibrogenesis at the hemodynamic level.

At the cellular level, sinusoidal pressure elevation induces stretch forces within the per-sinusoidal cells that include hepatic stellate cells (HSC), endothelial cells, hepatocytes and macrophages. Notably, fibroblasts and HSCs are known for a long time to contract and to respond to mechanic forces [63–65]. Taken together, the concept of SPH postulates, that collagen deposition is a result of pressure-elevation. The so-called pericellular fibrosis is not in contrast to SPH. **Pericellular fibrosis** describes collagen deposition around single ballooned hepatocytes and is commonly observed in heavy drinkers. This pericellular fibrosis could be also explained by a pressure-stretch force concept. In contrast to perisinusoidal fibrosis, pressure inside the ballooned hepatocyte causes stretch forces in pericellularly aligned HSC or fibroblasts and finally lead to mechanically induced collagen deposition. Thus, both intravascular and intracellular pressure can cause stretch forces at the hepatocyte membrane with consequent stretching of HSC and/or elevation of cellular stiffness.

A Novel Link Between Enhanced RBC Turnover and Shear Stress to RBCs in ALD?

This novel link could consist of the **increased mechanic stress to red blood cells during the arterialization of the liver**. It is also postulated that the typical laboratory finding of cirrhotic livers, an increased AST/ALT ratio and a slight GGT elevation [66] is indicative for the stage of arterialization [49]. It is long known from patients with artificial heart valve implantation, that mechanic stress can increase RBC turnover or even cause direct hemolysis with AST elevation. As discussed in Chap. 41 on "AST levels in ALD", AST/GOT seems to be primarily derived from RBCs (and not mitochondria) and there is enough clinical evidence (see also Table B.4) that RBC turnover further increases with fibrosis stage. Although speculative, the predominant perivenular hepatic injury could be due to **shear stress damage of RBCs in arterialized livers** when entering the liver through the artery. The perivenular bed would then be the first vascular section with decreased blood flow which would increase the likelihood to take up damaged RBCs either by **erythrophagocytosis** or **efferocytosis** (see also Fig. 49.7 and Figs. A.31, A.34, A.35). This would be in line with the **uptake of bilirubin in the perivenular region by OATP1B3**. Nrf2 could be primarily seen to orchestrate degradation of RBCs (see Figs. A.60 and A.75) as it controls transport, conjugation and glutathione

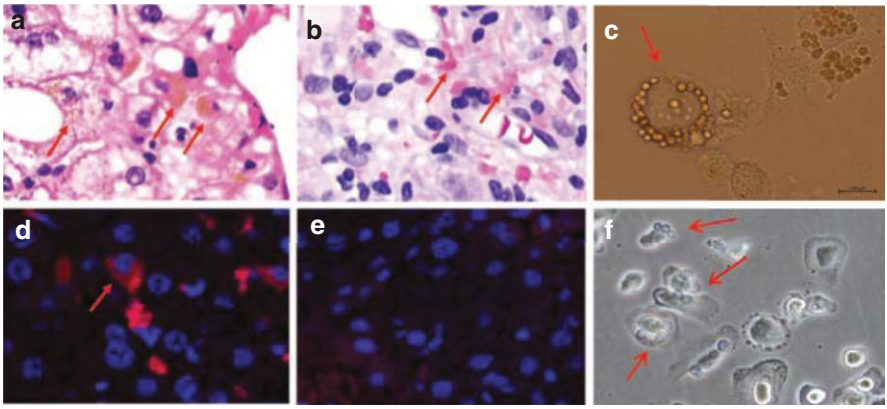

Fig. 49.7 Histological indications of hepatocellular heme degradation in alcohol-related liver disease or during hemolysis. (**a**) HE stain of a liver section from a heavy drinker. Note the intrahepatocellular bilirubin accumulation in ballooned hepatocytes. (**b**) HE stain of a liver section from a patient with ALD. RBCs are seen in red. Some hepatocytes seem to show ingested RBCs suggestive for efferocytosis. (**c**) Efferocytosis of oxidized human RBCs in cultured human hepatoma cells (huh7). Data can also be reproduced in primary hepatocytes. (Zheng C and Mueller S, 2023, unpublished) (**d**, **e**) RBC autofluorescence in a mouse phenylhydrazine model of mild hemolysis (**d**). Control livers are shown in (**e**). Note that heme autofluorescence is also seen inside hepatocytes, partly in the cytosol, but also in vesicles. Nuclei are stained blue. More studies are needed to comprehend RBC processing by hepatocytes under (patho) physiological hemolysis (**f**) Erythrophagocytosis of human oxidized RBCs by human macrophages (THP1) (Mueller S, unpublished)

metabolism, among many other pathways linked to RBC and heme degradation. With RBC turnover, cholesterol, phospholipid and triglyceride metabolism come into play which could be tightly linked to bile acid synthesis and bile formation. Highly fascinating, RBC turnover either through erythrophagocytosis or efferocytosis could provide novel links between iron metabolism, cytosol and mitochondria. As proposed above, RBC could be not only taken up by hepatocytes, but could also fuse with mitochondria, which contain high levels of iron. Such a potential fusion is highly interesting with regard to evolutionary conserved pathways, since mitochondria are considered ancient bacteria and bacteria require iron for growth and have developed sophisticated strategies to acquire iron. Not by chance, enzymes and molecules such as AST, peroxiredoxins such as PRX2 or glutathione are both existing in RBCs and mitochondria. These considerations open also other novel interactions such as the role of the malate aspartate shuttle to transport NADH from the cytosol into mitochondria. It also remains fascinating to study why, at least to some extent, heme degradation and heme synthesis are strictly separated in humans. Finally, as already mentioned above, the role of HO1-released carbon monoxide could profoundly link energy and oxygen metabolism with heme degradation. I truly hope that detailed and concerted mechanistic studies will soon shed more light on all of these exciting and potential options that go far beyond simple ethanol metabolism but the heart of life per se.

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Chapter 50
Ethanol Metabolism

Sebastian Mueller

Abstract Ethanol, an alcohol found in nature and in alcoholic beverages, is metabolized through complex catabolic pathways. Ethanol is amphiphile and, thus, distributes in all compartments. In addition, there are no negative feedback loops, so that the organism cannot escape from ethanol oxidation. This chapter introduces to major metabolic pathways and its consequences on energy metabolism, addiction, but also the development of alcohol-mediated diseases and cancer. The liver is the major elimination side of ethanol through alcohol dehydrogenases which convert it to the highly toxic and carcinogenic acetaldehyde. Acetaldehyde dehydrogenases ultimately transform acetaldehyde to acetic acid. This oxidation cascade also leads to an excess of NADH/lactate with important biochemical implications such as enhanced lipogenesis and decreased gluconeogenesis. Ethanol is also oxidized to acetaldehyde in the endoplasmic reticulum by the inducible cytochrome P450 system, especially the subtype CYP2E1. To a minor extent, catalase can also oxidize ethanol although its contribution in compartments such as red blood cells or brain are still poorly studied. The genetics of ethanol metabolism is increasingly uncovered, varies significantly between geographic regions and contributes to both the risk for alcohol-dependence and alcohol-related disease. Important features of ethanol metabolism such as the accumulation of fatty acids (steatosis) is considered a hallmark. Although, in contrast to carbohydrates, ethanol limits the availability of glucose, both lead to an enhanced energy flow through the mitochondrial respiratory chain, mitochondrial damage and enhanced lipogenesis. Thus, intermediary metabolism not only interlinks ethanol consumption, diabetes mellitus, and overweight to hepatic steatohepatitis but also tightly to addiction.

Keywords Alcohol · Ethanol · Steatohepatitis · Ethanol metabolism · Intermediary metabolism · NADH · Lactate · Addiction · Dependence · NAFLD · Gluconeogenesis · Lipogenesis · Energy metabolism · Glycogen

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Introduction to Ethanol Metabolism

Ethanol, an alcohol found in nature and in alcoholic drinks, is metabolized through a complex catabolic Ethanol is amphiphile and can dissolve both in water and lipid phases (see Fig. A.2). Ethanol is readily absorbed from the gastrointestinal tract. Major elimination side is the liver which can be seen after either functional or physical hepatectomy [1]. Using the elastographic methods to characterize fibrosis stages precisely, it has been recently shown that elimination of ethanol is decreased in higher fibrosis stages [2]. Thus, drinkers with manifest cirrhosis achieve both higher blood alcohol levels and biomarkers of ethanol such as ethyl glucuronide despite lower alcohol consumption [2] (see Table B.7). The extrahepatic metabolism of ethanol is relatively small [3]. However, extrahepatic metabolism remains important in order to understand ethanol-mediated organ damage e.g. in brain or red blood cells. Only 2–10% is eliminated through kidney and lungs while the rest is oxidized within the body, mostly in the liver.

As shown in Fig. 50.1, in the liver, ethanol is largely metabolized via alcohol dehydrogenases (ADH), cytochrome P450 (CYP) 2E1, and to a minor degree by catalase, resulting primarily in the generation of acetaldehyde. Acetaldehyde is further metabolized via acetaldehyde dehydrogenases (ALDH) to acetate. Acetate is

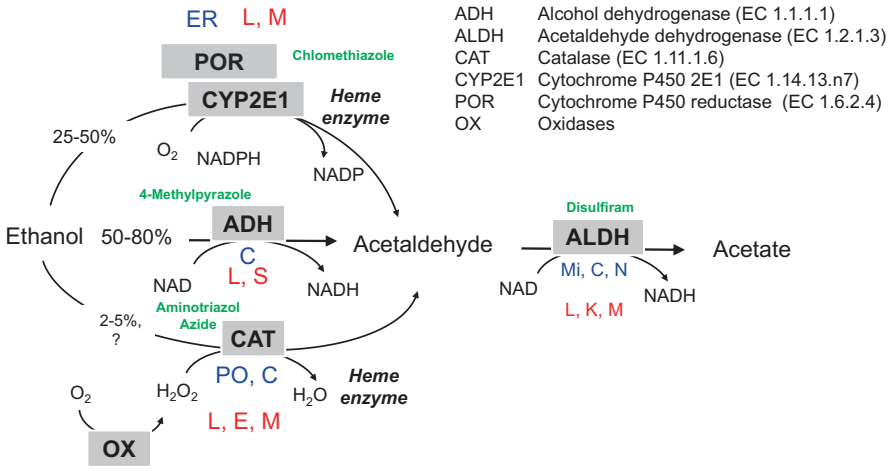

Fig. 50.1 Major enzymatic ethanol oxidation pathways. ADH and ALDH convert ethanol to acetic acid by transforming NAD to NADH. This causes an important shift of the redox potential and is responsible for many biochemical consequences including enhanced lipogenesis. Also note that CYPs require NADPH. They directly use oxygen which can lead to ROS formation through uncoupling. The proximity to carbon monoxide releasing HO1 in the endoplasmic reticulum has less well been studied but suggests a potential inhibitory interaction between CYPs and HO1. Abbreviations: subcellular localization are indicated in blue: *C* cytosol, *ER* endoplasmic reticulum, *Me* membrane, *Mi* mitochondria, *N* nucleus, *PO* peroxisome, inhibitors are given in green. Tissue distribution is given in red: *E* erythrocyte, *K* kidney, *L* liver, *M* many tissues, *S* stomach, enzyme abbreviations are provided in figure

then channeled as acetyl-CoA into the citric acid cycle. Since multiple isoenzymes exist for ADH and ALDH (see Figs. A.39, A.40, A.41) and some variants with reduced activity, ethanol metabolism varies considerably inter-individually. This results in different metabolic flows and the generation of different homeostatic acetaldehyde concentrations. Due to differences in enzyme presence and availability, human adults and fetuses process ethanol through different pathways.

Acetaldehyde is highly reactive, toxic, and carcinogenic, and the rate of its generation and of its degradation predicts the individual risk for organ toxicity and cancer development. This can be best seen in the high risk of local oropharyngeal cancers (see also Chap. 73–75 on cancer) in heavy drinkers. The oxidation cascade **tightly links addiction and alcohol dependence to general ethanol metabolism**. For instance, ADH variants with decreased activity increase the risk for addiction while variants with increased ADH and decreased ALDH activity result in acetaldehyde accumulation and rather prevent alcohol intake due to severe clinical symptoms. It seems that the brain-sensed joy primarily originates from ethanol and its intermediary metabolism while the negative consequences are mostly caused by the toxic ethanol oxidation intermediates.

In humans, several enzymes are involved in processing ethanol first into acetaldehyde and further into acetic acid and acetyl-CoA (see Fig. A.38). Once acetyl-CoA is formed, it becomes a substrate for the **citric acid cycle** ultimately producing cellular energy and releasing water and carbon dioxide (see Figs. A.43 and A.44). In addition to acetaldehyde, **reducing equivalents in the form of NADH** are also generated via the ADH reaction, resulting in a severe change of the intracellular redox state and leading to severe alterations of the intermediary metabolism. Due to its equilibrium with NADPH (see also Figs. A.46, A.47, NTH - $NAD(P)^+$ transhydrogenase), both reduced nicotine adenine dinucleotides (NADH and NADPH) are cumulating during ethanol metabolism leading to characteristic metabolic consequences such as enhanced lipogenesis, mitochondrial respiration, metabolism of galactose, serotonin, steroids, amines and other NADH dependent metabolism. NADPH is also required for the non-NADH mediated oxidation of ethanol by the microsomal multifunctional P450 cytochrome system in the endoplasmic reticulum, especially the subtype CYP2E1. CYP2E1 is not only induced by ethanol but also fatty acids, ketones and drugs and it can significantly contribute to ethanol elimination. CYPs are ubiquitously distributed in many tissues and are important for ω-1 hydroxylation of fatty acids but also have monooxygenase and epoxygenase activity. Although ethanol metabolism via CYP2E1 is quantitatively lower, it can be strongly induced by chronic alcohol consumption. Consequently, the rate of ethanol metabolism via CYP2E1 increases in the chronic alcohol abuser.

Ethanol metabolism via CYP2E1 also generates **reactive oxygen species (ROS)** through one electron reduction of oxygen, resulting in tissue toxicity and DNA damage (see Figs. A.67, A.68). Since CYP2E1 is not only responsible for ethanol metabolism, but also for the metabolism of various drugs, xenobiotics, and procarcinogens, alcohol metabolism can strongly interact with these substances which has clinical importance. Thus, ethanol metabolism is not only an important prerequisite

to explain ethanol toxicity in various organs such as the liver, but also plays an important role to explain the interaction with drugs, xenobiotics, procarcinogens, and retinoids.

Endogenous Ethanol Production and Evolution

Ethanol is produced endogenously during bacterial fermentation in the gut reaching up to levels of ca. 1 mM in the portal vein [4, 5]. Gastric bacterial overgrowth observed in atrophic gastritis may lead to the generation of ethanol within the stomach. In addition, Candida species may also generate ethanol, resulting in relatively high concentrations of blood alcohol up to 25 mM [6, 7]. The average human digestive system produces approximately 3 g of ethanol per day through fermentation. This is considered a potential evolutionary reason for the expression of ADHs both in stomach and liver. Regional differences of ethanol-metabolizing enzymes, however, suggest that the ability of humans to produce alcoholic beverages have also modulated the genetic background of ethanol metabolism. Catabolic degradation of ethanol is essential to life, not only in humans, but of most other organisms.

Certain amino acid sequences in the ethanol oxidation enzymes are highly conserved going back to the last common ancestor over 3.5 billion years ago [8]. Organisms including humans also produce several types of other alcohols in small amounts, primarily through fatty acid synthesis, retinol metabolism, glycerol lipid metabolism, and bile acid biosynthesis pathway, many of them in the liver. This may explain why the liver is also the main target organ of the negative side effects of ethanol metabolism. Importantly, **there is no feedback inhibition of ethanol metabolism** and most metabolizing enzymes are rapidly saturated. In other words, once exposed to ethanol, the metabolic machinery must metabolize it.

Energetic Considerations

As is also demonstrated in Fig. A.2, the exothermic reaction of the complete catabolism of alcohol yields about 1325 kJ or 317 kcal energy per mol [9]. With an molecular weight of 46 g/mol, this corresponds to 29 kJ or **6.9 kcal per gram ethanol**. Therefore, ethanol-bound energy is higher as the caloric content of sugars and proteins, but slightly lower as the one in fats. More complexity is added due to the different pathways to convert energy into heat or anabolic processes such as lipogenesis. Roughly, however, 5–6 L beer or 2–3 liters of wine are sufficient to cover the daily calory intake of a standard person (see also Figs. A.1, A.2 and A.3). In addition, in a standard person, at saturated levels, about **8 g pure alcohol are eliminated per hour** corresponding to about 230 kJ or 55 kcal per hour. Importantly, as already mentioned above, the organism cannot escape ethanol metabolism, and there is no negative feedback mechanism. In the 60ties of the last century there has been an intensive

debate to whether negative side effects of ethanol are due to caloric intake or ethanol per se. The now classical 4 year-long standardized feeding studies in non-primate monkeys by Charles Lieber could eventually establish once and for all that it is primarily the ethanol and its metabolism that causes liver damage [10]. As can be seen from the original data (see Table B.2) of the Heidelberg cohort of heavy but well-nourished Caucasian heavy drinkers, about **50% of their energy supply is covered by ethanol**. In this cohort, ca. 1200 patients consumed ca. 180-g pure alcohol per day during a heavy drinking period of about 14 years reaching a mean blood alcohol concentration of 1 °/°° (1 g/L). One can calculate from these data that this corresponds to a mean elimination of 7.5 g/h. With a mean size of 174 cm and weight of 78 kg (mean BMI 25.5), this corresponds to 0.01 g eliminated alcohol per kg body weight and hour. As is discussed at the end of this chapter, there are several emerging arguments to suggest that it is the energy availability of ethanol that, similar to excess carbohydrates, may explain the **steatohepatitis** caused by conditions such as chronic alcohol consumption, diabetes mellitus and overweight. In this concept, fat accumulation is rather seen as escape way to eliminate ethanol more rapidly than as a prerequisite for liver damage.

Ethanol Absorption and Ethanol Blood Levels

Blood alcohol concentrations (BACs) reflect gastrointestinal absorption, diffusion, metabolism, and unchanged excretion of ethanol. Thus, absorption of ethanol associated with increasing BAC has to be distinguished from elimination with decreasing BAC. At the end of alcohol absorption, a peak is detectable, which may change to a plateau if alcohol is further consumed continuously. As mentioned above, this plateau is reached in heavy drinkers e.g. from the Heidelberg cohort as described in Table B.2.

Alcohol is absorbed from the upper gastrointestinal tract by simple diffusion. Delayed gastric emptying and food in the upper gastrointestinal tract may lead to lower BAC, while a higher BAC is observed after gastrointestinal bypass surgery and after consumption of highly concentrated alcoholic beverages such as liquors compared to low concentrated beverages like beer and wine [11, 12]. Gastrointestinal absorption of ethanol depends on various factors, including the ethanol concentration of the beverage, blood perfusion of the stomach and duodenum, simultaneous food intake, rate of gastric emptying, body temperature, and menstrual cycle [11, 12]. Twenty percent of alcohol is absorbed from the stomach and 80% from the upper small intestine. In the gastric mucosa, alcohol can be metabolized by various ADHs. This is called gastric **first-pass metabolism (FPM)** of alcohol [13]. The rest of the ethanol enters the liver via the portal vein. Ethanol is metabolized to more than 90% in the liver after multiple passages through the liver, to 5–10% in the gastric mucosa, and approximately 3–5% of the orally absorbed ethanol is excreted unchanged through the lungs, skin, and kidneys [11, 12]. It should be noted that hemodynamics completely change in patients with liver cirrhosis where the hepatic artery increasingly takes over the blood supply of the whole liver. More of these

biomechanic considerations are discussed in the Chap. 49 on the "pathophysiology of alcohol and unexplained observations".

Calculation of Ethanol Elimination

A mathematical estimation of blood alcohol content is useful when blood alcohol is not currently detectable, or for the prediction of alcohol levels. While there are several ways to calculate it, the simplest is Widmark's equation [14–16]:

$C_o = A/[p \times r]$

C_o is the theoretical maximum concentration of alcohol in blood (mg/g). A is the amount of alcohol in the body (g). p is the body weight (kg). r is the correction factor corresponding to the ratio of total body water and blood water (0.6 for females and 0.7 for males).

Gender plays an important role in the total amount of water in the body. In general, men have less fatty tissue and a higher percentage of water (58%) than women (49%), thus the volume of distribution (V_d) for ethanol is higher in men. According to its partition coefficient (Poct/water is 0.1), ethanol is 10 times more soluble in water than in lipids. Thus, upon ingestion of the same amount of ethanol, the BAC will be higher in females than in males. The Widmark's equation has been improved subsequently by introducing individual r, based on the multiple linear regression equations:

for females:

rFI = 0.31223 – 0.006446 × body weight (kg) + 0.004466 × body height (cm)

for males:

rMI = 0.31608 – 0.004821 × body weight (kg) + 0.004632 × body height (cm).

There is no absolute accurate blood alcohol calculator because numerous factors influence the BAC, such as gender (male/female), rate of metabolism/elimination, health status, medications, drinking frequency, amount and the type of food in the stomach and small intestine, the time of food intake, and others [17].

Ethanol Oxidation to Acetaldehyde in Humans

Chemical properties of ethanol are listed in Fig. A.2. In human adults, ethanol is oxidized to acetaldehyde using NAD^+, mainly via the hepatic enzyme alcohol dehydrogenase IB (class I), beta polypeptide (ADH1B, EC 1.1.1.1). (see Fig. 50.1). The gene coding for this enzyme is located on chromosome 4, in 4q22 [12]. Members of this enzyme family metabolize a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. An actual complete list of the 7 ADHs is provided in Figs. A.39, A.40, A.41, A.42. ADH consists of several homo- and heterodimers of alpha, beta, and gamma subunits (see also Fig. A.42). It plays the major role in ethanol catabolism. Three genes encoding alpha, beta and gamma subunits are tandemly organized in a genomic segment as a gene cluster.

In human **embryos and fetuses,** ethanol is not metabolized via this mechanism as ADH enzymes are not yet expressed to any significant quantity in human fetal liver. In fact, induction of ADH starts after birth, and requires years to reach adult levels. Consequently, in fetuses, ethanol is metabolized at much slower rates by different enzymes from the cytochrome P-450 superfamily (CYP), in particular by CYP2E1. The low fetal rate of ethanol clearance is responsible for the important observation that the fetal compartment retains high levels of ethanol long after ethanol has been cleared from the maternal circulation by the adult ADH activity in the maternal liver [18]. CYPs, in contrast to ADH, directly use oxygen and uncoupled one electron reduction of oxygen can lead to the release highly reactive superoxide anion radicals that are able to initiate lipid peroxidation. These mechanisms render the fetus especially vulnerable to alcohol. The resulting **Fetal Alcohol Spectrum Disorders (FASD)** are discussed in detail in Part IV of this book (Chaps. 23–25).

Most of the alleles of ADH are single nucleotide polymorphisms. Table B.10 in the Appendix also shows kinetic parameters such as K_M and V_{MAX} for selected enzymes. Class I ADH – the major ADH in the liver –becomes quickly saturated at low millimolar ethanol concentrations. ADH4 contributes to ethanol oxidation at higher concentrations and is only expressed in the liver. ADH5 has virtually no affinity for ethanol with an extremely high K_M. ADH6 mRNA is also expressed in the liver, but the protein has not been isolated so far. ADH7 is primarily localized in the stomach and in the retina, and responsible for gastric FPM of ethanol and retinol oxidation [19].

ADH1B and ADH1C show polymorphisms, resulting in the production of enzymes with different kinetic properties and different ethanol-oxidizing capacities. There are three different ADH1B alleles that alter the amino sequence of the encoded β subunit. In both the β2 and β3 subunit, the amino acid substitution occurs at an amino acid that contacts with NAD. This substitution results in enzymes that have a 70- to 80-fold higher turnover rate than the β1 subunit [12]. ADH1B1 contributes ca. 20% to ethanol metabolism in the liver. ADH1C has also three alleles. While the ADH1B2 allele encodes for an enzyme that is approximately 40 times more active in producing acetaldehyde as compared to the enzyme encoded by the ADH1B1 allele, the ADH1C1 allele encodes for an enzyme with 2.5 times more acetaldehyde production as compared to the ADH1C2 allele.

This has severe consequences with respect to ethanol drinking behavior and ethanol-associated cancer development. With respect to alcoholism and liver disease, the presence of the ADH1B2 allele seems to be strongly protective, since individuals with this gene produce enormous amounts of acetaldehyde following alcohol ingestion [20–23]. Under these circumstances severe side-effects of acetaldehyde such as tachycardia, sweating, flushing, nausea, and vomiting occur (flush syndrome), and therefore these individuals avoid alcohol. With respect to ADH1C polymorphism, individuals homozygous for the ADH1C1 allele with a small but significant greater production of acetaldehyde do not show such side-effects. However, they seem to be at increased risk for the development of cancer of the upper aerodigestive tract, breast, and colorectum [19, 24–26]. ADH1C1 contributes approximately 40% to ethanol metabolism in the liver.

Metabolic consequences of the ADH reaction are either due to an increase in NADH or acetaldehyde. Production of NADH leads to a change in the cellular redox potential and has a severe influence on the intermediary cell metabolism. This is especially pronounced in the liver and include [27] a shift towards lactate and an inhibited gluconeogenesis, an increase in NADPH with **enhanced lipogenesis** [28], a change in transcriptional regulation by affecting C-terminal binding protein and the silent information regulator, resulting in enhanced histone acetylation and reduced deacetylation associated with epigenetic changes and activation of certain inflammatory genes [28] and, finally, an effect on signaling proteins such as NF-κB, c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase [28]. Clinical consequences of a change in the redox state are [27, 28]:

- Activation of the nuclear transcription factor SREBP-1c and inhibition of peroxisome proliferator-activated receptor-α, resulting in a stimulation of fatty acids and triglyceride synthesis, and inhibition of β-oxidation of fatty acids. As a result, fatty liver and also hyperlipoproteinemia type IV and V according to Fredricksen may occur.
- Decreased pyruvate and increased lactate concentrations in the liver. As a consequence, an inhibition of hepatic gluconeogenesis due to a lack of pyruvate with hypoglycemia may occur especially in individuals with liver disease in the fasted state. Also, lactic acidosis occurs followed by low urinary pH and increased tubular reabsorption of uric acid leading to hyperuricemia. The increase in lactate also stimulates hepatic stellate cells (HSCs) to produce collagen.
- A rapid depletion of hepatic glycogen due to enhanced glycogenolysis
- Disturbed porphyrin metabolism with the occurrence of secondary porphyria [29]
- Decreased production of testosterone in the Leydig cells of the gonads resulting in feminization (body fat, gynecomastia, body hair) [30].
- Reduced generation of UDP-glucuronic acid from UDP-glucose, and thus inhibition of hepatic glucuronidation of phenolphtalein, trichloroethane, and diethyldithiocarbamate [31].

The generation of acetaldehyde and acetate has several consequences that are shown in Table 50.1 [27, 28, 39]. Since ethanol metabolism primarily via ADH effects hepatic intermediary metabolism, the occurrence of various metabolic diseases is favored by chronic ethanol consumption, including hypoglycemia, hyperlactatemia (lactic acidosis), hyperuricemia, hyperhomocysteinemia, porphyria, and an altered testosterone to estrogen ratio. Ethanol competes with some substrates at the ADH binding site. Most importantly, the conversion of retinol to retinal and retinoic acid is inhibited in the presence of ethanol. This is one mechanism to explain low levels of retinoic acid in the liver after chronic ethanol consumption [40]. As is discussed at the end of this chapter, the important changes on intermediary metabolism may be the major reasons why ethanol leads to the same histological characteristics as an excess of carbohydrates does under condition of diabetes mellitus or overweight.

Table 50.1 Generation of acetaldehyde and acetate and its consequences

	References
Effects of acetaldehyde	
• Mitochondrial damage with alteration of the respiratory chain and decreased ATP production. As a morphological consequence hepatic megamitochondria may occur	[27, 28, 32, 33]
• Damage of the microtubular system with an altered secretion of proteins, such as albumin, transferrin, and very-low-density lipoproteins. As a morphological equivalent, ballooning of the hepatocyte may occur	
• A decrease in glutathione, and thus an alteration of the detoxification of xenobiotics and ROS	
• An inhibition of the nuclear repair systems with an enhancement of carcinogenesis	[34]
• A disturbed methyl transfer with decreased levels of the active methyl donor *S*-adenosylmethionine and an increase of homocysteine, which produces endoplasmic reticulum stress resulting in fatty liver, as well as a decrease in mitochondrial glutathione and increased apoptosis. As a consequence, membrane damage and hypomethylation of DNA may occur. Aberrant methylation causes an inflammatory response and tissue injury, and DNA hypomethylation may cause liver cancer	[35]
• Binding of acetaldehyde to proteins with generation of neoantigens, activation of the immune system, and production of antibodies	[36]
• Binding of acetaldehyde to DNA and generation of mutagenic DNA lesions	[37]
• Stimulation of fibrogenesis by activation of stellate cells	[27, 38]
Effects of acetate	
• Increased acetylation of histones associated with epigenetic changes (see above).	[39]
• Increased acetylation of certain compounds such as sulfanilamide	

Oxidation of Acetaldehyde to Acetic Acid

The second oxidation step by acetaldehyde dehydrogenases (ALDH) is also shown in Fig. 50.1 and Figs. A.40, A.41 show the actual, still growing list of the known ALDHs. Acetaldehyde is a highly unstable compound (see Fig. A.2) and an electrophile that readily engages in condensation reactions. It can also conjugate with glutathione and rapidly deplete this important antioxidative defense system [41]. Acetaldehyde is considered one of the major toxic intermediates of ethanol oxidation. A major ALDH is aldehyde dehydrogenase 2 family (ALDH2, EC 1.2.1.3, see also Figs. A.41 and A.42) which is found on chromosome 12, locus q24.2. Among the 19 known human ALDHs, mitochondrial ALDH2 and, to a lesser extent, cytosolic ALDH1 play a major role in acetaldehyde oxidation and elimination [42]. Both enzymes exhibit low K_M constants for acetaldehyde (i.e., 3.2 and 180 μM for human ALDH2 and ALDH1A1, respectively) [43]. Acetaldehyde is also considered to injure mitochondria and decrease the activity of ALDH2. Ultimately, this will further increase acetaldehyde levels and initiate a vicious cycle.

A coding variant known as ALDH2*2 allele leads to the substitution of Lys for Glu at position 504. This substitution results in a virtually inactive ALDH2 enzyme [12] which is present either in homo- or heterozygous form in almost 50% of Asians. For instance, in 10% of the Japanese population, this mutation is homozygous and associated with zero ALDH activity. These individuals cannot drink ethanol at all since they develop severe side-effects such as flushing, tachycardia, nausea, and vomiting. Forty percent of Japanese, however, are heterozygotes. They may consume alcohol with an ALDH2 activity of approximately 10–15% compared to those of normal Caucasians. They also develop a flushing syndrome, however, this can be tolerated, so that they continue to drink. As a result, acetaldehyde levels increase in the blood, in the liver, and in the saliva [6]. Since acetaldehyde is a carcinogen, these individuals have a high risk for alcohol-associated cancer development, such as cancer of the upper alimentary tract and the colon [44]. The presence of even a single ALDH2*2 allele is **strongly protective against alcohol dependence**. Compared with an individual carrying two active ALDH2*1 alleles and two copies of the normal ADH1B*1 allele, the odd ratios for the risk for alcohol dependence for a man carrying one inactive ALDH2*2 allele and two ADH1B*1 alleles is 0.33. If, in addition to the ALDH2*2 allele, the man also carries at least one overactive ADH1B*2 allele, the odds ratio declines further to 0.05 [12].

The protective effect of the ALDH2*2 allele can, however, be modulated by the environment. In Japan, the percentage of alcoholics with the ALDH2*2 allele has increased over the years from less than 3% to over 12% due to a sociological change of increased alcohol consumption [12]. ALDH2 can also be inhibited by various drugs leading to a flush reaction and Disulfiram has been used in alcoholics to obtain abstinence (see Fig. 50.1). Most recently, another human low-K_M ALDH has been characterized as ALDH1B1, being actively involved in ethanol metabolism, especially in the intestinal mucosa [45]. Similar to the ADH genes, many noncoding variations in the ALDH2 gene exist and several promoter polymorphisms in the ALDH1A1 gene affect gene expression *in vitro*.

Alternative Ethanol Oxidation in the Smooth Endoplasmic Reticulum

The **microsomal ethanol oxidizing system (MEOS)** is an alternate pathway of ethanol metabolism that occurs in the smooth endoplasmic reticulum in the oxidation of ethanol to acetaldehyde. While playing only a minor role in ethanol metabolism in average individuals, MEOS activity increases after chronic alcohol consumption. The MEOS pathway requires the CYP2E1 enzyme, part of the cytochrome P450 family of enzymes, to convert ethanol to acetaldehyde. Cytochrome P450 2E1 (abbreviated CYP2E1, EC 1.14.13.n7) is a member of the cytochrome P450 mixed-function oxidase system. It is involved in numerous physiological reactions and synthesis steps in humans, but also highly important for the

metabolism of xenobiotics. This class of enzymes is divided into a number of subcategories, including CYP1, CYP2, and CYP3 [46]. While CYP2E1 itself carries out a relatively low number of these reactions (~4% of known P450-mediated drug oxidations), it and related enzymes CYP1A2 and CYP3A4 are responsible for the breakdown of many toxic environmental chemicals and carcinogens, in addition to basic metabolic reactions such as fatty acid oxidations [47]. CYP2E1 is a membrane protein expressed in high levels in the liver, where it composes nearly 50% of the total hepatic cytochrome P450 mRNA [48] and 7% of the hepatic cytochrome P450 protein [49].

The activity of the MEOS is gender-dependent with higher activities in the male gender. Castration, ovariectomy, and substitution with sex hormones affect the MEOS activity. The MEOS activity decreases with age, and may depend on diets with higher activities following hypocaloric carbohydrate-deficient diets and lower activities following protein malnutrition [38]. The MEOS activity can also be induced by certain drugs. Major components of the MEOS are CYP2E1 and NADPH, cytochrome *c* reductase as well as phospholipids. The reaction occurs within the smooth endoplasmic reticulum and involves P450 reductase [13, 50, 51]. This protein transfers electrons to the CYP2E1 heme iron after first accepting them from the reduced NADPH. CYP2E1 catalyzes the oxidation of small organic compounds such as the production of glucose from ketones such as acetone during starvation [52].

The MEOS metabolizes not only ethanol, but also other primary aliphatic alcohols such as methanol, propanol, butanol, and pentanol, as well secondary alcohols such as isopropanol and tertiary alcohols such as *t*-butanol [27, 50, 51]. CYP2E1 also plays a role in several important metabolic reactions, including the conversion of ethanol to acetaldehyde and to acetate in humans [53]. In the conversion sequence of acetyl-CoA to glucose, CYP2E1 transforms acetone via hydroxyacetone (acetol) into propylene glycol and methylglyoxal, the precursors of pyruvate, acetate and lactate [54–56]. CYP2E1 also carries out the metabolism of endogenous fatty acids such as the **ω-1 hydroxylation** of fatty acids such as arachidonic acid, involving it in important signaling pathways that may link it to diabetes and obesity [17].

Thus, it can act as a **monooxygenase** to metabolize arachidonic acid to 19-hydroxyeicosatetraenoic acid (19-HETE), and as an epoxygenase to metabolize docosahexaenoic acid to epoxides [57]. 19-HETE is an inhibitor of 20-HETE, a broadly active signaling molecule, e.g. it constricts arterioles, elevates blood pressure, and it promotes inflammation responses. The EDP (see Epoxydocosapentaenoic acid) and EEQ (see epoxyeicosatetraenoic acid) metabolites also have a broad range of activities. In various animal models and in vitro studies on animal and human tissues, they decrease hypertension and pain perception; suppress inflammation; inhibit angiogenesis, endothelial cell migration and endothelial cell proliferation [58–61]. CYP2E1 is not regarded as being a major contributor to forming the cited epoxides but could act locally in certain tissues [61].

The ethanol metabolism via CYP2E1 produces first a *gem*-diol – an unstable product that disintegrates to acetaldehyde [52]. Since oxygen is used in this

CYP2E1-dependent reaction, ROS can occur. CYP2E1 also catalyzes the formation of hydroxyethyl radicals directly from ethanol. Importantly, when oxygen is used in the reaction, sometimes the reaction does not continue, and ROS may be generated [62–64]. The amount of CYP2E1 in the liver and in other tissue is variable and may vary up to eight-fold. Induction of CYP2E1 by chronic ethanol ingestion occurs not only in the liver, but has also been reported in the mucosa cells of the small and large intestine, in the pancreas, in the lung, and in the brain [65]. CYP2E1 is also induced by 4-methylpyrazole – an ADH inhibitor – and by acetone and free fatty acids, which may possibly be of importance in the pathogenesis of nonalcoholic fatty liver disease [66].

Chronic ethanol ingestion even at relatively low doses such as 40 g ethanol/day and even after a short period of time such as 1-week results in a significant induction of CYP2E1 which varies inter-individually [67]. The enhanced metabolism of ethanol after chronic alcohol consumption is due the induction of CYP2E1, and it is important to note that CYP2E1 activity needs NADPH and reutilizes reducing equivalents from the ADH reaction as NADPH from NADH. The mechanisms for regulating the enzyme concentration are complex. CYP2E1 is lower in the fed sate and higher during starvation or in obesity. In addition to control mechanisms for translation and transcription, an inhibition of CYP2E1 degradation by the ubiquitin–proteasome pathway may additionally contribute to the increase of CYP2E1 following ethanol consumption [68]. Thus, various factors contribute to the large variation of CYP2E1 after alcohol ingestion. The metabolic and clinical consequences of ethanol metabolism via MEOS are multiple and described in Table 50.2. The interaction with drugs is beyond the scope of this chapter and described in more detail elsewhere [72].

Table 50.2 Metabolic and clinical consequences of ethanol metabolism via MEOS

Effects of CYP2E1 (MEOS) induction	References
• Production of ROS including hydroxyl-ethyl radicals, superoxide anions, and hydroxy peroxide, which contribute to liver damage and cancer. ROS results in lipid peroxidation with lipid peroxidation products such as 4-hydroxynonenal or malondialdehyde. 4-Hydroxynonenal binds to DNA, forming highly carcinogenic exocyclic etheno–DNA adducts.	[69, 70]
• Interaction of the microsomal ethanol metabolism with the metabolism of various drugs, leading to decreased drug blood levels and increased drug toxicity.	[27, 38]
• Interaction of CYP2E1 ethanol metabolism with the metabolism of various xenobiotics and carcinogens, leading to increased toxicity and carcinogenesis.	
• Interaction of CYP2E1 ethanol metabolism with the metabolism of retinol and retinoic acid, leading to vitamin deficiency and increased toxicity, including enhanced carcinogenesis.	[69, 71]

P450 Subtype CYP2E1 and its Regulation

CYP2E1 exhibits structural motifs common to other human membrane-bound cytochrome P450 enzymes and is composed of 12 major α-helices and 4 β-sheets with short intervening helices interspersed between the two [17]. Like other enzymes of this class, the active site of CYP2E1 contains an iron atom bound by a heme center which mediates the electron transfer steps necessary to carry out oxidation of its substrates. The active site of CYP2E1 is the smallest observed in human P450 enzymes, with its small capacity attributed in part to the introduction of an isoleucine at position 115. The side-chain of this residue protrudes out above the heme center, restricting active site volume compared to related enzymes that have less bulky residues at this position [17]. Its hydroxyl group is well-positioned to donate a hydrogen bond to potential acceptors on the substrate, and its methyl group has also been implicated in the positioning of fatty acids within the active site [73, 74].

In humans, the CYP2E1 enzyme is encoded by the CYP2E1 gene [75]. As mentioned above, the enzyme has been identified in fetal liver, where it is considered to be the predominant ethanol-metabolizing enzyme, and may be connected to ethanol-mediated teratogenesis [76]. In rats, within 1 day of birth, the hepatic CYP2E1 gene is activated transcriptionally. CYP2E1 expression is easily inducible, and can occur in the presence of a number of its substrates, including ethanol [51], isoniazid, [51] tobacco, [77] isopropanol, benzene, toluene, and acetone [47]. For ethanol, specifically, there seem to exist two stages of induction, a post-translational mechanism for increased protein stability at low levels of ethanol and an additional transcriptional induction at high levels of ethanol [51]. CYP2E1 is inhibited by a variety of small molecules, many of which act competitively (see Fig. 50.1). Inhibitors include diethyldithiocarbamate [78], and disulfiram [79]. CYP2E1 and other cytochrome P450 enzymes can inadvertently produce reactive oxygen species (ROS) in their active site when catalysis is not coordinated correctly, resulting in potential lipid peroxidation as well as protein and DNA oxidation [17]. CYP2E1 is particularly susceptible to this phenomenon compared to other P450 enzymes, suggesting that its expression levels may be important for negative physiological effects observed in a number of disease states [17].

CYP2E1 expression levels have been correlated with a variety of dietary and physiological factors, such as ethanol consumption [80], diabetes [81], fasting [82], and obesity [83]. It appears that cellular levels of the enzyme may be controlled by the molecular chaperone HSP90, which upon association with CYP2E1 allows for transport to the proteasome and subsequent degradation. Ethanol and other substrates may disrupt this association, leading to the higher expression levels observed in their presence [84]. The increased expression of CYP2E1 in these health conditions is thought to contribute to their pathogenesis by production of ROS [17]. A study in rats revealed a eight- to nine-fold elevation of CYP2E1 with fasting alone, compared to a 20-fold increase in enzyme level accompanied by a 16-fold increase in total catalytic capacity in rats who were both fasted and given large quantities of ethanol for three consecutive days [85]. However, a previous study in humans with

detailed characterization of fibrosis stage by elastography revealed that other CYPs may also be expressed highly in drinkers. Of note, **expression of all CYPs drastically decreases once cirrhosis stage is reached** (Figs. A.65 and A.66). In fetuses, ethanol is instead metabolized at much slower rates by different enzymes from the cytochrome P-450 superfamily (CYP), in particular by CYP2E1. The low fetal rate of ethanol clearance is responsible for the important observation that the fetal compartment retains high levels of ethanol long after ethanol has been cleared from the maternal circulation by the adult ADH activity in the maternal liver [18]. CYP2E1 expression and activity have been detected in various human fetal tissues after the onset of organogenesis (ca. 50 days of gestation). Exposure to ethanol is known to promote further induction of this enzyme in fetal and adult tissues. CYPs, in contrast to ADH, directly use oxygen and uncoupled one electron reduction of oxygen can lead to the release highly reactive superoxide anion radicals that are able to initiate lipid peroxidation.

Ethanol Metabolism Via Catalase

Catalase is localized in the peroxisomes of cells, namely hepatocytes, and is able to oxidize ethanol to acetaldehyde by using H_2O_2 (Fig. 50.1). However, due to the low generation of H_2O_2 in the liver (typically at the 0.1 μM level), catalase seems not to contribute significantly to ethanol metabolism. The role of catalase in detoxifying ethanol has been extensively discussed in the past [5]. Physiological rate of H_2O_2 production has been estimated to represents 2% of the in vivo rate of hepatic ethanol oxidation. It has been further considered that catalase could account for 5% of the non-ADH mediated, pyrazole-insensitive ethanol oxidation. Conversely, in brain samples, the presence of the catalase inhibitor 3-amino-1,2,4-triazole induced a concentration-dependent reduction of the amount of acetaldehyde generated after incubation [86]. Moreover, the contribution of catalase for ethanol oxidation in red blood cells, another important side of this enzyme, has not widely been appreciated [87, 88]. As catalase activity is roughly comparable between liver and blood compartment but blood is at least 3 times larger in volume, RBCs could represent an estimated up to 6% of ADH-mediated ethanol oxidation and even up to 15% of the ADH-independent oxidation. Moreover, as shown in brain studies, catalase could play an important role in specific tissues locally. It also remains to be studied, in light of the new prospective all-cause mortality data presented in Part I of this book, how catalase-mediated ethanol metabolism interferes with the RBC metabolism and whether this impairs the physiological role of catalase as major H_2O_2 removing enzyme in human erythrocytes [88]. Recently, it has been suggested that catalase could participate in lactate-stimulated liver ethanol oxidation, where the addition of lactate generates hydrogen peroxide, which is then used by catalase to oxidize ethanol to acetaldehyde [89].

Nonoxidative Metabolism of Ethanol

Nonoxidative metabolism of ethanol includes the **generation of fatty acid ethyl ester (FAEE)** [90, 91], phosphatidyl ethanol (PEth) [92, 93] as well as ethyl glucuronide (EtG) and ethyl sulfate (EtS) [94, 95]. While FAEEs have been implicated in the pathogenesis of organ injury, especially in the pancreas, the later compounds have been used in forensic medicine as markers for chronic or acute alcohol ingestion. PEth is generated from phosphatidylcholine with phospholipase D. PEth has a high specificity for ethanol since it has a low rate of degradation. As already discussed above, however, degradation rate of PEth varies considerably between individuals. New work shows that PEth elimination depends on heme turnover and is enhanced in those drinkers with increased hemolysis [2]. EtG can be found in the urine up to 5 days after alcohol ingestion. EtG can also be determined in hairs (if more than 20 g ethanol/day is consumed). The detection of EtG and FAEE in hairs of more than 1 ng/mg demonstrates excessive ethanol consumption. Since EtG can be degraded by bacteria (urinary tract infection), the measurement of EtS seems superior. Other ethanol metabolites are ethylphosphate and ethylnitrite in very low concentrations. Additional information is further provided in Figs. A.77, A.78 and in the chapter on biomarkers.

Ethanol and Carbohydrate Metabolism—A Common Link to Metabolic Liver Disease?

Why can alcohol-related liver disease be replicated by diabetes and obesity? One of the major cause-specific deaths is liver related mortality with alcohol-related liver disease as one of the famous hallmarks of alcohol consumption. In 1995, more than 25 years ago, in a book edited by Pauline Hall and entitled “Alcoholic liver disease” [96], Peter Scheuer noted in the foreword that he did not understand why “there is an apparent latent period, supported by examination of serial liver biopsies, between the onset of heavy drinking and the development of steatohepatitis”. He further asked “why alcohol-related steatohepatitis is so very similar morphologically to non-alcoholic steatohepatitis (NAFLD)? [96]. Today, almost 30 years later, we have not yet found definite answers to these questions and, rather, a stagnation has been observed over the last two decades in our progress to better understand the underlying molecular mechanisms of ALD.

Moreover, ALD offers more unanswered questions, in addition to the above-mentioned stunning similarity between diabetes- and overweight-induced NAFLD. Almost no progress has been made in diagnosis and treating the often-fatal alcoholic hepatitis. Modest benefits are seen in only a fraction of patients with steroids. Although the microbiome has gained great attention, simple antibiotic treatment seems not to halt ALD. Moreover, the pathophysiology of ALD is often explained with quite complex and methodologically challenging mechanisms such

as reactive oxygen species that require deep physical, chemical and biochemical knowledge. On the other side, enzyme systems such as the p450 system localized in the endoplasmic reticulum are attributed to mediate major disease mechanisms but both knockout animals or pharmacological blockage show less convincing effects. In other words: What are the actual mechanisms of the alcohol-related liver disease and what does it have in common with NAFLD in the setting of overweight and diabetes?

Although the oxidation intermediated acetaldehyde is beyond any doubt crucial to explain alcohol-related disease mechanisms, it cannot be the major link to obesity and diabetes.

Rather, both alcohol, diabetes and overweight are characterized by an excess of energy. As can be seen from the original data (see Tables B.4, B.5, B.6, B.7, B.8, B.9) of the Heidelberg cohort followed-up over 15 years, heavy drinkers, despite having access to normal nutrition, cover about 50% of their energy supply by ethanol. In this cohort, in more than 1200 patients, in average, during a mean daily consumption of ca. 180-gram alcohol, a mean blood alcohol concentration of 1 °/°° (1 g/L) was reached. This corresponds to a **mean elimination of ca. 7.5 g alcohol per hour**. Based on a mean size of 174 cm and mean weight of 78 kg (BMI 25.5), this also corresponds to **0.01 g alcohol per kg body weight and hour**.

Although ethanol, comparable to sugars, only contains the three elements carbon, oxygen and hydrogen, it is neither chemically nor biochemically a carbohydrate and human metabolism is strikingly different. As mentioned above, ethanol is an energy supplier containing almost the double energy as compared to glucose (7 versus 4 kcal per gram). Similar to other physiological energy suppliers such as fatty acids or sugars, oxidation of ethanol leads to NADH which can be further used for mitochondrial respiration. However, in contrast to glucose and fructose, ethanol metabolisms rather **prevents gluconeogenesis** simply due to a balance shift towards reduced NADH (see Fig. 50.2). This **shifts the lactate/pyruvate ratio towards lactate** and, consequently, away from gluconeogenesis (see also Fig. 50.2). It should be also noted that typical ethanol metabolism starts already at 0.4 permille of blood ethanol concentration [97]. Through the **malate aspartate cycle**, reduced NADH is also shifted across the mitochondrial membrane and used for ATP production [98]. Peroxisomes seem also to have such a shuttle system so that peroxisomal ß-oxidation is also closely linked to the cytosolic intermediary metabolism [99].

Ethanol also provokes a fast and **efficient depletion of glycogen stores** (see Fig. 50.2). Although fundamental to ethanol biochemistry, it is still not clear whether these changes are responsible for rapid ethanol-mediated muscular fatigue as muscles obtain glucose from the liver through the Cori cycle. As already mentioned above, it is also highly intriguing that ethanol, which does not have any biochemical feedback loop in human cells, overwhelms the mitochondrial respiratory chain and may simply cause injury by uncoupling reactions leading to release of reactive oxygen species (ROS). Consequently, despite providing enough energy, ethanol metabolism leads to glucose deprivation and glycogen depletion which may become limiting for cells of the brain, red blood cells or muscle cells that are highly dependent on hepatic gluconeogenesis. In contrast to former reports, mitochondria seem

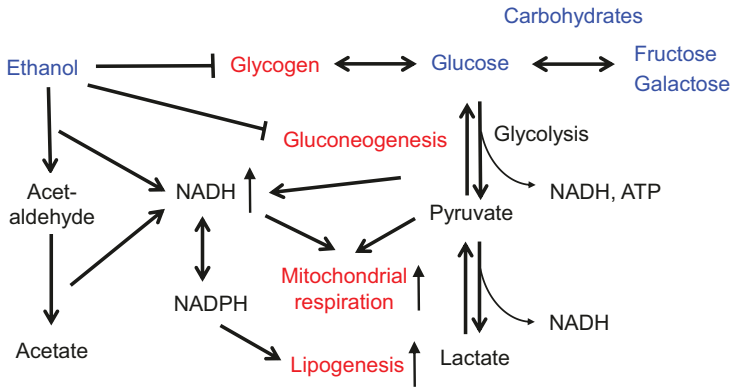

Fig. 50.2 Common pathways and differences of carbohydrate and ethanol metabolism. Both glycolysis and ethanol oxidation lead to formation of NADH. Cytosolic NADH can be transferred to mitochondria through the malate aspartate (Borst) shuttle. Both metabolic pathways also lead to lipogenesis, since NADH and the required NADPH are in balance. The major metabolic difference is that ethanol oxidation ultimately blocks gluconeogenesis most likely due to the redox changes with NADH excess and by shifting the lactate/pyruvate ratio towards lactate. Ethanol also causes rapid and significant glycogen depletion. Hence, glucose becomes limiting. With progression of ALD, however, blood glucose levels increase (see Table B.2). More studies are needed to fully comprehend the underlying hormonal and potentially hemodynamic mechanisms. The similarity of ethanol and carbohydrate metabolism consists in the excess and uncontrolled energy supply which may be one of the major driving forces of mitochondrial damage and liver inflammation. In addition, no metabolic escape exists for both excess carbohydrate and ethanol exposure except elimination by lipolysis or oxidation

first to increase respiration under conditions of ethanol. It would be an attractive scenario that the rapid and uncontrolled "fuel burning" in the cellular powerplants could ultimately be responsible for later observed mitochondrial damage e.g. through uncoupled redox reactions yielding to ROS, another established hallmark in alcohol-related liver and organ damage (see also chapter on "mitochondria and alcohol".

Glucose is central to energy consumption. Carbohydrates, lipids, and proteins can all ultimately break down into glucose, which then serves as the primary metabolic fuel of mammals and the universal fuel of the fetus. It serves as the major precursor for the synthesis of different carbohydrates like glycogen, ribose, and deoxyribose, galactose, glycolipids, glycoproteins, and proteoglycans [100]. However, in terms of evolution, access to small carbohydrates such as glucose, galactose or fructose has been always limiting to humans. Unlike glucose, which is directly metabolized widely in the body, fructose is almost entirely metabolized in the liver in humans, where it is directed toward replenishment of liver glycogen and triglyceride synthesis [101]. Ca. 40% of fructose is converted in liver to glucose, and about 25% is converted to lactate and ca. 20% is converted to glycogen [102]. Glucose and lactate are then used normally as energy to fuel cells all over the body [103].

Thus, it seems that although not being a carbohydrate, ethanol shares with sugars the immediate energy supply within the intermediary metabolism. They also share to some extent the lack of a negative feedback loop. As mentioned above for ethanol, the human metabolism can also not escape an excess of glucose or fructose and will metabolize it under excess conditions. In this scenario, fatty acid accumulation is the bodies only option to rapidly eliminate carbohydrates or ethanol and store the excess energy through lipogenesis in adipose tissue. Hence, in this context, fatty liver may rather be a bystander and a metabolic consequence to quickly remove the excess of energy but not the primary cause of steatohepatitis. Consequently, it is the **uncontrolled excess of rapidly-available energy that may link NAFLD with ALD**. Since lipogenesis, in this context, would be more a solution than a problem, fatty liver may not be the actual disease hallmark but rather the mitochondrial damage and inflammation due to excess energy supply and mitochondrial damage. The development of mitochondrial damage will later impair the mitochondrial ß-oxidation and, subsequently, further increase steatosis due to decreased fat elimination. More research on carbohydrate metabolism and its relation to ethanol and its hormonal control is needed. Ultimately, with regard to the terminology debate (see also Chap. 1), **metabolic (dysfunction) associated fatty liver disease (MAFLD)** [104] may not be optimal for paving a future path of better understanding the underlying mechanism. A potential more optimal alternative could be then broader term **"Metabolic Liver Disease" (MLD)** which would only encompass patients with signs of liver damage and fibrosis while fatty liver would be an important diagnostic feature but not necessarily part of the pathology. Finally, the intermediary metabolism would also provide a novel bridge to **interlink energy and ethanol metabolism to addiction**, whether it is food addiction or alcohol dependence. Here, more detailed studies a needed.

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Chapter 51
Modulation of Alcohol-Related Liver Disease by Obesity and Diabetes

Hannes Hegmar and Hannes Hagström

Abstract Current evidence suggests an interactive effect between a high consumption of alcohol, or ALD, and metabolic risk factors, associated with NAFLD, on the risk of development of cirrhosis. Patients with both a high consumption of alcohol and obesity or diabetes should therefore be considered a risk group for cirrhosis. Additional studies regarding the efficacy of screening for advanced liver fibrosis or cirrhosis in such risk groups are needed. The most effective, and established, methods to reduce the risk of progression of ALD is alcohol abstinence, and weight loss in NAFLD.

Keywords NAFLD · Alcohol-related liver disease · Diabetes mellitus · Prediction · Cirrhosis · Epidemiology

Introduction

The use of alcoholic beverages might have started as early as year 10,000 BC [1]. As such, alcohol consumption is highly integrated into most modern societies. The knowledge about the harmful effects of alcohol on the liver, however, is relatively new. Until the 1950s it was thought that malnutrition, and not alcohol itself, caused end-stage liver disease [2]. As opposed to alcoholic beverages, easy access to excess food is rather new to human history and has led to overnutrition in a substantial proportion of the population [3]. This excess in combination with a more sedentary lifestyle is the basis for the obesity pandemic currently affecting most developed and developing countries globally. Even though the harmful effects of obesity were

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known to the ancient Greeks and Hippocrates [4], the association between obesity and liver disease is also relatively new [3]. In 1980, a paper by Ludwig, et al., was published showing that patients abstaining from alcohol could have a histologic picture that mimicked alcohol-related liver disease (ALD). Most of those patients were obese and had diabetes mellitus type 2 [5]. Ludwig termed the condition non-alcoholic steatohepatitis (NASH) which today is part of the broader term non-alcoholic fatty liver disease (NAFLD). Besides the histologic resemblance, ALD and NAFLD share many disease traits, such as a broad spectrum of disease severity, from mild liver damage with steatosis to cirrhosis and end-stage liver disease. Since both ALD and NAFLD, which is closely related to obesity and diabetes mellitus type 2, constitute a large proportion of patients with advanced liver disease in many countries, attention to both diseases is required. In the USA, ALD was the most common indication for liver transplantation in patients without primary liver cancer in 2019, while the second most common indication was NAFLD [6].

Not all patients who drink alcohol, nor all patients with NAFLD, develop severe liver disease such as cirrhosis or hepatocellular carcinoma (HCC) [7–9]. Patients that do develop severe liver disease have certain specific risk factors, known and unknown, putting them at a higher risk. Emerging evidence regarding risk factors of a greater severity in both ALD and NAFLD associates with metabolic as well as genetic traits (see also book Chap. 52). Alcohol use disorder, obesity, and diabetes mellitus type 2, respectively, are identified as risk factors of progression to more advanced liver-related disease. However, there is a growing concern that a combination of these risk factors might act synergistically on the risk of developing liver disease, as well as its progression. This interaction therefore requires attention from both policymakers as well as health care professionals who meet these patients in their everyday care.

Definitions of Liver Disease Related to Alcohol, Obesity, and Diabetes

Since alcohol, obesity, and diabetes mellitus type 2 are independent risk factors for liver disease [10, 11], it is important to know how obesity and diabetes might modify the risk of developing more severe forms of liver disease in people with a high consumption of alcohol. Likewise, it is important to know how consumption of alcohol might modify the risk of liver disease in people with obesity and diabetes mellitus type 2, likely to have NAFLD. Since these conditions are all highly prevalent globally, it is obvious that many patients with ALD also are obese or have diabetes mellitus type 2.

When investigating the risk of liver-related outcomes based on alcohol consumption in obese and diabetic patients, many studies have investigated patients with non-alcoholic fatty liver disease, which is closely related to these conditions. The

definition currently separating ALD and NAFLD is the amount of alcohol consumed. Internationally, a daily intake of less than 20 g alcohol in women, and less than 30 g in men is usually recommended to define presence of NAFLD and rule out ALD in the setting of hepatic steatosis [12], whereas some countries and organizations recommend an even lower threshold, or total abstinence [13]. ALD is generally considered if the patient has an alcohol consumption above the recommended limits and other causes of liver diseases have been ruled out [14]. If alcohol consumption is within the recommended limits, NAFLD should be considered [15]. This cut-off is rather arbitrary. The terms NAFLD and ALD are umbrella terms which include different stages of disease severity. Presence of inflammation in ALD is termed alcohol-related steatohepatitis, or simply steatohepatitis, where the most severe form is termed alcoholic hepatitis (AH). More details are provided in book chapters xxx and xxx. However, it remains a central problem that it is difficult to measure alcohol consumption objectively, and definitions of ALD differ between studies, which must be taken into consideration when interpreting results of the available evidence. With regard to ethanol biomarkers, the reader is referred to chap. xxx.

Epidemiology of Alcohol-Related Liver Disease

It is believed that approximately 80% of adults in the Western world have been drinking alcohol sometime in their life, and that up to 60% of adults in the western world are active drinkers [16]. According to a World Health Organization report from 2018, the average consumption among active drinkers globally is 32.8 g of pure alcohol per day, or 15.1 L of pure alcohol annually, but there are large differences between populations [16]. Furthermore, there are sex differences, where men drink larger amounts of alcohol compared to women [16]. More details can be found in part I of the book.

Approximately 90% of patients who drink more than 60 g of alcohol daily, which equals five or six units depending on the definition of a unit, are thought to develop liver steatosis, but only around 20–30% develop significant fibrosis or cirrhosis [7–9, 17]. Still, ALD is attributable to roughly half of all deaths related to cirrhosis, corresponding to 607,000 deaths per year globally [16]. Apart from the total amount of alcohol ingested, the drinking patterns also seem to affect the risk, and heavy episodic drinking has been associated with an increased risk of ALD and mortality [18]. The global prevalence of regular heavy episodic drinking is estimated to 18% in people 15 years or older, where Europeans and Americans have a higher prevalence estimated to 26% and 22%, respectively [16]. Among current drinkers the prevalence of regular heavy episodic drinking is approximately 40% [16].

Epidemiology of Liver Disease in Obesity and Diabetes Mellitus Type 2

Overweight and obesity, defined as a body mass index (BMI) ≥25, and ≥30 kg/m^2, respectively has a prevalence of 39% globally. In total numbers, this equals to around 1.9 billion overweight people, of which 650 million are obese [19]. In the United States alone, the prevalence of obesity is 35% and is expected to reach 45% in 2030 [20]. Diabetes mellitus type 2 is also a disease of global concern with a prevalence of 10% in 2014 and it is expected to increase to 13% in 2030. The prevalence of diabetes is associated to that of obesity, and follows the increase of obesity prevalence closely [20]. Both diabetes and obesity are associated with NAFLD [21, 22]. Based on previous studies, approximately 70% of persons with obesity have NAFLD with an even higher proportion in those that undergo bariatric surgery (90%), and 55–70% of patients with diabetes mellitus type 2 have NAFLD [21–23]. The duration of obesity and diabetes likely affects risk of liver disease. A population-based study of 1.2 million men showed that a high BMI during late adolescence was associated with an increased risk of severe liver disease later in life [10]. The risk increased non-linearly without evidence of a threshold effect, suggesting that increasing one's BMI already from a low level is associated with some increase in this risk, although the risk in absolute terms was low for those with a mildly increased BMI (overweight). The risk was further enhanced if patients had diabetes mellitus type 2 compared to obese people without diabetes mellitus type 2 [10]. Similar findings have been seen in young women [24].

Diabetes mellitus type 2 has also been shown to increase the risk of severe liver disease [25]. When screened for liver disease with magnetic resonance imaging, almost 64% of patients with diabetes mellitus type 2 had liver steatosis, and 6% had cirrhosis [26]. An increased severity of liver disease in patients with diabetes mellitus type 2 and NAFLD was further seen in a meta-analysis, where 37% were estimated to have NASH, and 17% had advanced fibrosis [27]. That is of prognostic significance as the stage of fibrosis is the best predictor of severe liver disease and overall mortality in patients with NAFLD [28, 29]. This illustrates the increased risk of severe liver disease in patients with diabetes mellitus type 2. A recent meta-analysis combining epidemiological findings showed an association between obesity and type 2 diabetes mellitus and an increased risk of developing severe liver-related outcomes [30]. The combined evidence suggested an increased rate of developing severe liver disease of approximately 2.25 for persons with type 2 diabetes, and a more modest increase in this rate (1.20) in those with obesity [30].

Pathophysiology of Alcohol-Related Liver Disease

The metabolism of alcohol begins in the stomach, where alcohol is oxidated by gastric alcohol dehydrogenase [31]. More details are provided in chapters xxx. Alcohol which is not metabolized by the stomach enters the portal vein through

the lower intestinal tract. It finally reaches the liver where the majority is metabolized by hepatic alcohol dehydrogenase (ADH) and microsomal CYP2E1 [32, 33]. Hepatic ADH normally accounts for most of the ethanol metabolization, but oxidation within the CYP2E1 system can be increased in the setting of a high consumption of alcohol [33, 34]. However, this upregulation also increases the production of reactive oxygen species and DNA damage [35]. Furthermore, the intermediate product in alcohol metabolism, acetaldehyde, is highly reactive, a carcinogen and contributes to the development of ALD [36, 37].

The metabolism of alcohol also affects hepatic lipid metabolism [38]. An increased uptake of fatty acids and increased lipogenesis combined with a decreased fatty acid oxidation and decreased export of very low-density lipoprotein (VLDL) result in an accumulation of lipid droplets in hepatocytes [38]. This condition is then defined as alcohol-related fatty liver, or steatosis related to alcohol. This diagnosis has traditionally been made by liver histology, although currently biopsies are seldomly acquired in ALD. Fat accumulation in hepatocytes is a hallmark of the histologic picture of ALD, and is seen in the histology of most patients with ALD. For more details, see also the book chap. xxx on histology of ALD. ALD typically shows fat infiltration in hepatocytes in mild cases of disease, which can be either macro- or microvesicular [39, 40]. Steatohepatitis is a more severe form of the disease with inflammation and fibrosis. The histology in steatohepatitis displays, in addition to steatosis, lobular inflammation, focal necrosis with inflammatory infiltrates, hepatocyte ballooning, Mallory-Denk bodies, and different stages of fibrosis including cirrhosis [39–41]. Steatosis can be less pronounced in severe steatohepatitis or constitute of less than 5% of the parenchyma, and is therefore not a formal diagnostic criterion in alcohol-related liver disease [42].

Alcoholic Hepatitis

One of the most severe forms of alcohol-related liver disease is termed alcoholic hepatitis (AH) and more details are provided in chapters xxx and xxx. Briefly, AH usually evolves after a period of extended binge-drinking [14]. Cirrhosis is also frequently seen in patients with AH [14]. AH is primarily a clinical diagnosis and patients often present with jaundice combined with fever, weight loss, fatigue, malaise and malnutrition. Signs of decompensation such as ascites and hepatic encephalopathy can also occur. AH can resemble acute-on-chronic liver failure (ACLF) and bacterial infections, why other etiologies must be investigated before a final diagnosis can be made. A liver biopsy is seldom performed and a transjugular approach is recommended to avoid the risk of bleeding. Biopsy should be reserved for cases where the diagnosis is uncertain [14]. Patients with AH typically have a bad prognosis and 6 months mortality can be high as 30–40% [43].

Pathophysiology of Liver Disease in Patients with Obesity and Diabetes

In general, the histologic picture in ALD is not specific, and patients with NAFLD have a similar histologic presentation. Therefore, based on histology, ALD cannot be separated from NAFLD without additional information from laboratory reports or patient anamnesis [42]. There are some specific changes that have mostly been described in alcohol-related liver disease, such as sclerosing hyaline necrosis, alcoholic foamy degeneration among others [42]. However, these are nonspecific and not present in all patients. In patients with obesity and diabetes mellitus type 2 with a non-specific histologic picture of fatty liver disease, and where no secondary causes of liver disease are present, NAFLD is thought to be the cause of the histopathologic picture. The pathogenesis of NAFLD is complicated and beyond the scope of this chapter but is in principle caused by changes in metabolic pathways due to overnutrition. The major causes include increased substrate load directly from the diet, lipolysis of triglycerides in adipose tissue related to insulin resistance, and an increased flow of fatty acids to the liver, as well as increased de novo lipogenesis in hepatocytes [44, 45].

Non-alcoholic Steatohepatitis

Additional contributions to the progression of liver disease and liver inflammation include the formation of lipotoxic lipids, gut-derived lipopolysaccharides, reactive oxygen species, inflammasome activation, and injured hepatocytes which activate liver macrophages [46, 47]. Alone and in combination, this can form an inflammatory environment that trigger stellate cells in the liver to turn into myofibroblasts, these then produce extracellular matrix proteins and ultimately fibrosis [48]. Continuous progression of fibrosis can lead to cirrhosis, the common end-stage of all chronic liver diseases. Furthermore, the activity of the CYP2E1 enzyme has also been found to be induced by diabetes mellitus type 2 and obesity and could therefore contribute to changed alcohol metabolism, with increased oxidative stress and cell damage, in patients with these conditions [49]. An experimental study comparing microbiota in healthy children; obese children without sign of liver disease; or children with NASH found an abundance of ethanol-producing bacteria in the children with NASH, and also higher levels of endogen ethanol in blood [50]. These results have later been confirmed in another study [51]. Thus, low doses of endogenously produced ethanol could be a contributing factor for the progression of NAFLD to NASH and eventually fibrosis, although ethanol levels are significantly lower as those seen in ALD patients.

Genetics Modifiers of ALD and NAFLD

Genetic risk modifiers are present in both ALD and NAFLD, and are to some extent shared between the diseases, suggesting some degree of hereditability of these diseases. More details are provided in book chap. xxx. A study of more than 15,000 male twins showed that presence of alcohol-related cirrhosis was three times more common in monozygotic twins compared to dizygotic twins [52]. This indicates a correlation of genetic risk factors and progression of fibrosis in patients with ALD. Genome-wide association studies have identified mutations that have a strong correlation with ALD and cirrhosis in the genes PNPLA3, TM6SF2, and MBOAT7 [53–55]. The single-nucleotide polymorphism in the PNPLA3 gene, as well as the TM6SF2, have also been associated with NAFLD [56, 57]. Both PNPLA3 and TM6SF2 affect the hepatic lipid metabolism by retaining lipids in the liver through different pathways [58, 59]. A longitudinal study of a large cohort showed an association of mutations in PNPLA3 and an increased risk of liver cancer, hospital admission due to severe liver disease, and death [60]. Additional mutations in genes such as HSD17B13, MBOAT7, MARC1, GCKR and others have also been described [61, 62]. A deeper understanding of the pathophysiology and genetic predispositions are important and promising predictors of future liver-related events. However, currently no genetic data is routinely used in clinical practice.

Amount of Alcohol as a Risk Factor in ALD

ALD is caused by an excess intake of alcohol, and there is a direct association between the amount of alcohol consumed and the risk of developing ALD [63]. Data from meta-analyses suggest the risk of developing cirrhosis is present already in persons consuming more than 25 g of pure alcohol per day, although other studies found an increased risk even in persons consuming between 12 and 24 g of alcohol daily [64, 65]. Women are at an even greater risk of developing ALD, and even one drink a day is associated with an increased risk of developing cirrhosis [66, 67]. This could partly be explained by the higher proportion of body fat in women which leads to higher serum concentrations of ethanol, and by sex differences in gastric alcohol dehydrogenase [31]. A Danish study suggested that the largest risk of cirrhosis development was seen in patients drinking daily, and that this risk was higher in patients with a recent episode of drinking [68].

The presence of alcohol-related fatty liver itself is also a known risk factor of progression to fibrosis and cirrhosis, especially in patients that continue to consume alcohol [9]. The 5-year risk of alcohol-related cirrhosis is estimated to be 7% in patients with pure alcohol-related steatosis, while the 5-year risk of cirrhosis is around 16% in patients with steatohepatitis due to alcohol [69]. All together, these data suggest differences in risk of progression of ALD among people drinking alcohol that is not solely based on the amount of alcohol consumed.

The controlled attenuation parameter (CAP) is a surrogate for liver steatosis that is defined using an ultrasound-based method measuring the attenuation of ultrasound waves [70]. In patients consuming 5–7 drinks per week there was a significant association between the number of units of alcohol per week with steatosis, as defined by an increased CAP. This finding remained after adjusting for quantity and binge-drinking [71]. However, repeated measurements of CAP to investigate changes in liver steatosis might not be as accurate as methods based on magnetic resonance imaging, and its clinical use to monitor steatosis change over time is uncertain, especially in patients reporting periodical drinking, since abstinence of alcohol reduces the level of steatosis in the liver [39, 40, 72]. In a randomized controlled trial performed in healthy students consuming moderate amounts of red wine during 3 months, defined as 33 g daily in men and 16 g daily in women, no steatosis was induced as measured by magnetic resonance spectroscopy [73]. Altogether, this indicates that either a longer duration of consumption or higher dose of alcohol is needed to develop liver steatosis.

Amount of Alcohol as a Risk Factor in NAFLD

The evidence regarding the risk of developing liver disease, or worsening of known liver disease, in people consuming low to moderate amounts of alcohol is inconsistent. A study performed in patients with NAFLD found that moderate consumption of alcohol, defined as less than two drinks daily, was associated with less reduction of steatosis and less resolution of NASH in paired liver biopsies compared to patients who were abstinent from alcohol [74]. In patients with metabolic risk factors correlated to NAFLD, alcohol was a significant independent predictor of liver-related outcomes along with smoking, age, waist circumference, and insulin resistance [75]. A Mendelian randomization study used a mutation in the alcohol dehydrogenase gene as a proxy for long-term alcohol consumption in patients with NAFLD. Patients with the mutation reported less consumption of alcohol and did not have higher stages of fibrosis or a higher prevalence of steatohepatitis on biopsy compared to patients without this mutation [76]. Binge-drinking has been shown to be a risk factor of progression to advanced liver disease. Patients with biopsy proven NAFLD showed an association between heavy episodic drinking and fibrosis progression [77], which was also the result in a population based study where increased risk of decompensated liver disease was seen in patients with weekly and monthly binge drinking [78]. Further, binge-drinking and other behaviors associated with alcohol overconsumption has been showed to associate with increased risk for cirrhosis in healthy young adults later in life [79].

Other studies primary using a cross-sectional design have suggested a protective effect of low to moderate consumption in patients with NAFLD, compared to drinking no alcohol. Patients undergoing bariatric surgery had a reduced risk of non-alcoholic fatty liver disease if they reported a moderate alcohol consumption compared to patients with other drinking habits undergoing surgery [80]. It was

speculated that this was because of reduced insulin resistance. A meta-analysis included studies where patients with total abstinence from alcohol versus low to moderate alcohol consumption, defined as less than 40 g per day, were compared. It showed a protective effect of 31% from getting NAFLD, and a 50% protection from developing NASH in patients consuming low to moderate levels of alcohol [81]. In patients with NAFLD and concomitant diabetes, low to moderate alcohol consumption have been associated with improved insulin resistance and a lower risk of diabetes mellitus type 2 [82, 83]. Also, low to moderate alcohol was not associated with development fibrosis [84].

Several other cross-sectional studies have shown similar results with a reduced risk of advanced liver disease in patients with fatty liver drinking low to moderate amounts of alcohol [84–91]. However, there is a risk of bias in these studies due to methodological issues. Patients with known manifest advanced liver disease have, for example, been included and there is a risk of misclassification bias in patients that report current total abstinence, since many of them could previously have been drinking larger amounts alcohol, so called "sick quitters" [92]. Interestingly, one of the studies also measured phosphatidyl ethanol which is a validated marker for recent alcohol consumption [93–95]. Patients that had reported low to moderate alcohol consumption had an elevated phosphatidyl ethanol >0.3 μmol/L in 11% of the cases, which indicate a larger consumption than reported [90]. This is suggestive of either recall bias or dishonesty in the reported alcohol consumption. Furthermore, in 8300 patients with known liver steatosis, the risk of developing advanced liver disease, defined as liver-related hospital admission, liver cancer or liver-related death, based on low to moderate alcohol consumption was evaluated. The study excluded patients with manifest liver disease at baseline and current abstainers. They found a dose-dependent increase in the risk of incident advanced liver disease in persons consuming more than 10 g of alcohol per day [96].

Interaction Between Alcohol and Diabetes

Table 51.1 lists some important studies to analyze the modulating role of diabetes on ALD. A high chronic consumption of alcohol is associated with increased insulin resistance and the development of diabetes mellitus type 2 [83]. People with an alcohol use disorder have a higher risk of diabetes mellitus type 2 compared to people in the general population [99]. This is most likely due to the progression of insulin resistance seen in patients with ALD, where the insulin resistance is more severe in more advanced stages of fibrosis [61]. Progression to cirrhosis is more common in patients with diabetes and high BMI, compared to people without these diseases who drink the same high amount of alcohol [97].

Even without a known alcohol consumption, patients with diabetes and a severe insulin resistance progress to fibrosis at a higher rate than diabetic patients with other metabolic profiles [100], and an increased blood glucose level is associated with increased fibrosis [101]. Therefore, it is not surprising that a combination of

alcohol and diabetes should have a synergistic effect on the risk of liver disease. This synergistic effect was seen in a population-based study of 6700 persons with a follow-up time of 12 years. Persons with diabetes and a high alcohol consumption had a 20-fold increased rate of progression to liver cancer, hospital admission due to liver disease, or liver-related mortality compared to persons with little or no alcohol consumption and no diabetes. In contrast, there was a 3.5-fold increase in patients with high alcohol consumption alone, and a 2.5-fold increase in patients with diabetes alone [75]. Another study showed a similar synergistic effect where patients with liver steatosis and the metabolic syndrome, who drank excessive amounts of alcohol, had an increased mortality risk compared to people that did not drink excessive amounts of alcohol or did not have the metabolic syndrome [102]. The metabolic syndrome is closely related to insulin resistance, and diabetes was the only component in the metabolic syndrome that was significantly associated with increased mortality in this cohort [102].

Furthermore, patients with diabetes mellitus type 2 who carry mutations in the PNPLA3 gene have a more severe insulin resistance compared to patients with diabetes type 2 that do not carry this genotype [103]. In patients with ALD, presence of increased insulin resistance and mutations in PNPLA3 are associated with more advanced stages of fibrosis, and together with active alcohol consumption they are independent risk factors of inflammatory activity in the liver [61], which is a main driver in the fibrogenesis. Altogether, with the metabolic changes in ALD and diabetes, and the combined effect of alcohol and insulin resistance, screening for insulin resistance and diabetes in patients with manifest ALD might be valuable in clinical practice. Even genetic screening might be a future tool, used in combination with metabolic risk factors, to identify patients with ALD with increased risk of future cirrhosis.

Interaction Between Alcohol and Obesity

Table 51.1 also lists important studies to analyze the modulating role of obesity on ALD. Before the modulation of ALD by obesity is discussed in further detail the effect of alcohol on obesity should be taken into consideration, not the least because of the potential synergistic risk of liver disease between alcohol and obesity. Pure ethanol contains 7 kcal/g and heavy drinking is associated with weight gain [104]. The opposite effect with weight loss is usually seen in patients with severe alcohol use disorder and in cirrhotic patients with sarcopenia [105]. Consumption of 500 mL of beer daily has a weak association with weight gain, whereas a low consumption of wine has been associated with a possible protective effect on weight gain [106, 107]. As above, careful interpretation of such cross-sectional studies must be made, since residual confounding such as a better diet and more exercise might be present in those consuming wine. As mentioned above, obesity is defined by a BMI of 30 kg/m^2 or more, but adipose tissue differently distributed in the body, and a high waist-hip ratio are better predictors of liver disease than a high BMI, suggesting that

Table 51.1 Important studies to study the modulation of alcohol-related liver disease by obesity and diabetes

Reference	Setting and design	N patients	Key exposure	Key outcome	Key finding
Hagström [10]	Sweden, cohort study	1.2 M men	Body mass index	Liver-related outcomes from national registers	Higher BMI early in life associates with higher risk of cirrhosis. Further increase if presence of diabetes.
Jarvis et al. [30]	Multiple countries, meta-analysis	22.8 M	Body composition including BMI, diabetes	Liver-related outcomes	Meta-analysis finding an association between obesity (HR = 1.2) and diabetes type 2 (HR = 2.3) with future cirrhosis
Sahlman et al. [60]	Finland, cohort study	41,260	Patterns of alcohol consumption and metabolic, lifestyle-related, and anthropometric parameters	Liver-related outcomes from national registers	Strong synergism between alcohol and central obesity on the risk of liver-related outcomes
Israelsen et al. [61]	Denmark, cross-sectional	325 patients with alcohol-related liver disease	Metabolic and genetic parameters	Hepatic fibrosis defined by liver biopsy	Insulin resistance strongest risk factor for hepatic fibrosis in patients with ALD
Ajmera et al. [74]	US, cohort study	285 patients with biopsy-proven NAFLD with at least 2 biopsies	Questionnaire-defined alcohol consumption	Biopsy-defined improvement of hepatic steatosis and NASH	Modest alcohol use was associated with less improvement in steatosis as well as lower odds of NASH resolution, compared with no use of alcohol.
Åberg et al. [75]	Finland, cohort study	6732	Alcohol use and metabolic risk factors	Liver-related outcomes from national registers	Interaction between alcohol use and type 2 diabetes, higher risk for liver-related outcomes

(continued)

Table 51.1 (continued)

Reference	Setting and design	N patients	Key exposure	Key outcome	Key finding
Whitfield et al. [97]	UK, case-control study	1293 cases with ALD-cirrhosis and 754 controls with similar alcohol consumption but no cirrhosis	Alcohol use, life-style and metabolic risk factors	Cirrhosis defined as by clinical records	Patients with cirrhosis more commonly had type 2 diabetes and higher BMI
Liu et al. [98]	UK, cohort study	1.2 M women	BMI and questionnaire-defined alcohol consumption	Hospital admissions for cirrhosis	Alcohol and higher BMI interactively increases risk for cirrhosis. 17% of cirrhosis cases attributable to obesity, 42% to alcohol.

it is more important to consider abdominal obesity than only BMI [108]. This was recently also suggested in a paper where waist-hip ratio was also superior to blood-based scores such as FIB-4 and NAFLD fibrosis score in predicting the presence of hepatic fibrosis in a low-prevalence population [109]. Recent longitudinal studies in a Finnish cohort showed a synergistic interaction between high waist circumference or high waist-hip ratio and an alcohol consumption over 210 g per week in men and over 140 g per week with an increased risk of liver-related outcomes [60, 75]. Weekly binge drinking in patients with the metabolic syndrome has also showed a synergistic effect on the risk of developing advanced liver disease with complications such as ascites, esophageal variceal bleeding and hepatic encephalopathy [78]. Similar findings were seen in almost 10,000 British men during 29 years of follow-up. An increased risk of death from liver disease was found if drinking >15 drinks per week across all BMI categories, but the risk in obese men was even greater [110]. Abdominal obesity is further correlated with insulin resistance, which is an important driver of liver disease by increasing lipolysis from adipose tissue and shunting free fatty acids to the liver [111]. This could in part explain why estimations of pathologic fat distribution show a stronger association than BMI with development of liver disease.

In patients with biopsy-proven steatosis due to ALD, independent risk factors of alcoholic hepatitis and cirrhosis were being overweight during more than 10 years, female sex, and the duration of a risky consumption of alcohol [112]. There were similar findings in women consuming more than 150 g of alcohol per week, and the risk of developing cirrhosis was higher if they were obese compared to non-obese women [98].

Animal models have shown different potential pathways as a cause for the synergistic effects of alcohol and obesity on the development of steatohepatitis.

Moderate use of alcohol and obesity together cause an activation in macrophages which accentuates mitochondrial stress, and binge-drinking and obesity together induce the chemokine CXCL1, leading to infiltration of hepatic neutrophils which synergistically increase the risk of steatohepatitis [113, 114].

Risk of Hepatocellular Carcinoma in Patients with Alcohol-Related Liver Disease by Obesity and Diabetes

The risk of hepatocellular carcinoma is increased in patients with alcohol-related cirrhosis. It is estimated that 30% of the mortality in hepatocellular carcinoma is due to alcohol-related liver disease [115]. Diabetes mellitus type 2 and obesity are also independent risk factors of hepatocellular carcinoma [116, 117]. In patients with both alcohol and obesity as risk factors there seems to be a synergistic effect regarding the risk of hepatocellular carcinoma [118, 119]. The effect is also present in patients with diabetes and a high alcohol consumption [75, 120, 121].

Screening and Treatment of Risk Factors

Screening of at-risk individuals have gained more attention in recent years. The European Association for the Study of Liver (EASL) recommend screening for fibrosis with non-invasive methods in patients with potentially harmful alcohol consumption or metabolic risk factors such as diabetes and obesity, but not in an unselected general population [122]. A study investigating screening of liver disease using transient elastography in patients with heavy alcohol consumption or diabetes mellitus type 2 included 900 patients. A total of 230 (26%) patients had signs suggestive of fibrosis, and 27 (3%) had cirrhosis. The study also found that cirrhosis was significantly more common in obese patients with diabetes or heavy alcohol consumption compared to non-obese patients [123].

Alcohol might also indirectly affect the course of diabetes mellitus type 2 by an increased risk of non-adherence to the self-care recommendations and less compliance to anti-diabetic medications [124, 125]. Patients with type 2 diabetes drinking heavy or moderate amounts of alcohol were shown to report less self-glucose monitoring and were more likely to skip diabetes provider visits compared to non-drinkers [126]. At-risk alcohol consumption in patients with diabetes is often inadequately addressed but should be evaluated thoroughly in routine diabetes care [127].

The most effective, and established, methods to reduce the risk of progression of ALD is alcohol abstinence, and weight loss in NAFLD [14, 128, 129]. Treatment guidelines regarding contributing risk factors in ALD and NAFLD would be beneficial, but there is currently no consensus on this topic. The Asian Pacific Association

for the Study of Liver (APASL) as well as the European Society for Clinical Nutrition and Metabolism (ESPEN) recommend patients with diabetes mellitus type 2 and obesity and present liver disease to completely avoid alcohol [130, 131].

Summary

Current evidence suggests an interactive effect between a high consumption of alcohol, or ALD, and metabolic risk factors, associated with NAFLD, on the risk of development of cirrhosis. Patients with both a high consumption of alcohol and obesity or diabetes should therefore be considered a risk group for cirrhosis. Additional studies regarding the efficacy of screening in such risk groups are needed and eagerly awaited. The most effective, and established, methods to reduce the risk of progression of ALD is alcohol abstinence, and weight loss in NAFLD.

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Chapter 52
The Genetics of Alcohol-Related Liver Disease

Hamish Innes and Felix Stickel

Abstract Significant advances into the genetics of alcohol-related liver disease (ALD) have been made in the last two decades. Most notably, this includes the discovery of ten common genetic variants associated either with alcohol-related cirrhosis and/or hepatocellular carcinoma. These novel associations provide insight into the pathophysiology of ALD and have led directly to potentially new therapeutic targets, which are the subject of ongoing research. In addition, several genetic risk scores are now available to identify patients at high/low risk of developing cirrhosis, albeit their performance may not yet be adequate to enable risk stratification in the clinic. Prospectively, the increasing accessibility of population-level biobank data together with the emergence of whole genome sequencing data will likely lead to further discoveries in the years ahead. In this chapter, we will review the key genetic association studies performed so far in relation to ALD, discuss the potential clinical applications, and identify areas worthy of future research.

Keywords Alcoholism · Cirrhosis · Fatty liver disease · Heritability · Host genetics · Liver cancer · Polygenic risk score

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Heritability of Alcohol-Related Liver Disease

Alcohol-related liver disease (ALD) refers to a spectrum of partly overlapping liver tissue alterations with escalating severity attributable to excess alcohol use over time. Clinically, ALD ranges from simple steatosis in nearly all heavy drinkers through to life-threatening conditions such as cirrhosis and hepatocellular carcinoma (HCC) in only a minority of approximately 10–15% [1]. Global mortality from ALD is substantial, with ~350,000 people dying every year from alcohol-related cirrhosis alone [2]. In addition, alcohol consumption leading to ALD or as a cofactor in other non-alcohol-related liver diseases is a major driver of HCC, which causes ~800,000 deaths per year [3].

The quintessential risk factor for ALD is the total volume of alcohol consumed (i.e. greater total volume is associated with a higher ALD risk). However, other risk factors contribute too, including age, sex, diabetes, BMI, coffee intake, features of the metabolic syndrome and potentially drinking patterns and beverage types consumed [4–6]. As most of these factors are influenced by both genetic and environmental factors, it follows that ALD itself is a product of interaction between both genes and the environment (i.e. nature and nurture). Unfortunately, the relative importance of genes versus environment has not been widely quantified. The only significant study to investigate this issue was published >25 years ago by Reed et al. [7]. This analysis included data from 31,848 twins (i.e. 15,924 male twin-pairs), all within the US army veterans' health program. The authors quantified the heritability of three phenotypes: alcoholism, alcohol psychosis and cirrhosis. The overall prevalence of cirrhosis in the sample was 2.2%. The concordance of cirrhosis between twin-pairs was three times higher for monozygotic twins (16.9%) versus dizygotic twins (5.3%). Overall, Reed et al. found that 47% of the variability in cirrhosis status was due to genetic effects, with the remaining 53% attributable to the environment. Nevertheless, Reed et al. suggested that most of this genetic contribution reflected the genetic liability for alcoholism, rather than genetic liability for cirrhosis specifically [7].

Unfortunately, no comparable study has been performed since this landmark publication. Replication studies are fundamentally hampered by the low prevalence of cirrhosis among twins. Indeed, in the twins UK registry, there are only 16 individuals with a cirrhosis diagnosis, and none with HCC [personal communication: Victoria Vazquez; first March 2022]. Thus, meta-analysis of twin studies/registries will be useful to better understand the heritability of specific ALD phenotypes. Although meta-analysis has been performed in relation to alcohol use disorders [8], it has not yet been applied to the heritability of specific ALD phenotypes such as cirrhosis.

Genetic Association Studies: Overview

Although twin studies can establish whether a given phenotype is heritable, they do not indicate which specific genetic factors underpin this heritability [9]. This is the role of genetic association studies. In general, genetic association studies fall into two main categories. First, genome-wide-association studies (GWAS), which typically test up to millions of polymorphisms for association with a single phenotype. The hallmark of a GWAS is that it incorporates polymorphisms throughout the genome. In this way, GWAS is biologically agnostic insofar as it makes no prior assumption about which genetic regions influence disease [10]. However, this hypothesis-free perspective comes at a price. Namely, to guard against false-positive associations, one must use a very low p-value threshold to define statistical significance (typically $P < 5 \times 10^{-8}$). As a result, GWAS inherently favours the discovery of variants that: (a) exhibit very strong associations with the phenotype; or (b) are highly frequent in the population; or (c) exhibit a combination of the two. Rare variants that might very well influence the evolution of disease are less likely to be recognised and can be missed.

On the other hand, candidate gene association studies (CGAS) assess the association between single or a small number of specific variants with a given phenotype [11]. Candidate variants are typically selected on the basis of biological knowledge and/or the results from previous GWAS. A crucial point is that because fewer variants are considered, the p-value threshold used to define statistical significance is much higher, thus, statistical power is greatly increased.

In liver disease, CGAS studies are performed to assess if a variant-phenotype association identified in patients with a particular aetiology (e.g. NAFLD) is generalisable to patients with other aetiologies (e.g. ALD). One example is the discovery of rs429358 in *APOE* as a risk factor for alcohol-related HCC [12]. Also, the importance of rare variants (i.e. loci with a minor allele frequency < 1%) in liver disease is being increasingly recognised [13]. CGAS is the standard approach for investigating rare variant-phenotype associations, as rare variants are typically omitted from GWAS. Thus, new insight into ALD genetics will depend on the interplay between GWAS and CGAS approaches since both can be valuable if applied appropriately.

GWAS for Alcohol Liver Disease

Study Design Overview

Five robust GWAS studies have been performed for ALD phenotypes: three for cirrhosis [14–16] and two for hepatocellular carcinoma [17, 18]. All studies have used a case-control design with separate discovery and validation stages (Table 52.1). Effective sample sizes of the five GWAS have been substantial, ranging from 2942 to 10,209 patients. Crucially, study participants have been recruited from specialist

Table 52.1 Notable genome wide association studies performed for alcohol-related liver disease

Lead author(s)	Journal	Year	Country/ region	Phenotype	*N* cases	*N* controls	Effective sample size	Study design	Adjustment (discovery)	Exclusions:	Novel genes identified
Buch et al.	Nat Genet	2015	Europe	Cirrhosis	1860	2348	4151	*Case-control:* alcohol-related cirrhosis Vs alcohol exposure w/o significant liver disease)	Unadjusted	Non-European ancestry	*PNPLA3; TM6SF2; MBOAT7*
Innes, Buch et al.	Gastroenter-ology	2020	Europe	Cirrhosis	2574	300,489	10,209	*Stage 1*: GWAS on cirrhosis endophenotypes; *Stage 2*: case-control (alcohol-related cirrhosis Vs alcohol exposure w/o significant liver disease)	Age, sex, principal components	Non-European ancestry	*MARC 1; HNRNPUL1; SERPINA1*
Schwantes-An et al.	Hepatology	2021	Europe, US, Australia	Cirrhosis	1128	10,836	4087	*Case-control*: alcohol-related cirrhosis Vs alcohol exposure w/o significant liver disease	Alcohol use, age, sex, BMI, diabetes, principal components	Non-European ancestry	*FAF2*
Trepo et al.	Lancet oncology	2022	Europe	HCC	1649	2391	3904	*Case-control*: alcohol-related HCC Vs alcohol-related liver disease w/o HCC	PRINCIPAL components	Non-European ancestry	*WNT3A*
Buch, Innes et al.	Gut	2022	Europe	HCC	1214	1866	2942	*Case-control*: alcohol-related HCC Vs alcohol-related liver cirrhosis w/o HCC	Principal components	Non-European ancestry	*TERT*

liver centres across Europe, with case definitions based on strict clinical criteria. Moreover, controls have been selected from the same source population in which the study cases have arisen. These stringent selection attributes help to reduce bias. Another salient point is that in the three GWAS for alcohol cirrhosis [14–16], controls were defined not just by the absence of cirrhosis, but by the absence of any significant non-alcohol-related liver disease, such as chronic viral hepatitis and haemochromatosis. This represents an "extreme phenotyping" strategy designed to increase statistical power [19], and thus, maximise the number of statistically significant variant-phenotype associations identified for a given sample size.

The selection of controls requires careful consideration for a GWAS on HCC. This is because cirrhosis, being the strongest risk factor for development of alcohol-related HCC, is a major confounding factor. Thus, it is crucial that cases and controls are balanced with respect to cirrhosis status to ensure variants identified are directly implicated in hepatocarcinogenesis (as opposed to only modifying cirrhosis risk). In a recent HCC GWAS by Buch et al. [18], cases were tightly defined as cirrhosis with HCC, whilst controls were defined as patients with cirrhosis but without HCC. Thereby cirrhosis has effectively been "adjusted for" at the design stage of the analysis. On the other hand, in a GWAS by Trepo and coworkers, cases and controls with different fibrosis stages below the threshold of cirrhosis were included, and in their main analysis, fibrosis stage was not controlled for [17]. Such difference in case-control matching details can lead to different association results between studies unless a number of relevant sensitivity analyses are performed: (1) adjusting for fibrosis stage (F3-F4 vs F0-F2); (2) restricting the analysis to F3-F4 patients; and (3) comparing the association between novel variants with alcohol cirrhosis with data from publically accessible data repositories [14]. These sensitivity analyses are designed to act as a fail-safe to ensure novel associations with HCC were independent of cirrhosis status.

A significant limitation of all previous GWAS for ALD is that they have focused only on persons of Caucasian ancestry. As a result, their generalisability to other ethnic groups is unclear. On this point, an "ALD" GWAS in a Korean population was recently published by Kim et al. [20]. Unfortunately, in this study, ALD was defined by elevated serum liver enzyme tests as a surrogate of alcohol-mediated liver injury alone, which obviously is not a reliable approach. Indeed, a recent study by Innes et al. indicates that the majority of variants associated with liver blood tests are not actually associated with an objective measure of clinical disease [15]. Thus, studies like the one from Korea study should be considered hypothesis-generating at best, and a solid Asian GWAS on ALD is so far lacking.

Novel Identified Variants: Overview and Functional Impact

Previous GWAS have identified ten loci in total, all independently associated with alcohol-related cirrhosis and/or HCC (Table 52.2; Fig. 52.1). Striking are that many variant genes are integrally involved in lipid metabolism and trafficking, and therefore not surprisingly also associate with the progression of non-alcoholic fatty liver

Table 52.2 Detailed overview of the ten polymorphisms identified in previous genome-wide association studies for alcohol liver disease

Variant information						Phenotype association		Functional impact				
rsid	Nearest gene	Chr: position	Ref allele	Minor allele	MAF	Phenotype(s)	Direction of association	Annotation	Amino acid substitution	Impact on protein function	Evidence of regulatory function	Gene ontology enrichment analysis
rs738409	*PNPLA3*	22:43928847	C	G	0.26	HCC + cirrhosis	G allele ↑ risk	Exonic	I148M	Deleterious	None	
rs58542926	*TM6SF2*	19:19268740	C	T	0.07	HCC + cirrhosis	T allele ↑ risk	Exonic	E167K	Deleterious	None	
rs72613567	*HSD17B13*	4:87310241	A	AA	0.18	HCC + cirrhosis	AA allele ↓ risk	intronic	NA	NA	variant is a splicing QTL for HSD17B13 in liver	
rs2642438	*MARC1.*	1:220796686	G	A	0.19	cirrhosis	A allele ↓ risk	Exonic	A165T	Deleterious	None	
rs28929474	*SERPINA1*	14:94378610	C	T	0.02	cirrhosis	T allele ↑ risk	Exonic	E366K	Deleterious	None	
rs15052	*HNRNPUL1*	19:41307470	T	C	0.07	cirrhosis	T allele ↑ risk	3'UTR	NA	NA	Variant lies in enhancer region (GeneHancer ID: GH19J041294). "C" allele is associated with increased expression of TGFB1 in liver.	
rs2242652	*TERT*	5:1279913	G	A	0.17	HCC	A allele ↓ risk	intronic	NA	NA	None	
rs708113	*WNT3A*	1:228005052	A	T	0.40	HCC	T allele ↓ risk	intronic	NA	NA	Variant lies in cis regulatory element (ID: EH38E1428717)	
rs374702773	*FAF2*	5:176467130	(T)24	del(T)7	0.42	cirrhosis	NK	intronic indel	NA	NA	None	
rs641838	*TMC4*	19:54173068	C	T	0.43	cirrhosis	T allele ↑ risk	Exonic	G17E	Benign	Variant lies in enhancer region (GeneHancer ID: GH19J054172). "T" allele associated with ↓MBOAT7 and ↓TMC4 expression in liver.	

Cellular components:
The10 genes in this table are significantly enriched in lipid droplets (P=0.02) and endoplasmic reticulum (P=0.02)

Biological processes:
No significant enrichment

Molecular functions:
No significant enrichment

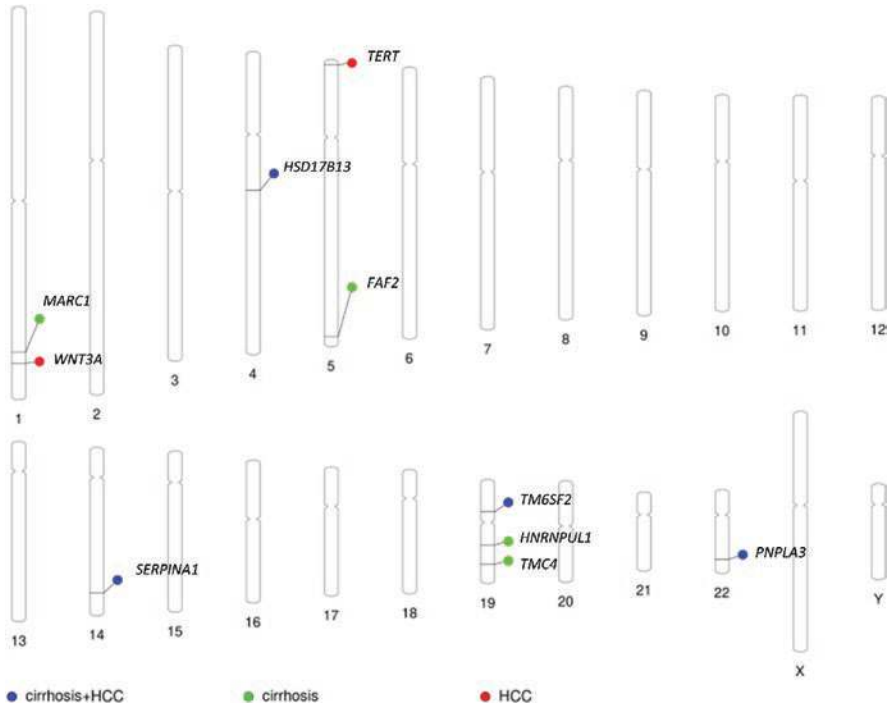

Fig. 52.1 Phenogram representation of loci associated with alcohol-related liver disease phenotypes. Numbers refer to chromosomes, X and Y to sex chromosomes

disease in the context of the metabolic syndrome. The allele frequencies for these variants are highly diverse, ranging from 2% (rs28928474:T in *SERPINA1*) through to 43% (rs641738:T in *TMC*). Importantly, loci that enhance the risk of ALD and cirrhosis in particular have been identified, as well loci that reduce the risk developing ALD, and are therefore considered protective (Fig. 52.2).

Half (5/10) of the identified loci represent missense variants lying in exonic regions, leading directly to an amino acid change in the corresponding protein. *In silico* bioinformatics tools can evaluate the impact of these amino-acid changes on the functioning of the protein product. One such tool – Polyphen2 [21] - predicts that 4/5 of these missense mutations have a deleterious impact on protein function. An exception to this is the rs641738 missense variant in *TMC*, which is predicted to be benign. However, as well as being an exonic variant in *TMC*, rs641738 also regulates the expression of proximal genes. In particular, data from the gene-tissue expression (GTEx) resource shows that rs641738 is associated with expression of *MBOAT7* in liver tissue [22]. Thus, *MBOAT7* is considered to be the causal gene underlying the association between rs641738 and cirrhosis, rather than *TMC*. Another interesting observation is that the rs224652:A missense allele in *MARC1* – despite being predicted to have a deleterious impact on MARC1 function – is in fact associated with a reduced risk of alcohol cirrhosis [15]. This suggests that inhibiting MARC1 may be a promising therapeutic strategy if the protein can be targeted.

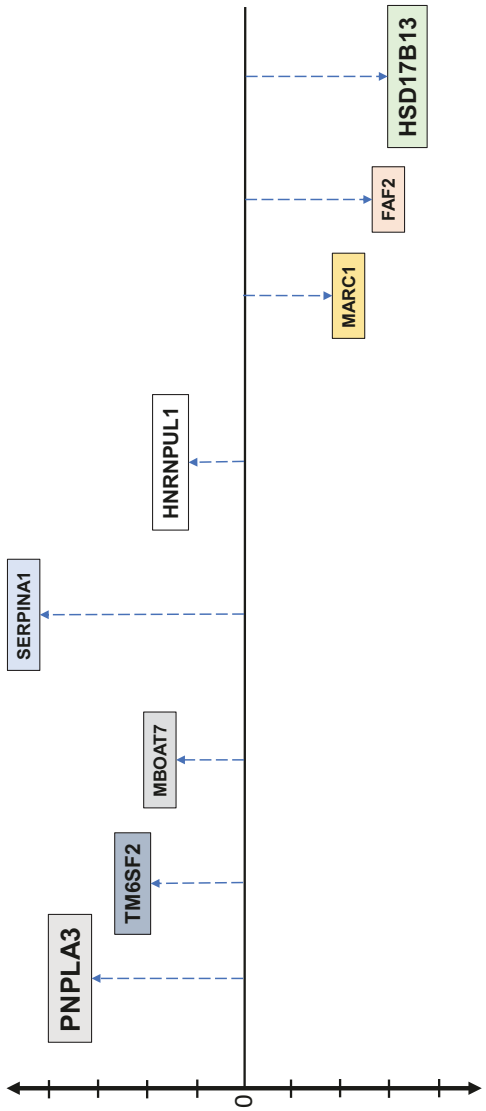

Fig. 52.2 Risk loci and their effect on ALD risk

The remaining 5/10 loci are located in non-coding regions of the genome. Although this means they do not modify protein composition, there is evidence that many exert regulatory functions, such as modifying the expression of proximal genes or splicing. For example, rs72613567 is in fact a splicing variant influencing the balance/abundance of HSD17B13 isoforms in liver tissue (Table 52.2). The rs15052 loci in *HNRNPUL1* lies in an enhancer region of the genome, and is directly associated with the expression of the transforming growth factor beta 1 protein in the liver, a potent profibrogenic cytokine produced by liver mesenchymal cells to stimulate collagen production [23]. Rs708113 also lies in an enhancer, and is associated with expression of mitochondrial genes (*IBA57* and *MRPL55*), albeit these associations are not evident in liver tissue. The rs2642438 variant in *TERT* is strongly associated with telomere length, a frequently altered pathway leading to hepatocarcinogenesis [24, 25]. On the other hand, no obvious regulatory function is apparent for rs374702773 in *FAF2*.

Table 52.3 gives an overview of identified risk loci and their known or putative functional implications.

Table 52.3 Risk loci for ALD and their known or suggested functional implications

Gene	Enzymatic function	Subcellular localisation	Cellular localization	Effect of the mutation
PNPLA3	Triglyceride hydrolase	Lipid droplets	Hepatocytes, mesenchymal cells	Missense mutation: Loss of function
TM6SF2	Unknown	Endoplasmic reticulum and ER-Golgi compartment	Hepatocytes	Retention of VLDL in hepatocytes
MBOAT7	Lysophosphatidyl-inositol acyl-transferase; phospholipid remodeling	Hepatocyte membranes	Hepatocytes	Lower expression
HSD17B13	Hepatic retinol dehydrogenase; activation of the retinoic acid receptor?	Hepatic lipid droplet	Hepatocytes	Insertion mutation: Loss of function
SERPINA1	Protease inhibition	Hepatocyte cytosol	Hepatocytes	Lack of protease inhibition: Loss of function
HNRNPUL1	Regulation of DNA transcription by dual DNA and mRNA binding	Endosplasmatic reticulum	Hepatocytes	Increase of TGFb1 expression
MARC1	Reduction of N-hydroxyl compounds; neutralization of acetaldehyde?	Outer mitocondrial membrane	Hepatocytes, adipose tissue	Gain of function
FAF2	Resistance to apoptosis	Cell membranes	T cells, eosinophils	Gain of function

Notable Findings from Candidate Association Studies

CGAS have succeeded in identifying novel variant-phenotype associations that have been overlooked by genome-wide analyses. In particular, a recent CGAS for ALD demonstrated an association between a missense variant in *APOE* (rs429358) and alcohol-related HCC [12]. Specifically, the rs429358:C allele (versus T allele) was shown to be associated with a reduced risk of HCC in patients with cirrhosis (OR:0.71; 95% CI: 0.61–0.84; $P = 2.9 \times 10^{-5}$). Previously, rs429358:C has been associated with a reduced risk of cirrhosis in patients with hepatitis C, and more recently with lower liver fat content in the UK biobank (UKB) community cohort study [26, 27]. However, the rs429358:C allele is best known for its deleterious impact on the risk of Alzheimer's disease. Indeed, homozygous carriers of rs429358:C are estimated to have >12-fold increased risk of AD relative to rs429358 T homozygotes [28, 29]. This highlights that in some cases, genetic risk factors for ALD exert significant pleiotropic effects. In the same CGAS, the authors also identified a second missense variant in *TM6SF2* (rs187429064:G) as being associated with alcohol-related HCC in cirrhosis patients [12]. Crucially, the rs187429064 locus is in complete linkage equilibrium with the better known rs58542926 locus, also in *TM6SF2*. Although the frequency of the rs187429064:G allele is very low at ~1% in Europeans, it's effect size with HCC is strong. Indeed, the magnitude of association between rs187429064:G and HCC (OR: 2.03) exceeds the equivalent association for rs738409 (in *PNPLA3*) and rs58542926 (in *TM6SF2*) [12].

Alcohol consumption is itself a heritable phenotype, most likely modified by multiple genetic determinants [30]. Previous CGAS have reported associations between loci that influence alcohol intake and ALD. Examples include rs1229984 in *ADH1B*, a gene that is directly involved in alcohol metabolism [31]. It is likely that such variants influence ALD only indirectly, for example through modifying alcohol intake. Nevertheless, these genetic predictors could be harnessed to build more effective risk stratification scores. However, until now, no risk-enhancing variant for alcohol drinking behaviour has been identified.

Clinical Applications

Treatment Discovery

Treatments for ALD have advanced only marginally over the last 50 years, with abstinence remaining the cornerstone of clinical management. Many candidate drugs for ALD have failed due to lack of efficacy [32], including colchizine, silibinine, polyenyl-phosphatidyl-choline, S-adenosyl-L-methionine, TNF-alpha inhibitors or propylthiouracil. Insight into the genetics of ALD could pave the way for innovative treatments for ALD by identifying novel drug targets (see also book chap. xxxx). Indeed, it has been shown that candidate drugs are more likely to

achieve regulatory approval if they are supported by data from genetic association studies [33, 34]. An example par excellence is the development of PCSK9 inhibitors to treat high cholesterol [35].

Data from previous ALD GWAS have already identified several promising therapeutic targets. Of these, PNPLA3 silencing has been a focal point of investigation [36]. In murine models, PNPLA3 silencing leads to reduced inflammation and fibrosis [37], and clinical trials are currently evaluating the safety of PNPLA3 silencers in human subjects [NCT04142424]. However, a fundamental concern about targeting PNPLA3 therapeutically is the potential for off-target effects. This is because the I148M variant exerts pleiotropic effects, particularly with respect to the risk of coronary artery disease. For example, in a general population cohort from Germany, the rs738409:G allele was associated with a higher risk of liver failure, but a lower risk of coronary artery disease and all-cause mortality [38]. Interestingly, a similar pattern may also be apparent for the E167K variant in TM6SF2 [39].

Beyond PNPLA3, the rs72613567 splicing variant in *HSD17B13* is also being widely investigated as a treatment target. Indeed, a phase 1 clinical trial is already underway for a therapeutic agent modifying HSD17B13 expression through RNA interference [NCT04565717]. The study completion date is expected for early 2023. Inhibition of the MARC1 mitochondrial protein could be another viable target given that loss of MARC1 function appears to protect against cirrhosis.

As well as identifying direct targets, GWAS can provide broader insight into the physiological pathways disrupted in disease progression. For example, using the gene ontology resource, it is possible to ascertain if the genes identified in previous ALD GWAS are enriched within specific cellular locations, biological processes, or molecular functions [40, 41]. This analysis indicates that relative to a random gene set, the protein products of the ten genes identified in previous ALD GWAS are more likely to be located in lipid droplets (60-fold enrichment; $P = 0.015$) and the endoplasmic reticulum (seven-fold enrichment; $P = 0.017$) (Table 52.2). These insights align with the therapeutic strategies currently being investigated in the field [36]. For example, the importance of lipid droplets in mediating lipid homeostasis and steatosis is well recognised in ALD and other forms of liver disease [42]. Similarly, ER stress triggers the unfolded protein response, leading to apoptosis and potentially hepatic steatosis onset [43]. Thus, agents acting to restore ER stress are of therapeutic interest.

Risk Prediction and Stratification

Why Risk Stratification Is Needed?

An unfortunate hallmark of ALD is that patients are typically not diagnosed until severe complications such as HCC or decompensated disease emerge [44, 45]. At this point, the damage incurred to the liver is usually advanced, potentially intractable and, thus, treatment options are limited. Therefore, prompt diagnosis of ALD

in the community is crucial to reduce the mortality burden from ALD. At present, clinicians have multiple opportunities to intervene early before patients develop alcohol-related cirrhosis [46]. However, because: (a) the size of the "at risk" population is vast (i.e. excess alcohol intake is relatively commonplace in the general population); (b) a majority of patients with excess alcohol intake show no progression to significant ALD and (c) health care resources are scarce, there is a clear need to focus the finite resources available on the highest risk patients. For these reasons, combating late diagnosis hinges on being able to stratify patients' risk in community/primary care settings [47, 48].

Risk stratification remains equally important even once patients have progressed into secondary care. Ultrasound surveillance for liver cancer is a case-in-point. Currently, clinical guidelines recommend all patients with cirrhosis should receive surveillance every 6 months [49], but in practice, surveillance is poorly implemented [50, 51], meaning that too few patients with HCC go on to be treated with curative intent [52, 53]. Support for "individualised" surveillance is growing [54–57], but this approach similarly hinges on the availability of robust and accessible risk stratification scores.

Risk Stratification through Polygenic Risk Scores

Current risk scores used in ALD are derived from routine blood tests, such as bilirubin, coagulation tests, albumin, and creatinine (e.g. MELD and Lille model for alcohol hepatitis). The falling cost of genotyping however, together with an increasing understanding of genetic predictors for ALD, means that for the first time, it may be feasible to utilise a new class of prognostic factors in clinical practice: namely, the polygenic risk score (PRS). At bottom, a PRS is simply a number reflecting an individual's lifetime genetic susceptibility to a disease. It is calculated by aggregating the effects conferred by multiple genetic variants on a given phenotype (hence "polygenic" as opposed to "monogenic") [58, 59]. PRSs can vary from one another in two main respects: (1) in terms of which genetic loci are included in the score; and (2) by the weighting assigned to each locus. Crucially, because a PRS is based on immutable germline DNA, it provides a measure of lifetime risk that is constant over time. Detailed tutorials explaining the methods used to generate a PRS are available [58, 59].

Studies have already begun to derive and evaluate PRSs for predicting cirrhosis and HCC. In particular, Whitfield et al. proposed a PRS for alcohol cirrhosis combining information on three genetic loci together with information on diabetes status [60]. All three genetic loci included were identified from previous GWAS: rs738409 (PNPLA3); rs10401969 (TM6SF2); and rs72613567 (HSD17B13) [14–16]. The authors reported that individuals with the highest PRS score had between 2.8 and 6.0 times greater risk of cirrhosis compared to individuals the lowest PRS score. The area under the curve (AUC) was between 0.62 and 0.67, indicating moderate discriminative ability. Another important contribution to the field is the HCC PRS developed by Bianco et al. [61]. This PRS is comprised of five variants associated with hepatic fat content: rs738409 (PNPLA3); rs58542926 (TM6SF2); GCKR;

rs641738 (MBOAT7); and rs72613567 (HSD17B13). Here, the AUC was 0.64, again indicative of moderate discrimination. High risk patients generally had >5 times odds of HCC versus lower risk patients. Other PRSs developed have reported similar results in terms of discriminative ability [14, 62, 63].

Clinical Translation of Polygenic Risk Scores

The increasing affordability of genetic sequencing has sparked hope that genetic data could soon be used routinely in liver clinics to advance patient management. However, like any risk score, utility hinges on whether it provides "new" prognostic information beyond what is already available to clinicians [64]. This perspective is crucial because clinicians already enjoy access to a diverse range of prognostic factors for liver disease, which are "free to use". For example, platelet count, alanine aminotransferase, aspartate aminotransferase, albumin, bilirubin etc. are all prognostic factors for ALD that are routinely available to clinicians. Thus, the real utility of a PRS depends less on its absolute performance, and more on its added-value relative to existing alternatives. This "added-value" perspective was explored recently in a study by Innes et al. [65]. The authors examined 20 routinely available risk scores, including the ALT:AST ratio, the fibrosis four index (FIB4) and the aspartate aminotransferase platelet ratio index (APRI). They found that majority of routine risk scores outperformed prediction using genetic data. For example, the C-index for the APRI score was 0.80, whereas the C-index for the PRS was 0.60. Secondly, they found that, for the best performing risk scores (e.g. APRI and Fib4), the addition of genetic risk data added only a very modest amount of "new" prognostic information. For example, the C-index for APRI alone was 0.804 versus only 0.809 when combined with genetic data. This suggests that current PRSs will not fundamentally change the risk stratification landscape. Comparable findings regarding the added-value of PRSs have also been observed in a specialist care context [66]. However, there are two caveats to point out regarding the interpretation of these studies. Firstly, the performance of PRSs are likely to improve as understanding of ALD genetics expands. Secondly, a PRS holds some practical advantages over traditional risk scores, which in some contexts, could outweigh differences in performance. For example, a PRS provides a measure of lifetime risk and so only needs to be calculated once. Conversely, risk scores derived from liver blood tests are dynamic and so must be periodically updated at relevant clinical milestones.

Future Research

Whilst considerable progress has been made in the past 10 years, there is still much to be done to better characterise the genetics of ALD. In particular, this includes translating novel discoveries into tangible patient benefits. In the last section of this review, we discuss specific topics/areas which are particularly likely to deliver research impact in the future.

Increasing Statistical Power

Statistical power is one of the most critical factors to consider when designing a genetic association study. This is because studies with low power are very unlikely to identify novel genotype-phenotype associations if they were missed by previous studies with greater statistical power. Thus, developing and applying new techniques to maximise statistical power will be an important theme of future research.

Meta-Analysis

Meta-analysis of individual genetic association studies is an important route to augmenting sample size, in order to increase statistical power [67]. Recently, this approach was used to verify the association between rs641738 (*TMC/MBOAT7*) and chronic liver disease phenotypes (cirrhosis, HCC, steatosis, etc) [68]. Other equivocal loci that would benefit from a meta-analysis approach include rs2954038 in the region of *TRIB1*. This variant has achieved borderline significance in previous association studies, and exhibits compelling evidence of regulatory function [14].

Exploiting Endophenotypes

An endophenotype is an intermediate variable on the causal pathway between a genetic variant and the disease of interest [69]. For example, elevated alanine aminotransferase (ALT) level is on the causal pathway between: a) rs738409:G in *PNPLA3*, and b) cirrhosis. This is because rs738409:G leads to liver cirrhosis, and in the process ALT levels (a marker of liver inflammation and damage) increase. Interestingly, the rs738409:G allele is easier to "discover" through a GWAS on ALT than a GWAS on cirrhosis. In other words, fewer patients would be required to detect rs738409 at a genome-wide level (i.e. $P < 5.0 \times 10^{-8}$) if ALT were the phenotype compared to if cirrhosis were the phenotype [14]. Essentially, this is because ALT is a continuous variable whereas cirrhosis is a binary phenotype; and in general, continuous variables provide greater statistical power than binary ones [70]. In this way, leveraging endophenotypes can considerably enhance the statistical power of a GWAS. An example of this endophenotype approach was recently illustrated in a GWAS by Innes and Buch et al. [14]. In stage 1, heavy drinkers from UKB were included in a linear regression GWAS against continuous measures of liver fibrogenesis (i.e. APRI, FIB4, Forns, ALT and AST). Loci significantly associated with one or more of these continuous fibrogenesis measures were brought forward into the stage 2 analysis, entailing direct testing for association with cirrhosis status in clinical cohorts. Using this approach, the authors were identified new variants (e.g. rs15052 in *HNRNPUL1*) that would not have been discovered using a conventional binary phenotype design. In the future, studies can extend this approach by leveraging a broader set of endophenotypes.

Harnessing Population-Based Biobanks

As discussed, future insights in ALD genetics will require increasing statistical power and hence increasing sample sizes. Large population-based biobanks will be critical in helping the field to achieve this. Biobanks are repositories of biological samples, stored at low temperatures. Samples are donated by members of the public who crucially also provide information about their health and risk factors for disease. In some cases, longitudinal data on health outcomes are available through linkage to national health registries. The UKB resource is an exemplar par excellence, and provides an excellent template for emerging biobanks to follow. In brief, UKB provides whole-genome sequencing data for 500,000 middle-aged people in the UK, together with detailed lifestyle, disease and biomarker data [70]. Proof of its utility is that UKB has actually supported (directly or indirectly) most of the recent genetic association studies performed so far relation to ALD [14, 15, 17] (see Table 52.1). Many countries are now seeking to develop their own population-level biobanks. Notable examples include: FinnGen (Finland) [71]; All of us (US) [72]; Our future health (UK) [73]; The million veteran program (US); 100,000 genomes (UK) [74]; and CanPath (Canada) [75]. Researchers who succeed in harnessing these population-based resources and integrating then with data from detailed clinical cohorts, are likely to achieve significant new insights.

Polygenic Risk Scores

Although current PRSs provide minimal "new" prognostic information [65, 66], their performance will improve as our understanding of ALD genetics grows. Transforming PRSs into clinically useful tools is likely to be an active area of future research.

One area that may bring about improvements is accommodating rare variants into existing PRSs. Thus-far, GWAS (and by implication PRSs) have focused exclusively on common variants with minor allele frequencies >1%. There is increasing recognition however that rare variants with high penetrance may be major contributors to genetic susceptibility for common diseases [76]. A recent study by Pelusi et al., comparing a PRS comprising rare variants versus a PRS comprising common variants alone, supports this view [77].

Multi-polygenic risk scores (MPS) are another promising avenue [78] The rationale behind this approach is that most disease events have multiple predictors, each of which can themselves be predicted through a PRS. Thus, an MPS is constructed by combining separate PRSs, each relating to a different predictor of the overall phenotype. For example, independent predictors of ALD cirrhosis, include alcohol intake, type 2 diabetes, obesity, telomere length and hepatic fat content. In this context, a MPS would combine five individual PRSs (i.e. one PRS for each predictor) into a single multivariate model. Previous studies generally show that MPSs exhibit better predictive performance than a single PRS alone [79, 80]. In particular, the

recent study by Sinnott-Armstrong et al. reported that an MPS for alcohol cirrhosis derived from PRSs of 35 urine/blood biomarkers exhibited better predictive performance (C-index: ~0.60) than a single PRS based on variants associated with alcohol cirrhosis alone (C-index: ~0.55) [80].

Multi-Omics

Previous studies focus overwhelmingly on the relationship between sequence variants and ALD risk. In the future, studies are likely to shift towards a broader "multi-omics" approach, e.g. considering genetics sequencing data together with data on gene expression and tissue-specific proteomics. Indeed, a very recent study by Niu et al. provides compelling evidence that a proteomics-based risk score outperforms existing diagnostic and prognostic risk models in ALD [81]. Scientific methods for standardising integration of multi-omics data are advancing at pace [82]. Cohorts where patients are characterised in terms of multi-omics data will soon become the new benchmark, and will yield deeper insights into ALD pathophysiology.

Multi-Ancestry Genetic Association Studies

An important limitation of current GWAS for ALD is that they are restricted to individuals of European ancestry. Going forward, multi-ancestry genetic association studies should be adopted. This is important if GWAS findings are to be successfully translated into ethnically diverse "real world" clinical populations. The implementation of PRS is a case-in-point for why GWAS must reflect the ethnic diversity of the "at risk" population. For example, it has been shown that the performance of PRSs - derived pre-dominantly from individuals of European ancestry - are suboptimal in people of non-European descent, particularly individuals of African ancestry [83].

Alcohol Hepatitis

No robust GWAS has been carried out to-date for alcohol hepatitis. This omission is notable given that alcohol hepatitis is a prominent feature of ALD, responsible for significant mortality [84]. In the future, GWAS are expected to emerge for this phenotype, which will provide broader insight into ALD genetics [85].

HCC Driver Genes

As with any form of cancer, HCC arises when healthy liver cells acquire mutations in key genes regulating cell division. In HCC tumours, the most frequently mutated genes are *TERT*, *CTNNB1* and *TP53* [86]. In their recent GWAS of ALD HCC, Buch et al. identified a new germline variant in *TERT* (rs2242652) [18]. This shows in principle that both hereditary and somatic mutations in HCC driver genes (in this case *TERT*) are associated with hepatocarcinogenesis. It is possible that equivalent patterns may hold for other HCC driver genes. Also, in the first French GWAS on HCC, it was reported that the lead variant (rs708113) was associated with somatic mutations in *CTNNB1* in HCC tumour cells [17]. Specifically, HCC patients with the rs708113 T allele were less likely to have somatic mutations in *CTNNB1* compared to carriers of the rs708113 A allele. Further research evaluating the interplay between germline polymorphisms and somatic mutations leading to HCC is warranted. One initial follow-up study could be to carry out a CGAS, systematically assessing the association between germline variants in HCC driver genes and HCC status.

Epistasis

Epistasis is defined as gene-gene interaction – i.e. where the association between a given genetic loci and a phenotype varies according to an individual's genotype at a second loci. Epistatic relationships are notoriously difficult to identify at a genome wide level. Firstly, because they require even greater statistical power than a conventional GWAS. Indeed, even for a very large interaction effect (i.e. the same magnitude as the main effect size), one's sample size would need to be inflated four-fold to detect the interaction effect with 80% power [87]. Where the interaction effect was more modest (i.e. half the size of the main effect), one's sample size would need to increased 16-fold [88]. Moreover, the computational resources required to screen for epistasis at a genome-wide level are another prohibitive factor. Consequently, studies typically only test for epistatic relationships where there is compelling biological information in support of this. Thus, little is known about the importance of epistasis in ALD at present. Currently, there is evidence that rs72613567 (*HSD17B13*) may interact with rs738409 (*PNPLA3*) to modify liver disease risk. Specifically, carriage of rs7613567:TA allele appears to attenuate the deleterious effect of the rs738409:G allele [89, 90]. Many more examples of epistasis are likely to exist. As statistical power increases, uncovering these relationships could be a source of great insight.

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Chapter 53
Mechanisms of Alcohol-Related Liver Cirrhosis

Honglei Weng, Yujia Li, and Steven Dooley

Abstract Liver cirrhosis-induced portal hypertension is a major cause for mortality in chronic liver diseases, including alcohol-related liver disease. Anatomically, liver cirrhosis is presented as septa separating hepatocellular nodules throughout the liver. Mechanistically, vascular injury-initiated congestive escalation is the central event to induce parenchymal extinction lesions and for the in-out imbalance of hepatic blood flow, which finally leads to portal hypertension. Parenchymal extinction leads to liver function insufficiency or even liver failure, while portal hypertension results in ascites, and mediates acute renal injury, hepatic encephalopathy, and gastrointestinal bleeding. In comparison with other etiologies, liver cirrhosis arising from long-term alcohol abuse impairs number and composition of gut microbiota and leads to pathological translocation of bacteria and bacterial endotoxins, thus stimulating systemic and liver inflammation. Patients with alcohol-related liver cirrhosis frequently develop spontaneous bacterial peritonitis, sepsis, and acute-on-chronic liver disease.

Keywords Alcohol-related liver cirrhosis · Vascular injury · Portal hypertension · Hepatocyte injury · Inflammation · Liver scarring

Introduction

Liver cirrhosis, severe alcoholic hepatitis and the development of liver cancer, in particular hepatocellular carcinoma (HCC), represent the major causes for mortality in patients with alcohol-related liver disease (ALD). HCC causes around 810,000 deaths annually, and ALD is responsible for approximately 30% of these HCC-related deaths [1], which far exceeds the number of HCC deaths caused by

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non-alcoholic fatty liver disease at this time. This chapter starts with an overview on the sequence of cellular events that underly liver damage and fibrosis with disease progression in general, and more specifically as it relates to ALD dynamics.

Pathophysiology

Cirrhosis is defined anatomically by the presence throughout the liver of hepatocellular nodules that are separated by septa [2]. Since its first use, the term "liver cirrhosis" has been a controversial issue [3]. In 1947, Himsworth once proposed to replace the term cirrhosis with "the sclerosed appearance of the liver as fibrosis" [4]. In 2012, an International Liver Pathology Study Group proposed that "this is an appropriate time to consider discontinuing the use of this term (cirrhosis)", given that "recent advances in the diagnosis and treatment of chronic liver diseases (CLD) have changed the natural history of cirrhosis significantly" [3]. However, as stated in the textbook *MacSween's Pathology of the liver*, 'cirrhosis' is a firmly entrenched term, likely to remain in common usage for the foreseeable future" [5]. This chapter describes conception evolution of liver cirrhosis and current knowledge of mechanisms underlying alcohol-related liver cirrhosis. Related chapters include Chaps. 7, 49, 54, 59 and 61.

Conception Evolution of Liver Cirrhosis

The definition of liver cirrhosis has been evolving particularly in the last three decades [6]. Wanless and Huang described evolution of the conception in the *MacSween's Pathology of the liver* [5]. There are three representative definitions in history of liver cirrhosis: (1) The presence throughout the liver of fibrous septa that subdivide the parenchyma into regenerating nodules; (2) The accumulation throughout the liver of confluent parenchymal extinction lesions; and (3) Cirrhosis is the collection of anatomic changes in the liver that result from the presence of widespread imbalance of hepatic blood flow where inflow exceeds the outflow capacity [5]. As explained in the textbook, point (1) of this conception is misleading because it denotes that fibrogenesis is the cause of cirrhosis. Point (2) reveals that parenchymal extinction is a special type of hepatocyte death required for the formation of cirrhosis. This definition leads to the question what causes or has caused parenchymal extinction. Point (3) arrives the core point of the pathogenesis of cirrhosis: it is the "in-out imbalance of hepatic blood flow" that results in parenchymal extinction and architecture distortion in the damaged liver. Point (3) also explains why the measurement of hepatic vein pressure gradient (HVPG) is a reliable tool to assess the progression of cirrhosis [7]. Thus, elucidating the cause and mechanisms of the "in-out imbalance of hepatic blood flow" is critical to understand the pathogenesis of liver cirrhosis.

Vascular Injury and Congestion

Vascular injury and congestion are central events in the pathogenesis of cirrhosis regardless of etiology. Given the central role of the in-out imbalance of hepatic blood flow in liver disease, vascular injury and subsequent vascular remodeling are the central events that result in liver cirrhosis and portal hypertension. In ALD, vascular alteration occurs at the early phase of liver damage. Capillarization of hepatic sinusoids, a term coined by Schaffner and Popper in 1963, is a phenomenon occurring in the perivenular zone following early alcohol-related liver injury [8]. Normal liver sinusoidal endothelial cells (LSEC) are characterized by the presence of pores (fenestrae). The fenestrae are 100–150 nm in size and are clustered in groups, which are termed as sieve plates [9]. In pathological conditions, for example alcohol stimulation, LSEC lose fenestrations and acquisition of a vascular phenotype. In the process of capillarization of hepatic sinusoids, the normal extracellular matrix, mainly type III collagen, in the space of Disse is replaced by mainly type I collagen, as well as basal lamina-like material containing laminin and type IV collagen, which are produced by activated hepatic stellate cells (HSC) [5].

It has been well recognized that maintenance of phenotype of fenestrated LSEC requires nitric oxide (NO), which is stimulated by VEGF secreted by hepatocytes and HSC [9]. VEGF stimulates NO release through endogenous nitric oxide synthase (eNOS) in LSEC [10]. During liver injury due to alcohol abuse, LSEC produce low production of NO due to increased binding to caveolin [11, 12]. Low NO levels in sinusoids diminish eNOS activity and thus result in capillarization of LSEC [11, 12].

Capillarization of hepatic sinusoids is also thought as the early feature of fibrosis. Although the detailed mechanisms remain largely unknown, the fenestrated LSEC play a crucial role in the prevention of HSC activation [13]. Capillarized LSEC lose capacity to maintain HSC quiescence [13]. Besides contributing to fibrosis, capillarization of hepatic sinusoids leads to additional two severe consequences: (1) Establishment of a significant barrier between the blood and the hepatocyte, which results in hepatocyte dysfunction and injury, as well as reduces the transport of solutes from the sinusoidal blood to the hepatocyte via the space of Disse. (2) Changing structure of the sinusoidal endothelial cells and localization of subendothelial basal laminas, which is associated with increased vascular resistance in the sinusoidal bed and the pathogenesis of portal hypertension.

In addition to capillarization of hepatic sinusoids, alcohol abuse leads to local vasculature including thrombosis, lymphocytic phlebitis, phlebosclerosis, veno-occlusive lesions and vascularized septa [14, 15]. These vascular alterations are essential to the formation of cirrhosis and portal hypertension. Wanless showed that obliterative lesions in hepatic veins and portal veins occurred in 70 and 36% of cirrhotic livers, respectively [15]. Goodman and Ishak described three types of venous lesions in ALD: (1) lymphocytic phlebitis; (2) phlebosclerosis; and (3) veno-occlusive lesions [16]. Levels of portal hypertension are associated significantly with the degree of phlebosclerosis and veno-occlusive change [16]. In addition,

Burt and MacSween found that the occlusive venous lesions may contribute to the atrophy of hepatic parenchyma and functional impairment [17]. To date, detailed mechanisms of how alcohol leads to lymphocytic phlebitis, phlebosclerosis and veno-occlusive lesions are largely unknown.

Recently, Wanless proposed a "vascular hypothesis", which clearly demonstrates the key role of vascular injury in the pathogenesis of cirrhosis in humans [2]. The hypothesis comprises four key conceptions: (1) a definition of parenchymal extinction, which emphasizes the importance of sinusoidal destruction; (2) a "congestive escalator" hypothesis, which explains how vascular obstruction occurs, beginning with sinusoidal endothelial cell injury, fluid translocation, and vascular compression by mechanics known as "compartment syndrome"; (3) a "nested cone model" of hepatic vein anatomy that predisposes to compartment syndrome in the human, and (4) a proposal for the mechanism of collagen formation in response to congestion ("congestive fibrosis") [2].

In 1990s, Wanless originally defined parenchymal extinction lesion (PEL) as a region with loss of contiguous hepatocytes [5]. Given the causality between loss of the local microvasculature and PEL, he recently revised the definition as "a region with focal loss of contiguous hepatocytes and adjacent microvascular structures" [2].

During CLD, vascular injury causes vascular obstruction which causes more vascular injury which causes more vascular obstruction in a positive feedback loop. Wanless defines such a loop as "congestive escalator" [2]. Vascular obstruction increases tissue pressure gradients and thus drives transudation of fluid into vessel walls and interstitial tissues, which results in further vascular obstruction. Vascular obstruction caused hepatocellular ischemia results in PELs with collapse of the architecture. Local vascular impairment delays tissue repair so that the regions of injury are repopulated by liver progenitor cells (LPC), which resides in smallest biliary tree [18]. Later, vascular obstructing lesions progresses from smaller branches to larger branches of the hepatic and portal veins, which leads to PELs larger and eventually merge into confluent septa. Collapse, delayed repair, and LPC-mediated regeneration constitutes hepatocellular nodules separated by septa and tissue distortion in cirrhosis [2].

Obstruction in hepatic vein (HV) and small portal vein is a prominent histologic feature in cirrhosis regardless of etiology [15–17, 19–21]. In a recent study, Wanless showed high-grade (>50%) obstruction of HVs in 15.0 and 66.5% of recognizable HVs in mild cirrhosis and severe cirrhosis, respectively. After correction for collapse of tissue, patent HVs were decreased by 59% in mild cirrhosis and 94% in severe cirrhosis. He also measured the involvement of small (20–100 μm) and larger HVs (≥100 μm). In patients with Laennec stage 4A cirrhosis, high-grade obstruction was found in 63.4% of small and 26.5% of larger HVs [2]. These data demonstrate an evolution that HV obstruction begins from small to larger HVs following advance of disease severity.

Following vascular obstruction, obliterative lesions in hepatic veins and portal veins occurred in severe cirrhotic livers [15]. The obliteration of hepatic veins explains the prominent congestive features in cirrhotic livers [2]. Many regenerative nodules have zones of sinusoidal congestion and collapse that lead to subdivision

and remodeling of nodules into two or more smaller nodules. The congested zones also contain red blood cells that have dissected into vein walls, causing luminal compromise. These features indicate that congestion is a mechanism causing progression from mild to severe cirrhosis [2].

Wanless further pointed out that the human liver is particularly vulnerable to this congestive escalator given the "nested cone" architecture in humans. Such a long and branched hepatic vein tree is susceptible to "compartment syndrome" effects [2]. He described the "nested cone" architecture as follows: "In normal human liver, the axial hepatic veins branch dichotomously 4–6 times at acute angles. Many small branches arise at right angles. Each hepatic vein branch drains a cone of parenchyma that is nested inside larger cones drained by larger branches. As collateral pathways are few, each cone has its own pressure environment modified by local venous obstruction and hyperemia. Distal branches have a longer path to the vena cava than side branches of larger axial branches. The shorter path would likely be associated with lower venous pressure. Obstruction is due to luminal reduction as well as external compression. The nested cone architecture facilitates the migration of external compression to involve larger vessels as adjacent tissue expands. In chronic hepatitis, there is widespread mild to moderate inflammation leading to local sinusoidal and venous obstruction. One cone has become more congested than others as its hepatic vein is compromised, with a local rise in pressure. Hyperemia and vascular leak has caused expansion of the affected cone that now is compressing adjacent cones, spreading the pressure rise to them. Vascular obstruction has extended to a larger vein causing three more small cones to expand with more compression of the cones on the right as well as higher in the tree" (Detailed description please see Fig 6 in [2]).

The consequence of extensive congestive injury is the "in-out-imbalance of hepatic blood flow", defined as inflow (hepatic artery and portal vein flow) exceeds the outflow capacity of the system (hepatic vein flow) [2]. Inflow is increased due to reactive hyperemia such as in response to inflammation, ischemia, portal vein obstruction, or shunting. Outflow is reduced by obstruction of hepatic and portal veins.

The in-out-imbalance of hepatic blood flow results in further elevated transmural pressure gradient, which causes endothelial injury, vascular leak, with transudation and edema [2]. Expansion of tissue compresses adjacent tissues including veins that leads to elevated in-out-imbalance and more vascular injury. Besides the in-out-imbalance of hepatic blood flow, an imbalance of vasoconstrictive and vasodilating agents in the intrahepatic circulation results in consistent net vasoconstriction inside the liver [22]. In the cirrhotic liver, the capillarized LSECs produce less nitric oxide [23]. Over time, congestive vasculopathy-induced in-out-imbalance and consistent intrahepatic vasoconstriction cause cirrhosis and portal hypertension [2, 24].

High levels of intrahepatic vascular resistance further lead to the development of splanchnic arterial vasodilation [25]. In contrast to the LSECs with compromised function to produce NO, extrahepatic endothelial cells-produced NO is increased in response to vascular shear stress, which results in vasodilation in the splanchnic capillary beds and arterioles [26]. Progressive splanchnic vasodilation initiates

complications of port hypertension such as ascites, acute renal injury, hepatic encephalopathy, and gastrointestinal bleeding [24]. To counteract progressive splanchnic vasodilation-induced systemic hypotension and arterial underfilling, neurohumoral vasoconstrictive systems such as sympathetic nervous system, renin–angiotensin–aldosterone system, and non-osmotic release of vasopressin are activated. Activation of these systems leads to an increase in plasma volume through sodium and water retention. The excessive plasma volume results in ascites. With the progression of cirrhosis, vasodilation increases and systemic blood pressure progressively decreases, which further activates vasoconstrictors factors. The intense vasoconstriction in the renal circulation causes hepatorenal syndrome [27].

With the progression of portal hypertension, new collateral channels are formed at the sites where systemic and portal circulation come together (such as at the gastrooesophageal junction) to shunt portal pressure [28]. In clinical, patients usually present as gastro-oesophageal varices. When the pressure in these varices exceeds the elastic capacity of the vessel wall, variceal bleeding occurs [24]. When hepatocyte functions such as urea cycle are severely compromised, portosystemic shunting induces hepatic encephalopathy given failing to clear gut-derived ammonia in cirrhotic patients [24].

Liver Cell-Based Mechanisms

The spectrum of ALD comprises steatosis, perivenular fibrosis, alcoholic foamy degeneration (microvesicular steatosis), alcoholic hepatitis, occlusive venous lesions, cirrhosis and hepatocellular carcinoma [5]. Among these pathophysiological alterations, occurrence of cirrhosis is a key step, which largely determines prognosis of a patient. On the other hand, development of cirrhosis is closely associated with early pathophysiological alterations. Besides vascular injury described above, hepatocyte death, inflammation, fibrosis, and extrahepatic pathophysiological alterations are additional important aspects of alcohol-related cirrhosis.

Alcohol-Related Hepatocyte Injury: Sublethal Hepatocytes

Intensive hepatocyte injury or even the loss of contiguous hepatocytes are dominant features of liver cirrhosis. In alcohol-related liver cirrhosis, long-term and excessive alcohol abuse induces hepatocyte apoptosis through oxidative stress and mitochondria stress [29, 30]. In addition, hepatocyte cytochrome P450 2E1 (CYP2E1)-mediated alcohol metabolism also result in hepatocyte necroptosis through receptor-interacting protein kinase (RIPK) 3 and mixed lineage kinase domain–like protein [31].

In addition to cell death, long-term alcohol insults leads to sublethal changes in hepatocytes, which is characterized by ballooning degeneration, Mallory-Denk

body formation and sclerosing hyaline necrosis [5]. Hepatocytes with ballooning degeneration and Mallory-Denk bodies reflect a progressive injury leading to lytic necrosis and are considered as sublethal changes [5]. These hepatocytes are not immediately dead. However, functions in these cells are largely compromised. Animal studies show that these sublethal changes may persist for weeks or even months [32].

When the loss of contiguous hepatocytes and sublethal hepatocytes extend to most of the liver tissue, the liver function is largely compromised, which contributes to the progression of liver cirrhosis. For example, insufficient release of albumin causes formation of ascites while the dysregulation of urea cycle plays a leading role in hepatic encephalopathy [24].

Inflammation and Innate Immunity in ALD

Different forms of inflammatory activities are present throughout the progression of ALD, starting with hepatocellular damage until end stage of disease, where it is characterized as non-resolving inflammation. Inflammation in ALD comprises multiple scenarios, including microbial dysbiosis, loss of barrier integrity in the intestine, hepatocellular stress and death, and inter-organ cell-to-cell communication. Multiple cell types are involved, including Kupffer cells (KC), the resident macrophages, infiltrating monocytes, neutrophils, the inflammatory subpopulation of hepatic stellate cells (iHSCs), LSECS, bile duct epithelial cells, as well as other cell types of the innate and adaptive immune system. Intercellular communication is mediated via the matrisome and cell secreted molecules, including miRNAs and extracellular vesicles. Importantly, the damaged and stressed hepatocytes themselves express chemokines and inflammatory mediators, and finally release damage-associated molecular patterns (DAMPs) during injury and death, which shape the immune and stromal response in the neighbourhood of the damaged region (Fig. 53.1).

In active drinkers, activation of tumor necrosis factor **(TNF)-α**, interleukin **(IL)-1β**, and nuclear factor **(NF)-κB** is found in liver tissue already at early disease stages. Double immunofluorescence staining localizes this proinflammatory response to activated, CD68-positive macrophages. In parallel, down-regulation of **IL-6**, abrogation of the signal transducer and activator of transcription 3 (**Stat3**) pathway, as well as blunted **cyclin D** expression in hepatocytes, reduced proliferation and increased hepatocyte apoptosis is found. Toll-like receptor **(TLR) 7**–interferon **(IFN)** axis in hepatocytes correlates with liver fibrosis markers and disease progression. Two weeks of abstinence attenuates the inflammatory response but does not allow recovery of the defective Stat3 pathway or effects fibrosis associated factors. Therefore, inflammation, activation of the TLR7–IFN axis, and inhibition of Stat3-dependent repair in early ALD pave the way for fibrosis development and ultimately disease progression [33].

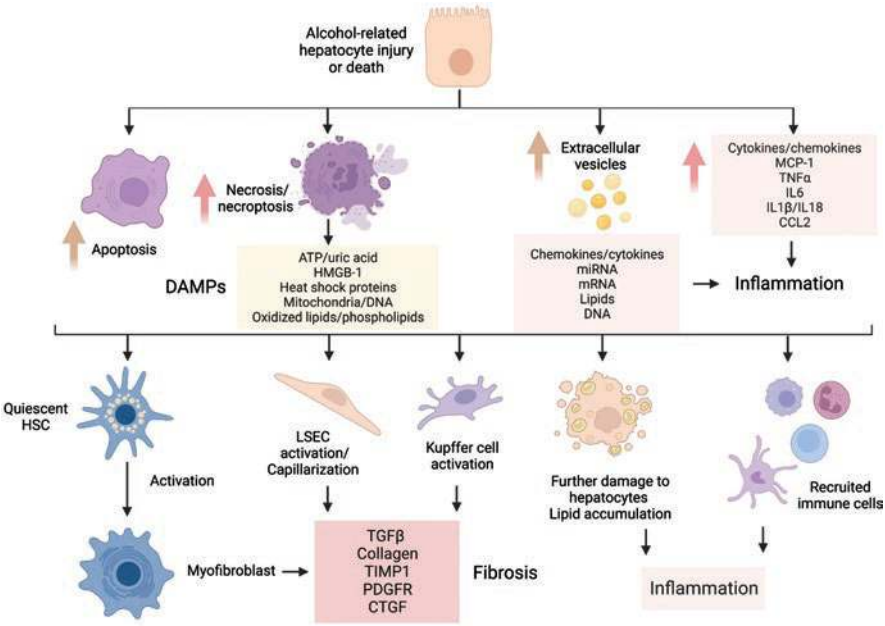

Fig. 53.1 Alcohol-related hepatocyte injury or death signals coordinate cells in the hepatic sinusoids. When alcohol-related hepatocyte injury occurs, the stressed, damaged and dying hepatocytes communicate with other cells that reside in the local hepatic sinusoids through releasing signals. These include apoptotic and necrotic damage associated molecular patterns (DAMPs; e.g. ATP/uric acid, HMGB-1, heat shock proteins, mitochondria/DNA, oxidized lipids/phospholipids), extracellular vesicles packaged with chemokines/cytokines, miRNA, mRNA, lipids or DNA, or directly secreted cytokines and chemokines including MCP-1, TNFα, IL6, IL1β/IL18, CCL2 and so on, therewith initiating and facilitating a protective wound-healing response with subsequent liver regeneration, or upon chronicity, orchestration of ALD progression with fibrosis and inflammation. The signals activate the non-parenchymal cells (NPCs) in the hepatic sinusoids to mediate. These responses include activation of quiescent hepatic stellate cells (HSCs) into myofibroblasts, activation of liver sinusoidal endothelial cells (LSECs) and vessel capillarization, as well as Kupffer cell activation, all of which lead to the secretion of fibrogenic factors like TGFβ, collagen, TIMP1, PDGF or CTGF, inflammatory factors like IL6, IL1β or TNFα, among others, and chemokines like CXCL1 or CCL2, to induce migration and immune cell infiltration to accordingly shape the tissue response to the injury. In the text, we review some of the mechanisms and signals by which hepatocytes and NPCs communicate and orchestrate the local environment to maintain a healthy liver or to develop an ALD

Alcohol metabolism also induces **IRAK4 (interleukin-1 receptor-associated kinase 4)** expression in hepatocytes, which is mediating an acute phase response and release of proinflammatory cytokines/chemokines, along with enhanced hepatocyte cell death. Pharmacological inhibition of IRAK4 kinase activity effectively attenuates alcohol-induced liver injury in mice [34]. Additionally, hepatocytic upregulation of MIF induces an inflammatory response with specific chemokine signatures, as observed in patients with severe AH [35].

Deregulation of the **Hippo/YAP** pathway with uncontrolled activation of YAP in hepatocytes is present in patients with severe AH. YAP activation in hepatocytes from AH patients leads to transdifferentiation towards a cholangiocyte program with loss of the hepatocyte identity, and results in an impaired regeneration in mice. Therapeutic inhibition of YAP activity in patients with AH interfered with hepatocyte transdifferentiation [36].

Hepatocyte **autophagy** is impaired in ALD and AH mouse models and human livers since both present with increased hepatic **p62** and **LC3-II** levels. Alcohol targets multiple steps in the autophagy pathway. Alcohol decreases **mTOR** and **Rheb**, correlates with increased **Beclin1** and **Atg7**, decreased lysosomal-associated membrane protein 1 (**LAMP1**) and lysosomal- associated membrane protein 2 (**LAMP2**). **miR-155**, which targets mTOR, Rheb, LAMP1, and LAMP2 in the autophagy pathway is increased by alcohol. miR-155-deficient mice are protected from alcohol-induced disruption of autophagy. As a result, alcohol impairs autophagic flux at the lysosome level, therewith promoting exosome release [37].

The **complement system** also belongs to the innate immune system and contributes to inflammation mediated liver injury and hepatic regeneration. Canonical factors C2, C4b, C4d, CFI and C5 are reduced in AH patients, whereas components of the alternative CFBa and CFD are increased. Therefrom, AH leads to profound disturbances of the complement and CFI and sC5b9 are valuable diagnostic and prognostic markers for disease severity and risk of mortality for AH patients [38].

Neutrophil infiltration is a frequent feature in severe AH. Immunohistochemistry of explanted livers now identified two variant severe AH phenotypes, one with high intrahepatic neutrophils, but low levels of $CD8^+$ T cells, and vice versa. RNA-Seq analyses demonstrated that neutrophil cytosolic factor 1 (NCF1), a key factor in controlling neutrophilic ROS production, was upregulated and correlated with hepatic inflammation and disease progression. Myeloid-specific deletion of the Ncf1 gene abolished ethanol-induced hepatic inflammation and steatosis in a mouse model of chronic-plus-binge ethanol feeding. Neutrophilic NCF1-dependent ROS promoted AH by inhibiting AMP-activated protein kinase (a key regulator of lipid metabolism) and microRNA-223 (a key anti-inflammatory and antifibrotic microRNA). In conclusion, two distinct histopathological phenotypes based on liver immune phenotyping are observed in severe AH patients, suggesting a separate mechanism driving liver injury and/or failure in these patients [39].

Neutrophils of patients with severe AH are associated with a defect in the **IL-33/ST2** pathway. This defect is associated with lower migration capacities and a higher probability of getting infected. Administration of IL-33 to the neutrophils partly restores this defect and may be effective at reducing the risk of infection in patients with severe alcoholic hepatitis [40]. Upregulated IRF3 increases apoptotic cell death of immune cells that promote the resolution of injury in a mouse model of alcoholic hepatitis [41].

IL-17A regulates inflammatory responses in macrophages (Kupffer cells and bone-marrow derived monocytes) and cholesterol synthesis in steatotic hepatocytes. IL-17 promotes alcohol-induced hepatocellular carcinoma. **IL-17** facilitates tumor necrosis factor/tumor necrosis factor receptor-mediated lipogenesis in

alcohol-damaged hepatocytes via activation of caspase-2-SP1-SREBP1/2-DHCR7 pathway [42].

Interleukin-22 (**IL-22**) biology and its roles of anti-apoptosis, anti-fibrosis, anti-oxidation, anti-bacterial infection and regenerative stimulation in protecting against liver injury in many preclinical models including several recently developed models such as chronic-plus-binge ethanol feeding, acute-on-chronic liver failure (ACLF), C-X-C motif chemokine ligand 1 (CXCL1) plus high-fat diet (HFD) (HFD + Cxcl1)-induced nonalcoholic steatohepatitis (NASH). Finally, clinical trials of IL-22 for the treatment of AH are also discussed, which showed some promising benefits for AH patients [43].

In summary, cellular fate and cell recruitment is orchestrated by the respective pro- or anti-inflammatory environment, either facilitating the wound healing response and liver regeneration, driving the devastating inflammation in the liver, or controlling bacterial infection. In late disease stages, physiological inter-organ crosstalk is heavily disturbed. A nicely illustrated summary of details on the aforementioned can be found e.g. in a recent review article by Bin Gao et al. [44].

Fibrosis in ALD

Accumulation of a fibrillar ECM is the consequence of repeated liver damage induced chronic wound healing reactions towards cirrhosis, the latter morphologically characterised by regeneration nodules of liver parenchyma cells encapsulated in fibrotic septa, and major changes in the vascular system. Initially, the deposited ECM is predominantly found in the subendothelial space of Disse, mainly consisting of collagen types I and III, which is paralleled with capillarisation of the sinusoids [45, 46]. Millions of individuals world-wide are affected by CLD, but only 25–30% progress to significant fibrosis and cirrhosis. Among others, daily alcohol intake is a strong predictor for disease progression. Importantly, in patients with CLD, progression to significant fibrosis in most cases needs a clinical course of between several years and decades, suggesting long latency periods [46, 47].

Depending on the disease etiology and type of insult, variant phenotypes of fibrogenesis are developing that all may progress towards cirrhosis [48]. Biliary fibrosis with significant proliferation of reactive bile ducts and activation of periportal myofibroblasts leads to ECM deposition in the portal–parenchymal interface, forming portal to portal bridges of scar around the liver lobules, preserving the functional linkage between central veins and portal tracts until late disease stages. Viral hepatitis causes portal–central bridging necrosis with corresponding ECM deposition, forming portal to central scar walls. Moreover, viral infections present with interface hepatitis, additionally resulting in portal-to-portal septa and septa ending blind in the parenchyma. This type of scarring leads to a rapid disruption of the vascular connections with the portal system and earlier development of portal hypertension. Central to central septa, also termed “reversed lobulation” [49], are forming due to targeted damage of the hepatocytes located around the central vein,

due to the zonated expression of Cyp2E1, which is responsible for mediating DILI. Besides, venous outflow problems (e.g. during chronic heart failure) lead to that kind of fibrosis pattern. Alcohol-related and metabolic liver diseases display a very distinct fibrosis pattern, where the deposition of ECM is concentrated around the sinusoids and around groups of hepatocytes, termed perisinusoidal, resulting in a so-called chicken-wire archetype. The different fibrosis patterns are induced in dependency of the region of tissue damage, the concentration of pro-fibrogenic mediators, and the prevalent pro-fibrogenic mechanism(s) [45, 47].

Sinusoids are the microvascular units in the liver and the space between hepatocytes and LSEC is termed space of Disse, where the HSCs are located throughout the liver lobules. The physiological basal membrane-like ECM in these spaces is required for physiological cell functions and an efficient metabolic exchange between the sinusoids and the hepatocytes. Hepatic sinusoids originate in the portal tracts from branches of the portal vein and the hepatic artery. The portal tracts further comprise bile ducts and lymphatic ducts as structural elements, and portal fibroblasts and bile duct epithelial cells as cellular components.

Extrahepatic Manifestations of Liver Cirrhosis

The Gut-Liver Axis

Besides intrahepatic disturbances, alcohol-related liver cirrhosis causes severe extrahepatic pathophysiological alterations that contribute to disease progression. The most predominant alterations are microbiota dysregulation-induced pathological bacterial translocation and systemic inflammation. Bacterial translocation is defined as translocation of bacteria and-or bacterial products from the gut to mesenteric lymph nodes [50]. In a healthy individual, bacterial translocation between the gut and the liver is a physiological phenomenon orchestrated by intestine barrier and host immunity [51]. There are three structural and regulatory mechanisms controlling microbiota in healthy condition: (1) stratification of the microbiota by the mucus barrier; (2) intestinal epithelial and the gut-vascular barriers; and (3) immune system control of the microbiota [52]. Excessive alcohol consumption induces gut dysbiosis, impairs the intestinal barrier and increases intestinal permeability, which leads to the gut-associated lymphatic tissues undergoing immune response aiming to eliminate invading bacteria and bacteria products [50]. Patients with chronic alcohol abuse show intestinal overgrowth of aerobic and anaerobic microorganisms, reduced bacterial diversity, and a shift in phyla towards a greater abundance of Proteobacteria and lower abundances of Bacteroidetes and Firmicutes, as well as of Lactobacillus species [53]. In addition to the microbiome alterations, the gut mycobiome such as fungi is also altered in patients with chronic alcohol abuse, which is associated with the severity of liver damage [54]. Alcohol intake impairs gut barrier and increases intestinal permeability at any stage of the disease [55]. Both dysbiosis

and damage of intestinal epithelial cells induce intestinal and systemic inflammation, which plays a critical role in pathological bacterial translocation and the progression of cirrhosis. In alcohol-related liver cirrhosis, pathological bacterial translocation, including bacterial overgrowth and deficiency in secretory and mechanical barrier function, triggers immune response in the gut-associated lymphatic tissue and the liver [56]. These events may lead to spontaneous bacterial peritonitis and acute-on-chronic liver failure (ACLF), a clinical situation characterized by decompensation, organ failure and high short-term mortality. More information can be found in the respective chapter within this book. This dysbiosis, which is present at different sites of our body, is highly associated with bacterial infections, systemic inflammation and poor outcomes. The dysbiosis-dependent constant stimulation of the immune system causes immune dysregulation in cirrhotic patients. The role of dysbiosis, unfavorable microbiota profiles, in the process of liver cirrhosis at the level of the gut and body system have been summarized in several elegant reviews [57–59].

Systemic Inflammation

The importance of systemic inflammation has been recognized and a systemic inflammation hypothesis was proposed by Arroyo and colleagues recently. The hypothesis suggests that "systemic inflammation through an impairment of the functions of one or more of the major organ systems may be a common theme and act synergistically with the traditional mechanisms involved in the development of acute decompensation" [60]. It has been well recognized that immune dysfunction commonly occurs in cirrhotic patients. Cirrhosis-associated immune dysfunction comprises immunodeficiency and systemic inflammation [56]. Immunodeficiency in cirrhosis refers to impaired local immune surveillance function of the liver, reduced synthesis of pattern recognition receptors, and damage at the systemic level of immune response cell function [56]. Cirrhosis-associated systemic inflammation is a dominant feature that is closely associated with consequence of cirrhosis [56]. Patients with alcohol-related liver cirrhosis constantly face challenges from both pathogen-associated molecular patterns (PAMPs) derived from dysregulated gut bacteria and damage-associated molecular patterns (DAMPs) from dead hepatocytes. Given the long-term alcohol consumption-induced gut dysbiosis, bacteria, fungi, and viruses from the gut lumen translocate to the gut-associated lymphatic tissue and the liver and thus result in systemic inflammation. Excessive systemic inflammation plays a leading role in the occurrence of ACLF [61]. Recently, Albillos et al. divided cirrhosis-associated immune dysfunction into two immune phenotypes: (1) the low-grade systemic inflammatory phenotype in the stage of compensated cirrhosis or clinical decompensation with no organ failure and (2) the high-grade systemic inflammatory phenotype in patients with ACLF [61]. In patients with high-grade inflammation, intense immune paralysis following systemic response syndrome increases the risk of infections and worsens prognosis [61].

In summary, vascular injury and congestion are central events in the pathogenesis of liver cirrhosis. Injury of hepatocytes, inflammation, fibrosis and pathological bacteria translocation and systemic inflammation contribute to the progression of this old and severe clinical syndrome. To date, detailed molecular mechanisms underlying liver cirrhosis are largely unknown. Further intensive investigations are required in the future.

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Chapter 54
Alcoholic Fibrosis/Cirrhosis and Its Reversibility

Massimo Pinzani

Abstract A consistent percentage of subjects with alcohol abuse develop alcohol-related liver disease (ALD) with the development of liver tissue fibrosis and cirrhosis often associated with hepatocellular carcinoma (HCC). Alcoholic steatohepatitis (ASH) is critical in driving a fast progression of ALD towards cirrhosis. Concurrent factors include age, gender, ethnicity and coexisting clinical conditions such as obesity and diabetes. Development of fibrosis in ALD is characterized by a perisinusoidal/pericellular pattern and by the central pro-fibrogenic role of hepatic stellate cells. In this context, a major pro-fibrogenic and pro-inflammatory role is played by reactive oxygen species (ROS), acetaldehyde, the main metabolite of ethanol, and other reactive aldehydes deriving from membrane lipid peroxidation. A key role is also attributed to bacterial translocation due to increased gut permeability with increased circulating levels of LPS correlating with the severity of hepatic injury. In general terms, it is likely that in ALD the stage of fibrosis, and not the extent of fatty deposition and inflammatory infiltration, determines the long-term outcomes including hepatic and extra-hepatic clinical outcomes. There is extensive evidence that abstinence can rapidly lead to total resolution of liver steatosis with a minimal or no impact on liver fibrosis. However, this is associated with evident clinical benefits particularly in patients with overt cirrhotic decompensation. When compared with patients with HCV-related cirrhosis with sustained viral response (SVR) following treatment, the clinical benefit of abstinence is unlikely to be due to a faster and more comprehensive regression of tissue fibrosis in ALD cirrhosis, and possibly relies in a difference in the mechanisms responsible for the development and the aggravation of portal hypertension. Indeed, ALD is likely characterized by key tissue mechanisms of early resolution which are not necessarily related to significant fibrosis and cirrhosis regression but rather to unique features of portal hypertension in ALD.

Keywords Alcohol-related liver disease (ALD) · Alcoholic steatohepatitis (ASH) · Cirrhosis · Fibrosis · Hepatic stellate cells · Portal hypertension

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Introduction

Alcohol consumption is a major risk factor for chronic liver disease and liver-related deaths in both industrialized and developing countries. Modern research on alcohol-related liver disease (ALD) has its foundation in the pioneer work of Lieber et al., who, in the early 1960s introduced experimental models to show that alcohol is a true hepatotoxin causing hepatocellular damage, and that ALD is not simply caused by malnutrition [1]. Overall, these early studies, as well those published in the following decades, demonstrated that ethanol metabolism-associated oxidative stress, glutathione depletion, abnormal methionine metabolism, ethanol-induced increase in intestinal permeability with endotoxin leakage followed by the activation of Kupffer cells have important roles in the pathogenesis of ALD [2–7].

In broad terms, liver fibrosis, similarly to other forms of tissue fibrosis occurring in chronic inflammatory diseases, is characterized by a chronic wound-healing response to a chronic tissue damage. Tissue scarring, which is the result occurring in general after several years of tissue injury, is not necessarily the worst outcome. Indeed, it is the best compromise to ensure continuity in the tissue structure although at the cost of a progressive loss of function associated to negative mechanical consequences. In very superficial terms it is also assumed that liver fibrosis develops in chronic liver disease (CLD) due to different causes of parenchymal damage (hepatocellular and/or cholangiocellular) according to the same cellular and molecular mechanisms irrespective of the etiology (viral, metabolic, toxic, autoimmune and cholestatic). However, there are important etiology-dependent features which are crucial to understand how fibrosis evolves into cirrhosis in different CLD and how fibrosis can be reversible or irreversible in cirrhotic liver.

General Cellular and Molecular Mechanism of Liver Fibrosis

The main effectors of hepatic fibrogenesis are hepatic stellate cells (HSCs). HSCs are liver-specific pericytes localized in the space of Disse, that, once activated, develop into highly proliferative fibrogenic myofibroblasts [8, 9]. Although HSCs are the main source of myofibroblasts in the liver, other cell types contribute to the pool of fibrogenic myofibroblasts in chronic liver disease. Portal myofibroblasts, located around bile ducts, also play a fibrogenic role particularly for the development of biliary fibrosis [10, 11]. Activation of HSCs is stimulated by damaged and apoptotic hepatocytes through several converging routes. These include: i) disruption of the normal ECM of the space of Disse because of hepatocyte damage and inflammatory infiltration, ii) release of reactive oxygen species (ROS) and other fibrogenic/pro-inflammatory mediators, and iii) recruitment of immune cells, which in turn mediate HSC activation and stimulate collagen secretion through release of cytokines and chemokines. Following the initial activation of HSCs, cytokines secreted by HSCs and by cells of the inflammatory infiltrate, provide signals that

maintain HSC activation/survival and promote the accumulation of fibrillar extracellular matrix (ECM). This leads to a vicious circle, in which mutual stimulation between inflammatory and pro-fibrogenic cells drives hepatic fibrogenesis [12].

The present knowledge concerning the cellular effectors of hepatic fibrogenesis derives from studies performed on primary culture or immortalized cell lines of human or rodent HSC as well as of immortalized rat portal myofibroblasts [13, 14]. The major phenotypic responses and their role can be summarized in the following points [15]:

1. *Synthesis and remodelling of the ECM.* Increased synthesis of ECM components is a disease hallmark in progressive CLD, with TGF-β1 playing a major role in the production of fibrillary collagens (mainly type I and III), laminin and fibronectin. A key counterpart of this feature is the reduced expression of genes involved in ECM remodelling, resulting in increased expression of tissue inhibitors of metalloproteinases (TIMPs) and the consequent inefficient removal of excess fibrillary collagen by metalloproteases (MMPs). Several other mediators have been proposed to be involved, including: (i) reactive oxygen species (ROS) released by injured hepatocytes or overproduced as a consequence of activation of NADPH-oxidase isoforms, associated to the interaction of growth factors, cytokines and other active peptides with their cognate receptors; (ii) aldehydic products like acetaldehyde (during ethanol metabolism) or 4-hydroxy-nonenal, the most relevant aldehydic end-product of lipid peroxidation; (iii) several growth factors, ligand peptides and the relative signalling pathways.
2. *Proliferation, survival, and migration.* Activated HSCs and myofibroblasts are highly proliferating cells because of increased availability of mitogenic growth factors released by surrounding cells in the profibrogenic environment and increased expression of the relative receptors. Indeed, the pro-fibrogenic microenvironment in CLD is characterized by a markedly increased expression of mitogens such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), thrombin, keratinocyte growth factor, connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF). Altogether, these mitogenic signals are also responsible for an increased cell survival and resistance to apoptotic stimuli, thus leading to a diffuse and persistent hyperplasia of pro-fibrogenic cells within the diseased liver tissue. Most part of the above growth factors and cytokines also promote cell migration according to concentration gradients which progressively develop in the pro-fibrogenic microenvironment and greatly contribute to tissue scarring.
3. *Pro-inflammatory role.* Activated HSCs are characterized by increased gene expression and release of proinflammatory mediators such as the chemokines CCL2 and CCL21 as well as IL-1β following activation of NLRP3 inflammasome. In these terms they can actively contribute to perpetuate inflammatory response and regulating and/or modulating interactions with cells of innate and adaptive immunity.
4. *Pro-angiogenic role*. Activated HSCs have been reported to synthetize and release proangiogenic mediators like vascular endothelial growth factor A

(VEGFA), Angiopoietin-1 or-2, PDGF-BB and hedgehog ligands. This feature strongly links neo-angiogenesis with the fibrogenic progression since HSCs express receptors for these soluble factors and support the formation of fibrotic septa typical of cirrhotic liver.

Specific Mechanism of Liver Fibrosis in ALD

ALD is characterized by a spectrum of histopathological lesions that range from simple steatosis to alcoholic hepatitis or alcoholic steatohepatitis (ASH). Tissue fibrosis and cirrhosis are key features with a significant risk to develop hepatocellular carcinoma (HCC). ASH is believed to be critical in driving a fast progression of ALD towards cirrhosis and to increase the risk of decompensation and liver failure [16]. ALD shares with non-alcoholic steatohepatitis (NASH) the pattern of perisinusoidal/pericellular fibrosis development and the key pro-fibrogenic role of HSCs [17, 18]. Interestingly, although alcohol abstinence can favour recovery from fatty liver, alcoholic hepatitis, and sinusoidal fibrosis, thus improving the outcome for cirrhosis, no significant regression of established cirrhosis in ALD patients has been convincingly documented [16].

The main pathogenetic determinants of liver fibrosis in ALD are illustrated in Fig. 54.1. In ALD, chronic hepatocellular injury and cell death are closely related to the oxidative ethanol metabolism by alcohol dehydrogenase and the ethanol inducible CYP2E1 cytochrome P450 isoform. This metabolic pathway leads to the

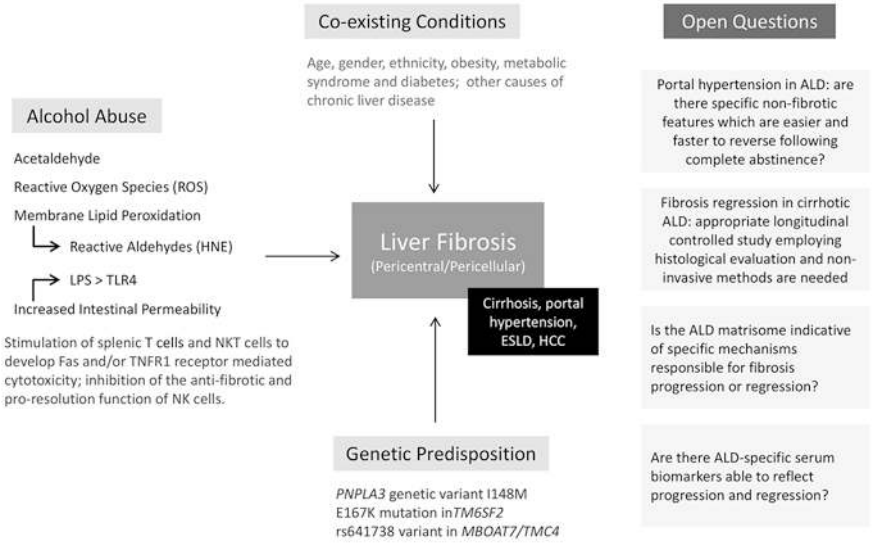

Fig. 54.1 Alcoholic liver fibrosis: pathogenetic determinants and open questions

formation of acetaldehyde and ROS, and to the consequent oxidative stress-mediated injury mainly through lipid peroxidation affecting the integrity of mitochondria and ER membranes [19–21].

Acetaldehyde is produced mainly by hepatocytes and acts on HSCs in a paracrine manner by directly inducing the expression of collagen type I in HSCs via activation of multiple signalling pathways and transcription factors. In addition, acetaldehyde reacts rapidly with cellular components, producing adducts such as malondialdehyde, 4-hydroxynonenal, and malondialdehyde-acetaldehyde, which further contribute to HSC activation and support their pro-fibrogenic role [22, 23]. ROS are the major determinants of ER stress and, together with acetaldehyde, of alcohol-induced steatosis. These features are associated to AMPK downregulation and inhibition of PPAR-α expression via SREBP1c stimulation [16]. DAMPs, released following necrotic cell death, trigger macrophage and neutrophil activation, in a context were senescence (via NK cells) and autophagy act as major regulators of liver inflammation [24].

A major role in the pathogenesis of ALD is also attributed to bacterial translocation due to increased gut permeability with increased circulating levels of LPS correlating with the severity of hepatic injury. LPS not only stimulates Kupffer cells to produce reactive oxygen species and cytokines, that subsequently promote activation of HSCs, but also directly activates HSCs via TLR4 [25]. LPS can also activate TLR4 signalling in liver sinusoidal endothelial cells with the promotion of angiogenesis. Collectively, TLR4 signalling in HSCs, Kupffer cells and endothelial cells provides a major contribution to inflammation, fibrogenesis and angiogenesis in ALD [26].

Adaptive immunity might also contribute to ALD progression, with chronic alcohol consumption leading to increased levels of antibodies directed against lipid peroxidation products. These antibodies can activate an adaptive immune response, likely by stimulating splenic T cells and NKT cells to develop Fas and/or TNFR1 receptor mediated cytotoxicity towards hepatocytes [27]. Finally, ethanol itself can suppress the anti-fibrotic and pro-resolution function of NK cells, which is believed to operate through IFN-γ secretion and the related elimination of activated HSC [28].

Liver Fibrosis in ALD: Incidence, Predisposition, and Natural History

There is a direct relationship between the quantity of alcohol consumed and the risk of ALD [29]. The risk of developing alcohol-related cirrhosis is increased with chronic alcohol consumption of 12–24 g/day compared to not drinking alcohol. Chronic alcohol abuse of more than 60 g/day, results in the development of fatty liver in most individuals with a progression to ASH in 10–35% [30]. In the context of ASH, fibrosis progression can be associated with episodes of alcoholic hepatitis which are key fibrogenesis accelerators. Overall, these numbers suggest that most

alcohol abusers will not develop severe disease with progression to advanced liver fibrosis and cirrhosis.

It is well established that age, gender, ethnicity and coexisting clinical conditions such as obesity and diabetes, are predisposing factors to the development of fibrosis and cirrhosis in ALD. Alcoholism is regarded to be a familial disorder since individuals with a familial history of alcohol abuse are more susceptible to develop alcohol dependence. Irrespective of this, the predisposition to develop ASH and fibrosis seems to be linked to genetic risk loci which are in common with NASH [31]. Along these lines, the non-synonymous genetic variant I148M in the *Patatin-like phospholipase domain containing-3* (*PNPLA3*) gene has emerged as the major risk factor for chronic liver disease progression in general, but particularly in ASH and NASH. In addition, a variant causing an amino acid change (E167K) in the *Transmembrane 6 superfamily member 2* (*TM6SF2*) gene, has been associated with the development and the severity of NASH and likely ASH. More recently, the rs641738 variant in the *Membrane bound O-acyltransferase domain containing 7-Transmembrane channel-like 4* (*MBOAT7/TMC4*) locus has been related to a higher risk of cirrhosis in alcohol abusers and with liver disease progression in NAFLD [32, 33].

It is increasingly established that these genetic variants favour fat accumulation in hepatocytes. In the case of ALD, prolonged ethanol exposure impairs insulin sensitivity with the consequent increase of free fatty acids from the adipose tissue and de novo lipogenesis. In this context, steatosis can be exacerbated by genetic modifiers such as the *PNPLA3 148 M* variant, which increases hepatic triglyceride content upon accumulation of the mutant protein on the surface of the lipid droplets. In addition, the 148 M variant impairs the amount of VLDL released, thus worsening fat deposition. Since the presence of these genetic risk loci is associated not only with the development of steatosis but also with the fibrogenic progression of chronic liver diseases, it is possible that they directly influence cellular and molecular pathways more relevant for fibrogenesis. For example, *PNPLA3* is strongly expressed and synthesized even in primary HSCs, and catalyses the hydrolysis of retinyl esters, regulating retinol release. The I148M could hamper the dismissal of retinol and lipids from intracellular lipid droplets, resulting in a more pronounced fibrogenic phenotype [34]. Current work from our laboratory, employing transcriptomic analysis, confirms that *PNPLA3 I148M* variant is associated with impaired mitochondrial function and antioxidant response in 3D cultured human HSCs and liver tissue from patients with NAFLD [35]. These findings tend to suggest that, in the presence of the *PNPLA3 I148M* variant, the handling of the antioxidant response in fibrogenic cells such as HSCs is markedly impaired, thus leading to an exaggerated fibrogenic response in the presence of acute and chronic oxidative stress conditions.

In addition to genetic determinants, epigenetic modifications have been identified in parenchymal and non-parenchymal cells in the liver and contribute to steatosis, inflammation, and oxidative stress following chronic exposure to excess ethanol. These modifications are hereditable and impact on gene expression without altering nucleotides sequence. Examples include DNA methylation, histone modifications and RNA silencing by microRNAs (miRNAs) [31].

In terms of natural history of ALD, progression from steatosis to cirrhosis is observed at 3%/year and from steatohepatitis (which is a histological diagnosis rather than the clinic syndrome of alcoholic hepatitis) to cirrhosis at 10%/year. This suggests that steatohepatitis is the progressive form of liver disease and is probably a prerequisite for the development of cirrhosis. The risk of HCC in ALD is lower than in cirrhosis due to chronic viral infection with a cumulative HCC incidence of 2.9 per 100 patient-years [36].

Reversibility of Fibrosis and Cirrhosis in ALD

The issue of fibrosis regression/reversibility of cirrhosis originates from evidence obtained in animal models upon the discontinuation of the cause of liver damage or following treatment with a putative antifibrotic agent. While tissue fibrosis in the absence of major changes in the hepatic angio-architecture is potentially reversible upon discontinuation of the cause of damage, the regression of cirrhosis is questionable. Regardless, evidence of cirrhotic regression has been reported in CLD of different etiologies. However, when performing an accurate analysis of the results of these studies, the only prudent conclusion is that, in most cases, there was a variable degree of fibrosis regression in cirrhosis but not a clear reversal of cirrhosis [37, 38]. In particular, there is no convincing evidence that major abnormalities of the intrahepatic vasculature typical of human cirrhotic liver can effectively regress. Along these lines, the available evidence suggests that the so-called veno-portal adhesions and evident "arterialized" sinusoids persist even in cases of extensive fibrosis regression [39].

Similarly, to what shown in NASH, it is also likely that in ALD the stage of fibrosis, and not the extent of fatty deposition and inflammatory infiltration, determines the long-term outcomes including hepatic and extra-hepatic clinical outcomes. However, this distinction is at least in part questionable in ALD, where the mainstay of treatment is alcohol abstinence. There is extensive evidence that abstinence can rapidly lead to total resolution of liver steatosis with a minimal or no impact on liver fibrosis. However, this is associated with evident clinical benefits particularly in patients with overt cirrhotic decompensation. Indeed, abstainers' probability of survival has been reported to be 87% compared to 55% in persistent drinkers within an observation period of about five year [40]. Therefore, the benefit of abstinence appears somehow disproportionated when compared, for example, to patients with HCV after obtaining a DAA-induced sustained viral response. This difference is unlikely to be due to a faster and more comprehensive regression of tissue fibrosis in ALD cirrhosis when compared to HCV-related cirrhosis, and possibly relies in a difference in the mechanisms responsible for the development and the aggravation of portal hypertension. Importantly, when compared to HCV or HBV cirrhosis, in ALD cirrhosis sinusoidal pressure is generally higher, hepatic venous pressure gradient better reflects portal pressure, the portal flow perfusing the liver is reduced despite an increase in liver weight, the prevalence of reversal portal blood flow is

higher, and a patent paraumbilical vein is a more common finding. In addition, signs of hyperdynamic circulations, such as an increased cardiac output and decreased systemic vascular resistance, are more pronounced. Besides, alcohol consumption can acutely increase portal pressure and portal-collateral blood flow. Alcoholic cardiomyopathy, another pathological consequence of prolonged alcohol misuse, may contribute to the hemodynamic changes occurring in alcohol-related cirrhosis [41]. Along these lines, Klein et al. showed that after 1 year of abstinence, portal vein pressure and the size of esophageal varices almost halved [42]. It is conceivable that abstinence, although not significantly affecting the amount and distribution of liver tissue fibrosis within the early time frame when the clinical improvement occurs, is able to reduce factors influencing portal pressure rapidly and efficiently in ALD cirrhosis such as tissue edema and hepatocyte swelling, which are characteristic of ALD and may represent rapidly reversible causes of intrahepatic resistance to portal inflow [43].

Conclusions

In conclusion, in the absence of other hepatic co-morbidities, liver fibrosis and cirrhosis due to alcohol abuse present with unique features which are relevant for the clinical management and the overall treatment. Of particular interest is the clinical evidence of a rapid and persistent clinic improvement observed in many patients following alcohol abstinence that may disentangle key tissue mechanisms of early resolution which are not necessarily related to significant fibrosis and cirrhosis regression but rather to unique features of portal hypertension in ALD. Along these lines, some of the key open questions concerning liver fibrosis in ALD are summarized in Fig. 54.1. See also related Chaps. 7, 49 and 53.

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Chapter 55
Alterations in Methionine Metabolic Pathway in the Pathogenesis of Alcohol-Related Liver Disease

Kusum K. Kharbanda

Abstract Alcohol-related liver disease is a major worldwide health care problem. Findings in multiple laboratories, including ours, have demonstrated that ethanol consumption impairs several of the multiple steps in hepatic methionine metabolism, leading to progressive liver injury. Ethanol consumption predominantly inhibits the activity of the vital enzyme, methionine synthase, which catalyzes homocysteine remethylation to form methionine. By way of compensation, chronic ethanol consumption increases the activity of betaine homocysteine methyltransferase. This enzyme catalyzes an alternate pathway in methionine metabolism by utilizing intracellular betaine to remethylate homocysteine to form methionine, thereby maintaining adequate levels of S-adenosylmethionine, the key cellular methylating agent. However, after extended periods of ethanol feeding, alternate pathway for remethylation cannot be maintained, likely due to depletion in hepatic betaine levels. This condition, in turn, results in a decrease in hepatocyte S-adenosylmethionine, while increasing the levels of two toxic metabolites, S-adenosylhomocysteine and homocysteine. These changes cause serious functional consequences, which include lower activities of essential methylation reactions critical to normal liver function and activation of endoplasmic reticulum-dependent stress response. The ultimate outcome of these consequences is enhanced fat deposition, increased apoptosis, accumulation of damaged proteins and alterations in various signaling pathways, all of which, if sustained, cause progressive liver damage.

Of all the therapeutic modalities currently used to attenuate ethanol-induced liver injury, betaine has been shown to be one of the most effective in a variety of experimental models of liver disease. Betaine, as a methyl group donor, remethylates homocysteine, thereby removing both toxic metabolites (homocysteine and

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S-adenosylhomocysteine) and restoring S-adenosylmethionine to reverse/prevent many hallmark features of alcohol-induced liver damage. Thus, betaine is a promising therapeutic agent that relieves methylation and other defects associated with alcohol use disorders.

Keywords Alcohol-related liver disease · Betaine · S-adenosylmethionine · S-adenosylhomocysteine · Homocysteine · Methyltransferases

Abbreviations

ALD	Alcohol-related liver disease
BHMT	Betaine-homocysteine-methyltransferase
CBS	Cystathionine-β-synthase
ER	Endoplasmic reticulum
GAMT	Guanidinoacetate methyltransferase
GCL	Glutamate cysteine ligase
GCLC	Catalytic subunit of GCL
GCLM	Modifier subunit of GCL
GSH	Glutathione
ICMT	Isoprenylcysteine carboxyl methyltransferase
K_i	Inhibitor affinity constant
K_m	Michaelis constant
MAT	Methionine adenosyltransferase
MS	Methionine synthase
MTHF	N^5-methyltetrahydrofolate
MTHFR	5,10 methylenetetrahydrofolate reductase
NAC	N-acetylcysteine
PEMT	Phosphatidylethanolamine methyltransferase
PIMT	Protein L-isoaspartate methyltransferase
PRMT	Protein arginine methyltransferase
SAH	S-adenosylhomocysteine
SAHH	S-adenosylhomocysteine hydrolase
SAM	S-adenosylmethionine
SREBP-1	Sterol regulatory element binding protein-1
VLDL	Very-low-density lipoprotein

Introduction

Alcohol-related liver disease (ALD) is one of the most serious medical consequences of ethanol misuse. Clinical and experimental models demonstrate that ethanol administration leads to many adverse functional and structural changes in

hepatic and extrahepatic organs and is associated with over 200 diseases [1]. Alcohol's hepatic effects are the most well characterized, as the liver is one of the most susceptible organs to its toxic effects. Alcohol (a.k.a. ethanol or ethyl alcohol) misuse produces a wide spectrum of hepatic lesions ranging from simple steatosis (fatty liver) that with continued drinking usually progresses to steatohepatitis, fibrosis, cirrhosis and even hepatocellular cancer [2]. These lesions reportedly can occur independently, in combination, or sequentially, with progression from steatosis through alcoholic hepatitis to established cirrhosis. Multiple factors and co-cofactors have been implicated in the pathogenesis of alcohol induced liver injury (reviewed in [3]). In particular, the interaction of ethanol with nutrients and/or their metabolism is believed to contribute significantly to the pathology observed in ALD. In this context, the effects of ethanol administration on methionine metabolism pathway have been studied, based on findings from the early 1950s, that ethanol consumption during dietary methionine or choline deficiency accelerates the onset of liver pathology. Therefore, investigations have focused on ethanol-induced perturbations in hepatic methionine metabolism to gain a clear understanding of the pathogenic mechanisms and to devise appropriate therapeutic interventions.

Methionine Metabolism

Methionine is an essential amino acid, well known for being the initiator (NH_2-terminal) amino acid during cellular protein synthesis. It also serves major roles through its metabolism, which fuels several metabolic pathways. Methionine metabolism is mainly comprised of the methionine cycle and the transsulfuration pathway.

The primary importance of the metabolic cycle is to conserve methionine so that S-adenosylmethionine (SAM), a universal methyl donor, can be generated. This reaction is catalyzed by methionine adenosyltransferases (MAT I and III) both liver-specific enzymes. The *MAT1A* and *MAT2A* genes, respectively encode the MAT catalytic subunits, α1 and α2. The α1 subunit organizes into dimers (MATIII) or tetramers (MATI). The α2 subunit is found in the MATII isoform. A third gene *MAT2B*, encodes beta, a regulatory subunit, that regulates MATII activity by lowering the inhibitor affinity constant (K_i) for SAM and the Michaelis constant (K_m) for methionine. The *MAT1A*-encoded enzyme (MATα1) is mainly expressed in liver hepatocytes and is associated with differentiated phenotype. The *MAT2A*-encoded isozyme (MATα2), expressed in non-parenchymal cells and extrahepatic tissues is associated with rapid growth and de-differentiation [4]. SAM, at its physiological concentration of 60 μM feedback inhibits MATα2 but it minimally inhibits MATα1 at this concentration. Therefore, MATα1 is considered responsible for maintaining adequate SAM levels, whereas MATα2 minimally contributes to the SAM pool in liver cells [4]. Under normal conditions, most of the 6–8 g of SAM generated per day in a healthy human adult serves as a methyl donor for more than 85 percent of all transmethylation reactions that occur in this organ. In SAM-dependent

methylation reactions, a methyl group is transferred from SAM to a variety of acceptors to form methylated acceptors that play vital roles in maintaining important functions in the liver. The other product of the methylation reaction is S-adenosylhomocysteine (SAH), which is hydrolyzed to adenosine and homocysteine by S-adenosylhomocysteine hydrolase (SAHH). Although the equilibrium constant of the SAHH-catalyzed reaction strongly favors SAH synthesis over homocysteine synthesis, the efficient removal of homocysteine and adenosine by multiple pathways (indicated in Fig. 55.1) allows homocysteine synthesis to proceed so that it is rapidly converted to methionine or to glutathione (GSH).

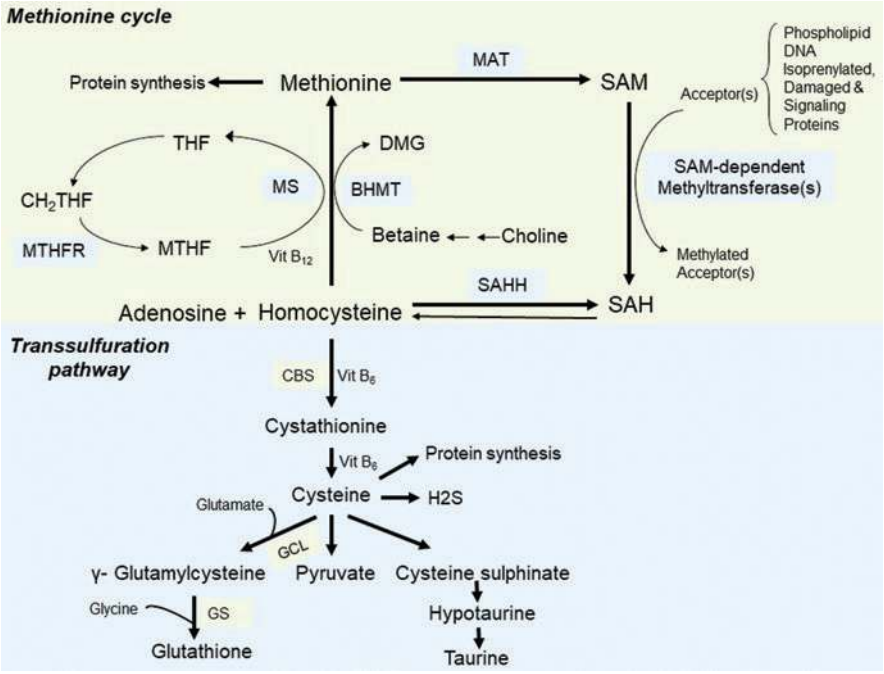

Fig. 55.1 Reactions of methionine metabolism in the liver. There are four main participants of this pathway, which are methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and homocysteine. Methionine adenosyltransferase (MAT) converts methionine to SAM, which then serves as a methyl-group donor substrate for methyltransferase-catalyzed reactions. The other product of these reactions is S-adenosylhomocysteine (SAH) which is hydrolyzed to adenosine and homocysteine by S-adenosylhomocysteine hydrolase (SAHH). The thermodynamics of this latter reaction favors the formation of SAH if the products (adenosine and homocysteine) are not removed. The methionine cycle is completed when homocysteine is remethylated back to methionine by B_{12}-dependent methionine synthase (MS) using 5-methyltetrahydrofolate (MTHF) as a substrate. MTHF is derived from dietary folate and from endogenous 5,10, methylenetetrahydrofolate (CH_2-THF) by way of its reductase, 5,10 methylenetetrahydrofolate reductase (MTHFR). Homocysteine can also be remethylated to methionine by an alternate pathway in the liver via betaine homocysteine methyltransferase (BHMT)-catalyzed reactions with the formation of dimethylglycine (DMG). Homocysteine can also be catabolized through the transsulfuration pathway initiated by B_6 dependent cystathionine β-synthase (CBS) to generate glutathione (GSH). See also related Appendix Figs. A.49, A.50, A.51 and A.52

There are two pathways in liver that participate equally in converting homocysteine to methionine [5]. One pathway utilizes N^5-methyltetrahydrofolate (MTHF), methionine synthase (MS) and vitamin $B_{12.}$ MTHF is derived from dietary folate and from endogenous 5,10, methylenetetrahydrofolate by way of its reductase, 5,10 methylenetetrahydrofolate reductase (MTHFR). Through the action of MS, a methyl group is transferred from MTHF to vitamin B_{12} to form methylcobalamine. The methylcobalamine in turn transfers the methyl group to homocysteine to produce methionine. In organs such as liver, kidney, pancreas, the eye lens, there is an additional pathway that is folate-independent for remethylating homocysteine. This pathway, catalyzed by betaine-homocysteine methyltransferase (BHMT), is of equal importance as the folate-dependent pathway in these organs. BHMT utilizes choline oxidation derivative, betaine, as a source of methyl group in the reaction to generate methionine and dimethylglycine [5].

Key enzymes of the methionine cycle (except MATIII) share several properties including a low K_m for sulphur-containing substrates, down-regulation by dietary methionine as well as inhibition by methionine and/or SAM. On the other hand, owing to the high K_m of MATIII for methionine, liver is the only tissue capable of synthesizing additional SAM when the concentration of methionine becomes excessive [6].

Homocysteine can also be utilized for the synthesis of cysteine and its derivatives, GSH, taurine and sulfate via the transsulfuration pathway. In this pathway, homocysteine is irreversibly catabolized by the action of cystathionine β-synthase (CBS). The tight regulation of homocysteine metabolism depends on different affinities for homocysteine by MS, BHMT and CBS. While MS and BHMT have high affinities for homocysteine ($K_m < 0.01$ mmol/L), CBS has a > 100-fold higher K_m (>1 mmol/L). Therefore, at low homocysteine concentrations, methionine conservation is favored whereas at higher homocysteine concentrations, the transsulfuration pathway is favored [7]. Through the action of CBS, homocysteine and vitamin B_6 are converted to cystathionine, which is further split to cysteine and α-ketobutyrate by cystathionase, another pyridoxal phosphate-containing enzyme. Cysteine is then incorporated into reduced glutathione (GSH) through several metabolic steps. A rate-limiting enzyme in GSH biosynthesis is glutamate cysteine ligase (GCL), which synthesizes gamma-glutamyl-cysteine. GCL holoenzyme is a heterodimer, consisting of the catalytic subunit (GCLC) and the modifier subunit, GCLM [8]. Gamma-glutamyl-cysteine may be produced by the holoenzyme as well as GCLC; however, the presence of GCLM decreases the K_m for ATP and glutamate to increase the K_{cat} for gamma-glutamyl-cysteine synthesis [8].

GSH is the most abundant non-protein thiol that protects cells against endogenous and exogenous electrophiles and it functions as a major antioxidant. The enzymes of the transsulfuration pathway also share common properties, such as the significantly higher K_m values for the sulphur substrate and induction of these enzymes by dietary methionine and SAM [6, 9].

Since transmethylation reactions and GSH levels regulate various cellular processes and antioxidant status, respectively, changes in methionine metabolism have far reaching detrimental effects in precipitating liver damage.

Defects in Hepatic Methionine Metabolic Pathway Following Ethanol Consumption

Using different animal models and human studies, it is widely recognized that multiple steps in hepatic methionine metabolism are influenced by ethanol consumption.

Effects on MS and BHMT: The first change that occurs by day 6 in livers of male rats fed the Lieber-DeCarli liquid ethanol diet is that MS activity declines by about 50 percent [10, 11]. The latter decrease still persists after 4 weeks of feeding [12]. Similar results have been reported by other investigators using other animal models of ethanol feeding, including female rats and micropigs [13–16]. There is also a decline in MS protein and mRNA that encodes MS in the livers of cirrhotic patients [17], and these changes have been reproduced in animal ethanol feeding models [18, 19]. Furthermore, *in vitro* studies have shown that the activity of purified MS enzyme is significantly and irreversibly inhibited by incubation with the primary ethanol metabolite, acetaldehyde [20]. This could be another contributing mechanism for a decrease in MS activity observed after alcohol exposure.

Another consequence of ethanol ingestion on the methionine metabolic pathway is that there is an adaptive increase in BHMT activity in male rats, which is seen as early as 1 week of ethanol feeding [21]. Subsequent studies showed that this rise in enzyme activity is due to an increase in BHMT protein and the mRNA that encodes BHMT [12]. Similarly, increases in BHMT mRNA are also reported from array analyses of human ALD livers [22]. However, this adaptive increase in BHMT is not observed in mouse models of ethanol exposure [23], which may be the reason for more severe liver injury seen in murine species with the same duration of ethanol exposure as that of rats [23].

The sustained increase in BHMT activity in livers of ethanol-fed rats is protective, in that, by utilizing endogenous betaine helps maintain hepatic SAM levels for up to 8 weeks of ethanol exposure [12, 24, 25]. This occurs despite impaired methionine production via the MS-catalyzed pathway. However, after 8 weeks of ethanol feeding SAM levels decline significantly [11]. This is likely due to depletion of intrahepatic betaine reserves [21, 24, 26] and hence the failure of induced levels of BHMT to compensate for impaired MS-mediated catalysis. Trimble *et al* also reported an adaptive increase in BHMT activity in rats on ethanol diet for 2 weeks but reported an earlier decrease in hepatic SAM after 2 weeks of ethanol feeding [13]. However, because female rats were utilized in their study, the discrepancy may be due to gender differences in hepatic betaine reserves and the inability of adaptive BHMT induction to maintain SAM levels. Rapid declines in SAM levels occur in ethanol-fed micropigs and mice, species in which ethanol-induced BHMT induction does not occur [15, 19]. Depletion of hepatic SAM is also reported in baboons following prolonged periods of ethanol feeding [27].

Effects on methionine adenosyltransferase (MAT) expression/activity: Declines in hepatic levels of SAM also occur because alcohol feeding reduces MAT

activity, the only enzyme that synthesizes SAM from methionine. Patients with alcoholic hepatitis and alcohol-related cirrhosis exhibit diminished hepatic *MAT1A* mRNA expression, lower MAT activity and impaired SAM biosynthesis [28–30]. Similar results were also reported for ethanol-fed micropigs [15, 16]. However, Alvarez *et al* reported normal levels of *MAT1A* mRNA in human cirrhotic livers, as compared to controls [31]. They postulated that post-translational modifications of critical cysteine residues (present only in liver specific MAT) by nitrosative or oxidative stress are responsible for lower MAT activities observed by other investigators. Contrary to these studies, a two-fold increase in hepatic mRNAs encoding both MAT1A and MAT2A occurs in rats after 9 weeks of intragastric ethanol feeding. But, despite similar increases in these mRNAs the protein levels of non-liver MATs were robustly induced [32]. Subsequent studies revealed that liver pathology is associated with a switch from MATα1 to MATα2, causing an overall decrease in MAT activity and SAM depletion [33]. The switch of MATα1 to MATα2 during alcohol exposure is associated with pro-proliferative states [33] that may promote the hepatocellular carcinoma phenotype [34]. Alcohol-induced loss of MATα1 may also have other detrimental consequences unrelated to decreased SAM levels. This is because (i) MATα1 targets the nuclear compartment where it acts as a transcriptional co-factor influencing the activity of several transcription factors [35, 36] and (ii) MATα1 negatively regulates CYP2E1 expression by promoting its degradation through the ubiquitin-proteasome system (UPS) [37]. MATα1 has recently been identified as a mitochondrial-targeted protein that is not only an additional source of mitochondrial SAM, but physically interacts with other mitochondrial proteins involved in the citric acid cycle, oxidative phosphorylation, and fatty acid β-oxidation to preserve mitochondrial function [30]. Ethanol depletes mitochondrial MATα1 via casein-kinase 2 (CK2)-mediated phosphorylation resulting in mitochondrial dysfunction and promoting ALD pathogenesis [30].

Effect on GSH levels and enzymes of transsulfuration pathway: The inactivation of liver-specific MAT has also been correlated to the depletion of reduced GSH levels. This has been observed in some models of ethanol-induced liver injury in rodents and baboons as well as in patients who are alcohol-dependent [27, 38, 39]. The decrease in GSH was suggested to be due to GSH efflux into plasma, decreased GSH synthesis, and accelerated GSH metabolism. Interestingly, there was no change in mRNA encoding CBS, the first enzyme of the transsulfuration pathway [19]. Rather, there was an increase in GCL activity [39]. Yet, reduced GSH levels were observed despite the induction in GCL activity and expression of the two GCL subunits [39]. This was believed to be caused by alterations in other factors important in determining the steady-state GSH level [39].

Notably, a marked and selective decrease in the intramitochondrial GSH pool was reported in rats chronically fed ethanol using either the Lieber-DeCarli diet for 4–6 weeks or by intragastric feeding for 3–16 weeks [40–46]. This reduction in GSH was attributed to defective transport of GSH into mitochondria following ethanol consumption that occurs preferentially in mitochondria of perivenous hepatocytes, which surround the central vein, an area of the liver lobule where

alcohol-induced liver injury initiates. The mechanism underlying the transport defect appears to be due to changes in kinetic parameters of the GSH carrier, which, in turn, is sensitive to changes in the physical properties and fluidity of the inner mitochondrial membrane [47, 48]. Further, *in vitro* studies documented that metabolically derived acetaldehyde causes depletion of mitochondrial GSH pools in a time- and dose-dependent fashion, without any change in cytosolic GSH levels [49]. These changes, like those reported in livers of long-term ethanol-fed rats [40–46], were ascribed to increased mitochondrial microviscosity due to enhanced cholesterol deposition. However, one laboratory reported no mitochondrial GSH depletion after chronic ethanol feeding [50], but instead, a rise in mitochondrial GSH. This finding was corroborated in unpublished work from Kharbanda laboratory [51]. Yet, others reported no change in mitochondrial GSH after alcohol exposure [19, 52]. Despite these controversial findings, likely related to ethanol exposure models, species and/or analytical assays, the pathogenic role of GSH depletion in ALD remains to be fully defined.

However, a series of recent studies, using a mouse model of GSH deficiency demonstrate that the status of chronic oxidant stress (resulting from GSH deficiency) may be beneficial. *GCLM*-null mice have about 15% of normal GSH levels in their livers [53] and despite exhibiting increased oxidant stress are resistant to alcohol-induced lipotoxicity [54]. Mechanistic studies revealed that *GCLM*-null mice exhibit constitutive activation of liver AMP-activated protein kinase (AMPK) signaling and nuclear factor-erythroid 2 related factor 2 antioxidant response. In addition, these mice display acetyl-CoA enrichment, diversion of acetyl-CoA flux from lipogenesis to alterative metabolic pathways, an elevation in glutamate levels, and inductions of the glucuronate pathway and nucleotide biosynthesis [54, 55]. Taken together, the molecular and metabolic features observed in the livers of these mice reflect low GSH-elicited hepatic reprogramming to cope with alcohol-induced cellular stress.

Effects on Homocysteine levels: Experimental and clinical studies of chronic alcohol consumption have consistently documented elevated plasma homocysteine levels [15, 19, 56–62]. That this increase was due to an ethanol-induced impairment in remethylation of homocysteine in hepatocytes was verified by observations of higher secretion by hepatocytes from ethanol-fed rats compared with those of pair-fed controls [63]. Additionally, when isolated hepatocytes were challenged with a methionine load, much higher levels of homocysteine were released from hepatocytes of ethanol-fed rats [63]. The latter-described conditions were used because supplemental dietary methionine elevates plasma homocysteine levels [64] and methionine loading in fasting patients has been used to stress the homocysteine remethylation pathways to detect disturbances in methionine metabolism [65].

Effects on Hepatic SAH Levels: Recent investigations have addressed whether ethanol consumption influences intrahepatic SAH levels. Results have consistently shown a ~ two-fold rise in hepatic SAH levels after ethanol exposure in several experimental models [12, 13, 15, 19, 32, 66, 67].

Detrimental Consequences of Altered Hepatic Methionine Metabolism

Of all the alterations in methionine metabolism by chronic ethanol consumption, the most detrimental changes in liver cells are: (i) increased levels of homocysteine and (ii) Reduction in SAM:SAH ratio, which occurs due to rising hepatocellular SAH levels, which may or may not be accompanied by decreases in hepatic SAM levels.

Defects in Crucial Methylation Reactions: The intracellular SAM:SAH ratio is an important metabolic indicator of cellular methylation status and is also called the methylation potential. A decrease in SAM:SAH ratio in the presence of increased SAH is associated with impaired activities of many of the 120 SAM-dependent methyltransferases [68]. These occur because SAH has a high affinity for binding to the catalytic region of many SAM-dependent methyltransferases, thereby enabling it to act as a potent end product inhibitor; the K_i values for SAH are in the submicromolar to low micromolar range. In fact, recorded K_i for SAH are often lower than the K_m for SAM for many of the methyltransferases [68]. The ethanol induced perturbations in the SAM to SAH levels specifically impairs methyltransferase(s)-catalyzed addition of a methyl group to diverse biologically active molecules that normally serve vital roles in biosynthesis, regulation, repair and detoxification [68]. This ultimately results in compromised liver function and progressive liver injury. Of particular interest is the inhibition of several important enzymes. These are phosphatidylethanolamine methyltransferase (PEMT), isoprenylcysteine carboxyl methyltransferase (ICMT), protein L-isoaspartate methyltransferase (PIMT), protein arginine methyltransferase (PRMT), guanidinoacetate methyltransferase (GAMT) and lysine methyltransferase. PEMT catalyzes the three sequential transfers of methyl groups to phosphatidylethanolamine to generate phosphatidylcholine, an important constituent of very-low-density lipoproteins [69–71]. ICMT is involved in carboxyl methylation of isoprenylated proteins [72], a step that is crucial for activation of these proteins to enable their participation in anti-apoptotic signaling pathways [73]. PIMT is required for catalyzing the repair of isoaspartyl sites of spontaneously damaged proteins, thereby preventing the consequences of their accumulation, as these atypical aspartyl residues compromise the biological function and modify the immunogenicity of the protein [74–77]. PRMT catalyzes the addition of monomethyl or dimethyl groups to the guanidine nitrogen atom of arginine. These methylated arginine proteins regulate protein-protein interactions and signaling events [78]. GAMT catalyzes the transfer of a methyl group from SAM to guanidinoacetate to form creatine in the liver [79] that is transported to distal "creatine requiring" organs where it serves as an important cytosolic phosphorylation potential buffer and energy shuttle [80]. Lysine amino acids on several histones and non-histone proteins are methylated by specific lysine methyltransferases, which modulate protein activity, stability, localization, and/or interaction, resulting in specific downstream signaling and biological outcomes [81]. Our studies have shown that decreased activities of these enzymes results in fat accumulation [12], elevated

apoptosis [82, 83], accumulation of damaged proteins [84, 85], altered signaling [86], reduced creatine synthesis [87] and impaired proteasome activity [88], respectively—all of which are characteristic features of alcohol-related liver injury.

Homocysteine Toxicity: High homocysteine levels contribute to a variety of diseases including cardiovascular disease, diabetes, seizures and neurodegenerative disorders [89]. However, recent studies indicate that SAH is a more reliable predictor of cardiovascular disease than homocysteine [90].

Regarding, its effect on the liver, Torres et al. suggested a possible role of homocysteine in liver fibrosis, based on their observations that increased collagen production and induction of tissue inhibitor of metalloproteinases occurs in cultured hepatic stellate cells exposed to homocysteine [91]. Seminal studies conducted ~20 years ago revealed that ethanol-induced hyperhomocysteinemia activates endoplasmic reticulum (ER) stress-dependent apoptosis and lipid synthesis in hepatocytes [59]. Of 1176 toxicology-related genes examined, they reported increases in glucose-regulated proteins-78 and -94, growth arrest/DNA damage-inducible protein 153 and caspase-12, all indicating that an ER stress response was prominent among the alcohol-responsive genes. Sterol regulatory element binding protein (SREBP-1) and HMG-CoA reductase were also enhanced by alcohol administration [59, 92]. Evidence from ethanol-fed micropigs also supports a correlation between the ER stress response and pathogenesis of alcohol-related liver injury [93]. Homocysteine also enhances production of several proinflammatory cytokines, it generates a procoagulant state and it increases oxidant stress [94–96]. All these hyperhomocysteinemia-induced changes may explain the cellular defects in the liver [19, 59, 97, 98] and brain atrophy observed with ethanol exposure [99].

Interestingly, in alcohol-dependent patients the plasma homocysteine concentration is associated with the degree of alcohol craving and hazardous and harmful patterns of its consumption [100]. Taken together, it appears that alcohol induced hyperhomocysteinemia in addition to causing organ toxicity simultaneously enhances alcohol consumption by increasing the severity of craving in a circular self-reinforcing mechanism.

One question that needs to be clarified is which of the two conditions (decreased intracellular SAM:SAH ratios or elevated homocysteine levels) is more critical to the pathogenesis of alcohol-related liver injury. Arguably, increases in both SAH and homocysteine following ethanol exposure are related. Higher homocysteine levels result in higher levels of SAH by mass action effects via the SAHH reaction, where the equilibrium favors the condensation of homocysteine and adenosine to form SAH (Fig. 55.1). However, some of the reported effects of these two conditions are diverse. Even though both hyperhomocysteinemia and altered SAM:SAH ratios appear to play a role in producing fat accumulation and apoptosis, previous research suggested differing mechanisms of action. Specifically, the altered SAM:SAH ratio appears to predominantly inhibit VLDL secretion via impaired PEMT activity, thereby generating steatosis [12, 101, 102], while hyperhomocysteinemia likely promotes fat accumulation by enhancing lipid synthesis [59].

We portend that elevated SAH levels have a greater impact in the pathogenesis of liver injury. This is because more than 85% of all SAM-dependent transmethylation

reactions occur in the liver. While not all methyltransferases are affected by low SAM:SAH ratios, some of the very important ones including PEMT, PIMT, PRMT, GAMT and lysine methyltransferases, have a lower K_i for SAH compared to the K_m for SAM [68]. All these methyltransferases have a broad range of functions [68], there is a myriad of detrimental consequences when their activities and that of many other critical SAM-dependent methyltransferase are inhibited in the liver. Indeed, the ethanol-induced decline in SAM:SAH ratio via inhibiting specific methyltransferases causes the generation of specific hallmark feature of ALD [12, 82–88, 101] as shown in Fig. 55.2. Further, studies have shown that ethanol-induced DNA and histone methylation changes alter the expressions of several susceptible genes [103–105] and epigenetic memory in relation to liver pathology and Mallory bodies [106, 107]. These studies add a new dimension to potential consequences of defective methylation.

The impact of elevated hepatocellular SAH was further demonstrated by conducting *in vitro* studies by exposing isolated hepatocytes to agents that selectively elevate intracellular SAH levels (without affecting homocysteine levels), such as after exposure to adenosine or deazaadenosine [82, 83]. In such a setting, increased

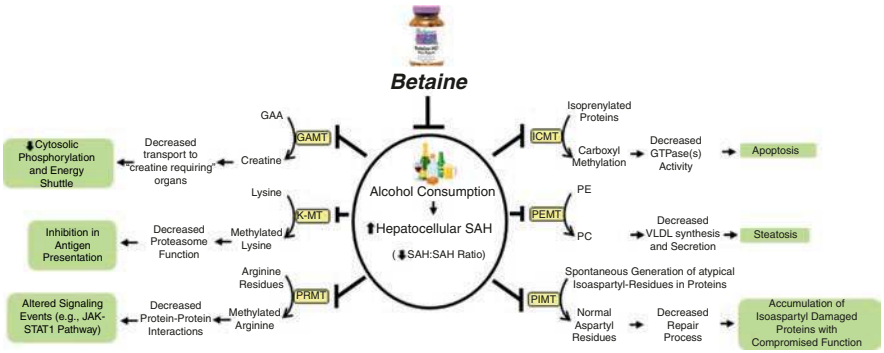

Fig. 55.2 Current scheme of our working hypothesis. Increased hepatocellular S-adenosylhomocysteine (SAH) levels generated by chronic ethanol exposure can negatively affect the activities of the several methyltransferases, phosphatidylethanolamine methyltransferase (PEMT), isoprenylcysteine carboxyl methyltransferase (ICMT), protein-isoaspartate methyltransferases (PIMT), protein arginine methyltransferase (PRMT), guanidinoacetate methyltransferase (GAMT) and lysine methyltransferase. These methylation defects, in turn, lower the synthesis and secretion of very-low-density lipoproteins (VLDL), impaired activation of GTPases, diminished protein repair processes, decreased protein-protein interactions due to reduced arginine methylation, reduced guanidinoacetate methylation and impaired lysine methylation on critical proteins (histones, proteasome), respectively. These deficiencies ultimately contribute to the development of steatosis (fatty liver), increased apoptosis (cell death), accumulation of isoaspartyl-damaged/dysfunctional proteins, altered signaling events, reduced creatine synthesis in the liver and its transport to "creatine-requiring" organs and impaired proteasome activity. Betaine administration prevents the increase in SAH and also prevents these ethanol-induced pathologies. The consequences of alterations in DNA and histone methylation, which influence gene expression and epigenetic memory as well as effects of elevated SAH on many other crucial methylation reactions are not depicted in this figure

apoptosis, steatosis, damaged protein accumulation, altered signaling, reduced creatine synthesis and impaired proteasome activity as seen after alcohol consumption, was observed [82, 83, 85–88]. Recent studies, using similar approaches to selectively elevate intracellular SAH levels, showed that the rise in intracellular triglycerides seen was accompanied by decreased lipolysis and increased expression of factors involved in lipogenesis and fatty acid mobilization [108].

Further support for the role of increased SAH in hepatic injury comes from case-reports of patients with inherited SAHH deficiency. The low liver SAHH activity in the first two cases was reported of brothers who exhibited over 100-fold higher SAH levels that accompanied histological macrovesicular steatosis, "piece-meal" necrosis, and signs of hepatitis and moderate fibrosis. These changes occurred despite only slight elevations in their plasma homocysteine levels [109, 110]. There are other case reports of two sisters with inherited SAHH deficiency that were born with fetal hydrops and had developmental abnormalities [111]. While SAHH deficiency typically presents in infancy, it can remain asymptomatic in childhood but when manifests in adulthood is associated with advanced liver damage, including early onset of HCC [112].

Interestingly, SAHH has recently been shown to have other functions including regulating DNA methylation maintenance at replication sites, mRNA cap methylation at transcriptionally active chromatin regions [113] and controlling circadian gene transcription by interacting with the core clock regulator [114]. This latter aspect illustrates a yet unexplored connection between alcohol-induced circadian rhythm disruption and SAHH.

Role of Altered Methionine Metabolism in Extrahepatic Organs that Promote ALD Pathogenesis

Recent studies demonstrate that ethanol-induced perturbation of hepatic methionine metabolism also occurs in adipose tissue and intestine, two organs whose dysfunction promotes and exacerbates ALD pathogenesis (reviewed in [115–118].

Ethanol consumption elevates adipose SAH levels and consequently decreases adipose SAM:SAH ratio [119, 120]. Mechanistic *in vitro* studies revealed that selective increase of SAH levels in adipocytes caused increased secretion of pro-inflammatory cytokines, accelerated lipolysis to increase the release of free fatty acids while decreasing the production and release of protective adipokines. All these effects mimic those seen after alcohol consumption *in vivo* [120]. Similarly, when intestinal cells were exposed to the intracellular SAH elevating agent, it caused a significant decrease in the localization of the important tight junction protein, occludin, to the apical junctional complex [121]. The consequent loss of tight junction integrity accompanied reduction in transepithelial electrical resistance and increased dextran influx—two endpoints frequently used for measuring epithelial

barrier function [122–124]. Collectively, these studies indicate that alcohol-induced alterations in the methionine metabolic pathway in adipose tissue and the intestine, contributes to the pathogenesis and progression of ALD.

Treatment Strategies

Elevated hepatic levels of SAH or homocysteine are both rectified by inclusion of betaine in the diet [63, 66]. These findings revealed that betaine is a promising therapeutic agent. Furthermore, betaine, by reversing/preventing elevated SAH and hyperhomocysteinemia, also prevents downstream consequences such as steatosis, apoptosis, accumulation of damaged proteins and signaling defects in the liver. These protective effects of betaine have been shown in a variety of rodent models of alcohol toxicity [12, 25, 59] as well as *in vitro* studies [63, 66, 82, 125]. While betaine is well known as an intracellular osmolyte [126], its protective effects in the liver are exerted through the BHMT-catalyzed reaction [82]. Although findings in other laboratories, including ours do not show restoration of GSH levels after betaine treatment [19, 51], there have been reports that betaine treatment may alleviate both the ethanol-induced decline of hepatic GSH and the elevation in oxidant stress [127–129].

Similarly, SAM administration reportedly prevents alcohol-related liver injury, which has been reported rather widely [130–134]. But there are several disadvantages of using SAM to ameliorate liver injury: Because of its rather high molecular weight and its low membrane permeability, SAM has very low bioavailability if given orally or parenterally [135]. Another disadvantage is SAM's prohibitive cost.

Additionally, it should be noted that studies in our laboratory demonstrate that betaine treatment generates SAM *in vivo,* eliminating the need for exogenous SAM supplementation. Specifically, we have shown that betaine feeding doubled hepatic levels of SAM in control rats and increased the levels of SAM four-fold in ethanol-fed rats [12]. This occurs despite continued MS inhibition by ethanol which is not improved by betaine treatment [25]. More important, unlike SAM treatment as reported above, the consumption of betaine by humans has had no adverse effects [126, 136].

Although antioxidants in general, have shown little benefit in the treatment of alcoholic hepatitis, a double-blind randomized control trial of patients with severe alcoholic hepatitis treated with N-acetylcysteine (NAC) and corticosteroids, demonstrated improved 28-day survival, primarily related to lower infection rates. However, no long-term benefits were observed [137]. A meta-analysis of 22 randomized control trials noted that the addition of NAC to corticosteroids may be superior to corticosteroids alone for reducing short-term mortality [138]. Further studies using NAC in alcoholic hepatitis are reportedly underway.

Summary and Future Studies

Ethanol consumption alters multiple steps in hepatic methionine metabolism. These alterations result principally in a decrease in the vital liver metabolite, SAM, while two toxic metabolites, homocysteine and SAH rise. The decline in SAM and increase in SAH leads to a plethora of detrimental functional consequences in the liver particularly those affecting hepatic methylation reactions. Betaine has the unique ability to remethylate homocysteine, thereby restoring hepatic SAM levels and lowering SAH levels. Thus, by correcting defective methylation and decreasing homocysteine levels, betaine prevents or attenuates alcohol-induced steatosis, apoptosis, protein damage, and altered signaling events. In addition, betaine treatment can also prevent ethanol-induced adipose dysfunction and increased gut barrier permeability [139].

To date, no clinical trials have been conducted for treatment of ALD with betaine. However, SAM has been used in several clinical studies, but the outcomes have been unclear and its efficacy in liver diseases continues to be debated [140, 141]. In the future, new treatment modalities for ALD should consider supplementation with betaine which will likely prove to be a promising protective agent. For further reading, see also related chapters encompass Chaps. 49, 58 and 66 on e.g. bone marrow toxicity and the management of alcoholic hepatitis.

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Chapter 56
Mitochondria and Alcohol

Sandra Torres, Paula Segalés, Laura Conde de la Rosa, Carmen Garcia-Ruiz, and Jose C. Fernandez-Checa

Abstract Alcohol-related liver disease (ALD) is a spectrum of liver alterations both at the structural and functional levels that begins with the deposition of lipids in hepatocytes defining the first stage of steatosis, which can progress to alcohol-related steatohepatitis and culminate in cirrhosis and hepatocellular carcinoma. The deleterious effects of alcohol consumption in liver function are determined by the oxidative metabolism of ethanol, which triggers multiple mechanisms, including oxidative and endoplasmic reticulum stress, disruption of methionine metabolism and alterations in mitochondrial function that contribute to the progression of ALD. Being mitochondria essential hubs for energy production and metabolism, alterations of mitochondria at the structural and functional levels are considered a driving force for the onset of steatosis and its progression towards more advanced stages of ALD. Thus, understanding the mechanisms that trigger the alterations of mitochondrial function may open novel opportunities for the identification of poten-

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tial therapies to manage ALD. In the present chapter, we briefly outline the players involved in mitochondrial dysfunction and their impact in ALD, which may stand as promising new targets for intervention.

Keywords Alcohol · Hepatotoxicity · Mitochondria · Oxidative stress · Glutathione

Abbreviations

OGC, SLC25A11	2-oxoglutarate
APAP	Acetaminophen
ASMase	Acid sphingomyelinase
ATP	Adenosine triphosphate
ADH	Alcohol dehydrogenase
ASH	Alcoholic steatohepatitis
ALD	Alcohol-related liver disease
ALDH2	Aldehyde dehydrogenase 2
AMPK	AMP-activated protein kinase
CO_2	Carbon dioxide
CK2	Casein kinase
CYP2E1	Cytochrome P450 2E1
CYP27A1	Cytochrome P450 family 27 subfamily a member 1
CYP7A1	Cytochrome P450 family 7 subfamily a member 1
DAMPs	Damage-associated molecular patterns
DIC	Dicarboxylate
DRP1	Dynamin-related protein 1
ETC	Electron transport chain
ER	Endoplasmic reticulum
ERAD	ER-associated degradation
FA	Fatty acid
GI	Gastrointestinal
GSH	Glutathione
Gpx	Glutathione peroxidase
HO-1	Heme oxygenase
HSC	Hepatic stellate cell
HCC	Hepatocellular carcinoma
HMGB1	High-mobility group box 1 protein
H_2O_2	Hydrogen peroxide
HHCy	Hyperhomocysteinemia
iNOS	Inducible nitric oxide synthase
IMM	Inner mitochondrial membrane

ICAM-1	Intercellular adhesion molecule-1
IL	Interleukin
IMS	Intermembrane space
KCs	Kupffer cell
LPS	Lipopolysaccharides
LDL	Low-density lipoprotein
mTOR	Mammalian target of rapamycin
MAT	Methionine adenosyl transferase
MS	Methionine synthase
MEOS	Microsomal Ethanol-Oxidizing System
MAMs	Mitochondria-associated membranes
mtDNA	Mitochondrial DNA
mGSH	Mitochondrial GSH
Mfn1/2	Mitofusin 1 and 2
MSP	Mitochondria shaping proteins
NAC	N-acetyl-L-cysteine
NAD^+	Nicotinamide adenine dinucleotide
NASH	Nonalcoholic steatohepatitis
Nrf-2	Nuclear factor erythroid 2-related factor 2
Opa-1	Optic atrophy 1
OMM	Outer mitochondrial membrane
OXPHOS	Oxidative phosphorylation
O_2	Oxygen
PA	Palmitic acid
PAMPs	Pathogen-associated molecular patterns
PP	Periportal
PV	Perivenous
Prx	Peroxiredoxin
PPARs	Peroxisome proliferator-activated receptors
PIN1	Peptidyl-prolyl cis/trans isomerase
(PAI)-1	Plasminogen activator inhibitor
PDGF	Platelet derived growth factor
ROS	Reactive oxygen species
SAM	S-adenosylmethionine
StARD1	Steroidogenic acute regulatory protein 1
SREBPs	Sterol regulatory element-binding proteins
SOD	Superoxide dismutase
Trx2	Thioredoxin2
TGF-β	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
UPR	Unfolded protein response
VLDL	Very-low-density lipoprotein
VDAC	Voltage-dependent ion channel

Introduction

Alcohol-related liver disease (ALD) is the most prevalent type of chronic liver disease in the world (see also part I of the book). Disproportionate alcohol drinking is a global healthcare concern with immense social, economic, and clinical consequences, accounting for up to three million deaths per year worldwide according to the World Health Organization. Excessive alcohol consumption over decades harms practically every organ in the body. However, the liver undergoes the earliest and the greatest tissue impairment grade from excessive drinking since it is the principal organ involved in ethanol metabolism [1]. Ethanol is an important source of energy, with 7.1 kcal (29.7 kJ) per gram, an amount that exceeds the energy content of carbohydrates or proteins. On average, ethanol represents half of the caloric intake of a chronic drinker, displacing normal nutrients, which causes malnutrition, and vitamin deficiencies. In addition, malnutrition is also due to malabsorption and nutrient impaired hepatic metabolism, as a consequence of a direct hepatotoxic effect of ethanol, as recognized over 6 decades ago [2].

Although ALD pathogenesis is still incompletely known, which has limited the availability of effective therapies, there have been important milestones in the identification of mechanisms involved in ALD progression, from the initial stage of hepatic steatosis towards its final stage of hepatocellular carcinoma (HCC). The oxidative metabolism of alcohol triggers a variety of players that elicit an unbalance between the biogenesis and catabolism of lipids, what translates in the initial stage of hepatic steatosis, that along with other mechanisms, such as oxidative and endoplasmic reticulum (ER) stress, disruption of methionine metabolism and mitochondrial dysfunction, contribute to the development of advanced stages of ALD, such as alcohol-related steatohepatitis (ASH), characterized by hepatocellular injury, inflammation and fibrosis. The combination of these ethanol-derived mechanisms with other players, including innate immunity, epigenetics and inflammasome activation drive the onset towards the final stage of ALD, such as alcohol-related HCC development.

Clinical and Histological Characteristics of ALD

Stages

ALD is worldwide recognized as a complex disease provoked by alcohol excessive intake over years comprising a range of stages including simple steatosis, steatohepatitis, cirrhosis and end-stage HCC (Fig. 56.1), although an overlap between them can generally be observed [3–5].

An accumulation of fat in the liver is induced by just a few days of drinking large volumes of alcohol. Fatty liver, the earliest liver response to alcohol abuse, rarely

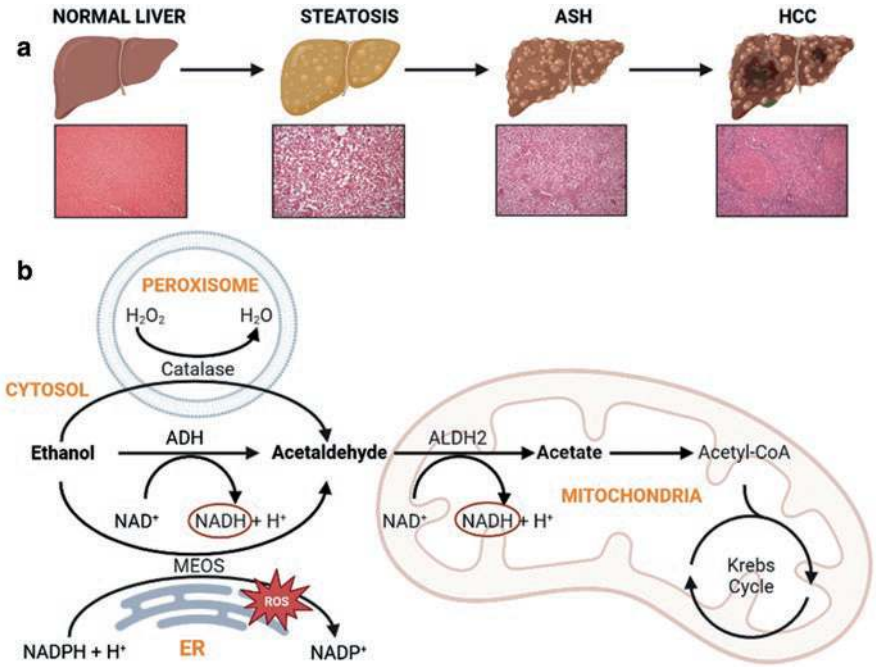

Fig. 56.1 (**a**) Stages of ALD. Alcohol intake induces a wide spectrum of hepatic lesions. Fatty liver (steatosis) is the earliest liver response to alcohol abuse. Although it develops in more than 90% of heavy drinkers, rarely causes any symptoms and it is often reversible with abstinence or moderation in alcohol intake. Continued alcohol consumption induces the progression of ALD to liver inflammation (ASH), fibrosis and even HCC. (**b**) Alcohol metabolism pathways in hepatocytes. Ethanol is metabolized mainly in hepatocytes of the liver. The enzymes ADH, the main ethanol detoxification pathway located in the cytosol, and ALDH2, located in the mitochondria, catalyze sequential oxidations, which convert ethanol to acetate, forming two mole equivalents of NADH. The major inducible pathway in ethanol metabolism is the CYP2E1, a major component of the MEOS, located in the endoplasmic reticulum, which oxidizes ethanol in the presence of molecular oxygen (O_2) to acetaldehyde and transforms reduced NAD phosphate (NADPH) to NADP+ and generates water. Peroxisomal catalase is a minor hepatic pathway of ethanol metabolism that utilizes H_2O_2 to oxidize ethanol to acetaldehyde and water. Ethanol metabolism elevates ROS production, which contribute to oxidative stress and can interact with other cellular molecules forming adducts (proteins, lipids or DNA). *ADH* alcohol dehydrogenase, *ALDH2* aldehyde dehydrogenase 2, *ASH* alcoholic steatohepatitis, *CYP2E1* cytochrome P450 2E1, *H_2O_2* hydrogen peroxide, *HCC* hepatocellular carcinoma, *MEOS* microsomal ethanol-oxidizing system, *ROS* reactive oxygen species

causes any symptoms and it is often reversible with abstinence or moderation in alcohol intake [6]. Alcohol promotes hepatic fatty acid (FA) uptake, FA oxidation impairment, induction of *de novo* lipid synthesis and neutral lipid storage, inhibition of lipid export and lipid droplet catabolism, driving hepatic fat accumulation.

Alcoholic steatohepatitis (ASH) is a potentially serious condition that can be caused by alcohol after years of heavy consumption. It is associated with hepatocyte ballooning and reactive oxygen species (ROS) production [7, 8]. In addition, excessive alcohol intake induces intestinal bacterial overgrowth, endotoxins accumulation and increased intestinal permeability by impairing the intestinal barrier function, which allow the translocation of bacterial products from the intestine to the liver, driving the activation and recruitment of inflammatory cells to this organ, and increasing inflammation. Approximately 20–40% of patients with steatosis present liver biopsies with additional histological changes showing hepatocellular damage accompanied with fibrosis and inflammation, indicative of ASH. Unlike ASH, which can be reversible upon abstinence or moderating alcohol drinking, severe alcoholic hepatitis (AH or sAH), characterized by massive inflammation and multiple organ failure is a life-threatening illness with a high probability of death within a few months of diagnosis [9, 10].

Alcohol-related liver fibrosis and cirrhosis are advanced stages in ALD and occur when the liver has been inflamed for a long period of time, driving to scarring and loss of function. Excessive and long-term alcohol oxidation damages hepatocytes structure, causing microtubule dysfunction and disruption in the transport of nutrients. Moreover, hepatic stellate cell (HSC) activation is a key step in the pathogenesis of alcoholic liver fibrosis, in which collagen synthesis is induced and extracellular matrix proteins accumulate. This event compromises the ability of the liver to detoxify and metabolize xenobiotics and it becomes more sensitive to medications and continued alcohol drinking. Cirrhosis stage is irreversible, and although exacerbated organ damage can be controlled by preventing continued alcohol intake, the only treatment available to increase life-expectancy is liver transplantation [11]. The onset of alcohol-related cirrhosis with obesity, diabetes and active alcohol drinking or viral-hepatitis increases the odds of developing ALD-driven HCC [12–15].

Risk Factors

The probability to develop ALD increases with augmenting daily alcohol intake with a threshold of 12–22 g/day in women and 24–46 g/day in men [16], although correlation is not dose-dependent [17]. Among heavy drinkers up to 90% develop steatosis, but only a minority progresses to steatohepatitis, and 10–20% eventually suffer cirrhosis. Many factors influence the development of ALD, in particular age [18], gender, pattern, duration and type of alcoholic beverage consumed, and ethnicity factors [19–22]. Other associated risk factors include nutritional factors [23], obesity [24], iron overload, concomitant infection with viral hepatitis [15], smoking and genetic factors. More details are find in other parts of the book, especially in Part IV.

Hepatic Zonal Pattern in ALD

Several liver pathologies display specific patterns that can be attributed to the functional zonation of hepatocytes [25]. ALD develops in a zonal pattern beginning from the pericentral or perivenous (PV) area, which disseminate into the periportal (PP) zone as the disease progresses [26–28]. ALD progression starts with the accumulation of lipid droplets, promoting hepatic steatosis. Alcohol-promoted lipid accumulation might be faster in PV regions than in PP zone due to the increased expression of lipogenesis genes and reduced expression of FA β-oxidation genes in PV hepatocytes compared to those in PP regions [25, 28]. Accordingly, in models of ethanol intoxication in rats, the overload of lipid droplets is detected in first place in the PV zone compared to the PP area [29]. Analogous PV zonation patterns have been observed in human ALD patients [30]. Furthermore, an elevated activity of the key alcohol metabolizing enzymes alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1) has been found in the PV zone, not only in basal conditions but also after long-term alcohol intake [31]. Thus, alcohol detoxification principally occurs in the PV area being the most affected zone in early stages of the disease. Thus, it is of critical importance to consider liver zonation when investigating specific liver diseases.

Hepatic Alcohol Metabolism and Pathological Effects

Alcohol Metabolism

Alcohol is a polar substance soluble in both water and lipid. After being consumed, it is absorbed through the gastrointestinal (GI) tract into the blood circulation. Following absorption in the GI, only 2–10% of total consumed alcohol is directly removed through the lungs, kidneys and sweat in its untransformed form. Thus, more than 95% of the alcohol ingested will experience metabolic processing in the liver.

Hepatocytes are responsible for alcohol metabolism, as they possess the principal ethanol oxidizing enzymes, ADH, which resides in the cytosol, and CYP2E1, which is located in the smooth ER. Hepatocytes also express high catalase levels, an enzyme located in peroxisomes.

In the liver, ADH is the most catalytically effective ethanol-oxidizing enzyme. ADH oxidizes ethanol using nicotinamide adenine dinucleotide (NAD+) as a cofactor, producing reduced NAD+ (NADH) and acetaldehyde, which is highly reactive and toxic. Acetaldehyde can covalently bind to proteins [32], lipids [33], and nucleic acids [34] to produce acetaldehyde adducts, which, in turn, can affect the structure and function of these macromolecules [35, 36], promoting mutations and carcinogenesis [37]. To minimize acetaldehyde toxicity, hepatocytes quickly oxidize it to acetate via aldehyde dehydrogenase 2 (ALDH2) inside mitochondria, producing

NADH and acetate. The elevated production of NADH by both ADH and ALDH2 reduces the cellular redox potential (intra hepatocyte NAD+ /NADH ratio), which alters from oxidative metabolism toward reductive synthesis, favoring the formation of FAs and causing fatty liver development [38].

Microsomal Ethanol-Oxidizing System (MEOS) is an alternative pathway to oxidize ethanol to acetaldehyde, which depends on cytochrome P450 (CYP450) enzymes, particularly CYP2E1. The catalytic efficiency of CYP2E1 is substantially slower than that of ADH, but it has a ten-fold higher affinity towards ethanol. Under normal physiological conditions, CYP2E1 oxidizes a small amount of ethanol (about 10%) into acetaldehyde. Remarkably, CYP2E1 is an inducible enzyme and its hepatocellular content increases during chronic ethanol intake [39, 40], accumulating in the smooth ER. CYP2E1 induction has diverse important effects in heavy drinkers, as they develop a metabolic tolerance to alcohol. In addition, together with acetaldehyde CYP2E1 also generates ROS, such us hydroxyethyl radicals, superoxide anions and hydroxyl radicals. Constant ROS production overcomes the detoxifying capacity of the liver driving to oxidative stress. Indeed, different animal studies described that chronic ethanol intake reduces the activities and/or amount of diverse antioxidant enzymes [41–43]. The dynamic imbalance of antioxidant systems affects the crucial antioxidant regulatory gene nuclear factor erythroid 2-related factor 2 (Nrf-2) in ALD [44], superoxide dismutase (SOD), glutathione (GSH), catalase, peroxidase-1, metallothionein, and heme oxygenase (HO-1) [45, 46]. Moreover, ROS endure secondary reactions with proteins and unsaturated lipids, which, in turn, react with each other and acetaldehyde exacerbating oxidative stress and triggering an immune response [47, 48]. Of clinical relevance, the induction of CYP2E1 by chronic alcohol drinking can accelerate/enhance xenobiotic metabolism, especially from drugs that are actively biotransformed by the CYP2E1 like acetaminophen (APAP) [49]. This convergence of alcohol and APAP on CYP2E1 metabolism implies that alcohol drinking can sensitize to APAP hepatotoxicity.

The third metabolic pathway system that relies on NADPH for alcohol oxidative metabolism to acetaldehyde involves the enzyme catalase, which, in the presence of ethanol, uses hydrogen peroxide (H_2O_2) to form acetaldehyde [50, 51]. Ethanol metabolizing role of catalase is minor in the liver, but has an important function in the brain [52] (Fig. 56.1).

Pathological Effects

The pathogenesis of ALD is multifactorial and involves several events such as steatosis, hepatotoxicity and inflammation. Chronic alcoholic consumption affects primarily hepatocytes and other liver cells, such as activated HSCs that proliferate and produce collagen that contributes to liver alcoholic fibrosis [53] (Fig. 56.2). In the following section, we will briefly describe the main effects of ethanol metabolism.

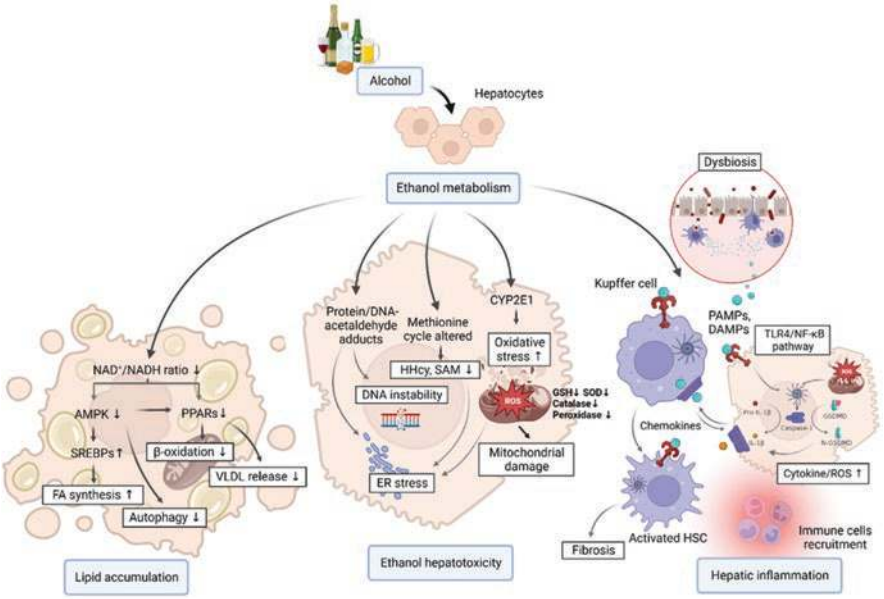

Fig. 56.2 Mechanisms involved in ALD. Lipid accumulation, hepatotoxicity and inflammation are the multiple effects involved in the development of ALD. Ethanol metabolism produces a decrease in NAD^+ /NADH ratio, an increment of SREBPs, and a reduction of AMPK, PPARs and autophagy pathways, leading to FA synthesis, and a decrease of beta-oxidation and VLDL release in hepatocytes. Furthermore, ethanol-induced protein and DNA-acetaldehyde adducts formation causes DNA instability and ER stress. In addition, the methionine cycle is altered with the reduction of SAM levels and HHcy. Moreover, the increase of CYP2E1 expression produces an increment of ROS and ends up in mitochondrial damage. Not only the hepatocytes are affected by the alcohol metabolism, the enteric dysbiosis produces PAMPs and DAMPs that activate KCs and HSC cells and cause the recruitment of immune cells producing hepatic inflammation that can progress to advanced stages of ALD. *ALD* alcohol-related liver disease, *AMPK* AMP-activated protein kinase, *CYP2E1* cytochrome P450 2E1, *DAMPs* damage-associated molecular patterns, *ER* endoplasmic reticulum, *FA* fatty acid, *HHCy* hyperhomocysteinemia, *HSCs* hepatic stellate cells, *KCs* kupffer cells, *PAMPs* pathogen-associated molecular patterns, *PPARs* peroxisome proliferator-activated receptors, *ROS* reactive oxygen species, *SAM* S-adenosylmethionine, *SREBPs* sterol regulatory element-binding proteins, *VLDL* very-low-density lipoprotein

Lipid Accumulation

One of the earliest responses of the liver during alcohol consumption is the lipid accumulation that involves molecules and regulatory pathways related to lipid synthesis, oxidation and very-low-density lipoprotein (VLDL) release [51]. Among the myriad well-known key players in lipid alteration by alcohol abuse are the decrease in NAD^+/NADH ratio, the increment of the expression of sterol regulatory element-binding proteins (SREBPs), the downregulation of peroxisome proliferator-activated receptors (PPARs) and AMP-activated protein kinase (AMPK), and in long-term ethanol consumption a decrease in autophagy is promoted.

A decreased NAD^+/NADH ratio is a main direct consequence of alcohol intake. The coenzyme NAD^+ is used as a cofactor for the transfer of hydrogen in the oxidation of ethanol to acetaldehyde and acetate and produces NADH. Since many of the enzymes of FA oxidation are pyridine nucleotide dependent NAD^+/NADH ratio promotes triglyceride accumulation in the liver by reducing FA oxidation and enhancing FA synthesis [54–56].

Another ethanol-impaired signaling pathway involved in hepatic steatosis is the downregulation of PPARs. PPARs are members of steroid/retinoid nuclear receptor superfamily involved in the regulation of lipid and lipoprotein levels, and consequently the transcription of genes involved in the esterification and VLDL release to be oxidized in the mitochondria, peroxisomes and microsomes. Once the heterodimer with retinoid-X receptor is formed, PPARα binds to DNA and interferes in the transcriptional activity and decreases PPARα target genes related to VLDL export, overall leading to the accumulation of FAs [57–60].

Another important player in ethanol-induced steatosis is the induction of SREBPs. SREBPs are a family of transcription factors that regulate the synthesis of FAs, triglycerides and cholesterol. These transcription factors undergo a proteolytic processing involving a crosstalk between the ER and Golgi. SREBPs are ER-resident proteins and ER stress caused by alcohol intake (see below) promotes the generation of mature SREPPs. SREBP-1 plays an important role regulating the genes of hepatic triglyceride synthesis [61] and SREBP-2 is responsible for regulating genes of cholesterol metabolism [62]. Activation of SREBP-1 by ethanol feeding and the production of acetaldehyde promote the synthesis of FAs in the liver which results in FA accumulation in hepatocytes [61, 63].

In addition to the outcomes described above, through the downregulation of AMPK, chronic ethanol exposure downregulates PPARα, decreasing FAs beta-oxidation and inflammation by acting on acetyl Coenzyme A carboxylase and carnitine palmitoyltransferase [64], which complement the lipid storage effect of alcohol intake [65–67]. Moreover, AMPK can be activated by ROS and promotes cell survival by inducing autophagy, mitochondrial biogenesis and increasing the expression of the genes involved in antioxidant response. Autophagy is a genetically programmed degradation of damaged organelles and proteins, with mammalian target of rapamycin (mTOR) as a key regulator [68, 69]. Chronic ethanol feeding decreases AMPK and thus, reduces autophagy activity and consequently it produces lipid accumulation through an impaired lipophagy [70, 71].

Hepatic Inflammation

Hepatic inflammation has a central role in the pathogenesis of ALD. Ethanol feeding causes imbalance of intestinal flora that produces gut-derived pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS), from the bacterial overgrowth and enteric dysbiosis. Alcohol consumption also causes hepatocyte death, producing damage-associated molecular patterns (DAMPs), such

as mitochondrial DNA (mtDNA) and high-mobility group box 1 protein (HMGB1) [72]. DAMPs and PAMPs activate the innate immunity and the release of cytokines and chemokines from KCs, macrophages and neutrophils. Aside from this, the alcohol metabolism occurs primarily in the gram-negative bacteria and intestinal epithelial cells, leading to acetaldehyde accumulation, which damages the intestinal barrier. In addition, alcohol also increases adaptive immune responses such as protein adducts with acetaldehyde and ROS.

PAMPs derived from gut microbiota and DAMPs from damaged cells are recognized by Toll-like receptors and stimulate the production of a large number of nuclear transcription factors (e.g., NF-κB) and inflammatory cytokines, but also induce overproduction of NO by the inducible nitric oxide synthase (iNOS), and this disrupts the barrier integrity and its function [73]. The increase of endotoxins that can across the intestinal barrier can activate KCs, HSC and other inflammatory cells, which produce pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin IL-1β, IL-17, IL-6, MCP1, CXC chemokines, osteopontin and inflammatory factors and free radicals [3]. The neutrophils migration is a histological hallmark in ALD, promoted by the chemokines produced by KCs and HSC, which have an increased expression of intercellular adhesion molecule-1 (ICAM-1) on the surface and is accompanied by E-selectin expression on sinusoidal endothelial cells [74]. The recruitment of neutrophils by KCs, induces the release of key fibrosis markers from HSC, such as transforming growth factor beta (TGF-β), platelet derived growth factor (PDGF), IL-1β and TNFα, among them, overall leading to a fibrogenic response, mitochondrial dysfunction, intrahepatic inflammation and hepatocyte damage [75, 76].

Mechanisms of ethanol's Deleterious Effects

Chronic alcohol consumption affects different molecules, pathways and subcellular compartments that play significant roles in alcohol-derived hepatotoxicity. Acetaldehyde is the first metabolite derived from the oxidative metabolism of ethanol. Acetaldehyde is extremely toxic and a carcinogen by its reactivity to form adducts with proteins and DNA, causing damage to hepatocytes. Acetaldehyde can bind to structural and functional proteins, such as albumin, tubulin, collagen and microsomal enzymes, leading to a defective assembly of microtubules, protein excretion and enzymatic activity. In advanced ALD, these protein-acetaldehyde adducts are found in HSC and myofibroblasts, being associated with fibrosis and cirrhosis [77]. As mentioned above, chronic alcohol use results in oxidative stress through the CYP2E1 metabolism not only producing acetaldehyde but also generating ROS, which induce ER stress and increase hepatocyte sensitivity to TNF-α [78].

Besides acetaldehyde, ethanol metabolism recruits and activates other important pathways involved in the progression of ALD from its initial stage of steatosis.

– *Oxidative stress and antioxidant systems*: In physiological conditions, the homeostasis of ROS involves different cellular pathways and functions. In

chronic alcohol users, the sustained oxidative metabolism directly induces an excessive accumulation of ROS in the hepatocytes, such as H_2O_2 and superoxide anions. Moreover, alcohol-induced ROS are also generated through inflammation and the recruitment of immune cells and the generation of pro-inflammatory cytokines. Furthermore, ROS can bind proteins and generate neoantigens that induce a host immune response [79]. As indicate above, in alcohol exposure, the Nrf-2, a factor that regulates the expression of antioxidant proteins, is upregulated to counterbalance the stimulation of ROS generation due to the induction of important antioxidant genes, such as SOD, GSH, catalase, peroxidase-1, metallothionein and heme oxygenase [11, 44, 45]. However, the constant generation of ROS due to chronic alcohol intake can exceed the antioxidant strategies leading to the onset of oxidative stress. In addition, increased prooxidant species (e.g. H_2O_2) can also generate an unbalance between antioxidant enzymes, such as MnSOD and the GSH redox cycle in mitochondria, resulting in a net onset of oxidative stress [80].

– *Methionine cycle*: Methionine is an essential amino acid, substrate for methionine adenosyl transferase (MAT1A) to produce S-adenosylmethionine (SAM), which is the methyl donor for methylation reactions (DNA, proteins, lipids and histones) and polyamine synthesis (see also respective chapter on methionine metabolism in Part IV and biochemical pathways in Appendix). In ALD, methionine metabolism is affected by decreased activity of the enzymes MAT1A and methionine synthase (MS), causing a reduction of SAM, hyperhomocysteinemia (HHcy) and with resultant nucleotide imbalance and DNA instability [81]. The reduction of SAM levels impact in the liver function [82]. In transsulfuration reactions, homocysteine is metabolized to produce cysteine and GSH, the principal antioxidant for the defense against oxidative liver injury [83]. HHcy has been shown to activate ER stress in the pathogenesis of ALD [84], promoting apoptosis, steatosis and inflammation [85, 86].
– *ER Stress*: The ER regulates posttranslational protein processing, trafficking and maturation of membrane and secretory proteins. ER stress is induced in conditions of accumulation of unfolded or misfolded proteins in the ER lumen, thereby leading to lipid accumulation, inflammation and cell death [87]. ER stress triggers unfolded protein response (UPR) that acts to inhibit protein synthesis and to increase the efficiency of chaperone proteins. Ethanol exacerbates ER stress responses by different mechanisms such as acetaldehyde-protein adduct formation, oxidative stress and alcohol-induced HHcy, and promotes liver damage [88]. Ethanol aggravates palmitic acid (PA)-induced ER stress response in primary rat hepatocytes and in a high-fat diet-treated mouse model [89]. Furthermore, other studies showed that CHOP knockout mice, one of the ER stress and apoptosis involved genes, have an attenuated liver damage and a reduced steatosis after ethanol exposure [90]. As mentioned above, ER stress is also a trigger for the activation of transcription factors SREBPs, and thus, besides promoting liver damage and inflammation, alcohol-induced ER stress also contributes to the accumulation of lipids.

– *Mitochondrial damage*: Alcohol overconsumption alters mitochondrial function in the liver, which has a wide-range impact due to the crucial role of these organelles in energy production and metabolism [91], which is described in the following section.

Mitochondria and ALD

Mitochondria are the energy centers of the cell designed to generate energy for myriad cell functions and critical hubs for metabolism and a strategic center of cell fate decisions. Mitochondria functions are affected by the oxidative metabolism of alcohol, and hence this event is considered as a causal role in the progression of ALD. In the following sections, we will briefly review the properties and structure of mitochondria and summarize what functions of mitochondrial physiology are altered in ALD.

Mitochondrial Properties and Structure

Mitochondria are specialized compartments found in most eukaryotic organisms. Their size is variable but the area is commonly between 0.75 and 3 μm^2. This organelle is known for being the main source of energy metabolism within cells. Their main function is to convert O_2 and nutrients into carbon dioxide (CO_2) and water in order to produce adenosine triphosphate (ATP), the main energy source of the cells.

To accomplish these roles, mitochondria rely on a cross-talk between nuclear genome and the mitochondrial circular DNA (mtDNA). Most of the mitochondrial proteins that sustain mitochondrial function derive from the nuclear DNA, with the mtDNA encoding for just a handful of components of the respiratory chain. The mtDNA shows substantial similarity to bacterial genomes and it is hypothesized to originate from an endosymbiotic event occurring 1.5 billion years ago in which an archaea host engulfed an α-proteobacterium ancestor. Mitochondria have retained their small genome throughout evolution in order to drastically improve cell energy production. Human mtDNA is a 16.5 kb circular dsDNA lacking introns and residing within the mitochondrial matrix [92, 93].

Structurally, mitochondria are composed of two membranes and two compartments, each one carrying out specialized functions [94, 95].

Outer mitochondrial membrane (OMM): the outer membrane separates mitochondrial content from the cytosol. Regarding membrane composition, the OMM is smooth and very similar in lipid composition to eukaryotic cell membranes. It is mainly formed by phospholipids of unsaturated FAs such as phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol. Another main component of this membrane are the membrane proteins called porins. Their main function is to allow the freely passage of ions and small molecules in order to allow mitochondrial

signaling events. The main transporter of these small molecules from the cytosol to the intermembrane space is the voltage-dependent ion channel (VDAC) [96]. The import of larger proteins (more than 5000 Da) required to be tagged with an amino-terminal sequence, which binds to a subunit called translocase that actively moves the proteins into the mitochondria. An example of this receptor system is the commonly known as TOM20 [97].

The outer membrane also hosts a number of enzymes with a wide variety of functions. In addition, the OMM establishes membrane contact sites with other subcellular compartments, including the ER, lysosomes, peroxisomes, endosomes, melanosomes, lipid droplets and the plasma membrane.

Inner mitochondrial membrane (IMM): Unlike OMM, the IMM is fairly impermeable to solutes due to the lack of porins, and this event requires specific carriers for transport into the matrix. This property is exemplified by the transport of GSH from cytosol to mitochondrial matrix (see below), which is of significant relevance for ALD. IMM has a unique lipid composition, characterized by low cholesterol content and enriched in cardiolipin (10–20%). This exclusive lipid of the IMM is characterized by four unsaturated fatty acyl chains and is essential in the performance of mitochondrial energy generation in the respiratory chain and in the maintenance of mitochondrial IMM structure [98]. While impermeable to most solutes, the IMM is permeable to O_2, CO_2 and water. The IMM is characterized by its invaginations called cristae that penetrate the mitochondrial matrix providing a large surface area for chemical reactions to occur. The cristae of the IMM are the principal site of oxidative phosphorylation (OXPHOS), since they host all the complexes implicated in mitochondrial respiration, including the mitochondrial respiratory chain and the ATP synthase. Hence, the primary function of the IMM is linked to bioenergetics, where the free energy stored in the reducing equivalents produced in the Krebs cycle is converted into ATP. Cristae organization ensures the optimal conditions for ATP production, minimizing the diffusion of metabolites, protons and ADP during respiration. Apart from being a chemical barrier, IMM is also an electrical insulator since it insulates the membrane potential generated by the action of the enzymes of the electron transport chain (ETC) [99].

Intermembrane space (IMS): This is a ~ 20 nm space that lies between the OMM and IMM. All matrix proteins imported into the mitochondria from the cytoplasm must pass through the OMM and IMM and therefore also through the IMS. Because the OMM is freely permeable to small molecules, the concentrations of ions and sugars in the IMS is more similar to that of cytosol. However, the protein composition of this space is different from the protein composition of the cytosol. It has a high concentration of protons due to their pumping by the ETC. This region allows oxidative phosphorylation to occur in it.

Mitochondrial matrix: This is the innermost compartment surrounded by the IMM. It contains a highly concentrated mixture of enzymes, mitochondrial ribosomes, RNA and several copies of the mtDNA. Most of the crucial metabolic pathways for mitochondrial function (Krebs cycle, FAs β-oxidation, alternative bile acid synthesis and steroid synthesis) take place in this compartment. It is a site for the

production of ATP with the help of the enzyme ATP synthase present in the IMM and is also the site of organelle DNA replication, transcription and protein biosynthesis.

Alterations of Mitochondrial Function in ALD

Mitochondrial Dynamics and ALD

Mitochondria are highly complex organelles, which can also move along the cytoskeleton and regulate their morphology by fusion and fission in a process named mitochondrial dynamics. This process is important not only for maintaining mitochondrial performance but also for coordinating metabolism and cell signaling [94]. Mitochondria move to specific destinations by attaching to the microtubular apparatus. Their proper distribution within the cell allows the correct execution of mitochondrial functions. Apart from being transported within the cell, mitochondria can also modulate their shape with fusion and fission events. The balance between fission and fusion has critical roles in maintaining mitochondrial homeostasis in response to metabolic or environmental stresses, and is linked to cell division, apoptosis, and autophagy. A high fusion activity leads to mitochondrial elongation, promotes the capacity of OXPHOS and allows redistribution of mtDNA between damaged and healthy mitochondria. This fragmentation and shape of mitochondria are precisely controlled by mitochondria shaping proteins (MSP). These include mitochondrial proteins such as Mitofusin 1 and 2 (Mfn1/2), Optic Atrophy 1 (Opa1) and the cytosolic dynamin-related protein 1 (Drp-1). These proteins influence not only the shape of mitochondria, but also the functions and cellular signaling cascades. Mfn1/2 and Opa1 are the proteins involved in the process of organelle fusion, whereas cytosolic Drp1 has a main role in mitochondrial fission [100–102].

One of the earliest features of mitochondrial structure alterations reported in patients with ALD was the presence of **megamitochondria** (see also Appendix Figs. A.33, A.34 and A.35), which were associated with a mild form of liver disease [103]. The significance of this characteristic feature was not understood until the uncovering of the machinery responsible for the regulation of mitochondrial dynamics. In an elegant study, using cell lines and knockout mice Palma et al., uncovered a critical role for Drp-1 in shaping the size of mitochondria and in the control of alcohol-induced liver injury [102]. While exposure of VL-17A cells exhibited hyperfragmentation of mitochondria, the deletion of Drp-1 in this cell line prevented this effect and increased cell growth. Moreover, mice with liver-specific Drp-1 deletion exhibited the presence of megamitochondria and decreased alcohol-induced liver injury, lending strong support for a role of Drp-1 in the control of mitochondrial dynamics in ALD. In line with these findings, patients with alcoholic hepatitis exhibited increased expression of Drp-1, findings that paralleled the outcome reported in human precision-cut liver slices exposed to increases doses of alcohol

[101]. Thus, overall, these findings suggest that alcohol intake promotes mitochondrial hyperfragmentation, which translates in a more severe state of liver injury, and indicate that the modulation of mitochondrial dynamics can be a novel target for ALD management.

Mitochondrial Respiration and ALD

Exposure to ethanol has been demonstrated to alter mitochondrial OXPHOS. Early studies in rats revealed that oral alcohol intake decreases mitochondrial respiration (state III) and lowered the respiratory control ratio (state III/state IV) in isolated mitochondria [105]. The findings were accounted for by the downregulation of the synthesis of subunits of the main respiratory complexes such as NADH dehydrogenase, cytochrome b-c1 (Complex III) and the ATP synthase complex (Complex V) [104]. Although these pioneering findings in alcohol-exposed rat liver mitochondria have documented impaired respiration due to different mechanisms [105], recent findings in mice fed alcohol showed opposing effects, in which alcohol feeding either in oral or in intragastric models increased state III respiration in liver mitochondria from mice fed alcohol [106]. Interestingly, the increase in state III respiration was more robust in the intragastric alcohol-feeding model, and correlated with enhanced liver injury, compared to the milder outcome seen in the oral alcohol intake model. This finding was associated with enhanced levels of complexes I, IV and V incorporated into the respiratory chain and reflected the effects of alcohol in increasing the expression of PGC1α, a master regulator of mitochondrial biogenesis. While the effect of alcohol intake in eliciting enhanced mitochondrial biogenesis remains to be formally demonstrated, these findings suggest that the stimulatory effect of alcohol intake in mitochondrial function is related to the replenishment of NAD^+ from NADH oxidation to accelerate the oxidative metabolism of ethanol. Feeding the electrons from NADH to the respiratory chain to oxidize NADH to NAD^+ in mice mitochondria would stimulate the generation of ROS from the enhanced consumption of O_2 in the ETC. Of interest, the increased stimulation of respiration in mice versus rats parallels the species-dependent sensitivity towards alcohol induced liver injury, being greater in mice than in rats, and parallels the enhanced replenishment of NAD^+ from NADH in the mitochondria, suggesting that an increased respiration couples alcohol intake with enhance metabolism and subsequent ROS generation. The relevance of the putative alterations of mitochondrial respiration to human ALD remains to be determined. Although systematic studies addressing the status of liver mitochondrial function in patients with ALD have not been established, there has been indirect evidence suggesting decreased mitochondrial function in patients with ALD. Using assays determining the decarboxylation state of ketoisocaproate as a surrogate way to measure mitochondrial function [107], pioneering observations indicated that alcoholic patients exhibited reduced peak exhalation of $^{13}CO_2$ from 2-keto[1-^{13}C]isocaproic acid while aminopyrine breath test and galactose elimination capacity were not altered, suggesting that the

impaired mitochondrial ketoisocaproate decarboxylation is not a consequence of decreased functional hepatic mass. Moreover, ALD in patients with active drinking was associated with the expression of a mtDNA deletion fragment in human liver tissue [108, 109]. Thus, these findings indicate that alcohol intake is associated with the disruption of mitochondrial respiration, which parallels the susceptibility towards ALD progression.

Mitochondrial Membrane Composition in ALD

As lipid composition determines membrane structure and affects the function of embedded proteins, it is conceivable that alcohol intake impacts in the lipid composition of mitochondria. Two major changes in the mitochondrial lipid composition of liver mitochondria from rodents fed alcohol have been described, such as an increase in the cholesterol content and reduced cardiolipin status. The cholesterol/phospholipid molar ratio is a critical determinant of the fluidity of membrane bilayers and the accumulation of cholesterol disrupts lipid organization and decreases the transition of liquid-ordered to liquid-disordered phases of membranes, leading to a higher membrane rigidity and alteration of the mitochondrial membrane proteins [110]. As described below, alcohol consumption increases the mitochondrial cholesterol levels due in part to the stimulated expression of a specific carrier involved in the trafficking of cholesterol to mitochondria. This event has important functional consequences mainly derived from the alteration in mitochondrial membrane fluidity, which impairs the action of specific carriers located in the IMM that affects the strategic antioxidant defenses, exemplified by the loss of mitochondrial GSH (mGSH) content (see below). In addition, early studies indicated that chronic alcohol intake affects the homeostasis of cardiolipin [111], which is known to play an essential role in the maintenance of cristae and in the function of respiratory chain organization [98]. While these changes have been mainly reported in experimental animal models, whether or not alterations in mitochondrial cholesterol and cardiolipin status contribute to human ALD remains to be further explored.

Mitochondrial DNA in ALD

As shown above, chronic alcohol ingestion and subsequent oxidative metabolism perturbs mitochondrial structure and function and hence promotes mitochondrial ROS generation (in part due to increased leakage of electrons from the transport chain to molecular oxygen and decreased antioxidant defenses), which can target macromolecules, including mtDNA. As mtDNA encodes for 13 components of the respiratory chain complexes, alcohol-induced mtDNA damage via mitochondrial ROS stimulation can impair mitochondrial respiratory chain [112]. Besides, alcohol decreases mitochondrial protein synthesis by impairing mitochondrial ribosomes and ROS irreversibly oxidize intramitochondrial proteins [104]. The inactivation of

proteins is critical for mitochondrial function and this contributes to alcohol-induced liver injury. Consistent with the susceptibility of mtDNA to ROS attack, it has been described in patients with ALD the presence of mtDNA deletion fragments, which likely reflects the severity of disease progression.

Mitochondrial ROS Production and Impaired Antioxidant Defense

Although the oxidative metabolism of alcohol is known to generate ROS as subproducts, as described above, the regeneration of NAD^+ from NADH, which is required for continued alcohol metabolism, feeds electrons to the respiratory chain. One of the consequences of this process is the increase of electron transfer directly to molecular oxygen to generate superoxide anion [113]. As mitochondria are the main consumers of oxygen, the likelihood to stimulate superoxide anion increases with the burden of oxidizing alcohol, particularly by CYP2E1. Moreover, although CYP2E1 is associated with the ER, it has been also shown to be present in mitochondria [114, 115] and potentiates alcohol injury by stimulating mitochondrial ROS generation. Although the main line of defense against superoxide anion generation in mitochondria is MnSOD, its status in ALD is controversial and not well-defined with studies reporting an increase, decrease or not change in expression/activity [116–118]. The action of MnSOD on superoxide anion results in the formation of hydrogen peroxide, which although is not strictly a free radical it is a potent oxidant that can generate reactive radicals through the Fenton reaction. The detoxification of hydrogen peroxide occurs mainly through the GSH redox cycle, for which the level of reduced GSH is essential, as well as by peroxiredoxin (Prx)-III, the Prx isoform located exclusively in mitochondria, which upon oxidation by hydrogen peroxide is reconstituted by thioredoxin2 (Trx2) [119]. Since mitochondria do not synthesize GSH *de novo* from its constituent aminoacids, mitochondria rely on cytosol GSH after being transported into the mitochondrial matrix to act as the cofactor for GSH peroxidase (Gpx) to detoxify hydrogen peroxide as well as other fatty acids-derived peroxides [105]. The transport of GSH into mitochondria has been a subject of intense investigation, with recent findings showing a role for the dicarboxylate and particularly 2-oxoglutarate (OGC, SLC25A11) transporters playing a key role in the import of GSH into mitochondria from cytosol. Importantly, the enrichment of mitochondria in cholesterol lowers mitochondrial membrane fluidity and affects the function of OGC to transport GSH (see below), resulting in mGSH depletion. Although alcohol induces mGSH depletion [105, 119], the status of Prx-III is not well-established raising the question about the relative importance between mGSH vs Prx-III in the detoxification of hydrogen peroxide in ALD [120, 121]. While differences in K_m for hydrogen peroxide, catalytic efficiency and K_{cat} between mGSH/Gpx and Prx-III/Trx-2 may exist, it has been shown that depletion of mGSH results in Trx-2 oxidation [122], which limits the reduction of oxidized

Prx-III, compromising the Prx-III/Trx-2 system to continue elimination of hydrogen peroxide.

Endoplasmic Reticulum (ER) Stress and Mitochondria Crosstalk in ALD

As mentioned above, ER stress is known to regulate hepatic steatosis via SREBPs activation, and hence ER stress plays a pivotal role in metabolic liver diseases, including ALD. The ER is an organelle responsible for the synthesis, folding, maturation and secretion of proteins and lipids and Ca^{2+} homeostasis. Disruption in protein folding or alterations in lipid homeostasis results in the activation of the unfolded protein response (UPR) that selectively increases the transcription of chaperones (GRP78/BiP) and the ER-associated degradation (ERAD) processes [123–125] with the ultimate goal to restore homeostasis. Alcohol intake induces ER stress through the onset of different mechanisms, including the generation of ceramide via acid sphingomyelinase (ASMase) activation, the generation of acetaldehyde due to the formation of protein adducts in the ER and the onset of oxidative stress [126]. A critical mechanism in alcohol-induced ER stress is the perturbation of the methionine metabolism with the subsequent increase in homocysteine levels (see above). In line with the role of homocysteine in mediating alcohol-induced ER stress, it has been shown that feeding mice with betaine decreased HHcy preventing alcohol-mediated ER stress, hepatic steatosis and liver injury [63].

Besides the impact of ER stress in protein and lipid homeostasis, ER stress can regulate mitochondrial function. The disruption of Ca^{2+} homeostasis in the ER is translated in mitochondria via the import of this cation, which promotes mitochondrial membrane permeability transition and subsequent cell injury [111]. ER and mitochondria exhibit physical contact through mitochondrial-associated membranes (MAMs), as specific membrane subdomain encompassing ER and mitochondria bilayers, which serve as a freeway for the movement of ions (e.g. Ca^{2+}) and lipids. In this regard, besides the movement of phospholipids synthesized in the ER moving to mitochondria, ER stress can signal the accumulation of cholesterol in mitochondrial membranes through the upregulation of steroidogenic acute regulatory protein 1 (StARD1), an OMM protein responsible for the transport of cholesterol into IMM, which has been recently described to be regulated by ER stress [120] and has emerged as a critical player in metabolic liver diseases, including nonalcoholic steatohepatitis (NASH) and more recently in ALD [127, 128]. As mentioned before, disruption in methionine metabolism is a key player in ALD pathogenesis via decreased MAT1A and MS expression and increased homocysteine levels, which is causally linked to ER stress. An additional link between methionine metabolism and mitochondrial cross-talk has been recently described in

ALD. Although MAT1A is found in the cytosol and nucleus, recent findings uncovered that MAT1A is localized in mitochondria, where it preserves mitochondrial proteome and promotes mitochondrial function [129]. Alcohol feeding to mice and liver samples from patients with ALD results in a striking decrease in the localization of MAT1A in mitochondrial mediated by the isomerase peptidyl-prolyl cis/trans isomerase (PIN1) and the casein kinase (CK2). Blocking PIN1-MAT1A interaction increased mitochondrial MAT1A levels and protected against alcohol-induced mitochondrial dysfunction and fat accumulation. Whether the beneficial effects of targeting MAT1A in mitochondria is due to the local generation of SAM levels to promote higher methylation and increased expression of mitochondrial proteins remains to be established. This is of particular interest vis a vis the known transport of SAM from cytosol to mitochondria by a specific carrier that is insensitive to the changes in mitochondrial membrane fluidity [130].

Inflammasome and Mitochondria Crosstalk in ALD

As discussed in the Sect. 3.2.2., pathogenesis of ALD involves inflammasome activation [131]. The disruption of the intestinal barrier integrity that allows the translocation of DAMPs and PAMPs to the circulation leads to the activation of the inflammasome cascade in the liver cells. Inflammasomes are a group of intracellular multicomplexes (NLRP3, caspase-1, IL-1β) located in the cytosol, which detect the PAMPs and DAMPs, such as ATP, pore-forming toxins, RNA viruses, cholesterol crystals, uric acid, and amyloid β [132, 133], and produce the release of the pro-inflammatory cytokines, such as IL-1β and IL-18 [134, 135]. There are different hypotheses regarding the activation of NLRP3 inflammasome, among them the release of ROS by damaged mitochondria produced by excessive electron flow [136], inhibited mitophagy [137], mtDNA oxidation [132]. In the context of the disruption of the balance between antioxidants and free radicals/oxidants, the onset of net ROS generation in mitochondria increases the risk of damaging mitochondrial components, such as the oxidation of mitochondrial DNA (ox-mtDNA) which are released to the cytosol and bind NLRP3 inflammasome [138]. The NLRP3 inflammasome complex activation is produced in MAMs, where cardiolipin is found and translocates from the IMM to the OMM and triggers the activation of the NLRP3 [139]. Furthermore, MAMs constitute the site where the transfer of lipids and Ca^{2+} influx between ER and mitochondria occurs mediated by VDAC [140] and facilitates the assembly of the NLRP3 inflammasome [141]. In this regard, the link between alcohol metabolism and NLRP3 activation is mediated via mitochondrial dysfunction, since mitochondria contributes to the oxidative metabolism of alcohol, the onset of ROS generation and oxidative stress, consequently the crosstalk between mitochondria and inflammasome contributes to the progression of ALD and emerges as a potential target for intervention.

Mitochondrial Cholesterol and Alcohol

Cholesterol is an important component of cell membranes, which determines membrane physical properties. Despite this structural role, cholesterol plays an important functional role by the regulation of multiple signaling pathways [142]. Cells meet their need for cholesterol either through the diet or synthesized *de novo* in the ER in the so-called mevalonate pathway. Once synthesized in the ER or delivered into lysosomes from diet-derived low-density lipoprotein (LDL), cholesterol is distributed to different membrane bilayers, particularly plasma membrane and mitochondria. Although the latter pool of cholesterol is considerably lower compared to the presence of cholesterol in the plasma membrane, the mitochondrial cholesterol plays important physiological roles, including the synthesis of bile acids in the liver in an alternative pathway to the classic one regulated by cytochrome P450 family 7 subfamily a member 1 (CYP7A1). The trafficking of cholesterol to mitochondria for metabolism is governed by StARD1, whose expression increases in metabolic liver diseases, such as NASH and ALD [143]. StARD1 is located in the OMM and has a cholesterol-binding domain. Being regulated by ER stress, alcohol consumption leads to the upregulation of StARD1, which has been shown to act as the rate-limiting step in the mitochondrial metabolism of cholesterol into oxysterols and bile acids. Thus, the induction of StARD1 by alcohol exceeds the capacity of cytochrome P450 family 27 subfamily a member 1 (CYP27A1) to initiate cholesterol metabolism into 27-hydroxycholesterol resulting in the next accumulation of cholesterol in the IMM. The accumulation of mitochondrial cholesterol results in a wide-range impact on mitochondrial function due to perturbation of mitochondrial membrane fluidity, which affects the activity of: (a) IMM solute carriers, such as the SLC25A11, involved in the transport of cytosolic GSH into mitochondrial matrix, which cause mGSH depletion [144, 145], and (b) oxidative phosphorylation by causing defective assembly of respiratory chain super-complexes [146] (Fig. 56.3). Although the activity of the SLC25A11 carrier has been shown to be sensitive to cholesterol-mediated changes in membrane fluidity resulting in impaired transport of GSH into the mitochondrial matrix [105, 145, 147], a novel mitochondrial carrier SLC25A39 has emerged as a putative GSH transporter [148]. However, whether SLC25A39 is sensitive to the disruption of membrane fluidity imposed by the accumulation of cholesterol or not remains to be established, and constitutes an essential mark for the mGSH transport activity. As ALD exhibit zonal dependent features, as mentioned above, it has been reported that the depletion of mGSH is preferentially seen in the PV area of alcohol-fed mice [149]. Interestingly, this outcome reflects a zonal-dependent induction of StARD1 by alcohol intake in which mice fed alcohol exhibit a preferential expression of StARD1 in PV hepatocytes [150]. While the direct determination of mitochondrial cholesterol levels in human ALD has not been determined, there was evidence indicating that patients with ALD exhibit increased expression of StARD1 [120].

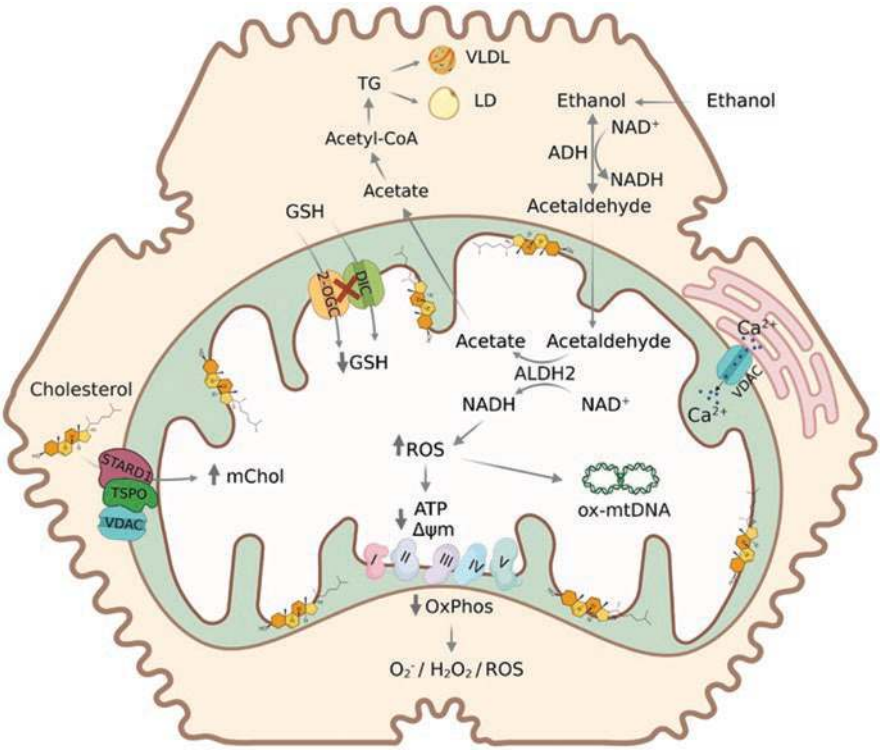

Fig. 56.3 Mitochondrial alterations in ALD. Ethanol is metabolized in the liver by ADH enzyme which converts it into acetaldehyde. This molecule is metabolized to acetate in the mitochondria by the ALDH2. Acetate is then converted to Acetyl-CoA which can be the energy source for other tissues or can be stored as triglycerides. The reaction catalyzed by the ALDH2 produces NADH that increases ROS production. ROS oxidize the mtDNA and interfere with the ETC. Ethanol intake increases the cholesterol into mitochondria through StARD1 protein. Cholesterol increases the membrane rigidity altering the membrane proteins and also the antioxidants (GSH) transport into mitochondria in order to reduce ROS. All this contributes to ALD progression. *ADH* alcohol dehydrogenase, *ALDH2* aldehyde dehydrogenase 2, *ETC* electron transport chain, *GSH* glutathione, *mtDNA* Mitochondrial DNA, *ROS* reactive oxygen species, *StARD1* steroidogenic acute regulatory protein 1

Conclusions and Future Perspectives

From the pioneering observations of the presence of megamitochondria in patients with ALD, it has become clear that mitochondria are not only involved in the metabolism of alcohol but are also targets of alcohol-derived metabolites. Mitochondria are important hubs for the energy production and metabolism and hence alterations in mitochondrial function are likely an important culprit of ALD pathophysiology. The nature of the alterations in mitochondria by alcohol consumption is multifactorial and involves changes at the structural and functional levels, most of which have

been described in experimental models of the disease. Although the translational side of these findings in patients with ALD has been limited there have been important developments, in which perturbations of mitochondrial dynamics has been described in human alcoholic hepatitis, a severe form of ALD, with an increased expression in the levels of Drp-1 that likely contribute to the hyperfragmentation of mitochondria and correlated with disease severity. At the functional level, limited information regarding the status of mitochondrial respiration in patients with ALD is available although indirect assays of mitochondrial functional activity indicated a possible impaired mitochondrial performance in human ALD, an aspect that deserves further effort and progression in the future. The increased expression of StARD1 in liver samples from patients with ALD [120] suggests that two important features of ALD seen in experimental models, namely increased mitochondrial cholesterol accumulation and mGSH depletion, are likely present in human ALD. Giving the role of mGSH in the detoxification of hydrogen peroxide and ROS attenuation, strategies boosting the mitochondrial pool of GSH may be of potential interest. Unfortunately although the role of N-acetyl-L-cysteine (NAC) has been shown to be ineffective in human ALD, NAC is not expected to recover the mitochondrial pool of GSH due to the defective transport from cytosol GSH, suggesting that other permeable GSH prodrugs, such as SAM may be more effective in treatment [151]. In addition to decreasing mitochondrial membrane fluidity, increased StARD1 expression may contribute to enhanced burden of bile acids production. Consistent with this possibility, which needs to be explored in the future, recent observations indicated increased total and conjugated bile acids in alcoholic hepatitis in patients with alcoholic hepatitis, although paradoxically this output did not derive from *de novo* synthesis based on a decreased expression of CYP7A1 and C4 serum levels [152]. Thus, although significant progress needs to be made to define the causal role of mitochondrial alterations in human ALD, the availability of new genetic models with liver-specific StARD1 deletion and humanized models with StARD1 overexpression may be useful to increase translational application of the impact of increased StARD1 in ALD pathogenesis. For related information on mitochondria and ALD see also Chap. 49 and Appendix Figs. A.33, A.34, A.36, A.43, A.74 and A.76.

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Chapter 57
Hepatic Iron Overload in Heavy Drinkers: Molecular Mechanisms and Relation to Hemolysis and Enhanced Red Blood Cell Turnover

Sebastian Mueller, Johannes Mueller, Siyuan Li, Chaowen Zheng, and Cheng Chen

Abstract About half of all heavy drinkers show pathological hepatic iron overload that is part of the developing alcohol-related liver disease (ALD). The underlying mechanisms have been remained largely obscure until now. We here review previous work, but also present novel long-term studies on iron parameters both in heavy drinkers but also animals and in vitro models. We show that enhanced red blood cell (RBC) turnover and hemolysis is strongly associated with mortality in heavy drinkers and seems to be a key mechanism responsible for the long-observed iron accumulation in alcohol drinkers. In line with this, ca. 50% show an enhanced erythrophagocytosis and ineffective erythropoiesis as evidenced by increased elevation of the hemoglobin-haptoglobin scavenger CD163 and serum ferritin. In general, and in contrast to former acute ethanol exposure models in mice, heavy drinkers have elevated hepcidin levels as compared to non-drinking human controls. Our preliminary data both in humans and animal indicate that hepcidin is primarily upregulated due to continued physiological heme turnover rather than excessive inflammation or cytokine production. This regulatory loop is not only disrupted by HFE mutations, but also cirrhosis development or severe hemolysis that causes toxic hepatocyte iron overload. There is also first indications that ethanol blocks erythropoiesis. Taken together, enhanced RBC recycling provide a first comprehensive but complex rational for iron overload in ALD linking iron metabolism tightly to liver, blood and bone marrow. The data also provide new insights into our understanding of alcoholic hepatitis.

Keywords Alcohol-related liver disease · Iron overload · Hemolysis · Erythrophagocytosis · Hepcidin · CD163 · Red blood cell

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Introduction

About half of all heavy drinkers show pathological hepatic iron overload that is part of the developing alcohol-related liver disease (ALD) [1–3]. The underlying mechanisms have been remained largely obscure until now. We here review previous work, but also present novel long-term studies on iron parameters both in heavy drinkers but also animals and in vitro models. We show that enhanced red blood cell (RBC) turnover and hemolysis is strongly associated with mortality in heavy drinkers and seems to be a key mechanism responsible for the long-observed iron accumulation in alcohol drinkers. In line with this, ca. 50% show an enhanced erythrophagocytosis and ineffective erythropoiesis as evidenced by increased elevation of the hemoglobin-haptoglobin scavenger CD163 and serum ferritin. In general, and in contrast to former acute ethanol exposure models in mice, heavy drinkers have elevated hepcidin levels as compared to non-drinking human controls. Our preliminary data both in humans and animal indicate that hepcidin is primarily upregulated due to continued physiological heme turnover rather than excessive inflammation or cytokines. This regulatory loop is not only disrupted by HFE mutation, but also cirrhosis development or severe hemolysis that causes toxic hepatocyte iron overload. There is also first indication that ethanol blocks erythropoiesis. Taken together, enhanced RBC recycling provide a first comprehensive but complex rational for iron overload in ALD linking iron metabolism tightly to liver, blood and bone marrow. The data also provide new insights into our understanding of alcoholic hepatitis.

Hepatic Iron Overload in Alcohol-Related Liver Disease

Gradual hepatic iron accumulation over years is an important key feature of alcohol-related liver disease (ALD) [1–3]. Hepatic iron accumulation and chronic alcohol consumption have been associated for a long time [4] and it has been recently confirmed in larger cohorts with non-invasive iron detecting methods [5]. Iron also represents an independent factor for disease progression and long-term survival [6]. Already a first look at typical laboratory parameters of a large cohort of heavy drinkers (see Table 57.1) shows that e.g. serum ferritin levels are higher than 1000 ng/mL in more than 20%. Other iron-related parameters such as transferrin or transferrin saturation are also changed significantly.

Figure 57.1a shows a Prussian blue stain with typical pathological iron deposits in a patient with ALD. Figure 57.1b shows a more quantitative analysis of the histological iron stain from 156 liver biopsies in heavy drinkers. The percentage of positive iron stains is shown separately for macrophages (Kupffer cells) and hepatocytes. The data show that ca. half of both cell types show hepatic iron loading. When looking into more details, macrophages always seem to have more iron load. Figure 57.1c shows how often pathological iron stain is found in both cells or isolated in either macrophages or hepatocytes.

Table 57.1 Typical laboratory parameter findings in heavy drinkers

Parameter	Units	Normal range	Normal (F0–2)		Fibrosis F3–4	
			Mean	Patholog (%)[a]	Mean	Patholog (%)[a]
Demographics and drinking history						
Male gender			71.30%		68.20%	
Duration of heavy drinking	Years	0				
Death in mean 3.8 years	Yes = 1	%	12.50%		34.90%	
Liver-related death		%	16.20%		56.80%	
Laboratory						
Erythrocytes	/pL	4.5–5.9	4.8	47.50%	3.9	76.50%
Hemoglobin	g/dL	12–16	14.5	6.10%	12.8	33.00%
Hemoglobin<10 (anemia)	g/dL	12–16	14.5	0.80%	12.8	12.90%
Bilirubin (total)	Mg/dL	<1.2	0.7	11.10%	3.3	50.30%
Bilirubin (indirect)	Mg/dL	<0.3	0.3	33.70%	0.7	55.20%
LDH	U/L	<250	223.6	26.30%	260.5	41.80%
Haptoglobin	g/L	0.3–2.0	1.5	2.90%	1.2	15.20%
CD163	Ng/mL	<800	1118	63.00%	2218.8	94.40%
Iron-related parameters						
Ferritin>150	Ng/mL	50–150/400	567	75.80%	674.4	75.90%
Ferritin>400	Ng/mL	50–150/400	567	41.70%	674.4	50.30%
Ferritin>1000	Ng/mL	50–150/400	567	17.20%	674.4	25.60%
Serum iron	Ug/dL	95–158	129.2	25.60%	117.9	25.10%
Transferrin	g/dL	2.0–3.6	2.5	2.30%	2	44.80%
Transferrin saturation	%	16–45	40.7	31.10%	48.3	44.50%
Iron stain macrophages	0–2	0	0.6	43.00%	0.632	46.10%
Iron stain hepatocytes	0–2	0	0.575	40.50%	0.526	36.80%
Liver iron concentration (AAS)	Mg/g dw	<0.8	1.4	5.60%	1.3	19.60%

[a] Underlined if decreased

Data are obtained from the Heidelberg cohort of heavy drinkers and stratified according to fibrosis stage (n = 1185). More parameters are shown in Tables B.1 and B.2 in the Appendix. Note that ca. 20% have ferritin levels >1000 ng/mL and more than 10% of F3/4 patients develop anemia

Figure 57.1d shows hepatic iron levels as assess with a non-invasive method (room temperature susceptometry [5]) in a large cohort of 154 ALD patients as compared to patients with non-alcoholic fatty liver disease (NAFLD) and patients with genetic iron overload. These data also confirm that ALD patients have significantly higher hepatic iron levels as compared to controls. Figure 57.1e further demonstrates that, in difference to common believe, hepatic iron not linearly increases with disease progression and fibrosis stages, but shows a peak at F1–2 fibrosis, while reduced iron levels are observed in patients with F4 cirrhosis. A similar pattern is observed when looking at serum ferritin levels (Fig. 57.1f).

In ALD, in previous reports, histological iron accumulation has been identified as independent risk factor both for survival and HCC development [7] comparable

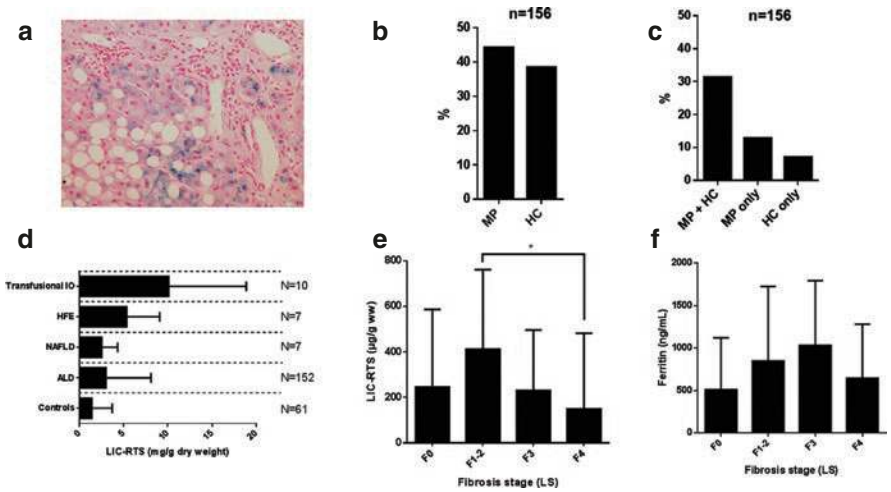


Fig. 57.1 Hepatic iron accumulation in ALD. (**a**) Liver stain with iron marker Prussian blue indicating pathological iron accumulation in hepatocytes and macrophages in a typical patient with ALD (counter stain Nuclear Fast Red). (**b**) Percentage of ALD patients with pathological iron deposits in liver biopsies for macrophages (MP) and hepatocytes (HC). Almost 50% have pathological iron overload, macrophages (Kupffer cells) slightly more than hepatocytes. (**c**) Percentage of pathological iron overload in both MP and HC, only MP and only HC. hepatocytes. (**d**) Comparison of liver iron concentration (LIC) as measured with non-invasive room temperature susceptometry (RTS) in patients with ALD, non-alcohol-related liver disease (NAFLD), hemochromatosis (HFE) and transfusional iron overload (IO). Both LIC-RTS (**e**) and serum ferritin levels (**f**) show characteristic changes with fibrosis stage in ALD. They increase from F0 to F3, but a decrease in F4 (cirrhosis)

to patients with hereditary iron overload [8]. These observations have led to intensive research activities aimed at better understanding molecular iron homeostasis, since iron is not only essential for hemoglobin synthesis, many iron containing enzymes and essential metabolic functions but also known to be highly toxic. To maintain an adequate iron supply, humans and other vertebrates have evolved effective mechanisms to conserve and finely regulate iron concentration, storage, and distribution to tissues. The carcinogenic potential of tissue iron accumulation is attributed to Fenton-like reactions which occur in the presence of reduced, ferrous iron and H_2O_2, yielding to highly reactive hydroxyl radicals [2]. In addition, excessive iron accumulates in lysosomes that originates from auto-phagocytosed ferritin and hemosiderin. It often leads to fragile membranes via lipid peroxidation and subsequent lysosomal dysfunction with the loss of free iron into the cytoplasm [9]. Taken together, the actual role of iron (in particular non-protein Fe complexes) and how alcohol favors iron accumulation is still under intensive investigation. Interestingly, recent preliminary data from our ongoing prospective, long-term mortality study in heavy drinkers has identified masked hemolysis, enhanced RBC turnover and ineffective erythropoiesis as important hitherto underestimated feature and predictor (see also book chapter on mortality and bone marrow toxicity). These new

insights also shed new light on our understanding of disturbed iron balance in patients with ALD. Consequently, besides established molecular pathways of iron regulation, this chapter will also introduce the very likely role of an impaired red blood cell cycle in heavy drinkers that seems to be the most likely reason for hepatic iron overload and other know changes of serum iron parameters in drinkers.

Control of Iron Homeostasis and Red Blood Cell Recycling

In the last decades, an enormous progress has been made to better comprehend the molecular mechanisms of iron regulation and homeostasis at the systemic and the cellular level [10, 11]. The human body contains ca. 5 g iron, of which ca. 2.5 g is used in the oxygen-carrying hemoglobin of erythrocytes (Fig. 57.2) and ca. 2.0 in liver, bone marrow and macrophages in the iron-storage protein ferritin and iron-containing proteins such as cytochromes. The liver serves as interim iron storage organ and can store up to 1 g. Circa 0.4 g are devoted to cellular proteins and enzymes in other cells. Iron is typically stored (ferritin, transferrin, enzymes) in the ferric state (Fe^{3+}) but crosses membranes through transporters in the highly reactive reduced, ferrous state (Fe^{2+}). Iron circulates bound to transferrin to be released to all organs/tissues through transferrin receptor 1. The transferrin bound ferric iron is relatively small only representing 2–3 mg. Most iron (20–25 mg) is recycled by

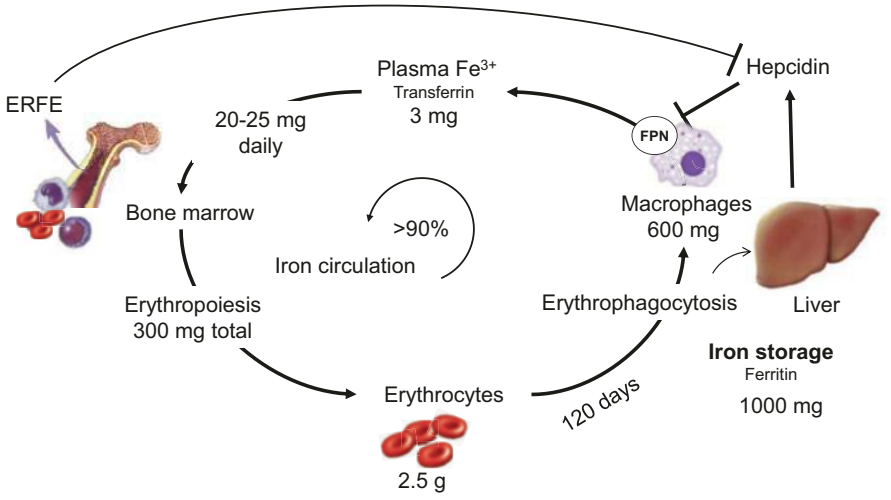


Fig. 57.2 Iron homeostasis and utilization in the body. Dietary iron is absorbed in the duodenum and binds to transferrin. Iron is then delivered to the bone marrow for erythropoiesis the major utilization pathway. Senescent RBCs are phagocytosed by macrophages (erythrophagocytosis) and ca. 90% of iron is recycled for heme synthesis. Excess iron is stored in ferritin in the liver. Regulation of iron metabolism by hepcidin and factors which influence hepcidin expression is also shown

macrophages, which phagocytize senescent red blood cells (RBC). In addition, as will be shown later in this chapter, hepatocytes are also able to directly uptake senescent RBCs, a function termed efferocytosis (see below). Most of this recycling occurs mainly in spleen and liver through the reticuloendothelial system, which initiates erythrophagocytosis. Macrophages can also directly recycle iron for new RBC production in the bone marrow. The daily uptake of dietary iron by duodenal enterocytes compensates for net loss or iron and is relatively small with 1–2 mg [10]. In contrast to general believe, iron is not only lost through cell desquamation and blood loss but also through the bile and urinary tract [12]. Excess iron is stored in ferritin of hepatocytes and macrophages as a reserve. As shown in Fig. 57.2, altogether about 90% of iron is recycled from senescent erythrocytes that typically have a mean survival of 120 days [13]. In other words, and considering that an average individual has 5 L total blood (2.5 g iron, ca. 0.5 mg per ml blood) ca. 40 mL blood are recycled every day which equals to ca. 20 mg iron. Likewise, these 20 mg of iron are required daily for erythropoiesis in the bone marrow.

Cellular Regulation of Iron

Each cell tightly controls iron homeostasis through sophisticated mechanisms. For instance, erythroid cells as well as all other cell types depend on the delivery of iron via the iron carrier serum **transferrin**, a glycoprotein with two affinity sites for ferric iron. Transferrin binds with high affinity to cell surface transferrin receptor 1 (TfR1) and with lower affinity to transferrin receptor 2 (TfR2) [14]. Genetic deletion of TfR1 in mice demonstrates its endocytic role and ability to import iron into several cell types [15]. In non-erythroid cells, iron is safely stored in ferritin complexes or can be incorporated into hemoglobin of erythrocytes, being later reused for various synthesis pathways [16]. Ferric iron stored in ferritin complexes (non-toxic form) must be subsequently released for biological use via lysosomal degradation of ferritin [17]. This mechanism of autophagy dominates during iron deficiency and is mediated by the nuclear receptor coactivator 4 (NCOA4) [18]. It has been described that NCOA4 interacts with ferritin heavy chain targeting ferritin for degradation. In iron overloaded cells, NCOA4 expression decreases leading to suppression of ferritin autophagy [19]. A recent study described the retention of iron within ferritin in NCOA4 KO mice, which led to iron-deficiency anemia, highlighting the important role ferritin autophagy mechanism on cellular and systemic iron homeostasis [20]. At the cellular level, central regulators of iron homeostasis are controlled post-transcriptionally by **iron responsive proteins** (IRP1 and IRP2) which are able to bind to the **iron responsive elements** (IREs) of the RNA encoding for various iron-related proteins. While binding to the IRE located in the 5′ untranslated region of the mRNA results in a translational inhibition, the bind of IRPs to the 3′ untranslated region stabilizes and protects the transcripts from degradation. In particular, IRPs can cause upregulation of TfR1 or suppress the translation of mRNA encoding

other proteins involved in iron metabolism, such as ferritins or ferroportin [21]. During cellular iron deficiency, IRPs are in the active form (apo-IRP) and this results in TfR1 induction stimulating the acquisition of iron from plasma Tf. In contrast, to counteract iron overload, IRPs become inactive (holo-IRP) for IRE binding leading to degradation of TfR1 mRNA and translation of ferritin mRNA [22]. The interactions IRE-IRP allow an autonomous independent control of iron homeostasis at the individual cell level. TfR1 expression is also regulated at the transcriptional and translational levels [23, 24]. Interestingly, IRP1 is regulated in a complex manner by various ROS linking iron homeostasis to oxygen metabolism [25–27].

Systemic Iron Control by Hepcidin

Systemically, iron is mainly controlled by the **hormone hepcidin** (Fig. 57.2). This 25 amino-acid peptide is primarily expressed in hepatocytes and to a lesser extent in macrophages [28, 29] representing about 10% of total serum hepcidin [30]. By binding to the unique iron exporter, **ferroportin**, hepcidin efficiently inhibits duodenal iron absorption, iron recycling from macrophages and iron mobilization from hepatic stores. Hepcidin blocks the iron efflux into the plasma by binding ferroportin and consequently inducing the phosphorylation, internalization and lysosomal degradation of the complex by the proteasome [31]. Deletion of hepcidin in mice or hepcidin deficiency in humans results in severe hepatic iron overload, increased serum iron levels and loss of iron in macrophage stores, caused by hyperabsorption of iron [32]. In contrast, transgenic overexpression of hepcidin causes decreased serum iron leading to anemia by blocking the iron absorption in enterocytes and the release of iron from the hepatic stores [33, 34].

The levels of circulating hepcidin are mostly controlled at the transcriptional level. Hepcidin promoter activity can be induced by iron signals, including serum iron concentrations and liver stores, or by inflammatory signals and suppressed during increased erythropoietic activity. In general, the transcriptional control of hepcidin by iron occurs via the bone morphogenic protein-SMAD (BMP/SMAD) pathway. High circulating concentrations of transferrin-bound iron (Tf-Fe) are the extracellular signal for transcriptional induction of hepcidin [35]. Tf-Fe modulates the interaction between the transferrin receptors (TFR) 1 and 2 and **hemochromatosis protein** (**HFE**) by inhibiting the binding of HFE to TFR1. Consequently, HFE stabilizes activin receptor-like kinase 3 (ALK3), which activates BMP/SMAD signaling cascade [36]. Increased Tf-Fe [37] concentrations can also promote the association between HFE and TFR2 that can further form a membrane complex with the BMP the co-receptor HJV, promoting hepcidin transcription via BMP/SMAD pathway [38]. Besides the iron, inflammatory signals as TGF-β, activin B and BMPs are also inductors of BMP/SMAD signaling while matripase-2 and furin act as suppressors by cleavage of the cell surface hemojuvelin (HJV) protein [39–43].

Hepcidin Regulation by Bleeding, Erythropoiesis, and Inflammation

Figure 57.3 shows the major pathways of hepcidin regulation. More details about iron homeostasis and erythropoiesis are provided in book chapter on bone marrow xxx. A complex interplay exists between liver and blood system and require a fine-tuned coordination that allows the maintenance of iron homeostasis. Increased **erythropoiesis**, caused by exposure to high altitude, anemia, or other physiological conditions, is so far described as the major inhibitory stimuli of hepcidin synthesis. Increased erythropoietin (EPO) release by the kidney is the major erythropoietic factor, which has been also implicated in downregulating hepcidin [44]. Despite many efforts over the last decade, there are still many open questions on how EPO suppresses hepcidin. Years ago, several studies have associated the suppression of hepcidin with two **erythroid regulators (GDF15 and TWSG1)**, which are normally increased during erythropoiesis. However, the direct link between these proteins and hepcidin regulation is still missing [35]. A more recent study has identified another erythroid regulator as part of the hepcidin-EPO axis, called **erythroferrone (ERFE)** [45, 46]. ERFE has been identified as main erythroid regulator of hepcidin and, in contrast to many other hepcidin stimuli but seems not to be responsible for the complete hepcidin suppression (see Fig. 57.2) [47]. When the release of erythropoietin from the kidney stimulates the production of new red blood cells, it also

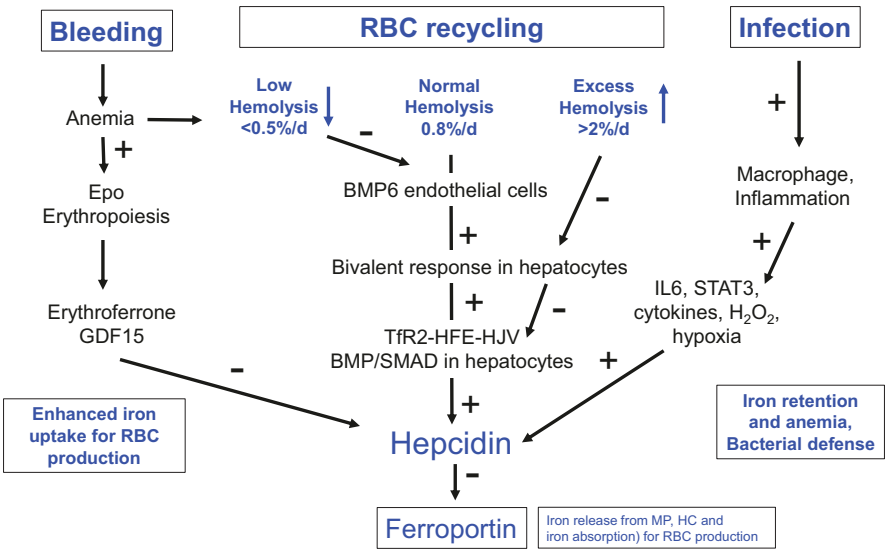


Fig. 57.3 Important signaling pathways on hepcidin: Bleeding, red blood cell recycling and inflammation/infection. Note that RBC recycling seems to control basal hepcidin levels which is HFE dependent and also depends on the rate of heme turnover. While physiological low hemolysis stimulates hepcidin, severe hemolysis strongly suppresses hepcidin

increases the synthesis of ERFE in bone marrow erythroblasts. Increased ERFE then suppresses hepcidin synthesis, thereby mobilizing cellular iron stores for use in heme and hemoglobin synthesis. Recent mechanistic studies have shown that ERFE suppresses hepcidin transcription by inhibiting bone morphogenetic protein signaling in hepatocytes. During ineffective erythropoiesis, pathological overproduction of ERFE by an expanded population of erythroblasts suppresses hepcidin and causes iron overload, even in non-transfused patients. Finally, it has been recently demonstrated that excess non-toxic iron and heme, *in vitro* and in the absence of erythroid cells, is able to suppress hepcidin expression [48]. In contrast, low levels of heme stimulate hepcidin both in hepatocytes and macrophages, which is further potentiated through endothelial derived BMP6 [49].

The induction of hepcidin during **inflammation or infection** constitutes an important evolutionary conserved mechanism known as "anemia of chronic disease" (ACD) see also Fig. 57.3). This mechanism describes a host defense response against invading extracellular pathogens by decreasing the availability of iron, an important bacterial growth factor. In ACD, IL-6 plays a key role as a major upstream regulator via STAT3 pathway leading to hepcidin induction [50]. Recently, H_2O_2 has been suggested as additional important inflammatory cofactor and second messenger capable of upregulating hepcidin by activation of the STAT3 signaling cascade [51]. In particular, hepcidin can be strongly induced by exposing hepatoma cells to sustained H_2O_2 concentrations similar to that released by inflammatory cells [51]. Subsequent studies have confirmed the role of STAT3 in the H_2O_2-mediated hepcidin induction [52, 53]. Other studies reported contrary findings and demonstrated a suppression of hepcidin in alcohol-fed mice by ROS [54]. However, no mechanistic details were provided, and we had earlier shown that the concentration of peroxide is crucial for hepcidin transcription [51]. While low levels induce hepcidin, toxic levels drastically block hepcidin most likely through unspecific inhibition of the transcription machinery. In the context of ALD, H_2O_2 has been shown to have a complex, concentration-dependent and bivalent action on hepcidin. Since ethanol metabolism strongly affects hepatic oxygen homeostasis, liver hypoxia is thought to have an important impact on hepcidin regulation *in vivo* and *in vitro* [55].

Erythrophagocytosis and Efferocytosis

Erythrocytes are either be directly phagocytosed by macrophages – termed **erythrophagocytosis**- or released toxic heme is bound to either haptoglobin (Hp) or, after release of heme, hemopexin (Hpx) and internalized through the hemoglobin- haptoglobin (Hb-hp) complex-CD163 or the heme-hemopexin (Heme-Hx) complex-CD91 [56, 57]. As we will describe later, hepatocytes are even able to quickly uptake oxidized red blood cells. The exact underlying mechanisms and how RBCs reach hepatocytes are not fully understood. During erythrophagocytosis, **heme oxygenase (HO)** catalyzes the enzymatic degradation of heme and produces equimolar amounts of carbon monoxide (CO), biliverdin and iron [58–61]. In a coupled

reaction, biliverdin is converted into bilirubin (BR) via biliverdin reductase [62] (see Fig. 57.4a). Of the two genetically distinct HO isoforms, HO2 is constitutively expressed and mainly found in brain and testis [63], whereas the inducible HO **isozyme HO-1** is expressed at low levels in most cells and tissues [60]. HO-1 is markedly up-regulated by its substrate heme and a variety of oxidative stress stimuli [64, 65] and HO-1 induction has been considered a general adaptive response to protect against the toxicity of oxidative stress [66–69]. Of note, Nrf2 is a major upregulator of HO1 that also orchestrates the transcriptional induction of various enzymes of the hepatic elimination and detoxification phases 0–3. More details are shown in a scheme in Fig. A.75. HO-1 deficient mice develop a chronic inflammatory disorder and are highly vulnerable to an experimental sepsis induced by the classical proinflammatory mediator endotoxin [70]. Second, these animals exhibit a marked hemosiderosis of solid organs such as the liver and kidney [71]. Finally, the liver is also the only location for bilirubin glucuronidation and excretion through canaliculi

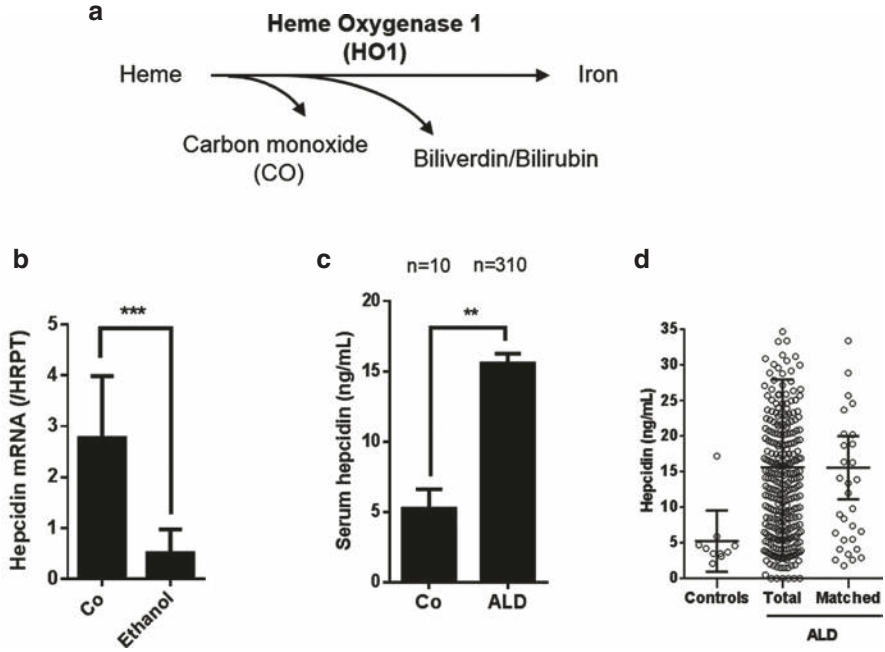


Fig. 57.4 (**a**) **Heme degradation by HO1.** Notably, the heme binding respiratory chain-blocker carbon monoxide CO is produced and toxic iron is release. In addition, bilirubin is ultimately conjugated in the liver and excreted through the biliary system. Bilirubin and bile acids can both cause eryptosis and hemolysis at higher concentrations. (**b**) Strong suppression of hepcidin mRNA in mice in response to acute ethanol exposure. Liver hepcidin expression in untreated (white) and 10% ethanol, gavage-fed male 129/Sv mice for 24 h as determined by real-time PCR (n = 7). (**c**) Elevated serum hepcidin levels in heavy drinkers as compared to controls. Serum hepcidin in healthy controls (n = 10) and ALD patients (n = 310) from the Heidelberg ALD cohort. (**d**) Hepcidin is also elevated when drinkers are matched for age and gender

and bile ducts. Mechanic obstruction of canaliculi or small bile ducts or direct hepatocyte damage through e.g. alcoholic liver injury/ballooning will also cause accumulation of bile content including bilirubin and bile acids. It is often overlooked that hepatocytes are also able to "phagocytose" larger vesicles and even whole cells [72].

Present Concepts of Iron Overload in ALD and Ethanol-Mediated Dysregulation of Hepcidin

Both acute and chronic alcohol exposure have been shown to suppress hepatic hepcidin expression in rodents [73]. As will be discussed later, these early reports of acute alcohol exposure models in rodents are in contrast to human data [1]. In mice, acute exposure to ethanol for 24 h rapidly suppresses hepcidin [74]. Figure 57.4b shows confirmative data of these reports in an acute binge model in mice for 24 hours from our laboratory. We still saw suppressed hepcidin level in a chronic ethanol model after 4 weeks. Only after 4 months, hepcidin was induced as compared to controls (not shown). In these acute ethanol models in mice, it has been further shown that ethanol downregulates hepcidin promotor activity and the DNA binding activity of CCAAT/enhancer-binding protein alpha (C/EBP alpha) but not C/EBP beta in mice [54] leading to downregulation of hepcidin gene transcription thereby increasing duodenal iron transport. Recently, it has been shown that alcohol exerted different effects on TGF-β-mediated SMAD2 activation and BMP-mediated SMAD1 and SMAD5 activation [75]. Other data suggest the simultaneous inhibition of BMP-mediated SMAD activation and stimulation of TGF-β-mediated SMAD activation by alcohol in the involvement of hepcidin suppression by alcohol *in vivo*. However, doubt has been shed on publications showing suppression of hepcidin by oxidative stress [76], as these conditions were later shown to non-specifically suppress the transcription machinery while physiological low hydrogen peroxide levels even increased hepcidin [51, 77].

As already mentioned above, in our large cohort of heavy drinkers, we could not confirm suppression of hepcidin. Figure 57.4c shows that serum hepcidin levels from 300 heavy drinkers is actually increased as compared to a non-drinking control cohort (for patient characteristics see Appendix Table B.1) [1]. Of note, mean hepcidin of non-drinkers were in the range of levels reported for normal population [78]. This is also the case if drinkers and non-drinkers were matched for age (Fig. 57.4d). Of note, inflammation and elevated cytokines cannot be the sole explanation for the upregulation of hepcidin as CRP or white bloods are only moderately induced, if at all. As will be later discussed many positive upstream regulators are not correlated in these patients. In conclusion, the observed short-term inhibition of hepcidin mRNA levels by acute and high alcohol exposure cannot be recapitulated at the serum level in heavy drinking humans. As will be discussed later, low level of

hemolysis during normal RBC turnover continuously stimulates hepcidin in the absence of virtual inflammation. Erythrophagocytosis can also be studied in vitro [1]. These data also show that hepcidin is induced in response to oxidized RBCs or heme at low concentrations (below 2% hematocrit). At higher concentrations, hepcidin expression will be suppressed.

Iron Markers and Mortality in Heavy Drinkers

New preliminary data of our ongoing long-term prospective mortality study in heavy drinkers show that hemolytic anemia is one of the most important confounders of long-term mortality. Details of the ongoing study are discussed in book Chap. 7 on mortality. Briefly, in the current interim analysis, information of survival status was obtained in 786 patients that had presented from 2007 to 2022 with a mean daily consumption of alcohol of 184 g/day. Mean observation time was 3.8 years and mean duration of heavy drinking was 14.0 years. During the observation time, 159 patients (20%) had passed away. More details are provided in the chapter on mortality and in the appendix (Table B.3). The cause of death could be clarified in 47%. In 34%, the death was liver-related. Of most interest within the context of this chapter, signs of anemia were associated with long-term mortality. Table 57.2 shows an extract of parameters that are correlated with mortality. Parameters of the blood compartment are marked in red, while iron-associated markers are marked in blue. Among the three major markers of anemia (hemoglobin, RBC counts, hematocrit), RBC count was best associated with an increased mortality, suggesting that not only the amount of hemoglobin production, but also the number of cells are important. For more details, the reader is referred to the book chapter on bone marrow toxicity in this book. Interestingly, markers of anemia were better correlated with death than known other prognostic markers such as albumin or INR.

Bilirubin is also highly associated with death, and it is also often forgotten that bilirubin is the major end product of heme degradation. Multivariate analysis confirmed that low RBC count is an independent predictor of death. Table 57.2 also suggests that the anemia is primarily due to hemolysis as levels of the hemolytic enzyme LDH, the iron marker ferritin and the end-product of heme production bilirubin were all positively and significantly associated with long-term death. Moreover, death also correlated highly with a large size of RBCs (MCV), typical hallmark of drinkers. To further confirm the nature of the anemia we measured in the serum of a representative sub-cohort the precursor of conjugated bilirubin, the unconjugated or indirect bilirubin and the soluble hemoglobin-haptoglobin scavenging receptor CD163. Both showed the highest correlation with death ($r \sim 0.25$) only being surpassed by RBC count and AP. Interestingly enough, although ferritin

Table 57.2 Univariate Spearman rho correlation analysis with mortality status in 786 heavy drinkers after a mean observation time 3.8 years. Detailed table is provided in Appendix (Table B.10)

Spearman rho correlation with status dead (1 or 0)		
Parameter	r	p
Liver stiffness (kPa)	0.299	**6.0E-17**
Erythrocytes (/pL)	– 0.281	**1.6E-15**
Signs of cirrhosis (US) (0 or 1)	0.275	**4.1E-14**
AP (U/L)	0.269	**2.4E-14**
Bilirubin indirect (mg/dL)	0.258	**4.9E-03**
Transferrin (g/L)	– 0.257	**6.2E-11**
CD163 (ng/mL)	0.256	**6.8E-04**
Hematocrit (%)	– 0.252	**1.2E-12**
LDH (U/L)	0.244	**4.6E-07**
Bilirubin total (mg/dL)	0.242	**9.4E-12**
Ascites (US) (1 or 0)	0.233	**1.3E-10**
Hemoglobin (g/dL)	– 0.232	**6.5E-11**
Albumin (g/dL)	– 0.229	**1.2E-08**
Age (years)	0.204	**1.0E-08**
Platelets (/nL)	– 0.192	**6.8E-08**
MCV (fL)	0.192	**1.4E-06**
CRP (mg/L)	0.175	**1.0E-06**
GGT (U/L)	0.121	**7.6E-04**
Spleen size (cm)	0.117	**3.0E-03**
AST (U/L)	0.111	**1.9E-03**
HDL cholesterin (mg/dL)	– 0.103	**1.2E-02**
Hepcidin (ng/mL)	– 0.094	1.7E-01
Protein total (g/dL)	– 0.093	**1.6E-02**
CK (U/L)	– 0.091	1.0E-01
Ferritin (ng/mL)	0.076	**3.7E-02**
Haptoglobin (g/L)	– 0.070	1.4E-01
CAP (dB/m)	0.060	1.9E-01
Hepatic steatosis (US) (0-3)	0.035	3.9E-01

Parameters are sorted in descending order according to the absolute value of the regression coefficient r. Parameters of the blood compartment are marked in red, while iron parameters are marked in blue. Note that mortality in heavy drinkers is significantly associated with signs of hemolytic anemia (see also Chap. 7)

levels were still significantly associated with mortality, hepatic iron deposition either assessed by histology, atomic spectroscopy or non-invasive means was not associated with mortality as reported earlier (not shown). Thus, in hour cohort of heavy drinkers, we cannot confirm a direct relation between hepatic iron overload and mortality. However, the enhanced RBC turnover highly suggests that it is related to hepatic iron overload.

Hepatocyte Iron and Serum Hepcidin Levels in Heavy Drinkers

To better understand the induction of hepcidin in heavy drinkers, we first studied associated parameters with histological iron deposition in hepatocytes, the primary source for hepcidin.

As shown in Table 57.3, hepatocyte iron load, as measured histologically by Prussian blue stain, is indeed slightly negatively correlated with RBC count (although not significantly). Table 57.3 also shows that iron content in hepatocytes is associated with iron load in macrophages, other markers of iron overload such as ferritin or liver iron as measured by atomic absorption spectroscopy (AAS, the gold standard). Most importantly, hepatocyte iron is highly positively correlated with both levels of serum hepcidin and hepcidin mRNA. In Table 57.4, finally, we analyzed serum hepcidin levels and its association with other parameters in subcohort of 304 heavy drinkers. Positively and significantly associated parameters are shown on the left, negatively correlated parameters on the right. As seen, serum hepcidin levels highly correlate with hepcidin mRNA but also with several absolute markers of iron content such as hemoglobin, histological iron in hepatocytes and macrophages or total hepatic iron as measured by AAS. No conclusive associations were seen with cytokines such as IL6, although considered one of the strong and positive upstream regulators of hepcidin through the STAT3 pathway. Signs of liver cirrhosis were clearly negatively associated with hepcidin whether diagnosed by ultrasound or histology. In this analysis, the erythrophagocytosis marker CD163 was also negatively associated with hepcidin, and no clear association was found with ERFE,

Table 57.3 Univariate Spearman Rho correlation of hepatocyte iron with different parameters

Spearman Rho correlation with histological iron stain in hepatocytes			
Parameter	r	p	N
Iron-macrophages 0–3 (histology)	0.612	1.1E-17	159
Serum ferritin (ng/mL)	0.501	2.2E-09	126
Iron in atomic absorption spectroscopy (mg/g dry weight)	0.625	3.4E-07	55
Serum hepcidin (ng/mL)	0.318	2.4E-04	129
HIF2alpha mRNA/b2mg	−0.462	1.3E-02	28
Systolic pressure (mmHg)	−0.287	2.3E-02	62
12-HETE (ng/g)	0.511	3.0E-02	18
Hepcidin mRNA/b2mg	0.408	3.1E-02	28
TfR1 mRNA/b2mg	−0.407	3.2E-02	28
Ballooning 0–2 (histology)	0.170	3.2E-02	159
Transferrin saturation (%)	0.267	5.8E-02	51

Parameters are sorted in descending order according to the absolute value of the regression coefficient r (n = 159). Number of available parameters are shown in the right column. Note that hepatocyte iron correlates highly with macrophage iron, serum ferritin and hepcidin

Table 57.4 Univariate Spearman rho correlation with hepcidin

Spearman Rho correlation with serum hepcidin (n = 304)							
Positive correlation				Negative correlation			
Parameter	r	p	N	Parameter	r	p	N
Hepcidin mRNA/ b2mg	0.682	**9.0E-05**	27	Reticulocytes (°/°°)	−0.720	**2.9E-02**	9
Hepcidin mRNA/ GADH	0.615	**6.3E-04**	27	HGF-1 (pg/mL)	−0.678	**1.5E-02**	12
IL-6 (pg/mL)	0.546	6.6E-02	12	EGR1 mRNA/b2mg	−0.559	**2.5E-03**	27
Ferritin (ng/mL)	0.382	**3.6E-11**	280	ABCG2 mRNA/b2mg	−0.495	**1.2E-02**	25
ALAS1 mRNA/ GAPDH	0.374	5.4E-02	27	TfR1/b2mg	−0.446	**2.0E-02**	27
Iron stain macrophages 0–3	0.356	**3.5E-05**	129	Transferrin mRNA/ b2mg	−0.445	**2.0E-02**	27
Iron stain hepatocytes 0–3	0.318	**2.4E-04**	129	ABCB11 mRNA/b2m	−0.439	**2.8E-02**	25
Liver iron -AAS (mg/g dw)	0.301	**4.0E-02**	47	Serum PINP (ng/mL)	−0.409	**4.8E-04**	69
Albumin (g/dL)	0.223	**1.5E-03**	200	TfR1 mRNA/GAPDH	−0.390	**4.5E-02**	27
Hemoglobin (g/dL)	0.206	**3.2E-04**	302	Chevallier fibrosis score	−0.301	**1.1E-03**	114
Haptoglobin (g/L)	0.184	**2.5E-02**	148	Serum PAPP-A (ng/ mL)	−0.299	5.4E-02	42
ALT (U/L)	0.181	**1.6E-03**	304	Fibrosis stage (LS1)	−0.235	**4.5E-04**	220
Erythrocytes (/pL)	0.175	**2.3E-03**	302	Ascites (0 or 1)	−0.219	**1.6E-04**	291
				Signs of cirrhosis (US) (0 or 1)	−0.212	**3.7E-04**	279
				AST/ALT ratio	−0.193	**7.4E-04**	304
				CD163 (ng/mL)	−0.135	**3.9E-02**	235

Positive associations are shown on the left, negative correlations are shown on the right. Note that serum hepcidin is tightly associated with hepcidin mRNA and parameters of iron compartments such as ferritin, histological iron stains or hemoglobin. Parameters are sorted in descending order according to the absolute value of the regression coefficient r, since this allows better direct comparison. Hepcidin is negatively associated with erythropoiesis (reticulocyte count) and signs of fibrosis

considered an important negative bone marrow derived regulator of hepcidin. Taken together, the data confirm that parameters of high iron load are positively and highly correlated with hepcidin. These findings match the current understanding of hepcidin as iron sensor. If the organism is loaded with iron (high hemoglobin levels, high ferritin levels or other iron parameters) then a high hepcidin prevents further uptake of iron through the duodenal compartment. The robust negative association of hepcidin with signs of cirrhosis is more complicated and not simply due to suppression

of protein synthesis or transcription. As e.g. shown in the Table B.25 for alpha2-macroglobulin (a2-MG), the cirrhotic liver maintains protein synthesis capacities for specific pathways, in this case alpha macroglobulin is higher in those with anemia who have progressed more. Most likely, a2-MG compensates for decreased albumin availability to maintain oncotic pressure. Alternatively, it could be due important hemodynamic changes in cirrhotic patients with a switch of the major blood supply from portal vein to hepatic artery and, consequently, with a disruption of the normal BMP6-hepcidin axes.

Macrocytic Anemia and Ineffective Erythropoiesis in Heavy Drinkers

To get more insights into potential causes of the anemia, patients were grouped (Tables 57.5, 57.6) according to the size of RBCs **mean corpuscular volume (MCV)** in three groups (microcytic <80, normocytic 80–96 and macrocytic >96). Compared to a normal population, hemoglobin levels values were about 20% lower, although only 20% of all patients full-filled criteria of anemia (<12.5 g/dL). Macrocytic group represents one third (31.8%) and it showed the highest mortality. In this group, mortality was three times as compared to patients with normocytic RBCs (31.1 vs 11.6%). Expectedly, the group with microcytic anemia, classically representing iron-deficiency anemia, had the lowest iron level. Important **hematopoietic parameters** such as levels of folic acid, erythropoietin (EPO), vitamin B12 were usually all in the normal range. In the macrocytic group, however, levels of EPO were highest and in the upper normal range, while B12 levels slightly exceeded upper normal levels. Finally, as a direct group, reticulocyte count as a direct measure of hematopoietic activity was only increased in this group.

The macrocytic group also showed further **evidence of hemolysis**. CD163, indirect bilirubin and LDH was highest while in this group haptoglobin was lowest. Based on levels of CD163 and ferritin in Tables 57.5, 57.6, ca. 50% of all drinkers show an increased erythrophagocytosis. Thus, 65.6% of all women and 46.3% of all men have elevated ferritin levels. Altogether, in 52.7%, and elevated ferritin was seen, in most cases due to ineffective erythropoiesis and enhanced heme turnover. Notably, enhanced erythrophagocytosis is not only observed in the macrocytic group (59.6%) but also in the normocytic and microcytic group (32.5 and 38.5%). In addition, ferritin levels were highest in the macrocytic group. Consequently, this laboratory represents a typical configuration of **hemolytic anemia with enhanced, but ineffective erythropoiesis**. With today's ethical and practical limitation of performing isotope RBC labeling studies in drinkers, we also used a chemical pulse chase approach to estimate RBC removal based on the specific alcohol marker phosphatitdylethanol (PEth). RBC represent the major phospholipid pool in blood

Table 57.5 Hematopoietic and iron parameters in heavy drinkers grouped according to RBC size (MCV). Complete data are provided in Appendix Table B.29

Groups	Units	Normal	P*	High MCV	Normal MCV	Low MCV	All
				>96	80–96	<80	
MCV	fL	80–96	***	101.7	90.4	65.2	93.4
Percentage	%			31.80%	65.70%	2.50%	100.00%
Hemoglobin	g/dL	>12.5	***	13.4	14.4	12.1	14
Anemia fraction				28.4%	13.0%	33.7%	19.7%
Erythrocytes	/pl	4.5–5.9	***	3.7	4.5	4.7	4.3
Hematocrit	%	40–53	***	37.8	40.8	35.8	39.8
All-cause mortality			***	31.10%	11.60%	20.00%	
Parameters of hematopoiesis							
Vitamin B12	Pmol/L	145–596		616.2	494.4	341	524.5
Folic acid	nmol/L	>7.1	**	10.7	17.3	8.6	15.3
Epo (erythropoietin)	mIU/mL	6–15	**	11.7	6.2	0.5	8
Reticulocytes	°/°°	8–25	***	27.0	15.4	16.8	19.5
Parameters of iron metabolism							
Ferritin	ng/mL	>400/150	***	853.4	484.1	272.1	594
Elevated ferritin fraction				62.9%	38.0%	20.0%	44.9%
Transferrin	g/L	2–3.6	***	2	2.5	2.6	2.4
Serum iron	µg/dL	95–158		129.1	122.2	103.7	123.9
Transferrin saturation	%	16–45	***	49.5	38.9	32.6	42.1
Hepcidin	ng/mL	1–55	**	13.9	17.1	24.4	15.8

P* comparison between high and normal MCV

Note, that 31.8% of all drinkers show macrocytosis. Almost 30% of them have clear signs of anemia, corresponding to hemolytic anemia in the light of elevated ferritin levels. Hemolytic anemia is not due to the lack of folic acid and vitamin B12. Potential other causes are related either directly to RBC toxicity or bone marrow toxicity. Lowest levels of hepcidin are seen in the macrocytic group

and PEth is specifically formed in the presence of ethanol catalyzed by erythrocyte phospholipase D. Indeed, PEth elimination was highest and increased by 24% in the macrocytic group [79]. This is further underlined in Fig. 57.5, were RBC count, ferritin and CD163 levels are depicted for all three MCV groups. Figure 57.6 finally shows reticulocyte count for all three MCV groups. The reticulocyte count is an accepted measure of direct erythropoietic activity. As shown in Fig. 57.6, ALD patients with macrocytosis show an elevated erythropoiesis. Considering the fact that these patients have decreased hemoglobin levels and other indicators for hemolysis, these data further clearly confirm that alcohol causes ineffective erythropoiesis. Consequently, the enhanced RBC turnover could be considered as novel and important reasons for hepatic iron overload.

Table 57.6 Parameters of hemolysis and liver parameters in heavy drinkers grouped according to RBC size (MCV)

Groups	Units	Normal	P*	High MCV	Normal MCV	Low MCV	All
				>96	80–96	<80	
MCV	fL	80–96	***	101.7	90.4	65.2	93.4
Percentage	%			31.8%	65.7%	2.5%	100.0%
Parameters of erythrophagocytosis/hemolysis							
CD163	ng/mL	<1500	***	1945.0	1325.8	1149.8	1686.3
Elevated CD163 fraction	ng/mL		***	59.6%	32.5%	38.5%	44.7%
Bilirubin indirect	mg/dL	0.2–0.8	*	0.57	0.4	0.37	0.46
LDH	U/L	<250	***	268.9	223.8	210.6	238.5
Haptoglobin	g/L	0.3–2.0	*	1.3	1.5	1.7	1.4
Liver parameters							
Liver stiffness (fibrosis)	kPa	< 6 kPa	***	27.8	12.8	17.1	17.7
CAP (steatosis)	dB/m	<240	*	283.7	268.4	286.2	294.1
AST	U/L	<50	***	118.9	84.8	81.3	95.6
ALT	U/L	<50	Ns	66.9	68.2	60.3	67.1
GGT	U/L	<60	***	601.5	304.9	348.3	400.3
AP	U/L	40–130	***	134.3	101.9	123.9	112.8
Bilirubin total	mg/dL	<1.3	***	2.5	1.1	0.8	1.6
Albumin	g/dL	3.4–5.4	***	4.1	4.4	4.1	4.3

P* comparison between high and normal MCV

Patients with signs of hemolytic anemia (high MCV, signs of hemolysis, decreased hemoglobin) have a three-time increased mortality. Hemolytic anemia is also tightly linked to liver damage. Although signs of hemolysis occur already prior to the onset of liver damage, progressing cirrhosis further deteriorates RBC turnover

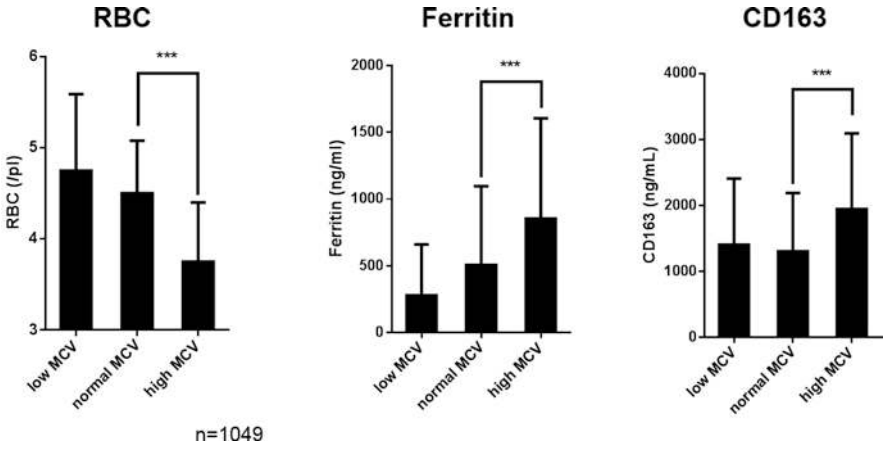


Fig. 57.5 Evidence for hemolytic anemia and elevated RBC turnover in heavy drinkers with enlarged RBC size (MCV). Patients with high MCV have a lower RBC count (anemia), elevated levels of the iron storage marker ferritin and the erythrophagocytosis marker CD163

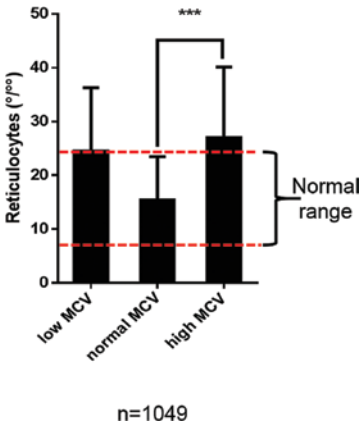


Fig. 57.6 Increased erythropoiesis (elevated reticulocyte count) in heavy drinkers with enlarged RBC size (MCV). Reticulocyte count is a direct measure of erythropoietic activity. Heavy drinkers with enlarged MCV show an enhanced erythropoiesis while patients with normal sized RBCs have normal RBC production. Note that patients with microcytic anemia (2.5%) also have increased erythropoiesis being in the upper normal range. These findings are strong indicators of an ineffective erythropoiesis in a large fraction (34%) of heavy drinkers

Is Hepcidin Involved in Iron Dysregulation in Drinkers?

As shown above, long-term heavy drinkers have upregulated hepcidin but not really elevated markers of inflammation nor a tight and conclusive association between hepcidin and markers of inflammation. To gain further insights into ethanol-mediated iron overload and the role of hepcidin, we now focused on the 52.7% patients with ineffective erythropoiesis and elevated ferritin levels has mentioned above. We divided this group into patients with normal hemoglobin and with anemia. In other words, the sub-group with normal hemoglobin seems to be able to efficiently recycle iron into de novo production of RBCs while the anemic groups fail to do so. Table 57.7 shows the data. Parameters are sorted according to the P values between both cohorts. Next to RBC count, parameters such as albumin, transferrin but also liver stiffness are among the most discriminative parameters. Direct parameters of hemolysis such as indirect bilirubin, transferrin saturation and CD163 are also significantly higher in the anemia cohort. The table underlines that, due to ineffective erythropoiesis, the anemia group seems to be overwhelmed with the recycling of damaged or fragile RBCs. It remains to be discussed whether albumin as bilirubin carrier and transferrin as iron carrier are suppressed due to liver toxicity or whether this is a specific adaptive response. There are more arguments for the latter. First, proteins such as alpha macroglobulin are increased in these patients. Second, serum iron is significantly decreased (111 vs 148 μg/dL) in the anemia group despite macrocytosis. This highly suggests that the bone marrow is overwhelmed with erythropoiesis under the toxic, alcohol-exposed environment. It also explains the elevated transferrin saturation which is not due to total iron increase in the serum compartment but rather reduced transferrin.

Table 57.7 Heavy drinkers with ineffective erythropoiesis and elevated ferritin (ca. 50% of all drinkers) are grouped according to anemia status. Note that suppression of important carrier proteins (albumin, transferrin) but also liver stiffness show the most significant differences between both groups. The anemia group also demonstrates higher levels of hemolysis as shown by CD163, LDH and indirect bilirubin

			Anemia		No Anemia		T TEST
Parameter	Units	Normal range	Mean	N	Mean	N	P
Hemoglobin	g/dL	13.5–17.5	11.36	133	14.98	362	3.8E-101
Erythrocytes	/pL	4.5–5.9	3.28	133	4.47	362	5.5E-76
Albumin	g/dL	3.82–5.92	3.64	94	4.45	283	1.0E-26
Transferrin	g/L	2–3.6	1.58	97	2.27	299	8.5E-23
Ascites	0 or 1		0.36	125	0.05	337	3.4E-20
Liver stiffness	kPa	<6	36.01	128	16.59	349	3.4E-16
AP	U/L	40–130	169.59	132	111.27	361	1.6E-13
Bilirubin total	mg/dL	<1.3	4.43	133	1.41	359	2.8E-13
INR		0.85–1.15	1.21	132	1.00	360	1.7E-12
Serum iron	µg/dL	59–158	111.26	120	148.51	323	4.9E-09
Status death	0 or 1		0.40	96	0.18	249	1.2E-05
MCV	fL	80–96	99.07	120	94.79	300	1.6E-04
Bilirubin indirect	mg/dL	<0.8	1.01	30	0.40	82	3.1E-04
ALT	U/L	<50	70.19	133	99.92	362	2.0E-03
LDH	U/L	<250	304.36	90	253.74	200	4.6E-03
Transferrin saturation	%	16–45	59.07	93	50.71	284	5.5E-03
Transferrin	g/L	2–3.6	1.89	6	2.64	20	9.8E-03
Ferritin	ng/mL	30–400	1248.31	133	1075.76	362	1.0E-02
Age	Years		55.20	133	52.54	361	1.0E-02
CD163	ng/mL	<800	2041.76	44	1675.11	80	4.8E-02
M65	U/M	<400	1694.48	69	1313.29	227	6.1E-02
GGT	U/L	<60	733.50	133	596.94	359	7.6E-02
M30	U/L	<200	940.59	69	745.35	227	1.2E-01
Platelets	/nL	150–360	181.05	133	188.91	362	3.6E-01
AST	U/L	<50	132.73	133	143.41	362	4.0E-01
Folic acid	nmol/L	>7.1	11.59	12	12.02	21	8.8E-01
Vitamin B12	pmol/L	145–596	653.15	13	663.96	22	9.4E-01

T-Test was performed, and parameters are sorted according to P values in ascending order. Note that suppression of important carrier proteins (albumin, transferrin) but also liver stiffness shown the most significant differences between both groups. The anemia group also shows higher levels of hemolysis as shown by CD163, LDH and indirect bilirubin

To better understand the underlying signaling of hepcidin, we further studied several important hormones such as EPO, but also BMP6, ERFE and cytokines, all involved in hepcidin regulation. The results are shown in Table 57.8, but the data remain largely inconclusive and it seems that not a single factor controls hepcidin expression. Hepcidin is slightly lower in the anemia group but BMP6, an important upregulator, is suppressed, and ERFE, and important negative regulator, is also suppressed. Moreover, IL6, also a strong upregulator of hepcidin, is also suppressed while IL8 is slightly increased. Of

Table 57.8 Hepcidin levels and important upstream regulators of hepcidin in heavy drinkers with ineffective erythropoiesis (ca. 50% of all drinkers) divided according to anemia status

Parameter	Units	Normal range	Anemia		No anemia		T test
			Mean	*N*	Mean	*N*	P
Hepcidin levels							
Hepcidin	ng/mL	–	16.90	*49*	20.58	*95*	1.2E-01
Hepcidin mRNA	mRNA	–	0.90	*2*	1.20	*11*	3.5E-01
Iron compartment							
Hemoglobin	g/dL	13.5–17.5	11.36	*133*	14.98	*362*	**3.8E-101**
Serum iron	µg/dL	59–158	111.26	*120*	148.51	*323*	**4.9E-09**
Ferritin	ng/mL	30–400	1248.31	*133*	1075.76	*362*	**1.0E-02**
Intracellular iron							
Pigmented macrophages	0–1	–	0.59	*27*	0.39	*52*	8.5E-02
Iron stain Kupffer cells	0–4	–	1.07	*27*	0.75	*51*	1.1E-01
Iron stain hepatocytes	0–4	–	0.96	*27*	0.70	*51*	2.0E-01
Important hepcidin regulators							
ERFE	ng/mL	–	0.39	*15*	1.30	*29*	3.0E-01
BMP6	ng/mL	–	0.10	*15*	0.35	*30*	2.8E-01
TNF alpha	pg/mL	–	5.72	*6*	3.03	*16*	1.2E-01
IL-8	pg/mL	–	104.30	*18*	66.08	*34*	1.3E-01
IL-6	pg/mL	–	28.48	*16*	125.85	*30*	1.3E-01
IL-1b	pg/mL	–	20.85	*16*	103.35	*30*	1.8E-01
PRX2 ox/red	rel units	–	0.86	*5*	2.69	*5*	2.8E-01
Nox4	rel units	–	1.00	*6*	2.20	*5*	1.3E-01
Erythropoiesis							
EPO1	mIU/mL	–	13.19	*6*	7.42	*27*	1.0E-01
Reticulocytes 1	°/°°	8–25	28.57	*7*	18.00	*12*	1.1E-01
Reticulocytes 2	°/°°	8–25	41.50	*4*	18.00	*6*	**2.0E-02**
Erythrocytes 1	/pL	4.5–5.9	3.28	*133*	4.47	*362*	**5.5E-76**

Several categories are shown, and parameters are sorted according to P values in ascending order within the category. Note that hepcidin is slightly lower in the anemia group that has higher intracellular iron levels. Also note that erythropoiesis boosts after alcohol detoxification (reticulocyte count 2)

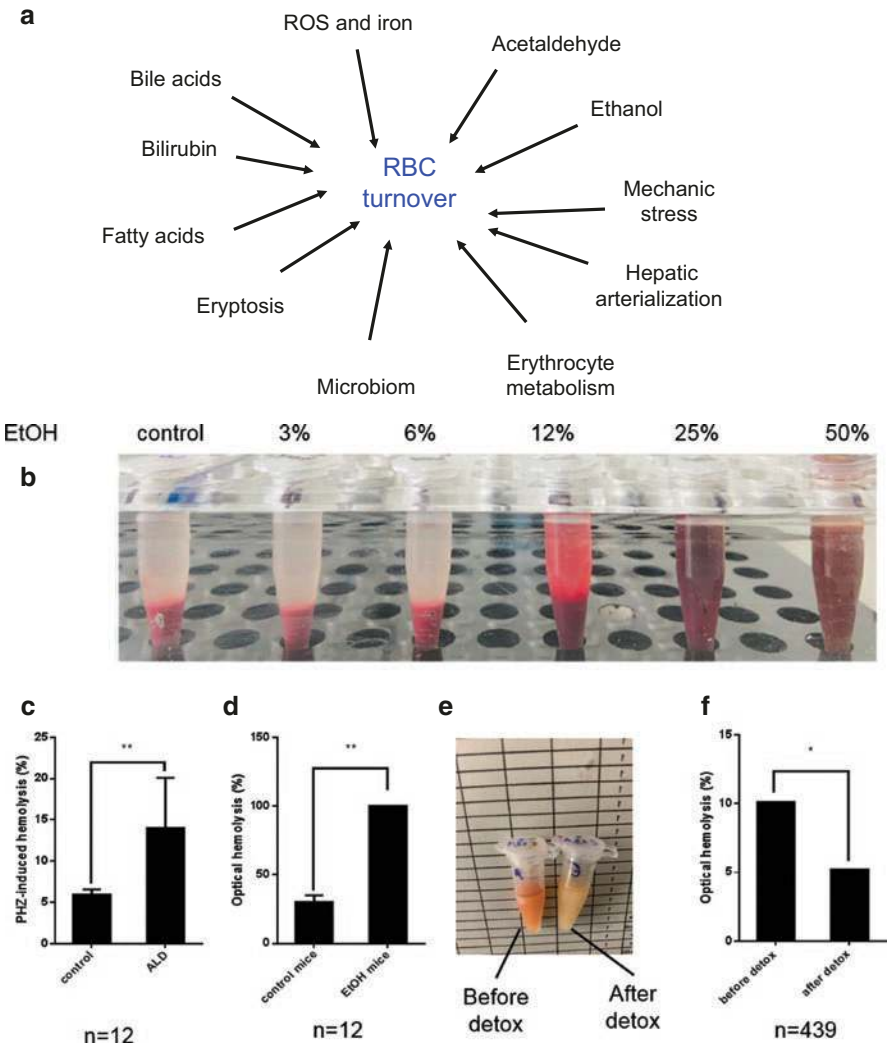
note, erythropoiesis boosts after 1 week of alcohol detoxification as shown by doubling of reticulocytes. Consistently, histological iron is higher both in macrophages and hepatocytes. Although rather complex, these findings allow the conclusion that, due to ineffective erythropoiesis and enhanced RBC turnover, both macrophages and hepatocytes are loaded with iron. In response, specific carrier proteins are downregulated rather than in a non-specific manner, to prevent toxic iron overload.

Potential Mechanisms of Hemolytic Anemia in Heavy Drinkers

It is known for a long time that alcohol consumption can result in structural and metabolic abnormalities of the erythrocyte membrane leading to hemolytic anemia varying from very mild to severe [80]. Reasons for hemolytic anemia are disorders altered erythrocyte cytoskeleton proteins, an increase of lipid fluidity by oxidative stress [81] and reduced phosphate levels (hypophosphatemia) [82]. In drinkers, an abnormal structure of erythrocyte surface can cause dysfunctional and heteromorphic echinocytes, such as stomatocytes or spur cells [80]. These spur cells with an irreversible rigid membrane will be prematurely removed by spleen macrophages [83, 84]. Spur-cell associated hemolysis is observed in ca. 3% of chronic heavy drinkers ultimately leading to progressive fatal anemia [82]. However, these changes have been not associated with mortality so far besides the novel mortality data presented in Table 57.2.

Several studies and experiments were performed to learn more about hemolysis in our cohort of heavy drinkers. Figure 57.7a demonstrates that ethanol can directly lyse fresh human RBCs as compared to e.g. hypoosmotic or mechanic shear stress conditions [57]. This is due to the amphiphilic properties of ethanol (see Fig. A.2) as we strictly controlled osmolality in these experiments. Although the concentrations of 10% are quite high, they could be readily achieved during binge drinking of high percentage beverages, at least in some blood compartments. Moreover, it has been recently shown that ethanol itself or enhanced levels of bile acids and conjugated bilirubin are able to induce eryptosis or prime RBCs for erythrophagocytosis [1, 85, 86]. Besides these direct hemolytic erythrophagocytosis-priming effects of

Fig. 57.7 RBC turnover and various signs of hemolysis in human RBCs of heavy drinkers. (**a**) RBC turnover is complex and affected by multiple factors. (**b**) Direct exposure of human RBCs with ethanol (EtOH) for 24 h. Note, that hemolysis or modification only occurs at quite high levels of ethanol (ca. 10%) which, however, may be achieved during binge drinking in some compartments using high percentage liquors or wine. (**c**) RBCs of heavy drinkers are more fragile in response to hemolytic stress by phenyl hydrazine or mechanically during blood taking. RBCs from both heavy drinkers and healthy volunteers (each cohort n = 6) were in silico treated with the hemolytic agent phenyl hydrazine (PHZ) for 60 minutes and hemolysis was measured by absorption spectroscopy in the supernatant. Hemolysis rate of ALD patients was significantly higher than in healthy controls. It indicates that RBCs seem to be generally more fragile in drinkers. (**d**) Presence of optical hemolysis in serum samples of ethanol-treated mice. Six mice were treated for 4 weeks with ethanol (see above). (**e**) Example of optical signs of hemolysis in a serum sample from a heavy drinker before and 1 week after alcohol detoxification. (**f**) Significantly lower optical hemolysis in sera after alcohol withdrawal in a large study on optical signs of hemolysis in serum samples before and after alcohol detoxification. Statistical analysis of frozen serum samples form n = 439 heavy drinkers prior and 1 week after alcohol detoxification from the Heidelberg heavy drinker cohort collected between 2007 and 2022. It is assumed that, in this large cohort of patient sample, mean mechanic stress to RBCs was similar both prior and after alcohol withdrawal. The data suggest that 1 week of alcohol detoxification already improves ethanol-mediated RBC fragility



ethanol, it can be also demonstrated that blood from heavy drinkers is less resistant to either direct chemical or mechanic hemolytic stress. Figure 57.7b shows a significant increase of ex vivo phenyl hydrazine (PHZ)-induced hemolysis in RBC preparations from heavy drinkers and controls. Hemolysis was directly measured photometrically after 60 min incubation with PHZ. These results are especially convincing since the test measures the overall fragility of RBCs towards a standard stress situation but does not detect the specific cause of hemolysis (membrane rupture, reduced antioxidative defense, hypophosphatemia etc.).

We also studied in our cohort of heavy drinkers another phenomenon of hemolysis. If RBCs from heavy drinkers or ethanol-treated animals should be more vulnerable to mechanic stress, routine blood taking should also causes more hemolysis as compared to healthy controls. This is shown in Fig. 57.7d which demonstrates the presence of optical macroscopic hemolysis in serum samples of ethanol-treated mice Fig. 57.7e in serum samples before and 1 week after alcohol detoxification in n = 439 heavy drinkers prior and 1 week after alcohol detoxification. It is assumed that, in this large cohort of patient sample, mean mechanic stress to RBCs was similar both prior and after alcohol withdrawal. The data suggest that 1 week of alcohol detoxification already improves ethanol-mediated RBC fragility.

We could also show that 4 weeks of chronic ethanol exposure significantly upregulates CD163 in mouse livers (Fig. 57.8a, b). The importance of the hemochromatosis gene HFE is underlined in Fig. 57.8c. Here, almost no hepcidin upregulation is observed a mouse model of hemolysis (PHZ). In other words, heme recycling seems to require an adequate hepcidin response that controls the iron release both from macrophages and hepatocytes through the iron exporter ferroportin. Figure 57.9 finally shows that normal human RBCs (Fig. 57.9a) develop spur-like phenotypes under conditions of oxidative stress (Fig. 57.9b, copper sulfate treatment) and area readily ingested by macrophages (Fig. 57.9c, human THP1 macrophage). They are also ingested by hepatocytes (Fig. 57.9d), a process called efferocytosis and not studied very well. We have been able to show RBC efferocytosis both in human cancer-derived hepatocytes but also in primary mouse hepatocytes. It remains unclear how

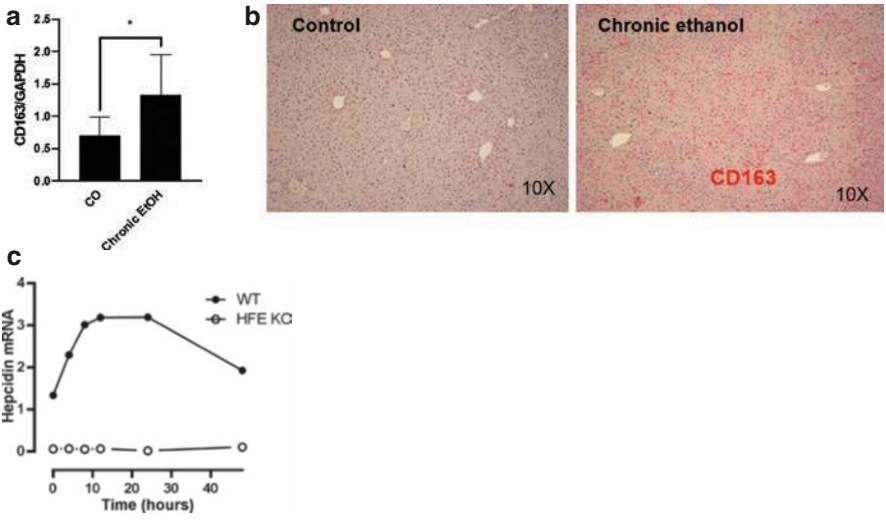


Fig. 57.8 Induction of the hepatic hemoglobin-haptoglobin scavenger CD163 in a chronic alcohol exposure model. (**a**) CD163 Western blot in mouse liver after 4 weeks of chronic alcohol feeding (n = 7). (**b**) Representative example of a CD163 immunostaining (red, Vectastain) and HE background stain. Note that CD163 is expressed in the liver (Kupffer cells) underlining the importance of hepatic erythrophagocytosis next to spleen erythrophagocytosis. (**c**) In wild type mice, mild hemolysis by phenyl hydrazine induces hepcidin mRNA which is completely blunted in the absence of HFE (HFE KO mice)

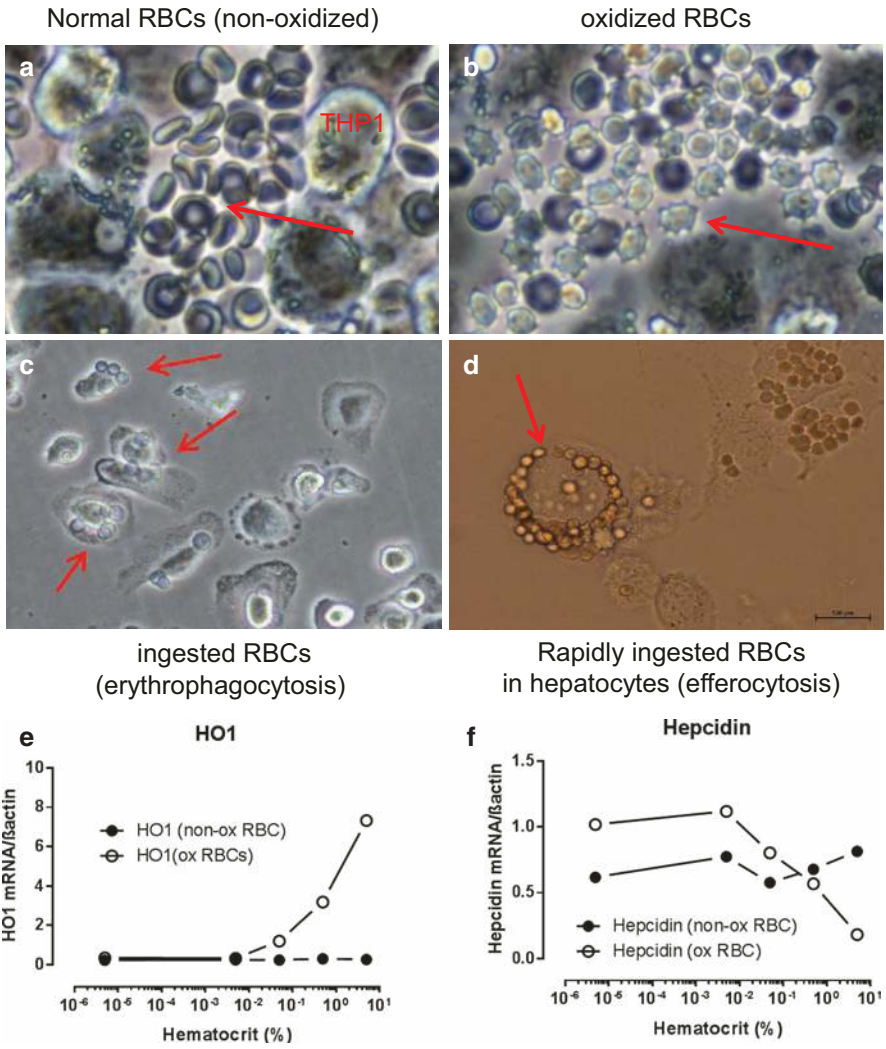


Fig. 57.9 Erythrophagocytosis in vitro of oxidized human erythrocytes. (**a**) Control RBCs (red arrow) and co-cultured human THP1 macrophages. (**b**) Morphological changes (spur cells, red arrow) of RBCs in the presence of copper sulfate-induced oxidative stress after 120 minutes (**c**) Erythrophagocytosis of oxidized human erythrocytes (red arrow, oxidized by copper sulfate) by THP-1 cells. (**d**) **Efferocytosis** of oxidized RBCs by hepatocytes. Huh7 cells were exposed for 60 min to oxidized human RBCs. RBCs are also rapidly ingested by hepatocytes which is demonstrated by aligning around the cell nucleus. Ingestion can also be seen in primary hepatocytes, followed by a strong HO1 response (not shown). Efferocytosis has been less studied in liver science. (**e**) Induction of HO-1 mRNA and (**f**) hepcidin mRNA in THP1 macrophages with increasing amounts of oxidized (white circles) and non-oxidized (black circles) RBCs. THP1 cells were exposed to oxidized RBCs (shown as %hematocrit). No HO1 induction is seen with non-oxidized control RBCs. Note that hepcidin mRNA is suppressed at high hemolysis rates

RBCs are directly taken up through the fenestrated endothelium in liver sinusoids. Finally, Fig. 57.9e, f demonstrates the expression of both hepcidin and HO1 during in vitro erythrophagocytosis with oxidized human RBCs using the macrophage cell line THP1. The figures demonstrate that only oxidized RBCs induced phagocytosis and HO1. It also demonstrates, the hepcidin is induced only at lower heme levels but suppressed and higher concentrations. The underlying mechanisms that are independent of bone marrow factors such as ERFE have been recently discussed [1, 48, 49]. It should be finally mentioned that alcohol directly interferes with the RBC production in the bone marrow and the hematopoietic stem cells. These mechanisms are more discussed in the chapter about ethanol-mediated bone marrow toxicity.

Clinical Implications of Ethanol-Induced Hemolysis for Hepatic Iron Overload

The prospective mortality analysis in heavy drinkers identifies hemolytic anemia as major prognostic factor (see Fig. 57.10). This is also implications for hepatic iron overload, long being recognized as typical feature in ALD. Since ca. 1% of the blood (ca. 40 mL, 20 mg iron) are recycled every day though the spleen-liver axis, this system becomes highly vulnerable to liver insults, since the heme degradation products needs to be eliminated by hepatocytes. Since the iron-carrier capacity of transferrin only allows to carry 2–3 mg undergoing receptor-mediated endocytosis and exocytosis, an enormous microtubule activity is required. This is

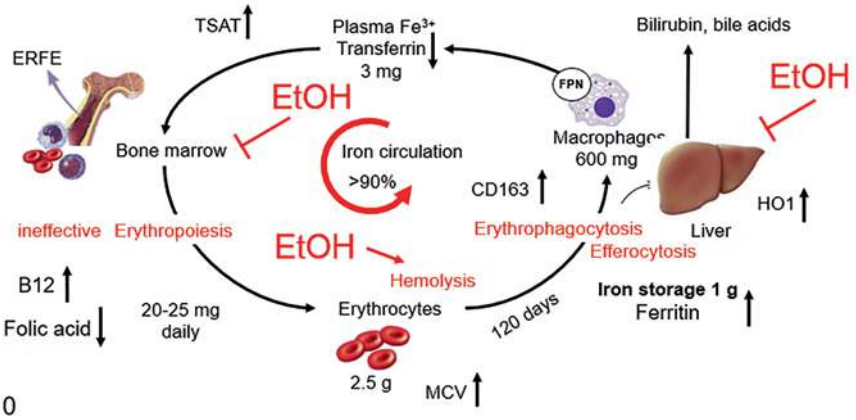


Fig. 57.10 Summarizing scheme of hematological and iron changes in heavy drinkers. Generally, RBC turnover is enhanced. Ethanol interferes with RBC turnover at three major sides: it blocks hematopoiesis, primes RBCs for erythrophagocytosis and interferes with hepatic iron handling. Major consequences are elevated ferritin and MCV, suppressed transferrin and low RBC count. Hemolytic anemia is one of the major factors associated with long-term mortality in heavy drinkers

also relevant for bilirubin transport which requires albumin binding and endocytic uptake within hepatocytes. Our clinical data show that ca. 50% of all heavy drinkers show some sign of ineffective erythropoiesis, meaning enhanced RBC turnover and heme release. In those patients were the hepatocyte is further impaired by ethanol, the enhanced bilirubin and iron recycling will cause an additional insult. As shown in Table 57.9, hemolysis as measured by CD163 levels is highest associated with hepatocellular bile acid production. Although a direct link between hemolysis and bile acid production has not been described and many explanations may account for this observation, it is quite intriguing that enhanced RBC turnover may not only be associated with release of bilirubin but also modulated bile formation, as RBCs are the major source of cholesterol and phospholipids in blood which are both also released and synthesized by hepatocytes, next to bile acids.

The link to RBCs has also shed new light on the hepcidin studies by us and others. Both, in vitro and in vivo, mild, physiological hemolysis causes a non-inflammatory hepcidin upregulation. Under conditions of severe hemolysis such as thalassemia, hepcidin is paradoxically inhibited. This has been intensively discussed in [1]. In our believe, the total understanding of iron homeostasis in heavy drinkers requires the cellular, organ and systemic considerations. Thus, the in Fig. 57.1 described lower iron levels in cirrhotics are very likely caused by an uncoupling of the spleen-liver axis under conditions of portal hypertension, where most of the

Table 57.9 Parameters that are positively (left) and negatively (right) associated with the soluble (serum) hemoglobin-haptoglobin scavenger receptor CD163

Positive Spearman rho	CD163		Negative Spearman rho	CD163	
	r	p		r	p
Bile acids (µmol/L)	0.757	3.4E-07	APO A1 after detox (mg/dL)	**−0.772**	5.9E-07
Liver stiffness (kPa)	0.670	2.5E-33	APO A1 (mg/dL)	**−0.639**	1.6E-13
Reticulocytes after detox (°/°°)	0.647	8.3E-02	Albumin (g/dL)	−0.497	3.4E-12
Bilirubin indirect (mg/dL)	0.626	2.5E-07	Transferrin (g/L)	−0.455	3.8E-11
Maddrey	0.580	7.9E-23	Hemoglobin (g/dL)	−0.254	5.6E-05
Bilirubin total (mg/dL)	0.562	8.2E-22	Hemopexin (mg/mL)	−0.236	4.0E-02
M30 (U/L)	0.547	1.8E-20	Serum iron (ug/dL)	−0.067	3.0E-01
AST/GOT (U/L)	0.533	1.5E-19			
Reticulocytes (°/°°)	0.451	1.2E-01			
ERFE (ng/mL)	0.436	1.0E-04			
MCV (fL)	0.345	9.1E-08			
CRP (mg/L)	0.323	2.3E-07			
Ferritin (ng/mL)	0.289	4.2E-06			
ALT (U/L)	0.255	5.1E-05			

Positively associated markers are linked to hemolysis (indirect bilirubin, AST), erythropoiesis and liver damage while negatively associated markers are hemoglobin or important carrier proteins that are all synthesized in the liver. Suppression may be rather due to an adaptive response than toxicity since not all liver-synthesized proteins are suppressed (e.g. macroglobulin)

portal blood will bypass the liver and only has access to hepatocytes through multiple, by far less efficient systemic cycles through the hepatic artery. Consequently, the iron-carrying transferrin and bilirubin-carrying albumin will have less access to hepatocytes. Ultimately, since RBC fragility seems to be the major causes of iron disturbances, future therapeutic measures should be more target at the complex interplay of iron homeostasis but at stabilization of RBCs and, potentially, the bone marrow. As will be discussed in the chapter about ethanol-mediated bone marrow toxicity, alcohol detoxification at least transiently, deteriorates in some patients RBC fragility and hemolysis. The causes for this are not directly alcohol related and require further studies.

Conclusions

About half of all heavy drinkers show pathological hepatic iron overload. We here demonstrate that enhanced RBC turnover is strongly associated with mortality in heavy drinkers and seems to be a key mechanism responsible for the long-observed iron accumulation in alcohol drinkers (Fig. 57.10) (see also Chap. 7 on mortality in heavy drinkers). In line with this, ca. 50% show an enhanced erythrophagocytosis and ineffective erythropoiesis as evidenced by increased CD163 elevation and ferritin in the serum. In general, and in contrast to former acute ethanol exposure models in mice, heavy drinkers have elevated hepcidin levels. Our preliminary data both in humans and animals indicate that hepcidin is primarily upregulated due to continued physiological heme turnover. This regulatory loop is disrupted by HFE mutation, cirrhosis development or severe hemolysis. There are also first indications that ethanol blocks erythropoiesis (see also Chap. 58 on bone marrow toxicity). Taken together, RBC recycling provides a first comprehensive but complex rational for hepatic iron overload in patients with ALD. The data also provide new insights into our understanding of alcoholic hepatitis.

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Chapter 58
Ethanol-Mediated Bone Marrow Toxicity and Impaired Erythropoiesis: Implications for Alcohol-Related Liver Disease

Sebastian Mueller and Marina Scheller

Abstract Alcohol-related liver disease (ALD) has been long associated with changes of red blood cells (RBC) such as macrocytosis with an elevated mean corpuscular volume (MCV). However, their implications for prognosis and disease progression have been poorly studied and understood. First preliminary data from the Heidelberg prospective study on long-term mortality in heavy drinkers has identified macrocytic hemolytic anemia as important confounder of survival in drinkers. This suggests an important role of bone marrow toxicity and connects hematopoiesis to the development of liver disease. The enhanced RBC turnover is not primarily due to vitamin or hormone deficiency but rather to enhanced RBC degradation and bone marrow injury, eventually resulting in so-called ineffective erythropoiesis. Preliminary studies in mice show that chronic alcohol exposure for 4 weeks drastically suppresses the stem cell niche while, in a compensatory manner, erythroblast maturation is increased. Of note, in humans, alcohol withdrawal initially deteriorates ineffective erythropoiesis, worsens anemia, and increases MCV. This either suggests that chronic ethanol-mediated damage to the stem cell compartment needs further repair or that additional conditions such as toxic iron overload additionally contribute to bone marrow damage. These observations have important clinical implications. There are first indications that the novel findings are not only relevant for ALD but also the rare but often fatal alcoholic hepatitis. The observations are also related to Zieve syndrome, a rare but severe form of hemolysis during heavy alcohol consumption.

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Introduction to ALD

Alcohol-related liver disease (ALD) is the most frequent cause of severe liver disease in Europe. Based on the WHO database, more than 40% of the liver deaths are attributed to alcohol [1]. The number of performed liver transplantations for patients with ALD-related cirrhosis has increased over the past two decades, both in Europe and in the USA [2, 3]. Despite the high burden of ALD, it is regrettable that most patients with ALD are diagnosed in a stage of decompensation. As shown in Figs. B.7 and B.8, ALD includes a wide spectrum of lesions ranging from steatosis to steatohepatitis, progressive liver fibrosis, cirrhosis and its complications [1]. Although steatosis occurs in >90% of heavy drinkers (see e.g. Table B.9, it is estimated that only 10–20% will develop cirrhosis [4]. Genetic and non-genetic factors also modify both individual susceptibility and the clinical course of ALD [5], however, the underlying mechanisms are not completely understood. Most animal studies have been performed in rodents with chronic alcohol intake (e.g. Tsukamoto-French model or Lieber-DiCarli diet). However, these models induce only moderate liver disease and severe fibrosis, while liver damage usually develops after an additional insult by another toxic agent. Few studies have been performed so far in livers from patients with ALD. These translational studies are needed to develop novel targeted therapies for these patients [5].

ALD has been long associated with changes of red blood cells (RBC) such as macrocytosis with an elevated mean corpuscular volume (MCV). However, their implications for prognosis and disease progression have been poorly studied and understood. First preliminary data from the Heidelberg prospective study on long-term mortality in heavy drinkers has identified macrocytic hemolytic anemia as important confounder of survival in drinkers (see also Chap. 7). This suggests an important role of bone marrow toxicity and connects hematopoiesis to the development of liver disease. The enhanced RBC turnover is not primarily due to vitamin or hormone deficiency but rather to enhanced RBC degradation and bone marrow injury, eventually resulting in so-called **ineffective erythropoiesis**. We here discuss first preliminary data in a mice model of chronic alcohol exposure for 4 weeks demonstrating a drastic suppression of the stem cell niche while, in a compensatory manner, erythroblast maturation is increased. Of note, in humans, alcohol withdrawal initially deteriorates ineffective erythropoiesis, worsens anemia, and increases MCV. This either suggests that chronic ethanol-mediated damage to the stem cell compartment needs further repair or that additional conditions such as toxic iron overload additionally contribute to bone marrow damage. The observations have important clinical implications. There are first indications that the novel findings do not only have implications for ALD but also the rare but often fatal

alcoholic hepatitis (see also Chaps. 64–68). Whether the **Zieve syndrome**, a rare but severe form of hemolysis during heavy alcohol consumption, is caused by bone alcohol-mediated marrow damage remains an open question [6].

Production and Recycling of Red Blood Cells

In the last decades, an enormous progress has been made to better comprehend the molecular mechanisms of iron regulation and homeostasis at the systemic and the cellular level [7, 8] (see also Chap. 57). The human body contains ca. 5 g of iron, of which ca. 2.5 g is used in oxygen-carrying hemoglobin of red blood cells (RBC) (Fig. 58.1) corresponding to 0.5 mg iron per ml of blood. Another 2.0 g is used in liver, bone marrow and macrophages, mostly in the iron-storage protein ferritin and iron-containing proteins such as cytochromes [9]. The liver serves as interim iron storage organ and can store up to 1 g. Circa 0.4 g are devoted to cellular proteins and enzymes in other cells. Iron is typically stored (ferritin, transferrin, enzymes) in the ferric state (Fe^{3+}) but crosses membranes though transporters in the highly reactive reduced, ferrous state (Fe^{2+}). Iron circulates bound to transferrin to be released to all organs/tissues through transferrin receptor 1. The transferrin bound ferric iron is relatively small only representing 2 mg. Most iron is recycled by macrophages, which phagocytize senescent RBCs, a procedure termed **erythrophagocytosis**. Most of RBC recycling occurs mainly in the spleen and liver through the

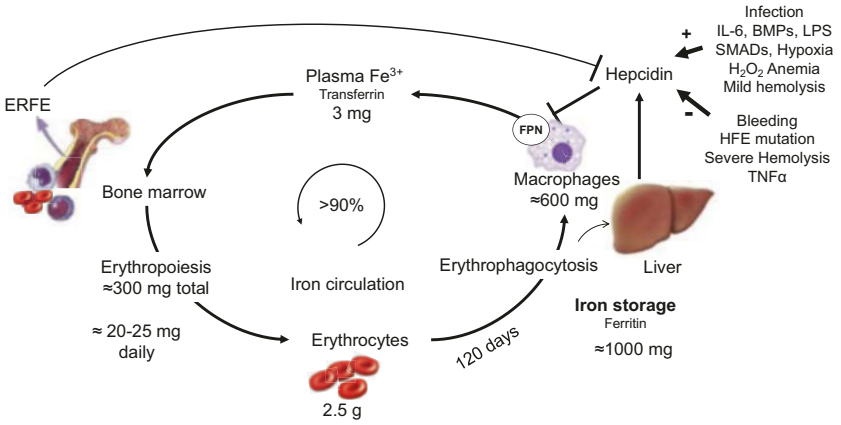

Fig. 58.1 System iron homeostasis and utilization in the human body. Dietary iron is absorbed in the duodenum and binds to transferrin. Iron is then delivered to the bone marrow for erythropoiesis the major utilization pathway. Senescent RBCs are phagocytosed by macrophages (erythrophagocytosis) and ca. 90% of iron is recycled for heme synthesis. Excess iron is stored in ferritin in the liver. Bone marrow-derived ERFE (erythroferrone) is an important repressor of hepcidin. Also note that mild hemolysis (physiological RBC turnover) stimulates hepcidin while severe hemolysis causes massive suppression

reticuloendothelial system. Macrophages can also directly recycle iron for new RBC production in the bone marrow [10].

The daily uptake of dietary iron by duodenal enterocytes compensates for net loss or iron and is relatively small with 1–2 mg [7]. Interestingly, in contrast to general believe, according to earlier observations, iron is not only lost through cell desquamation and blood loss but also through the bile and urinary tract [11]. As shown in Fig. 58.1, altogether about 90% of iron is recycled from senescent RBCs that typically have a mean survival of 120 days [10]. In other words, and considering that an average individual has 5 L total blood (2.5 g iron), ca. 40 mL blood are recycled every day which equals ca. 20–25 mg iron. Likewise, these 25 mg of iron is required for *de novo* erythropoiesis in the bone marrow [12].

During erythrophagocytosis, iron is recovered from the degradation of hemoglobin and heme by hydrolytic enzymes in the phagocytic vesicles. Iron is further released from heme in the endoplasmic reticulum by heme oxygenase-1 (HO-1) and then again redistributed to tissues via serum transferrin (see Fig. 58.1) [12]. More information about HO1, its upstream regulation and potential intracellular interactions are shown in Figs. A.73, A.74, and A.75. Besides the spleen, liver macrophages (Kupffer cells) are also an important side of erythrophagocytosis. In addition, liver is also the only location for bilirubin glucuronidation and excretion through canaliculi and bile ducts (see Fig. A.60). Mechanic obstruction of canaliculi or small bile ducts or direct hepatocyte damage through e.g., alcoholic liver injury/ballooning will also cause accumulation of bile content including bilirubin and bile acids.

Control of Iron Homeostasis during Erythropoiesis

More details about iron homeostasis and ALD are provided in book Chap. 57 on iron and ALD. A complex interplay exists between the liver and blood system requiring a fine-tuned coordination of iron homeostasis. Cells of both the blood compartment and the liver participate in iron homeostasis through specific functions. For instance, erythroblasts are specialized in iron uptake, macrophages and hepatocytes in iron export and iron storage. May be not by chance, both hepatocytes and macrophages share the expression of important iron related proteins and signaling cascades such as HO-1, ferritin, ferroportin and secretion of hepcidin. There are important differences, however, how hepcidin is regulated in both cell types [13] and respond to oxygen-derived molecules [14]. Hepatocytes also release almost 10 times more hepcidin than macrophages [14].

Absence or blockage of erythropoiesis or bone marrow failure, e.g., under conditions of aplastic anemia, myelodysplastic syndrome etc., lead to accumulation of iron in the blood that saturate the buffering capacity of serum transferrin and result in non-transferrin-bound highly reactive forms of iron. The excess iron in all these cases is derived from senescent RBCs that mainly accumulate in liver macrophages and later hepatocytes as mentioned above (see Fig. 58.2). Studies in animal models

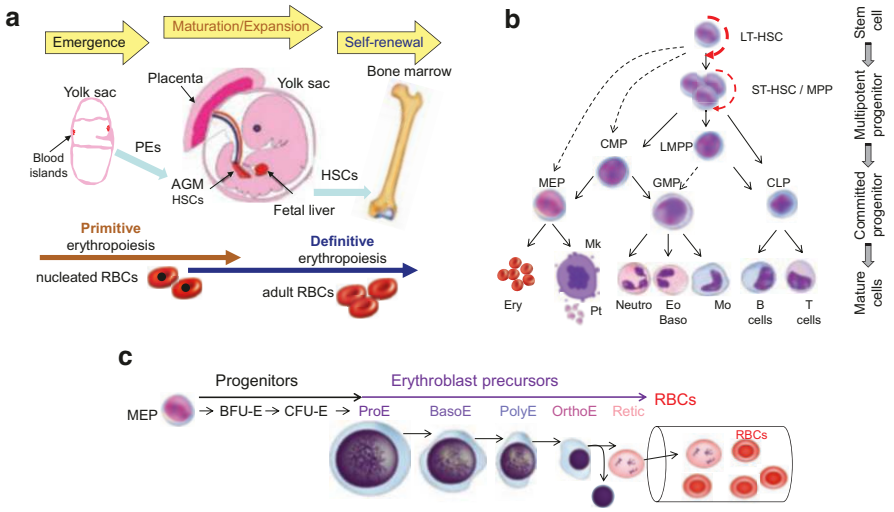

Fig. 58.2 Hierarchical organization of fetal and adult hematopoiesis. (**a**) There are three developmental stages of hematopoiesis in mammals. The first – the emergence of primitive erythroblasts (PE) in yolk sac blood islands, presence of nucleated erythrocytes, so called, primitive erythropoiesis. In the second – maturation and expansion of the HSC that emerges from the aorto-gonad mesonephros (AGM). Third – the self-renewal HSC migrates to fetal liver and to the adult BM, producing definitive erythroblasts. (**b**) Classical (solid line) and alternate (dotted) models of the adult haematopoietic hierarchy. In the classical model, the HSCs gives rise to either a CMP or CLP. The CMP then differentiates into either a GMP or MEP. These progenitors differentiate into mature cells of distinct lineages. Alternate pathways (dotted lines): HSCs were shown to differentiate directly into CMP, MEP and megakaryocytes. HSC can also differentiate into a lymphoid primed multipotent progenitor (LMPP) lacking any megakaryocyte erythroid potential. (**c**) Erythroid differentiation in the mouse: from MEPs to mature RBCs. *BFU-e* burst-forming unit, erythroid, *BM* bone marrow, *CFU-e* colony-forming unit, erythroid, *CLP* common lymphoid progenitor, *CMP* common myeloid progenitor, *GMP* granulocyte monocyte progenitor, *HSCs* hematopoietic stem cell, *MEP* megakaryocyte-erythroid progenitor, *MPP* multipotent progenitor, *RBC* red blood cell

[15, 16] and humans [17, 18] have shown that hepcidin expression is dependent on the degree of erythropoiesis, showing a dominance of the erythropoiesis over the storage regulator [19, 20]. Altogether, ineffective erythropoiesis strongly suppresses hepcidin, induces excess iron absorption finally leading to hepatic iron overload comparable to hereditary iron overload. The underlying mechanism, however, are still poorly understood.

Erythroferrone (ERFE) has been identified as important negative erythroid regulator of hepcidin and, in contrast to many other hepcidin stimuli (see Fig. 58.1) [21]. When the release of erythropoietin from the kidney stimulates the production of new RBCs, it also increases the synthesis of ERFE in bone marrow erythroblasts. Increased ERFE then suppresses hepcidin synthesis, thereby mobilizing cellular iron stores for use in heme and hemoglobin synthesis. Recent mechanistic studies have shown that ERFE suppresses hepcidin transcription by inhibiting bone

morphogenetic protein signaling in hepatocytes. In ineffective erythropoiesis, pathological overproduction of ERFE by an expanded population of erythroblasts suppresses hepcidin and causes iron overload, even in non-transfused patients. However, besides ERFE, we could recently demonstrate that excess of non-toxic iron, *in vitro* and in the absence of erythroid cells, is also able to suppress hepcidin expression [22].

Organization of Fetal and Adult Erythropoiesis

Hemoglobin-producing RBCs are the terminally differentiated end-product cells of a lineage-restricted erythroid progenitor which undergo differentiation and enormous expansion to cover the daily needs of $\sim 2 \times 10^{11}$ new erythrocytes [23]. There are large similarities between humans and mice, the latter often used for RBC development studies. Developmentally, there are two types of RBCs - embryonic and adult (Fig. 58.2a). In mice, the early embryonic erythroid cells arise in the yolk sac from the mesodermal cells. They are nucleated and short-lived cells [24]. This developmental stage known as primitive erythropoiesis, shows more immature, less differentiated erythroblasts or megaloblasts that are not pluripotent and self-renewing (Fig. 58.2a). The yolk sac blood islands contain primitive nucleated erythrocytes that disappear from the circulation very quickly during the embryonic-to-fetal transition period resulting in macrocytic and enucleated erythrocytes. Erythromyeloid progenitors (and subsequently hematopoietic stem cells, HSCs) from the aorta-gonad-mesonephros (AGM) region will migrate to the fetal liver to produce the first definitive erythrocytes (Fig. 58.2a). Similarly, in humans, definitive hematopoiesis from HSC and hematopoietic progenitors (HPCs) first in the AGM region, later in the fetal liver, until the 4th–fifth months of pregnancy. During embryonic and fetal development, hematopoiesis takes place in different organs: the yolk sac, the aorta–gonad mesonephros region, the fetal liver, the spleen, and bone marrow (Fig. 58.2a).

The hematopoietic function of the fetal liver is especially related to its erythroid function. Finally, HSCs from fetal liver migrate and colonize the bone marrow (BM) at birth, where they provide lifelong production of definitive RBCs. While the spleen, in addition to BM, remains an important additional erythropoietic organ in mice, the BM remains the **major place for steady-state adult erythropoiesis** in humans. Only under erythroid stress conditions, mainly as a compensatory mechanism under conditions of impaired BM function, spleen and liver of both mice and humans are used to expand the erythropoietic capacity [25]. This process is called extramedullary hematopoiesis (EMH).

Maturation from erythroid-committed precursors is called terminal erythropoiesis. It starts with HSCs in the adult BM (Fig. 58.2b, c). HSCs possess the unique ability to both self-renew and to generate multipotent (MPPs) and common myeloid progenitors (CMP) or common lymphoid progenitors (CLP) (Fig. 58.2b). The classical hierarchical relationship is, however, currently challenged by alternate differentiation pathways (Fig. 58.2b, dotted arrow) [26–28]. The committed granulocyte

monocyte progenitors (GMPs) differentiate into mature granulocytic and monocytic cells. Megakaryocyte erythroid progenitors (MEPs) further differentiate into erythroid precursor cells with distinct morphologies (Fig. 58.2c).

Burst forming unit-erythroid (BFU-E) cells are progenitors that possess colony-formation capacity in response to growth factors in methylcellulose culture *in vitro*. These multi-subunit colonies (or bursts) contain thousand hemoglobin-harboring cells and appear after 5–8 days (mouse) or 10–14 days (human) in culture. Later, more mature erythroid progenitors consisting of small colony- forming units-erythroid (CFU-E) colonies of 16–125 cells appear further after 2–3 days (mouse) or 5–8 days (human) of culture. Erythroblasts (EP) differentiate gradually reducing in cell and nuclear size, to proerythroblasts (ProE), basophilic erythroblasts (BasoE), polychromatophilic erythroblasts (PolyE), and orthochromatic erythroblasts (OrthoE) (Fig. 58.2c) [29, 30]. Specifically, one orthochromatic erythroblast undergoes an asymmetric division and divides into two cells, one containing the nucleus with a small cytoplasm, and one enucleated reticulocyte, which later matures into a red blood cell [30]. Normal and pathological erythropoiesis can be successfully analyzed using fluorescence-activated cell sorting (FACS) techniques based on changes in the expression of cell surface markers on mouse and human erythroid cells at different stages of erythropoiesis.

Alcohol-Related Toxicity on the Hematopoietic System

As shown in Table 58.1, alcohol can cause damage to hematopoietic cells both directly and indirectly [31–33]. The direct toxic targets of excessive alcohol include most of bone marrow cells, blood stem cells and precursors, mature erythrocytes, white blood cells, and platelets [31]. Impairment affects not only process of blood cells production but also changes bone marrow cell morphology. The most commonly observed effects of morphological changes are **large vacuoles** in erythroid and megakaryocytic lineages especially in early RBC precursor cells which usually emerge in the pronormoblasts within 7 days after heavy alcohol intake and disappear within 1–2 weeks after abstinence [34–37]. Culturing normal marrow cells in nutrient medium with alcohol can induce cytoplasmic vacuolization as well, and the proportion of cells developing vacuoles appears to correlate with the concentration of alcohol [37]. Of note, the hematologic alterations occur despite the concomitant administration of pharmacologic doses of folic acid [38]. To a lesser extent, vacuoles also develop in the granulocyte precursors of heavy drinkers.

The precise mechanisms underlying vacuole development in blood cell precursors is currently unknown but seems to be an indication for cell stress and reflects an adaptive response for cell survival, ultimately leading to different forms of cell death [39, 40], however, its role in survival versus apoptosis remains unclear. Moreover, alcohol induces oxidative stress, disrupts protein production in the ER, and inhibits ubiquitin-proteasome activity in cells [41–43]. These negative effects of alcohol on cell functions may potentially contribute to the formation of vacuoles.

Table 58.1 Reported effects of ethanol and its metabolites on erythropoiesis and RBCs

Molecules	Targets	Effects	Ref.
Ethanol	Intestine	Folate deficiency	Medici et al. [45]
Ethanol	Erythroid precursors	Vacuolization and cell death	Roselle et al. [36] Yeung et al. [37]
Ethanol	Erythroblasts	Iron deposit and ring sideroblasts, sideroblastic anemia	Pierce et al. [100] Lindenbaum et al. [98]
Ethanol	Erythrocytes	Elevated MCV, macrocytic anemia	Seppä et al. [94] Maruyama et al. [91]
Acetaldehyde	Hemoglobin	Forming adducts with hemoglobin A	Stevens et al. [106]
Acetaldehyde	DNA	Blocking DNA translesion	Yu et al. [72]
ROS	Erythrocytes	Disorder of erythrocyte cytoskeleton, stomatocytes and spur-cells	Morse et al. [107] Fukuda et al. [108] Koch et al. [109]
ROS	Erythroblasts	Disturbing the enucleation	Zhao et al. [110]
Fatty acid ethyl ester	Erythrocytes	Incorporating into the membrane and causing hemolysis	Tyulina et al. [111]

It is possible that vacuolization directly primes erythroid precursors to death, thereby contributing to macrocytic anemic of chronic drinkers combined with nutritional deficiency especially folate [44, 45]. Cytoplasmic vacuoles, however, can be also seen in several other clinical settings such as copper deficiency/zinc toxicity, antibiotic treatment, myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML) [46–48]. Thus, blast vacuoles predict poor overall survival in AML patients undergoing induction chemotherapy [49]. Another consequence of alcohol cell toxicity is cell death via the apoptosis pathway. Although human and murine embryonic (ESCs), neural (NSCs) and mesenchymal stem cells (MSCs) are sensitive to the induction of apoptosis by alcohol administration, HSCs appear to be more resistant [50]

The other deleterious effect of alcohol abuse associates with myelosuppression, disruption of homeostasis of granulopoiesis and impaired functional activities of granulocytes [51–53]. This represents an underlying mechanism for defects in immune defenses in alcohol-dependent patients with severe bacterial infections, particularly pneumonia and septicemia reviewed in [50, 53, 54]. Analysis of alcoholic patients with severe neutropenia has shown that early granulocytic progenitors do not mature in bone marrow, and neutrophil stores are emptied faster than in healthy controls [31]

The cytotoxic effect of alcohol also affects the development of platelets. Although the abnormal alcohol-related cytotoxic effects on platelets development have been adequately described, a systematic review of the relationship between alcohol and thrombocytopenia is still lacking, and the postulated hypotheses are not adequately supported by research evidence [55].

Overall, chronic, and acute alcohol exposure affects multiple hematopoietic cell populations and their functions, indicating the crucial role of HSCs, which should be able to self-renew and restore the entire blood system under alcohol-related toxicity. Normally, HSCs reside in a quiescent state in specialized niches in the bone marrow, which represent an interdependent network of endothelial, osteolineage cells, pericytes, reticular (CAR) cells, MSCs, fat cells [56, 57]. Alcohol may impact key aspect of stem cell biology, namely maintenance of self-renewal and differentiation, indirectly fashion by altering function and maintenance of the cells in the bone marrow niche. These causes increase in osteocyte apoptosis and accumulation of lipid droplets within the osteocytes, which has been associated with the reduction of bone mass and decreased bone formation observed in alcoholics [58–60]. Such alcohol-induced adipogenic activity in the BM niches leads to osteopenia and pro-inflammatory environment and mobilization [61].

Ethanol-Derived Metabolites Potentially Involved in Bone Marrow Toxicity

Besides the direct influence of alcohol on bone marrow cells, the alcohol-derived metabolites such as acetaldehyde (AA) and reactive oxygen species (ROS) leads to enormous toxicity. The toxic effects of AA are caused by its ability to interact with proteins, peptides and other biomacromolecules in the cell to form harmful adducts [62, 63]. The formation of AA-induced adducts on the erythrocyte membrane is associated with ethanol-induced an abnormally large number of enlarged erythrocytes in the blood, called macrocytosis, and have been found both in blood and bone marrow of patients with ethanol-induced erythrocyte abnormalities [64].

Alcohol-induced ROS (see also Figs. A.35, A.67, A.68) modify erythrocyte membranes which leads to destabilization [65]. Normally, ROS decreases in erythroblasts in later stages when the cells are preparing for enucleation. However, in heavy drinkers, ROS accumulation of terminal erythropoiesis will interfere with the initiation of enucleation [66]. Furthermore, alcohol metabolism generates a variety of nitrogen oxygen species (NOS) radicals (see also Figs. A.35, A.67, A.68), which significantly alter erythrocyte membrane fluidity, membrane bound proteins, enzymes and transport mechanisms [65, 67].

Compared to alcohol effects per se, its metabolites, AA and ROS are mutagenic and carcinogenic due to induction of DNA damage [68, 69]. The obvious manifestations of AA-induced DNA damage are severe cytogenetic abnormalities, sister chromatid exchanges (SCEs), and chromosomal aberrations, including translocations and rearrangements [70, 71]. In particular, AA reacts with DNA to primarily form a dysfunctional adducts which ultimately and efficiently block DNA synthesis [72]. It also binds to proteins critical for DNA repair and DNA methylation, and to the antioxidant glutathione, causing both differentiation defects in stem cells as well as genomic damage that incites cellular aging and carcinogenesis [69, 73].

With regard to the hematopoietic system, it has been demonstrated that AA affects survival of HSCs but not their progenitors in the absence of aldehyde dehydrogenase (*Aldh2)* [74]. *At the same time,* AA and endogenous aldehydes in the absence of the protective role of the Fanconi anemia DNA repair pathway (*Fancd2*-loss) and *Aldh2* leads to engraftment defects and a severe depletion of the HSC pool and their progeny, including B cells and granulocyte-macrophage progenitors [70, 74]. Indeed, deficiency in aldehyde dehydrogenase 2 (ALDH2) expression and function in patients of East Asians (*ALDH2*2* genetic polymorphism) has been found to be associated with marrow failure, with both an increased risk of sporadic aplastic anemia and more rapid progression of Fanconi Anemia [75]. This points to important crosslinks between alcohol detoxification pathways and DNA repair mechanisms in triggering severe phenotypes of HSCs and their blood lineages. Furthermore, formaldehyde produced by the metabolism of methanol, which may be also present in alcoholic beverages at small amounts, exerts a genotoxic effect on HSCs [76]. In addition to AA, ROS also affects HSC function, induced hematopoietic aging linking elevated ROS levels and impaired DNA repair mechanisms [77] (Fig. 58.3). Taken together, alcohol derived AA acts as a genotoxic agent that can induce mutations in HSCs. Alcohol does not destroy healthy mature circulating cells, it gradually ruins HSCs and blood cells factory, resulting in bone marrow failure.

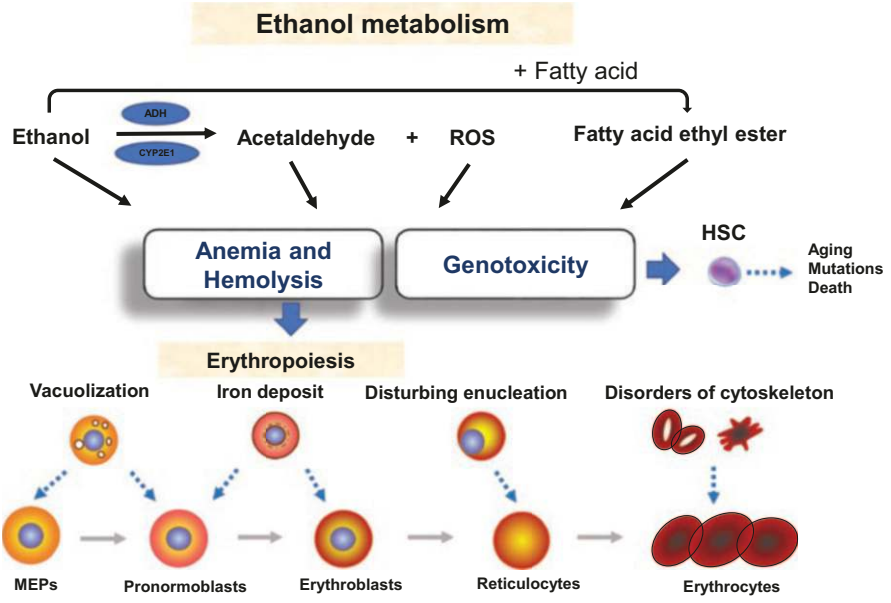

Fig. 58.3 Ethanol metabolism and bone marrow toxicity. Ethanol and its metabolites acetaldehyde, reactive oxygen species (ROS) and fatty acid ethyl ester interfere with erythropoiesis and cause damage to stem and precursor cells such as erythroblasts. This leads to vacuolization of MEPs and pronormoblasts, iron deposit in erythroblasts and malfunctional enucleation of reticulocytes

In steady state conditions, high and low oxygenated areas present in the BM microenvironment create conditions for a differential production of ROS in different niches. These niches contain undifferentiated HSCs with low levels of ROS and high self-renewal potential [78–80]. Conversely, ROS high areas contain more cycling, HSCs and progenitors that show bias toward myeloid differentiation, like aged mice *in vivo* [78]. Increased ethanol-induced ROS-stress may leads to reduced stem cell regenerative potential, excessive differentiation, resulting in stem cell "exhaustion", loss of ability to efficiently replenish progenitor pools that finally contribute to bone marrow failure. ROS seem well tolerated in "young" HSCs with highly proficient DNA repair, and thus have few long-term deleterious effects. However, "aged" HSCs, with less efficient repair become more sensitive to ROS levels, accumulate DNA damage [81] that lead to increased genome instability.

In summary, alcohol consumption causes impairment of structure, signaling, metabolism, proliferation, and differentiation of hematopoietic cells. HSCs and the upstream multipotent progenitors are more resistant to the negative effects exerted by ethanol and acetaldehyde in comparison to the myeloid and the downstream progenitor cells [82] which is thought to be related to aldehyde dehydrogenase (ALDH) activity [83–85]. Whether the Zieve syndrome, a rare but severe form of hemolysis during heavy alcohol consumption, is caused by alcohol-mediated bone marrow damage remains an open question [6].

Alcohol-Related Effects on Erythropoiesis

When ethanol was administered to human volunteers in doses equaling 46 to 66 per cent of caloric intake, and excellent protein and vitamin intake was maintained, vacuolization of bone-marrow pronormoblasts developed [38]. Their presence appeared to be dose-related. Vacuolation of promyelocytes was seen less consistently, and only with the larger doses. The hematologic alterations occurred despite the concomitant administration of pharmacologic doses of folic acid [38]. It directly damages erythroid precursors with vacuolization [33, 34, 37], thereby contributing to macrocytic anemic of chronic heavy drinkers combined with nutritional deficiency especially folate [44, 45]. Nucleated bone marrow cells metabolize ethanol [86] and the metabolites can also exert negative effects on erythropoiesis by disrupting and/or impairing the structural integrity, signaling regulation, metabolism, survival, proliferation, as well as differentiation of hematopoietic tissue [70, 87, 88].

Macrocytic Anemia and Sideroblastic Anemia in Heavy Drinkers

Excessive alcohol consumption is one of the most common causes of **macrocytosis (an elevated mean corpuscular volume of erythrocytes - MCV**) and non-megaloblastic macrocytic anemia [89, 90]. Alcohol detoxification can improve the

elevated MCV [91]. On the other side, it is also commonly observed that MCV can remain elevated for months despite abstaining from alcohol. Neutrophil hypersegmentation is a useful sign of folate depletion in patients [92, 93], whereas serum folate concentrations are not reliable. The Michigan Alcoholism Screening test and obtaining γ-glutamyl transferase (GGT) levels are most sensitive tests for detecting heavy drinkers and alcohol dependence with macrocytosis (see also Chap. 37), who may also exhibit other typical symptoms such as gynecomastia, caput medusae, and jaundice [94]. Significant folate depletion in alcoholism has been described as important reason for macrocytosis [95, 96]. The normal production and maturation of erythroid precursor cells require folic acid and other B vitamins [31]. Although ethanol itself has been considered as the main reason of folate deficiency in alcoholics [93], there are conflicting results and we and others have not seen a stringent association between an elevated MCV deficiency of folic acid and vitamin B12 (see also text to Table 58.3).

Sideroblastic anemia is characterized by the emergence of ring sideroblasts in bone marrow [97, 98]. In some patients with alcoholism, iron cannot be properly incorporated in hemoglobin. These pathological erythroblasts which have particular iron accumulation in perinuclear mitochondria are called ringed sideroblasts [99] and cannot further develop into functional erythrocytes. Acquired sideroblastic anemia is a common complication in heavy drinkers [100]. Furthermore, alcoholic sideroblastic anemia is often associated with **myelodysplastic syndrome** [101]. It has been shown that ring sideroblasts usually disappear within 1 week of abstinence [31].

Preliminary Lessons from the Prospective Survival Cohort of Heavy Drinkers in Heidelberg: Hemolytic Anemia as an Important Prognostic Marker

Mortality data are essential to identify important confounders of disease progression. So far, no prospective long-term data on survival are available for patients with ALD. In 2007, the Heidelberg Center for Alcohol Research started to prospectively enroll heavy drinkers. Most patients are presenting for alcohol detoxification allowing to also collect data after ca. 1 week of alcohol withdrawal. The study is still ongoing and covers now almost 15 years (see also Chap. 7). In an interim analysis, information of survival status was obtained in 786 patients that had presented from 2007 to 2022 with a mean daily consumption of alcohol of 184 g/day. Mean observation time was 3.8 years and mean duration of heavy drinking was 14.0 years. During the observation time, 159 patients (20%) had passed away. More details are provided in Chap. 7 on mortality. The cause of death could be clarified in 47%. In 34%, the death was liver-related. As shown in Table B.10, **signs**

of anemia were associated with long-term mortality. Among the three major markers of anemia (hemoglobin, RBC counts, hematocrit), a long RBC count was especially associated with an increased mortality. Interestingly, these markers were better than known other prognostic markers such as albumin, INR, or bilirubin. Multivariate analysis confirmed that low RBC count is an independent predictor of death.

Anemia in response to chronic alcohol exposure can have multiple causes ranging from iron deficiency due to blood loss up to inflammation. However, as discussed in the chapter on mortality, anemia rather shows typical characteristics of hemolytic anemia meaning that RBCs are destroyed faster than they can be made. Thus, levels of the hemolytic enzyme LDH, the iron marker ferritin and the end-product of heme production bilirubin were all positively and significantly associated with long-term death. Moreover, death also correlated highly with a large size of RBCs (MCV), the typical hallmark of drinkers. To further confirm the nature of the anemia we measured in serum of a representative subcohort the precursor of conjugated bilirubin, the unconjugated or indirect bilirubin and the soluble hemoglobin-haptoglobin scavenging receptor CD163. Both showed the highest correlation with death ($r \sim 0.25$) only being surpassed by RBC count and AP. In conclusion, long-term follow up in our prospective cohort of heavy drinkers identifies signs of **hemolytic anemia** as predominant predictor of death.

To get more insights into potential causes of the anemia, patients were grouped (Tables 58.2 and 58.3) according to the size of RBCs (MCV) in three groups (microcytic <80, normocytic 80–96 and macrocytic >96). Compared to a normal population, Hb values were about 20% lower, although only 20% of all patients full-filled criteria of anemia (<12.5 g/dL). The macrocytic group represents one third (31.8%) and it showed the highest mortality. In this group, mortality was three times as compared to patients with normocytic RBCs (31.1 vs 11.6%). Expectedly, the group with microcytic anemia, classically representing iron-deficiency anemia, had the lowest iron level. Important **hematopoietic parameters** such as levels of folic acid, EPO, B12 were usually all in the normal range. In the macrocytic group, however, levels of EPO were highest and in the upper normal range, while B12 levels slightly exceeded upper normal levels. Finally, reticulocyte count as a direct measure of hematopoietic activity was only increased in this group.

The macrocytic group also showed further **evidence of hemolysis**. CD163, indirect bilirubin and LDH was highest while in this group haptoglobin was lowest. Based on CD163 levels in in Table 58.2 and 58.3, 44% of all drinkers show erythrophagocytosis. Notably, enhanced erythrophagocytosis is not only observed in the macrocytic group (59.6%) but also in the normocytic and microcytic group (32.5 and 38.5%). In addition, ferritin levels were highest in the macrocytic group. Consequently, this laboratory represents a typical configuration of **hemolytic**

Table 58.2 Various parameters in heavy drinkers grouped according to RBC size (MCV)

Groups	Units	Normal	P*	high MCV	normal MCV	low MCV	All
		Range		>96	80–96	<80	
MCV	fL	80–96	***	101.7	90.4	65.2	93.4
Percentage	%			31.8%	65.7%	2.5%	100.0%
Hemoglobin	g/dL	>12.5	***	13.4	14.4	12.1	14
Anemia fraction				28.4%	13.0%	33.7%	19.7%
Erythrocytes	/pL	4.5–5.9	***	3.7	4.5	4.7	4.3
Hematocrit	%	40–53	***	37.8	40.8	35.8	39.8
All-cause mortality			***	31.1%	11.6%	20.0%	
Parameters of hematopoiesis							
Vitamin B12	pmol/L	145–596		616.2	494.4	341.0	524.5
Folic acid	nmol/L	>7.1	**	10.7	17.3	8.6	15.3
Epo	mIU/mL	6–15	**	11.7	6.2	0.5	8.0
Reticulocytes	°/°°	8–25	***	27.0	15.4	16.8	19.5
Parameters of iron metabolism							
Ferritin	ng/mL	>400/150	***	853.4	484.1	272.1	594
Elevated ferritin fraction				62.9%	38.0%	20.0%	44.9%
Transferrin	g/L	2–3.6	***	2.0	2.5	2.6	2.4
Serum iron	µg/dL	95–158		129.1	122.2	103.7	123.9
Transferrin saturation	%	16–45	***	49.5	38.9	32.6	42.1
Hepcidin	ng/mL	1–55	**	13.9	17.1	24.4	15.8

P* comparison between high and normal MCV
Note, that drinkers with signs of hemolytic anemia (high MCV, signs of hemolysis, decreased hemoglobin) have a three-time increased mortality. Hemolytic anemia is also not due to the lack of folic acid and vitamin B12. Potential other causes are related either directly to RBC toxicity or bone marrow toxicity

anemia with enhanced, but ineffective erythropoiesis. Finally, we studied the effect of alcohol detoxification on RBC markers. As shown in Fig. 58.4, both anemia (RBC count and hemoglobin) and RBC size (MCV) further deteriorate despite increased reticulocytes/erythropoiesis after ethanol detoxification. This either suggests that chronic ethanol-mediated damage to the stem cell compartment needs further repair or that additional conditions such as toxic iron overload contribute to hemolysis. The observation has important clinical implications as alcohol withdrawal can have negative side effects and a sudden withdrawal from alcohol may put some patients at risk. Besides the molecular mechanisms of the further pronounced ineffective erythropoiesis after alcohol detoxification, it remains to be studied whether this phenomenon contributes to the rare but often fatal alcoholic hepatitis.

Table 58.3 Various parameters of erytrhophagocytosis and liver in heavy drinkers grouped according to RBC size (MCV)

Groups	units	normal	P*	high MCV	normal MCV	low MCV	All
				>96	80–96	<80	
MCV	fL	80–96	***	101.7	90.4	65.2	93.4
Percentage	%			31.8%	65.7%	2.5%	100.0%
Parameters of erythrophagocytosis/hemolysis							
CD163	ng/mL	<1500	***	1945.0	1325.8	1149.8	1686.3
Elevated CD163 fraction	ng/mL		***	59.6%	32.5%	38.5%	44.7%
Bilirubin indirect	mg/dL	0.2–0.8	*	0.57	0.40	0.37	0.46
LDH	U/L	<250	***	268.9	223.8	210.6	238.5
Haptoglobin	g/L	0.3–2.0	*	1.3	1.5	1.7	1.4
Liver parameters							
Liver stiffness (fibrosis)	kPa	< 6 kPa	***	27.8	12.8	17.1	17.7
CAP (steatosis)	dB/m	<240	*	283.7	268.4	286.2	294.1
GOT	U/L	<50	***	118.9	84.8	81.3	95.6
GPT	U/L	<50	Ns	66.9	68.2	60.3	67.1
GGT	U/L	<60	***	601.5	304.9	348.3	400.3
AP	U/L	40–130	***	134.3	101.9	123.9	112.8
Bilirubin total	mg/dL	<1,3	***	2.5	1.1	0.8	1.6
Albumin	g/dL	3.4–5.4	***	4.1	4.4	4.1	4.3

P* comparison between high and normal MCV

Hemolytic anemia is also tightly linked to liver damage, as shown by elevated transaminases, bilirubin and liver stiffness. Although sigs of hemolysis occur already prior to the onset of liver damage, progressing cirrhosis further deteriorates RBC turnover. CD163, the hemoglobin-haptoglobin scavenging receptor is also highest in the high MCV group

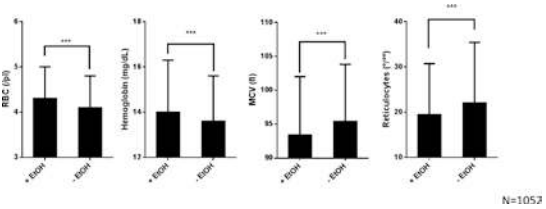

Fig. 58.4 Red blood cell parameters after alcohol detoxification. Note that both anemia (RBC count and hemoglobin) and RBC size (MCV) further deteriorate despite increased reticulocytes/erythropoiesis after ethanol detoxification. This either suggests that chronic ethanol-mediated damage to the stem cell compartment needs further repair or that additional conditions such as toxic iron overload play a role. The observation has important clinical implications as alcohol withdrawal can have some negative side effects. Besides the molecular mechanisms of the further pronounced ineffective erythropoiesis after alcohol detoxification, it remains to be studied whether this phenomenon contributes to the rare but often fatal alcoholic hepatitis

Alcohol-Related Effects on the Hematopoietic Stem and Progenitor Cells: Preliminary Data from an Ethanol Mouse Study *In Vivo*

Despite the considerable amount of research in this area, many questions remain unanswered of how alcohol-related stress impact the hematopoietic stem/progenitor cells function and their terminal differentiation. Due to its relevance, we are here sharing first preliminary findings of alcohol effects on hematopoietic system in a murine alcohol model. In this model, we focus on cellular aspects between alcohol consumption, iron overload and erythropoiesis. We explored two different established murine models of alcohol exposure. In the first [102], animals, C57BL/6N mice were fed 20% ethanol in water for 2–4 h in a dark cycle (DID-model). The other explored the classical isocaloric Lieber-DeCarli (LD) diet with alcohol (4.5 g/kg) [103]. Although, total body wight was not changed, ethanol tended to increase the liver/body weight ratio in both models (Fig. 58.5a), which was in difference to [104]. Four weeks after alcohol exposure, a significant increase of fat content as

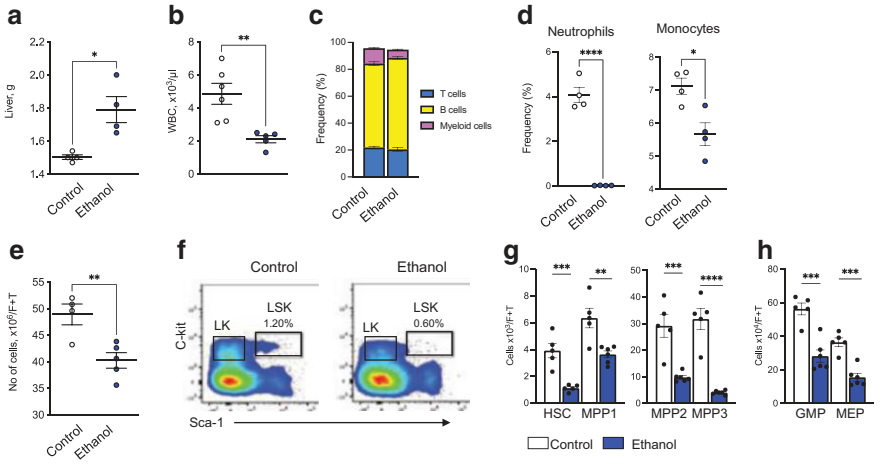

Fig. 58.5 Hematopoietic changes in a chronic murine ethanol model. A classical isocaloric Lieber-DeCarli diet with alcohol (4.5 g/kg) was applied for 4 weeks. (**a**) Elevation of liver weight and (**b**) Decrease of WBCs in peripheral blood (**c**) Frequency of the major differentiated cell lineages: B-, T- and myeloid cells in the peripheral blood of control and ethanol-treated mice (**d**) reduced frequency of the neutrophils and monocytes in the peripheral blood (**e**) Decreased total number of nucleated cells in the bone marrow as indication of toxic ethanol effects on stem und progenitor reservoir. (**f**) Representative FACS plot with gating strategy of stem and progenitor cells (LSK) from control versus ethanol-treated mice (**g**) Reduction number of HSCs and further undifferentiated MPPs. (**h**) Interestingly, ethanol-treated mice produced less of more committed myeloid GMPs and megakaryocytic-erythroid progenitors (MEPs). Abbreviations: *CLP* common lymphoid progenitors, *CMP* common myeloid progenitors, *GMP* granulocyte monocyte progenitors, *HSC* hematopoietic stem cell, *LSK* progenitor cells, *MEP* megakaryocyte erythroid progenitors, *MPP* multipotent stem cells, *Sca-1* stem cell antigen-1

assess by oil red O stain was observed but no signs of inflammation, iron changes or fibrosis (data not shown). On the other hand, level of blood AST were significantly increased after 4 weeks in the LD mice, confirming the development of liver injury in LD mice.

The hallmark of progressive alcoholic toxicity on the hematopoietic system in humans is anemia, neutropenia and thrombopenia. Four weeks post alcohol feeding, "chronic" mice showed a **remarkable decrease of WBCs** in peripheral blood (Fig. 58.5b). Multilineage contribution within WBCs showed reduction of frequency and number of myeloid cells, especially mature neutrophils and circulating monocytes, showing higher sensitivity to ethanol-related toxicity compared to B- and T-lymphoid lineages (Fig. 58.5c, d), confirming results by others [103].

The total number of nucleated cells also in the bone marrow was reduced showing toxic effect on stem und progenitors' reservoir (Fig. 58.5e). Determination of HSCs and progenitors in various developmental stage using phenotypic surface marker as described [105] showed reduction number of HSCs and further undifferentiated MPPs (Fig. 58.5f, g). Interestingly, LD and DID mice produced less of more committed myeloid GMPs and megakaryocytic-erythroid progenitors (MEPs) (Fig. 58.5h).

Thus, chronic ingestion of alcohol affected the early stem and progenitor compartment, especially the myelo-erythroid line. Ethanol-related effects were analyzed more precisely on differentiation stage of erythroid progenitors from proerythroblast till reticulocytes (Fig. 58.6a FACS and b picture, morphology of cells). Erythroid progenitors and their maturation can be monitored by the differential expression of CD44 and TER119 in mouse (Fig. 58.6a). Earlier megakaryocyte–erythroid progenitors (MEPs) lack TER119, but downstream erythroid progenitors, proerythroblast are $Ter119^{+}$ $CD44^{+}$ (Fig. 58.6a, b Fraction I). Accordingly, further differentiated stage may subdivide into downstream progenitor populations as II (basophilic erythroblasts), III (polychromatic erythroblasts), IV (orthochromatic erythroblasts) and V (reticulocytes) (Fig. 58.6a, b, plot of CD44 versus FSC (reflecting the cell size) of the TER119 positive cells). Our quantitative analysis revealed that alcohol drastically reduce number of all nucleated erythroblasts (I II III IV) (Fig. 58.6c). These findings demonstrate the decreased erythropoietic activity of bone marrow under stress conditions. Of note, within the nucleated erythroblast population, the ProE (I), Baso (II), Poly (III), and Ortho (IV) maintain the 1:2:4:8 ratio (Fig. 58.6d, left and e), confirming a normal progression of erythropoiesis in bone marrow. However, under chronic alcohol abuse, the ratio changed to 1:2:9:26 (Fig. 58.6d, right and e), showing intensive proliferation/mitotic division under alcohol-induced stress (shifting in the direction of enucleated erythrocytes). Surprisingly, proportion of enucleated erythrocytes/reticulocytes (V), measured in BM were slightly increased but number of mature RBCs in peripheral blood was unchanged (Fig. 58.6f, g).

In conclusion, these preliminary data in an *in vivo* ethanol mouse model show that chronic **alcohol exposure drastically decreases the number of phenotypically identified stem and early progenitors**. Our study also confirms the deleterious effects of chronic alcohol on early myeloid progenitors to mature circulating

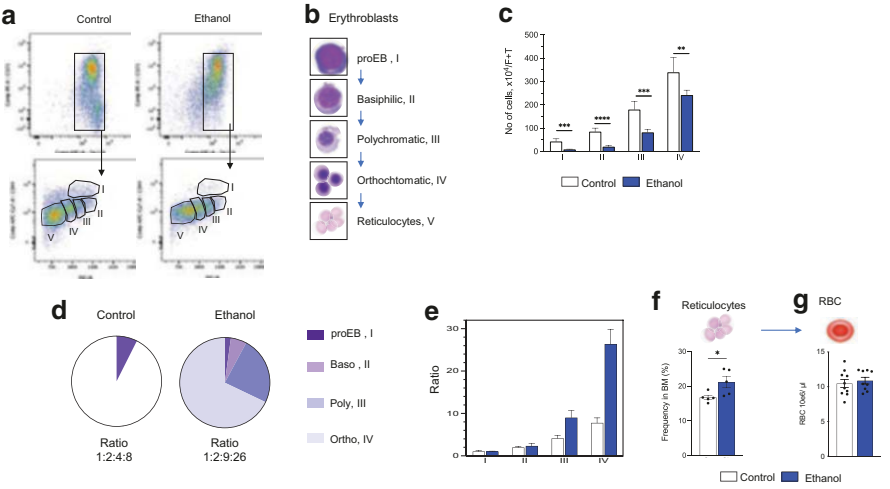

Fig. 58.6 Chronic ingestion of alcohol affects erythroid progenitor compartment. (**a**) Erythroblast differentiation stage of erythroid progenitors from proerythroblast till reticulocytes (stage I-V) can be analysed by FACS using expression of CD44 and TER119. (**b**) Morphology of maturation types I-V based on expression of CD44 and TER119 in mouse. MEPs downstream erythroid progenitors, proerythroblast (ProE, stage I) are $Ter119^{+}$ $CD44^{+}$. Further differentiated stage subdivide into downstream basophilic erythroblasts (Baso), II, polychromatic erythroblasts (Poly), III, orthochromatic erythroblasts (Ortho), IV and reticulocytes, V. (**c**) Quantitative analysis shows that alcohol drastically reduces number of all nucleated erythroblasts (I, II, III, IV). (**d** and **e**) Within the nucleated erythroblast population, the ProE (I), Baso (II), Poly (III), and Ortho (IV) maintain the 1:2:4:8 ratio (6**d**, left and **e**), confirming a normal progression of erythropoiesis in bone marrow. However, under chronic alcohol abuse, the ratio changed to 1:2:9:26 (**d**, right and **e**), showing intensive proliferation/mitotic division under alcohol-induced stress (shifting in the direction of enucleated erythrocytes). (**f** and **g**) Proportion of enucleated erythrocytes/reticulocytes (V), measured in BM were slightly increased but number of mature RBCs in peripheral blood was unchanged. Abbreviations: *CLP* common lymphoid progenitors, *CMP* common myeloid progenitors, *GMP* granulocyte monocyte progenitors, *HSC* hematopoietic stem cell, *LSK* progenitor cells, *MEP* megakaryocyte erythroid progenitors, *MPP*, multipotent stem cells, *Sca-1* stem cell antigen-1

granulocytes and monocytes. Erythroid cells were sensitive to alcohol exposure, developmental stages and maturation were disrupted, showing intensive proliferation in the late developmental stage from polychromatic to orthochromatic differentiation stage.

Conclusions

Enhanced RBC turnover seems to be a key mechanism responsible for the long-observed iron accumulation in alcohol drinkers. Although major ethanol-metabolites such as acetaldehyde and ethanol are able to cause hemolysis, quite

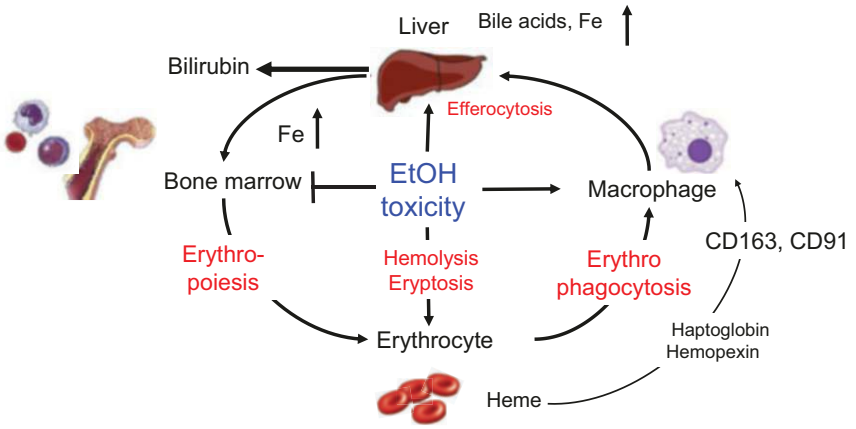

Fig. 58.7 Present model of potential interactions of ethanol with the red blood cell cycle. Ethanol increases RBC turnover by both enhancing degradation and erythropoiesis. Hepatocytes can also contribute to RBC recycling by direct efferocytosis, a process that is still poorly understood. Also note that folic acid levels decrease during ethanol-mediated ineffective erythropoiesis while B12 levels increase (see Table A2a)

high concentrations are necessary that may only be transiently reached e.g. in the portal vein (see also Chap. 57). However, many other causes of hemolysis are present in heavy drinkers and may be related to an altered RBC metabolism, a weakened erythrocyte antioxidant defense system by ethanol, priming for eryptosis, erythrophagocytosis or efferocytosis and, finally, at later stages of ALD, related to toxic effects of accumulating bile acids, bilirubin, hypalbuminemia and hypoosmolality. There are first indications that **bone marrow toxicity**, most likely at the **stem cell compartment**, contributes to this impaired RBC synthesis. It could also explain why MCV is elevated in ALD and considered a hallmark of chronic alcohol consumption for many decades. Of note, MCV remains elevated for several weeks despite complete alcohol withdrawal. Vitamin analysis by us and others also indicated that the seemingly first explanation, deficiency of either vitamin B12 or/and folic acid, are not the cause of MCV elevation in heavy drinkers. These observations also suggest that alcohol itself, so it may be the trigger for stem cell impairment, is not directly involved. One potential hypothesis could be the concept that iron accumulation itself could cause stem cell compartment toxicity as **iron overloaded erythroid precursor** cells have been observed in drinkers for a long time. Figure 58.7 schematically illustrates how alcohol may interact with the RBC life cycle. The observations are also related to Zieve syndrome, a rare, poorly understood but severe form of hemolysis during heavy alcohol consumption [6].

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Chapter 59
The "Matrisome" and Alcohol-Related Liver Disease

Gavin E. Arteel

Abstract The progression of ALD represents a spectrum of disease stages ranging from simple steatosis, or fatty liver, to inflammation and necrosis (steatohepatitis), and ultimately, to fibrosis and cirrhosis. Unfortunately, severe ALD is usually first diagnosed when the patients show symptoms of severe liver dysfunction, where no therapies have been proven effective. Although the classic meaning of the ECM referred to only proteins directly involved in generating the ECM structure, such as collagens, proteoglycans and glycoproteins, the definition of the ECM is now broader and has been coined the 'matrisome'. The matrisome is a dynamic compartment that comprises a diverse range of players that work bi-directionally with hepatic cells to regulate overall homeostasis. However, when these responses are dysregulated, the changes to the ECM can be maladaptive. The most well-recognized example of ECM dyshomeostasis in the liver is that of hepatic fibrosis. Although end-stage collagenous scarring of an organ/tissue is often considered synonymous with ECM remodeling, this remodeling is actually a key factor in early stages of injury and restitution from injury and much more diverse than simple collagen accumulation. The purpose of this review is to explore the role (or potential role) of ECM dyshomeostasis across the earlier spectrum of pathology caused by alcohol consumption prior to fibrosis (i.e., steatosis, cell death and inflammation) and to discuss new approaches and opportunities of to discover and leverage this understanding to prevent and treat ALD.

Keywords Extracellular matrix · Alcohol-related liver disease · Steatosis · Inflammation · Degradome

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Introduction

The Need for Better Strategies to Prevent and Treat Alcohol-Related Liver Disease

According to the National Survey on Drug Use and Health conducted in 2015, 86.4% of U.S. adults report consuming alcohol at some point in their lives [1], and the value is >50% Worldwide [2]. Alcohol (mis)use is reportedly the seventh leading risk factor for both death and disability-adjusted life years (DALYs) lost globally [3, 4]. A major burden of alcohol consumption and misuse is caused by alcohol-related liver disease (ALD). ALD impacts millions each year and is now the leading indication for liver transplantation in the US [5]. The incidence of ALD has been increasing across all ages and sociodemographics since 2008, with young adults (25–34) being the most rapidly growing group [6]. The latter point is alarming, as ALD is historically viewed as a disease that first manifests in middle age [7].

The progression of ALD is well-characterized and is actually a spectrum of liver diseases, that range from simple steatosis, or fatty liver, to inflammation and necrosis (steatohepatitis), and ultimately, to fibrosis and cirrhosis [8]. Moreover, alcohol-related hepatitis (AH) is an acute clinical syndrome that can occur at any time during the progression of ALD with a dismal survival rate [9, 10]. Although the prevalence of subclinical liver damage in heavy drinkers is nearly 100%, only a fraction of this at-risk population will later develop clinically relevant ALD [9]. Unfortunately, severe ALD is usually first diagnosed when the patients show symptoms of severe liver dysfunction (i.e., decompensation) very late in disease progression, where no therapies have been proven effective [10]. As a consequence, the overall prognosis of ALD has not improved in decades.

Over 65 years ago, there was a paradigm shift in the field of atherosclerosis, another pathogenic process characterized by lipid dysmetabolism, chronic inflammation and damage, and at the end-stage, by collagenous scarring. Specifically, atherosclerotic disease was no longer considered an unavoidable and untreatable condition of the elderly, but rather a chronic lifelong disease with identifiable and preventable risk factors that manifest at a young age [11]. This shift was driven by a landmark study in which Enos et al. [12] demonstrated that 77% of autopsied US soldiers killed in action during the Korean War already showed significant remodeling of their coronary arteries, indicative of early stage atherosclerosis. Moreover, a fraction of these young soldiers already had nearly complete vessel occlusion and were at high risk for clinically relevant cardiovascular disease later in life [13]. Research and therapy for atherosclerosis now focus on mechanism(s) of disease development prior to collagenous scarring of the plaque, as these targets more responsive to therapeutic intervention [14]. A major research goal in the alcohol-related liver disease field is to achieve a similar paradigm shift from treatment to prevention/intervention by better understanding the mechanism(s) of disease progression and risk. This understanding would in principle improve early detection, inform prevention efforts and identify novel protective “theragnostic” strategies.

The Hepatic Extracellular Matrix and the “Matrisome”

The hepatic extracellular matrix (ECM) is most accurately depicted as a dynamic compartment that comprises a diverse range of players that work bi-directionally with hepatic cells to regulate overall homeostasis. Although the classic meaning of the ECM referred to only proteins directly involved in generating the ECM structure, such as collagens, proteoglycans and glycoproteins, the definition of the ECM is now broader, and includes all components associated with this compartment, including ECM affiliated proteins (e.g., collagen-related proteins), ECM regulator/modifier proteins (e.g., lysyl oxidases and proteases) and secreted factors that bind to the ECM (e.g., TGFβ and other cytokines) [15]. This updated definition has been coined the ‘matrisome’ [16]. Although the canonical function of the ECM is structural, it is also a key storage unit for signaling molecules (e.g., growth factors and cytokines), as well as serving as a sensing mechanism for outside-in signaling and vice-versa [17, 18].

In solid organs, the ECM is separated into two distinct structural components: the interstitial matrix and the basement membrane [19]. Interstitial matrix proteins (e.g., fibrillar collagens, elastins and fibronectins) form networks that provide support to the overall superstructure that shapes and encapsulates the liver [20]. In most solid organs, the basement membrane is a thin, electron-dense sheet of mostly collagens that forms the foundation for epithelial and endothelial cells attachment and growth [18]. In contrast to other tissues, where the basement membrane is a true barrier between the epithelial/endothelial cells and the adjacent parenchymal cells, the basement layer in the liver is fenestrated and much more loosely organized [20]. Although it possesses similar ECM as more clearly-defined basement membranes [21], this region acts more as a structural and biochemical “sieve” that facilitates bidirectional exchange of proteins and xenobiotics between the sinusoidal blood and hepatocytes [18]. Although it is clear that liver does not have a *basal lamina*, whether or not the ECM found in the space of Disse should be considered a basement membrane is a subject of a histological, rather than functional, debate [19].

Balance and Imbalance of ECM Turnover in the Liver

The ECM responds dynamically to stress and changes. Under ideal conditions, these responses assist in maintaining organ homeostasis and help mediate appropriate responses to injury/stress. This coordinated dynamic response is most likely best illustrated by subcutaneous wound healing, in which the tightly regulated deposition and remodeling of the ECM not only mediates wound closure, but also restitution and repopulation of the wound with replacement cells [22]. However, when these responses are dysregulated, the changes to the ECM can be maladaptive [23]. For example, ‘aging’ of the ECM (i.e., increased crosslinking) is hypothesized to contribute to dysfunction in several organ systems, including the liver [24–27].

The most well-recognized example of ECM dyshomeostasis in the liver is that of hepatic fibrosis, which is the common end pathology of almost all chronic liver diseases [20]. Although end-stage collagenous scarring of an organ/tissue is often considered synonymous with ECM remodeling [28], this remodeling is actually a key factor in early stages of injury and restitution from injury and much more diverse than simple collagen accumulation [29]. The hepatic ECM also responds rapidly and dynamically to insult, even after acute injury. Indeed, we and others have shown dynamic transitional changes to the hepatic ECM that appear to be key to the normal response to acute injury and recovery, as well as setting the stage for chronic disease [18, 30]. Homeostasis in ECM is mediated by a balance in the production of ECM, as well as in the degradation of existing ECM by matrix metalloproteinases (MMPs) [31]. Even in cases where there is a net increase in ECM in liver (e.g. fibrosis) overall turnover is also increased [32].

The impact of the ECM and (to a certain extent, the matrisome) on hepatic fibrosis has been covered heavily in the literature [33, 34]. The purpose of this review is to explore the role (or potential role) of ECM dyshomeostasis across the earlier spectrum of pathology caused by alcohol consumption prior to fibrosis (i.e., steatosis, cell death and inflammation) and to discuss new approaches and opportunities of to discover and leverage this understanding to prevent and treat ALD.

Hepatic ECM Changes and Steatosis Caused by Alcohol

The liver plays a central role in lipid metabolism for the entire organism. There is intricate crosstalk between other organs involved in lipid metabolism and the liver that is controlled by a complex interplay of hormones, nuclear receptors, intracellular signaling pathways and transcription factors. Under homeostatic conditions, hepatic lipid flux maintains relatively low concentrations of lipid pools (e.g., free fatty acids, triglycerides and cholesterols). However, dysregulation of this flux can cause lipids to accumulate in hepatocytes and lead to steatosis.

The first and most common hepatic change caused by alcohol consumption is steatosis, or fatty liver [35]. The prevalence of steatosis is essentially 100% in those who regularly consume alcohol at pharmacodynamically-relevant concentrations [5]. Fat accumulation can be both macrovesicular (having one large fat droplet per hepatocyte and lateral displacement of the nucleus) or microvesicular (many small fat droplets per hepatocyte) [36]. Alcohol-induced steatosis is rapidly and readily reversible upon cessation of alcohol consumption. Steatosis can also be clinically ‘silent,’ and can exist in the absence of increases in any other index of liver damage (e.g., plasma transaminases) in individuals who chronically misuse alcohol. For these reasons, steatosis was originally viewed as an inert pathology in ALD (and in other fatty liver diseases). However, more recent studies have suggested that

blunting or preventing steatosis could help attenuate the progression of ALD; in fact, the degree of steatosis is an early predictor of overall disease severity [37]. Therefore, although other factors clearly mediate the overall risk of developing severe liver disease, steatosis may not be as inert a pathology as originally thought [35].

Alcohol directly and indirectly impacts numerous aspects of hepatic lipid flux that ultimately leads to lipid accumulation. The simplest example is that alcohol metabolism itself directly causes steatosis. Concentrations of alcohol can easily reach the mM range in the portal/hepatic circulation during alcohol consumption. In the process of metabolizing ethanol to acetate, two equivalents of reduced NADH are generated per equivalent of ethanol oxidized. This metabolism robustly increases the ratio of NADH:NAD^+ within the cell, which then favors inhibition of fatty acid β-oxidation in the liver (see also Chap. 50). Furthermore, ethanol metabolism also increases the rate of esterification of fatty acids [38]. The net effect is to favor TG and other lipid pool accumulation in the hepatocytes. However, the impact of alcohol exposure on lipid metabolism is far more complex than simple redox inhibition of β-oxidation (see [35] for review).

ECM/Matrisome and the Impact on Lipid Metabolism and Steatosis

The understanding of the role ECM/matrisome proteins in the induction of steatosis caused by alcohol is anecdotal and sparse at this time. It is known that models of ethanol-induced steatosis (e.g., chronic liquid diet feeding to rodents) is sufficient to cause robust increases in proteins associated with the matrisome in the liver [39]. It is also known that select ECM components (e.g., osteopontin, thrombospondin, fibrin(ogen) and periostin) appear to have a direct influence on lipid metabolism in experimental models of fatty liver diseases [40–43]. Moreover, signaling molecules that are stored in the matrisome and proteolytically released during remodeling/injury (e.g., hepatocyte growth factor) have a direct influence on hepatic lipid metabolism [44]. However, the functional impact and mechanisms by which ECM/matrisome components influence these changes is, at best, incompletely understood.

In contrast to the liver, the impact of ECM/matrisome on lipid metabolism in other organs/diseases is more clearly understood and can be used to leverage parallel assumptions fatty liver disease. For example, Baker et al. [45] determined that decellularized ECM from adipose tissue of diabetic patients was sufficient to drive a similar diabetic phenotype (i.e., decreased glucose update and lipolysis) in naïve adipocytes cultured *ex vivo*. Although the mechanisms by which ECM/matrisome alter metabolism are incompletely understood, these mechanisms fall generally into the categories of physical/mechanical effects and altered ligand/receptor interactions.

Physical/Mechanical Impacts of the ECM/Matrisome on Lipid/Glucose Metabolism

As mentioned above, the hepatic ECM/matrisome qualitatively and quantitatively responds rapidly to injury/stress. These changes can impact the elasticity of the ECM and organ [46], and injury directly increases ECM stiffness in organs [47–50]. The cell has several signaling mechanosensory pathways that drive phenotype changes in cellular function in response to the ECM changes. The best understood pathway involves the indirect linkages of the ECM to actin cytoskeleton via integrin receptors in focal adhesions on the surface of the cell [51]. However, actin-independent signaling via integrins have also recently been identified (e.g., via focal adhesion kinases) as well as integrin-independent pathways [52]. Interestingly, the latter (integrin-independent) pathway links ECM stiffness to key players hypothesized to be critical in alcohol-related steatosis and ALD (e.g., AMPK and SREBP-1 [53–55]).

Ligand/Receptor Interactions

The interaction between cells and the surrounding ECM can also impact downstream signaling cascades that mediate metabolic pathways [56]. This control can be at mediated via altering receptor affinity, or changes to downstream signaling cascades. Under basal conditions, receptors for these mediators are generally dispersed on the plasma membrane in lipid/lipoprotein-rich regions (i.e., lipid rafts); the relatively close proximity of receptor monomers facilitates ligand binding, receptor dimerization and subsequent downstream signaling [57]; ECM proteins contribute to this 2-dimensional organization on the plasma membrane [58]. Signal integration between ECM receptors and extracellular signaling factors also varies with interactions with the ECM stratum. This influence of ECM on signaling has best been described for cellular responses to growth factors, and is categorized as concomitant signaling, collaborative activation, direct activation, amplification and negative regulation [59, 60]. Injury impairs growth factor signaling, in part by altering the make-up of the ECM surrounding the cell [61]. Moreover, ECM interactions qualitatively and quantitatively influence the response of TLR and TNFα signaling [62].

In addition to fibrillar ECM, the matrisome contains components and mediators that may influence energy/lipid metabolism within the liver. For example, matrix metalloproteinases (MMPs) and related proteinases cleave a myriad of substrates that may impact cellular metabolism (e.g., lipoproteins and growth factors) that can directly or indirectly impact steatosis in the liver, and recent work suggest a direct metabolic effect of these proteases by targeting key complexes (e.g., mitochondria) involved in metabolism [63–65]. The ECM protein osteopontin has been shown to impact steatosis in both ALD and NAFLD [41]. Recent work has indicated that

thrombin activation and its canonical receptor, PAR-1 contribute to hepatic steatosis in experimental models of non-alcoholic fatty liver disease (NAFLD) [66, 67].

Taken together, there are at least anecdotal indications that ECM remodeling directly and indirectly impacts metabolism, and by extension steatosis. The magnitude, mechanisms and impact of these changes are generally incompletely understood in fatty liver diseases. This developing field that should more specifically be explored in more detail in the context of ALD.

Hepatic ECM Changes and the Regulation of Cell Viability

The accumulation of hepatic damage caused by alcohol is characterized by an increase in cell death. Interestingly, the magnitude of hepatocyte death appears to be out of balance with that of inflammation compared to non-steatotic liver injury. This observation has led to the understanding that hepatocytes and other noninflammatory hepatic cells appear to be sensitized to intercellular signals (e.g., cytokines) to favor downstream signaling pathways that lead to cell death [68–70]. In the past few decades, the binary concept of “cell alive/cell dead” has been demonstrated to woefully overly simplistic, with a myriad of processes that can mediate cell death of different phenotypes (e.g., apoptosis, necroptosis and pyroptosis) [71]. Alcohol exposure appears to increase the likelihood of almost all of these regulated cell death (RCD) pathways [72].

Work derived predominantly from the field of morphogenesis and development indicate that specific ECM proteins have key roles in cell viability and death [23, 73, 74]. Indeed, recent interest on the impact of ECM on hepatic cellular phenotype has increased dramatically, especially in the concept of ex vivo “liver-on-a-chip” platforms, as well as biomatrices for injury restitution [75, 76]. In toto, connection of the cells to basement membrane proteins (e.g., collagen III & IV, laminin and elastin) appear to be key for cells to maintain their normal viability and function [23]. Serum levels of several of fragments of these basement membrane proteins are elevated in ALD and other fatty liver diseases, and may represent increased turnover of these critical ECM compartments [77]. This effect can be mediated via ECM components acting directly as a ligands, or indirectly via altering the binding of other growth factors to their receptors.

In addition to serving as direct ligands that mediate and alter life/death signaling cascades, the ECM/matrisome serves as a reservoir of signaling molecules, including growth factors. The ECM sequesters and stores these molecules via charge affinities with glycosaminoglycans (GAG) [78]. Although this affinity is sufficiently strong to be metastable, ECM turnover activates and releases these mediators [79, 80]. Moreover, recent work indicates that several ECM peptide fragments are biologically active and can impact cellular viability and function (i.e., “matrikines”) [81]. The localized release of these mediators creates a gradient that acts as a ‘homing signal’ to the origin of the injury [80, 82]. In addition to storing/releasing growth

factor ligands, signal integration from ECM-binding receptors can alter growth factor signaling cascades, both positively and negatively [59, 61].

Integrins are heterodimeric proteins that are mainly responsible for facilitating interaction between the ECM and surrounding cells [83]. Integrins play a myriad of roles within the body that may directly or indirectly impact cell viability [84, 85]. Appropriate activation is critical for normal cellular survival, as well as for regulated cell death [74]. Dysregulated integrin signaling has been demonstrated to be involved in hepatic injury and disease progression in a wide variety of liver diseases [86, 87]. There are also several non-integrin receptors involved in signaling between the ECM and the cell. For example, CD44, a type I transmembrane glycoprotein with over 20 different isoforms, has been demonstrated to be involved in liver injury [88]. CD44 has also been implicated in the resolution of injury by facilitating the migration of hematopoietic stem cells to the injured liver [89].

Taken together, several of the effects of alcohol exposure on cell viability could, in principle, be mediated via changes to the ECM/matrisome and/or its partnered signaling pathways. What is lacking is an integrative analysis linking these potential interactions, as has been done extensively in other organ diseases. More research should also focus on this issue.

Role of the ECM/Matrisome in Hepatic Inflammation

As mentioned above, given the poor prognosis of treating late-stage liver disease, much of current research focuses on identifying at-risk individuals and preventing the progression of the disease during earlier phases, especially inflammation. Inflammation plays a central role in ALD and involves both the innate and adaptive immune responses [90, 91]. Inflammation in ALD is characterized by a chronic, low-grade inflammatory condition, in which innate immune cells are “primed”, and adaptive immune surveillance and tolerance is dysregulated [92–94]. It is this vicious cycle of cell damage/death and inflammation, when it overwhelms the repair/recover responses of the liver, which leads to the chronicity of ALD.

The role of the ECM in fibrotic liver injury has been heavily studied. This is not surprising, given that liver disease is often clinically asymptomatic until this later stage [95]. The ECM defines properties permissive and/or instructive to inflammation and changes to the ECM directly and indirectly alter this response. Although some areas of research have a deep understanding of the roles of the ECM in inflammation (e.g., subcutaneous wound healing), this is a developing field in the context of liver disease [22]. Work by this group and others have shown that the inflammatory response to hepatic damage involves several of the ECM proteins found in subcutaneous wound healing, such as fibrin, osteopontin, and fibronectin [96–99]. These histologically undetectable changes to the matrisome appear to resolve after acute injury [30, 39]; with chronic injury, the transitional matrisome is replaced by collagenous scarring in the liver, which is parallel to mechanisms associated with subcutaneous wound healing [22].

The involvement of the ECM/matrisome in inflammation has several mechanisms that overlap with those described previously for steatosis and cell death. For example, the ECM also serves as a source of ligands to inflammatory cells and can alter inflammatory cell phenotypes [100]. The matrisome also serves as a reservoir of cytokines and chemokines that are rapidly released to attract components of the inflammation/wound healing response [100]. Many of these reactions are also mediated by ECM receptors (see above) [84, 85, 101]. Specific matrikines released by degradation of the ECM can also be directly proinflammatory via interacting with specific ligand receptors and/or pattern recognition receptors [102–104]. Lastly, the clustering of integrins into focal adhesions that directly communicate with the cytoskeleton likely mediates the impact of ECM rigidity on the inflammatory response [105].

There are also functions mediated by ECM/matrisome that are unique to this portion of pathologic spectrum of ALD. For example, interaction between leukocytes and the ECM is critical for the process of leukocyte adhesion and transmigration to sites of inflammation/injury [56, 106]. Leukocyte surfaces contain ECM receptors/adhesion molecules that direct their migration through interaction with the ECM [107]. Regulation of these receptors is important for the rapid change between adhesive and nonadhesive states of immune cells [107]. The interaction between the ECM and cell infiltration is bidirectional; as leukocytes integrate structural and biochemical cues from the ECM, they in turn release matrix-degrading proteases [108] which alter the extracellular composition and allow for easier cell migration. Degradation of the ECM during inflammation can also expose self-antigens (e.g. the basement membrane ECM, collagen V) that can be used to promote infiltration of immune cells that are normally tolerant to the liver [109].

Inflammation and ECM remodeling can become a feed-forward cycle [100]. The ECM facilitates immune cell migration and differentiation, while immune cells trigger new ECM deposition and proteolytic remodeling. Proteases subsequently cleave ECM producing proinflammatory degradation products. These processes can be adaptive, but aberrant ECM and bioactive degradation products perpetuate inflammation in a maladaptive response, such as in chronic hepatic inflammation [110]. Given the key role of the ECM in mediating inflammation, it is not surprising that this compartment also plays key roles in the resolution of inflammation (i.e., catabasis; [111–113]).

Regulation of Regeneration by the ECM/Matrisome

The ability of the liver to regenerate after damage is a unique mechanism to recover from injury and protect against damage accumulation. Hepatic regeneration is a tightly organized process that is sensitive to perturbation. Indeed, the development of chronic liver diseases, including ALD, is almost universally present on the background of genetic/acquired impairment of regeneration and restitution from injury [114, 115]. The hepatic ECM plays a key role in liver regeneration. As discussed

above, it serves as a storage reservoir for preformed growth factors such as HGF. The storage capacity of these growth factors is so large that temporally controlled knockouts of HGF do not phenotype until this reservoir is depleted [116].

Hepatic regeneration can be organized into three phases: priming, proliferation and growth termination [117]. Hepatic regeneration requires a coordinated restructuring of the ECM/matrisome in all phases to facilitate de novo proliferation of surviving liver cells in response to injury [117]. This remodeling not only removes physical barriers to growth during the priming phase [118], but also breaks connections to ECM proteins that restrict proliferation under normal conditions (e.g., endostatin) [119]. Significant ECM synthesis and remodeling is also required to build the new infrastructure and superstructure on which the recently divided cells will populate during the growth termination phase [117]. Little is known about the influence of ethanol on ECM turnover that is critical for normal liver regeneration and this question should be the topic of future studies.

Senescence and aging of the liver have been extensively studies in the context of chronic liver diseases, including ALD. Aging is a not only a known risk factor for chronic liver disease, but the chronic cycle of injury and recovery leads to premature aging of organ [120]. As mentioned above, "aging" of the ECM is also a key factor in the loss organ elasticity associated with normal or premature senescence [24–27]. Some studies have suggested that there may be a direct link between ethanol-induced senescence and altered ECM/receptor signaling in chronic liver diseases and aging. For example, hepatic fibrosis is associated with a redistribution of laminin ECM away from the perisinusoidal space to the fibrous septa [121] and that this effect is associated with impaired growth factor (e.g., HGF) release [122]. The potential specific contribution of ethanol-altered ECM to premature aging of the liver and replicative senescence has not been yet investigated.

Summary and Future Prospects

Chronic liver diseases, including ALD, are usually clinically silent until very late stages, when the organ starts to decompensate. This is an especially important clinical need, as the potential reversibility of the disease decreases as disease severity progresses. There are various elastographic (e.g., transient elastography) and scoring (e.g., FIB-4) approaches that have good negative predictive values (NPV; i.e., low false negative rate), but do not have as strong positive predictive values (PPV; i.e., relatively high false positive rate) (e.g., [123]). The end result is that although these approaches are good at predicting who does not have active hepatic fibrosis, there are several false positives (i.e., high liver stiffness without active fibrosis) in the "at risk" group. Moreover, although these approaches are generally good at detecting hepatic fibrosis, they are less sensitive and specific for earlier stages of disease progression (e.g., inflammation) [124]. What is needed are more minimally-invasive approaches to detect disease progression accurately at earlier stages of development.

As mentioned above, *de-novo* synthesis of ECM proteins is a key factor during any stimulus of the wound healing response. Indeed, changes in biomarkers of collagen synthesis/deposition have been employed to serve as predictors of fibrosis severity. For example, the precursor of Type III collagen (PRO-C3) has been identified to have areas under the receiver operating characteristics curve (AUROC) values for predicting liver disease severity that are superior to imaging and/or scoring approaches; the improved AUROCs were largely driven by better PPVs compared to the imaging and scoring approaches [125, 126]. These approaches may also be favorable for ECM remodeling associated with earlier stages of ALD development.

Another possible source of new biomarkers is based on indices of ECM turnover. The degradation of ECM proteins is almost always induced simultaneously with upregulation of de novo synthesis during ECM remodeling. The accumulated signal of these peptide fragments of the ECM is part of the peptidome, and more specifically, the degradome [127–130]. The latter subset of the peptidome has generated key interest in some areas of human health as possible (surrogate) biomarkers for disease. Degradomic analysis of cancer metastasis, and by extension overall patient outcome, has garnished significant interest [131]. The rationale is that metastasis and tumor growth require significant remodeling of the normal and cancerous interstitial space, which can lead to alterations in the degradome profile in biological fluids. Similar approaches are beginning to be applied for liver diseases. For example, the peptidome has been shown to predict liver disease severity and outcome in HBV infection [126]. The study of the peptidome is a discipline related to proteomics, but with significant methodological and analytical differences [127, 132]. For example, as the original structure of the peptides is of interest, samples are not trypsin digested prior to mass spectrometry (MS) analysis, as is the case for bottom-up proteomic approaches. This difference originally limited peptide identification, as the available databases were based on trypsin-digested peptide fragments [128]. However, the development of peptidome-specific identification and analysis tools has addressed this concern [128, 129]. This approach could yield discovery of new biomarkers for earlier stages of ALD development.

This chapter summarized the roles (or potential roles) ECM plays in the progression of ALD, well before fibrotic scarring. What should become clear is that although the general understanding of the potential role of the ECM in all biochemical/cellular facets related to the initiation and progression of ALD, with the exception of the interaction between the ECM and inflammation, the specific role of the ECM in these processes is poorly established in the literature. A major goal of this review is to therefore highlight the key gaps in our understanding. This information can yield new mechanistic insight into improving the landscape of interventive strategies to treat and/or prevent the progression of ALD in the early stages of disease development, vis-à-vis atherosclerosis. However, to leverage this new understanding for interventive approaches in ALD, the early diagnosis of those truly at risk for severe ALD must be improved. Again, the ECM, and detection of its turnover, may be a fertile field to develop new and improved approaches in this area (Fig. 59.1).

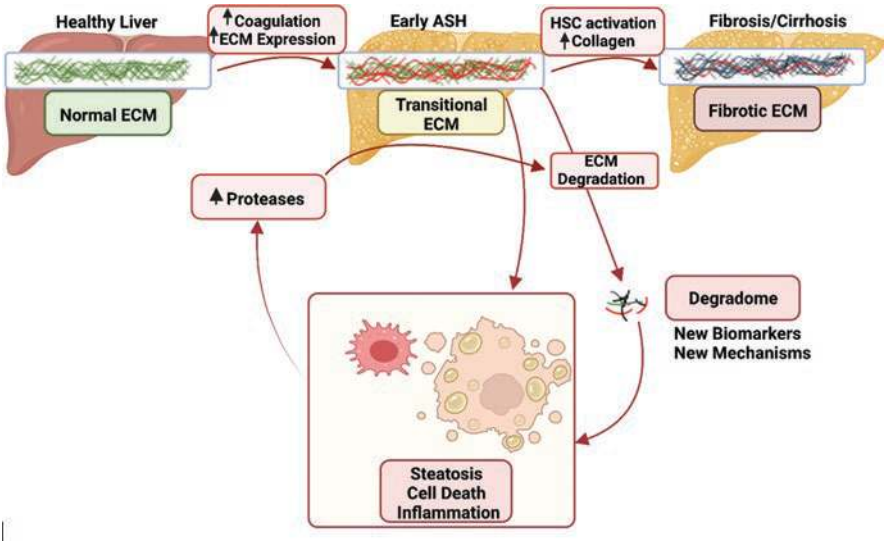

Fig. 59.1 Working hypothesis on the role of the ECM in the initiation and progression of alcohol-related liver disease (ALD). The progression of ALD is well-characterized and is actually a spectrum of liver diseases, that range from simple steatosis, or fatty liver, to inflammation and necrosis (steatohepatitis; "Early ASH"), and ultimately, to fibrosis and cirrhosis [8]. It is known that even early alcohol-induced liver injury causes formation of a transitional ECM. This transitional ECM may contribute to steatosis, cell death and inflammation. The transitional ECM often resolves after removal of the insult and may contribute to the recovery from that insult. With continued injury, the transitional matrix may progress to a fibrotic matrix, which can also resolve under some conditions. Even during ECM accumulation in response to acute/chronic injury, there is a net increase in turnover of the matrisome, yielding degraded ECM products ("degradome"). These degraded protein peptides may serve as biomarkers for disease development, as well as potentially driving disease progression

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Chapter 60
MicroRNAs and Alcohol-Related Liver Disease

Kristina Perez, Jing Ma, Nazmul Huda, Zhihong Yang, and Suthat Liangpunsakul

Abstract MicroRNAs (miR) are small non-coding RNAs that bind to specific mRNA targets and promote their degradation or inhibit translation. In this book chapter, we reviewed the roles of multiple miRNAs, which are involved in the pathogenesis of alcohol-related liver disease (ALD). We also briefly discuss the roles of miRNAs as the mediators for inter-organ crosstalk and the development of ALD. We provided clues for the potential clinical applications of miRNAs as the prognostic markers and the future perspectives on the use of miRNA-based strategy for the treatment of patients with ALD.

Keywords miRNA · Pathogenesis · Alcohol-related liver disease

Introduction to Micro RNAs

MicroRNAs (MiRNAs) are evolutionarily conserved small single stranded non-coding RNA with around 18–24 nucleotides long [1]. While their size is relatively small, micro-RNAs are widely diverse and account for over millions of possible sequence combinations between their adenine, guanine, cytosine, and uridine base pairs [2]. Given this diversity, miRNA can be highly tissue and cell type specific, therefore adding to their importance in regulating intracellular pathways and in disease pathogenesis. The structure of miRNAs is dependent on the abundance of

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adenine-uridine rich base pairings within their sequence [3]. Rich sequences harboring these pairs contribute to shorter half-lives of the miRNA [3]. MiRNA is first formed from the 60–70 nucleotide long RNA hairpin precursor [3]. In addition, RNA polymerase II are highly implicated in the synthesis of miRNA [4]. These clusters of miRNA precursors then undergo cleavage events that involve either dicer in the nucleus or drosha in the cytoplasm [5]. After generation, the exportin 5/RanGTP complex exports the pre-miRNA to mature in the cytoplasm. After maturation in the cytoplasm, the miRNA disperses in terms of their specificity and plays an important role in post-translation modifications.

MiRNAs play various roles in the post-transcriptional regulation of genes. Due to their small size, miRNAs require a minimum of 7 complimentary nucleotides to bind to their target and begin regulation [6]. MiRNAs bind to mRNAs by partial base pairing (partial complementarity) at the 3′-UTR (untranslated region); however, the interaction at the 5′- UTR, coding sequence or promoter region has also been reported [4, 7] The binding at the UTR target on the mRNA leads to translational inhibition [4]. In this review, we will focus on the reported roles of miRNAs in the pathogenesis of ALD.

Alcohol as Regulator of miRNA Biogenesis

The direct mechanisms between ethanol and microRNA dysfunction remain under investigation. However, recent studies provide growing evidence for the involvement of ethanol in the expression of mature miRNAs. One study showed that long-term alcohol use can impact miRNA profiles within mouse brains [8]. Prenatal ethanol exposure can also alter mature miRNA expression in fetal mouse brains [8]. Chronic alcohol induction illicits an upregulation of miRNA-155 in liver macrophages and miRNA-212 in the gut which contributes to disease progression through downregulation of gut tight junction proteins [9]. Mechanism studies revealed that ethanol may interfere with microRNA synthesis by altering activation of transcription factors (TF) and/or epigenetic modifications of DNA and DNA-associated histone complexes, including methylation (Me) and acetylation (Ac) [10]. Ethanol may reduce epigenetic DNA methylation, therefore leading to altered gene expression, including those that synthesize microRNAs [10].

MiRNAs as Mediators for Inter-Cellular Crosstalk in ALD Pathogenesis

While the studies of miRNAs in ALD pathogenesis focus on the role of miRNAs in regulating the target genes within each specific cell type, such as hepatocytes or Kupffer cells, miRNAs can be secreted into the extracellular space within

extracellular vesicles (EVs). Once secreted, miRNA-containing EVs can be uptaken by neighboring cells or enter the circulation to regulate the target genes in other tissues or organs. In the liver, parenchymal and non-parenchymal cells can release miRNA-containing EVs. Multiple miRNAs, such as miR-122 and miR-155, have been shown to be released from hepatocytes in ALD [11]. The pathogenesis of ALD involves the cross talk among multiple organs, which eventually leads to hepatic inflammation and metabolic alterations [12–14]. MiRNA-containing EVs likely are the key players in connecting the cross talk among several tissues, such as the gut, liver, adipose tissue, and brain, leading to the development of ALD.

Clinical Implications of miRNAs in Patients with ALD

Due to the myriad functions of miRNAs in ALD pathogenesis, circulating miRNAs have been extensively studied as potential biomarkers for patients with ALD. Circulating miRNAs are relatively stable and resistant to the degradation by RNases [15, 16]. Numerous studies have shown the superiority of several miRNAs as biomarkers for diagnosis and prognostic indicators for several liver diseases [17]. A recent study showed the use of miRNAs as prognostic markers for AH patients [18]. The following circulating serum miRNAs are found to be markedly reduced in such patients, miR-30b-5p, miR-20a-5p, miR-146a-5p, and miR-26b-5p [18]. Pathway analysis of the potential targets of these miRNAs revealed that the genes are related to DNA synthesis and cell-cycle progression pathways, including ribonucleotide reductase regulatory subunit M2 (RRM2), cyclin D1 (CCND1), cyclin D2 (CCND2), MYC proto-oncogene (MYC), and phorbol-12-myristate-13-acetate--induced protein 1 (PMAIP1) [18]. MiR-26b-5p and miR-30b-5p inhibit the 3′-UTR luciferase activity of RRM2 and CCND2, and miR-20a-5p reduces the 3′-UTR luciferase activity of CCND1 and CCND2 [18]. Among these miRNAs, the expression of serum miR-20a-5p, miR-146a-5p, and miR-26b-5p, are associated with mortality in AH patients [18].

Specific Micro RNAs in ALD

miRNA-21

MiR-21 is highly conserved and located on chromosome 17 [19]. It is one of the most abundant miRNAs and ubiquitously expressed in various tissues [20]. MiR-21 can be found in the cytosol, extracellular vesicle, and in multiple tissues such as liver, lung, kidney, and peripheral blood [21, 22]. Like other miRNAs, miR-21 regulates its mRNA targets by its interaction with the 3′ UTR binding leading to

post-transcriptional gene silencing. There are multiple gene targets for miR-21, illustrating its important role in regulating intracellular homeostasis [20]. MiR-21 controls cholesterol and triglyceride metabolism by targeting 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) [23]. The level of hepatic miR-21 is significantly increased in animal models of mice fed with ethanol via intragastric ethanol feeding or Lieber Decarli ethanol feeding [24]. An induction of hepatic miR-21 after alcohol feeding is mediated by the IL-6/STAT3 axis and that the IL-6/STAT3 pathway mediates alcohol-induced liver injury by modulating apoptosis, cell proliferation, and cell survival [24]. The upregulation of miR-21 is also in parallel with the inflammatory responses in the livers [25]. Dysregulation of miR-21 expression has been implicated during hepatic stellate cell (HSCs) activation [26]. The miR-21 mediated hepatic inflammation is secondary to the Von Hippel-Lindau (VHL)/ NF-κB signaling pathway in hepatic stellate cells (HSCs) [25]. The lack of miR-21 *in vivo* suppresses the production of several pro-inflammatory cytokines, such as monocyte chemoattractant protein-1 (MCP-1), Interleukin (IL)-1β, and IL-6 in human HSCs during alcohol-induced liver injury [25]. MiR-21 is also involved in alcohol-induced liver injury through its effect on hepatic regeneration secondary to TGF-β/SMAD/SMURF signaling in activated HSCs. Inhibition of miR-21 activity followed by the stimulation of the TGF-β leads to the changes in the expression of components of the TGF-β signaling pathway [26].

miR-26a

MiR-26a is conserved across vertebrates, and has a differential effect on different types of cancer, either a tumor suppressor or a tumor promoter [27, 28]. MiR-26a also regulates IFN-β anti-inflammatory pathway, and hepatocyte proliferation [29, 30]. MiR-26a is dysregulated during the autophagic process; overexpression of miR-26a induces autophagic activity [31]. In an ethanol-induced acute liver injury mouse model, miR-26a down-regulated the expression of two mitogen-activated protein kinases (MAPK) inhibitors, dual specificity phosphatase 4 and 5 (DUSP4 and DUSP5) [31]. The inhibition of DUSP4 and DUSP5 by miR-26a lead to an activation of MAPK and Beclin-1, the autophagy mediator [31]. Taken together, miR-26a promoted autophagic activity by activating Beclin-1 and protects against alcohol-induced liver injury by an augmentation of lipid droplet degradation [31]. Additionally, long term ethanol binge had significantly decreased miR-26a expression in the hippocampus compared to control counterparts in rats during mid/peri puberty [32]. This downregulatory effect resulted in increased Brain Derived Neurotropic Factor (BDNF), however, this effect lasted immediately after binge and did not persist through late puberty [32].

miR-27a

The expression of miR-27a is significantly increased in the monocytes in the presence of alcohol in vitro [33]. The circulating extracellular vesicles (EVs) from plasma of alcoholic hepatitis patients showed a markedly increase miR-27a compared with healthy controls [34]. Alcohol-induced miR-27a plays an important role in the differentiation and M2 macrophage polarization of human monocytes [33]. Over-expression of miR-27a in monocytes enhances IL-10 secretion through the activation of ERK signaling pathway by inhibiting ERK inhibitor sprouty homolog 2 (sprouty2) in monocytes [33]. MiR-27a also modulates phagocytosis by targeting CD206 expression on monocytes [34].

miR-34a

The miR-34 family comprises of three members; miR-34a is transcribed from chromosome 1, and miR-34b and miR-34c are co-transcribed from chromosome 11 [35]. While mechanisms that address the role of miR34b/c in alcohol induced liver injury are sparse, research suggests that upregulation of miR34b/c may protect against liver fibrosis [36]. MiR-34a is an important regulator of tumor suppression. It regulates the expression of multiple target proteins involved in cellular differentiation, the cell cycle, and apoptosis [37]. MiR-34a is upregulated in neural crest cells exposed to ethanol treatment, affecting the inhibition of neural differentiation by targeting autophagy pathway [38]. Alcohol use disorder is also associated with the upregulation of this miRNA in the hippocampus [35]. The hepatic expression of miR-34a is markedly increased in patients with alcohol-related hepatitis and in ethanol-fed mice [37, 39]. In the liver, alcohol causes the loss of methylation on the miR-34a promoter, resulting in an increase in its expression in ethanol-fed mice, compared to that of controls [39]. The underlying mechanism of miR-34a-mediated alcohol induced liver injury is by the regulation of cellular remodeling and proliferation [39]. These effects are mediated through its target genes, *CASP2* (Caspase 2) and *SIRT1* (Sirtuin 1), two known regulator genes of apoptosis and tissue remodeling [39]. An increase in hepatic miR-34 expression is also associated with enhanced cellular senescence especially in hepatic stellate cells in ethanol-fed mice [40].

miR-122

MiR-122 is transcriptionally regulated by several transcription factors including hepatocyte nuclear factor (HNF)1α, HNF4α, HNF3β, and CCAAT/enhancer-binding protein (C/EBP)α [17, 41]. MiR-122 is considered a liver-specific miRNA,

and its expression is activated during embryogenesis and liver development [42]. MiR-122 target genes involve in cellular proliferation and differentiation [42]. One of the important targets is a transcriptional repressor of genes, *CUTL1,* which plays an important role in cell cycle progression [42]. MiR-122 suppresses liver fibrosis by targeting connective tissue growth factor (*CTGF*) gene [43]. It promotes hepatic lipogenesis via inhibiting the LKB1/AMPK pathway by targeting Sirt1 [44]. Chronic exposure of alcohol (0.5% v/v ethanol in aquarium water) increases the expression of hepatic miR-122 in the zebrafish in parallel with hepatic lipid accumulation [45]. The hepatic expression of miR-122 is decreased while hypoxia-inducible factor 1 alpha (HIF1α), the known miR-122 target is increased in ALD patients and in mice fed with ethanol containing diet [42]. The gain of miR-122 function ameliorates alcohol-induced liver injury by reducing several inflammatory cytokines, such as monocyte chemoattractant protein-1 (MCP-1) and IL-1β, and liver fibrosis [42]. Mechanistically, alcohol inhibits miR-122 transcription via alternate splicing of grainy head-like 2 (Grhl2) transcription factor [42].

miR-125b

MiR-125b mediates NF-κB-induced inflammatory response by targeting TNFα induced protein 3 (TNFAIP3) [46]. The upregulation of this miRNA by estrogens protects against hepatic steatosis by decreasing fatty acid uptake and triglyceride synthesis in non-alcoholic fatty liver model [47]. MiR-125b also has the anti-fibrotic property by regulating GLI family zinc finger 3 (Gli3) [48]. While miR-125b may implicate in the pathogenesis of non-alcoholic fatty liver disease and hepatic fibrosis, its role in ALD pathogenesis is not clear. It has been suggested that chronic alcohol treatment *in vitro* may not cause changes in miR-125b levels [49]. MiR-125b was reduced in hepatocytes of alcohol-fed mice, while miR-155 was upregulated [50]. MiR-125b protects against ethanol-induced apoptosis in neural crest cells and mouse embryos by targeting Bak 1 (BCL2 Antagonist/Killer 1) and PUMA (p53 upregulated modulator of apoptosis) [51].

miR-129

Downregulation of miR-129-5p improves alcohol-induced barrier dysfunction of Caco-2 human intestinal epithelial cells [52]. Downregulation of miR-129-5p by long non-coding RNA NEAT1 increases the expression of paternally expressed gene 3 (PEG3) and aggravates liver injury and fibrosis via NF-κB-induced hepatic stellate cell apoptosis [53]. An inhibition of NEAT1 leads to an increase in miR-129-5p, ameliorates lipid metabolism, and restrains inflammatory responses in ethanol-treated AML-12 cells. A similar observation is observed in ethanol-fed

mice when an inhibition of NEAT1 or an elevation of miR-129-5p promotes liver function and alleviates hepatocyte apoptosis and hepatic inflammation by targeting suppressor of cytokine signaling 2 (SOCS2) [54].

miR-155

The expression of miR-155 is relatively low in the normal liver [17]. However, its level is altered during liver injury [17]. MiR-155 is highly expressed in immune cells and regulates hepatic lipid metabolism, inflammation, and fibrosis [17, 55, 56]. In the *in vitro* experiments, chronic alcohol treatment of RAW 264.7 macrophages causes an increase in the expression of miR-155, and alcohol pretreatment augments lipopolysaccharides (LPS)-induced miR-155 expression [57]. An increase in the miR-155 expression after alcohol treatment is associated with an increased in TNFα production, though the stabilization of TNFα mRNA in macrophages [57]. Alcohol induced hepatic miR-155 expression at the transcriptional level via the toll-like receptor4 (TLR4) pathway [49]. Alcohol-induced miR-155 inhibits negative regulators of the TLR4 pathway leading to an increase in susceptibility of Kupffer cells in response to LPS [58]. MiR-155 mediates alcohol-induced steatosis by enhancing the proliferator-activated receptor response element (PPRE) and PPARα binding [49]. The deficiency of miR-155 attenuates alcohol-induced steatosis, hepatic macrophage and neutrophil infiltration, and hepatic oxidative stress [49].

miR-181b

MiR181b-3p is a potential negative regulator of TLR4 signaling in Kupffer cells from ethanol-fed rats [59]. Mechanistically, it regulates the expression of importin α5 and translocation of p65 subunit of NFκB in Kupffer cells [59]. The expression of miR-181b-5p is increased in ethanol-fed rat [60]. The loss of miR-181b-5p upregulates protein inhibitor of activated STAT 1 (PIAS1) to inhibit hepatic oxidative stress and inflammatory response by inhibition of protein arginine methyltransferase 1 (PRMT1) in ethanol-fed rats [60]. MiR-181b also mediates hepatic steatosis by targeting SIRT1 [61].

miR-182

The hepatic expression of miR-182 level is associated with disease severity and short-term mortality in patients with alcohol-related hepatitis [62]. An increase in the level of hepatic miR-182 in patients with AH is not consistently observed in

ethanol-fed animal model [62]. Its expression is comparable in animals treated with carbon tetrachloride (CCl_4) or ethanol alone [62]. The combination between CCl_4 and ethanol induces a slight, but significant increase, in its expression [62]. MiR-182 is mainly expressed in ductular reaction cells and hepatocytes. It promotes hepatocellular injury and hepatic inflammation by downregulation of predicted targets, solute carrier family 1 member 1 (SLC1A1) and cofilin1, and an upregulation of inflammatory and cell cycle genes such as C-C Motif Chemokine Ligand 20 (CCL20), C-X-C Motif Chemokine Ligand 1 (CXCL1), IL-8, and Cyclin D1 [62]. MiR-182 can also target forkhead box protein O1 (FOXO1) in mediating ALD pathogenesis [63].

miR-199

Liver sinusoidal endothelial cells (LSEC) derived from ethanol-fed rats exhibit a significant increase in endothelin-1 (ET-1), hypoxia-inducible factor-1α (HIF-1α), and inflammatory cytochemokines transcripts compared with control rat LSEC; the process involves the activation of NADPH oxidase [64]. As for the upstream, the activation of ET-1 by ethanol is secondary to the binding of hypoxia response element to its ET-1 promoter [64]. The inhibition of miR-199 by ethanol leads to an increase in HIF-1α and ET-1 expression [64].

miR-200

miR-200 is abundantly expressed in the liver and involved in cellular proliferation, hepatic fibrosis, and hepatocellular carcinoma [16, 65]. Hepatic expression of miR-200 is significantly increased in ethanol-treated AML-12 cells and ethanol-fed mice [66]. An induction of miR-200 leads to hepatocyte apoptosis and liver injury by targeting Zinc Finger E-Box Binding Homeobox 2 (ZEB2) [66].

miR-212

As previously mentioned, one of the key mechanisms of alcohol-induced liver injury is the impairment in gut permeability leading to bacterial translocation. Alcohol increased the expression of miR-212 in intestinal epithelial cells. Alcohol-induced miR-212 inhibits the expression of zonula occludens 1 (ZO-1), a major intestinal tight junction protein, leading to gut leakiness [67].

miR-214

One of the pathways associated with alcohol-induced oxidative stress is the glutathione pathway; the glutathione reductase (GSR) catalyzes glutathione disulfide (GSSG) into reduced glutathione [68, 69]. Cytochrome P450 oxidoreductase (POR), a flavin-containing electron donor for all microsomal cytochrome P450, acts as an antioxidant through association with heme oxygenase-1 (HO-1) [69, 70]. MiR-214 can bind to the 3'-UTR of GSR and POR genes and down-regulate their respective protein expression in both an *in vitro* model and ethanol-fed rats [69].

Ethanol-induced oxidative stress through the activation of miR-214, the process which may be regulated by long noncoding RNA UCA1 [69, 71].

miR-217

Ethanol up-regulates miR-217 in part through acetaldehyde during ethanol metabolism in AML-12 cells, ethanol-fed mice, and zebrafish model [45, 72]. MiR-217 promotes ethanol-mediated inhibition of SIRT1 and lipin-1α, a nucleocytoplasmic shuttling protein [72]. It also regulates genes associated with lipogenic enzymes or fatty acid oxidation [72]. In addition to the role of miR-217 in mediating alcohol-induced steatosis, ethanol also exacerbates LPS-mediated up-regulation of miR-217 and promotes the release of pro-inflammatory cytokines in RAW 264.7 macrophage cell line and primary Kupffer cells [73]. MiR-217 also plays an important role in the generation of TGF-β or reactive oxygen species (ROS) in macrophages [73]. MiR-217-mediated inflammatory responses are secondary to an activation of NF-κB and nuclear factor of activated T Cells 4 (NFATc4) [73].

miR-223

MiR-223 is highly expressed in neutrophils and is considered a neutrophil-specific miRNA. It plays an important role in attenuating neutrophil maturation and activation [74]. The expression of miR-223 in peripheral blood and hepatic neutrophils is markedly induced after chronic-plus-binge ethanol feeding in mice [75]. The induction of miR-223 by ethanol is through transcriptional regulation and not from the direct effect of ethanol or its metabolite [75]. *MiR-223*$^{-/-}$ mice are more susceptible to chronic-plus-binge-induced liver injury with an increase in hepatic neutrophil infiltration and ROS production in the liver and neutrophils [75]. The p47phox is predominately expressed in neutrophils and plays a key role in generating oxidative

burst [76]. Mechanistically, miR-223 down-regulates neutrophilic p47phox via the inhibition of IL-6 [75]. MiR-223 also plays an important role in mediating acute-on-chronic alcohol-induced liver injury in aging mice [77]. Neutrophilic Sirt1 and miR-223 expression are significantly reduced in age compared with young mice [77]. Deletion of the *Sirt1* gene in myeloid cells including neutrophils exacerbates chronic-plus-binge ethanol-induced liver injury and inflammation and down-regulates neutrophilic miR-223 expression [77]. SIRT1 promotes deacetylation of C/EBPα, a key transcription factor regulating miR-223 biogenesis, and increases the neutrophilic miR-223 expression [77].

miR-291b

Tollip is a negative regulator of the MyD88-dependent pathway of TLR2 and TLR4 signaling in Kupffer cells [78]. Chronic ethanol feeding increases the expression of miR-291b, which can target the 3′-'UTR on Tollip gene resulting in a decrease in the expression of Tollip [78]. This decreased expression of Tollip contributes to enhanced TNFα expression in response to activation of TLR2/TLR4 [78].

Let-7

The alteration of the let-7/Lin28 axis is associated with mesenchymal phenotypic changes and ALD progression [79]. A reduction of let-7, particularly let-7a and let-7b, is associated with HSC activation in ethanol-fed mice [79]. Let-7 (especially let-7b) is an endogenous ligand of TLR7; TLR7-let-7 pathway contributes to alcohol-induced hepatic inflammatory process in ethanol-fed mice and in patients with AH [80]. A brief summaries of selected miRNAs and their respective gene targets in mediating ALD are shown in Fig. 60.1 and Table 60.1.

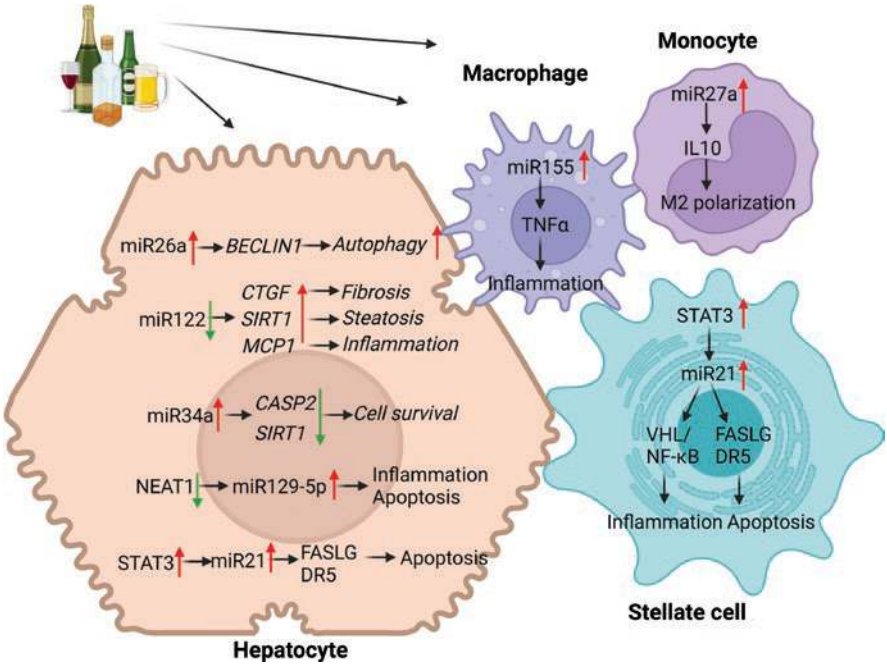

Fig. 60.1 Brief summary of selected miRNAs and their respective gene targets in mediating ALD

Table 60.1 Major miRNAs and their respective target genes in ALD pathogenesis

miRNAs	Targets	References
miR-21	FASLG, DR5, Crebl2	[24]
miR-26a	DUSP4, DUSP5	[31]
miR-27a	Sprouty2, CD206	[33]
miR-34a	SIRT1, CASP2	[37, 39, 40]
miR-122	HO-1, HIF-1α, GRHL2	[11, 42, 81–83]
miR-125b	Gli3	[46–48]
miR-129	PEG3, SOCS2	[53, 54]
miR-155	TNFα, SHIP1, SOCS1, IRAKM, C/EBPβ	[50]
miR-181b	Importin α5, PRMT1, SIRT1	[59–61]
miR-182	SLC1A1, cofilin 1, FOXO1	[62, 63]
miR-199	ET-1	[64]
miR-200a	ZEB-2	[66]
miR-212	ZO-1	[67]
miR-214	POR, GSR	[69]
miR-217	SIRT-1	[72]
miR-223	$p47^{phox}$, IL-6	[75]
miR-291b	Tollip	[78]
Let-7b	lin28	[79]

Conclusions and Future Perspectives

To date, multiple miRNAs are reported to be involved in ALD pathogenesis. These miRNAs have multiple functions through their regulation of the target genes leading to the alterations in lipid metabolism, inflammation, and fibrosis. MiRNAs have the potential to serve as biomarkers in predicting outcomes of patients with ALD. They also are attractive targets for therapeutic strategies. Several miRNAs are being evaluated in pre-clinical studies in patients with liver diseases [84]. The understanding of basic molecular mechanism of specific miRNAs in ALD may pave the way in the development of novel miRNA-based therapeutics for patients with ALD.

Conflict of Interest None of the authors have any conflicts of interest with this work.

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Chapter 61
Intestinal Barrier and Pathogen-Associated Molecular Patterns (PAMPs) in the Development and Therapy of Alcohol-Related Liver Disease

Finn Jung, Annette Brandt, and Ina Bergheim

Abstract Alcohol consumption is still among the leading causes of liver damage world-wide. Molecular mechanisms of the development of alcohol-related liver diseases (ALD) are still not fully understood, however, several studies suggest an important role of alcohol-mediated changes in intestinal barrier function and the interaction with the liver (gut-liver-axis) seems to be critical for the development of ALD. This book chapter focuses on the present knowledge and understanding of the altered intestinal barrier function subsequently leading to an increased permeation of pathogen-associated molecular patterns (PAMP) followed by an activation of pattern recognition receptors like toll-like receptors (TLR) in the development of ALD.

Keywords ALD · Intestinal permeability · Toll-like receptors · Ethanol · Endotoxin · Tight junctions · PAMP

Abbreviations

ALD	Alcohol-related liver disease
ALDH	Aldehyde dehydrogenase
CD14	Cluster of differentiation 14
CYP2E1	Cytochrom P450 2E1
HIF	Hypoxia-inducible factors
IgA	Immunoglobulin A
iNOS	Inducible nitric oxide synthase
IRF3	Interferon regulatory factor 3
LBP	Lipopolysaccharide binding protein

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LPS	Lipopolysaccharide
M cells	Microfold cells
MAL	MyD88 adaptor-like
MAPK	Mitogen-activated protein kinase
MD2	Myeloid differentiation factor 2
MLC	Myosin light chain
MLCK	Myosin light chain kinase
MTP	Microsomal triglyceride transfer protein
MyD88	Myeloid differentiation primary response 88
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PAI-1	Plasminogen activator inhibitor-1
PAMP	Pathogen-associated molecular patterns
PRR	Pattern recognition receptors
Reg3	Regenerating islet-derived protein 3
TIR	Toll-interleukin-1 receptor
TIRAP	Toll-interleukin 1 receptor domain containing adaptor protein
TLR	Toll-like receptor
TNF-R1	Tumor necrosis factor receptor 1
TNFα	Tumor necrosis factor α
TRAM	TRIF-related adaptor molecule
TRIF	TIR-domain-containing adapter-inducing interferon-β
ZO-1	Zonula occludens-1

Introduction

An interaction of the liver and the intestinal bacteria and/ or bacteria-derived compounds has been discussed for several decades. For instance, already in the 1950s, studies demonstrated that germ-free animals were protected from the development of liver necrosis [1]. Furthermore, the concomitant treatment with antibiotics like aureomycin protected animals with diet-induced steatohepatitis from the development of cirrhosis [2]. Results of these studies also suggested that absorbable antibiotics were markedly less efficient in delaying the development of cirrhosis when compared to non-absorbable once [3]. In line with these findings, case report documented development of steatohepatitis in a patient suffering from small bowel diverticulosis and altered bacterial composition in the small intestine [4]. In the second half of the last century, increasing evidence was found linking changes in the composition of the gut microbiota and the function of the gut barrier to liver health but also to the development of liver diseases of various etiologies. About 70% of the liver's blood inflow consists of venous blood derived from the intestine via the portal vein. In line with this, recent studies further indicate that the liver not only receives nutrients from the intestine but also substances derived from the intestinal microbiota that enter the bloodstream when the intestinal barrier is disrupted [5]. This book chapter focuses on the present knowledge and understanding of the interplay of intestinal barrier function, pathogen-associated molecular patterns (PAMPs) and the development of alcohol-related liver diseases (ALD).

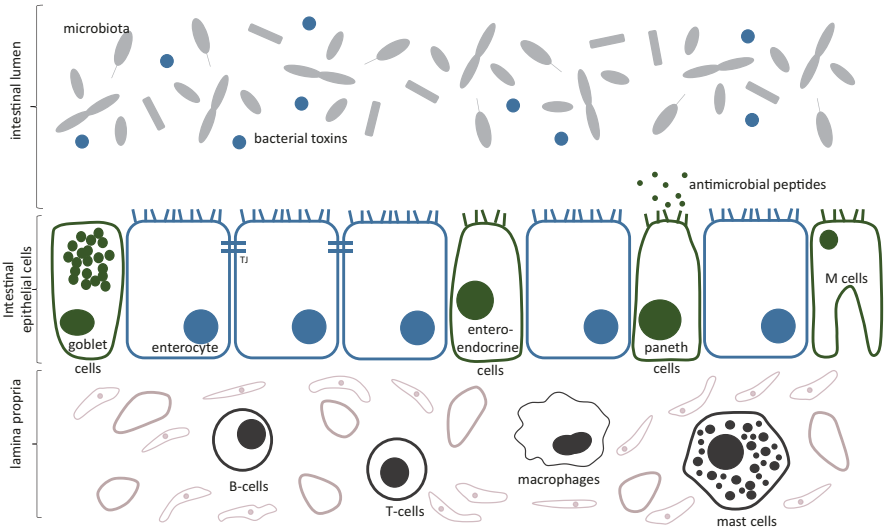

Fig. 61.1 Schematic overview of the intestinal barrier. The physical barrier consists of multiple different cells types including enterocytes, goblet cells, Paneth cells, microfold cells (M cells) and enteroendocrine cells, while the immunological barrier located in the lamina propria interacts with the intestinal barrier. Modified after [6–9]

Intestinal Barrier: Structure in Health

As depicted in Fig. 61.1 the intestinal barrier is composed of several interacting layers forming a complex structure. Apart from its function in nutrient digestion and absorption, the intestinal barrier also functions as physical barrier in the prevention of the entry of pathogens and PAMPs from intestinal lumen to circulation. For instance, an impaired barrier function or already minor changes in regulation of the interplay of the epithelial, microbial, biochemical, or immunological barrier already contribute to the development of liver disease such as ALD but also metabolic liver diseases (NAFLD) and even cognitive decline (for overview see [10–12]). In the following, we will describe key components of this complex structure thought to be critical in the development of alcohol-related impairment of intestinal barrier function with a specific focus on the epithelial layer.

Intestinal Epithelial Layer

Along with a stable microbiota the mucus layer can be considered as `first line defense´ against external injuries (for overview see [6, 13]). Herein, the microbiome not only contributes to the digestion of nutrients and production of vitamins but is also critical for shaping the immune system (for overview see [14, 15]). For instance,

studies in germ-free mice have shown that intestinal microbiota determines the number of peyer's patches, lymphoid follicles and in general, the number of immune cells, e.g., immunoglobulin A (IgA)-producing plasma cells or $CD8^+$ and $CD4^+$ T cells [16].

The core of the intestinal barrier is constituted of the epithelial cell layer composed of multiple different cell types, e.g., enterocytes, enteroendocrine cells, goblet cells, Paneth cells and microfold cells (M cells) (see also Fig. 61.1). All these cells which differentiate from pluripotent intestinal stem cells located in the crypts [17] contribute to the complex interplay of nutrient absorption, maintaining mucosal barrier function and secreting immunological mediators (for overview see [18–21]). Epithelial cells in the small intestine of humans typically have a turnover of ~3.5 days [22]. Through junctional complexes comprised of tight junctions on the luminal, apical side and adherence junction and desmosomes towards the basolateral side, intestinal epithelial cells are tightly connected (for overview see [23]). Studies indicate that tight junction proteins are key components in the control of paracellular transport in both the small and large intestines of the resulting semipermeable barrier (also see [21]). The permeation of ions and other substances is facilitated through this semipermeable barrier, whereas the translocation of noxious molecules like that of bacterial endotoxin is very limited in healthy subjects [13, 21, 23, 24]. Tight junctions which are composed of transmembrane proteins like claudins, occludin and junctional adhesion molecule have been shown to interact with peripheral membrane proteins such as the zonula occludens (ZO-1) (for overview see [24]). For the latter results of *in vitro* studies suggest that the protein connects with the cytoskeleton of the epithelial cell via F-Actin [25]. *In vitro* studies have also demonstrated an interplay of tight junctions and the actin-myosin cytoskeleton being critical in maintaining the paracellular barrier integrity [26] with myosin light chain kinase (MLCK) as key regulatory kinase [26] . Thus, activation of myosin light chain (MLC) by MLCK results in a restructuring of peri-junctional F-actin which in turn leads to a reorganization of occludin and ZO-1 and subsequently an increased permeability [27]. In addition, posttranslational phosphorylation of occludin seems to be critical for interconnecting tight junctions and, therefore, controlling the intestinal barrier function [28]. For example, it has been shown that, depending upon the phosphorylation site, tyrosine phosphorylation mitigates the interconnection of occludin-ZO-1 leading to a destabilization of tight junctions, whereas the phosphorylation of serine and threonine residues of occludin enhances the cohesion of tight junction proteins [29–31]. Also, results of more recent *in vivo* and *in vitro* studies suggest that a posttranslational nitration enhances the ubiquitin-dependent proteolytic degradation of tight junctions [32]. In contrast, studies in occludin knockout mice have shown that these animals display histological abnormalities in various tissues while tight junctions in intestinal tissue were found to be `normal´ in these mice [33]. This suggests that, in addition to tight junctions, other mechanisms may also be critical in regulating intestinal barrier function and compensate for loss of occludin. For instance, besides peptide hormones such as ghrelin, peptide YY, and cholecystokinin, glucagon-like peptide 1 secreted by

enteroendocrine cells in response to nutrient exposure (for overview see [34, 35]) and glucagon-like peptide-2 may also contribute to maintaining intestinal barrier function [36]. Further studies are needed to fully elucidate the complex interplay underlying the function of the intestinal barrier and, in particular, the function of the tight junction proteins therein.

Mucus and Immunological Barrier

Intestinal epithelial cells are covered by a thick mucus layer mainly synthesized by goblet cells and composed of glycosylated mucin proteins such as mucin-2 which is considered the main mucin in human intestine [6, 13, 37, 38]. Mucin-2 knockout mice have been shown to develop colitis and suffer from an impaired intestinal barrier function [39]. Furthermore, so called transmembrane mucins like mucin-1, -3, -4, -12, -13, 16 and -17 have been shown to carry out barrier and signaling functions (for overview see [38]). In addition, antimicrobial peptides secreted by Paneth cells are also important for maintaining the intestinal homeostasis [40]. Transgenic mice with a reduced number of Paneth cells have been reported to suffer from an increased penetration of commensal as well as pathogenic bacteria into the intestinal wall [41]. Secretion of antigen-specific IgA by B cells has also been shown to decrease intrusion of bacteria into the intestinal mucosa. Studies have also shown that dendritic cells which can be activated by epithelial M cells (located in follicle associated-lymphoid tissue as part of Peyer's patches) contribute to the antigen uptake from the intestinal lumen (for details please refer to [42, 43]). A loss of IgA has been shown to be associated with an impaired intestinal barrier function *in vivo* [44] and M cells may act as an entrance for pathogens (for overview see [43]).

Pattern Recognition Receptors Related Signaling Cascades

Alterations of the intestinal microbiota and a dysfunctional intestinal barrier can both result in an increased permeation of microbial compounds and even whole bacteria but also of viruses, fungi, parasites, and archea (for overview see [45]). In 1989, the concept of Pattern Recognition Receptors (PRR) recognizing pathogen-associated molecular patterns (PAMPs) was introduced with PAMPs subsequently activating both innate and adaptive immunity [46]. PRRs consist of a large variety of receptors including Toll-like receptors (TLRs), Nucleotide-binding oligomerization domain-like receptors, C-type lectin receptors and Retinoic acid-inducible gene I-like receptors [45]. In the following, TLRs will be in the focus since these are the main receptors that recognize intestinal bacteria and results of rodent and human studies suggest that the activation of TLRs, namely of TLR4, the most studied TLR, is critical in the onset and progression of ALD (for overview also see [47, 48]).

TLRs are not only expressed in innate immune cells like monocytes, macrophages and dendritic cells but also in non-immune cells such as epithelial cells and fibroblasts. Upon their cellular localization, the so far 10 TLRs described in humans can be distinguished in cell surface TLRs and intracellular TLRs. While TLR1, TLR2, TLR4, TLR5. TLR6 and TLR10 are localized in the cell surface, TLR3, TLR7, TLR8 and TLR9 are found intracellularly in the endosome [49] (for schematic overview see Fig. 61.2). However, recent studies also indicate that TLR3, TLR7 and TLR9 may occur at both sites, cell surface and intracellularly, and while this process is not yet fully understood it could contribute to endosomal TLR-mediated activation of immune response (for overview see [52]). TLRs located at the cell surface have been shown to predominantly recognize components of microbial membranes like lipids, lipoproteins, and proteins. TLR2 generally forms heterodimers either with TLR1 or TLR6. It has been shown to recognize a variety of PAMPs from Gram-positive bacteria such as lipoproteins, peptidoglycan, lipoteichoic acid but also lipoarabinomannan from mycobacteria zymosan from fungi, and tGPI-mucin from *Trypanosoma curzi* as well as hemagglutinin protein from measles virus [53]. For the recognition of some of the PAMPs by TLR2, a delivery through cluster of differentiation 14 (CD14) has been shown to be crucial [54]. Until recently, most studies suggested that TLR2 is mainly involved in mediating inflammatory

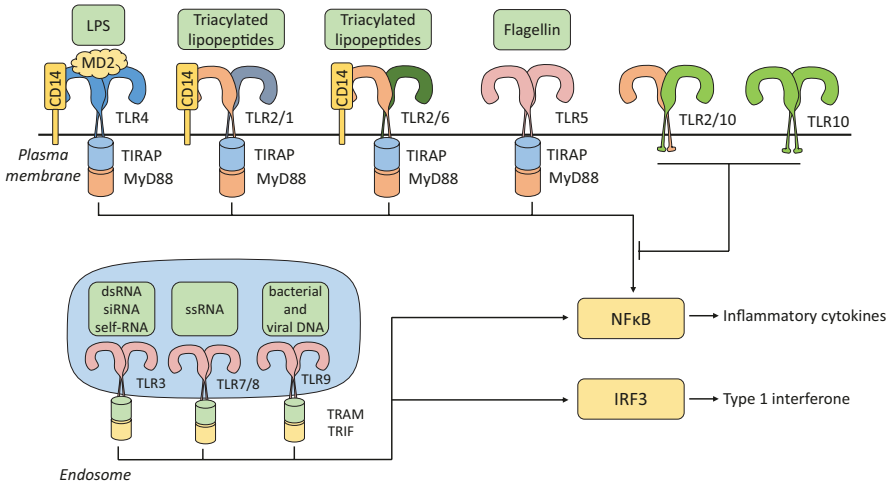

Fig. 61.2 Schematic overview of toll-like receptor signaling and their respective ligands. TLR1, −2, 4, −5, −6 and −10 are bound to the cell membrane and activated by the recognition of their respective ligands (TLR1/2/6: triacylated lipopeptides, TLR4: lipopolysaccharides, TLR5: Flagellin) whereas TLR10 exhibits anti-inflammatory properties by inhibiting MyD88-dependent signaling. TLR3, −7, −8 and −9 are endosome-bound and recognize bacterial and viral RNA and DNA fragments. Once activated by their respective ligands inflammatory cytokines like TNFα are released through the TIRAP/MyD88-dependend activation of NFκB or Type 1 interferon by IRF3. *IRF3* interferon regulatory factor 3, *MyD88* myeloid differentiation primary response 88, *NFκB* nuclear factor kappa-light-chain-enhancer of activated B cells, *TIRAP* toll-interleukin 1 receptor (TIR) domain containing adaptor protein, *TLR* toll-like receptor. Modified after [50, 51]

responses. However, results of more recent study suggest that TLR2, through the recognition of specific bacteria, may also modulate the intestinal barrier function and intestinal mucosal serotonin production [55, 56]. Further studies are needed to fully unravel the role of TLR2 in the latter.

Lipopolysaccharides (LPS) found in the outer-wall of Gram-negative bacteria have been identified to be recognized by TLR4 which forms a complex with myeloid differentiation factor 2 (MD2) (for overview see [57]). Somewhat similar to TLR2, LPS is also delivered to the TLR by CD14 [58]. Furthermore, results of several studies suggest that the lipopolysaccharide binding protein (LBP), a soluble plasma protein shown to bind LPS, is also involved in the delivery of LPS to the TLR4-MD2 complex [59]. However, there are also contradictory studies suggesting that LBP might not be involved in the recognition of LPS by the TLR4-MD2 complex [60]. TLR5 has been shown to be activated by flagellin [61]. While being a pseudogene in mice due to an insertion of a stop codon, in human, TLR10 being most homologous to TLR1 and TLR6 [62], has been suggested to interact with TLR2 in the sensing of triacylated lipopeptides and other agonists of TLR1 [62]. Also, more recent results suggest that TLR10 may exhibit anti-inflammatory properties (for overview see [63]). For instance, it can inhibit myeloid differentiation primary response 88 (MyD88) dependent and independent pathways [64]. The intracellularly located TLRs sense nucleic acids of bacteria and viruses but also self-nucleic acids in diseases like autoimmunity [65]. Specifically, TLR3 has been shown to recognize viral double stranded RNA (dsRNA), small interfering RNA, and self-RNA of damaged cells (for overview see [49, 66]) while TLR7 and 8 recognized single-stranded RNAs [66]. TLR9 has been shown to recognize bacterial and viral DNA being rich in unmethylated CpG-DNA motifs [66].

Upon sensing their respective ligands, TLRs differentially engage with members of the family of toll-interleukin-1 receptor (TIR) domain-containing adaptors such as MyD88, TIR domain-containing adapter-inducing interferon-β (TRIF), toll-interleukin 1 receptor domain containing adaptor protein/ MyD88 adaptor-like (TIRAP/MAL), or TRIF-related adaptor molecule (TRAM). Herein, MyD88 engages with all TLRs with the interaction leading to an activation of NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) and mitogen-activated protein kinases (MAPKs) and subsequently the induction of pro-inflammatory cytokines (for overview also see [45, 49]). TIRAP has been suggested to be a sorting adaptor recruiting MyD88 to TLR2 and TLR4 but has also been shown to be involved in signaling of TLR9 (for overview also see [45, 49]). The recruitment and activation of TRIF by TLR3 and TLR4 results in the activation of an alternative pathway that leads to the activation of interferon regulatory factor 3, NFκB and MAPKs resulting in an induction of type I interferon and proinflammatory cytokines. Herein, TRAM seems to be only recruited to TLR4 but not TLR3 linking TRIF and TLR4. Taken together, depending upon the adaptor protein employed, activation of TLR signaling can be divided in two main signaling pathways: the MyD88-dependent and the TRIF-dependent pathways (for overview also see [45, 49]).

Alcohol-Related Alterations of Intestinal Barrier Function and the Translocation of Bacterial (Endo) Toxins: Current Knowledge

In the following, some of the key findings regarding the interaction of alcohol with the intestinal barrier function and subsequently the translocation of bacteria and bacterial wall-compounds are summarized. Effects of alcohol on intestinal microbiota are summarized elsewhere in this book (book Chap. xxx) and are therefore only briefly touched in the following section.

PAMPs and TLRs in ALD

The groups of Christiane and Christian J. Bode but also Ronald G. Thurman and his team have reported already more than 30 years ago that in humans and rodents' alcohol-related liver disease is associated with elevated bacterial endotoxin levels in blood [67, 68]. It has also been shown that, in patients with ALD, bacterial endotoxin levels are positively correlated with intestinal permeability [69] and disease severity [70]. However, in the latter study, bacterial endotoxin levels were assessed in a mixed study population of patients with alcoholic and non-alcoholic cirrhosis. Results of several animal and human studies have by now confirmed these findings (for overview see [71]). Studies have also suggested that even the intake of one high-dose (> 20 g raw ethanol in one setting) is sufficient to increase bacterial endotoxin levels in peripheral blood in humans [67, 72]. In animals, even after one acute ingestion, this increase in bacterial endotoxin levels was found to be associated with the accumulation of fat in the liver [73]. In support of these data, we could recently show that not only liver parameters like activity of ALT and AST as well as γ-GT, liver stiffness and CAP are markedly reduced within 1 week of total abstinence in patients with alcohol-related liver disease but that this also goes along with a decrease of ligands of TLR2 and TLR4 in serum [74]. Furthermore, serum protein levels of zonulin and I-FABP were also almost similar to those found in healthy controls after 1 week of abstinence in these patients.

In the liver, gut derived bacterial endotoxin but also other PAMPs derived from Gram-positive bacteria and fungus as well as viruses, have been suggested to contribute to the development of ALD (for overview see [75]). Jun et al. even reported that the prevalence of cirrhosis was related to the presence of bacterial DNA in peripheral blood [76]. However, no details were provided in this study regarding etiology of cirrhosis. In line with this, it has been shown that besides TLR4 also other TLRs are induced in rodent liver upon chronic high intake of alcohol [77]. Furthermore, employing mice deficient of TLR4, TLR2 and TLR9, it has been shown that an activation of these TLRs not only plays a pivotal role in inflammatory processes associated with the development of ALD but also the hypermetabolic state e.g., increased hepatic oxygen uptake and accumulation of lipids after acute

and chronic alcohol exposure [78–80]. Indeed, in settings of acute and chronic alcohol intake, it was demonstrated that the activation of TLR4-dependent signaling cascades results in an induction of tumor necrosis factor α (TNFα) [81, 82], eventually impairing insulin signaling, inducing plasminogen activator inhibitor-1 (PAI-1) and subsequently hepatic growth factor/cMet-dependent signaling [83] (for schematic overview see Fig. 61.3). In further support of the hypothesis that an increased translocation of PAMPs and subsequent activation of Kupffer cells is critical for the development of ALD, treatment with both, non-resorbable antibiotics and Kupffer cell toxic Gadolinium(III)-chloride, has been shown to almost completely abolish hypermetabolic alterations after acute high alcohol ingestion in rodents [79].

In confirmation, various studies suggest that ethanol consumption is associated with an altered intestinal microbiota composition (for overview see [85] and Chap. X of this book). Studies have shown that the number of anaerobic and aerobic bacteria is significantly higher in jejunal juice of alcoholics than in controls. Herein, alcoholics were found to have a incidence of Gram-negative bacteria and endospore-forming bacterial correlating closely with the pH found in gastric juice [86]. Employing a hydrogen breath test, changes in microbiota composition in small intestine have been reported to be more prevalent in alcoholics than in controls [87]. Other studies showed that changes in microbiota composition in small-intestine is frequently associated with the presence of cirrhosis regardless of the cause of liver

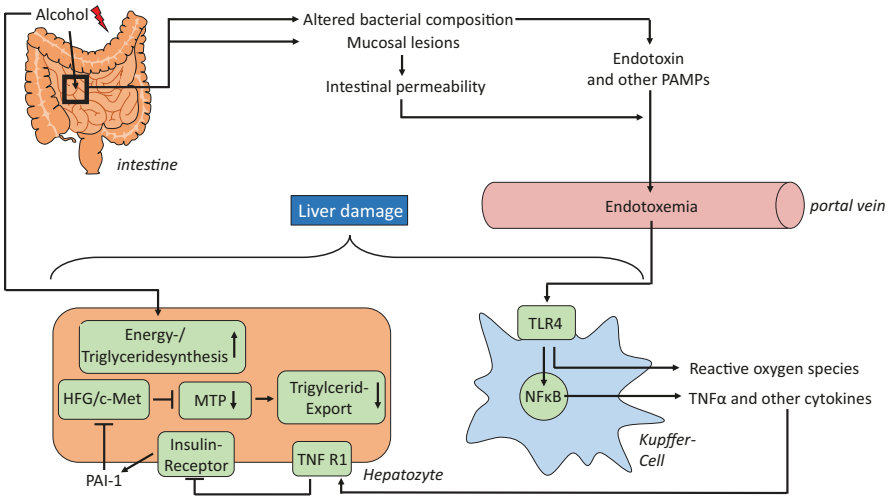

Fig. 61.3 Schematic overview of the role of gut-derived PAMPs in the development of alcohol-related liver disease. Through alcohol induced alterations of bacterial composition and intestinal permeability, gut derived PAMPs like endotoxin cross the intestinal barrier and are recognized by Kupffer cells in the liver. Reactive oxygen species and pro-inflammatory cytokines like TNFα are released and contribute to inflammatory processes and impairments of triglyceride export in the liver. *MTP* microsomal triglyceride transfer protein, *PAMP* pathogen-associated molecular patterns, *TNF-R1* tumor necrosis factor receptor 1, *TNF-α* tumor necrosis factor α. Modified after [84]

damage [88–92]. In a study assessing colonic bacterial composition in patients with alcohol dependence with and without liver disease, it was shown that altered colonic microbiota were correlated with higher blood endotoxin levels [93]. Finally, some studies suggest that patients with alcoholic cirrhosis may have a distinctly altered functional composition of the fecal microbiome e.g., a depletion of functional genes involved in nutrient metabolism including amino acids, lipid and nucleotide metabolism (for overview see [85]).

Effect of Acute and Chronic Alcohol Intake on Intestinal Mucosa

Both, chronic and acute high-dose-intake of ethanol have been reported to lead to losses of epithelial cells from the villi tips and even to hemorrhagic erosions in the lamina propria (for overview also see [94]. Furthermore, studies employing chronic feeding models in rodents have shown that mucus layer and expression of certain mucins like mucin 2 but also antimicrobial peptides like cathelicidin-related antimicrobial peptide, regenerating islet-derived protein 3β (Reg3β) and Reg3γ are altered in distal small intestine [95]. While some of the studies suggest that these alternations may not only be related to alcohol intake but also fatty acid composition of the diet [96, 97], a dysfunction of Paneth cells as major source of antimicrobial peptides [40] seems to be critical in the development of alcohol-related intestinal barrier dysfunction and subsequently ALD. For instance, it has been reported that overexpressing Reg3γ being inducible by Interleukin-22 [98] in intestinal epithelial cells restricts bacterial colonization of mucosal surface and subsequently translocation of bacteria and the development of ALD [95]. Furthermore, recently, it was reported that an oral supplementation of human beta defensin-2 can attenuate liver injury in mice and that this was associated with alterations of multiple bacterial genera in feces [99].

However, studies in Cytochrom P450 2E1 (CYP2E1) knockout mice fed ethanol also suggest that ethanol metabolism itself may be critical in the induction of alcohol-related intestinal barrier dysfunction. In these studies, serum endotoxin levels were markedly lower in knockout mice than in wild-type animals being also associated with lower inducible nitric oxide synthase (iNOS) induction and less mucosal damage [100]. Furthermore, it has also been reported that a disruption of ethanol metabolism through knocking out acetaldehyde dehydrogenase (ALDH) may enhance not only the development of ALD but also the disruptive effects of ethanol in intestinal barrier function [101]. Indeed, when being exposed chronically to ethanol ALDH (+/−) mice showed a greater permeability in large and small intestine along with a marked redistribution and disruption of tight and adherent junction proteins [101]. Somewhat in line with these results, studies in differentiated Caco-2 cells being a model of small intestinal epithelial cells suggest that expression of

tight junction proteins can also be diminished through direct effects of ethanol, e.g., ethanol metabolites and/ or metabolic alterations associated with the metabolism of ethanol e.g., the shift of NAD^+ $NADH^+H^+$ or the formation of nitric oxide [102, 103]. Specifically, studies employing cell lines have shown that, through NFκB-dependent signaling cascades, alcohol activates iNOS in enterocytes and that the resulting increase in nitric oxide and peroxynitrate can add to the ethanol-related disruption of intestinal barrier and loss of tight junctions [104, 105]. In line with these findings, it was also shown, that a concomitant treatment with iNOS inhibitors not only attenuated the development of ALD but also alcohol-induced intestinal barrier dysfunction and endotoxemia [106]. Other studies also suggest that intestinal barrier function is highly dependent on a `normal´ microcirculation of the underlying vasculature system [107]. Moreover, acute and chronic intake of alcohol is associated with impairment of the intestinal microcirculation [108] and a decreased expression of hypoxia-inducible factors (HIF) HIF1α and 2α seem to be involved [109, 110]. Taken together, these data suggest that alcohol, probably through its alcohol dehydrogenase- or CYP2E1-dependent metabolism, may also affect intestinal barrier function independently of its effects on intestinal microbiota composition.

Conclusion

Animal and human studies suggest that besides alterations of intestinal microbiota composition (for overview also see Chap. X of this book) impairments of intestinal barrier function and subsequently an increased permeation of PAMPs are critical in the development of ALD. Studies further suggest that abstinence is associated with a rather rapid change of intestinal microbiota composition and intestinal barrier function. So far, however, only a limited number of studies have addressed this question. Although the pharmacotherapeutic targeting of the intestinal barrier function and/or TLRs with pro- or prebiotics or drugs appears as an attractive molecular strategy to treat ALD in early stages, more experimental studies and clinical trials are needed.

Author's Disclosures The authors declare that they have no conflict of interest.

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Chapter 62
Microbiota and Alcohol-Related Liver Disease

Alina Popescu and Felix Bende

Abstract Alcohol-related liver disease (ALD) comprises different histopathological changes in patients with excessive alcohol intake ranging from alcohol-induced steatosis to alcoholic steatohepatitis, and liver cirrhosis. The presence of advanced fibrosis or cirrhosis in these patients is the main predictor of long-term survival. The gut microbiota plays an important role in the pathogenesis and progression of ALD, and its role within the progression to liver cirrhosis and hepatocellular carcinoma has been intensively studied. Several changes in ALD patients' microbiota were documented starting with an increased dysbiosis, an increased intestinal permeability, and an increased small-intestinal bacterial overgrowth. These considerable gut flora abnormalities present in ALD patients may affect their liver disease's natural course and may represent potential targets for intervention in the management of these patients. Despite an significant progress in this field of medical research, more data are needed to fully understand the implications of microbiota in the pathogenesis and progression of ALD. This chapter briefly describes and discusses some of the key observations.

Keywords Alcohol-related liver disease · Microbiota · Dysbiosis · Liver cirrhosis · Alcohol use disorders

Introduction

The human microflora, also known as "microbiota", includes multiple species of germs (bacteria, fungi, bacteriophages or viruses) that colonize the skin, the genitourinary system, the respiratory system and the digestive tract. The highest microbial

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density is found in the intestine where it forms the intestinal microbiota. The gut microbiome includes about 1000 different species of bacteria, totaling a weight of about 1 kg [1]. The stomach and small intestine are less populated by bacteria, the vast majority of them colonizing the large bowel. At this level, there are normally over 10^{14} microorganisms [2]. The vast majority belongs to two groups, either gram-positive *Firmicutes* or gram-negative *Bacteroides* [1, 2]. However, there are other species, less abundant, but important for homeostasis, among which *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* should be mentioned [2]. The intestinal microbiome is characterized by a great diversity of germs. Important bacterial species are *Clostridium coccoides* (C. coccoides)-*Eubacterium rectale*, *Clostridium leptum* (C. leptum), *Bacteroides-Prevotella*, *Bifidobacterium* and *Atopobium* [3]. The intestinal microbiota is in symbiosis with the human body, it is accepted by it (it has "compatibility"), it has functions (defense, immunity, digestion and metabolism), it communicates with the intestinal epithelium and with other systems, including the central nervous system ("gut-brain interactions"), behavior similar to that of any organ. For these reasons, the microbiom is sometimes considered an "organ" often forgotten in medical practice [1]. The intestinal microbiome contains 100 times more genes, compared to the human genome [4]. It has multiple functions that are not yet fully understood.

First, it forms an external barrier ("barrier effect"), preventing pathogenic germs from colonizing the intestinal mucosa. Intestinal epithelial cells create a physical barrier between intraluminal microbes and the intestinal tissue. In addition, they produce a mucus layer and secrete antimicrobial proteins, such as secretory immunoglobulin A (Ig A), which limit the exposure of epithelial cells to microbial agents [5]. Clostridium difficile-associated colitis is an example of losing this barrier function [4]. Second, the microbiome has metabolic and energetic functions ("metabolic organ"), producing energy from undigested residues in the small intestine ("recovers residues"). Under the action of the bacterial flora, short-chain fatty acids are converted largely to butyric acid, with a beneficial role for colonocytes, as well as vitamin formation. Thus, vitamin K is mainly derived from intestinal microbiota) [5].

Third, the maturation and education of the intestinal immune system is unthinkable without the microbiome through continuous, direct and indirect stimulation, and the production of a chronic, physiological, mild inflammation ("low-grade physiological inflammation"), which constantly keeps the immune system on alert, maintaining a perfect symbiosis with the microbiota ("innate and adaptive immune responses"). The microbiota ensures homeostasis of the immune system, so that the body tolerates microorganisms. Dysregulation of the interaction between the host and the microbiota can lead to an inadequate or exaggerated inflammatory reaction, with increased mucosal permeability.

In healthy subjects, the microbiome aids at maintaining a balance between the immune tolerance of the host and the permanent stimuli coming from the existing flora and its metabolic products [4]. Microbial recognition using antigen-presenting cells (for example dendritic cells, DC) and epithelial cells, is achieved by identifying "microbial-associated molecular patterns" (MAMPs), using "toll-like"

receptors (TLR), capable of detecting a multitude of bacterial components, such as lipopolysaccharides (LPS), lipoproteins, CpG DNA, but also through "nucleotide-binding oligomerization domain (NOD)-like" (NLR) receptors, which recognize peptidoglycan molecules from the bacterial cell wall [6]. In healthy subjects, the pro-inflammatory pathways associated with TLRs and NLRs are suppressed by inhibitors, both of human nature and bacterial origin such as Cyclooxygenase-2 (COX-2) inhibitors, LPS, A20, peroxisome proliferator-activated receptor-γ (PPAR-γ), nuclear factor-κB (NF-κB) inhibitor IκB-α, interferon-α/β (IFN-α/β), interleukin-10 (IL-10), TGF-β, and eicosanoids] [7, 8]. On the other hand, the tolerance mechanisms of the commensal microbiota offer protection against the inappropriate inflammatory response, but also against the invasion of pathogenic flora [4]. So far, however, the mechanisms by which the commensal flora is tolerated by the host, at the expense of pathogenic germs, is not fully known [4].

Gut Microbiota Changes in Alcohol-Related Liver Disease

Alcoho-related liver disease (ALD) comprises different histopathological changes in patients with excessive alcohol intake ranging from alcohol-induced steatosis, to alcoholic steatohepatitis, which features hepatocytes necrosis, inflammation and potential evolution to fibrosis, and liver cirrhosis. ALD is the major cause of chronic liver diseases worldwide, accounting for approximately 27% of liver-related deaths [9]. While simple alcohol-induced steatosis is present in almost all heavy drinkers, it is estimated that ca. 10–20% of these subjects will eventually develop liver cirrhosis [10]. The presence of advanced fibrosis or cirrhosis in these patients is the main predictor of long-term survival [11]. The gut microbiota plays an important role in the pathogenesis and progression of ALD, and its role in the progression to liver cirrhosis and hepatocellular carcinoma has been intensively studied.

Thus, several changes in ALD patient's microbiota were documented starting with an increased *dysbiosis*, an *increased intestinal permeability*, and an *increased small-intestinal bacterial overgrowth* [12]. Alcohol-induced gut ***dysbiosis*** is an important feature of ALD that modulates disease progression [13]. Increased metabolism of ethanol in the gut promotes gut dysfunction (a decreased function of tight junction proteins and adhesion junction proteins) and small bowel bacterial overgrowth, generating a leaky gut. When the intestinal barrier is weakened due to dysfunctional tight junctions, microorganisms such as bacteria and fungi may translocate to the blood stream and reach the liver via the portal vein. These bacterial products interact with Toll-like receptors (TLRs) on the surface of the hepatic cells, which leads to inflammation, with the activation of the nuclear factor kappa-B pathway, which releases pro-inflammatory cytokines and chemokines, and the development of liver injury [14]. Chronic alcohol consumption also increases the production of its main toxic metabolite acetaldehyde, promoting mitochondrial dysfunction and oxidative stress perpetuating liver injury [13].

Gut-Liver Axis in Alcohol-Related Liver Disease

Around 15–20% of people with alcohol use disorders (AUDs) may develop ALD [15]. Progression of the disease can vary considerably and intestinal microbiota have been suggested to causes this variability in ALD risk [16–18]. ALD progression, however, may be influenced by coexisting depression and other psychiatric illnesses, as well as abnormalities in circadian rhythms. These factors could interact and skew the microbiological data as well as negatively affect intestinal permeability [19]. In a recent study that included 48 patients with AUDs with ALD (n = 19) and without ALD (n = 28), as well as 18 healthy control persons, the stool and mucosa-associated colonic microbiota were examined [17]. There was no difference between the groups with AUDs, although higher serum levels of endotoxin were observed in both AUD groups. In addition, patients with AUDs (with or without ALD) had significant overlaps in the general distribution of the microbiom. The relative abundance of the family Bacteroidaceae was found to be highest in the healthy control group and lowest in the AUD patients with ALD. However, colonic mucosa dysbiosis was not entirely linked with ALD in AUD patients.

Another study also found differences in the amounts of microbial metabolic products including short-chain fatty acids (SCFAs) and sulfides as well as a decline in antioxidant fatty acids in patients with AUD when compared to healthy control individuals [20]. In this study, however, ALD status was not characterized. Leclercq et al. Studied in 60 patients with AUD whether alcohol-mediated dysbiosis of the microbiome can be reversed [16]. Interestingly, only 40% showed dysbiosis were restored to levels of control persons after 3 weeks of complete alcohol abstinence. Ruminococcaceae abundance also increased during this time. Even after alcohol cessation, increased intestinal permeability was associated with higher levels of anxiety, despair, and alcohol craving.

It is also important to better understand how the gut-brain axis functions in individuals with AUDs and ALD. The interfaces between the liver, intestine, and brain are caused by inflammatory cytokines and direct neuronal connections that can be bidirectional, and these factors all significantly affect the prognosis as a whole. ALD is most severe in patients with endstage cirrhosis or alcoholic hepatitis. These patients not only have extremely poor clinical outcomes, a multifold increased risk of infections, complications due to protal hypertension and acute-on-chronic liver failure, but they also clearly show changes in the composition and function of their microbiota tightly associated with the presence of liver injury. Even in the absence of alcohol drinking, patients with alcohol-related cirrhosis show more gut dysbiosis and endotoxemia than non-alcoholics [21]. Gut microbiota profiles of patients with alcoholic cirrhosis with a cognitive impairment have more Enterobacteriaceae and less Lachnospiraceae and Ruminococcaceae [22].

Patients with cirrhosis who continue to drink alcohol have lower levels of native taxa and functional microbiota in their colonic and duodenal mucosa and feces [23]. These changes are accompanied by increased secondary bile acid synthesis and enterohepatic bile acid circulation [23]. A surge in secondary bile acids can

undermine the already weakened intestinal barrier, impair cell membrane integrity, and accelerate alcohol-related gut-liver axis damage [24]. Another study that analyzed the gut microbiota's composition and activity in cirrhotic and noncirrhotic patients revealed that cirrhosis patients had more oral-derived microbiota and Lactobacillaceae in their stool than the others [25]. These changes could be explained by the high prevalence of periodontitis, altered salivary microbiota, use of proton pump inhibitors, and low gastric acid in these patients [26, 27]. Earlier studies of gut microbiota in cirrhosis patients have also seen an increase in Lactobacillaceae, which was linked to the therapeutic use of lactulose [28].

In alcoholic hepatitis (AH), typically having already manifest cirrhosis, increased Enterobacteriaceae and Streptococcaceae groups have been associated with disease severity [29]. The same study observed a rise in secondary bile acids with progressing AH. Patients with AH exhibited the lowest relative abundance of commensal Akkermansia muciniphila [30]. An impaired immune response was found in another ALD study that compared circulating microorganisms in patients with AH, cirrhosis without AH, and healthy controls [31]. Fusobacteria abundance was higher in drinkers than in healthy controls, but lower in those with severe AH. Bacteroidetes showed the opposite distribution, with the highest relative abundance in healthy non-drinkers. In contrast, patients with severe AH exhibited the greatest endotoxemia. In summary, these data demonstrate that ALD patients have considerable abnormalities in their gut flora, which may affect their liver disease's natural course.

Gut-Brain Axis in Alcohol-Related Liver Disease

AUD effects on the brain range from acute intoxication to personality, behavioral changes to dementia. Hepatic encephalopathy and nutritional deficits can further deteriorate the brain reserve and function [32]. The gut-brain axis as a whole must be considered when evaluating the gut as a potential pathway by which brain function is altered in patients with AUDs [19]. In individuals with early AUD, a correlation between increased intestinal permeability and symptoms of depression, anxiety, and alcohol seeking has been identified [16]. Even after alcohol withdrawal, patients with high intestinal permeability remained depressed, and anxious. In primary mental illnesses such as depression and schizophrenia, the gut-brain axis can be affected also in the absence of alcohol misuse [33]. Systemic inflammatory mediators, ammonia, and endotoxemia exacerbate neuroinflammation in AUD as a result of dysbiosis. Patients with alcohol-related cirrhosis are more likely to experience persistent cognitive impairment than those with cirrhosis unrelated to alcoholism, which may have an impact on their ability to carry out everyday activities [22]. However, more research is required to better understand the interaction between gut and brain at the molecular level and to discriminate between "unspecific" toxic specifically mediated effects.

ALD Therapies Involving the Microbiome

ALD inpatients with probiotics (Bifidobacterium bifidum and Lactobacillus Plantarum 8PA3) for 5 days increased levels of potentially beneficial bacteria [34]. Along with these findings, liver enzymes alanine aminotransferase, aspartate aminotransferase, and -glutamyl transpeptidase improved. In a study by Han et al., 117 patients with AH from four centers were randomly assigned to a probiotic regimen comprising Bacillus subtilis and Enterococcus faecium or a placebo for 7 days [35]. Serial plating showed that probiotics reduce stool E. Coli, endotoxemia and liver-related enzymes, but not inflammatory cytokines. Fecal microbiota transplantation (FMT) has been explored for several years to restore or change the microbiome [36]. In a study on patients with steroid-resistant AH followed-up for 1 year, FMT patients had a higher overall survival rate (87.5 vs. 33.3%) as compared to non-FMT patients [37]. In another open-label trial, male AH patients were treated with FMT, nutrition, corticosteroids, or pentoxifylline. Compared to other interventions, FMT improved 90-day survival (75 vs. 38% for steroids, 29% for nutrition, and 30% for pentoxifylline) and gut microbiota composition and functionality [38].

Conclusions

In ALD, the development of liver injury is tightly connected to the gut flora. There is increasing evidence that specific alterations in the human gut microbiota may modulate liver disease progression. However, more large-scale, long-term and randomized clinical trials are required to confirm these initial observations.

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Chapter 63
Alcohol Potentiates HIV-Induced Hepatotoxicity Via Induction of Lysosomal Damage in Hepatocytes

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Abstract Life expectancy in people living with HIV (PLWH) has increased due to effective antiretroviral therapy (ART). Consequently, they survive longer, which increases co-morbidities. Liver disease is one of the leading co-morbidities in PLWH representing 18% of non-AIDS mortality. In addition, the incidence of alcohol abuse is twice as high in PLWH as compared to the general population. Generally, ethanol metabolism has been shown to aggravate the HIV-mediated liver damage. It enhances the accumulation of HIV in hepatocytes, which further aggravates oxidative stress and lysosomal damage/dysfunction. When oxidative stress is high, hepatocytes undergo apoptosis with the release of apoptotic bodies (ABs), while low oxidative stress causes exosome release from these cells. Engulfment of either ABs or exosomes by liver macrophages induces liver inflammation, and internalization of these extracellular vesicles (EVs) by hepatic stellate cells (HSC) promotes fibrosis development. The proposed anti-fibrotic therapy in PLWH with alcohol-use disorders (AUD) includes antioxidants, nanoparticles to block activation of pro-fibrotic pathways in HSC, and Obeticholic Acid (OCA).

Keywords Hepatocytes · HIV · Ethanol · Acetaldehyde · Lysosomal damage · Apoptotic bodies · Exosomes · Hepatic stellate cells · Macrophages

Introduction

A special interest in HIV-associated liver damage is supported by the fact that 40 million people are living with HIV globally, with 1.2 million of them in the US. While life expectancy has increased due to effective antiretroviral therapy (ART), people living with HIV (PLWH) survive longer, which has increased the risk of co-morbidities. Liver disease is one of the leading co-morbidities representing 18% of non-AIDS mortality [1, 2]. In fact, the liver plays a major role in the clearance of circulating viral particles from the blood, as was shown for Simian Immunodeficiency Virus (SIV) [3]. Liver transaminases are frequently elevated in the sera of HIV-infected patients even in the absence of accompanying viral hepatitis [4]. HIV-1 infects hepatocytes, but this infection is either low or latent [5, 6]. There is an association between HIV RNA content and liver fibrosis [6]. Hepatocytes are found in the proximity of actively replicating HIV in Kupffer cells (KC), sinusoidal endothelial cells (LSEC), stellate cells (HSC), and $CD4^+$ T cells. While hepatocytes seem non-permissive and are not harmed by HIV mono-infection, their exposure to the virus triggered by HCV co-infection or other second hits may lead to increased cell death [4, 7, 8]. The major turnover of damaged hepatocytes is mediated by liver macrophages, which regulate hepatocyte damage and fibrotic replacement. Modern ART efficiently blocks active viral replication. Still, it does not eliminate latently infected cells with HIV DNA integrated into the host genome, thereby improving disease outcomes without halting disease progression [9] with increased age-dependent comorbidities, including liver diseases [10].

Why HIV-infection has such an impact on liver cells? Hepatocytes are generally exposed to high levels of HIV because the liver is scavenger of activated immune cells that replicate the virus. Highest levels of HIV are found in gut-associated lymphoid tissue, and in contrast to peripheral blood mononuclear cells, HIV persists in this tissue despite of ART [11] [12]. HIV is typically brought to liver with portal blood, infecting Kupffer cells [13] and accessing hepatocytes. Although long-term ART is claimed as a major reason for hepatotoxicity [14], hepatotoxic effects maybe observed even in the absence of ART to be attributed to damaging effects of HIV by itself, especially when HIV-infection is potentiated by second hits, like alcohol.

HIV-Infection in Hepatocytes: Effects of Ethanol Metabolism

The above reasons prompted us to study the combined effects of HIV and ethanol metabolites on the survival of HIV-infected hepatocytes. For this purpose, we used ethanol-metabolizing primary human hepatocytes (PHH) and Huh7.5-CYP2E1 (designated as RLW) cells, which efficiently oxidize ethanol by overexpressed CYP2E1 but lack of alcohol dehydrogenase (ADH), a major generator of acetaldehyde. PHH were exposed to 50 mM ethanol and RLW cells- to the acetaldehyde-generating system (AGS), which contains ethanol as a substrate, yeast ADH as a source of the enzyme, and NAD^+ as a co-factor. The aforementioned system mimics Ethanol metabolism in PHH and was successfully used in prior studies [15–17]. While hepatocytes were usually not considered HIV-permissive cells, surprisingly, we found that after pre-exposure of PHH to 50 mM ethanol for 24 h followed by HIV infection for 3 days, they express more HIV gag RNA and HIV gag protein, p24 than the cells infected without ethanol pre-exposure [18]. These effects were seen when HIV- and ethanol-induced hepatocyte death was prevented by co-treatment with pan-caspase inhibitor (PCI). In fact, under the specified conditions, cells undergo rapid apoptosis, as became evident from M30 and caspase 3 measurements. To clarify whether HIV gag RNA detected in hepatocytes is inside of cells or is just attached to cell membrane from outside, after pre-treatment of RLW cells with AGS followed by exposure to HIV, the membrane-associated binders were removed by low acid stripping [19]. Then HIV gag RNA was repeatedly measured by RT-PCR. However, no difference in the levels of HIVgag RNA expression was observed between these two protocols indicating that HIVgagRNA is not cell membrane-associated [18]. We next studied the HIV entry receptors on hepatocytes because these cells do not express canonic CD4 receptors. We assumed that HIV_{ADA} enters via CCR5 receptor (HIV-co-receptor). To prove it, we blocked CCR5 receptor on infected either with HIV alone or HIV combined with AGS liver cells by Maraviroc, a specific CCR5 receptor blocker. While AGS potently increased HIVgag RNA expression, this does not happen in the presence of Maraviroc, indicating that CCR5 may serve as a receptor for viral entry to hepatocytes. The blocking effects of Maraviroc have also been shown by others for HIV infection in hepatocytes with no ethanol metabolite exposure [6].

The entry via CCR5 receptor requires the trafficking of HIV via an endosomal compartment with low pH, to which HIV is highly sensitive [20]. Moreover, ethanol has been shown to increase the pH of the endosomal-lysosomal compartment [21], thereby allowing HIV to survive. This becomes evident from lysoSensor experiments, in which the combination of AGS and HIV changed pH in RLW cells to neutral, which became visible due to appearance of a blue staining in hepatocytes [22]. Thus, it is important to understand if an increase in HIVgag RNA and HIV proteins under ethanol treatment in hepatocytes is due to increased viral replication or due to the accumulation of these HIV particles in hepatocytes. To address this, we measured the effects of ethanol/AGS on HIV RNA, HIV DNA and integrated HIV DNA. These measurements were performed in hepatocytes protected from HIV-ethanol-triggered cell death by exposure to pan-caspase inhibitor as well as in the absence of this inhibitor. While exposure to pan-caspase inhibitor just increased HIV RNA expression in HIV-infected hepatocytes treated with ethanol, we were unable to quantify HIV DNA and integrated HIV DNA in these cells in the absence of pan-caspase inhibitor, which suggests that reverse transcription from HIV RNA to HIV DNA and HIV DNA integration are associated with hepatocyte apoptosis induction. In the literature, there are several very controversial reports on HIV DNA integration to hepatocyte genome in the absence of AGS: although some authors demonstrated HIV DNA integration and low levels of productive infection in hepatocytes, others do not support it [6, 23, 24]. We indeed observed that caspase 3 cleavage, as well as cleaved cytokeratin 18, were enhanced by co-exposure of PHH/RLW cells to Ethanol/r AGS and HIV in a MOI-increasing fashion, and this apoptosis was attenuated by azidothymidine (AZT), a reverse transcriptase inhibitor. This also indicates that the reverse transcription from HIV RNA to HIV DNA initiates the mechanism of cell death [18, 25]. Thus, we deal with abortive replication. Since the full HIV replication cycle in hepatocytes does not take place due to rapid apoptosis.

The kinetic studies on HIVgag RNA, p24 and cleaved caspase 3 in HIV-infected RLW cells either exposed or not to AGS indicated that AGS treatment stabilized HIV gag RNA and protein in hepatocytes, which, in turn, induced oxidative stress and consequent apoptosis [18, 25, 26].

Stabilization of HIV Proteins in Hepatocytes Exposed to Ethanol Metabolites Due to Lysosomal Dysfunction

We observed the accumulation of HIV proteins (namely, p24) in RLW cells exposed to lysosomal inhibitor, bafilomycin as well as to the combination of HIV and AGS [18]. This means that suppression of lysosome function by HIV infection and AGS treatment prevents HIV protein degradation by lysosome leading to protein

stabilization in the cells, which might induce oxidative stress. In fact, cell exposure to AGS decreased cathepsins B and L (lysosomal enzymes) activities in HIV-infected hepatocytes [27]. The same treatments caused co-localization of lysosomal protein, LAMP1 with Gal3, thereby indicating lysosomal leakage. As known, the leakage of cathepsins and their co-localization to organelles damage these organelles and induce apoptosis [28]. In HIV-infected hepatocytes, leaked cathepsin B co-localized with mitochondrial outer membrane protein, TOM 20, and this becomes even more visible after exposure of RLW cells to AGS [27]. Furthermore, caspase 3 cleavage in HIV^+ AGS-treated RLW cells was reversed in the presence of caspase 9 inhibitor, suggesting that co-localization of cathepsin B with mitochondria may, in turn, induce mitochondrial leakage with the release of cytochrome C to activate intrinsic apoptosis. Another reason for apoptosis induction is related to enhanced expression of the lysosomal protein, DRAM1, which is co-localized with BAX and has p53 as its downstream target [29, 30]. This overexpression is observed when HIV-infected hepatocytes are treated with AGS [27]. Induction of apoptosis, as well as the reduction in cathepsin activities in HIV-infected hepatocytes exposed to ethanol metabolites can be reversed by N-acetyl cysteine (NAC) and thus, is related to oxidative stress. While the pathogenic importance of lysosomal leakage has already been shown for steatohepatitis of both alcoholic and non-alcoholic origins [31–33], previously, this has never been implemented into mitochondrion-dependent pathologic mechanism of HIV-ethanol metabolism -triggered hepatotoxicity.

Damaged lysosomes can be easily removed and replaced if lysosomal biogenesis is not impaired. However, it has been shown that the activation of lysosomal genes is significantly reduced in RLW cells infected with HIV and exposed to AGS [27]. The reason for that is an impaired translocation of a master regulator of lysosomal biogenesis, transcription factor EB (TFEB), from the cytosol to the nucleus to activate lysosomal genes. It appears that, in HIV-infected hepatocytes, acetaldehyde induces acetylation of tubulin, thereby contributing to impaired TFEB trafficking via microtubules [27]. This mechanism is not unique for TFEB and has been demonstrated for trafficking of other transduction factors in alcohol-exposed hepatocytes [34, 35]. In addition, our study [27] revealed that in HIV^+ hepatocytes, AGS increases the levels of ZKSCAN3, a repressor of lysosomal gene transcription, which prevents activation of lysosomal biogenesis. These *in vitro* findings were supported by *in vivo* studies on liver-humanized mice injected with HIV and pair-fed either control or ethanol liquid diets. In fact, ethanol suppresses LAMP1 expression and cathepsin activities in these mice, which was accompanied by activation of oxidative stress as evident from the increased TBARS (malondialdehyde) activity in liver tissue. The findings summarized here are presented on Fig. 63.1. More details have been described in [27]. In addition, we could not exclude that HIV + ethanol induced oxidative stress damages lysosomes and cell death not only in the liver but in other organs, such as the pancreas, thereby promoting metabolic changes and dysregulation [36].

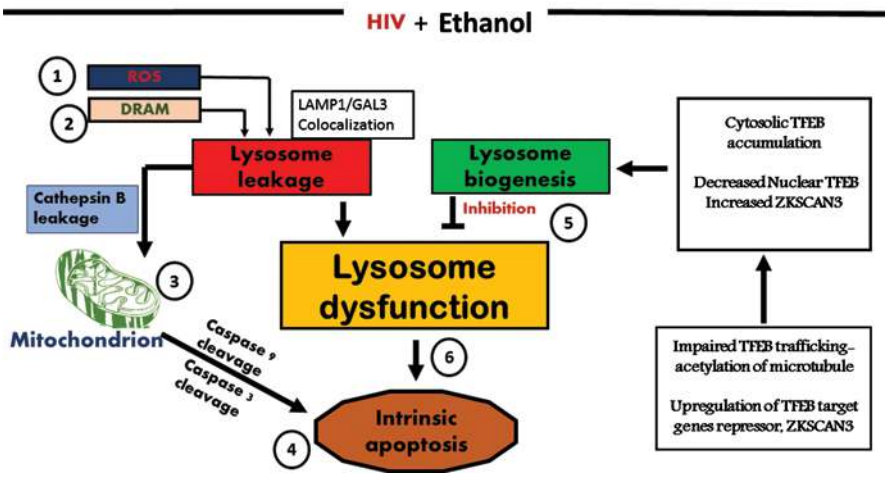

Fig. 63.1 Role of lysosomal rupture/dysfunction in HIV-and ethanol metabolism -induced apoptosis (1) Combined treatment with HIV and AGS triggers ROS and acetaldehyde release, which mediates lysosome leakage; (2) HIV/ethanol metabolism triggers the release of DRAM1, which also induces lysosome leakage; (3) Cathepsin B leaked out from damaged lysosome and diffused into the mitochondrion to initiate the intrinsic apoptotic pathway; (4) Caspases 9 and 3 become cleaved, leading to hepatocyte apoptosis; (5) Alcohol metabolites inhibit lysosome biogenesis factor TFEB, hence impairing the compensation of damaged lysosomes; (6) Both lysosome damage and impaired lysosome biogenesis lead to HIV–ethanol-metabolism-induced lysosome dysfunction, which triggers apoptosis

Exposure of HIV-Infected Hepatocytes to Ethanol Promotes the Release of Extracellular Vesicles (EVs)

Extracellular vesicles (EVs) are small membrane-bound vesicles, which contain proteins, lipids, and nuclear acids as a part of their cargo. These vesicles are subdivided into three groups: exosomes, micro-vesicles, and apoptotic bodies. Inside cell cytoplasm, the intraluminal vesicles are formed by components of the endosomal-sorting-complex-required-for-transport (ESCRT) machinery, lipids, and tetraspanins (e.g., CD63, CD81, etc.). When the multivesicular bodies (MVB) dock and fuse with the plasma membrane, the intraluminal vesicles are released as exosomes [37]. The mechanisms by which MVB sort either to the plasma membrane or to lysosome are unclear. It is possible that the suppression of lysosome function, which is observed in HIV-infected hepatocytes exposed to ethanol metabolites, may cause not only cell death [27], but also promotes exosome release from activated alive cells [22]. In fact, the outcome depends on the levels of oxidative stress induced by both hits, HIV and ethanol: if oxidative stress is low and cells can manage it, they try to compensate for it by releasing exosomes carrying excessively expressed harmful proteins not degraded properly by lysosome; however, if oxidative stress is high, this can lead to apoptotic cell death and release of apoptotic bodies (AB) [38].

Indeed, in pro-apoptotic conditions in the absence of pan-caspase inhibitor, where hepatocytes were under prolongated exposure to HIV and AGS, exosome release was diminished [22].

Many sαtudies are focused on the roles of liver-derived EVs in the induction of inflammation and fibrosis because of cell-to-cell EV-mediated crosstalk between parenchymal and non-parenchymal liver cells in the settings of exposure to ethanol [38–41]. MicroRNAs as a part of EV cargo can also be used as the biomarkers of Alcohol-Associated Liver Disease (ALD) [42]. However, so far, the information on liver EVs and their cargo released from cells exposed to HIV and alcohol is very limited. In our hands, AB formation may contribute to liver fibrosis progression in alcohol-abusing PLWH, and the characterization of the cargo of AB derived from HIV-and AGS-exposed hepatocytes demonstrated an increased content of HIVgag RNA, p24, Nef, and TAT proteins as well as oxidative modification of proteins with malondialdehyde (MDA) [43]. Furthermore, internalization of these AB by HSC suppresses innate immunity (activation of Interferon-stimulated anti-viral genes by IFNα), but triggers IL-6-induced activation of JAK-STAT3 pathway, which is survival for HSC [43]. As shown, AB engulfed by macrophages induce inflammasome activation, while engulfment by HSC causes pro-fibrotic activation [18]. Interestingly, a similar situation was observed in HCV infection [44], indicating that from this standpoint, there is no difference in disease outcomes when AB internalized by non-parenchymal cells were generated from hepatocytes either replicating or just accumulating the viruses. The pro-fibrotic effects of AB were hepatocyte-specific because we observed an activation of pro-fibrotic markers in HSC when AB were prepared from HIV-infected hepatocytes, but not HIV-infected immune cells [18]. Currently, we cannot explain why engulfment of only hepatocyte AB promotes pro-fibrotic activation in HSC; however, we anticipate that HIV proteins combined with so far non-identified hepatocyte-specific markers may serve as a driving force of this process. In non-HIV/alcohol studies, AB were also claimed as the trigger or fibrosis development [45], but HIV component in these AB significantly enhanced pro-fibrotic HSC activation [18]. Importantly, along with large EVs (AB), exosomes derived from HIV-infected hepatocytes exposed to ethanol metabolites also up-regulate pro-fibrotic activation of HSC [46].

Those proteins, which are not degraded by lysosomes, may be secreted with exosomes. Partially, it is related to posttranslational modification of subjected to degradation HIV proteins named ISGylation, which is interferon (IFN)-inducible [47]. ISGylated HIV proteins are prone to lysosomal cleavage, but ethanol by suppressing IFN type 1 signaling, interferes with protein ISGylation, thereby promoting their stabilization and release with exosomes [48]. It has also been shown that lysosome inhibition with various chemicals, such as bafilomycin A1 increases EV secretion [49]. In fact, we observed exosome secretion in HIV- and ethanol-exposed hepatocytes, which was corroborated by bafilomycin treatment [22]. Here, in primary human hepatocytes (PHH), EV secretion was induced by each ethanol and HIV; however, the combined treatment provided the strongest effect. These *in vitro* results were supported by *in vivo* data. To this end, using pan-exosome isolation kit, EVs from plasma of FRG-KO liver-humanized mice were quantified by

Nano-tracking analysis (NTA), and the highest levels of hepatocyte-specific EVs were in mice fed ethanol diet and injected with HIV (HIV injection has been used since human hepatocytes only abortively replicate HIV) [22]. In these studies, oxidative stress served as EVs trigger since exosome secretion was attenuated by NAC co-treatment. Also, there was a direct association between increasing lipid peroxidation marker, TBARS (MDA) in liver tissue and liver EVs release. Furthermore, the results of next generation sequencing (NGS) from the same study predicted that hepatocytes exposed to HIV and ethanol activated stress-regulated genes and provided lysosome dysfunction in HIV and ethanol-treated PHH, potentially increasing exosome release from these cells [22]. In addition, in-silico data revealed that PHH exposed to ethanol-HIV enhanced upstream regulators targeting genes associated with oxidative stress, lysosomal dysfunction and exosome secretion. Specifically, we observed the increased expression of genes associated with alcoholic hepatitis (CRP, ICAM1, FMO5, CD109, CHI3L1, ERRFI1, HAO2, MT1M, KLF9, RHOB, PER1, TSC22D3) and HIV-1 gene (gag, pol, vpr, nef, and env). PHH-EVs were characterized by Transmission Electron Microscopy, NTA (NanoSight), and western blot, confirming their cup shape, size around 100 nm consistent with exosomes, and the enrichment of ALIX and TSG101 throughout the tested samples, respectively. In addition, the expressions of hsa-miR16–2; hsa-miR-27a, hsa-miR-501 and hsa-miR-99A miRNAs were significantly altered in PHH-EVs in ethanol and HIV group from their controls. The network analysis revealed the targets of miRNAs which were associated with of HIV1 (ATP5G3, ABCC4, SLCO5A1, AFG3L2, OTUD3, SFXN4 and ERP27) and liver disease (PTER, PAMR, MAPKAPK3 and PEX5L) progression [50].

Circulating macrophages infiltrate liver during organ injury and replenish the resident macrophage population, Kupffer cells (KC) [51]. As shown, ethanol increases HIV-1 replication in macrophages by causing DNA damage, CYP2E1 elevation and decreased expression of antioxidants [52]. The polarization into M1 cells results in a decreased expression of HIV DNA. Thus, while M1 phenotype marker, TNFα is an activator of HIV replication, some studies indicated its protective role in HIV infection by stimulating the production of RANTES and decreasing CCR5 expression in macrophages [53]. A recent study demonstrated that miR-99a expression is negatively correlated with inflammation by targeting TNFα, showing that overexpression of miR-99a prevented M1 phenotype activation and promoted a phenotype switching to M2 [54]. Alcohol-HIV treatment of PHH caused upregulation of four EV-miRNAs (miR-99a, miR-16, miR-122 and miR-17HG) and downregulated ten miRNAs [38]. KEGG pathway analysis demonstrated that metabolic pathways, such as the MAPK signaling pathway, ubiquitin-mediated proteolysis, the insulin signaling pathway etc., were significantly upregulated by miRNAs in hepatocytes from the HIV^+ alcohol group compared to either alcohol or HIV-only groups. Mapping of miRNA target genes on KEGG disease database identified pathways associated with liver disease i.e., alcohol-related disorders, insulin resistance, fatty liver, fibrosis, and inflammation in HIV^+ ethanol and HIV- treated

groups. The internalization of hepatocyte-exosome by macrophages induced anti-inflammatory TGFß and ARG1, as well as downregulated expression of pro-inflammatory IL-1ß, IL-6, and TNFα markers in the latter cells.

Treatment Approaches for Prevention of HIV-Ethanol-Induced Liver Fibrosis Development

Treatment of alcohol abusing PLWH with modern and less hepatotoxic ART will be important to limit the supply of HIV coming to the liver and hepatocytes. However, HIV does not replicate in hepatocytes, and in combination with ethanol, HIV proteins are stabilized in hepatocytes to induce oxidative stress. While intensive apoptosis is initiated by exposure of hepatocytes to HIV and Ethanol metabolites, it will not be beneficial to treat alcohol abusing PLWH with anti-apoptotic drugs because the prevention of apoptosis in HIV-infected hepatocytes may potentially induce HIV DNA integration to the hepatocyte genome. In fact, the prevention of HIV and AGS- induced apoptotic hepatocyte death by exposure to pan-caspase inhibitor caused accumulation of cells with integrated HIV DNA [18]. Thus, a major treatment strategy should be directed to prevent oxidative stress. In prior studies, when HIV-Ethanol-exposed hepatocytes were treated with antioxidants, such as NAC, we observed the reduction in lysosomal damage and restoration of cathepsin activities, which are supposed to reverse stabilization of HIV proteins in liver cells, thereby suppressing intrinsic apoptosis in hepatocytes [27]. Usually, the bioavailability of NAC in vivo is limited, but many other potent antioxidants can be used in a complex with ART therapy in alcohol abusing PLWH. One of the optional antioxidants shown efficiency in alcohol-induced liver injury is a pro-methylating agent, betaine [55].

The major strategy for prevention of liver fibrosis development in PLWH with alcohol use disorders (AUD) is to block the activation of HSC, the regulators of fibrosis progression. We found that Obeticholic Acid (OCA) reduces HIV markers expression in hepatocytes and thus, attenuates HIV-AGS-induced hepatocyte death, thereby, reversing pro-fibrotic activation in HSC (based on Col1A1 and TGFβ mRNA levels) [56]. The treatment with OCA also restores proteasome and lysosome functions by scavenging ROS and suppressing oxidative stress in hepatocytes [56]. Many other approaches to block HSC pro-fibrotic changes by nanoparticle delivery [57] are under investigation for PLWH with AUD.

In conclusion, ethanol metabolism significantly potentiates toxic events in HIV-exposed hepatocytes. It promotes liver inflammation/fibrosis progression due to hepatic parenchymal-non-parenchymal (macrophages, HSC) cell communications via HIV protein-containing EVs, ABs and exosomes. Schematically, it is illustrated by Fig. 63.2.

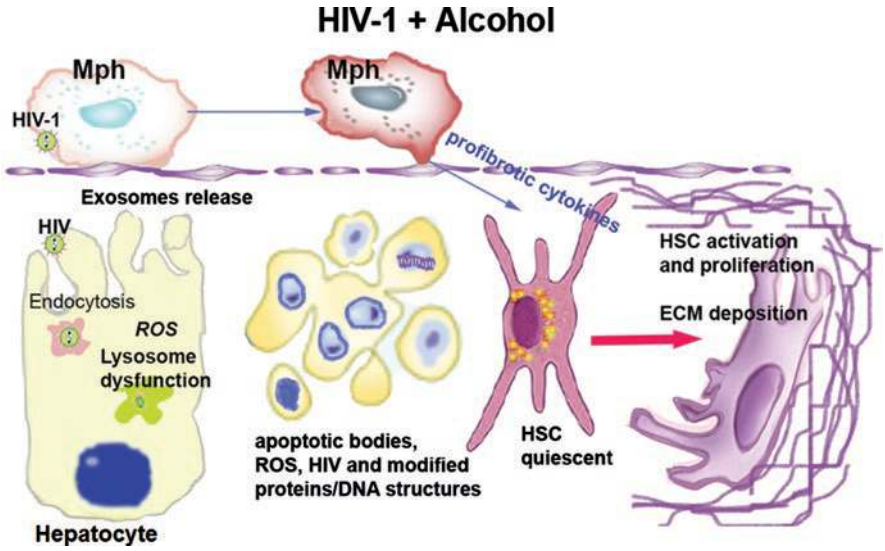

Fig. 63.2 Scheme of EV-mediated crosstalk between parenchymal and non-parenchymal liver cells in the settings of HIV and alcohol exposure

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Part X
Severe Alcoholic Hepatitis

Chapter 64
Pathophysiology of Alcoholic Hepatitis: Emerging Role of Enhanced Red Blood Cell Turnover

Sebastian Mueller

Abstract This chapter introduces to the book section on alcoholic hepatitis (AH) and the emerging role of hemolytic anemia and enhanced red blood cell (RBC) turnover. AH is a rare but prognostically one of the most severe complications in heavy drinkers, with a 90 day mortality almost reaching 50%. Present diagnostic and therapeutic options are very limited and the molecular mechanisms are poorly understood. Only a minor fraction of patients benefits from steroids with regard to short-term but not long-term mortality. Based on novel prospective mortality data in heavy drinkers, ineffective erythropoiesis and macrocytosis anemia are identified as important, so far unrecognized confounder of liver damage and mortality. Preliminary data also indicate that these mechanisms contribute to AH. Of note, alcohol detoxification initially deteriorates blood parameters suggesting that additional, non-alcohol related factors also contribute to RBC changes. The novel insights link AH and liver damage to the blood and bone marrow compartment. It is hope that these first data will stimulate the search for novel targeted therapies. It will especially be interesting to further dissect the close interaction of RBC turnover and liver and the role of iron in various forms of hepatocyte cell death such as apoptosis, pyroptosis and ferroptosis.

Keyword Alcoholic hepatitis · Alcoholic steatohepatitis · Alcoholic liver cirrhosis · DAMPs · PAMPs · RBC · Hemolysis · CD163 · Haptoglobin · Hemopexin · Macrophage · Mortality

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Introduction to Severe Alcoholic Hepatitis and Current Challenges

Alcoholic hepatitis (AH) sometimes also described as severe alcoholic hepatitis (sAH) is considered one of the prognostically most severe complications in heavy drinkers, with a 90 day mortality almost reaching 50%. Figure 64.1 shows an overall scheme of the natural cause of ALD, its complications such as AH, cirrhosis, HCC but also highlights the role of non-ALD related-death in heavy drinkers. Despite continued discussions, AH originates in this figure solely from cirrhosis, according to the evidence provided below. As mentioned in part I of the book, ca 200 pathological entities are associated with alcohol consumption. Although it seems that the liver is the major target organ of alcohol, important other organ systems include the heart, bone marrow, immune system, red blood cells and brain. It should be appreciated more that these organ systems interact not only in the stage of liver cirrhosis, but much earlier.

Although AH is rather rare among drinkers (ca. 2%), the natural course is often tragic as patients are not really addicted to alcohol, and they typically have even stopped drinking for several days or weeks prior to admission to the hospital. However, they eventually face rather limited therapeutic options and, due to organ shortage and societal considerations, will not have access to liver transplantation in the following 6 months.

For these reasons, an especial scientific interest has been generated for the last five decades in order to better understand the underlying pathology and to develop targeted therapies. Unfortunately, with a good portion of honest self-reflection, we must admit that improvements have been very limited. Moreover, confusion and debates continue even among experts about definition, diagnostic measures and

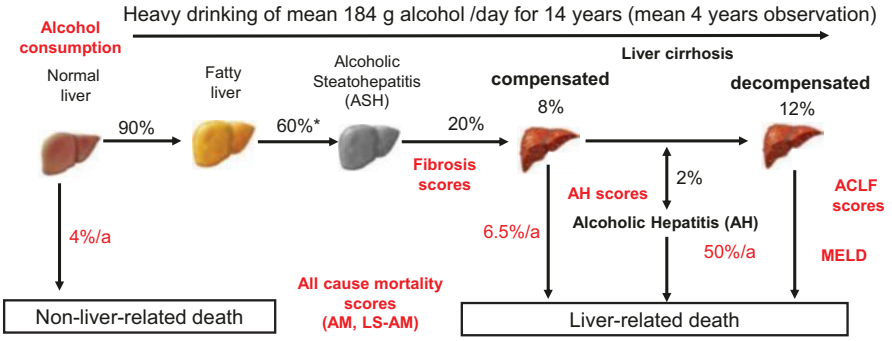

Fig. 64.1 Progression of liver diseases from the Heidelberg cohort of heavy drinkers (n = 1078). Score for several endpoints are given in red. Note that AH and decompensated liver cirrhosis show similar per annum mortality. Percentage numbers are related to 100% of initial drinking cohort. See also Tables B.1 and B.2 and Chap. 7 on mortality in Part I. Signs of inflammation* are seen in 78% in the liver biopsy cohort, and in 42–62% using biochemical markers in necrosis/inflammation/damage

therapeutic algorithms. Discussions also continue about the strict discrimination of AH from "conventional histological alcoholic steatohepatitis" which affects e.g. almost 80% in the Heidelberg cohort of heavy drinkers (see also Table B.9). In fact, in liver biopsy specimen, the pathologist cannot discriminate between simple alcohol-related liver disease, alcoholic hepatitis (AH), acute on chronic liver failure (ACLF) or non-alcohol-related liver disease (NAFLD).

In such a situation, it may be advisable to rather stop for a minute to ask what potential and fundamental confounders have been missed or overlooked? For this reason, this book contains one part just devoted to alcoholic hepatitis and closely related topics such as ACLF in order to cross-read and collect the state of the art to renew and inspire discussions for future directions. Topics of the following chapters include diagnosis, therapy, relation to ACLF and role of biopsy. This introductory chapter is, in addition to several comments in the general chapter on the pathophysiology of ALD, a brief discussion of potential missing links and introduces the novel and emerging role of the red blood cell (RBC) turnover in heavy drinkers with all its consequences.

What Are Major Unresolved Problems in Understanding AH?

1. Despite many randomized studies and metanalyses, only **steroids** remain an option for a small fraction of patients with AH, having only a very limited, short-term benefit. Thus, the last larger trial (STOPAH) only found a benefit for 28-day mortality for steroids (14% vs 17% mortality as compared to placebo) but no differences after 90 days or 1 year [1].
2. Despite numerous original articles and reviews on the role of intestinal translocation of bacteria, the role of pathogen-associated molecular patterns (PAMPs) and their role in liver inflammation, it remains difficult to comprehend **why then antibiotics are not effective in AH**. With a sobered view, it can only mean that bacteria are just playing a bystander role and increasingly contribute to systemic inflammation and organ failure in the final stages. This is also somehow reflected in the definition and terminology of ACLF that highlights the important role of these features in all endstage liver diseases (see also Chap. 67). There is no controversy that in these clinical stages, bacteria find ideal conditions for growth.
3. On the other side, it is a typical clinical finding, that both AH patients but also patients with decompensated liver cirrhosis with ongoing and proven viral or bacterial infections typically do not show pronounced clinical signs of infection such as fever or parameters of inflammation. While this can be explained by an "exhaustion" of the immune system, additional, potentially **more relevant mechanisms specific to alcohol and AH may have been overlooked**.
4. As mentioned above, the **striking similarity in liver histology between AH, ACLF, ALD and NAFLD** is unsatisfying and it is strongly asking for a novel rational to plausibly explain why all these clinically different entities result in the same histological picture.

5. Although normally not directly related to AH, there are many laboratory features of ALD patients that have been quietly and generally accepted. Consequently, they are not discussed anymore although still poorly understood. This includes the typical "liver parameters" such as levels of AST, ALT but also AP, GGT and even bilirubin. The latter is often seen as sign of a liver damage while it is less frequently appreciated that bilirubin is primarily derived from heme degradation. As is discussed in the Chap. 41 on AST, **AST seems to be mainly derived from RBCs** and not hepatocytes. It also remains unclear why a typical ALD-associated parameter such as GGT is only elevated in some patients and the role of AP is also poorly understood. Moreover, although known for many decades, the reason why heavy drinkers show an **elevated RBC size (MCV)** has been not really addressed so far.

Present Understanding of AH

Precent concepts to understand AH within ALD are nicely described in Chap. 67 by R. Jalan, but also some other chapters on ALD in part IX in this book. Accordingly, a systemic inflammatory state is regarded as important driver for AH [2, 3]. Proteases, oxidative molecules, cytotoxic cytokines, prostaglandins, and leukotrienes, among other mediators, are consequently released by activated immune cells, leading to further worsening of the tissue damage [4]. Two main components have been observed to drive this intense systemic inflammation in ACLF, PAMPs and damage-associated molecular patterns (DAMPs) [3] (see also Chaps. 61 and 62). PAMPs represent molecular structures that are expressed by different pathogens and microbial agents. In contrast, DAMPs are circulating intracellular molecules following death or damage of the host cells, albeit without infection as a triggering agent. PAMPs and DAMPs bind to specific Pattern Recognition Receptors (PRRs) such as Toll like Receptors (TLR) located on peripheral innate immune cells [5]. Receptor binding activates down-stream signaling pathways that ultimately lead to the increased transcription and release of inflammatory cytokines with induction of severe systemic inflammation [6].

Emerging Role of RBC Turnover in ALD

One plausible explanation for the many frustrating attempts to shed more light into the pathophysiology of AH is very likely due to the endstage situation. There is good agreement among experts that AH requires several years to develop during heavy drinking. The sometimes emotionally discussed questions whether AH only develops in cirrhotics may distract from the fact that AH requires at least a fibrosis stage higher than F2. In fact, in our Heidelberg cohort of heavy drinkers, we have never seen any AH in patients with normal liver stiffness.

One major recent inspiration has come from the analysis of the **first 15-year lasting prospective mortality study in heavy drinkers** (see Chap. 7). Importantly, the study design tried to be as unbiased as possible recruiting patients that presented to the hospital for alcohol detoxification and not patients with organ-specific clinical symptoms such as jaundice.

Besides routine laboratory parameters and abdominal ultrasound, liver stiffness was measured initially in all patients. The study newly **identifies hemolytic anemia as important driver of all-cause mortality** while also ruling out typically associated confounders such as deficiency of vitamin B12 and folic acid. As is discussed in more detail in the Chaps. 57 and 58 on iron and bone marrow changes in drinkers, alcohol causes an environment of enhanced RBC degradation but also, in a compensatory manner, enhanced erythropoiesis. While this is compensated for many years it eventually leads to anemia in a significant portion of heavy drinkers and at least in half of them to 20% reduction of the hemoglobin mass. The degradation is sometimes not directly obvious as LDH, haptoglobin or indirect bilirubin are still in the normal range.

Is Heme Degradation Causing Transformation to an Erythrophagocytosis Macrophage M2?

However, when specifically studying markers of heme-turnover or erythrophagocytosis such as CD163, RBC turnover starts from the very beginning of drinking in more than 50% of drinkers (see also Chap. 37). On the other side, once cirrhosis has been established, hemolysis increases further. It will eventually lead to so-called ineffective erythropoiesis that further aggravates hemolysis through release of bilirubin and/or bile acid accumulation. It should also be noted that the mechanisms of RBC turnover are quite diverse ranging from simple RBC erythrophagocytosis triggered by membrane oxidation/apoptotic changes up to complete hemolysis with release of hemoglobin or even free heme, a known and important DAMP [7]. Figure 64.2 provides an overview of some of the **important RBC recycling pathways**. It should be also mentioned that RBC degradation can activate TLR4 through toxic heme but is normally and rather safely recycled by direct receptor mediated erythrophagocytosis (CD163 and CD91). This process is still not completely understood nor the fact that also hepatocytes are able to uptake intact RBCs, a process termed **efferocytosis**. Some more details are discussed and presented in Chap. 57 on iron and ALD. Even in the case of uncontrolled RBC lysis (hemolysis), liver-produced scavenging proteins such as haptoglobin or hemopexin are rapidly secreted to bind either hemoglobin or heme and to internalize and further degrade them through the receptors CD163 and CD91.

As is further discussed in Chap. 58 on bone marrow and alcohol, RBC turnover is not only caused by degradation. Alcohol seems to directly effect erythropoiesis in the bone marrow causing most likely imperfect production of fragile RBCs. Thus,

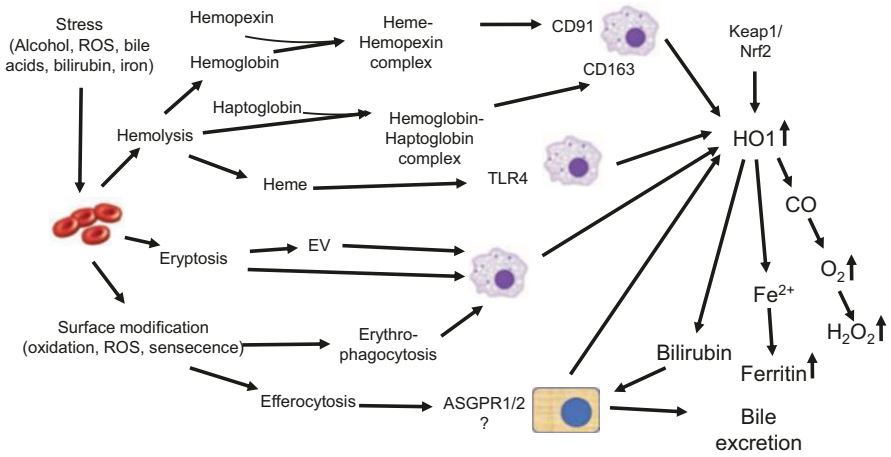

Fig. 64.2 Pathways of red blood cell turnover triggered by alcohol or related conditions. *EV* extracellular vesicle

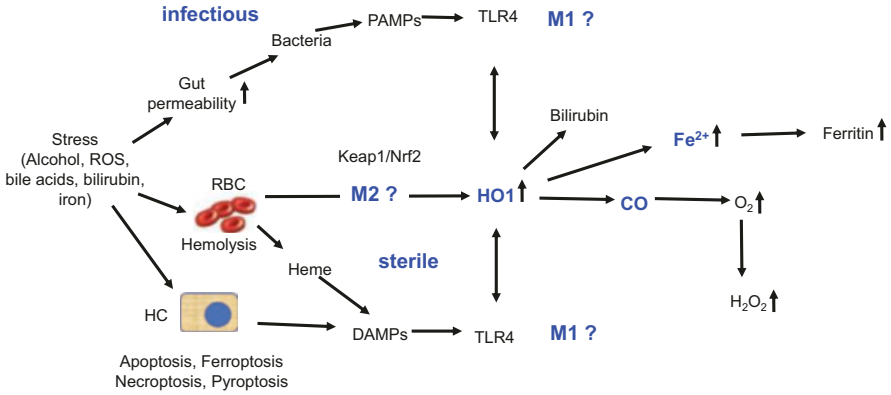

Fig. 64.3 Difference between alcohol-triggered RBC-turnover and TLR4 activation by DAMPs, PAMPs and bacteria. About 40 mL whole blood are daily recycled by macrophages and the liver, far exceeding the load of bacteria. Moreover, this physiological process has unique properties including the release of iron and carbon monoxide (CO). It remains to be studied whether heme turnover allows a better discrimination between the long established M1 and M2 macrophage state

elevated MCV is a result of ineffective erythropoiesis and hemolysis. More studies are needed to explore the molecular effects of alcohol on the hematopoietic stem cell niche. Importantly, abstaining from alcohol normalizes RBC count and MCV, but normalization requires longer than e.g. the normalization of liver parameters (see also Chap. 57 on iron and ALD).

Finally, Fig. 64.3 shows the difference between alcohol-triggered RBC-turnover and TLR4 activation by DAMPs, PAMPs and bacteria. About 40 mL whole blood are daily recycled by macrophages and the liver, far exceeding the load of bacteria. Moreover, this physiological process has unique properties including the release of iron and carbon monoxide (CO) which has important effects on macrophage and hepatocytes metabolism. As is highlighted in Fig. A.76, carbon monoxide is an efficient heme binding molecule that may interact with important heme enzymes such as CYP p450 system, catalase, mitochondrial cytochromes of the respiratory chain. This all should result in an increase of cellular oxygen levels that can now serve other enzymes such as oxidases (NOX1, NOX4, DUOX) allowing for fascinating but complex molecular interactions at the cellular, sub-cellular and interorgan level. In the case of peroxisomal catalase, carbon monoxide should directly lead to peroxide elevation. It remains to be studied whether heme turnover allows a better discrimination between the long established M1 and M2 macrophage state. More specific and mechanistic studies are urgently needed.

Interaction of Heme Degradation, Release of Iron and Hypoxia Signaling

The release of iron is also noteworthy since it will interfere with the hypoxia signaling pathways including HIF1 alpha. HIF1 is targeted for degradation by prolyl hydroxylases (namely PHD2) that contain soluble iron in their reactive center. Iron elevation will increase PDH2 activity (see also Figs. A.70, A.71, A.72), downregulate HIF1 and eventually stop many energy and metabolic pathways. It is often overlooked that the HIF1-PHD2 loop always results in HIF1 degradation and rather loop disrupting signals are important to control HIF1 than oxygen itself [8] (see also Fig. A.72). Alpha-Ketoglutarate is another important substrate of PHD2 linking it to the Krebs cycle and, hence, to alcohol metabolism or transaminase reactions (see Figs. A.43, A.44, A.48, A.70, A.71, A.72).

In conclusion, based on recent prospective mortality data, enhanced RBC turnover is a so far underrecognized important feature in heavy drinkers that drives liver damage and mortality. Consideration of RBCs and hemolysis is able to explain several key features in patients with ALD, including elevated AST levels (see chapter on AST), typical iron changes in patients with ALD, and, finally, development of macrocytic anemia with elevated MCV. More studies are required to better understand the interplay between RBC recycling and liver damage. Preliminary data suggest that hepatocyte damage is closely related to enhanced heme turnover, and hepatocyte death resembles a recently introduced type of death called ferroptosis [9–12].

Table 64.1 Spearman Rho correlation of AH status (n = 25) with total cohort of heavy drinkers (n = 1063)

Spearman Rho correlation with AH status	AH		
	r	p	N
14.15-epoxyeicosatrienoic acid (ng/g)	−0.465	**2.5E-02**	23
5-HEPE (ng/g)	0.465	**2.5E-02**	23
Apoptosis aC3 (0 or1)	0.424	**6.4E-03**	40
Megamitochondria (0 or 1)	0.371	**1.9E-06**	156
Ascites (0 or 1)	0.366	**7.7E-36**	1087
RDW-SD (/fL)	0.357	**1.9E-02**	43
Folic acid (nmol/L)	−0.316	**2.0E-02**	54
Vitamine B12 (pmol/L)	0.294	**1.9E-02**	63
CYP2E1 score (immunostain)	−0.277	**3.9E-02**	56
Mallory hyaline (0 or 1)	0.275	**5.0E-04**	157
Bilirubin indirect (mg/dL)	0.270	**1.8E-05**	246
Signs of cirrhosis (US)	0.262	**3.2E-18**	1070
Bilirubin total (mg/dL)	0.245	**2.4E-17**	1163
INR	0.237	**2.8E-16**	1163
Liver stiffness (kPa)	0.235	**1.7E-15**	1116
HDL cholesterol (mg/dL)	−0.231	**3.7E-12**	881
Transferrin (g/L)	−0.221	**4.1E-11**	872
Leukocytes (/nL)	0.220	**3.3E-14**	1163
AST/ALT	0.220	**3.5E-14**	1163
CD163 (ng/mL)	0.217	**6.9E-04**	241
Albumin (g/dL)	−0.216	**6.3E-11**	897
CRP (mg/L)	0.215	**1.3E-13**	1159
Erythrocytes (/pL)	−0.213	**2.3E-13**	1163
Hematocrit (%)	−0.199	**8.5E-12**	1162
M65 (U/L)	0.194	**3.7E-07**	675
M30 (U/L)	0.191	**5.7E-07**	675
Hemoglobin (g/dL)	−0.190	**7.1E-11**	1161
Cholesterol (mg/dL)	−0.188	**1.3E-09**	1027
APO A1 (mg/dL)	−0.186	6.4E-02	100
PTT (sec)	0.185	**2.8E-09**	1013
AP (U/L)	0.185	**1.9E-10**	1162
Ballooning (0–2)	0.173	**3.0E-02**	157
HbA1C (%)	−0.172	**8.0E-07**	814
Kleiner fibrosis score (0–4)	0.171	**3.3E-02**	156
Liver size (cm)	0.141	**1.2E-05**	955
MCV (fL)	0.141	**1.2E-05**	959
AST/GOT (U/L)	0.140	**1.5E-06**	1163
Spleen size (cm)	0.129	**7.0E-05**	945
Status dead (0 or 1)	0.102	**4.6E-03**	768

Table 64.1 (continued)

Spearman Rho correlation with AH status	AH		
	r	p	N
Hepatic steatosis (US) (0–3)	0.066	5.7E-02	843
Transferrin saturation (%)	0.060	8.4E-02	844

Note that parameters were first sorted according to P value and then absolute r value in descending order. Number of available parameters are shown in right column. Importantly, markers of hemolysis are significantly associated with AH but also deficiency in folic acid and lipidomics parameters

Preliminary Evidence for Enhanced RBC Turnover and its Association with AH

First preliminary data indicate that AH is indeed associated with enhanced RBC turnover. In this first approach, we analyzed all 25 cases (1.9%) with proven AH (see Tables B.21, B.22) from our Heidelberg cohort of heavy drinkers (n = 1063) and performed Spearman Rho correlation of AH status. Data are shown in Table 64.1. Here, clearly, markers of hemolysis or enhanced RBC turnover such as CD163 are significantly associated with AH but also deficiency in folic acid and lipidomics parameters and genotypes. We next also compared these 25 AH cases with a cohort of 30 cases with alcoholic cirrhosis matched for histological fibrosis. Only 7/25 (28%) of AH cases had a liver biopsy, 2 with F3 fibrosis and 5 with F4 cirrhosis. All patients in the cirrhosis group had histologically proven liver cirrhosis F4. Consequently, fibrosis score was slightly higher in the cirrhosis cohort (see Table 64.2). According to Table 64.2 which shows parameters that are different between these two cohorts, leukocyte count but also signs of hemolysis were higher. In addition, liver stiffness was also significantly higher but both parameters were in the cirrhotic range (69 v 52 kPa). These first data suggest to us that development of AH is associated with enhanced RBC turnover as described above. As is discussed in more detail in Chap. 57 on iron and ALD, **MCV and anemia deteriorate after alcohol detoxification**. This means that the sudden stop of alcohol consumption interferes with the complex blood-bone marrow-liver axis in a way that, at least transiently, ineffective erythropoiesis worsens. These insights provide a first idea to better target the problem in patients in AH since they usually voluntarily abstained from alcohol prior to hospital admission and this may aggravate, at least in some patients, complications. It further means that not alcohol alone, but additional confounders are responsible. One potential explanation could be that the sudden removal of the toxic blockage of ethanol on physiological processes such as apoptosis [13] or cell regeneration or division (see also Chap. 49 on the pathophysiology of ALD) could both **unchain regeneration in the bone marrow and liver**, potentially in an iron overloaded toxic environment, that causes production of fragile RBC and, unintentionally, aggravates ineffective erythropoiesis (see also Chap. 58).

Table 64.2 Comparison between AH and alcoholic cirrhosis matched for histological fibrosis

Parameter	Units	Alcoholic hepatitis (AH)	T-Test	Alcoholic cirrhosis
		Mean	P	Mean
MELD		20.1	**2.3E-06**	12.4
Quick	%	52.2	**2.5E-06**	74.4
Leukocytes	/nL	13.6	**1.5E-05**	8.5
Ascites	0 or 1	0.9	**2.8E-05**	0.3
Maddrey DF		40.6	**5.2E-05**	13.2
AH criteria		0.4	**5.4E-05**	0.0
Transferrin	g/L	1.2	**1.8E-04**	1.9
Bilirubin (total)	Mg/dL	9.7	**2.4E-04**	2.9
PNPLA3 CC	0 or 1	0.6	**3.0E-04**	0.1
INR		1.7	**3.1E-04**	1.2
Liver stiffness	kPa	69.1	**4.8E-04**	52.2
Fibrosis (Kleiner)	0–4	3.7	**6.3E-04**	4.0
CRP	Mg/dL	30.0	**6.4E-04**	12.3
Bilirubin indirect	Mg/dL	1.9	**2.2E-03**	0.4
Sodium	Mmol/L	131.2	**3.6E-03**	135.4
Erythrocytes	/pl	3.1	**4.0E-03**	3.6
Cholesterol	Mg/dL	137.5	**4.3E-03**	187.3
HDL cholesterol	Mg/dL	18.3	**4.5E-03**	43.9
Hematocrit	%	31.1	**5.2E-03**	36.2
Hyaloronan	Ng/mL	1304.9	**5.8E-03**	458.6
Diabetes	0 or 1	0.0	**7.3E-03**	0.3
Platelets	/nL	196.1	**8.0E-03**	144.3
Megamitochondria	0 or 1	0.4	**1.1E-02**	0.1
Hemoglobin	g/dL	10.9	**1.4E-02**	12.5
Mallory hyaline	0 or 1	1.0	**2.1E-02**	0.5
PNPLA3 GG	0 or 1	0.0	**3.1E-02**	0.3
14.15-EET	Ng/g	3244.5	**3.4E-02**	8040.7
Protein (total)	g/dL	6.5	**3.7E-02**	7.1
13.14-EDP	Ng/g	929.3	**3.8E-02**	1896.4
ABIC	Rel units	7.7	**4.9E-02**	7.0
10.11-EDP	Ng/g	1147.2	5.3E-02	1869.0
5-HEPE	Ng/g	583.9	5.4E-02	252.3
16.17-EDP	Ng/g	771.1	5.7E-02	1453.7
CD163	Ng/mL	3567.7	6.2E-02	2424.2
TM6SF2 CC	1 or 0	0.9	6.5E-02	0.7
Albumin	g/dL	3.3	7.5E-02	3.7
APO A1	Mg/dL	57.6	9.4E-02	101.0

25 patients with AH were histologically matched with 30 patients with alcoholic liver cirrhosis. Only 7/25 (28%) had a liver biopsy, 2 with F3 fibrosis and 5 with F4 cirrhosis. All patients in the cirrhosis group had histologically proven liver cirrhosis F4. Note that AH score was higher in the AH group, Leukocyte count and signs of hemolysis.

Table 64.3 Correlation of ferroptosis marker ACSL4 (Long-chain-fatty-acid—CoA ligase 4) with various clinical and laboratory markers from a heavy drinking cohort

Parameter	Category	ACSL4/β-actin		
		r	p	N
ERFE (ng/mL) (erythroferrone)	Special laboratory	0.943	**4.8E-03**	6
CD163 (μg/mL)	Special laboratory	0.886	**1.9E-02**	6
Liver iron conc. (RTS) (μg/g ww)	Iron	0.729	**2.1E-03**	15
Sex (1: Male)	General information	−0.567	**7.3E-03**	21
Haptoglobin (g/L)	Special laboratory	−0.516	5.9E-02	14
APRI	AST/platelets	0.600	**1.4E-02**	16
Mallory hyaline (0 or 1)	Histology	0.517	**1.6E-02**	21
Bilirubin (μmol/L)	Score	0.502	**4.0E-02**	17
Lobular inflammation (0–3)	Histology	0.452	**4.0E-02**	21
MBOAT7 CC (0 or 1)	Genes	0.451	**4.6E-02**	20
Alcohol consumption (g/day)	Alcohol	−0.442	5.8E-02	19
Maddrey	Score	0.433	6.4E-02	19
Ballooning (0–2)	Histology	0.428	5.3E-02	21

ACSL4 mRNA was assessed in liver biopsies from heavy drinkers using Western blotting and subsequent densitometry. Parameters were first sorted according to P value and then, in descending order, according to the absolute correlation coefficient (Spearman Rho correlation). Numbers of samples are indicated in the far-right column. ACSL4 converts free long-chain fatty acids into fatty acyl-CoA esters, preferentially arachidonate. Note that this ferroptosis markers is highly correlated with markers of hemolysis or iron (CD163, liver iron, haptoglobin, ERFE), liver damage (Mallory hyaline, lobular inflammation) and directly with the Maddrey score. Maximum 21 samples were available for analysis. P is given in bold if smaller than 0.05

In this context, it is highly interesting that ACSL4, which is Long-chain-fatty-acid-CoA ligase 4 and involved in **ferroptosis**, is also highly associated with hemolysis in the Heidelberg cohort of heavy drinkers (preliminary data in Table 64.3 and Table B.39). ACSL4 converts free long-chain fatty acids into fatty acyl-CoA esters, and thereby plays a key role in lipid biosynthesis and fatty acid degradation. This isozyme preferentially utilizes arachidonate as substrate. Lipidomics data combined with ACSL4 data on ferroptosis are still very limited but suggest a positive association with AH. More data are needed on this topic.

Future Directions

Enhanced RBC turnover could be one of the long thought important and less well appreciated pathomechanisms involved in ALD and AH. Since the amount of protein turnover by macrophages during physiological erythrophagocytosis is by far much higher as compared to PAMP- and DAMP-mediated macrophage uptake, heme degradation in the release of iron and carbon monoxide must play an important role. The accumulating iron in liver of ALD patients is most likely derived from RBC recycling. It remains to be studied how hepatocytes actually get access to this

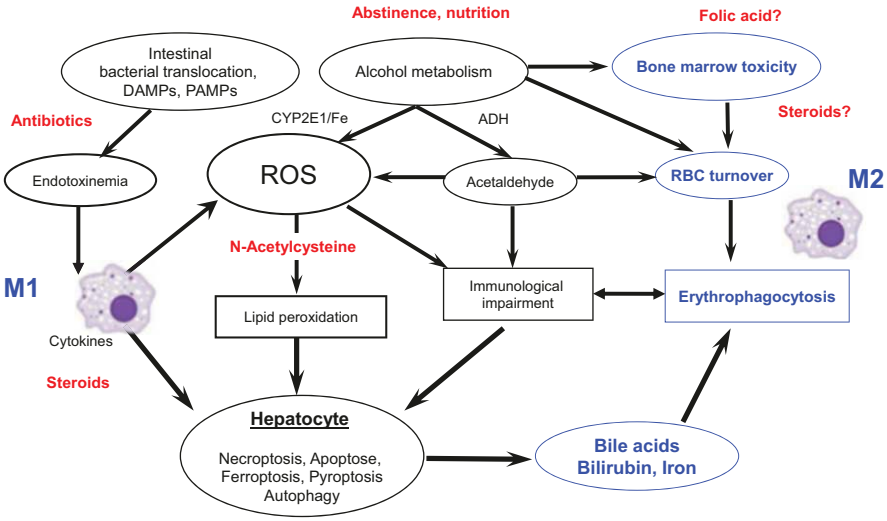

Fig. 64.4 Emerging role of enhanced RBC turnover and hemolysis and the conventional concept of AH pathophysiology. Established pathomechanisms are given in black with corresponding therapeutic measures in red. RBC turnover is highlighted in blue. There are indications that the inflammatory macrophage (M1) results from the signaling events on the right while heme turnover may generate M2-like macrophages with an important role of HO1

iron. Obviously, it is not only transferred through transferrin since hepatocytes can directly uptake erythrocytes and transferrin is typically downregulated in these patients. However, it remains completely unknown how they get access to RBCs and which chemokines and chemotactic factors are involved. As has been already discussed in the pathophysiology Chap. 49 of ALD, direct efferocytosis of RBCs by hepatocytes could also explain the release of bilirubin in these cells. It also needs to be studied why hemolysis is highly associated with hepatocyte synthesis of bile acids (see Table B.24) and how the lipids from the RBC membrane (phospholipids, triglycerides and cholesterol) are processed by hepatocytes and whether this contributes to steatosis or even the ballooning and foamy degeneration, described in more detail in Chap. 38 on histology by C. Lackner. In this context, it is also quite exciting to see, that heme degradation by HO1 occurs in the ER where also P450 CYPs are located, heme enzymes, that should be blocked by HO1-mediated release of carbon monoxide. Consequently, heme degradation could block ethanol oxidation by CYPs and interfere with the metabolism of lipids and steroids performed by CYPs. Hence, a new molecular link between steroid metabolism and the therapeutic action of steroids in AH could exist (Fig. 64.4).

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Chapter 65
Diagnosis and Staging of Disease-Severity in Symptomatic Alcoholic Hepatitis

Christophe Moreno and Delphine Degré

Abstract Alcoholic hepatitis should be suspected in patients with a recent onset of jaundice and with excessive chronic alcohol consumption. Diagnosis is based on clinical presentation and typical laboratory findings (AST/ALT ratio > 1.5, AST > 50 UI/L, AST and ALT <400 IU/L, total serum bilirubin >5 mg/dL). A liver biopsy is useful to confirm the diagnosis and exclude other diagnosis, but is not routinely performed in clinical practice in many centers. Different prognosis tools aiming to estimate the risk of short-time mortality and to determine whether the patients should be treated with a specific therapy, have been developed. The most used in clinical practice are the Maddrey discriminant function and the model for end-stage liver disease.

Keywords Alcoholic hepatitis · Jaundice · Liver biopsy · Prognosis · Maddrey discriminant function · MELD score

Introduction

Excessive alcohol consumption is one of the leading causes of liver disease worldwide (see also part I of this book). According to the World Health Organization's (WHO) 2014 report, harmful alcohol use causes approximately 3.3 million deaths per year, corresponding to among 6% of all death [1]. Alcohol-related liver disease (ALD) presents a broad spectrum of disorders including steatosis, alcoholic hepatitis (AH), cirrhosis and development of hepatocellular carcinoma. AH is one of the most severe manifestation of ALD associated in severe forms with high morbidity and mortality [2]. The incidence of AH has been difficult to estimate and varies

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worldwide. In United States, admissions for AH were found to have increased to 0.83% of all admissions in 2010 [3]. In Denmark, the incidence of AH for the period 1999–2008 rose from 37 to 46 per million persons per year in men and 24 to 34 per million persons per year in women [4]. However, estimating the burden of AH is difficult since the incidence assessment is based on diagnostic coding [5].

Definition of Symptomatic Alcoholic Hepatitis

AH is a clinical syndrome characterized by recent onset of jaundice in patients with excessive chronic alcohol consumption. Some patients have ceased drinking alcohol but a period of less than 60 days of abstinence before the onset of jaundice is generally observed [6]. Jaundice is often associated with other symptoms such as fever, malaise, malnutrition or other signs of liver decompensation (i.e. ascites, encephalopathy or bleeding).

Diagnosis of Symptomatic Alcoholic Hepatitis

Diagnosis of AH is based on clinical presentation and typical laboratory findings, and imaging should exclude biliary obstruction. Blood analysis of AH patients typically shows hyperbilirubinemia (> 5 mg/dL), AST > 50 IU/L with an AST/ALT ratio typically greater than 1.5. The AST and ALT levels usually do not exceed 400 U/L distinguishing AH from other diseases such as ischemic hepatitis or drug induced liver injury. In severe forms, a prolonged prothrombin time, hypoalbuminemia and decreased platelet count are frequently observed [2]. When AH is suspected based on clinical and laboratory analysis, a liver biopsy can be useful to confirm the diagnosis. Typical histological findings are described in more detail in chap. xxx of this book. They show steatohepatitis including steatosis, hepatocyte ballooning, an inflammatory infiltrate with neutrophils, bilirubinostasis and fibrosis with a typical chicken-wire pattern [7]. However, liver biopsy is not routinely performed in many centers. The main restrictions to perform liver biopsy in routine clinical practice are routine access to transjugular liver biopsy (indicated in the presence of ascites, coagulation disorders and low platelet count), potential risks, the cost of the procedure and the absence of well-validated grading systems. Recently, National Institute on Alcohol Abuse and Alcoholism (NIAAA) Alcoholic Hepatitis Consortia recommendations have defined AH patients for inclusion in clinical studies as follows: definite AH defined as clinically diagnosed and biopsy proven, probable AH defined as clinically diagnosed but not biopsy-proven; and possible AH as clinically diagnosed but not biopsy-proven with potential confounding factors [6]. Confounding factors include possible ischemic hepatitis (e.g., severe upper gastrointestinal bleeding, hypotension, or cocaine use within 7 days), possible drug-induced liver injury, uncertain alcohol use assessment, atypical laboratory tests

(e.g., AST < 50 IU/L or >400 IU/L, AST/ALT ratio < 1.5), and blood markers of auto-immunity. Based on these definition, which still need to be validated, a liver biopsy is mandatory in possible AH but not absolutely mandatory in cases of probable AH. In clinical practice, we routinely performed a transjugular liver biopsy in suspected severe AH, because of long term experience, easy access to the procedure, and absence of significant complications. However, in centers without easy access or without experience in this procedure, applying the NIAAA criteria is an acceptable alternative and should probably be applied in many centers. The challenge in clinical practice is to identify cases without the typical clinical or laboratory features of AH and the potential confounders, in order to avoid to expose patients without AH to corticosteroids or other specific therapies.

Noninvasive tests for AH diagnosis are sorely needed. Cytokeratins have emerged as serum biomarkers of hepatocyte damage. Circulating fragments of cytokeratin-18 (CK-18), and the main constituent of Mallory-Denk bodies, termed M65 and M30 are of particular interest in AH. A recent study reported higher levels of total and microvesicle-bound M65 and M30 in the circulation of patients with biopsy-proven AH [8]. Although promising, these biomarkers need further investigation before recommending their routine use.

Assessment of Symptomatic AH Severity

Several laboratory-based prognostic models are available for assessing severity and prognosis of AH (see Table 65.1). The aims of these scores are first to estimate the probability of short-term mortality and second to determine whether a specific therapy is indicated (i.e. corticosteroids). The Maddrey discriminant function (mDF) which uses bilirubin and prothrombin time, is frequently used for predicting the

Table 65.1 Variables included in the different prognostic scores for assessing severity in alcoholic hepatitis

Scores	Bilirubin	PT/ INR	Creatinine	Age	Albumin	Urea	WCC	ΔBilirubin	Cut-off
mDF	+	+							Severe >32
MELD	+	+	+						Severe ≥21
GAHS	+	+		+		+	+		Severe ≥9
ABIC	+	+	+	+					Low risk ≤6.71 Severe >9
Lille score	+	+	+	+	+			+	<0.45 >0.56

The Lille score with change of bilirubin after 1 week identifies those who benefit from continued steroid treatment

ABIC age-bilirubin-international normalized ratio-creatinine, *GAHS* glasgow alcoholic hepatitis score, *INR* international normalized ratio, *mDF* maddrey discriminant function, *MELD* model for end-stage liver disease, *PT* prothrombin time, *WCC* white cell count.

severity of AH and for assessing the need for corticosteroid (CS) therapy [9, 10]. The limitations of this score include its use of prothrombin time expressed in seconds, which is not standardized between clinical laboratories and the absence of renal function assessment, as acute kidney injury is a strong predictor of mortality in AH [11]. A cut-off value above 32 identified patients with severe AH in whom CS are usually initiated. In the absence of treatment, 1-month mortality of patients with severe AH was 50% in early studies but decreased to approximately 20% in more recent studies [12]. Patients with non-severe AH defined as mDF < 32 have less than 10% risk of 1-month mortality. However, recent reports have shown rather low 1- and 5-year survival rates of 80% and 50%, respectively, for patients with symptomatic non-severe AH who were admitted with liver decompensation [13]. The term "non-severe alcoholic hepatitis" seems therefore inappropriate for patients with symptomatic AH and mDF < 32. This study also showed that non-commitment to a strict alcohol abstinence is associated with a worse outcome in this AH population.

The model for end-stage liver disease (MELD) score, which includes bilirubin, international normalized ratio (INR) and creatinine levels, is also useful for predicting 30- and 90-day mortality in patients with AH. Patients with a MELD score ≥ 21 have a 90-day mortality of 20% [14], and is generally accepted as a cut-off for indicating CS.

The Glasgow alcoholic hepatitis score (GAHS) and the Age, serum Bilirubin, INR, and serum Creatinine (ABIC) score are other scores used to predict short-term mortality in patients with AH [15–17]. The variables included in the scoring systems for AH and the cut-offs used to determine the severity of the disease are shown in Table 65.1. Although the mDF remains the most commonly used score in clinical studies and in many centres, the MELD, GAHS and ABIC scores appear to have a slightly better efficacy for predicting short-term mortality in patients with severe AH and are used according to the local practice of each centre [18].

More recently, baseline neutrophil-to-lymphocyte ratio (NLR) has been suggested as a parameter that may help to stratified risk and likelihood of CS response in patients with AH. Patients with low (<5) and high (>8) NLR values do not appear to benefit from CS treatment. In the modified GAHS (mGAHS) NLR has been incorporated in place of the white cell count to improve the discriminatory power of the score [19]. However, validation studies are needed before NLR can be recommended for routine clinical practice.

Some histological features are also associated with severity and mortality in AH. Specifically, degree of fibrosis, neutrophil infiltration, type of bilirubinostasis and the presence of megamitochondria have been found to be independently associated with 90-day mortality and are included in the Alcoholic hepatitis Score (AHHS). This score identifies patients with low (0–3 points), moderate (4–5 points) and high (6–9 points) risk of death within 90 days (3%, 19%, and 51%, respectively) [20]. Limitation of this score is the requirement of liver biopsy and significant interobserver variability among pathologists [21].

The gene-signature plus MELD (gs-MELD) combines the baseline liver expression patterns of 123 genes with the MELD score to discriminate patients with poor

and good 90-day survival. Patients with a gs-MELD score greater than 2.66 were considered to have a poor prognosis [22]. However, the need for liver biopsy limits its clinical application. Recently, a plasma protein-based surrogate of the gene signature was developed and combined with the MELD score. The high-risk plasma-signature (ps)-MELD score was associated significantly with death or liver transplantation within 90 days [23].

The presence of acute kidney injury, infection or systemic inflammatory response syndrome (SIRS) are associated with mortality in patients with severe AH [11, 24, 25]. Acute-on-chronic liver failure (ACLF) defined as an acute deterioration of liver function combined with single or multiple organ failure is frequent during the course of severe AH and is associated with high mortality. In clinical practice, it is important to understand that ACLF is not a specific disease but rather a clinical syndrome caused by different precipitating factors (alcoholic hepatitis and infection are the most frequent causes of ACLF in Western countries). In the setting of AH, the main interest of ACLF is its prognostic value. Indeed, the ACLF scoring system, was recently studied as a strong predictor of short-term mortality in AH patients. The 28-day cumulative incidence of death in patients with and without ACLF were 54% and 10.4%, respectively (30.8%, 58.3% and 72.4% for ACLF-1, ACLF-2 and ACLF-3 respectively) [26].

Prognostic factors of long-term outcome in AH patients have been less well studied. Abstinence seems to be the most important predictive factor for long-term survival in patients with AH [13, 27, 28]. Improvement in long-term survival after AH should thus include secondary prevention strategies to promote complete alcohol abstinence.

New biomarkers, such as serum transferrin or circulating hepatocyte-derived.

extracellular vesicles seem to be promising biomarkers for prediction of mortality in AH patients but validation studies are needed before recommending their routine use [29, 30].

In conclusion, alcoholic hepatitis should be suspected in every patient with recent onset of jaundice and excessive alcohol consumption. Diagnosis is based on clinical presentation, typical laboratory findings, exclusion of biliary obstruction and can be confirmed by liver biopsy. Maddrey Discriminant function and MELD score are the most frequently used scores in clinical practice and in clinical trials. A mDF > 32 and/or MELD ≥21 identifie patients at high risk of death in the short term and are used to indicate specific therapy.

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Chapter 66
Management of Severe Forms of Alcoholic Hepatitis

Alexandre Louvet

Abstract Severe alcoholic hepatitis (AH) is one of the most fatal liver complications of chronic alcohol use and occurs predominantly on a background of alcohol-related cirrhosis. Severity is defined by Maddrey's and/or MELD scores. Medical treatment is based on a 28-day course of prednisolone at 40 mg/day and therapeutic response is evaluated at day 7 using the Lille score, which integrates baseline biological features and evolution in bilirubin after 7 days of steroids. Infection is a frequent event which requires a systematic screening at admission in all patients (urine and blood culture, ascitic fluid examination, chest X-ray). In case of response to treatment (Lille<0.45), prednisolone is continued for a total of 28 days. Conversely, non-responders take no benefit of steroids and treatment is stopped at day 7. Survival of non-responders is poor at 6 months (approx. 30–40%) and no medical option has been proven efficient in improving outcome. Liver transplantation can be offered to a minority of patients, experiencing their first liver decompensation, and carefully selected in a multidisciplinary approach. Several new molecules are being tested either alone or in combination with steroids and progress in the management of these patients is expected soon.

Keywords Alcohol · Liver failure · Corticosteroids · Survival · Infection · Transplantation

Introduction

Alcoholic hepatitis (AH) is one of the most severe manifestations of alcohol-related liver disease (ALD) (see also book Chaps. 64 and 65). It is typically a clinical diagnosis in patients with sudden onset of jaundice, previous periods of heavy drinking. Often, patients present to the hospital with sustained jaundice despite several days

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or weeks of abstinence [1–3]. Histology shows typical signs of ALD (described in detail in book Chap. 38). Elementary features comprise cell damage with changes in hepatocyte (ballooning), cytoplasm eosinophilic condensations (Mallory-Denk bodies) and inflammatory infiltrate mostly with neutrophils [3]. The clinical severe form of AH should be discriminated between the more often observed histological steatohepatitis. Terminology is still somewhat confusing, and the international debate continues to better describe the various clinical and histological entities.

It is also important to underline that these histological features are not specific for an alcohol etiology, especially because ballooning and Mallory-Denk bodies can also be found in patients with metabolic fatty liver (see also chapter on histology 38). Conversely, infiltrates with neutrophils are less frequently observed in patients with metabolic syndrome without excessive alcohol consumption. Of note, the precise definition of AH on liver biopsy needs international consensus because the number of elementary lesions required for a definite diagnosis has not been fully validated by a panel of experts. More specifically, there is no consensus as to whether the three histological lesions mentioned above must be present or whether two are sufficient. Given that such histological lesions occur in patients with excessive drinking for several years, it is not surprising that, besides inflammation, other alcohol-related liver abnormalities are also present, such as steatosis (triglyceride accumulation in the cytoplasm secondary to heavy and recent alcohol consumption) and/or a certain degree of fibrosis, which is often closely associated with the duration of heavy alcohol consumption.

Besides the clinical features, AH diagnosis is confirmed on liver biopsy, which implies that such alcohol-related inflammation can cover several clinical entities. While some patients have no symptoms except those related to excessive alcohol consumption, the severe form of AH is often accompanied by clinical signs of liver decompensation (jaundice, ascites and/or encephalopathy). The definition of disease severity (i.e. severe alcoholic hepatitis) is based on biological scores (see below). When liver biopsy is performed (mostly using the transjugular route given that coagulation disorders and ascites are often present in severe alcoholic hepatitis), cirrhosis is common: more than 90% of patients with severe AH included in randomized controlled trial with liver biopsy required have histological cirrhosis [4].

In patients with a background of alcohol-related cirrhosis, AH can be difficult to discriminate from other causes of decompensation. Over the last years, a new entity **termed acute-on-chronic liver failure** (ACLF) has been described. This wording encompasses a spectrum of different clinical diseases and is defined by the number of organ failures [5]. ACLF is not a specific disease but rather a clinical syndrome caused by different precipitating factors (infection, AH, drug-induced liver injury, viral hepatitis, etc.). given that most patients with ACLF present with jaundice, it can be difficult to differentiate AH from other causes of liver decompensation. Liver biopsy is certainly useful in this setting. However, if biopsy is not available, a careful analysis of clinical and biological profile of patients can help. Typically, patients with AH have a moderate elevation of transaminases, a recent onset of jaundice, no fever, etc. (see Chap. 65). The fact that jaundice precedes all other signs of ACLF

(renal fever, sepsis, etc.) argues for AH as the precipitating event, the other entities being more consequences of AH than causes of liver dysfunction.

Clinically, severe AH is suspected in case of a recent onset of jaundice in a heavy drinking patient [1–3]. Given that several factors apart from AH can cause jaundice in patients with excessive alcohol drinking, liver biopsy is considered the gold standard for disease confirmation [1–3]. Differential diagnoses include hemolysis, infection, drug-induced liver injury, acute viral hepatitis (especially caused by hepatitis E virus), red cell transfusion (which can cause jaundice, especially in patients with cirrhosis), etc. However, liver biopsy may be difficult to make, mostly in non-tertiary centers and in some countries in which access to this technique is uneasy. Routine biochemistry shows moderately elevated transaminases, elevated GGT (closely related to time from last drinking), altered coagulation parameters and high bilirubin, predominantly conjugated [3, 6]. In addition, a certain degree of acute kidney injury is often seen. As mentioned below and in the chapter dedicated to diagnosis and prognosis (see book Chap. 65), several biological scores can be used to define severe AH and most of them use liver function as key elements for calculation. If liver biopsy is not available, a careful analysis of events by a trained practitioner can help to rule out other diagnoses (see above). In cases of clinical uncertainty, liver biopsy is very useful.

Medical Treatment of AH: Steroids and the Lille Model

Several pharmacological options have been tested to treat severe AH, although most of them have failed in showing a clinical benefit. Nowadays, there is a consensus to restrict pharmacological treatment to patients with severe AH, mostly defined by a Maddrey's discriminant function equal or greater to 32. Both the European Association for the Study of the Liver (EASL) and the American association for the Study of Liver Diseases (AASLD) support this recommendation in their guidelines [1, 2]. The reason for not treating patients with a less severe disease (i.e. those with a Maddrey's discriminant function lower than 32 is the lack of an effective and specific treatment perspective. Conversely, in patients with Maddrey's discriminant function greater than 32, a 28-day of prednisolone improves short-term survival which has been supported by several randomized controlled trials and meta-analyses [7–11]. Thus, corticosteroid treatment significantly decreased risk of death within 28 days compared with controls (hazard ratio [HR] 0.64 [7]. Prednisolone is mostly given orally at a dose of 40 mg daily for 4 weeks, regardless of patient weight or body mass index. Other treatments have failed in improving survival at 1 month, for example pentoxifylline [4, 7, 11] or antioxidants drugs [7, 12, 13].

Although prednisolone remains the only medical therapy option to treat severe AH, it is not an ideal drug. Indeed, some patients will do not improve their liver function upon treatment and their risk of death is very high, related to persistent liver decompensation and injury. An early identification of these patients unlikely to take benefit from corticosteroids is a major challenge to clinicians in order to stop

treatment and consider other therapeutic options. Since patients with severe AH have hepatic decompensation, persistent liver impairment is strongly associated with outcome and most patients at risk of dying will succumb from a lack of improvement in liver function [14–17]. Thus, not surprisingly, response to treatment is mostly defined by a decrease in bilirubin after some days of challenge with prednisolone. This early change in bilirubin level (ECBL) is defined by the presence of absence of a decrease of total bilirubin within the first 7 days of prednisolone therapy [17]. The absence of a bilirubin decrease (i.e. absence of ECBL) is strongly associated with death and the risk of dying within 6 months following treatment initiation is higher than 60%. Although this criterion is simple and easy to apply in clinical practice, it lacks sensitivity since some patients, despite an ECBL, will not show a bilirubin decrease upon treatment sufficiently strong enough to minimize the risk of liver events and of death. Based on mortality and treatment response data, consequently, the Lille model has been developed which integrates several prognostic markers including the magnitude of change (either decrease or increase) in bilirubin after a challenge of 7 days by prednisolone [16]. Other prognostic parameters of the Lille score include age, presence of a renal insufficiency, albumin, total bilirubin and prothrombin time at baseline (i.e. at the beginning of prednisolone). Once combined, the final Lille model ranges from 0 to 1, the lower score being associated with the best prognosis. The 0.45 cut-off classifies patients into responders (Lille <0.45) who have an acceptable survival rate at 6 months (>80%) and non-responders (Lille ≥0.45) who have a very high risk of mortality at 6 months (>60–70%). In non-responders, most deaths are related to liver dysfunction and occur generally within the first 2 months [16]. Outcome can also be predicted by the combination of the Lille model at day 7 with the MELD score at baseline [18]. This approach can help to calculate survival at 2 and 6 months and adapt therapeutic management. Such prediction of survival is especially useful when liver transplantation is considered (see below).

In order to refine prediction of survival, a definition of treatment into three groups can be proposed according to two other cut-offs (0.16 and 0.56). Patients with a Lille score at 7 days <0.16 are called complete responders and their risk of dying is minimal while partial responders (0.16 < Lille<0.56) have an intermediate survival [9]. The subgroup of patients with a null response (Lille ≥ 0.56) have a poor prognosis at short term, and prednisolone should be stopped after 7 days in them. Indeed, patients who have been randomized in clinical trials testing steroids vs. placebo have not shown any survival benefit in patients with Lille ≥0.56 treated with prednisolone as compared to controls who did not receive steroids [9]. Both EASL and AASLD recommend stopping prednisolone after 7 days if the Lille score is equal or greater to 0.56 [1, 2]. It has also been suggested that the Lille score can be calculated after 4 days of prednisolone by using the same cut-offs [19] but this has not been largely confirmed by independent studies. While prednisolone has to be stopped in non-responders, patients who have a therapeutic response, either complete or partial, continue treatment for a total of 1 month and can be discharged. After 1 month of treatment, steroids can be stopped without tapering [1, 2]. While prednisolone

improves survival rate at 1 month as compared to placebo, the survival benefit disappears at 3 and 6 months and trials and meta-analyses do not show a survival benefit over placebo at these time points [7]. Thus, evaluation of new therapeutic options is required.

Non-steroid Medical Treatment of AH

Given that corticosteroids are associated with a significant number of treatment failures, some strategies have tried to combine other drugs to prednisolone. Unfortunately, no combined therapy has been able to increase short-term survival. It must be borne in mind that patients treated with placebo have nowadays a better survival as compared to studies performed in the 70s and 80s. This is related at least in part to a better management of sepsis and of renal failure. Pentoxifylline was thought to decrease the incidence of hepatorenal syndrome [20] but this did not result in a better survival when combined to prednisolone [4]. Intensive enteral nutrition and antioxidants yielded similar disappointing results [21]. N-acetylcysteine was tested for 5 days in an intravenous protocol similar to that used for acetaminophen poisoning, but this did not reach statistical significance, although a trend toward a better survival was observed in the study for patients treated with the combined therapy with prednisolone [13]. Several other medical options have been tested recently. A corticosteroid-free combination of an IL-1β inhibitor, called anakinra, pentoxifylline and zinc has failed in improving survival [22]. An FGF-22 agonist, called F-652, has shown promising results in an open-label phase 2 trial in terms of safety [23] but its clinical benefit must be confirmed in larger trials. At present, only prednisolone is recommended to treat severe AH.

Management of Infectious Complications in AH

Due to the severe liver dysfunction in AH patients, the risk of systemic infections is very high [24]. Prospective studies have shown that 25–30% of patients admitted with severe AH have infections, either patent or identified by a systematic screening [25, 26]. This risk of infection is not due to steroid treatment but can be observed before steroid initiation. Common sites of infection are lung, blood, ascites and urine. This high risk of infection which can be observed without any clinical symptoms is a strong argument to systematically and extensively screen patients with suspected AH at admission for infections. Screening should include chest X-ray, urine and blood cultures and ascites fluid examination. Lung infections are of particular interest because they are associated with a high short-term mortality and a lower probability to receive steroids [26]. Risk factors for pneumonia include a history of smoking, male gender and encephalopathy. In case of suspicion of lung

infection, chest CT-scan can be recommended because it can help diagnose infection [26]. If infection improves after initiation of antibiotics, patients can safely be treated subsequently with prednisolone and their prognosis is similar to that observed in patients admitted without infection [25].

Management of Alcohol Abstinence

Abstinence from alcohol is required as in other forms of decompensated alcohol-related cirrhosis. There is also a general consensus to recommend complete abstinence [2]. However, alcohol recurrence is observed in about 30% of patients [14, 27]. While survival at short term (less than 6 months) is not influenced by alcohol recurrence, it clearly impacts survival at medium and long term. Not surprisingly, alcohol relapse is seen more often in the most heavy drinkers because alcohol dependence is more prevalent in patients with the highest daily alcohol consumption [14]. Thus, addiction management is crucial in patients with severe AH and should be based on a multidisciplinary approach, in close collaboration between hepatologists and addiction specialists. Despite the absence of specific studies in the field of AH, we recommend that addiction management be started as soon as possible, preferably during hospital stay. It must however be emphasized that the use of medications to treat alcohol use disorder in patients with decompensated liver disease should be further studied. Indeed, only baclofen at low doses (less than 30 mg/day) has shown an efficacy in a randomized controlled trial in patients with decompensated cirrhosis [28] with an acceptable safety profile. At higher doses, a cohort study has suggested baclofen might be used in patients with cirrhosis [29], however high doses were not frequently used in this cohort and the absence of a control arm somewhat limits the conclusions of this work. Of note, while baclofen is used in France [30], it is not approved in many countries, especially in the USA (no approval was received from the FDA). Other medications such as acamprosate, naltrexone and disulfiram must not or should not be used in patients with decompensated cirrhosis [30]. Recently, a panel of French experts has elaborated in their guidelines the body of evidence surrounding the efficacy and safety use of medications to treat alcohol use disorder [30].

Liver Transplantation for Patients with Severe Alcoholic Hepatitis

Alcohol-related liver disease has become the first indication for liver transplantation in Western countries [2]. Most patients are transplanted for decompensated cirrhosis and/or for hepatocellular carcinoma. For patients with alcohol-related cirrhosis (without AH), liver transplantation is normally only offered after an significant

period of abstinence [31]. This recommendation is based on the fact that alcohol cessation generally improves liver function [32], especially in the first 3 months. Of note, however, improvement after abstinence is mostly seen in patients with mild liver dysfunction [33] while an important proportion of patients with a more profound liver failure will continue to worsen despite alcohol cessation [33].

On the other hand, because of organ shortage, liver transplantation cannot be offered to all patients with decompensated cirrhosis and some selection rules must be applied. From an addiction point of view, it seems relevant to propose liver transplantation to candidates with the lowest probability of alcohol recurrence after liver transplantation. Indeed, while return to alcohol drinking does not affect graft and patient survival in the first 5 years following liver transplantation [34], long-term outcome of patients is drastically affected by alcohol recurrence after 5 years [35]. Unfortunately, selection of transplant candidates is still challenging, since we lack strong predictors of alcohol relapse after liver transplantation and despite the identification of important predictors of continued drinking such as lack of family support, young age, other addictions, poor compliance, mental disorders, relatives drinking excessively [36, 37].

Not surprisingly, one of the most often used tools to select "good" candidates (i.e. patients with the lowest probability of alcohol recurrence after liver transplantation) for transplantation is the length of sobriety. It has been shown by several studies that a longer period of abstinence before liver transplantation was associated with a lower probability of alcohol relapse and the "6-month rule" has been established in order to select a patient for transplantation [38, 39]. Although the 6-month rule is a simple tool, it cannot be regarded as optimal. For instance, the 6-month rule leads to excluding patients who have a good addiction profile but who have stopped alcohol for a shorter period of time. This can be quantified by the prognostic capacity of the 6-month rule to predict alcohol consumption after liver transplantation. Indeed, several studies have shown that patients who were abstinent for at least 6 months before transplantation had a lower probability of drinking alcohol after transplantation [38, 40]. This suggests that the 6-month rule has a good specificity to identify patients less likely to return to alcohol drinking. However, the sensitivity is poor (less than 60%), and many candidates are turned down but would be abstinent after liver transplantation despite a period of sobriety before entering the waiting list of less than 6 months [38, 40].

With respect to AH, liver transplantation is a special challenge since patients are, by definition, not abstinent, or only short-term abstinent at their admission to the hospital. Given that medical treatment fails in improving survival in about 40% of patients (overall survival of patients with severe AH is around 60% and response to treatment is only observed in 60% of cases), a very significant proportion of patients are at risk of death in the weeks/months following their admission for severe AH. In addition, as pointed out earlier, in non-responders to corticosteroids (see above), no alternative treatment has been established so far. The lack of pharmacological option to treat these non-responders has led the French consensus on liver transplantation in 2005 to propose the evaluation of an early access to liver transplantation in a subgroup of carefully selected patients [31].

Based on the French consensus, French and Belgian teams have tested the access of an early liver transplantation in patients identified as non-responders to medical therapy [41]. Although the selection process was not protocol-based, patients had to experience their first episode of decompensation of alcohol-related liver disease (any decompensation, not only AH) and a consensus of the transplantation team was required. This consensus was reached after a discussion with the patient and his/her relatives about the absence of severe coexisting psychiatric disorders and an agreement by patients to adhere to lifelong total alcohol abstinence. Not surprisingly, the presence of close supportive family members was also an important parameter of patient selection. In this pilot study, 26 patients with severe AH not responding to medical treatment were transplanted and survival at 6 months and 2 years was much better than that of not-transplanted controls [41]. An acceptable low proportion of patients developed evidence of alcohol recurrence after liver transplantation (3 patients relapsed during the follow-up) and this important step has led other teams to evaluate this accelerated access to transplantation for severe AH.

Given the notion that early access to liver transplantation raises some important ethical issues [42], this procedure must be validated and endorsed by regulatory agencies. The American consortium ACCELERATE has gathered data from several centers in the USA and have reported good survival after early liver transplantation for AH [43]. The risk of alcohol relapse in this cohort can be estimated at around 15–20%. More recently, the same authors performed a modeling study which demonstrates that early liver transplantation provides the most important survival benefit over a delayed procedure [44]. No prospective controlled studies had been performed until the publication of the French and Belgian study QuickTrans [45] which has compared early liver transplantation for AH not responding to medical therapy to transplantation for alcohol-related cirrhosis, with a primary endpoint on 2-year alcohol consumption. Controls were included following the 6-month rule while patients with severe AH were selected using a dedicated algorithm based on a large evaluation which included a consensus meeting gathering members of the hepatology, surgery and addiction teams after several interviews of the patient and the family members. This trial has concluded that alcohol recurrence in patients treated with early transplantation was not non-inferior to standard liver transplantation with 6 months of abstinence. Alcohol consumption was recorded using the TLFB agenda [46, 47] and any evidence for alcohol recurrence was seen in 33.8% of patients early transplanted for AH and in 24.7% of patients transplanted for alcohol-related cirrhosis. This difference was not significant, but patients who had been early transplanted for AH had a higher rate of heavy alcohol consumption after transplantation than patients with alcohol-related cirrhosis. In the two groups, survival at 2 years was very high, close to 90% without differences between the two arms.

Despite encouraging results regarding survival, more data are required to understand the drivers of alcohol recurrence after liver transplantation. Some factors such as a young age [43] or the history of alcohol consumption (legal issues, attempts of prior rehabilitation, illicit drug use, etc.) [48] have been identified but they must be

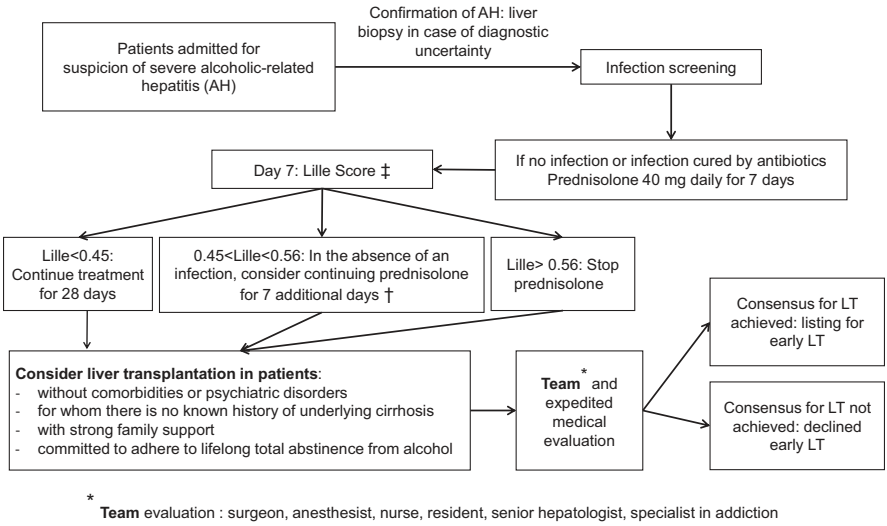

Fig. 66.1 Algorithm for management of patients with severe alcoholic hepatitis. †There is no consensus in patients with 0.45 < Lille<0.56 to continue or stop prednisolone. In patients with no sign of infection, we propose to consider a further challenge by steroids for 7 additional days, but this is not evidence-based and this is only an expert opinion. ‡Lille score formula: Exp(−R)/[1 + Exp(−R)], where R = [3.19–0.101 × age (in years) +0.147 × albumin (in g/L) +0.0165 × evolution in bilirubin (in μmol/L) −0.206 × renal insufficiency −0.0065 × bilirubin (in μmol/L) −0.0096 × prothrombin time (in seconds)]

validated at a large level (see also Book Part III). At present, there are some important discrepancies between countries in terms of policies towards early transplantation for AH [49]. Conversely, although some reluctance from the general public was expected but a recent study has shown that most people were in fact neutral toward or in favor of this procedure [50]. Further addiction data are also required to adapt addiction management after transplantation. An algorithm of medical management of patients with severe AH including LT is given in Fig. 66.1.

Conclusion

Severe AH is a life-threatening condition which requires specific management by a dedicated team. While medical treatment is still based on corticosteroids, new pharmacological agents are currently tested. In case of non-response to medical treatment, no drug has been proven efficient and early liver transplantation can be proposed in a subgroup of patients selected using a multidisciplinary approach. It is hoped that a better understanding of the underlying molecular mechanisms of AH will help to develop novel targeted therapies and optimize the management of AH (for more details see also Chap. 64).

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Chapter 67
Mechanisms of Recovery from and Strategies for Survival of Severe Alcoholic Hepatitis and ACLF

Ahmed Y. E. Ibrahim and Rajiv Jalan

Abstract Acute on Chronic Liver Failure (ACLF) is a syndrome characterised by rapid deterioration of liver function, multiple organ failure and high short term mortality rate. Several precipitating factors can be implicated in the development of ACLF. The most common triggering factors encountered in the western countries are bacterial infections and alcoholic hepatitis. Severe alcoholic hepatitis (SAH) is by far the most devastating and life-threatening form of alcohol-related liver diseases (ALD). ACLF can be identified at any point during course of SAH, either at the first presentation or during follow up. Although different trigger-specific pathophysiological mechanisms are involved in the development of ACLF, the clinical outcomes in SAH induced ACLF is comparable to other precipitating factors. This chapter focuses on the interactive relationship between SAH and ACLF and provides insights on potential mechanisms of survival from these two clinical syndromes.

Keywords Acute on chronic liver failure · Alcoholic hepatitis · Liver transplantation · Organ recovery

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Introduction

Acute on chronic liver failure (ACLF) is a syndrome that occurs in patients with acute decompensation (AD) of liver cirrhosis, characterised by multiorgan dysfunction and high risk of short-term mortality [1]. The findings of the CANONIC study have paved the way for the European Association for the Study of the Liver–Chronic Liver Failure (EASL-CLIF) consortium to provide a clear definition of ACLF. This definition is based on the results of prospective evaluation of 1343 patients hospitalized with AD [2]. Organ failures are identified using EASL-CLIF Consortium criteria which forms the basis of the diagnosis of ACLF [3] (Table 67.1). Although, differing views regarding the diagnostic criteria have been proposed by the Asian Pacific Association for the Study of the Liver (APASL) and The North American Consortium for the Study of End-Stage Liver Disease (NACSELD) groups, the definition proposed by the EASL-CLIF Consortium and criteria have been best validated [4]. The mortality rate of ACLF patients is about 30% to 50% and is closely related to the number and severity of organ failure/dysfunction. Without liver transplantation, the 28-day mortality rate among patients with ACLF grade 1, grade 2 and grade 3 was estimated to be 23%, 31% and 74% respectively compared to 1.9% in patients with decompensated cirrhosis but without ACLF [5] (Table 67.2). The global prevalence of ACLF among hospitalized patients is estimated to be 35% in a systematic review and metanalysis which included 43,206 patients with ACLF and 140,835 patients without ACLF [6].

Alcoholic hepatitis (AH) is a clinical syndrome of jaundice and liver failure that generally occurs after years of heavy alcohol consumption (mean intake, approximately 100 g per day) [7]. For more details see also book Chaps. 65 and 66 on diagnosis and treatment of AH in this book part. The typical age at presentation is 40–60 years [8]. The severity of this syndrome can markedly influence the clinical

Table 67.1 Clinical criteria for the diagnosis of ACLF

Organ/system	Subscore = 1	Subscore = 2	Subscore = 3
Liver	Bilirubin <6 mg/dL	Bilirubin ≥6 mg/dL and < 12 mg/dL	Bilirubin ≥12 mg/dL
Kidney	Creatinine <1.5 mg/dL Creatinine 1.5–1.9 mg/dL	Creatinine ≥2 mg/dL and < 3.5 mg/dL	Creatinine ≥3.5 mg/dL or renal replacement
Brain (west-haven grade for HE)	Grade 0	Grade 1-2	Grade 3–4
Coagulation	INR <2.0	INR 2.0–2.4	INR ≥2.5
Circulatory	MAP ≥70 mm Hg	MAP <70 mm hg	Vasopressor requirement
Respiratory	$PaO_2/FiO_2 > 300$ $SpO_2/FiO_2 > 357$	PaO_2/FiO_2 201–300 SpO_2/FiO_2 215–357	$PaO_2/FiO_2 \leq 200$

Adapted from [3]
FiO_2 fraction of inspired oxygen, *INR* international normalized ratio, *MAP* mean arterial pressure, *PaO_2* partial pressure of arterial oxygen, SpO_2 oxygen saturation as measured by pulse oximetry

Table 67.2 Mortality of ACLF patients according to its severity

Patient group	Prevalence over 1287 patients (%)	28-day Mortality (%)	Assigned grade
Absence of organ failure	68.3	4.4	Absence of ACLF
Single non-kidney organ failure without KD or BD	9.9	6.3	
Single KF	6.7	18.6	ACLF-1
Single non-kidney organ failure with KD or BD	4.2	27.8	ACLF-1
Two organ failures	7.5	32	ACLF-2
Three organ failures	1.9	68	ACLF-3
Four to six organ failures	1.4	88.9	ACLF-3

Adapted from [2]
BD brain dysfunction, *KD* kidney dysfunction, *KF* kidney failure

outcome. Presenting with a Maddrey's Discriminant Function (mDF) ≥32 [9], it is the most life-threatening form of alcohol-related liver diseases (ALD), a clinical entity defined as severe alcoholic hepatitis (SAH) [10]. The occurrence of ACLF is frequent during SAH. ACLF can be identified at the time of first presentation with AH but can also develop during the course of medical management of this disorder [11]. From the outline above, the rates of survival at 28-days and 3 months, is related to the severity of ACLF at presentation.

This review will briefly describe the main pathophysiological factors and organ immunopathology known to be associated with AH-related ACLF and explore how these are restored in relation to recovery of organ function either spontaneously or with liver transplantation.

Pathophysiological Basis of AH-Related ACLF and its Evolution during Recovery

This section describes the underlying pathophysiologic mechanism associated with ACLF and explores whether targeting the underlying mechanisms can lead to the recovery from ACLF. Although not all the data described here are from AH-associated ACLF, most can be validated even in this group.

Systemic Inflammation

The accumulating understanding of the pathophysiological basis of ACLF indicates that a systemic inflammatory state is the main driver of widespread tissue damage and organ dysfunction, a state that is overexpressed in AH-related ACLF [12, 13]. The term "immunopathology" refers to the immune-mediated tissue damage that

can be caused by effectors of systemic inflammation such as cytokines and chemokines. Proteases, oxidative molecules, cytotoxic cytokines, prostaglandins (PGs), and leukotrienes (LTs), among other mediators, are consequently released by activated immune cells, leading to further worsening of the tissue damage [14].

Two main components have been observed to drive this intense systemic inflammation in ACLF, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [13]. PAMPs represent molecular structures that are expressed by different pathogens and microbial agents. In contrast, DAMPs are circulating intracellular molecules following death or damage of the host cells, albeit without infection as a trigging agent. PAMPs and DAMPs bind to specific Pattern Recognition Receptors (PRRs) such as Toll like Receptors (TLR) located on peripheral innate immune cells [15]. Receptor binding activates down-stream signalling pathways, leading to the increased transcription and release of inflammatory cytokines with induction of severe systemic inflammation [16].

Taking the alcohol model into account, AH-related ACLF can exhibit both sterile and non-sterile pathways of systemic inflammation. Chronic alcohol consumption can lead to disturbance of the gut microenvironment in the form of intestinal barrier disruption, dysbiosis and impaired release of antimicrobial peptides [17–19]. The defective gut barrier permits translocation of bacterial PAMPs, particularly lipopolysaccharide (LPS) which could then reach the liver where they are recognized by TLRs expressed in hepatic Kupffer cells (see also Chap. 61). The end result is induction of inflammation with over production of proinflammatory chemokines and cytokines such as IL-8 and TNF-α [8, 20]. LPS-induced TNF-α may cause hepatocyte necrosis [8] resulting in the release of DAMPs that further aggravates the already existing inflammation. ACLF is characterised by a cytokine storm where there are markedly increased pro-inflammatory (TNF, IL-6, IL-8) and anti-inflammatory (IL-10, IL-1RA) cytokines, soluble markers of macrophage activation (sCD163 and mannose receptor) [21], C-reactive protein, and white blood cells in the plasma of ACLF patients [2]. The importance of systemic inflammation is highlighted by the temporal clinical correlates with the severity of systemic inflammation observed. Patients who showed clinical improvement of their disease had decreasing levels of these markers compared to increasing levels among those who were deteriorating [13].

The next important question is whether targeting the intense systemic inflammation at various levels would influence the outcome of ACLF syndrome. To answer this question, ACLF-simulating rodent models were used to study the therapeutic benefits of TAK-242, a molecule known to have inhibitory effects on TLR4 activation, a key component in driving systemic inflammation. This study showed that inhibiting TLR4 signalling with TAK-242 ameliorated organ injury and systemic inflammation [22]. One of the key PAMPs that plays a pivotal role in ACLF development is LPS. Targeting LPS with recombinant alkaline phosphatase in rat model of bile duct ligation with LPS co-administration has shown significant reduction in TLR4 expression with improvement of the systemic inflammation and organ recovery [23]. Blocking DAMPs signalling is another method of modulating the systemic inflammation (see later).

These findings suggest that there are three key characteristics in the relationship between systemic inflammation and ACLF. First, the development of ACLF is associated with intense systemic inflammation. Second, this relationship seems to be dynamic rather than static, with improvement in systemic inflammation being parallel to clinical improvement in ACLF and vice versa. Finally, the course and prognosis of the illness can be improved by using agents that target certain inflammatory checkpoints.

Immune Deficiency

In addition to the intense systemic inflammation in ACLF, the syndrome is also associated, paradoxically, with immune deficiency at both humoral and cell mediated levels [24, 25]. Three main factors are implicated in the dysfunctional innate immune response in patients with liver cirrhosis; reduced production of acute phase proteins, defective complement system and hypoalbuminemia, all of which are features of hepatocellular insufficiency [26, 27]. In ACLF, the situation is even worse with impaired gut barrier and, circulatory and endothelial dysfunction adding more to the already existing defect [14].

The immune status in ACLF patients is not exclusively restricted to one phenotype, rather it exhibits a spectrum fluctuating from immunosuppressed, immunomodulated, tolerogenic to hyperinflammatory prototypes. The fact that this cohort of patients are living with nearly constant hyperinflammatory state with over production of inflammatory mediators (e.g., galectin-3, IL-6, TNFα, IL-10) [13, 28] and lipid mediators (e.g., PGE2), which can lead to restraining of the innate immune response and giving the steering wheel to immunomodulatory cells with subsequent immunosuppression [29, 30] indicates that the two states can coexist within the same patient at the same time.

Therapeutic interventions targeting gut dysbiosis may provide a clue to induce recovery of the immune paralysis in ACLF. In experimental models as well as human studies, intestinal cleansing with non-absorbable antimicrobials was associated with restoration of dendritic cell function and TNF-α production [31]. This approach is currently being investigated in patients with decompensated cirrhosis to evaluate safety and efficacy of using rifaximin and simvastatin in prevention of ACLF development (LIVERHOPE project, EU H2020). Yaq-001 is an oral non-absorbable synthetic carbon compound which showed promising results in animal studies investigating its effects on the gut microbiome. This study also demonstrated that the use of Yaq-001 was associated with positive impacts on monocyte function [32].

The high energy demanding systemic inflammatory state in ACLF is not met by equivalent energy production, due to mitochondrial dysfunction with subsequent metabolic switch to the cytosol [33]. This metabolic switch is associated with implications on glutamine, which is known to be involved in maintaining immune system

health. In vitro inhibition of glutamine synthesis was associated with recovery of dysfunctional monocytes in ACLF [34].

Disturbed phagocytic and bactericidal functions of monocytes was found to be correlated with higher expression of MERTK pathway in ACLF patients. The ex vivo function of the monocytes was restored by pharmacological inhibition of MERTK with UNC56915 (Calbiochem/Millipore, UK) [28]. Similar defects were identified in the bactericidal properties of neutrophils from ACLF patients. These defects were attributed to impaired N-formylmethionine-leucyl-phenylalanine-induced myeloperoxidase release due to defective AKT (protein kinase B)–p38 MAPK (mitogen-activated protein kinase) pathway signalling. Ex vivo activation of the TLR7/TLR8 pathway using the agonist CL097, could overcome these defects, and induce recovery of the neutrophilic functions [35].

The idea of simulating the immune paralysis using serum from ACLF patients, and the in vitro restoration of cell function utilising immunological targeted techniques raise the possibility that cell reprogramming-induced immunodeficiency may be reversible and point to novel therapeutic targets [36].

Portal Hypertension

Reduced activity of hepatic endothelial nitric oxide synthase (eNOS) eventually leads to increased hepatic vascular resistance [37]. This phenomenon is exaggerated in ACLF, since systemic inflammation can induce endogenous eNOS regulatory proteins, such as NOSTRIN, caveolin-1, and asymmetric dimethylarginine (ADMA) [38–40]. The resulting increase in hepatic vascular resistance promotes rise in the portal pressure with decreased hepatic blood flow in patients with ACLF compared to those with alcohol related stable or decompensated cirrhosis without ACLF [41]. Additionally, reducing systemic inflammation by blocking TNFα with infliximab in AH patients resulted in significant reduction in portal pressure [42]. The PREDICT study provided evidence that portal hypertension is important in the pathogenesis of ACLF [43]. However, it is unclear at present whether the increased portal pressure observed in ACLF is a cause or a consequence.

Metabolic Dysfunction

Significant alterations in the main metabolic pathways as evidenced by accumulating levels of certain blood metabolites in the course of systemic inflammation in ACLF patients provide further understanding of the role of metabolic dysfunction in general and mitochondrial dysfunction in particular in the pathogenesis of ACLF syndrome, and their contribution to the development of organ failures [33].

Defective urea cycle enzymes with significant portosystemic shunting can lead to increased plasma levels of ammonia in patients with liver cirrhosis [44]. In

addition to neurotoxicity, hyperammonaemia has been found to be involved in immune dysfunction, activation of hepatic stellate cells proliferation and sarcopenia [45, 46]. In patients with AH, increased blood ammonia at admission was an independent predictor of in-hospital mortality rates [47]. Lowering blood ammonia levels has been shown to improve survival in ACLF patients regardless the presence of HE [48], indicating that ammonia level is not only correlated with organ failure, but also with ACLF prognosis.

Organ Immunopathology

Liver

It has been shown that circulating markers of hepatocyte death are markedly elevated in AD and continue to rise as ACLF progresses. Even though apoptosis occurs in ACLF, several studies demonstrated that other non-apoptotic forms of cell death that are known to be more immunogenic, predominate [22, 23, 49]. It was therefore not surprising that targeting apoptosis in patients with ACLF was not successful [50]. The inflammasome, which cleaves and activates gasdermin proteins, is responsible for the activation of the caspase family of proteins during cell pyroptosis, a type of immunogenic programmed cell death [51]. This pathway has been shown to be activated both in patients as well as models of ACLF [52]. Strategies inhibiting this pathway using limonin or disulfiram in experimental models promoted liver recovery and cell death reduction [53, 54]. The other non-apoptotic, immunogenic pathway that is observed in ACLF is necroptosis. Plasma levels of RIPK3, a marker of necroptosis was elevated in ACLF and could be used to identify patients transitioning from “no ACLF” to “ACLF”. Inhibition of RIPK1 activity in a rodent model of ACLF prevented hepatocyte death [55]. These findings provide multiple levels of evidence not only about mechanisms of liver injury in ACLF, but also about the potential therapeutic targets that may improve recovery and survival in these patients.

Kidneys

Renal dysfunction in the context of ACLF seems to be complex whereas pre-renal causes or hepatorenal syndrome may be implicated in some cases, acute tubular necrosis is the predominant underlying pathology in others [56, 57]. The presence of renal dysfunction in ACLF was correlated with higher levels of systemic inflammatory markers rather than plasma renin concentrations, a marker for systemic circulatory disturbance, suggesting two important concepts; firstly the deleterious effects of systemic inflammation on the kidneys is mediated predominantly by non-hemodynamic mechanisms [58], and secondly renal dysfunction in ACLF patients is more likely to be organic rather than functional disorder. Renal histology in ACLF

patients showed significant tubular cell death, which was associated with increased renal expression of TLR4 in humans [59], and RIPK1 and RIPK3 in animal models. Targeting RIPK1 was protective against renal tubular cell death, [55]. These observations help to explain the higher likelihood of non-response to terlipressin and albumin and potential irreversibility of kidney injury [60] if the acute tubular necrosis does not recover in weeks.

Brain

Although data from human studies are lacking, it has become clear that the pathogenesis of hepatic encephalopathy is complex, and although hyperammonemia and systemic inflammation are the key underlying mechanisms, neuroinflammation and neuronal cell death are important features specially in ACLF [61, 62]. Additionally, in about 5% patients with ACLF, severe cerebral oedema, indistinguishable from that seen in acute liver failure can be found [63]. Recovery from ACLF either spontaneously or with liver transplantation leads to resolution of brain dysfunction but complete reversibility is questionable [64–67].

Reduction in ammonia concentration and severity of inflammation are key metrics defining recovery. Drugs targeting ammonia such as ornithine phenylacetate and L-ornithine L-aspartate have been shown to result in faster resolution of hepatic encephalopathy, which correlates with the reduction in ammonia levels [68–70]. In those with refractory hyperammonaemia, veno venous hemofiltration as a method of removing ammonia [71] should be considered to enhance recovery.

Circulation

Systemic vasodilatation with reduction of systemic vascular resistance and mean arterial pressure (MAP), are frequently encountered in ACLF patients [41]. Cardiac dysfunction is manifested by inability to increase cardiac output despite fluid resuscitation and the attendant vasodilation. Sepsis, in addition to sterile inflammation together impact on endothelial function to produce systemic hypotension [72]. Although the mainstay of therapy are vasoconstrictors using noradrenaline and fluid expansion with albumin, removal of circulating inflammatory molecules with plasma exchange or other liver support devices such as molecular adsorbents recirculating system reduces inotrope requirements and improves blood pressure [73–76]. This argues for better understanding of mechanisms underlying hypotension to allow recovery. The mechanism underlying cardiac dysfunction is not clear and there are no available strategies to enhance cardiac contractility. It is uncertain if cardiac dysfunction persists or recovers completely with recovery from ACLF.

Respiration

The inflammatory sequelae of ACLF and/or lung infections contribute to respiratory failure in the ACLF population. Proinflammatory cytokines and nitric oxide hyperproduction could participate in the high incidence of acute respiratory distress syndrome (ARDS) observed in ACLF [77]. Alterations of consciousness due to hepatic encephalopathy increase the risk of aspiration pneumonia. Tense ascites reduces basal lung expansion [78]. Ventilation with low tidal volume strategy seems to be the best approach to manage ARDS related respiratory failure in ACLF [79]. Even with the best practice, ACLF patients with respiratory failure requiring mechanical ventilation still have considerable 1-year mortality rate (89%) [80].

The one-year post transplant survival in patients with ACLF grade 3 who did not require mechanical ventilation before liver transplantation is 85.4% compared to 75.3% among those who required it, suggesting that the need for mechanical ventilation at time of liver transplantation can significantly influence the outcomes after liver transplantation [81]. Whether ACLF itself contributes to respiratory failure or it is a consequence of critical illness is unknown and whether it recovers fully with resolution of ACLF requires more studies.

Coagulation

Coagulation status in patients with liver disease is governed by a balance between procoagulant and anticoagulant pathways, a balance that is lost in ACLF. The predominance of one pathway over the other depends on many factors and superimposed conditions [82]. As sepsis and systemic inflammation are often closely associated with the syndrome, thrombocytopenia due to consumption and disseminated intravascular coagulation are features of the syndrome [83, 84]. Whether endothelial dysfunction and release of tissue factor to drive this is the mechanism remains to be determined. A shift of the coagulation profile towards greater risk of bleeding is manifest particularly when the patients are placed on extracorporeal systems or undergo invasive procedures. [83, 85, 86].

Assessment of the coagulation status in patients with liver diseases using the standard laboratory tests (PT, APTT, INR, and bleeding time), has some limitations in terms of accurate measurement of the potential risk of bleeding [87], which may have implications on clinical decision making in critical situations. Therefore, incorporating viscoelastic tests into clinical practice can minimize the unnecessary transfusion of coagulation factors and other blood products based on the accurate data obtained [88]. In general terms, the severity of coagulopathy tends to improve with treatment of sepsis [89] and resolution of ACLF but coagulation disturbance consequent on synthetic failure of the liver remains.

Considerations in the Management of AH-Related ACLF Allowing Recovery

General Outline

The current approach to the management of AH-related ACLF is primarily based on identifying the underlying disease, precipitating factor (severe AH in our case), type and severity of organ dysfunction/failure and treat accordingly. The patients should be managed in an enhanced care area, a high dependency unit or an ICU, dependent on their requirement for organ support [90]. The general organ support strategies will not be described here except to highlight the importance of early, aggressive monitoring for infection and rapid treatment using broad spectrum antibiotics according to pre-defined local practice [91, 92].

Specific Strategies

Steroids

The current guidance for management of SAH recommends prednisolone as a first line treatment [93]. This guidance mainly relies on the results of meta-analyses investigating the value of steroids as a rescue therapy in this life-threatening condition [94, 95]. A large randomized controlled study (STOPAH) concluded that irrespective of using corticosteroids, the 90-day mortality for patients with SAH was about 30% [96], suggesting that there was no added survival benefit from using steroids in this cohort of patients. Whether corticosteroids improve the short-term survival of patients with SAH complicated by ACLF is unclear [97]; current data suggest a lower rate of response in patients with ACLF grade 2 and 3 (42 and 8%, respectively) [96, 98]. On the other hand, the risk of infection increases markedly with increasing severity of ACLF leading many to consider ACLF a contraindication to steroids [99].

Granulocyte Colony Stimulating Factor (G-CSF)

G-CSF is produced mainly by monocytes, macrophages, endothelial cells, fibroblasts, astrocytes, and several immune cells in response to injury or infection [100]. Impaired liver regenerative capacity is the main driver for the use of G-CSF in patients with SAH [101]. The therapeutic effects of G-CSF have been examined in several small single centre studies, some of which have shown overwhelming beneficial effects [102]. However, in a large, multicentre, carefully monitored clinical trial, G-CSF failed to confirm these previous data suggesting that G-CSF should not be used for the treatment of ACLF outside clinical trials [103].

Extracorporeal Liver Support (ELS)

The result of the systemic inflammation and immune dysfunction in patients with ACLF is accumulation of toxins and cytokines, which drive organ dysfunction/failure. If these toxins and cytokines can be removed via a liver support system, this may theoretically aid in replacing or complementing the work of a failing liver [104]. This idea to some extent, is similar to the concept of ECMO or renal dialysis, where the ultimate goal is to prolong the patient survival [105]. Based on this mechanism, there are two types of ELS systems available, artificial system (MARS, Prometheus, SPAD and HepaWash) and bioartificial system (HepatAssist and ELAD) [74, 106, 107]. The findings from studies on the artificial systems have shown promising results, where MARS was associated with improved outcomes in patients with grade 3 and 4HE, better short-term transplant free survival and improved biochemical parameters. In patients with MELD score > 30, Prometheus system has been proven to significantly improve the survival at both 28 and 90 days [108]. However, neither the artificial nor the bioartificial devices have been shown to improve overall survival of patients with ACLF, therefore further studies are needed prior to implementation in current clinical practice [109].

Albumin

Albumin use in spontaneous bacterial peritonitis, HRS and large volume paracentesis, has been associated with reduction of hypovolemia related complications and mortality [110]. Besides, albumin is well known to have other non-oncotic features such as homeostatic, antioxidant, immunomodulatory and endothelial stabilizing and toxic binding capabilities [111]. These unique features of albumin have made its use in ACLF become an interesting research area. However, clear data about the dosage, duration and frequency of administration are still lacking in patients outside of these conditions [112].

INFECIR-2 study provides novel insights into the effect of administration of human albumin on systemic inflammation in patients with decompensated cirrhosis [113], but the ATTIRE study did not improve clinical outcomes when albumin was used to treat acutely decompensated cirrhosis [114]. The ANSWER study, in which the aim was to prevent acute decompensation using long-term albumin administration, survival benefits were observed [115]. The MACHT study on the other hand, in which long term albumin was trialled, was negative for survival [116]. However, both studies provided useful data on the dose and frequency of albumin administration to attenuate systemic inflammation and improve cardiocirculatory dysfunction. The PRECIOSA study, which is currently running, is powered to determine the actual role of long-term albumin therapy [117]. However, implementation of this approach in real-world settings will be challenging given the cost and utilization of clinical services [112].

Liver Transplantation

If multiorgan supportive efforts fail to achieve clinical and biochemical improvement, liver transplantation would represent the last solution available for patients with ACLF. The overall 5-year survival rate after liver transplantation ranges between 74 and 90% [118]. The main challenge is the pre-transplant disease severity where multiple organ failure and bacterial infections usually supervene, leading to high mortality rate within the waiting list reaching about 50% [119].

The rate of delisting or mortality within 28 days of listing is about 44% in patients with ACLF grade 3. These findings should raise many questions about the best time to transplant those severely ill patients. Early liver transplantation within 30 days in this cohort, is associated with significant improvement in overall survival compared to the standard supportive care. The decision of when to transplant these sick patients is governed by many factors. However, the most important equation here is to weigh the benefit of waiting for good quality grafts against the risk of mortality while waiting for too much [81].

Looking further into the long-term outcomes of this intervention, analysis of the United Network for Organ Sharing (UNOS) registry for liver transplantation from 2004 to 2017 revealed that post-transplant 5-year survival was lowest among patients with ACLF grade 3 (67.7%) compared with the other patient groups (75%-79%, $P < 0.001$). However, the majority of deaths amongst ACLF grade 3 patients occurred during the first year post-transplant and then became comparable with other groups for the following 4 years [120]. These findings suggest that patients with ACLF grade 3 continue to have high mortality until 1 year post transplantation [120]. In the ELITA/EF-CLIF collaborative study, it was estimated that 1-year post-transplant survival rate in ACLF patients was about 80%, reflecting the importance of liver transplantation as a rescue therapy in this cohort of patients with high short term mortality rate. Poor prognostic factors that could be identified in this large international multicentre study included serum lactate >4 mmol/L, need for renal replacement therapy at time of liver transplantation and MDRO infections while on the waiting list [121].

Although the most widely accepted practice for liver transplantation in ALD follows an alcohol abstinence interval strategy, early liver transplantation in SAH has been proved to improve the clinical outcomes and survival [122, 123]. In ideal world, a decision to transplant a patient with SAH should be preceded by clear determination of unsatisfactory spontaneous recovery probabilities to avoid the unnecessary risks associated with LT. In a single-centre retrospective study, younger age, lower index INR, and lower peak MELD scores were found to be associated with higher likelihood of spontaneous recovery from SAH [124]. However, relying on MELD score may underestimate the risk of mortality in severely ill ACLF patients with multiple extrahepatic organ failure [125, 126]. On the other hand, identification of the risk of mortality based on ACLF grade, can provide a prognostication tool in patients with SAH [98], which can aid in better prioritisation in the setting of organ allocation.

Future Perspectives

The concept of ACLF as a distinct clinical entity has been validated world-wide with emerging clarity on the diagnostic and prognostic criteria for this syndrome. Alcoholic hepatitis is the most important cause of ACLF in the western world. With this clinical characterisation of the syndrome has emerged a deeper understanding of the pathobiology, which has led to the recognition of several therapeutic targets. The importance of the gut in shaping the immune dysfunction of cirrhosis and knowledge about the intestinal microbiome led to studies targeting the gut using Yaq-001, fecal microbiota transplantation and bacteriophages [32, 127–129]. The role of cell transplantation is maturing, and clinical trials of mesenchymal and hepatic progenitor cells are underway [130, 131]. The failure of the MARS and ELAD devices and the better understanding of the pathophysiology has led to the development of novel liver support devices such as DIALIVE and Cytosorb, which have shown promise in early studies [132, 133]. Finally, liver transplantation, a treatment approach for the sickest patients shows huge promise but there are many unanswered questions. These are being addressed in the CHANCE study (NCT04613921), which is a global multicentre international study.

In conclusion, when patients with SAH fulfil ACLF criteria, their prognosis is determined by organ failures, and it is likely that the novel therapeutic approaches targeting multiple domains in ACLF will also be effective on SAH patients with ACLF.

Conflict of Interest Rajiv Jalan is the inventor of OPA, which has been patented by UCL and licensed to Mallinckrodt Pharma. He is also the founder of Yaqrit Discovery, a spin out company from University College London, Hepyx Limited and Cyberliver. He had research collaborations with Yaqrit Discovery. The other author has no conflicts of interest to declare.

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Chapter 68
The Role of Liver Biopsy and Hepatic Venous Pressure Gradient in the Prognosis of Acute Alcoholic Hepatitis

Andreea Bumbu and Bogdan Procopet

Abstract The use of liver biopsy in diagnosing alcoholic hepatitis has been controversial. Usually is used via the transjugular approach due to frequent ascites and coagulation abnormalities. Due to low availability and the risk of complications, the real-life applicability is questionable. However, apart from the diagnostic certainty, the histology feature has prognostic relevance. The main counterargument against liver biopsy would probably be that treatment decision relies on laboratory findings demonstrating disease severity. Finally, the most considerable responsibility is the correct diagnosis and avoiding unnecessary corticosteroid treatment in patients without alcoholic hepatitis. Using only clinical criteria is challenging, especially because patients with severe alcoholic hepatitis associate bacterial infection and both conditions may precipitate acute-on-chronic liver failure. Recently, the proposal to classify the condition as definitive, probable or possible alcoholic hepatitis could standardize the clinical practice for diagnosis. Apart from biopsy, portal hypertension is essential in developing complications. The standard method for portal hypertension diagnosis is hepatic venous pressure gradient, which was extensively validated as a prognostic marker in liver disease. In alcoholic hepatitis, there is a single study that demonstrates the prognosis relevance in patients with alcoholic hepatitis. In these patients, an HVPG higher than 22 is associated with increased mortality.

Keywords Transjugular liver biopsy · Prognosis · Advanced liver disease · HVPG · Diagnostic accuracy

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Liver Biopsy: The Debate Is Still Open

The role of liver biopsy in diagnosing and managing alcoholic hepatitis (AH) has been controversial throughout the years. The main counterargument to its routine use as a precise diagnostic tool is its low availability of the transjugular approach, performed mainly in tertiary centers [1]. Therefore, even though the guidelines for clinical practice recommend it as the golden standard for the definitive diagnostic of AH, the real-life applicability is questionable. The later EASL guideline softened the indication for the cases of diagnostic uncertainty or if the disease staging must be precise, as in clinical trials (grade A1) [2]. However, the diagnostic certainty based only on clinical and paraclinical criteria is illusory because the clinical picture of decompensated alcoholic liver cirrhosis and severe alcoholic hepatitis are perfectly superposable and sometimes associated.

Transjugular liver biopsy is usually the preferred route because, frequently, these patients manifest severe ascites and prolonged conventional coagulation tests. Although it is well tolerated, it remains an invasive procedure, and periprocedural complications can occur. However, significant complications that include intraperitoneal bleeding through capsular perforation, cardiac arrhythmias, perforation of the hepatic artery, pseudoaneurysm, or haemobilia are rare, accounting for 0.5–0.8% of the cases. The most frequent minor complications are bleeding at the puncture site or abdominal pain [3, 4]. Recent reports are consistent with previous publications. Thus, a recent study on 1321 TJLBs showed an overall major and minor complication rate of 1% and 9.5%, respectively. There was no difference in the incidence of minor and major complications between different subgroups of patients classified according to varying ranges of platelets and INR [5]. This follows the new evidence that conventional coagulation tests do not correctly reflect the hemostasis of patients with advanced liver diseases [6].

The suspicion of AH diagnosis is based on clinical criteria, which involve the recent onset of jaundice in a patient with a recent history of alcohol heavy abuse, combined with the laboratory findings. The most typical laboratory features are hyperbilirubinemia, increased transaminases with the dominance of AST more than twice the level of ALT, but usually not higher than 300 UI/L, and neutrophilia [2]. Over the years, the severity of the condition was assessed based on a different combination of variables related directly to liver function. The oldest severity score, the Maddrey discriminant function (DF) based on bilirubin and prothrombin time, is still recommended to select patients that could benefit from corticosteroids [7]. Newer scores, such as Model for End-stage Liver Disease (MELD) [8], Glasgow Alcoholic Hepatitis Score (GAHS) [9], or the Age, serum Bilirubin, INR, and Creatinine (ABIC) score [10], proved to be superior to DF by adding variables associated with short term prognosis [11]. Once the patients were under corticosteroid treatment Lille model, based on the early dynamic of the bilirubin after 7 days of treatment, prevents continuation of the treatment in patients without response [12, 13].

The certainty of the diagnosis is given by liver biopsy. The histological diagnosis is based on steatohepatitis, which typically includes microvesicular steatosis, hepatocyte ballooning, and polymorphonuclear neutrophils (PMN) infiltrate [2]. The histology features proved to have prognostic relevance, either in the short term, as the type of bilirubinostasis, presence of megamitochondria or neutrophils infiltration [14, 15], or in the long term, as the stage of fibrosis [16]. At the moment, two histology scores have been developed, the Alcoholic Hepatitis Histologic Score (AHHS) [14] and the Study of Alcohol-related LiVer disease in Europe (SALVE) histology system [15], and both seem to have prognostic relevance.

In this context, it would be relatively simple to follow an algorithm starting from the clinical suspicion of severe alcoholic hepatitis, confirming the diagnosis by liver biopsy and, according to the histology findings, to step further to the treatment. However, this algorithm is rather exceptional, being applied in expert research centers or the context of clinical trials. There is an ongoing debate about whether the biopsy is essential in managing severe AH. Maybe the most justifying and straightforward argument against liver biopsy is that the diagnosis and the severity are suspected based on laboratory findings. Moreover, the decision for the corticosteroid treatment and the assessment of the treatment's response is based mainly on the bilirubin levels. Thus, the liver biopsy would not influence the treatment decision despite the prognostic relevance of some histology features. The discussion is still open because the performances of the clinical criteria for an accurate diagnosis are not perfect. Differentiating between severe AH and decompensated cirrhosis seems impossible, and 25–30% of the clinically suspected patients have an alternative diagnosis on liver biopsy [17, 18]. The proportion of patients that may receive corticosteroid treatment without AH is unacceptable, especially knowing that corticosteroid treatment is associated with an increased risk of bacterial infections [19]. Moreover, 25–45% of patients with decompensated cirrhosis present with bacterial infections at admission [20, 21]. In the studies reporting insufficient accuracy, the clinical diagnostic criteria were not the most appropriate. In the study of Mookerjee et al., the clinical suspicion of AH was based on the SIRS criteria, and the overall accuracy was only 54%, with only 50% of the patients with SIRS criteria having AH and 40% of the patients without SIRS criteria having AH on liver biopsy [18]. It is not surprising that in the same study, the authors found that in patients with SIRS criteria but without AH, canalicular cholestasis was associated with bacterial infections and, consequently, with a worse prognosis. It is well known that both AH and bacterial infection trigger systemic inflammation and may precipitate acute-on-chronic liver failure (ACLF); therefore, SIRS criteria are an inappropriate diagnostic tool. An editorial to the Mookerjee et al. paper suggested that when applying clinical criteria, increasing the bilirubin threshold from 50 to 80 μmol/L will increase accuracy from 70–80 to 96% for AH diagnosis [22]. The rationale is that hyperbilirubinemia is the main feature that differentiates the AH from decompensated cirrhosis. In fact, higher bilirubin thresholds were used in trials where a biopsy was not a prerequisite for entry to the trial, as in the most recent large randomized trial (STOPAH trial) [23]. In this trial, 1103 patients were randomized to receive either prednisolone or pentoxifylline. While pentoxifylline did not improve

survival, prednisolone was associated with a reduction in 28-day mortality that did not reach significance and no improvement in outcomes at 90 days or 1 year. This is the most extensive negative trial that increased the doubts about the efficiency of the corticosteroid treatment, but is this enough to ban this treatment in severe AH? One of the significant drawbacks in all the studies dedicated to AH, including RCTs, is the heterogeneity of the included populations [24], and the STOPAH trial makes no exception. When reviewing the data, an essential hint regarding the population selection is to look closely at the placebo arms, which should reflect the natural history of the disease. Let's compare the populations in the STOPAH trial with the patients included in the individual data meta-analysis of Mathurin et al. [25]. They are pretty similar in terms of mean bilirubin or DF, even a little more severe in the STOPAH trial [62.6 ± 27.2 vs. 48.5 (45.5–51.3)]. However, the 28-day mortality rate in the placebo arms is 17% in the STOPAH trial vs. 38% in Mathurin's study. This means the failure of the STOPAH trial to correctly identify the patients with severe AH, despite the use of clinical criteria with a higher bilirubin threshold. There is no obvious explanation for this difference, and the improvement in healthcare over the years, including the management of malnutrition, is not enough. At least in part, a possible explanation would be the inclusion of patients without AH in the absence of liver biopsy. In our view, these results advocate for the mandatory use of liver biopsy in therapeutical trials, despite the risk of reducing the number of patients potentially included.

An excellent solution to overcome the risk of heterogeneity regarding the population to be included is the standardization proposed by The National Institute on Alcohol Abuse and Alcoholism (NIAAA)-funded Alcoholic Hepatitis Consortia as **Definite AH** (clinically diagnosed and biopsy-proven), **Probable AH** (clinically diagnosed AH without confounding factors) and **Possible AH** (clinically diagnosed but with potential confounding factors) [26] (see also Appendix Figs. A.9 and A.10).

The following clinical criteria proposed by NIAAA to diagnose alcoholic hepatitis (AH) in clinical trials, as outlined in reference [26]:

- Jaundice that has developed within the previous 8 weeks.
- Consumption of more than 40 g of alcohol per day for women or 60 g of alcohol per day for men, for at least 6 months, with less than 60 days of abstinence prior to the onset of jaundice.
- Aspartate aminotransferase (AST) levels greater than 50 IU/L, a ratio of AST to alanine aminotransferase (ALT) greater than 1.5, and both AST and ALT values below 400 IU/L.
- Total serum bilirubin levels greater than 3.0 mg/dL.
- In patients with confounding factors, confirmation of the diagnosis with a liver biopsy may be necessary.

Interobserver variability is a relatively constant counterargument against liver biopsy in diagnosing liver diseases. The data is limited regarding the diagnosis and assessment of the AH's histological severity. There is only a fair level of agreement

between the pathologists regarding the inclusion in different AHHS [14] for prognostic stratification of AH categories [27]. The lowest agreement was reported for identifying megamitochondria, while the highest was in fibrosis and steatosis. The standardization of the histological diagnosis using validated staging and grading scores should be helpful in clinical practice. It should also overcome the heterogeneity of the diagnosis criteria in clinical studies. Recently, the Study of Alcohol-related LiVer disease in Europe (SALVE) consortium proposed a new grading and staging system for AH that standardize the pathological report [15]. This new system seems reproducible and prognostically relevant for the histological assessment of disease activity and fibrosis in ALD.

In recent years, many non-invasive tools, especially liver and spleen elastography, have been validated for staging liver disease, diagnosing clinically significant portal hypertension, and assessing these patients' prognoses [28]. Because liver fibrosis, intrahepatic inflammation [29], and alcohol consumption increase liver elastography [30], using elastography to diagnose AH was not helpful. Moreover, due to ascites for the vibration-controlled transient elastography (VCTE), the feasibility is lower than other elastography methods [31]. The lack of non-invasive methods also represents an argument for using liver biopsy in the scenario of AH.

Patients without clinical criteria of severity (a discriminant function <32) and who would not need corticosteroid treatment but with histology of AH represent a particular subgroup of patients. A high proportion of them has a histology score of severe AH [32]. While the short-term mortality is very low (around 5%) compared to those with DF > 32, the long-term mortality is considerable, 20 and 50% at one and 5 years, respectively. Interestingly, the severity of the histology score does not predict survival. Still, in patients with an AHHS of 0–3, the 5 years mortality was 30%, while in those with AHHS >3, the mortality was around 50%. The factors independently associated with long-term survival were the presence of encephalopathy at baseline and long-term abstinence. There is a need for more data on this subgroup of patients and whether they should receive treatment, but what is certain is that they cannot be identified without biopsy.

Finally, besides the drawbacks and disadvantages of using biopsy in AH, there are certain benefits. Table 68.1 lists the main advantages and disadvantages of using liver biopsy in AH. It should be recommended in patients with possible AH with any atypical features or concurrent factors. To prevent confounding factors and

Table 68.1 The arguments and counterarguments for using liver biopsy in alcoholic hepatitis

The use of liver biopsy in alcoholic hepatitis	
Advantages	Disadvantages
Certainty of diagnosis	The transjugular approach is not widely available
Prognostic relevance	Percutaneous rarely possible
Concomitant HVPG measurement	Invasive
Lack of non-invasive methods	Risk of complications
	Do not contribute to treatment decision

heterogeneity, liver biopsy should be required in clinical trials. Moreover, serial biopsy in abstinence could give hints about disease regression and regeneration, which could represent a therapeutic target.

Hepatic Venous Portal Gradient: A Superstar in Liver Diseases but Still a Cinderella for Alcoholic Hepatitis

Hepatic venous portal gradient (HVPG) is the best method to diagnose portal hypertension (PHT). Over the years was extensively validated against hard clinical endpoints and is now considered a robust surrogate marker for the prognosis [33]. Any factor influencing the HVPG would be reflected in a change in prognosis, making the changes in HVPG a reliable end-point therapeutic trial [34]. An HVPG less than 5 mm Hg is considered normal, while a value between 5 and 10 mm Hg is considered mild PHT. HVPG over 10 mm Hg represents clinically significant portal hypertension (CSPH), which is associated with the risk of decompensation [35]. HVPG measurement can be performed easily together with TJLB without increasing the risk of complications.

Until now, only one study investigated the role of HVPG in patients with AH [36]. In this study, 60 patients with AH were compared with 66 patients with advanced alcoholic and viral cirrhosis listed for transplantation. Patients with AH had significantly higher HVPG values than cirrhosis, and an HVPG value higher than 22 mm Hg was associated with increased mortality among AH patients. A straightforward explanation for higher HVPG is AH could be the intrahepatic inflammation that characterizes the hepatitis process. However, there is no correlation between the degree of inflammation, fatty change, or occurrence of Mallory bodies [36]. These findings were also confirmed by Altamirano et al. They found no differences in HVPG measurements between patients with a definitive AHHS $\geq$5 points and a definitive AHHS <5 points (19.0 ± 6 mm Hg vs. 19.7 ± 6 mm Hg, respectively) [14].

Let's go back to the pathogenesis of the PHT. The main factors that increase the portal pressure are the amount of fibrosis (the mechanical compound) and the increase in intrahepatic vascular resistance (the dynamic compound) [37]. There is evidence that the thickness of fibrous septa is well correlated with HVPG [38–40]. Therefore, probably the new grading and staging proposed by the SALVE consortium [15], which classifies the cirrhosis stage according to the Laennec classification, could bring hints about the mechanism of very high HVPG in patients with AH. On the other hand, the dynamic compound should not be ignored in this context. AH is characterized by marked bacterial translocation and liver cell necrosis that will increase the systemic inflammation through the PAMPs and DAMPs pathway, in particular through TLR4 signaling, which will further activate hepatic stellate cells and will aggravate the endothelial dysfunction and finally will lead to an increase intrahepatic vascular resistance [41]. This hypothesis is of great interest

because intrahepatic vascular resistance may represent a therapeutic target in patients with AH.

Although there is limited data, it seems that HVPG correlates well with the prognosis of patients with AH. Further studies are needed to evaluate the mechanism of portal hypertension in these patients and whether it could be a therapeutic target. HVPG may be easily obtained during TJLB, thus, offering the possibility of having two standard methods for the diagnosis and the prognosis of patients.

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Part XI
Alcohol-Related Damage of Other Organs

Chapter 69
Skeletal Muscle and Adipose Tissue: Targets or Relays for Interorgan Axis in Alcohol-Induced Tissue Injury?

Liz Simon, Brianna L. Bourgeois, Jonquil M. Poret, and Patricia E. Molina

Abstract At-risk alcohol use is an independent risk factor for liver disease and type 2 diabetes and synergizes with an obesogenic environment additively increasing the risk of cardiometabolic disease. At the core of metabolic dysregulation is alcohol-induced cellular injury of the liver, pancreas, skeletal muscle (SKM), and adipose tissue (AT). This chapter focuses on the contribution of SKM and AT to alcohol-mediated metabolic dysregulation. SKM and AT are targets of alcohol-mediated dysregulation of glucose, protein, and lipid metabolism; aberrant extracellular matrix remodeling; bioenergetic adaptations; and impaired differentiation of muscle and adipose derived progenitors. Based on emerging evidence of interorgan communication as an important mechanism underlying alcohol-associated tissue injury, SKM and AT as relays are discussed. Secretion of soluble factors and extracellular vesicles are proposed as critical mediators of inter-organ communication contributing to metabolic dyshomeostasis associated with at-risk alcohol use. We provide insight into areas of research gaps that warrant systematic studies on how alcohol-mediated changes in mediators, particularly extracellular vesicles, and their bioactive cargo, mechanistically contribute to cardiometabolic disease. Research integrating these complex metabolic networks is imperative to elucidate their role and potential as targets for interventions to reduce comorbidities and improve quality of life among people with at-risk alcohol use.

Keywords Alcohol · Skeletal muscle · Adipose tissue · Metabolism · Interorgan communication · Adipokines · Myokines · Extracellular vesicles

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Introduction

At-risk alcohol use is a major global risk factor for preventable morbidity and mortality, significantly contributing to health care burden and economic costs [1]. More details about epidemiology are provided in part I of this book. People with at-risk alcohol use are at increased risk for psychiatric comorbidities, liver disease, and cardiometabolic disease. Moreover, clinical [2–4] and preclinical studies [5–7] demonstrate that at-risk alcohol use promotes metabolic dysregulation and is an independent risk factor for development of type 2 diabetes [8, 9] through yet unknown mechanisms.

Compelling evidence indicates that at-risk alcohol use leads to tissue injury. However, patterns of alcohol consumption, types, and amount of alcohol consumed complicate the classification of at-risk alcohol use [10]. While studies suggest a J-shaped curved in the relationship between the amount of alcohol consumed and risk for metabolic dysregulation [11, 12], any protective effects of moderate drinking are eliminated when adjusting for physical activity and health status [13]. Thus, while the beneficial impact of low to moderate alcohol consumption on metabolism is debatable, at-risk alcohol use, including binge drinking negatively impact metabolic homeostasis [10, 14, 15].

The mechanisms involved in alcohol associated metabolic dysregulation are likely due to alcohol metabolism and the resulting cellular alterations [10, 16, 17]. Acetaldehyde and acetate; metabolites generated during ethanol metabolism [10] can produce tissue injury by activating immune responses, and protein and histone acetylation that dysregulate gene and protein expression [18]. The altered NAD^+ to NADH ratio resulting from alcohol metabolism also adversely affects numerous cellular metabolic processes [16, 17]. Similarly, reactive oxygen species (ROS) generated by alternative alcohol metabolic pathways damage DNA and proteins and deplete antioxidant capacity in tissues. Reduction of cellular S-adenosylmethionine (SAM) levels resulting from increased oxidative stress contributes to epigenomic modifications like DNA hypomethylation [19]. More details are also provided in book Chap. 55.

These alcohol-mediated cellular and metabolic effects have been well characterized in alcohol-related liver injury [10]. However, increasing evidence suggests that metabolic tissues such as the pancreas, skeletal muscle (SKM), adipose tissue (AT) are also significantly impacted by at-risk alcohol use. The SKM and AT play key roles in the multidirectional network of metabolically active organs involved in maintaining metabolic homeostasis. This has led to novel explanatory models invoking inter-organ communication and interplay as contributing mechanisms of alcohol-induced pathology. This chapter will discuss how the SKM, and AT are targets of alcohol-induced tissue injury and emerging evidence suggesting a role for these tissues as relays of interorgan communication contributing to metabolic dysregulation (Fig. 69.1).

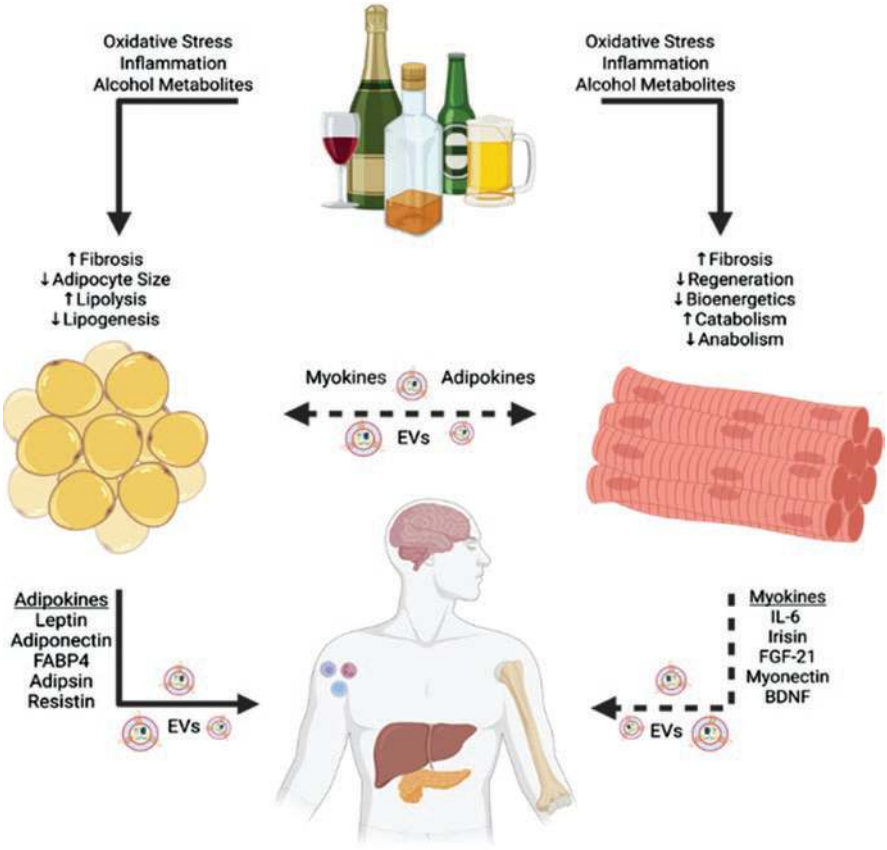

Fig. 69.1 Skeletal muscle and adipose tissue function as targets of alcohol-mediated tissue injury. Alcohol and alcohol metabolites, alcohol-induced oxidative stress, and inflammation, promote a profibrotic milieu and dysregulate metabolic capacity in both the tissues. Skeletal muscle and adipose tissue act as relays of interorgan communication by the release of extracellular vesicles, and myokines and adipokines, respectively. Evidence suggests alcohol-mediated changes in adipokines. However, whether alcohol alters skeletal muscle myokine levels or extracellular vesicle number or cargo from both the tissues is not known

Skeletal Muscle Is a Target of Alcohol-Induced Tissue Injury

At-risk alcohol use decreases functional SKM mass defined as loss of muscle mass or function (i.e. alcohol-induced myopathy). Clinically, alcohol-induced myopathy can be acute or chronic. Acute alcohol-induced myopathy occurs when an alcohol binge leads to myonecrosis, or the breakdown of muscle tissue. Muscle fiber contents can be released into circulation to cause rhabdomyolysis [20]. Rhabdomyolysis

results from increased intracellular calcium through membrane damage or impaired energy production and the increase in intracellular calcium leads to destructive cellular processes [21, 22]. Acute alcohol-induced myopathy can present with acute pain, swelling, and tenderness of the affected muscle and elevated circulating creatine kinase and myoglobin levels. In severe cases, acute kidney failure can occur [20].

Chronic alcohol-induced myopathy is more common than acute alcohol-induced myopathy and presents with progressive muscle weakness. Clinical and preclinical studies have demonstrated that chronic at-risk alcohol use leads to a reduction in functional muscle mass. Alcohol-fed mice have reduced gastrocnemius mass to total body mass ratio, and patients with alcohol-induced cirrhosis have reduced total muscle area as measured by computed tomography [23]. In athletes, alcohol consumption negatively impacts SKM recovery after strenuous exercise, and dose-dependently diminished muscle strength in men [24]. In a preclinical model of simian immunodeficiency virus (SIV)-infection, chronic binge alcohol (CBA) reduced thigh muscle area at end stage infection [25]. These reductions in SKM mass with at-risk alcohol use can be due to an imbalance in catabolic and anabolic signaling, increases in fibrosis, or impaired regenerative capacity, as described in the next section (Table 69.1).

Alcohol-Associated Dysregulation of SKM Anabolic and Catabolic Signaling

SKM is a highly adaptable organ that balances anabolic and catabolic signaling based on environmental cues such as growth factors, energy status, or mechanical strain. It plays a major metabolic role and is responsible for about 85% of insulin-mediated glucose utilization [53].

SKM mass is determined by the balance between protein synthesis and breakdown. Protein synthesis is driven by the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) signaling pathway, activated by insulin and insulin-like growth factor 1 (IGF-1) [54]. Activation of mTORC1 initiates S6 kinase 1 (S6K1) phosphorylation resulting in activation of ribosomal protein S6 and inactivation of eukaryotic initiation factor 4E-binding protein (4E-BP1). Both activation of S6K1 and inactivation of 4E-BP1 increase translational machinery allowing protein synthesis. Preclinical models show that chronic alcohol decreased the phosphorylation of mTOR and 4E-BP1 contributing to decreased protein synthesis [29]. Acute alcohol also attenuated refeeding-induced increases in protein synthesis and S6K1 phosphorylation [26]. Thus, both acute and chronic alcohol impair the activation of protein synthesis. Negative regulators of the mTORC1 pathway include AMP-activated protein kinase (AMPK) and Regulated in Development and DNA damage responses (REDD1 & REDD2). AMPK is activated by energy stress while REDD1 & REDD2 are activated by hypoxia, ROS, and glucocorticoid excess. While acute

Table 69.1 Summary of alcohol effects on skeletal muscle and adipose tissue

Summary of Alcohol's Effects on Skeletal Muscle	Refs #	Summary of Alcohol's Effects on Adipose Tissue	Refs #
Alcohol decreases anabolic signaling		*Alcohol decreases lipogenesis*	
• Chronic alcohol decreases phosphorylation of mTOR and 4E-BP1	[26]	• Chronic alcohol decreases PPARy expression	[27], [28]
• Acute alcohol attenuates refeeding-induced increase in S6K1 phosphorylation	[29]	• *In vitro* alcohol decreases ACC phosphorylation	[30]
• Acute and *in vitro* alcohol increase REDD1 and AMPK gene expression	[31]	• Alcohol decreases ACLY enzyme expression in VAT	[28]
		• *In vitro* alcohol decreases AMPK activity	[30]
Alcohol increases catabolic signaling		*Alcohol increases lipolysis*	
• Acute and chronic alcohol increase atrogin-1 expression	[32], [25]	• Chronic alcohol inhibits insulin anti-lipolytic effects	[27], [33]
• *In vitro* alcohol increases proteolysis through autophagy	[23]	• Chronic alcohol increases activation, expression, and activity of HSL	[27], [34], [35]
		• Chronic alcohol increases ATGL expression	[27], [28], [34]
Alcohol dysregulates ECM remodeling		*Alcohol dysregulates ECM remodeling*	
• Chronic alcohol increases hydroxyproline content	[36], [37]	• Chronic alcohol increases VAT collagen content in SIV infection	[38]
• Alcohol increases the expression of proinflammatory mediators	[39], [40, 41]		
• Chronic alcohol increases MMPs	[42]		
Alcohol decreases regeneration		*Alcohol increases inflammation*	
• Chronic alcohol increases *Tnfa* and decreases muscle fiber area in mice after injury	[41]	• Alcohol promotes macrophage polarization to M1 pro-inflammatory phenotype	[43], [44]
• Chronic alcohol decreases myogenic gene expression	[40], [45]	• Alcohol metabolism by CYP2E1 increases the expression of pro-inflammatory cytokines	[46], [47], [43]
• Chronic alcohol in non-human primates decreases myoblast differentiation	[45]		
Alcohol alters bioenergetic function		*Alcohol dysregulates adipokine profile*	
• *In vitro* alcohol decreases glycolytic function	[48]	• Chronic alcohol decreases VAT and SAT adiponectin	[33], [49]
• Chronic alcohol increases mitochondrial size	[50]		
• Chronic alcohol decreases mitochondrial gene expression and myoblast mitochondrial respiration in SIV-infection	[51], [52]		

and *in vitro* alcohol appear to increase REDD1 mRNA expression [31] and AMPK activation, respectively, chronic alcohol does not have the same effects. Thus, while impairment in various proteins in the mTOR signaling pathway appears to contribute to alcohol-induced decrease in protein synthesis, REDD1 and AMPK are unlikely mediators of alcohol-induced decreases in protein synthesis [55],

Loss of muscle mass may result from increased protein breakdown mediated by the ubiquitin proteosome pathways (UPP) and autophagy. Proteins destined for degradation are tagged with ubiquitin through action of E1 ubiquitin activating enzymes, E2 ubiquitin conjugating enzymes, and E3 ubiquitin ligases. Acute alcohol increased expression of two ubiquitin ligases, atrogin-1, and muscle ring finger 1 (MuRF1), in a rodent model but this was not associated with increased proteolysis [32]. In SIV infection, CBA increased atrogin-1 expression, dysregulated proteins in the UPP pathway, and increased proteasome activity [25]. Autophagy is activated during cellular stress to remove misfolded proteins or damaged organelles. Ethanol decreased murine myotube cell size and increased proteolysis, which was attenuated with an autophagy inhibitor but not a proteosome inhibitor suggesting that the increase in proteolysis was mediated by autophagy [23]. Overall, alcohol reduces SKM protein synthesis and increases autophagy, leading to a decrease in functional mass.

Alcohol Modulation of SKM Extracellular Matrix (ECM) Remodeling

The ECM contains capillaries and nerves, and surrounds SKM cells, or muscle fibers. The ECM provides a scaffold for SKM regeneration, and its structural integrity is maintained by fibroblasts that produce collagen. Abnormal increases in fibroblast activity or dysregulated ECM turnover can lead to aberrant SKM ECM remodeling, which is another contributor to reduced functional SKM mass.

Chronic alcohol administration in rats increased SKM collagen and hydroxyproline expression [36]. Similarly, CBA increased SKM collagen content as indicated by picrosirius red staining and hydroxyproline content in SIV-infection [37]. Alcohol-associated increases in SKM fibrosis are thought to be mediated by growth factor and cytokine expression and signaling, leading to an increase in fibroblast proliferation and activation. The release of profibrotic mediators such as transforming growth factor β (Tgfb1) from proinflammatory cells can increase SKM fibrosis [56]. In a transgenic HIV-1 rat model, chronic alcohol decreased SKM fiber area and increased myostatin and *Tgfb* gene expression [39]. Similarly, alcohol feeding during recovery from hind limb immobilization increased *Tgfb* and tumor necrosis factor α (*Tnfa*) expression [40] and increased collagen expression (unpublished data) (Fig. 69.2). Additionally, chronic alcohol increased SKM matrix metalloproteinases (MMPs), which can lead to pathologic remodeling of the ECM [42].

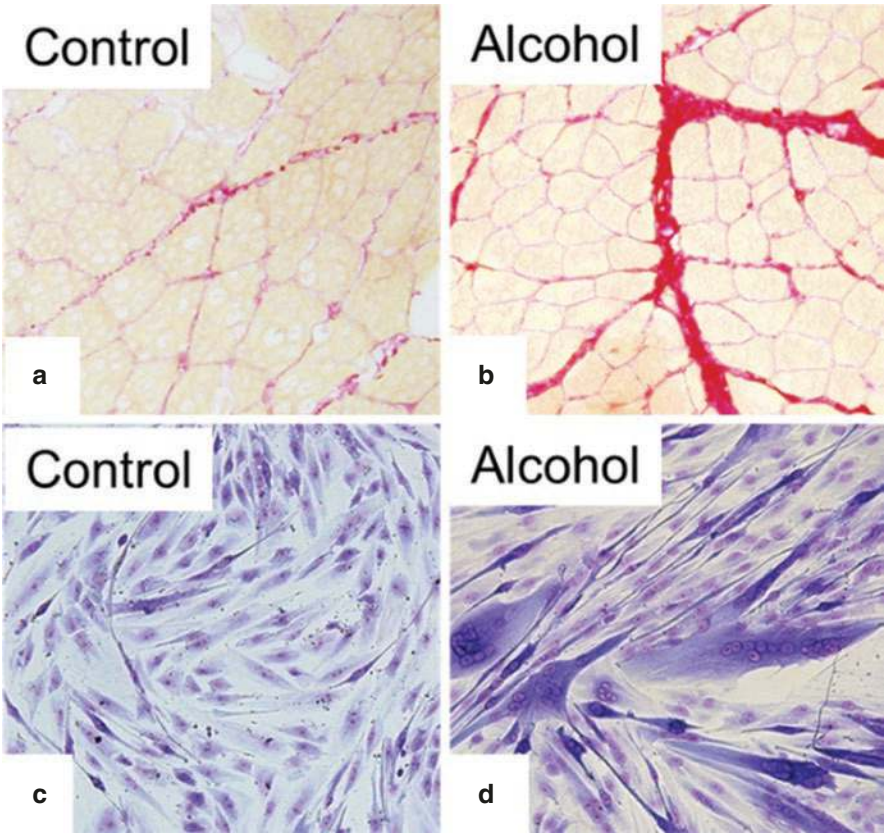

Fig. 69.2 Collagen content in skeletal muscle. Representative images of picrosirius red staining of quadriceps muscle in (**a**) Control (**b**) chronic alcohol fed rats. Representative images of Jenner-Giemsa staining of ex vivo differentiation of myoblasts isolated from (**c**) control (**d**) chronic in vivo alcohol administered nonhuman primates

Alcohol-Associated Decrease in SKM Regenerative Capacity

SKM has an exceptional regenerative capacity allowing it to replace loss or damaged tissue. SKM loss can occur with prolonged periods of disuse or damage that can occur routinely through exercise, or in the case of trauma or surgery. About 2–5% of nuclei in adult SKM are from satellite cells (SCs). SCs are quiescent stem cells that when activated can proliferate and differentiate in response to muscle injury. Impaired SKM regenerative capacity can also contribute to alcohol-associated loss of SKM functional mass. SCs express Pax7 and Myogenic factor 5 (Myf5) until activated to myoblasts, which express Myod and myogenin. Myoblasts are myogenic progenitor cells that can fuse with each other or damaged muscle fibers to regenerate the muscle tissue. Typically, muscle injury results in the recruitment of neutrophils followed by proinflammatory macrophages that secrete TNF-α,

interleukin 1β (IL-1β), and interferon γ (IFN-γ). This acute proinflammatory environment activates SCs and increases myoblast proliferation. Two to four days after injury, anti-inflammatory macrophages are recruited that secrete IL-4 and IL-10, which promote myoblast differentiation [57]. However, a chronic inflammatory state or increased oxidative stress can interfere with this physiologic process. While acute alcohol has anti-inflammatory effects, chronic alcohol exposure promotes a proinflammatory environment [58, 59] that can interfere with appropriate SKM regeneration [41]. These effects of alcohol on regeneration are evident in preclinical models where alcohol-fed mice have increased *Tnf*α 2 days after injury and fail to recover muscle fiber cross sectional area 2 weeks after injury [41]. Additionally, alcohol-fed rats have reduced expression of *Myod* 3 days after hind-limb immobilization [40] suggesting impairments in SC activation. Similar effects are reported in nonhuman primate models where CBA decreased myogenic gene expression and myoblast differentiation [45] (Fig. 69.2). Taken together, these data support a contribution of impairments in SKM regenerative capacity to reductions in functional SKM mass that accompany at-risk alcohol use.

Alcohol Decreases SKM Function

SKM must generate enough adenosine triphosphate (ATP) to meet energy demands. ATP in SKM is generated through creatine phosphate, glycolysis, and oxidative phosphorylation. The ability of SKM to uptake glucose from the blood is not only necessary for the generation of ATP but important in maintaining whole-body glucose homeostasis. Thus, any impairments in SKM glucose utilization have the potential to significantly affect SKM bioenergetics and functional homeostasis. Preclinical models suggest that alcohol impairs insulin-mediated glucose uptake likely through a decrease in GLUT4 translocation [60]. Furthermore, *in vitro* ethanol (50 mM) reduced glycolytic function while increasing oxygen consumption rate in primary rhesus macaque myoblasts suggesting a shift from glycolysis to oxidative phosphorylation and this was associated with decreased myoblast differentiation potential [48]. Whereas, ethanol (100 mM) decreased basal, ATP-linked, maximal respiration, and spare respiratory capacity [61, 62]. Chronic alcohol negatively impacts mitochondrial dynamics, and SKM mitochondria are larger in alcohol-induced muscle injury [2, 3, 50]. In people living with HIV, proton leak is higher in myoblasts from those with high Alcohol Use Disorders Identification Test (AUDIT) scores [63]. Additionally, CBA dysregulated mitochondrial gene expression and decreased myoblast mitochondrial respiration in SIV infection [51, 52]. Together, these reports strongly suggest a significant role for altered bioenergetics and mitochondrial dysregulation in alcohol-associated loss of functional SKM mass. Despite associations between alcohol use, myoblast bioenergetics, myoblast differentiation, and dysglycemia, the exact mechanisms for the decrease in functional SKM mass seen with alcohol-induced myopathy and its contribution to metabolic dysregulation are yet to elucidated.

Adipose Tissue Is a Target of Alcohol-Induced Tissue Injury

Adipose tissue (AT); together with SKM, the liver, and the pancreas, plays a vital role in regulating whole-body energy and glucose homeostasis. AT can be classified as brown, beige or white. Brown adipose tissue has high levels of mitochondria and is involved in energy expenditure. Beige adipose tissue is a mix of white and brown adipose tissue and is also involved in heat generation and energy expenditure but to a lesser extent than brown adipose tissue [64, 65]. White adipose tissue (WAT), considered an endocrine organ releases biologically active adipokines and cytokines and contribute to maintaining whole body energy homeostasis [66]. WAT tissue is classified based on anatomical location as either visceral AT or subcutaneous AT.

In rodents, chronic alcohol consumption decreased overall AT mass and adipocyte size [27]. In humans, chronic at-risk alcohol consumption is associated with decreased total adiposity [67] but increased visceral adiposity [68]. The elevated waist-to-hip ratio resulting from increased visceral adiposity is associated with worse metabolic clinical outcomes [69]. The alterations in AT mass and distribution associated with at-risk alcohol consumption are thought to result from alterations in lipid metabolism, impaired adipose ECM remodeling, adipose tissue inflammatory milieu, and/or impaired adipose tissue secretory phenotype (i.e., adipokines) as discussed in the next section.

Alcohol Dysregulates AT Metabolic Function

AT is central to lipid homeostasis and has a modest contribution (about 10%) to insulin-stimulated whole body glucose uptake/storage [70, 71]. AT mass is maintained by a balance between lipogenic and lipolytic pathways that predominate during the fed and fasted states, respectively. Alcohol directly and indirectly alters the balance of lipogenesis and lipolysis, dysregulating lipid homeostasis (Fig. 69.3, Table 69.1).

Accretion of AT mass involves lipogenesis from dietary triglyceride (TG) uptake and *de novo* lipogenesis from acetyl-coenzyme A (acetyl-CoA). In addition, glucose can be metabolized to glycerol 3-phosphate and participate in the synthesis of TGs. Alcohol regulates several components of the lipogenic pathway. Alcohol decreases AT expression of peroxisome proliferator- activated receptor gamma (PPARγ or PPARG), one of the most potent lipogenic stimulators [27, 28, 72]. Activation and binding of PPARG and CCAAT-enhancer-binding protein alpha (CEBPA) is central to the activation of adipogenesis. Chronic alcohol decreased CEBPA expression in rodent visceral AT, but not in subcutaneous AT or in 3T3-L1 cells [28]. Lipoprotein lipase (LPL) enables adipocyte lipoprotein uptake and hydrolysis to two free fatty acid (FA) molecules and one monoacylglycerol. Reports on the effects of alcohol on LPL expression and activity are incongruent, with some showing increased [73, 74],

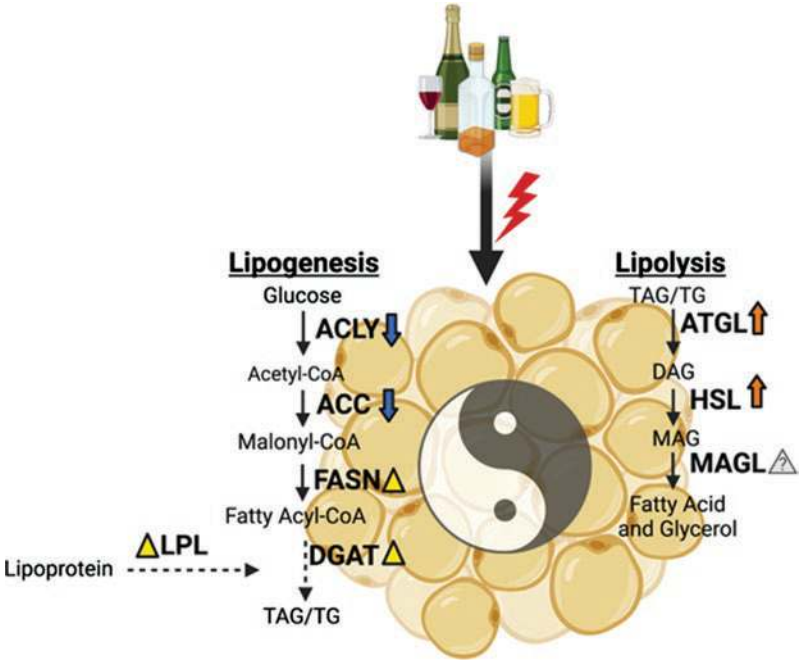

Fig. 69.3 Alcohol alters the balance of lipogenesis and lipolysis in adipose tissue. Alcohol decreases lipogenic enzymes, ATP-citrate lyase (ACLY) and Acetyl coA carboxylase (ACC). Alcohol is shown to increase, decrease or not affect fatty acid synthase (FASN), diacylglycerol acyl transferase (DGAT), and lipoprotein lipase (LPL) expression (Δ). Alcohol increases lipolytic enzymes, adipose tissue triglyceride lipase (ATGL), and hormone sensitive lipase (HSL) expression. These alcohol-mediated dysregulation of lipid homeostasis contributes to alcohol-related liver disease

decreased [75], or unchanged [76, 77] patterns. These differences may be attributable to dose and route of alcohol administration, experimental model, or tissue type.

A major hormonal regulator of AT homeostasis is insulin. Insulin stimulates FA and glucose uptake into adipocytes. Glucose participates in the lipogenic pathway via downstream oxidation to acetyl CoA or glycerol-3-phosphate. Several of the enzymes responsible for glucose conversion to a fatty acid substrate are modulated by alcohol. Before glucose is converted to fatty acyl-CoA (FA-CoA), it must undergo glycolysis to pyruvate and conversion to acetyl-CoA by ATP-citrate lyase (ACLY), which is decreased by alcohol in visceral AT but not in subcutaneous AT or in primary adipocytes [28]. The conversion of acetyl-CoA to malonyl-CoA is catalyzed by acetyl-CoA carboxylase (ACC), the rate-limiting enzyme of lipogenesis. *In vivo* alcohol decreased ACC stimulatory phosphorylation at Ser79 [30, 75]. Additionally, activity of AMPK, which is responsible for ACC phosphorylation, is also decreased with chronic alcohol [30, 78]. Activation of AT AMPK can also inhibit both basal and stimulated lipolysis, thus this alcohol-mediated decrease in AMPK activity may also lead to increased lipolysis. The conversion of

malonyl-CoA to FA-CoA in preparation for assembly of a TG molecule is catalyzed by fatty acid synthase (FASN). There is conflicting evidence of alcohol's effect on AT FASN expression or activity, including decreased FASN in visceral, but not in subcutaneous AT [28, 72]. Finally, after the conjugation of FA-CoA molecules to a monoacylglycerol (MAG) or glycerol-3-phosphate molecule, diacylglycerol acyl transferase (DGAT) catalyzes the addition of the last FA-CoA to form the full TG molecule. DGAT expression is either unchanged or decreased by chronic alcohol in visceral AT of mice [79]. Overall, most reported studies suggest alcohol decreases several lipogenic regulatory enzymes. These effects appear to be alcohol dose- and route-dependent, depot-specific, and model-specific.

Lipolysis occurs during times of starvation or extreme energy demands and AT mobilizes TG stores to release glycerol and FA for hepatic glucose production and SKM fatty acid oxidation, respectively. Chronic alcohol stimulates AT lipolysis and there is an associated increase in FA release [33, 80]. Circulating levels of FA may not directly reflect increased release from AT, because of lipid deposition in the liver, a likely mechanism involved in the pathogenesis of liver steatosis [27]. Beta-adrenergic receptor stimulation by sympathetic nervous system activation is a potent activator of lipolysis, while insulin inhibits the lipolytic pathway. Early studies provided evidence that epinephrine-stimulated lipolysis in rodent visceral AT was not affected by alcohol [81]. Additionally, studies provided evidence that alcohol diminished the inhibitory actions of insulin [82]. These data provided the foundation for later studies that revealed that alcohol's ability to increase lipolysis was principally attributable to insulin inhibitory effects [33, 83]. As with lipogenesis, there are also several regulatory checkpoints in the lipolysis pathway that are affected by alcohol. Activation of the beta-adrenergic receptors mediate an increase in intracellular cyclic adenosine monophosphate (cAMP) and chronic alcohol increased AT cAMP levels [77, 84, 85]. Activation of PKA by cAMP phosphorylates proteins involved in lipolysis, including perilipin and hormone sensitive lipase (HSL). Once phosphorylated, perilipin undergoes a conformation change allowing for lipid droplet hydrolysis by HSL. Additionally, PKA phosphorylates HSL at the Ser660 to increase its activity and remove fatty acids from TG to form a MAG molecule. Chronic alcohol increased phosphorylation, activity, or mRNA expression of HSL [27, 34, 35] and increased adipose tissue triglyceride lipase (ATGL), the principal rate-limiting enzyme in lipolysis, which catalyzes the removal of the first fatty acid forming DAG [27, 28, 34]. Insulin suppresses lipolysis by two main mechanisms: (1) phosphodiesterase-3B (PDE3B) activation and (2) protein phosphatase 1 (PP1) activation. Insulin decreases PKA activity by increasing PDE3B activity, a downstream mediator of the insulin signaling cascade. Activation of PP1 leads to the dephosphorylation of HSL and inhibition of lipolysis. Chronic alcohol does not affect PDE3B mRNA or protein expression in rodent visceral AT or visceral adipocytes [33]. However, chronic alcohol decreased PP1 phosphorylation in rodent visceral AT, while upregulating phosphatase and tension homologue (PTEN) and suppressor of cytokine signaling 3 (SOCS3) expression, major negative

regulators of insulin signaling [27]. Collectively, these data strongly suggest that inhibition of insulin signaling is a key mechanism underlying alcohol-induced adipose tissue lipolysis.

Alcohol Dysregulates AT ECM Remodeling

The AT ECM is composed of a complex network of fibrillar and non-fibrillar fibers that provide structural support and aids in differentiation, migration, and survival for normal tissue homeostasis. ECM fibers in the AT are not produced by one specific cell type but rather a combination of adipocytes and stromal cells, such as immune and progenitor cells [86]. Collagen is the most abundant AT ECM fiber and provides structural support for cellular functions [87]. Despite evidence indicating that ECM is an essential factor in maintaining tissue homeostasis and development of pathology (i.e., fibrosis), little is known about AT ECM physiology and pathophysiology. In the SIV-infected macaque model, CBA increased collagen content in omental AT, a visceral AT depot [38] (Fig. 69.4). To date, this is only study that has investigated alcohol's effects on AT ECM content. However, evidence from studies in other disease models indicate that AT fibrosis is a common pathophysiological response to tissue injury. For example, clinical studies show increased omental AT fibrosis in obese subjects compared to other adipose depots and compared to lean subjects [88–91]. Similarly, HIV and SIV increased AT collagen deposition and impaired ADSC differentiation in humans and cynomolgus macaques [92].

Alcohol Increases AT Inflammation

The alcohol metabolizing enzyme CYP2E1, is expressed in both visceral and subcutaneous AT [28, 93] and its expression is increased by alcohol [46, 94]. CYP2E1 mediated increased oxidative stress and increased endotoxins induce the expression of proinflammatory cytokines such as TNF-α, IL-6, and monocyte chemoattractant protein-1 (MCP-1), which directly contribute to the alcohol-induced inflammatory milieu in AT [43, 44, 46, 47]. CYP2E1 knockout mice show attenuated alcohol-induced increase in adipose pro-inflammatory cytokine expression, suggesting a role for the CYP2E1 axis in mediating adipose inflammation with alcohol [46]. Additionally, CBA in TLR4 knockout mice attenuated alcohol-mediated accumulation of proinflammatory AT macrophages [95]. In humans, AT inflammatory cytokine production is correlated with acute alcohol-related hepatitis [43]. Alcohol also induces a reversible shift in AT macrophage towards a M1 pro-inflammatory phenotype. One week of alcohol withdrawal alleviated macrophage infiltration in subcutaneous AT and transitioned AT macrophages toward a M2 anti-inflammatory phenotype [44]. In the SIV-infected macaque model, CBA increased immune cell infiltration in omental AT [38] (Fig. 69.4). Additionally, a role for lymphatic vessel hyperpermeability was revealed as a likely mediator of alcohol-induced

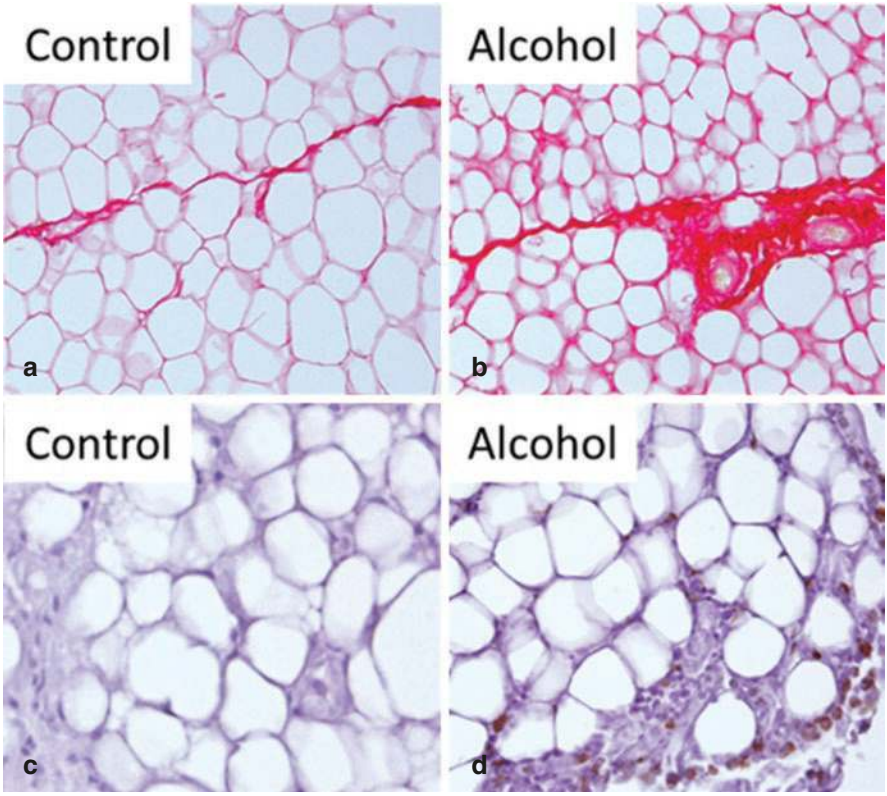

Fig. 69.4 Collagen content and immune cell infiltration in omental adipose tissue. Representative images of picrosirius red staining in (**a**) Control (**b**) chronic binge alcohol administered non-human primates. Representative images of HAM56 (macrophage) staining in (**c**) control (**d**) chronic binge alcohol administered non-human primates

inflammation in perilymphatic and mesenteric adipose tissue [96]. The effects of alcohol on AT hormonal, inflammatory, oxidative stress, and ECM environment significantly impact on AT homeostasis. The increasing reports of secreted factors as mediators of interorgan communication, strongly suggest this may play a role in multi organ alcohol induced tissue injury.

Skeletal Muscle and Adipose Tissue as Relays of Alcohol-Induced Tissue Injury

The maintenance of whole-body energy homeostasis relies on effective inter-organ communication. In the early 1900s, hormones were first identified as chemical messengers that allowed for communication between tissues, and more recently other mechanisms of intercellular communication such as cytokines and extracellular vesicles (EVs) have been recognized. Cytokines include growth factors,

interleukins, and interferons that are mostly secreted by immune cells. Adipocytes and muscle fibers also secrete cytokines (i.e., adipokines and myokines, respectively) into the extracellular space and impact neighboring and distal target cells. The importance of interorgan communication is highlighted in physiologic conditions of fasting, feeding, thermogenesis, and exercise [97] and likely contributes significantly to alcohol-related pathologies.

EVs are membrane bound nanoparticles that transport proteins, lipids, mRNA, and non-coding RNAs from the originating cell to a target cell are involved in intercellular communication. Their protective membrane prevents nucleases and proteases from degrading the bioactive cargo making them an effective vehicle for delivery. Three types of EVs have been identified: apoptotic bodies, microvesicles, and exosomes. These subtypes are difficult to distinguish experimentally so the term EV is preferred. Apoptotic bodies are released from cells undergoing apoptosis. Microvesicles directly bud from the cell membrane, a process which is initiated by translocation of phosphatidylserine to the outer leaflet of the cell [98]. Exosome biogenesis on the other hand, starts with the formation of an endosome. Intraluminal vesicles are formed through the invaginations of the endosome. These invaginations are created through either endosomal sorting complex required for transport (ESCRT)-dependent or ESCRT-independent mechanisms. The ESCRT-dependent pathway requires several ESCRT complex proteins including tumor susceptibility gene 101 (TSG101) and Alix that are often found associated with exosomes [99]. In the ESCRT-independent pathway, sphingomyelinase catalyzes the formation of ceramide from sphingomyelin to initiate the inward budding of the endosome. Once intraluminal vesicles are formed, the endosome is called a multivesicular body. Rab27a/b proteins along with actin, annexins, and tubulin transport the multivesicular body and allow for fusion to the cell membrane [98]. Once fusion occurs, the intraluminal vesicles are released into the extracellular space as exosomes.

Many of the studies on alcohol-mediated dysregulation in intercellular communication are in the context of liver disease. For example, the increased secretion of TNF-α from immune cells with at-risk alcohol use contributes to hepatocyte injury [100]. Additionally, patients with alcohol-related hepatitis have increased circulating EVs [101], and EVs from alcohol-treated hepatocytes can sensitize monocytes and contribute to alcohol-mediated liver damage [102]. Despite compelling evidence that SKM and AT secrete cytokines and tissue specific factors that are implicated in interorgan communication, there is a gap in the literature on how alcohol modulates these factors and thus affect metabolic regulation. Here we briefly discuss the existing literature on SKM and AT as relays of interorgan communication and how alcohol regulates these factors.

Mechanisms of SKM Communication

Myokines can directly act on a wide range of tissues including metabolic tissues such as AT, the liver, and pancreas. IL-6; the first myokine discovered, is released during exercise [103], increases lipolysis in AT, increases hepatic glucose

production, and stimulates pancreatic β-cell proliferation [104]. Two other myokines; irisin and fibroblast growth factor 21 (FGF-21), are secreted by SKM and contribute to the browning of white adipocytes and induction of thermogenesis [105]. Myonectin is another myokine released in response to glucose and fat intake and increases free fatty acid uptake into AT by increasing expression of fatty acid transporter proteins [106]. In addition to having direct metabolic effects on distant organs, myokines modulate immune cell function and the anti-inflammatory effects of exercise are at least partially mediated by increased IL-6, that has been shown to reduce TNFα secretion from endotoxin-stimulated monocytes *in vitro* [104].

SKM-derived EVs; positive for the membrane marker α-sarcoglycan [107], account for approximately 5% of circulating EVs [108]. Exercise transiently promotes the release of EVs into circulation [107, 109] and miRNAs in myotube-derived EVs silence genes in target myoblasts and promote myoblast differentiation [110]. Additionally, satellite cell EVs downregulate MMP-9 expression in myotubes [111] and Wnt-inducible signaling pathway protein 1 (Wisp1) expression in fibroadipogenic progenitor cells [112] during mechanical overload allowing for an ECM environment suitable for muscle hypertrophy. In addition, SKM EVs containing miRNA-1 can target AT and suppress transcription factor AP-2, a repressor of adrenergic receptor β3 expression, thereby affecting lipolytic gene expression [113]. Additionally, SKM-derived EVs from high palmitate diet fed mice affect pancreatic β-cell development *in vitro* [114]. While numerous studies suggest that SKM-derived EVs can be taken up by other metabolic cell types, most of this work is limited to *in vitro* studies. SKM EVs are believed to be mostly taken up by the liver and spleen and can also be found in the pancreas and other tissues [114].

Alcohol's effect on SKM communication mechanisms is not well characterized. Circulating levels of myokines such as IL-15 and TNF-α are higher in people with alcohol use disorder (AUD) [115, 116]; however, it is unclear if muscle is the main source of these cytokines. Additionally, brain-derived neurotrophic factor (BDNF), which is increased in muscle following exercise, is reduced in circulation in people with AUD and correlates with muscle strength [117]. And while we know SKM cells release EVs, how acute or chronic alcohol modulates EV release, and their paracrine or endocrine function is not known.

Mechanisms of AT Communication

Alcohol's produces marked alterations in adipokine levels. Adiponectin, a well-known insulin-sensitizing and anti-inflammatory adipokine [118] also has protective effects on pancreatic β-cell proliferation, apoptosis, and insulin secretion. Most preclinical studies show that chronic alcohol decreased circulating adiponectin levels [49, 119, 120]. Decreased adiponectin protein and mRNA in visceral AT [49, 119] and subcutaneous AT [33] can lead to deleterious effects on SKM and impair hepatic lipid metabolism [121]. Reports from clinical studies are incongruent. Some clinical studies report increased adiponectin levels with alcohol consumption [122–124], while others have shown a dose dependent decrease [125]. Leptin, another

important adipokine is secreted in direct proportion to AT mass and regulates several processes including food intake, energy expenditure, lipolysis, lipogenesis, and insulin sensitivity [126, 127]. Leptin protein levels in rodent visceral AT are reported to be increased [128] or unchanged [129] with chronic alcohol administration. Similar discordant effects of alcohol are reported for circulating leptin levels, with studies reporting decreased [130], increased [131] or unchanged [128] circulating concentrations in animal models and humans. Thus, the effects of alcohol on leptin are inconsistent and may be related more to changes in fat mass than direct effects of alcohol on leptin synthesis. The impact of chronic alcohol on resistin levels has not been extensively investigated. One study has shown chronic alcohol feeding in rats increased serum resistin and visceral AT resistin mRNA levels [132]. Another study showed higher resistin levels among alcohol-related liver disease patients compared to healthy controls [133]. Much remains to be characterized with respect to the effects of at-risk alcohol use on adipokine release. However, it is unlikely that alterations in adipokine release may be solely responsible for distant organ pathophysiology resulting from at-risk alcohol use.

An alternative mechanism through which adipose-secreted mediators impact on distant tissue pathophysiology is through the release of EVs [134, 135] containing bioactive cargo including adiponectin, perilipin A, FABP4 and miRNAs, proteins and mRNA [136]. Adipocyte-derived EVs mediate paracrine communication between adipocytes and macrophages inducing M1 pro-inflammatory macrophage polarization through a miR-155 mediated mechanism in high fat diet fed mice [137]. Adipocyte-derived EVs also modulate metabolic regulation through their effects on SKM, liver, and the brain. EVs derived from AT macrophages of obese mice showed a direct effect to impair insulin signaling in hepatocytes and myocytes, possibly through miR-29a, which targets PPARG [138]. Similarly, the effects of adipocyte-derived EV miR-27a to induce insulin resistance in SKM are also attributed to its suppression of PPARG [139]. EV miR-130b target muscle cells and reduce the expression of its target gene, PPARγ coactivator 1α (PGC1α), which plays a key role in lipid oxidation and mitochondrial function [140]. Adipose derived EV mir-155 also affects pancreatic β-cell proliferation and insulin secretion [141]. Alcohol-fed mice have an adipocyte EV profile that differs from control mice [142], though the functional effect of these EVs has not been studied. Alcohol-mediated AT lipolysis and TG release can be reverse transported to the liver [27] demonstrating a role in adipose-liver crosstalk in alcohol-induced liver disease. Studies are warranted to fully understand the alcohol-mediated direct effects on adipokine profile and function, changes in EV bioactive cargo and function and its contribution to metabolic homeostasis.

Concluding Remarks

This chapter has largely focused on the contribution of skeletal muscle and adipose tissue to alcohol-mediated metabolic dysregulation; however, additional mechanisms significantly contribute to alcohol's damaging effects including that of the pancreas and liver. SKM and AT are targets of alcohol-induced cellular injury that contribute to dysregulation of metabolic processes (glucose homeostasis, protein balance and lipid metabolism), increased aberrant extracellular matrix remodeling, bioenergetic adaptations, and impaired differentiation ability of muscle and adipose derived progenitors. The diet composition, nutrient availability, gut microbiome, and its metabolites, all add layers of complexity to alcohol-induced end organ injury and metabolic dyshomeostasis. We now know that SKM and AT function as relays through the secretion of soluble factors and EVs. We speculate that these mechanisms of interorgan communication are disrupted with at-risk alcohol use contributing to alcohol-associated pathologies. Understanding the mechanisms of release and targeted uptake of EVs, the naturally occurring vehicles carrying multiple types of mediators warrant special attention and extensive investigation. There is a gap in the literature on how alcohol modulates SKM and AT EV biology, despite compelling evidence of EVs from both these tissues playing critical roles in metabolic homeostasis. Integrating multiorgan multiomics to identify mediators of interorgan communication and modulation by alcohol will provide insight of target cells, transporters, receptors, and signaling pathways that are differentially regulated, and can potentially provide therapeutic targets for alcohol-related metabolic dysregulation. Alcohol-mediated cellular injury of metabolically active tissues potentially synergizes with the obesogenic environment including diet, and sedentary lifestyle behaviors significantly increasing the risk of cardiometabolic and liver disease. Thus, research integrating these complex networks of factors are imperative to reduce health care burden and improve quality of life among people with at-risk alcohol use.

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Chapter 70
Alcoholic Cardiomyopathy: Pathogenic Aspects

Joaquim Fernández-Solà

Abstract In susceptible individuals, chronic ethanol consumption may cause progressive myocardial damage leading to alcoholic cardiomyopathy (ACM). This progressive effect of ethanol on the myocardium is dose-dependent, worsens with binge-drinking and is modulated by gender, race and some genetic polymorphisms (acetetaldehyde dehydrogenase 2, angiotensin-converting enzyme). Ethanol has synergistic myocardial damaging effects with other drugs (tobacco, cocaine) and other comorbid diseases (arterial hypertension, cirrhosis, malnutrition, vitamin deficiencies or ionic disturbances). At the molecular level, ethanol may damage several myocyte structures including membrane phospholipid composition, ionic receptors and channels, disturbs intracellular [Ca^{2+}] transients and damage sarcomere structural proteins disturbing excitation coupling and contractile function. The main histological lesions in ACM include myocyte apoptosis and necrosis causing myocytolysis and cell loss, which repair mechanisms compensate for by inducing myocyte hypertrophy and interstitial fibrosis. This limited remodeling process is regulated by cardiomyokines and growth factors (myostatin, IGF-1, FGF21, Metrnl). The final process of ACM is the result of ethanol dosage and individual predisposition and the prognosis depends on the degree of persistence in ethanol intake and the equilibrium between damage *vs* repair mechanisms. New strategies to minimize ethanol-related cardiac damage, avoid pathological myocyte hypertrophy and interstitial fibrosis and improve myocyte regeneration are addressed.

Keywords Ethanol · Alcohol · Heart damage · Alcoholic cardiomyopathy

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Abbreviations

ACM	Alcoholic cardiomyopathy
BAC	Blood alcohol concentration
CaMK	Calmodulin kinase
CMP	Cardiomyopathy
E/C	Excitation/contraction
EF	Ejection fraction
ETHANOL	Ethanol
LV	Left-ventricle
MR	Magnetic resonance
ROS	Reactive oxyen species
SR	Sarcoplasmic reticulum

Introduction

Alcoholic Cardiomyopathy—ACM—*(2020 ICD-10-CM Diagnosis Code I42.6)* is the disease manifestation of long-standing and additive deletereous efects of ethanol- ethanol- (also referred to as "alcohol" in this chapter) on the heart myocites in susceptible subjects [1–6]. In fact, this disease originates from the sum of repeated acute ethanol binge-drinking effects in addition to the persistence of chronic long-standing direct and indirect effects of ethanol and its metabolites on the myocardial tissue [7, 8]. Genetic, racial and gender factors as well as other comorbidities [9–12] and polytoxic misuse, spetially tobacco and cocaine [13, 14], can influence the course and intensity of ACM [6, 8].

In the case of the heart, the potential beneficial effects of low-dose alcohol on coronary artery disease and mortality coexist with the detrimental effects on the myocardium induced by the dose-dependent damaging effect of ethanol intake [15]. In view of the coexistence of other ethanol-related diseases (liver cirrhosiscirrhosis, dementia, arterial hypertension) with ACM, it would be recommendable to avoid any degree of alcohol consumption [16]. In addition, low-dose ethanol may increase the risk of cancer, neurological brain damage, and alcohol addiction [16–18], and therefore, even low-dose consumption should be discouraged in the general population. Accordingly, the only safe ethanol dose for the cardiovascular system is zero [6, 19], making it necessary to establish a balance in relation to alcohol counseling [15, 20].

At present, ACM should not be considered an isolated or independent disease, but rather as a part of systemic damage induced by ethanol misuse in a specific subject [8, 17]. In this sense, the presence of other alcohol-related comorbidities (i.e. liver cirrhosiscirrhosis, malnutrition or skeletal myopathy) is frequent and influences the presence and worsens the course of ACM [14, 21, 22]. In fact, the myocardium is one of the most susceptible targets of long-standing effects of ethanol and is involved in most of comorbidities produced by alcohol consumption.

Thus, ACM is more prevalent in alcohol misusers with other alcohol-related diseases such as liver cirrhosis than in those without [9, 22].

Recently, a new perspective in ACM has appeared due to the description of novel pathogenic mechanisms [8, 15, 23, 24]. It has been clear that ethanol not only damage myocytes but also is able to impair their repair mechanism, disturbing some local and also systemic cardiomyokines (CMK) and growth factors [25]. This produces a lower rate of myocyte repair mechanisms and diminishes adaptative mechanisms. In addition, it has recently been reported that alcohol consumption also alters the myocyte regenerative process, causing an additive and synergic damage [26, 27].

This review describes and discusses the global effects that ethanol exerts on the heart myocytes, and specifically, the so-called ACM, in relation to the description of new pathogenic data. More clinical data on ethanol and heart failure are provided in Chap. 71.

Historical Perspective of Alcoholic cardiomyopathy

Alcohol has been consumed as different beverages by almost all civilizations in human history. Therefore, the toxic deleterious effects of alcohol misuse on the heart have been recognized since ancient times [28]. In fact, in Greece, in the IVth century B.C, Hippocrates observed and described the development of "hidropressy", the equivalent of congestive heart failure in individuals with chronic high alcohol consumption. In these individuals, Hippocrates recommended complete avoidance of alcohol consumption, establishing the first documented treatment on ACM. After this early clinical observation, no other references appeared related to ACM until the nineteenth century. To the contrary, alcohol was usually recommended as a heart tonic beverage. At this time, detailed clinical descriptions in Germany and England reported the development of progressive congestive heart failure in beer drinkers. Otto von Bollinger described the "Munich beer heart" with fibrosis, hypertrophy, and fatty degeneration in postmortem cardiac tissue of alcoholics who consumed more than 400 liters of beer per year [29]. Curiously, in this period, it was suspected that this noxious effect was not related to alcohol itself but rather to beer contaminants as arsenic or beer additives, especially cobalt which was used as anti-foam agent. Similarly other etiological causes of ACM were proposed, with the hypothesis of vitamin deficiencies, especially thiamine in the context of occidental beriberi [30], selenium deficit, ionic (Na^+, K^+, P^{4+}, Mg^{++}) disturbances or nutritional deficiencies [31]. Until the second part of the twentieth century there was no scientific evidence of a clear and direct relationship between alcohol consumption and myocardial damage, leading to ACM in a dose-dependent manner related to the lifetime dose of ethanol consumed [32, 33]. In fact, it has been determined that the minimal cumulative dose of pure ethanol necessary to achieve ACM is 10 kg ethanol/kg body weight, but usually ranges from 20 to 30 kg ethanol/kg body weight.

More recently, specific groups of major susceptibility have been described, spatially in relation to race, ethnicity, gender, metabolic polymorphisms and genetically-mediated variants and the co-existence of other collateral factors for the development of ACM [12, 14, 34]. The consumption of other toxic substances such as tobacco or

cocaine in addition to alcohol has additional deleterious effects in ACM [35, 36]. ACM is not considered as an isolated disease, but is rather part of ethanol-related systemic damage and a consequence of the global biological response [9, 17, 37]. Similarly, the progressive pathogenic mechanisms of ACM have been described at a structural, biochemical and molecular level, improving our pathomechanistic understanding [8, 27]. Looking towards the future, new strategies to prevent and treat or minimize this disease are currently evolving [25, 38].

Epidemiology of Alcoholic cardiomyopathy

Alcohol is the sixth most relevant factor of global burden of disease and responsible for 5.3% of all deaths [39]. The global alcohol-attributable fraction (AAF) of cardiovascular (CV) death based on alcohol exposure measures (AAFs) is estimated 6.9% (95% CI: 5.4–8.4%). Alcohol is responsible of 21–36% of all cause of idiopathic dilated cardiomyopathy (CMP) [40]. In active alcohol consumers, the prevalence of clinical ACM ranges from of 0.25% in men to 0.43% in women (global mean 0.34%). Considering 10% of global population with excessive alcohol intake, the global number of subjects with ACM worldwide in 2020 was 2,650,000. These numbers would double if subclinical ACM were taken into account [41]. In clinical settings evaluating high-dose chronic alcohol consumers, the prevalence of subclinical left ventricular (LV) diastolic dysfunction is 33% [42] while that of systolic dysfunction usually with overt LV heart failure 13% [32].

The global mortality attributable for ACM per year is 41,400 subjects/year (range 16,200–27,900, 95% CI). The population-weighted mean crude ACM mortality rate was estimated at 8.4 deaths per 1,000,000 (95% CI: 7.4–9.3). The number of daily adjusted life years (DAYLYs) attributable to ACM in 2016 was 897,000 (corresponding to 9.7% of all alcohol attributable DAYLYs in CV disease). In a chronologic overview, comparing civil registries with 2015 data and the Global Burden of Disease 2020 study, a 3.71-fold increase in ACM mortality was observed, although there may be some gap in this estimation. The increase in ACM is related to the absolute rate of global alcohol consumption and not to changes in the disease itself [41].

Despite the clear epidemiological evidence demonstrating that ethanol consumption is not safe and increases health risk, consumption policies are not sufficiently effective [16, 43]. The need to establish more effective control of ethanol consumption is discussed elsewhere in this book [41, 44].

Natural History of Alcoholic cardiomyopathy

Due to the relevant genetic, gender and ethnic predisposition, the course of ACM is different according to each individual and medical care should be individualized [11, 45, 46]. ACM may develop though consumption of any type of alcohol

beverage such as beer, wine or spirits and has a linear dose-dependent relationship with the total lifetime dose of ethanol (TLDE) consumed by an individual [32, 47]. In global terms, the natural history of ACM begins with an asymptomatic phase in people from 35 to 55 years of age, predominantly in men [2]. Diastolic dysfunction is detected by echocardiography or magnetic resonance spectroscopy in one third of alcohol consumers with a TLDE >10 kg ethanol/kg body weight [42]. If the TLDE progresses, systolic dysfunction appears and is clinically relevant when the left ventricular ejection fraction (LVEF) is <50% [2, 5, 15]. During this period, around 20% of women and 25% of men with excessive alcohol consumption develop exertion dyspnea and orthopnea, leading to episodes of LV failure [48]. The clinical characteristics of LV failure in ACM are similar to other causes of LV heart failure such as idiopathic dilated CMP [49]. Signs of right ventricular or congestive heart failure with lower limb oedema or anasarca appear later in the course of the disease [5, 50, 51]. In addition, a diversity of arrhythmias may develop, atrial fibrillation being the most frequent [52] and ventricular tachycardia the most dangerous [50]. Arrhythmias are first related to episodes of binge drinking in the scenario of the holiday heart syndrome and also in end-stage disease causing sudden death [1, 53, 54]. In this stage, cardiac thrombi may appear as a rare but ominous finding [55]. More detailed characteristics of heart failure in ACM are discussed in book Chap. xxx of this book.

In alcohol misusers with ACM the most important prognostic factor is the maintenance of active ethanol consumption [56–58]. In patients with persistent alcohol intake >60 g/day, the reduction in the LVEF is maintained, leading to episodes of heart failure and arrhythmias, and frequent sudden death [51, 53, 59]. In contrast, in most patients with ACM who are able to achieve abstinence, the LVEF improves and returns to normal values (LVEF >50%) in half of the cases. Similarly, with complete abstinence, early LV diastolic dysfunction reverses within 6 months [60]. In individuals who are unable to achieve complete abstinence and maintain controlled drinking with a daily ethanol intake <60 g/day, the LVEF may improve, albeit to a lesser degree than individuals with complete abstinence. Therefore, while alcohol abstinence is the best goal, the LVEF can improve with controlled drinking (<60 g/day) in subjects unable to achieve abstinence [20, 57, 61]. Episodes of binge-drinking, defined as the consumption of four or more alcoholic beverages in women and five or more drinking in men in a period about 2 h [62], have been demonstrated to worsen the clinical course of ACM [63]. During binge drinking, alcohol blood levels reach 0.08 g or higher. QRS duration, systolic blood pressure, and New York Heart Association classification at admission provide independent prognostic information in patients with ACM [58].

Moderate alcohol drinking, considered as the consumption of 10–40 g /day in women (1–3 standard drinks) and 20–60 g/day (1.5–4 standard drinks) in men, is usually not considered cardiotoxic unless it is consumed over a large period of time, usually more than 10 years [2, 15, 64]. In fact, moderate alcohol consumption in healthy individuals has been related to lower risk of development of heart failure [2, 65]. However, it should be avoided in presence of other causes of cardiac disease (i.e. hypertensive, or valve disease), other systemic-related effects of ethanol or in the presence of alcohol use disorder or dependence [6, 66].

Other factors Influencing Alcoholic Cardiomyopathy

In addition to the direct effects of ethanol that can explain around 60% of the global effects in ACM [48], other factors may have a relevant influence on the development of ACM.

Gender

The absorption, distribution and metabolism of alcohol are clearly different in women compared to men [67–69], resulting in higher ethanol blood levels at the same ingested dose of ethanol and inducing a major propensity to myocardial damage). Functional proteomic analysis reveals sex-dependent differences in structural and energy-producing myocardial proteins in rat model of alcoholic cardiomyopathy [70]. In the follow-up of progressive ethanol intake, the decrease in LVEF over time is 30% steeper in women than in men [48]. This indicates that with the TLDE consumed, the prevalence of ACM in women is higher than in men [48, 68]. In has been described that women with heart failure due to ACM consume a significantly lower TLDE tan men [71], which would explain the greater susceptibility of women to the myocardial toxic effects of ethanol compared to men [48]. In ACM, there are also sex differences in certain co-occurring conditions among men and women. Women with ACM experienced more anxiety and depression tan men [72]. Pregnancy is a period were alcohol consumption should be discouraged because the high prevalence of fetal alcohol syndrome that may also involve heart diseases [73].

Ethnicity

Although ACM has been described in all races and ethnicities, racial predisposition to ACM is more prevalent in Asian and black people than in Caucasians [45, 74]. Acetaldehyde dehydrogenase 2 (ALDH2) gene mutations that increase the risk of developing ACM are present in around 50% of East Asians, with 40% being homozygous. This represents around 8% of the global world population with increased ACM risk [75, 76].

Genetic Polymorphisms

The involvement of dilated CMP-related genes has also been reported in ACM [11, 12]. ACE (angiotensin converting enzyme) gene polymorphism also influence ACM development. Alcoholic consumers with the ACE "*DD"* genotype presented a 16-fold excess risk of developing alcoholic CMP compared to the other *"ID* "or "*II*

“variants [77]. Carriers of the truncate variant of titin, a structural protein related to ventricle distensibility, are more susceptible to ACM development and are also associated with a worse LVEF [78]. Enzymatic polymorphisms in ALDH2 have been described to modulate the incidence and course of ACM [69, 75]. These individuals have ineffective acetaldehyde detoxification mechanisms and present with the clinical phenotype of Asian-alcohol facial flushing and tachycardia after alcohol consumption (see also book chapter within this book xxxx). They should completely avoid alcohol consumption because of the influence of ALDH2 polymorphism variants in mitochondrial ALDH2 alcohol metabolism during which toxic acetaldehyde is oxidized [76].

Malnutrition

The presence of protein or caloric malnutrition, vitamin deficiencies, especially thiamine deficiency causing beriberi heart disease [30] and ionic disturbances (Na^+,K^+,Ca^{2+}, Mg^{2+}) may interfere with and aggravate the course of ACM [6, 79]. Therefore, this should be avoided in order to improve ACM symptoms and prognosis. Correction of these deficiencies may help to stabilize the course of ACM and avoid acute heart failure or episodes of arrhythmia [6, 80].

Other Drugs

Co-abuse of alcohol with tobacco and cocaine increases global myocardial damage and the intensity of ACM and accelerates disease progression (13 ,35, 36,81, 82). In fact, two third of ACM patients are active smokers. Tobacco may act at the same targets as ethanol such as mitochondrial oxidative stress and apoptosis [13, 35, 36, 81, 82]. In the context of persistent polytoxic drug consumption, the global effect in ACM is clearly detrimental.

Other Systemic Effects of Ethanol

The presence of alcohol-related cirrhosis, malnutrition, chronic pancreatitis, peripheral neuropathy, skeletal myopathy, dementia of arterial hypertension can negatively influence the course of ACM [17, 22, 37, 66, 79]. This is due to the global and synergistic effect that ethanol exerts on the development of ACM e.g. water retention in cases of liver cirrhosis [6, 9]. Obesity is unusual in ACM (<5% of cases) and has no relevant synergistic effect on ACM. In contrast to alcohol-related liver damage, alcohol-dependent iron overload in the myocardium has not been shown to be relevant in ACM.

Ethanol Induction of Functional and Structural Myocardial Damage

Both in clinical and experimental studies, ethanol has been demonstrated to induce an immediate negative and dose-dependent effect on myocyte contractility [83, 84]. Over longer periods of time, ethanol also not only decreases contractility of myocytes but also increases excitability causing arrhythmias [24, 27, 34, 64, 85]. Episodes of binge-drinking are especially damaging in the course of the disease [62, 63]. The pattern of alcohol consumption is also more relevant than the type of alcohol consumed [7, 86]. Both the sum of acute, repeated intake and the duration of chronic abuse are likely to contribute to disease progression [1, 15].

Since ACM usually appears in middle-aged adults in the third to the fifth decades of life, it is assumed that a minimum period of time (>10 years) is necessary for the clinical development of this disease [1, 2]. Similarly, a minimum cumulative dose of ethanol is necessary to develop ACM [32]. Combining both factors, the minimum TLDE per kg of body weight necessary to develop of ACM is 7 kg ethanol/kg for women and 10 kg ethanol /kg for men. However, patients with overt ACM have usually consumed more than 25 kg ethanol/kg over a period longer than 25 years [15].

The first functional signs of ACM in these patients is the echographic detection of LV diastolic dysfunction in asymptomatic alcohol consumers [42]. This is evident by the presentation of echographic parameters of a mitral valve filling pattern (E/A ratio, the deceleration time of the E wave, the early and late velocities of the mitral annulus measured by tissue Doppler, the left auricular volume, the pattern of pulmonary vein flow, and the duration of reversed flow into the pulmonary veins during atrial contraction) [87]. Diastolic dysfunction usually precedes detection of systolic dysfunction. LV diastolic dysfunction may appear at TLDE as low as 5 kg/ethanol/kg of body weight [15]. Mirijello et al. [60] described altered E/e' ratio as characterization of early-ACM before the occurrence of other relevant echocardiographic alterations.

Among individuals who continue ethanol consumption, on third may develop LV systolic dysfunction, usually detected by echocardiography with a reduction in the LVEF lower than 50%. At this time episodes of LV heart failure may appear within a clinical spectrum similar to idiopathic dilated CMP [2, 5, 88].

At the histological level, this subclinical period may be accompanied by myocyte hypertrophy and oxidative LV damage and pro-apoptotic activation mechanisms. Myofibre size variability and disarray is one of the most relevant initial features of ACM [21, 89] (Fig. 70.1). As the disease progresses, focal necrosis of myocytes appears with increased oxidative damage and the development of nuclear apoptotic phenomena. This causes progressive myocytolysis and final myocyte loss that may involve more than 30% of the global myocardium [21, 90].

Advanced cases of ACM have evident variability of fibre-size with some presenting massive hypertrophy [91], as well as active myocyte necrosis and fibrosis that starts at the subendocardial level and progressively appears in the myocardial

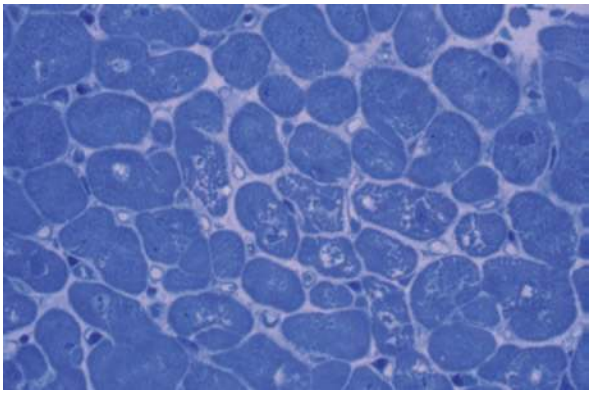

Fig. 70.1 Preclinical alcohol-induced structural myocardial damage. Left-ventricular heart biopsy from a patient with subclinical alcoholic cardiomyopathy. Moderate cellular and nucleic hypertrophy and myofibrillary disarray is apparent. Toluidine-blue staining of a semithin section, original magnification ×400. Permission obtained from Wiley Periodicals, Incl. © Urbano-Marquez A, Fernández-Solà, J. Effects of alcohol on skeletal and cardiac muscle. Muscle and Nerve. 2004; 30(6):689–707

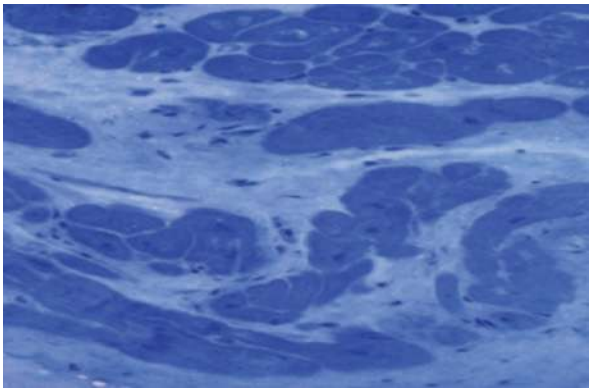

Fig. 70.2 End-stage alcoholic cardiomyopathy. Left-ventricular heart biopsy from a patient with end-stage alcoholic cardiomyopathy. Diffuse interstitial and subendocardial fibrosis, as well as cellular and nucleic hypertrophy, is apparent. Toluidine-blue staining of a semithin section, original magnification ×400. Permission obtained from Wiley Periodicals, Incl. © Urbano-Marquez A, Fernández-Solà, J. Effects of alcohol on skeletal and cardiac muscle. Muscle and Nerve. 2004;30(6):689–707

interstitium. In advanced cases, fibrosis may occupy more that 30% of the histological myocyte fraction [21, 92, 93] (Fig. 70.2).

In early stages of ACM, the deleterious effects of ethanol causing acute LV depression are reversible within hours or few days in subjects able to completely suppress ethanol intake [94, 95]. This acute reversibility is not present in advanced stages.

End-stage LV failure is defined as a LVEF <20%. The clinical situation is repetitive episodes of congestive heart failure, with oedema and anasarca [2, 50]. Arrhythmias are also frequent in this period, being atrial fibrillation the most frequent and ventricular extrasystole and tachycardia the most dangerous [52, 85]. At a histological level, diffuse fibrosis appears, substituting the death myocytes, which may be macroscopically evident with cardiac enlargement and focal scars together. In this phase, heart transplantation is the only effective measure, but is limited to subjects who achieve complete alcohol abstinence [96]. In long-term follow-up studies in ACM, mortality is related to progression of heart failure and the development of malignant arrhythmias [1, 85]. In this period, mortality is high (>10% patient/year) in the group of subjects withe persistent high-dose ethanol consumption >60 g ethanol/day.

In the course of ACM it is also important to determine the coexistence of other heart risk factors such as malnutrition, vitamin deficiencies, tobacco or cocaine consumption, and the presence of uncontroled diabetes mellitus, arterial hypertension and hypercholesterolemia that can further impair the course of ACM [6].

Pathophysiological Mechanisms in Alcoholic Cardiomyopathy

Ethanol and its metabolites, especially acetaldehyde and its protein adducts, produce a diversity of toxic effects on heart myocytes, most of which act in synergistic manner [8, 24, 34, 64, 97]. Figure 70.3 shows 14 different multiple-site ethanol targets in cardiac myocyte. These targets include modification of membrane composition and structure [98], disruption of ion channels and receptors [35, 83], interference in second messenger cell receptors [35, 99, 100], disruption of protein synthesis and composition [101], cytoskeletal structure and inter-cellular connections [100, 102], a reduction in cell energy mechanisms (mitochondria, carbohydrates) and the induction of pro-oxidative effects [7, 103]. All these processes disrupt myocyte excitation/contraction (E/C) coupling mechanisms and induce myocyte autophagy leading to progression to apoptosis and structural and functional cardiac myocyte damage with final cell loss [93, 104–106].

In experimental models of acute ethanol exposure, this damaging effect of ethanol is dose-dependent and reversible within minutes in the absence of ethanol [83, 107]. The direct effect of ethanol consumption on the myocardium accounts for 60% of the global risk to develop ACM. This is a long-standing and dose-dependent cumulative effect [32, 48]. These cummulative effects usually appear in subjects with a long-life consumption greater than 7 kg of ethanol per kg of body weight in men and 5 kg of ethanol per kg of body weight in women [15, 48]. The combination of the direct and indirect ethanol effects with other toxins, especially with tobacco and cocaine, or other additional risk factors (ethnicity, gender, ALDH2 or ACE gene polymorphisms) render ACM highly diverse [6, 10].

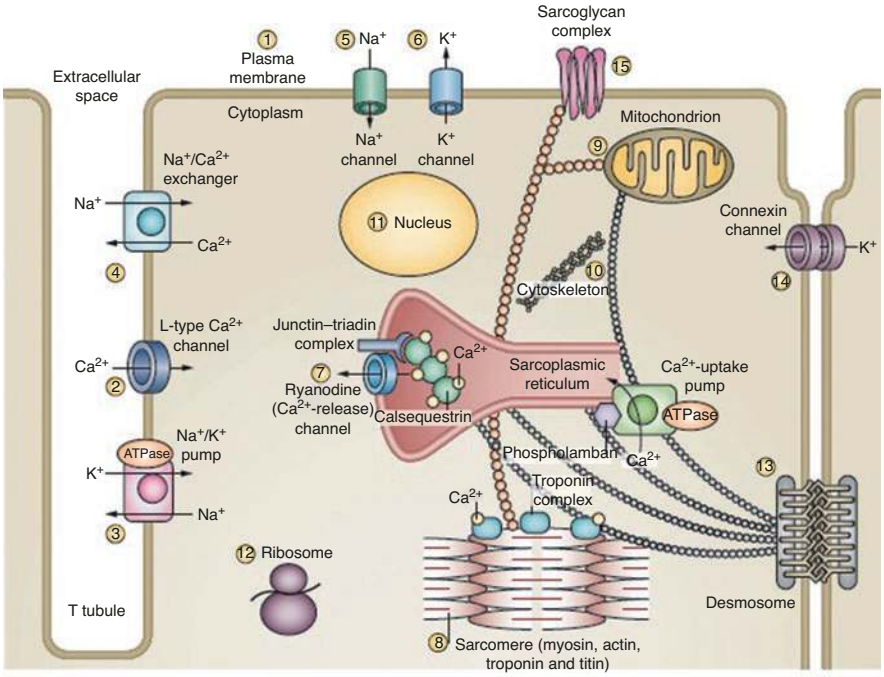

Fig. 70.3 Effects of ethanol on cardiomyocyte structure and organelles. Cardiac myocytes are excitable cells with complex signaling and contractile structures, and are highly sensitive to the toxic effects of alcohol on: (1) plasma membrane composition and permeability, signaling, and activation of apoptosis; (2) L-type Ca2 + −channel activity; (3) Na+/K+ ATPase channel activity; (4) Na+/Ca2+ exchanger activity; (5) Na + channel currents; (6) K+ channel currents; (7) ryanodine (Ca^{2+}- release) channel activity; (8) sarcomere Ca2+ sensitivity, excitation–contraction coupling, myofibrillary structure and protein expression; (9) several aspects of mitochondrial function, including respiratory complex activities; (10) cytoskeletal structure; (11) nuclear regulation of transcription; (12) ribosomal protein synthesis; (13) desmosomal contacts; (14) connexin channel communication; (15) sarcoglycan complex interactions. Permission obtained from Nature Publishing Group © Knollmann, B. C., Roden, D. M. A genetic framework for improving arrhythmia therapy. Nature. 2008;45:929–936

The coexistence of other cardiac risk factors in ACM such as arterial hypertension, and coronary or valve disease, is frequent, inducing an additional factor for increasing final myocyte damage [66]. The synchronic and multiple effects of ethanol on myocyte structure, energy production, oxidative stress, E/C coupling mechanisms, inflammatory response, and genetic control can explain the multiple-target synergistic effects of this substance [8, 14, 15, 34]. The combination of factors leading to cardiac damage demonstrates the need for personalized evaluation of the noxious effects of alcohol consumption and the development of ACM in each individual [14, 108].

Alcohol Damaging Effects on Myocytes (Table 70.1)

The Role of Acetaldehyde in Alcoholic Cardiomyopathy

Acetaldehyde is and active metabolite of ethanol oxidation, able itself to cause relevant cardiac toxicity and damage [109–111]. Lower quantities of this metabolite are produced in the heart compared as to the liver while systemic acetaldehyde production seems not to reach toxic heart concentrations [112]. At an experimental level, acetaldehyde directly impairs cardiac contractile function [113, 114], disrupts cardiac excitation-contraction coupling and promotes lipid peroxidation and oxidative damage [34]. Due to the evident direct cardiotoxic effect of both of these molecules [114], it is questionable whether cardiac damage is due to ethanol itself or to the effects of acetaldehyde [97, 111]. Elevated cardiac acetaldehyde exposure via alcohol dehydrogenase may exacerbate alcohol-induced myocardial dysfunction, hypertrophy, insulin resistance and endoplasmic reticulum stress [75, 115]. Acetaldehyde also activates a local myocardial renin angiotensin aldosterone

Table 70.1 Mechanisms of alcohol-induced heart damage

Mechanisms	Effectors
Interference with cell signaling and calcium transients	MAPK, TGF-β, PKC, PPARγ, MMPs, NF-κβ, PAI-1
Decrease in excitation-contraction coupling mechanisms	Intracellular $[ca]^{2+}$ transients, L-type Ca^{2+} channel
Induction of oxidative damage	ROS, SOD, acetaldehyde
Pro-inflammatory effect	IL-2, TNF-α, NF-κβ
Induction of apoptosis	FAS, TNF-α, TGF-β, Bax-Bcl-2, caspases 3,6
Induction of fibrosis	TLR-4, TGF-β
Protein-adduct formation	Protein-ethanol-adducts
	Malondialdehyde-DNA adducts
Disruption in protein synthesis	Decrease in ribosomal protein synthesis, actin, myosin, troponin, titin
Increased glycogen deposition	Glycogen synthase kinase-3β, PARP
Renin-angiotensin-aldosterone activation	Renin, angiotensin, aldosterone, p38 MAPK/Smad
Interference in hormone-growth factors	Myostatin, ghrelin, leptin, IGF-1
Interference in regulatory cardiomyokines	FGF21
Decrease in myocyte regeneration	Myostatin, IGF-1
Impairment of extracellular matrix turnover	Cytoskeletal structure, connexin channel, desmosome contacts
Imbalance between cardiac lesions/repair mechanisms	Cell apoptosis and necrosis increased myocardial fibrosis decreased myocyte regeneration

From: Fernandez-Solà J, Planavila A©. New treatment strategies for alcohol-induced heart damage. Int J Mol Sci. 2016;17:1651

system [111]. In fact, both molecules are able to increase myocyte oxidative and metabolic damage, decrease structural protein synthesis and heart contractility, leading to myocate autophagy [34, 105, 114]. Another pathogenic effect of acetaldehyde is its interaction with proteins, generating protein-adducts that are highly reactive and may induce additional inflammatory and immunologic heart damage, including DNA damage [116]. With its multiple actions, acetaldehyde can influence the pathogenesis of ACM in addition to the direct effect of ethanol [34, 112, 116].

[Ca^{2+}] Transients, Sarcoplasmic Reticulum Activation and Sarcomere Contractile Damage

The most relevant function of cardiac myocyte is heart contraction. As an excitable cell, this myocyte contraction is mediated by (E/C) coupling mechanisms, regulated by transmembrane electrically-induced Ca^{2+}transients, followed by activation of the ryanodine L-Type Ca^{2+} receptors, with final activation of the sarcomere actin/myosin coupling that produces myocyte contraction [83, 84, 107, 117]. Ethanol may damage these myocyte contractile mechanisms in a dose-dependent manner at different levels [15, 24]. This first transmembrane transient step is modified by the effect of ethanol on the membrane composition bilayer, that modifies its composition, permeability, signaling and activation mechanisms producing a disruption of transmembrane electrically-induced Ca^{2+} transients [107]. This involves plasma membrane L-type Ca^{2+} channel activity, Na^+/K^+ ATPase channel activity, Na^+/Ca^{2+} exchanger activity, Na^+ channel currents and K^+ channel currents [98] (Fig. 70.3).

The second step is at the sarcoplasmic reticulum level, at which ethanol specifically alters the ryanodine L-type Ca^{2+} receptor (RyR), producing a dose-dependent down regulation of sarcolemmal Ca^{2+} release and sarcomere Ca^{2+} sensitivity [34, 99, 104]. There is induction of sarcoplasmic reticulum Ca^{2+} leakage by CaMKII-mediated pathways downstream from reactive oxygen species (ROS) [24, 103]. Finally, the sarcomere E/C coupling contractile mechanism is also affected [118]. Chronic ethanol consumption down-regulates myofilament Ca^{2+} sensitivity [34, 104] and depressed structural and non-structural heart protein synthesis and degradation [118, 119]. This effect, if persistent, induces sarcomere structural damage expressed as sarcomere Z-line distortion and disruption of sarcomere contractille pattern with focal myofibre dissolution, causing myocytolysis, cell vacuolization and fiber disarray [34, 103, 118]. Titin is a sarcomere complex protein early affected by ethanol exposition, and a reduction in this protein leads to impairment in sarcomere relaxation and LV distensibility [120–122]. As a consequence of this progressive damage, first subclinical and later clinically evident diastolic dysfunction develops [42, 60]. This is followed by damage of contractile sarcomere proteins such as myosin, actin and troponin, causing progressive functional depression of myocyte contractility, inducing progression to systolic dysfunction and overt heart failure [50, 106, 120].

In addition, this transient intracellular [Ca^{2+}] disturbance also involve other intracellular [Ca^{2+}]$^{-}$ dependent organelles, as in the case of mitochondria or ribosomes [123–125]. Myocyte adaptative responses to ethanol-mediated toxicity leads to an up-regulation of myocardial L-type [Ca^{2+}] channel receptors, the activity of which is decreased in the presence of ACM [99, 117].

Mitochondrial Oxidative Damage and Energy Disturbance

The myocardium maintains the persistent sarcomere contractions requiring a high energy supply. Therefore, it has been supposed that alcohol could exert his damaging effect on the mitochondrial energy supply system by disruption of oxidative control mechanisms and ROS production [10, 103, 125]. At an ultrastructural level, the mitochondria of chronic alcohol consumers show typical structural changes, having the appearance of being swollen, the presence of megamitochondria and distortion of inner cristae [106, 126, 127]. At the functional level, ethanol decreases the activity of Complexes I, II and IV of the mitochondrial respiratory chain and alters the myocyte oxidative pattern [10, 109, 110], leading to subsequent glycogen deposition and cytoplasmic lipid droplet accumulation. Dysfunction on the transition pore in the inner membrane is also a key point in the mitochondrial effects of ACM [123]. Ethanol may also induce a mitochondrial-dependent apoptosis pathway with Bax and caspase activation [123, 128].

Inflammation

The myocardium is sensitive to the inflammatory effects of ethanol and the persistence of systemic inflammation increases cardiovascular risk [129]. At low doses (<20 g per day), ethanol has anti-inflammatory effects but at high doses (>60 g/day) it has a pro-inflammatory effect [17, 27]. It has been hypothesized that the cardiovascular effects of ethanol are mediated, at least in part, by inflammatory mechanisms [17, 27, 129, 130].

Ethanol may induce diverse pro-inflammatory effects increasing the levels of C-reactive protein, interleukins, pro-inflammatory cytokines, tumor necrosis factor, decreasing serum concentrations of fibrinogen and IL-5 and changing the levels of inflammasomes [131]. Specifically, microRNAs related to inflammation increase after beer consumption and decreased after non-alcoholic beer consumption [132].

As a consequence of this inflammatory process, increased oxidative stress develops with nicotinamide adenine dinucleotide phosphate oxidase activation, and lipid peroxidation, with glutathione and superoxide dismutase depletion. This may cause an increase of endothelial nitric oxide synthase expression, as well as endothelial and myocyte dysfunction [27, 131–133].

Cardiac Hypertrophy and Remodeling

When myocardium undergoes is challenged by toxins such as ethanol, a global remodelling adaptation process is induced, making the myocytes relatively resistant to the toxic effects of ethanol [134–136]. To minimize or repair the toxic effects of ethanol, heart myocytes develop functional and structural compensatory mechanisms [4, 34, 134]. Structural myocyte hypertrophy appears in the early stages of ACM to avoid contractile myocyte depression [2, 106]. If the toxic effects of ethanol persist, the sarcomere contractile system and myofibrillary composition is altered and myocitolysis progressively develops, causing heart ventricle wall hypertrophy and compensatory ventricle dilatation. This induces a dose-dependent decrease of cardiac output, inversely related to the TLDE consumed by the patient [19, 137]. Cardiac hypertrophy increases cardiomyocyte size and mass, induces cardiac remodeling and it is a key factor in the transition from a normal to a pathologic heart [15, 91].

Myocyte Apoptosis and Autophagy

The development of cardiomyopathy of diverse origin is usually accompanied by active myocyte apoptosis, a situation that induces progressive myocyte loss and myocardial functional and structural damage [134, 138]. This is also the case of chronic high-dose ethanol consumption in both clinical [128] and experimental settings [101]. The presence of apoptosis may be assessed by TUNEL staining and immunohistochemistry and caspase activity [128].

Ethanol can induce myocyte apoptosis by different mechanisms [139, 140]. One mechanism is direct damage and permeabilization of the mitochondrial membrane transition pore by physiological calcium oscillations. This produces the release of pro-apoptotic factors (cytochrome c) from the mitochondrial inter-membrane space to the cytosol [7, 123]. Another mechanism is independent of the mitochondrial pathway [139] and occurs by an extrinsic pathway, which involves cell surface death receptors [126, 141]. It has also been described that a specific microRNA, miR-378a-5p, is involved in the stimulation of cardiomyocyte apoptosis through ALDH2 gene suppression [105, 142].

In parallel to this ethanol- dependent induced apoptosis, ethanol also has additional effects in inhibiting anti-apoptotic molecules such as BCL-2 [118, 128, 139]. Several growth factors [101] and CMK [24, 71] might regulate this ethanol –induced myocyte apoptosis. Although the percentage of apoptotic myocytes is relatively low in ACM, the combined effect of a persistent decrease of myocyte proliferation results in absolute cell loss and decreased cardiac contractility [95].

In addition to apoptosis and necrosis, autophagy has recently been described as a possible relevant mechanism in ACM [101, 105, 138, 143]. Autophagy is a physiological mechanism that is responsible for the removal of cell debris and

defective organelles. This process is essential to the maintenance of cardiac homeostasis in both physiological and pathological conditions. At present, few data are available in relation to the involvement of autophagy and the pathogenesis of ACM [8, 23].

Cardiac Fibrosis and End-Stage ACM

When the heart is submitted to persistent myocyte apoptosis or necrosis by chronic ethanol consumption, the myocardium attempts to activate repair and regenerative tissue damage mechanisms [4, 144]. However, the regenerative capacity of cardiac myocytes is low leading to ineffective repair mechanism [90, 140]. In addition to direct myocyte damage, chronic ethanol intake also significantly decreases the myocyte regeneration capacity and increases the myocardial fibrogenic process [2, 15, 92]. Some CMKs such as FGF21 may regulate this process of alcohol-induced cardiac fibrosis [124, 145]. Ethanol treatment directly promotes cardiac fibroblast activation by stimulating transforming growth factor (TGF)-β release from fibroblasts. Inhibition of the action of TGF-β decreases the fibrogenic effect induced by ethanol treatment [146]. This results in the progressive development of subendocardial and interstitial fibrosis that is more evident in advanced stages of ACM in which fibrotic tissue may replace more than 30% of the myocyte ventricular fraction [21, 106]. This fibrotic myocardial process reduces heart elasticity and contractile capacity [42, 50, 64].

In the setting of end-stage ACM, progression of ethanol-induced myocardial damage and blockage of the heart's plastic and repair mechanisms limit myocardial remodeling that is apparently ineffective in this chronic longstanding scenario [50, 90, 136]. This situation usually appears after more than 20 years of high- ethanol consumption, when the total lifetime cumulative doses of ethanol exceeds of 20 kg ethanol/kg body weight [2, 15] and at which time the myocardium has been submitted to major structural damage. The histological pattern of diffuse myocyte necrosis with significant myocyte loss is compensated by fibre and nuclei hypertrophy of the remaining myocytes. This myocyte loss is replaced by subendocardial and interstitial fibrosis, which is functionally less effective [21, 106]. In this scenario, individuals present an LVEF <15% and have developed frequent episodes of congestive heart failure and ventricular arrhythmias leading to possible sudden death [52, 53, 85]. At the time of advanced myocardial damage, these subjects normally also develop other systemic ethanol-related diseases [6]. Thus, liver cirrhosis, digestive tract and neurological lesions and psychiatric disorders are usually present. Indeed, this systemic involvement contributes to worsening the prognosis of the patient [37]. In this end-stage scenario, the mortality is higher than 30% per year, mainly in subjects who continue with ethanol consumption [2, 88, 147].

The Effects of Alcohol on Myocyte Repair Mechanisms

Repair mechanism are crucial to maintain a normal cardiac function over time [50, 136, 141]. Ethanol not only damages myocyte structure and function, causing apoptosis and myocyte loss, but also decreases and alters the myocyte repair mechanisms at different levels [26, 50, 90]. In this setting, alcohol consumption impairs cardiac adaptation and remodeling mechanisms and causes additional heart damage [6, 24].

Thus, the structural damage of sarcolemmal E/C proteins is difficult to be repaired in the presence of ACM due to the inhibition that ethanol produces in myocyte structural protein synthesis at the ribosome level [103, 118]. Hypertrophy of the myocytes may be a compensatory phenomenon in early stages but has a limited effect in advanced stages [106]. The myocyte regenerative capacity, that is usually low, is clearly decreased in the presence of ACM [26, 148]. These factors limit the plasticity and myocardial remodeling mechanisms, resulting in progressive myocardial damage, especially if alcohol consumption persists [57, 134].

The Role of Cardiomyokines and Growth factors in ACM

Cardiomyokines (CMK) are autocrine and paracrine proteins with systemic and local protective effect and play a relevant role in intercellular connectivity within the myocardium [124, 149, 150]. At present, more than 60 CMK are described, 30 of which are biologically relevant**.** CMK act as metabolic regulators in lipid and carbohydrate metabolism, having a relevant role in the control of obesity, diabetes mellitus, exercise tolerance and energy expenditure. They coordinate the interorgan cross-talk and they are also involved in the modulation of heart remodeling and have a regulating role in the alcohol-induced heart damage [124].

Recently, a new CMK (FGF21, Metrnl) and several growth factors (myostatin, IGF-1, leptin, ghrelin, miRNA, and rho-associated protein kinase- ROCK- inhibitors) have been described as being able to regulate cardiac plasticity and decrease cardiac damage, and improving cardiac repair mechanisms, respectively [26, 124, 145]. Their effect may explain different adaptative mechanisms in ACM and may be of potential therapeutic use [151].

With respect to **myocyte hypertrophy**, FGF-21 and Metrnl have a protective role against excessive cardiac hypertrophy [124, 145, 152]. Myostatin, a member of the TGF-β family, is a potent inhibitor of muscle and heart growth and mediator on alcohol-induced myocyte hypertrophy [26]. It controls cell cycle progression, arresting G1 phase and inhibiting myoblast proliferation and terminal differentiation. Myostatin has antihypertrophic effects and its disruption causes increased myocyte mass with hypertrophy and hyperplasia and increased satellite cell stimulation and myocyte regeneration. Ethanol up-regulates heart myostatin expression

[119]. In human myocytes, the expression of myostatin is higher in alcohol consumers with CMP compared to those without CMP or to non-alcoholic controls. This increase in myostatin expression in alcoholic CMP is significantly higher compared to other causes of CMP, as hypertensive CMP [26].

Myocte proliferation, evaluated with the Ki-67 proliferation index, increases in different causes of CMP (alcohol, arterial hypertension or idiopathic) compared to those groups without CMP, with ACM showing a lower increase in this proliferation response. This explains an antiproliferative effect of ethanol on cardiac myocyte regeneration [26, 153]. Ethanol increases the activity of myostatin [26] and decreases IGF-1 myocardial expression, leading to inhibition of myocyte proliferation [145]. Metrnl has a pro-proliferative effect [152] and alpha-lipoic acid has a robust anti-hypertrophic and anti-remodeling effect that is mediated by the inhibition of C/EBPβ [25].

With regard to **heart fibrosis**, ethanol consumption down-regulates IGF-1 activity, increasing heart fibrosis [145]. As a protective effect, FGF-21, IGF-1, Metrnl and TGF-β antagonists have antifibrogenic effect in ACM [25, 93, 146]. Pharmacological inhibition of soluble epoxide hydrolase ameliorates chronic ethanol-induced cardiac fibrosis by restoring autophagic flux [154]. The potential use of CMK to control ethanol-induced damage in ACM aims to control oxidative disruption, myocyte hypertrophy, interstitial fibrosis and persistent apoptosis and increase myocyte regeneration. However, these new strategies have not yet demonstrated their real effectiveness in clinical trials, require further evaluation, and are not still approved for clinical use [6, 153].

The final cardiac status in ACM will be the consequence of an equilibrium between the intensity of damaging effects and the possibility of defense, plasticity, regeneration, and adaptation in each individual [23, 134, 150]. Thus, ACM is the result of dosage effect and individual predisposition to myocyte damage and the capacity of heart repair mechanisms [46, 134]. In the future, gene testing will be most likely performed in order to predict and individualize the degree of alcohol-induced cardiac damage [2, 11, 12].

Complementary strategies to decrease alcohol-induced systemic subclinical organ damage, avoid other cardiovascular risk factors [17, 37, 44], and improve nutritional health with the Mediterranean diet and vitamin or ion deficiencies supplementation may improve prognosis in ACM [25, 80].

In summary, total lifetime alcohol consumption produces progressive cardiac myocyte damage and loss through apoptosis but also partially inhibits myocyte proliferation by means of cardiomyokine and growth factor regulation. The final result supposes an imbalance in myocyte homeostasis, with a net decrease in total ventricular myocyte mass and progressive ventricular dysfunction as well as structural damage leading to end-stage cardiac failure.

Future Trends in Alcoholic Cardiomyopathy

Since the prevalence of alcohol consumption is not decreasing in Europe, especially among teenagers and young people [155], the prevalence of ACM is not expected to diminish in the near future [156]. In fact, the total alcohol consumption per capita (15+ years) is expected to increase until 2025 in half of the regions of the World Health Organization [41]. Therefore, according to the global alcohol consumption rates in Europe, ACM cases are expected to rise in the next years due to the direct dose–dependent effects of alcohol consumption on ACM [15].

Taking into account the expected increase in the number of cases of ethanol-induced ACM in the future, preventive strategies are needed to minimize the damaging effects on health and these should be personalized with a multidisciplinary approach combining control of alcohol-induced systemic damage, as well as antiinflammatory, antioxidant, antifibrotic and antiapoptotic preventive measures [24, 25, 44, 108]. Nonetheless, treatment of alcohol-use disorders to achieve abstinence is among the main goals on managing ACM [16, 61]. Strategies to control alcohol consumption would be useful to minimize the global burden of disease by ACM [61].

On the other hand, alcohol consumers able to diminish ethanol ingestion to a controlled drinking situation (ethanol consumption<60 g/day), will develop less myocardial damage compared to those consuming higher ethanol doses [57, 157]. In addition, the use of CMK in subjects not able to achieve abstinence may potentially reduce ongoing myocyte damage and improve cardiac remodelling [25]. Improvement in myocyte protein synthesis and reduction in degradation may be modulated by Valsartan, an angiotensin receptor antagonist that inhibited the RhoA-ROCK2-MYL pathway in rat model of ACM, and contribute to improving heart function [25, 158].

Gene therapies, targeting a defective gene or transcript, or ameliorating a genetic insufficiency are currently under study and may modulate downstream faulty protein products affected in CMPs [12, 24, 38]. Thus, miRNAs have a role in the regulation of oxidative-stress induced apoptosis, and selected mRNAs have regulatory effects on target gene expression with potential therapeuthic use in the control of heart apoptosis and damage [159]. Different variants of stem-cell therapy have been studied in an attempt to improve myocyte regeneration, albeit with limited success to date [160–162]. Heart transplantation is the final step in patients with end-stage ACM [96], but alcohol abstinence is a necessary requirement and only a minority (<15%) of ACM patients undergo heart transplantation [50].

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Chapter 71
Ethanol and Heart Failure: A Clinical Perspective

Adina Ionac

Abstract Ethanol consumption is the primary cause of alcoholic cardiomyopathy, known to affect only a fraction of drinkers. So far, it remains difficult to define a border between healthy and unhealthy alcohol ingestion either for risk of development of cardiac pathology or for worsening of pre-existing heart failure. The negative effects of ethanol on cardiac structures and function determines the decrease of systolic and diastolic left and right ventricular function and the clinical onset of heart failure. Diagnosis is primarily based on multimodality imaging and echocardiography. Ethanol has also an important role in arrhythmic complications, the most frequent and important being atrial fibrillation. However, it can also trigger life-threating arrhythmias or sudden cardiac death, especially in heavy drinkers or in binge drinkers. There is also evidence for other cardiac diseases influenced by alcohol such as coronary artery disease or systemic hypertension. On the other hand, controversies continue till today whether alcohol, in small quantities, has a cardioprotective or deleterious effect as improved lipid and glucose metabolism, anti-inflammatory effect, beneficial effect on endothelial function and lowered platelet aggregation have been reported.

Keywords Ethanol toxicity · Heart failure · Cardiovascular disease · Alcoholic cardiomyopathy · Multimodality cardiac imaging

Introduction

The cardiovascular system and the heart are probably the secondly most affected system by ethanol toxicity, after the gastrointestinal/ liver system [1, 2]. Cardiovascular disease is the leading cause of mortality in Europe (47%) and one of the main causes of death worldwide (31%) [3, 4]. It seems that in moderate

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consumers there is a mild cardiovascular risk, while heavy consumers have a high risk for cardiovascular disease. Exact borders are still difficult to delineate. For more details on epidemiology and alcoholic cardiomyopathy (ACM), the reader is referred to the respective chapters in part I and this part of the book and Chap. 70.

Defining the Concepts

Moderate consumers are those people who drink 3–9 drinks/week, corresponding to less than 20-gram pure alcohol per day [3]. It seems that a moderate amount of ethanol ingestion is beneficial or associated with a low risk for cardiovascular disease [3, 5]. Heavy consumers are those people who drink more than 9–10 drinks/week [3]. They have a high risk for cardiovascular disease, such as: systemic hypertension (SHT), angina pectoris, arrhythmias, even life-threating arrhythmias, and sudden cardiac death (SCD), systolic and diastolic left ventricular (LV) and sometimes associated with right ventricular (RV) dysfunction and, finally, global heart failure (HF). Even rather moderate amounts of ethanol ingestion can affect the function of the heart, most frequently being directly toxic for the myocardium, causing toxic dilated cardiomyopathy (DCM) [1–3] with its complications, most of them arrhythmias, including life threatening ones, and sudden cardiac death [6]. Generally, small doses of ethanol ingestion do not cause cardiac disturbances, but can be dangerous in some groups of population, such as children, adolescents, or women.

Mechanisms of Ethanol Cardiac Toxic Effects

Ethanol causes myocardial damage through multiple mechanisms and affects both the systolic and/or diastolic left ventricular function. The mechanisms that may cause myocardial damage are discussed in more detail in Chap. xxx of this part of the book. Briefly, they include:

- Direct toxic ethanol effects on the myocardium
- Ethanol toxic effect through its metabolites (acetaldehyde and ROS)
- Deficiencies of vitamins, minerals, or electrolytes (magnesium, potassium), which sometimes occur in heavy drinkers and may affect myocardium function
- Other toxic substances which sometimes contaminate alcoholic beverages may damage myocardium (cobalt, arsenic).

Direct toxic effects of the ethanol on myocardium cells are well documented: the alteration of sarcolemma membrane function (which leads to the decrease of calcium in the sarcolemma reticulum, and the inhibition of the sarcolemma adenosine triphosphate-dependent Na/K pump) and an altered mitochondrial function (through the decrease of mitochondrial respiratory rate). These processes determine myofibrillar degeneration and progressive fibrosis [3].

Moreover, they increase extracellular protein synthesis, accumulation of collagen in the extracellular matrix and progressively lead to the extracellular fibrosis [3]. Finally, these modifications will determine the alteration of ventricular systolic and diastolic function, in other words, the clinic syndrome of heart failure will develop. Ethanol also impairs the coupling of the excitation/contraction system. It can favour the development of atherosclerotic plaques and vascular disease such as coronary, carotid or peripheric artery disease [3]. Ethanol consumption must be stopped once cardiac ethanol-related diseases are diagnosed [7, 8]. In most cases, the symptoms are ameliorated although the disease will never completely disappear.

Alcoholic Cardiomyopathy: Definition and Diagnosis Evaluation

The association between alcohol consumption and HF remains controversial: **alcoholic cardiomyopathy** (ACM) may appear in cases of heavy alcohol consumption, while moderate alcohol intake may be associated with a lower risk of HF [8]. Limited data are available regarding the period of alcohol consumption. Most authors agree that more than 10 years are required for developing ACM while the amount of alcohol consumption is still under debate. As compared to alcohol-related liver disease, there are also gender differences in alcohol consumption: women develop ACM and HF at smaller amounts of alcohol consumption than men, and also after a shorter period. The most prevalent form of ethanol-induced heart damage is Alcoholic Cardiomyopathy. It was first described in heavy drinkers and already gained first clinical recognition by Hippocrates [9]. Ethanol induces toxic cellular effects followed by repair mechanisms, cardiac remodelling, and progressive systolic and diastolic LV dysfunction. ACM depends on dosage and individual predisposition, while management and prognosis will focus on abstinence or at least on controlled drinking [9, 10]. As mentioned above, the risk of ACM is higher in women than in men (for any given lifetime amount of alcohol) [9].

The definition of **Cardiomyopathy** was established many years ago in European and American Societies of Cardiology position papers and has remained almost unchanged until now: Accordingly, cardiomyopathy is "*a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction that usually (but not invariably) exhibit inappropriate ventricular hypertrophy or dilatation and are due to a variety of causes that frequently are genetic*" [11]. Despite many classifications, all authors agree strongly to "Dilated Cardiomyopathy" (DCM) which is defined by the dilation and decreased systolic function of the left ventricle and/or the right ventricle [11, 12]. The genetic or acquired causes are multiple and there is a permanent debate for a new classification according to the aetiology. The toxic effects of alcohol on the myocardium are recognized as one cause of DCM.

ACM typically presents with exertional dyspnoea, orthopnoea and paroxysmal nocturnal dyspnoea, palpitations, and fatigue. Although decreased systolic function has been long thought as major reason for HF, it is been learnt that an impaired diastolic function occurs in one third of heavy drinkers with otherwise normal systolic function. In many patients, however, systolic and diastolic dysfunction coexist. The specific diagnosis is challenging, and multimodality imaging (MMI) is the best solution. There are many studies, guidelines and position papers, which review the current knowledge on MMI diagnosis in ACM and they can also be applied to ACM-HF studies [13–19].

Accordingly, ACM is considered a non-ischemic dilated cardiomyopathy. Among the available imaging modalities, **transthoracic echocardiography** (TTE) is the method of choice for these patients. The assessment of ventricular function is essential and TTE is the first line for the assessment of LV and RV systolic and diastolic functions. The assessment of systolic function should include conventional data. For LV global systolic function, the established parameters are ejection fraction (EF), shortening fraction and stroke volume. The EF is defined as the percentage ratio between stroke volume and LV end-diastolic volume with a normal value higher than 55%. Despite the many limitations of EF, its use is strongly recommended since most doctors with easily understand its significance and implications, regardless of their training and medical background. The European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE) recommend the EF evaluation in two-dimensional (2D) echocardiography by the modified Simpson's method of discs and not in M mode echocardiography [20]. Calculation of EF by M mode echocardiography was originally based on the, clearly wrong, assumption that LV is a cube. Calculation of EF by the modified Simpson's formula consider the LV is an ellipsoid and LV size is calculated based on several LV sections. Although closely meeting reality, the best evaluation of EF is obtained by three-dimensional (3D) echocardiography using full-volume acquisition. However, even using 3D-EF evaluation, there may be situations with normal EF despite clinical signs of heart failure. This can be due to a particular LV architecture: the disposition of the myocardial fibrils in two layers with an oblique arrangement. Consequently, the global contraction is the sum of all radial, longitudinal and circumferential contractions. Studies have shown that the longitudinal contraction is first reduced while the radial and circumferential contraction increase in a compensatory fashion a global contraction remaining normal.

The **EF** is a parameter which should describe the global contraction and myocardial movement does not necessarily mean contraction. The term "deformation" should be better used for such cases. The movement of a myocardium point has velocity in systole and diastole. Strain is a dimensionless parameter which describes the percentual change of a myocardial segment length. By convention, it has a negative value in systole (the myocardium is thickening) and a positive value in diastole (the myocardium is lengthening). The strain will describe better the LV systolic function because it can evaluate separately the longitudinal, radial and circumferential contraction or the global contraction taking into account all three. The strain rate is defined as the rate at which the myocardium deformation ocurs [21–24].

Strain parameters can by evaluated by Tissue Doppler Imaging (TDI) and 2D and 3D speckle tracking echocardiography (STE). TDI can measure the velocities of myocardial movement and can calculate the strain and the strain rate based on the differences in two points of myocardial movement velocities. A major limitation is the fact that it is a Doppler-derived method and, consequently, it is angle dependent. This is also the reason why it measures the longitudinal velocities, respectively the longitudinal strain and strain rate [23]. For longitudinal LV systolic function evaluation, the pulsatile TDI measures the systolic velocity (S′) and for LV diastolic function, it measures the diastolic velocities (E′ in early diastole, in the same time with transmitral E wave; A′ in late diastole, in the same time with transmitral A wave).

Speckle tracking echocardiography is a method which is based on registration of acoustic points (named speckles) movement during the cardiac cycle. In contrast to Doppler-derived methods, it is not angle-dependent [23, 24]. The longitudinal systolic LV function is described by global longitudinal strain (GLS), which has been shown to have a superior sensitivity for LV systolic function evaluation, and a superior prognosis for HF as compared to the classical parameter LVEF [25]. The normal value is 20 ± 2% [20].

The diagnostic criteria for DCM rely on the identification of a LVEF <45% and/or a fractional shortening <25%, in association with a LV end-diastolic dimension >112% predicted value corrected for age and body surface area. However, because of the limitations of LVEF, a correct evaluation of LV systolic function must be extended to more other parameters derived from TDI, 2D and 3D speckle tracking and 3D echocardiography (Table 71.1) [22]. Systolic (S′) velocity measured by TDI, and global longitudinal strain (GLS) evaluated by 2D speckle tracking are robust parameters for evaluation of LV systolic dysfunction [21–24].

According to the American Society of Echocardiography [26–28] and the European Association of Cardiovascular Imaging [29], the assessment of diastolic function should include a multiparametric evaluation. In the patients with low EF, transmitral diastolic flow pattern is usually enough to identify the presence and the

Table 71.1 Parameters of left ventricular function

Echocardiographic technique	Parameters
Conventional TTE (2D, doppler)	Ejection fraction Fractional shortening Stroke volume Myocardial kinetics
Tissue doppler	Velocity (S′ wave) Strain Strain rate
Speckle tracking	Global longitudinal strain Radial function Circumferential function 3D speckle tracking
3D TTE	Ejection fraction Myocardial kinetics

2D two-dimensional, *3D* three dimensional, *TTE* transthoracic echocardiography

type of diastolic dysfunction [28, 29]. However, it should be verified by other parameters. Moreover, if the E/A ratio is ≤08 and E wave velocity > 50 cm/s or E/A ratio is between 0.8 and 2, it is mandatory to use other parameters for diastolic function evaluation [28]. The most important parameters are the values of early diastolic wave (E′) of mitral annulus movement measured by TDI, the E/E′ ratio, the left atrium volume index and tricuspid regurgitation velocity. The normal values are given in Table 71.2 while Fig. 71.1 shows an example of an evaluation of LV diastolic function in a patient with toxic dilatative cardiomyopathy and diastolic LV dysfunction. There are recommendations for other parameters as well such as: transmitral flow parameters (E wave, E/A ratio, E wave deceleration time and isovolumic relaxation time), the difference between transmitral A wave duration and pulmonary vein A wave duration, etc. (Table 71.2) [26–30].

The **right ventricular systolic function** should also be evaluated in patients suspected with ACM, and a low RV function is a predictor of high mortality [29]. The quantification of RV function is challenging due to its complex 3D shape [20, 31]. The most useful parameters are fractional area change (FAC) (normal value >35%), tricuspid annular systolic excursion (TAPSE) (normal value >17 mm), systolic (S′) velocity measured by TDI (normal value >9.5 cm/s), RV longitudinal strain measured by STE and EF measured by 3D echocardiography. In Fig. 71.2 there is an example of an evaluation of RV systolic function in a patient with toxic dilatative cardiomyopathy. Because all of these parameters have some limitations, a multiparametric evaluation of RV function is recommended [20, 31–33]. These parameters do not only have a diagnostic value, but are also prognostic. The RV longitudinal strain measured by STE seems to be the most valuable one, because it is angle independent and recent studies have shown its very good prognosis value [34–37].

Transthoracic echocardiography is considered the imaging modality of choice for mitral regurgitation severity and progression, which appears frequently secondary to mitral annulus dilation [38]. The evaluation must be also multiparametric and

Table 71.2 Parameters for left ventricular diastolic function [28, 29]

Echocardiographic parameter	Normal values
Transmitral E velocity	<50 cm/s
Transmitral E/A ratio	0.8–2
Septal annular E′ velocity (TDI)	>7 cm/s
Lateral annular E′ velocity (TDI)	>10 cm/s
LV E/ E′ (average of medial and lateral E′) ratio	<14
Left atrium volume index	≤34 mL/m^2
Transmitral A wave duration - pulmonary vein A wave duration	>30 ms
Tricuspid regurgitation velocity	≤2.8 m/s
Transmitral E wave deceleration time	160–240 ms
Isovolumic relaxation time	60–90 ms

TDI tissue doppler imaging

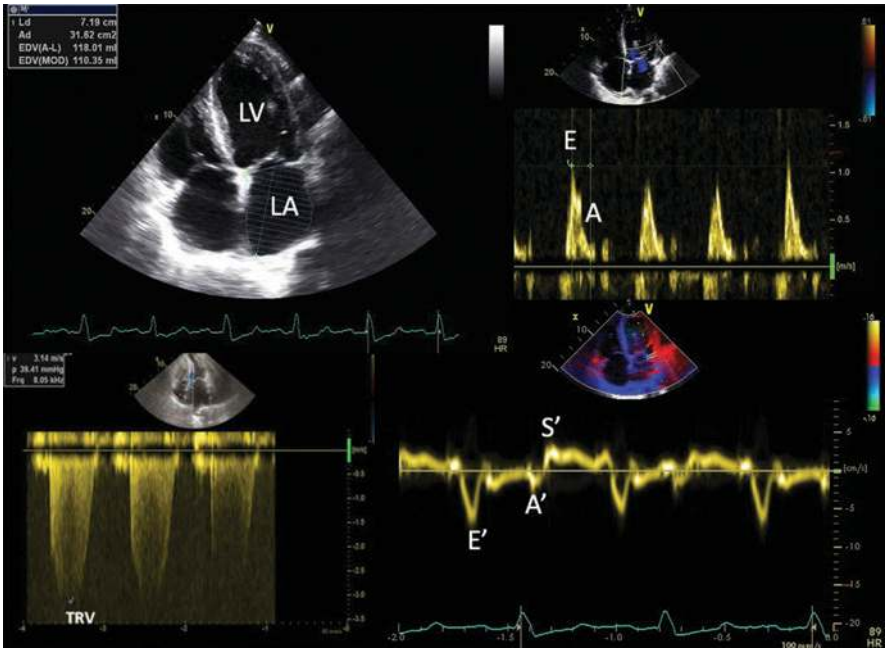

Fig. 71.1 Transthoracic echocardiographic evaluation of LV diastolic function in a patient with toxic dilatative cardiomyopathy. *LV* left ventricle, *LA* left atrium, *TRV* tricuspid regurgitation

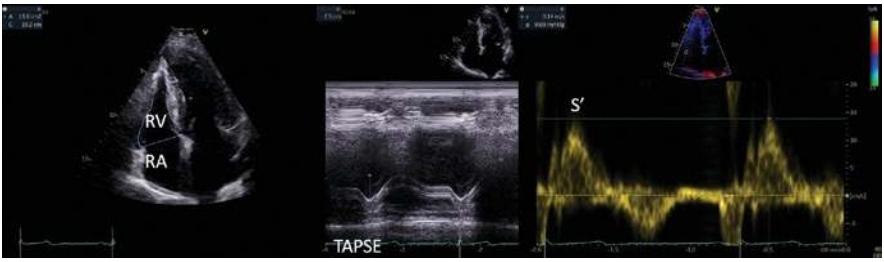

Fig. 71.2 Transthoracic echocardiographic evaluation of RV function in a patient with toxic dilatative cardiomyopathy. *RV* right ventricle, *RA* right atrium, *TAPSE* tricuspid annular systolic excursion

integrative. The severity evaluation is the same as for the primary mitral regurgitation, but it must take into account that the stroke volume is generally lower due to the systolic dysfunction, so the values for severe mitral regurgitation parameters are lower. Generally, a regurgitant orifice ≥40 mm^2 and a regurgitant volume >60 mL/beat will define a severe mitral regurgitation [38]. In ACM, the severe mitral regurgitation can be described by lower values: a regurgitant orifice ≥30 mm^2 and a regurgitant volume <60 mL/beat, or ≥45 mL/beat if flow conditions are very low [38].

Transthoracic echocardiography is useful as well for estimating the presence and the extent of mechanical desynchrony in the failing heart, and therefore it can serve as an aid to patient selection for cardiac resynchronization therapy [38]. **Cardiac magnetic resonance imaging** (CMR) may help in the assessment of LV dimensions and function whenever image quality is suboptimal with echo. Global longitudinal strain determine by CMR is a better parameter for risk stratification in DCM, than EF, but there is not a cut-off value yet and the technique is not routinely recommended [39]. Assessment of RV size and function should be evaluated by CMR as well. CMR has also the unique ability to non-invasively characterize the morphology and structure of the myocardium, making it an excellent diagnostic tool to differentiate the aetiologies of DCM. It can also detect myocardial perfusion defects. For an optimum treatment, it is very important to differentiate between ischemic and non-ischemic aetiology and, here, CMR is the best tool [39]. The technique uses late gadolinium enhancement (LGE). It can describe the places of scars, fibrosis or inflammation. In patients with ischemic DCM, the LGE (which describes fibrosis) appears subendocardial or transmural, according to the extension of myocardial infarcts. In contrast, in patients with non-ischemic cardiomyopathy, the LGE is located in the middle of the myocardium, has a patchy distribution, or there is no LGE [40].

In the past, it was assumed that fibrosis dies not appear in ACM, however, this has changed recently. The presence and pattern of distribution of LGE in ACM and idiopathic non-ischemic DCM showed a different LGE localization, mostly septal in ACM and lateral in idiopathic non-ischemic DCM, with different prognostic impact [40–42]. Importantly, presence of fibrosis is an independent prognostic factor for both DCM and ACM, and for risk evaluation of cardiac arrhythmias and sudden cardiac death. Here, the native T1 mapping is a valuable technique for ventricular arrhythmia risk evaluation in these patients and it is used for intracardiac defibrillators implantation indications [43]. Extracellular volume CMR evaluation has been reported to also have a good prognostic value for major cardiovascular events and heart failure decompensation [44, 45].

It is possible to use nuclear imaging and **computer tomography** (CT) imaging to complete the MMI diagnosis in ACM, but they rarely provide more data, so they are not routinely used. Cardiac CT is useful to exclude coronary artery disease. In conclusion, MMI evaluation in ACM is very important to introduce the optimum treatment and follow-up it, but it is also very important to appreciate the risk for cardiovascular events, for complications and to establish the prognosis. The best markers for prognosis in ACM are:

- LV EF $\leq$ 35%
- RV EF < 45%
- Abnormal GLS (determined by TTE and CMR)
- Advanced diastolic LV ± RV dysfunction
- Significant mitral regurgitation (secondary; the organic cause must be excluded)
- Presence, extension and pattern of LGE
- High T1 and extracellular volume values (by CMR) [41].

HF and Alcohol Consumption

With regard to the association between HF and alcohol consumption, most available data have been published for alcohol intake in patients with known HF. Several studies have suggested that moderate drinking in patients with known heart failure do not cause acute deterioration of cardiac function. In patients with ischemic left ventricular dysfunction, light to moderate drinking was even associated with decreased all-cause mortality [46]. However, other studies clearly suggest that ACM heart failure treatment requires complete abstaining form alcohol [1, 9, 47]. It remains unclear so far, whether abstaining from alcohol in patients with severe HF and ACM will reverse disease progression. There are published data, however, that patients with LV systolic dysfunction and moderate alcohol consumption have a lower mortality risk as compared with abstainers [48]. Other studies concluded that both reduction of alcohol intake or complete abstinence had similar effects on LV systolic function (LVEF) [49, 50].

Currently, the debate continues whether patients with HF should be advised to abstain from alcohol drinking. Regarding the genetic predisposition for ACM, very few studies have explored genetic association and ACM. There is a report showing an association between alcohol dehydrogenase gene polymorphisms, alcohol consumption and myocardial infarction risk, but no studies have shown directly association with heart failure or ACM [47].

Association of Ethanol and Artery Disease

There are multiple mechanisms and factors that may explain how low alcohol intake protects against coronary artery disease. It is known that ethanol improves lipid and glucose metabolisms. For instance, low ethanol consumption increases HDL cholesterol and apolipoprotein A1 and lowers LDL cholesterol and C-reactive protein blood level. Ethanol has also been shown to improve insulin sensitivity by increasing adiponectin. Seemingly paradox as compared to liver effects, it also modulates inflammatory processes by decreasing the level of C reactive protein, interleukin 6, PAI–1 and fibrinogen. All these mechanisms lead to a decreased atherosclerotic risk and an improved endothelial function. Other favourable effects are the activation of the fibrinolytic system and lowering of the platelet aggregation. Taken together, all these effects will reduce the risk for clot formation [3, 47].

Beneficial effects have also been attributed to wine for a long time, via improved endothelial function, reduced inflammation, and through the action of antioxidants [50]. Non-alcoholic substances in wine such as flavonoids and resveratrol may also play an important protective role. Another study, published in 2018, suggested that wine consumption increases coronary artery plaque calcification which could stabilize the atherosclerotic plaque reduce the risk of myocardial infarction [51]. It should be noted, however, that the seemingly beneficial effects of alcohol by various

studies on the development of coronary artery disease have been obtained under conditions of low alcohol consumption [50].

Controversies exist on whether alcohol has a direct cardioprotective effect on the ischemic myocardium. According to some studies, heavy drinkers have a high risk for coronary artery disease. However, this could be the cumulation of various confounders such as systemic arterial hypertension, hypertriglyceridemia and other cardiovascular risk factors such as smoking. On the other side, in low alcohol level drinkers, a decreased risk for myocardial infarction has been repeatedly shown, also called "French paradox" [50]. Mukamal and collaborators studied alcohol habits in people free of major illness and with low cardiovascular risk. They found that mild to moderate alcohol consumption was associated with a low risk for myocardial infarction [50]. The beneficial effects low alcohol consumption seem also to vary between countries, depend on other habits and are often traced back to non-alcoholic ingredients such as antioxidants as mentioned above. At present, it is generally accepted, that the dose of alcohol intake but not the type (wine, beer, spirits) are important for the risk of cardiovascular events.

Association of Ethanol and Systemic Arterial Hypertension

Ethanol is a poor risk factor for systemic arterial hypertension and the underlying mechanisms are poorly understood. Several mechanisms are discussed such as an increased sympathetic activity, stimulation of the renin- angiotensin-aldosterone system, increased intracellular calcium level, increased vascular reactivity, release of vasoconstrictors or inhibition of nitric oxide production from the endothelial bed. It has been also suggested that ethanol increases the level of catecholamines, renin, cortisol and aldosterone which all may contribute to elevated blood pressure [47, 52]. On the other side, it has been established that development and maintenance of high blood pressure by alcohol is dose-related while completely abstaining from alcohol normalize pressure values [52, 53]. In the Framingham study, an increase of 7 mm Hg in mean arterial pressure has been observed in heavy alcohol drinkers [54]. However, rendering it more complex, other publications have shown that moderate alcohol consumption lowers blood pressure values [55, 56].

Association of Ethanol and Arrhythmias

Arrhythmic complications can always appear in HF and ethanol consumption is associated with a variety of atrial and ventricular arrhythmias, from ectopic beats up to ventricular life-threating arrhythmias. The most common ethanol-induced arrhythmia is by far the atrial fibrillation and a linear relationship between atrial fibrillation risk and the dose of alcohol has been described [54]. Ethanol can be

arrhythmogenic via several mechanisms which are described in more detail in the book Chap. xxx [47]. Briefly, these mechanisms include:

- Alcohol consumption in the presence of other predisposing factors such as cigarette smoking, electrolyte disturbances
- Increased diuresis with accompanied disturbances of electrolytes
- Myocardial fibrosis
- Ventricular hypertrophy
- Ventricular dilation
- Decreased heart variability
- Autonomic dysfunction

Many episodes of arrhythmia and frequent atrial fibrillation occur after binge drinking, usually at weekends or on holidays [57]. Regarding sudden cardiac death (SCD), an U-shaped relationship between the dose of alcohol intake and the risk this severe complication has been described [58–61]. An increased all-cause mortality has been observed at consumption levels higher than 30 g/day [58, 60]. Doll and collaborators [60] published in 1994 a study which followed-up 12,000 males for up to 13 years and looked at all-cause mortality and alcohol drinking. They reported that people who consumed less than 29 g/day pure alcohol had a lower all-cause mortality than people who consumed more than 30 g/day pure alcohol, but also lower mortality than people who consumed no alcohol. So, "too little or too much alcohol intake was associated with a higher risk of death than moderate intake of alcohol" [58]. A metaanalysis which included more than 84 studies showed a low risk for coronary artery disease, ventricular life threating arrhythmias and sudden cardiac death in people with low-to-moderate alcohol consumption (less than 60 g/day) [61]. Interestingly, alcohol's beneficial effects on SCD were independent of its source [58–60].

Conclusions

At a high dose (more than 60 g/day for men and 40 g/day for women) and chronic consumption (more than 10 years), alcohol increases the risk for atherosclerosis and the risk of coronary, cerebral, and peripheral vascular complications. At these levels, it also increases blood pressure values, causes progressive myocardial fibrosis and development of alcoholic dilative cardiomyopathy with subsequent atrial fibrillation (the most frequent complication) up to ventricular life threating arrhythmias and sudden cardiac death. In contrast, low doses of alcohol consumption have a dual effect on the cardiovascular system. They are beneficial against coronary artery disease but can cause tissue damage at moderate to high doses. Therefore, many authors consider that the only safe ethanol dose for the cardiovascular system are zero ethanol levels. Prospective, randomized trials remain to be initiated to exactly determine whether one drink per day (or perhaps one drink every other day) reduces mortality and major cardiovascular events.

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Chapter 72
Wernicke-Korsakoff Syndrome

Alain Dervaux, Lisa Blecha, and Amine Benyamina

Abstract Wernicke-Korsakoff syndrome is a complication of thiamine (vitamin B1) deficiency, common in patients with alcohol use disorders. Up to 80% of patients with Wernicke's encephalopathy are undiagnosed and, thus remain untreated. This syndrome is classically described as a clinical triad consisting of confusion, ocular dysfunction (notably nystagmus and ophthalmoparesis) and ataxia. However, a minority of patients (16%) with Wernicke's encephalopathy present with the complete triad. According to the European Federation of Neurological Societies, the clinical diagnosis of Wernicke's encephalopathy in patients with alcohol use disorder requires two of the following signs: (1) dietary deficiencies, (2) ocular symptoms, (3) cerebellar dysfunction and (4) either an altered mental state or mild memory impairment. Wernicke encephalopathy is readily reversible if treated with adequate doses of parenteral thiamine, preferably within the first 48–72 h of the onset of symptoms. The overall safety of intravenous thiamine is very good.

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When untreated, Wernicke's encephalopathy can lead to coma or death, or progress to Korsakoff syndrome. Korsakoff syndrome is chronic and may be irreversible. It is characterized by cognitive and behavioral symptoms, including anterograde and retrograde memory impairments, executive dysfunction, confabulation, apathy, affective and social-cognitive impairments. Cognitive rehabilitation, including memory compensation techniques, as well as long-term thiamine supplementation, are currently recognized in the treatment of Korsakoff syndrome.

Keywords Alcohol · Alcohol use disorder · Wernicke-Korsakoff syndrome · Wernicke's encephalopathy · Korsakoff syndrome · Memory · Thiamine · Thiamine deficiency · Vitamin B1

Introduction

Wernicke-Korsakoff syndrome includes two different syndromes, Wernicke encephalopathy and Korsakoff syndrome. First described by Wernicke in 1881, it is characterized by a triad of eye movement disturbances, ataxia, and mental confusion [1]. Wernicke encephalopathy is acute and often reversible whereas Korsakoff syndrome is chronic and potentially irreversible. Postmortem histological analyses have provided evidence that Wernicke's encephalopathy occurs in about 1% of the general population (between 0.4 and 2.8%) and in 12.5–35.0% of patients with alcohol use disorder [1–3].

Thiamine (Vitamin B1) Deficiency

Wernicke's encephalopathy and Korsakoff syndrome are caused by thiamine deficiency resulting from malnutrition. Over 90% of Wernicke's encephalopathy is reported in patients with alcohol use disorders. Wernicke's encephalopathy may also occur in patients with nutritional deficiencies resulting from hyperemesis gravidarum, intestinal obstruction, bariatric surgery, cancer chemotherapy, hemodialysis or malignancies [1, 4].

Thiamine is one of 12 water-soluble vitamins. It plays a significant role in the maintenance of the nervous system [5]. Thiamine pyrophosphate, the biologically active form of thiamine, plays a key role in glucose metabolism and energy production [6]. Thiamine pyrophosphokinase is responsible for phosphorylating thiamine to its active co-enzyme form. This step requires magnesium as a cofactor, which may often be depleted in patients with alcohol use disorders [7].

The human body is incapable of synthesizing thiamine and, thus, the vitamin needs to be supplied by food intake [7]. A healthy person requires between 1 and 2 mg of dietary thiamine daily [3]. Human stores of thiamine are limited to 30–50 mg [2]. Inadequate intake, inadequate activation and/or decreased

absorption may result in thiamine depletion within 2.5–6 weeks [2, 3]. Wernicke's encephalopathy symptoms begin to appear when thiamine levels drop below 20% of optimal levels [2].

Alcohol consumption decreases thiamine intake, gastrointestinal absorption, and hepatic storage while it increases cellular utilization [3, 8]. Alcohol damages the mucosa of the gut, thus reducing intestinal thiamine uptake. Thiamine absorption can further be depleted by diarrhea or vomiting which are common in patients with alcohol use disorder [7]. Cumulatively, alcohol dependence leads to thiamine deficiency via the reduction of intake and uptake, as well as increased utilization [7].

Due to its comparatively increased energy requirements, the brain represents the primary site that is damaged by thiamine deficiency [3]. However, over time, deficiency can cause nerve damage, leading to alcoholic neuropathy, as well as immune dysfunction. Thiamine deficiency can also affect the cardiovascular systems resulting in high-output cardiac insufficiency, known as wet beriberi.

Wernicke Encephalopathy

Clinical Characteristics

Wernicke's encephalopathy is a medical emergency, most commonly seen in patients with alcohol use disorders. The diagnosis of Wernicke's encephalopathy is primarily clinical but also histopathological [9].

The classic triad of clinical manifestations of Wernicke's encephalopathy includes delirium, ophthalmoplegia (nystagmus and ophthalmoparesis) and ataxia [5]. In reality, the complete triad is present in only 8–17% of cases [4, 6, 10, 11]. The absence of the stereotypical presentation reinforces the misconception that Wernicke's encephalopathy is rare [4]. Most patients only present with delirium. Cerebellar symptoms are the most frequent followed by oculomotor dysfunction [6]. In the absence of large, prospective data, the precise prevalence of symptoms/signs of Wernicke's encephalopathy cannot be determined [4]. When left untreated, Wernicke's encephalopathy evolves to coma, and even death, or progresses to Korsakoff syndrome in approximately 80% of patients [4].

Delirium

Wernicke's encephalopathy is consistently characterized by altered mental status consisting of acute confusional state with often reversible clinical features [4]. Cognitive changes range from apathy and mild neurocognitive symptoms to severe symptoms, disorientation, indifference, inattentiveness, increased loss of consciousness, that may rarely evolve towards a comatose state [4].

Gait Ataxia

Gait ataxia includes a range of symptoms from mild gait abnormalities to a complete inability to stand [4]. In the Dingwall et al. study [10], ataxia was diagnosed based on the presence of one or more of the following symptoms: abnormal gait, upper or lower limb dysmetria or abnormal Romberg's test. Lesions to the anterior and superior vermis of the cerebellum are a primary cause of ataxia and dysarthria of the limbs. However, Chandrakumar et al. [6] stressed that vestibular paralysis and polyneuropathy also contribute to ataxic gait.

Ocular Dysfunction

Horizontal nystagmus is the most common oculomotor symptom in Wernicke's syndrome. While complete ophthalmoplegia is rarely observed, it is a pathognomonic symptom [12]. Thus, it has been suggested that the term "ocular" should replace ophthalmoplegia in the clinical triad [4]. Other ocular findings include: sixth nerve palsy, lateral rectus palsy, lateral gaze palsy, conjugate gaze palsy, internuclear ophthalmoplegia, sluggish reactions to light, ptosis, retinal hemorrhage, papilledema, anisocoria or miosis. Dingwall et al. [10] diagnosed the presence of oculomotor abnormalities when one or more of the following were present: nystagmus, abnormal range of eye movement or diplopia. Bilateral visual disturbances frequently occur together rather than alone [6].

Neuropathology

Chandrakumar et al. [6] have emphasized that the symptoms in patients with Wernicke's encephalopathy are a direct outcome of the neuropathological lesions in specific areas of the brain. These lesions follow a symmetric distribution among structures that surround the third and fourth ventricles and the aqueduct [4]. The most commonly affected structures are the mammillary bodies, in up to 80% of cases. Most patients have bilateral histopathological lesions in the dorsomedial thalamus which have been associated with reported memory loss in Wernicke's encephalopathy.

The ocular deficits are due to brainstem lesions affecting pons and the midbrain. However, with thiamine administration, the condition improves, as there is no significant damage to the nerve cells [6]. The ataxic gait is due to the lesions of the superior vermis of cerebellum. Vestibular apparatus damage in these patients further worsens the abnormalities with gait and stance [6]. Abnormal vital signs, characterized by respiratory distress, hypothermia, and hypotension, are due to brainstem lesions (vestibular and inferior olivary nucleus) [6].

Comorbidities of Wernicke Encephalopathy

Liver Diseases Comorbidities

Caine et al. [13] found significant overlap in clinical signs between hepatic encephalopathy and Wernicke's encephalopathy in patients with severe liver disease and alcohol use disorder. In a Spanish multicentric study, Novo-Veleiro et al. [14] found that 37% of patients diagnosed with Wernicke's encephalopathy and alcohol use disorders (n = 434) also had alcohol-related liver disease (ALD). In patients with ALD (n = 272) versus those without, a relationship was found between mortality and the presence of anemia, low level of consciousness and previous diagnosis of cancer. The presence of liver disease was also associated with less chance of full recovery: 27 patients with (17.8%) versus 71 (27.8%) without ALD (p = 0.03) [14].

Neurologic Comorbidities

Marchiafava–Bignami disease, pontine or extrapontine myelinolysis, acute pellagra encephalopathy (B3 depletion), traumatic brain damage, as well as head injury should be diagnosed prior to simply attributing cognitive dysfunction to ethanol neurotoxicity [1].

Psychiatric Comorbidities

Guirguis et al. [2] found that 1.85% of psychiatric inpatients (n = 486) had clinical signs of Wernicke's encephalopathy and 7% were at high risk for developing the disorder. However, Lin et al. [15] found that the frequency of Caine-positive Wernicke-Korsakoff syndrome was 12% among psychiatric inpatients, but only half used alcohol. Patients treated with high-dose thiamine showed clinically significant neurocognitive improvement. Few cases of schizophrenia-related Wernicke's encephalopathy have been published in the literature [16]. Depression represents an additional risk factor for malnourishment [17].

Caine's Criteria

Caine et al. [13] proposed operational criteria for the diagnosis of Wernicke's encephalopathy. These criteria require two of the following four signs: (1) dietary deficiencies, (2) oculomotor abnormalities, (3) cerebellar dysfunction, and (4) either an altered mental state or mild memory impairment [13].

1. Dietary deficiencies include undernutrition (body mass index <2 SD below normal), history of grossly impaired dietary intake or abnormal thiamine status [1].
2. Oculomotor abnormalities include ophthalmoplegia, nystagmus, or gaze palsy [1].
3. Cerebellar dysfunction includes unsteadiness or ataxia, abnormal past pointing or dysdiadokokinesia [1].
4. Altered mental state includes disorientation in two of three fields: confusion, coma, or abnormal digit span memorization. Mild memory impairment include failure to remember two or more words in the 4-item memory test or impairment on more elaborate neuropsychological tests of memory function [1].

The criteria were designed to differentiate Wernicke's encephalopathy from other cognitive disorders, including hepatic encephalopathy, in patients with alcohol use disorders [13]. Using the proposed criteria, sensitivity for the diagnosis of Wernicke's encephalopathy was improved to 100% versus 31% with the classic triad [13]. Differential diagnosis of Wernicke's encephalopathy either alone or with amnesia (Wernicke-Korsakoff syndrome) or hepatic encephalopathy improved from 22 to 85% using these criteria [13]. Patients who met one criterion in the presence of alcohol use disorders, medical morbidity or psychiatric disorders were considered to have a high risk for Wernicke's encephalopathy [2]. Nonspecific signs and symptoms of malnutrition may include but are not limited to any of the following: reported loss of appetite, living alone or homelessness, weight loss, nausea and/or vomiting [1, 2].

Caine's et al. diagnostic criteria have shown high sensitivity (94%) and specificity (99%), thus several authors (e.g. [1, 2]), as well as the European Federation of Neurological Societies (EFNS) [11] strongly recommend using them for diagnosing Wernicke's encephalopathy in both alcohol-dependent and non-alcohol-dependent patients. Ritz et al. [18] found that 16% of patients with alcohol use disorders "without complications", presented two Caine criteria and half of them presented one criterion.

It is noteworthy that clinical presentation can differ greatly in individuals with Wernicke's encephalopathy [3]. Above all, it remains a clinical diagnosis. Diagnostic testing (whether imaging or laboratory) should not delay thiamine administration in individuals suspected of having Wernicke's encephalopathy [3, 4]. Treatment for Wernicke's encephalopathy is frequently administered prior to diagnostic confirmation [3]. Many authors consider reversal of clinical signs upon treatment with thiamine to be the best argument in favor of an antemortem diagnosis of Wernicke's encephalopathy. Chandrakumar et al. [6] suggested that patient improvement following thiamine therapy was considered a good diagnostic strategy.

Neuroimaging Characteristics

Numerous magnetic resonance imaging (MRI) studies have typically shown symmetrical signal intensity alterations of the mammillary bodies, thalamus, fornices, midbrain and periaqueductal-periventricular grey matter area [11, 19, 20]. Finding

these alterations on MRI strongly supports a clinical diagnosis of Wernicke's encephalopathy [2]. These signal changes are seldomly seen in the chronic stages of Korsakoff's syndrome. While MRI imaging is not recommended in the diagnosis of Wernicke's encephalopathy, it could be used to rule out other, alternative diagnoses [6]. For instance, lesions to the corpus callosum on MRI should raise suspicion of Marchiafava-Bignami disease [4].

About 60% of patients present typical lesions on MRI scans [3, 4]. MRI has a 53% sensitivity and a 93% specificity in detecting Wernicke's encephalopathy [3]. Wernicke's encephalopathy is by far the most frequent cause of lesions to the mammillary bodies in humans [9]. Mammillary body atrophy is frequently observed in MRI scans within a week of encephalopathy onset [6]. Similarly to clinical signs, damage to brain areas observed in these scans varies widely from person to person [3]. Computed tomography is not a reliable test for Wernicke's encephalopathy [4]. Diagnostic imaging should not delay thiamine administration in individuals suspected of having Wernicke's encephalopathy [3, 4].

Thiamine Blood Dosage

Thiamine levels can be measured using high-performance liquid chromatography [4]. However, Wernicke's encephalopathy cannot be diagnosed solely on the basis of thiamine concentration, as there is not a specific threshold below which all individuals develop the disease [6]. Blood dosage is neither sensitive nor specific for diagnosing active disease [4]. Since the majority of the blood's thiamine is contained in red blood cells, a less common but more accurate method involves detecting monophosphorylated and dephosphorylated thiamine in these cells [4]. Direct measurement of thiamine pyrophosphate or thiamine via high performance liquid chromatography has been shown to be more precise and robust [3]. However, these tests are not available in most clinical laboratories and treatment must not be delayed to obtain these results [3].

Wernicke Encephalopathy: Challenges and Pitfalls

Up to 80% of all cases of Wernicke's encephalopathy are undiagnosed and therefore go untreated [9, 10, 28]. This is due to variable clinical presentations, symptom overlap with other neurological conditions, a lack of sensitive laboratory tests and a low incidence of oculomotor symptoms [10, 11, 13]. Altered mental state, the most common symptom of Wernicke's encephalopathy, may be confused for inebriation, withdrawal delirium, hepatic encephalopathy or a number of other neurological conditions [8]. Early signs and symptoms of Wernicke's encephalopathy are vague and nonspecific. Patients report nausea, vomiting, weight loss and sometimes memory loss [2]. In addition, administering glucose prior to thiamine can cause or exacerbate Wernicke's encephalopathy.

Many conditions encountered in patients with alcohol use disorders can mimic Wernicke's encephalopathy. The most frequent include acute alcohol intoxication, acute alcohol withdrawal syndrome or delirium tremens, hepatic encephalopathy, and head injuries. Exclusion of these comorbid pathologies may prove difficult in everyday clinical practice [1, 12]. There are symptom overlaps with other alcohol induced disorders such as acute hypoglycemia, alcohol-induced seizures, subdural or intracranial bleeds induced by head injury, or when alcohol-induced seizures result in head injury and/or cerebral hypoxia [12].

Kopelman [12] stressed that multiple factors can underlie the etiology of alcohol-related brain lesions such as recurrent head injury and its complications, recurrent seizures and cerebral hypoxia, recurrent hypoglycaemia, and chronic hepatic toxicity/cirrhosis. Both smoking and substance misuse are commonly associated with alcohol misuse and can cause or exacerbate brain pathology. Other medical conditions may mimic Wernicke's encephalopathy including stroke, drug overdose, particularly nonmedicinal use of prescription medications, benzodiazepine withdrawal, anticholinergic induced delirium, other encephalopathies, hyperammonemia from valproic acid, central nervous system infections, CO intoxications, electrolyte disturbances (sodium, calcium, magnesium, phosphate), endocrine disturbances (thyroid, parathyroid, pancreas, pituitary, adrenal), seizures, especially nonconvulsive status epilepticus, psychiatric conditions, cardiac, kidney, and liver failures [4].

In psychiatric patients, Wernicke's encephalopathy can mimic major depressive disorder with psychotic or catatonic features, psychotic disorders, or dementia [2]. Clinicians, especially those working in the emergency setting, need to be aware of the clinical variability of Wernicke's encephalopathy because most patients initially present to the emergency department and the disorder remains underdiagnosed [4].

Treatment of Wernicke's Encephalopathy

Early recognition of Wernicke's encephalopathy is vital, as rapid treatment can restore cognitive and ocular function. Indeed, thiamine is relatively inexpensive and safe; rapid administration has been reported to prevent progression of Wernicke's encephalopathy to irreversible deficits of Korsakoff syndrome [4, 5, 21]. Mortality was drastically reduced with acute administration of thiamine [10]. Unfortunately, the living conditions and socioeconomic status of patients with alcohol use disorders may be responsible for extended treatment delays [1].

Thiamine Treatment

Primary treatment includes timely administration of thiamine. When treated effectively, within hours after encephalopathy development, a full recovery is likely to occur. Even after a delayed initiation of treatment for a few days, a complete

recovery is still possible following thiamine replacement [1, 5]. The response to thiamine administration on ocular findings is quite predictable and constant. Failure of recovery should alert the physician to consider alternative diagnoses [4].

There is no consensus on the effective dose of thiamine, route of administration, daily dose frequency, or the duration of the treatment [4]. The traditional recommendation is a parenteral thiamine dosage greater than or equal to 100 mg/day [22]. Oral administration of thiamine is normally insufficient to treat Wernicke's encephalopathy since patients require a higher daily dosage of thiamine [22]. For instance, Thomson et al. [23] suggested regimens include high-dose thiamine (≥500 mg) prescribed intravenously three times a day, for 2–3 days initially with additional doses based on clinical response.

Smith et al. [5] conducted a review to determine the optimal thiamine dosage in alcohol-induced Wernicke's encephalopathy. Six publications including 138 patients were evaluated. Clinical diagnostic criteria varied significantly between publications. Doses ranged from 100 to 1500 mg intravenous thiamine and up to 300 mg IM thiamine. All patients who received thiamine experienced symptom improvement [5]. Ambrose et al. [24] conducted a trial using five thiamine dosing regimens ranging from 5 to 200 mg IM administered daily. Patients who received the highest doses experienced the most rapid resolution of symptoms and demonstrated higher mental acuity. Patients with hepatic encephalopathy may have an additional risk of Wernicke's encephalopathy and should be treated with parenteral thiamine [13]. Later case studies have shown that 100–250 mg doses of parenteral thiamine did not prevent death and that Korsakoff's psychosis developed in 56–84% of patients upon later follow-up [19].

High-dose parenteral administration also facilitates passive diffusion of thiamine across the blood-brain barrier [4]. Parenteral thiamine is capable of rapidly correcting depleted stores, thus achieving therapeutic plasma levels that assist in reversing neurological symptoms [5]. Some observations have reported the development of symptoms in patients taking high doses of oral thiamine [6]. In terms of parenteral administration, the intravenous route is preferred over intramuscular. Pharmacokinetic studies have documented that the plasma half-life of thiamine is only 96 minutes. Thus, administering thiamine two to three times daily might achieve better bioavailability than a single dose [4].

These recommendations are not based on controlled trials [4]. A Cochrane review by Day et al. [25] identified only two double-blind, randomized, controlled trials (RCT) on thiamine administration in Wernicke's encephalopathy of adequate quality. The review concluded that there was insufficient data from the available clinical studies to recommend an optimal therapeutic regimen that clinicians could use for treatment or prophylaxis in patients with alcohol dependence [6]. Recently, Dingwall et al. [10] conducted two double-blind, parallel group, randomized controlled trials to determine optimal thiamine dose required in asymptomatic but "at-risk" alcohol misuse patients (history of heavy alcohol use in the past 3 months with nutritional risk but without neurological symptoms), and in symptomatic alcohol misuse patients with Wernicke's encephalopathy (two or more clinical signs of oculomotor abnormalities, ataxia, confusion, or nutritional risk). There was no clear

benefit of high dose thiamine over intermediate or lower doses, over the time intervals examined in the treatment and prevention of cognitive and neurological abnormalities related to Wernicke's encephalopathy [10]. The authors concluded that if replicated in other randomized trials, such a finding may change clinical recommendations [10].

A literature review reported that it was reasonable to consider a minimum of 72 h of treatment with a high dose as likely to achieve complete resolution of symptoms [5]. If patients continue drinking alcohol, prophylactic thiamine supplementation should be administered indefinitely.

Parenteral thiamine is underutilized in patients with alcohol use disorders and risk factors for Wernicke's encephalopathy. Education is needed to enhance thiamine prescription and evaluation of risk factors for Wernicke's encephalopathy in this population.

Hypomagnesemia is also common in alcohol-dependent patients and can contribute to the development of Wernicke's encephalopathy, as thiamine requires magnesium as a cofactor [2]. In the presence of hypomagnesemia, patients with suspected Wernicke's encephalopathy may be unresponsive to parenteral thiamine. Thus, those who are at potential risk should have their magnesium level checked and be supplemented via oral or parenteral administration [2, 4, 22].

Prophylaxis

Substitution of parenteral thiamine in individuals with suspected Wernicke's encephalopathy is a well-established treatment regimen, but available guidelines are widely variable [1]. The use of thiamine as prophylaxis is widespread internationally. Many hospitals use thiamine administration prophylactically for patients with alcohol use disorders [3]. When a suspected case of Wernicke's encephalopathy is administered glucose, it is recommended that thiamine infusion be given before or conjointly with glucose load to prevent the exacerbation of symptoms [4, 6]. Sinha et al. [4] suggested that oral administration of thiamine 100 mg, three times daily may be reserved for prophylactic treatment of patients with low clinical suspicion. This is based on standard clinical practice since insufficient evidence exists in the literature to support this recommendation**.** Placebo-controlled thiamine substitution trials in treating suspected Wernicke's encephalopathy are needed, but would be unethical by modern standards [8].

Standardized National Guidelines

Pruckner et al. [8] conducted a review of 14 treatment guidelines for patients with alcohol use disorders in order to identify recommendations for the use of thiamine, including the recommendations of the American Psychiatric Association, the

American Society of Addiction Medicine (ASAM), the Österreichische Gesellschaft für Neuropsychopharmakologie und Biologische Psychiatrie (ÖGPB), the British Association for Psychopharmacology (BAP), the French Alcohol Society, the Italian Society on Alcohol, the National Institute for Health and Care Excellence (NICE), the Substance Abuse and Mental Health Services Administration (SAMHSA), the Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde (DGPPN), the German Guidelines for Austria, Germany and Switzerland, the World Federation Federation of Societies of Biological Psychiatry (WFSBP), the Mental Health Gap Action Programme (WHO mhGAP) Guidelines of the World Health Organization, and the Australian Government Department of Health and Ageing (AGDHA). Pruckner et al. [8] concluded that although specific modalities and indications varied considerably, high-dose treatment with parenteral thiamine in several daily doses should be considered a state-of-the-art procedure.

The most detailed recommendations were those of the BAP and the AGDHA. The BAP "Guidelines for the Pharmacological Management of Substance Abuse" recommended oral thiamine administration of (>300 mg/day) in healthy uncomplicated heavy drinkers during withdrawal. For patients at high risk for developing Wernicke's encephalopathy, thiamine should be administered IV or IM for 3–5 days or until no further improvement can be seen [22]. If Wernicke's encephalopathy is suspected or established, the treatment regimen should be >500 mg of thiamine IV or IM three times a day for 3–5 days, followed by 250 mg thiamine administered parenterally once daily for another 3–5 days minimum [22].

Little information on thiamine treatment was provided in APA, ASAM, ÖGPB (2013), French Alcohol Society, NICE, SAMHSA, and WHOmhGAP.

Safety Profile of Thiamine

Thiamine is very well tolerated regardless of administration route or dosage [4]. Adverse reactions to thiamine are rare. Thomson et al. [26] reviewed the previously released data on adverse reactions to parenteral thiamine in the treatment of Wernicke's encephalopathy. They retrieved 10 anaphylactic reactions from 5,431,235–6,651,947 patient-days of treatment. They concluded that the risk-benefit ratio for administration is favorable given the potential severity of brain damage in Wernicke-Korsakoff syndrome. Pruritus and local irritation may occur [6].

Korsakoff Syndrome

Korsakoff described the syndrome between 1887 and 1891. According to Arts et al. [1], Korsakoff syndrome is a residual syndrome in patients who suffered from Wernicke encephalopathy but did not receive immediate and adequate treatment with thiamine replacement therapy.

Clinical Characteristics

Korsakoff syndrome is characterized by memory impairments, in particular of episodic memory, disordered cognition, executive dysfunction, confabulation, disorientation, apathy, flattened affect, lack of illness insight, in the context of alcohol abuse and malnutrition. These symptoms are chronic and may be irreversible [3]. Cognitive impairments may have a significant impact on a patient's daily life, and memory impairments can heavily impact their sense of self and identity.

As a core characteristic for Korsakoff syndrome, memory impairments primarily relate to declarative memory. Within this domain, both episodic memory – explicitly remembered, personally experienced events specific to time and place - and semantic (fact-related) memory are affected [1]. In each subdomains, there are significant deficits in anterograde and retrograde memory. Patients are unable to acquire new information [27]. Overall intelligence, attention, immediate memory and implicit or procedural memory generally remain intact [28]. Segobin and Pitel [29] highlighted the central role of neuronal loss within the thalamus, especially the anterior thalamic nuclei, in amnesia associated with alcohol use disorders and Korsakoff syndrome.

Korsakoff syndrome is also characterized by behavioral and affective impairments [1].

Among these, apathy is a characteristic and fundamental symptom, blunted or detached affect, irritability, emotional over-reaction, and confabulation [1, 28]. Confabulation refers to the emergence of memories of experiences and events that are incorrect in place and time, or never took place [30, 31]. Patients may fabricate stories in the setting of clear consciousness. Confabulations can be spontaneous or provoked. Rensen et al. [31] validated the Nijmegen-Venray Confabulation List (NVCL), an observation scale for quantifying both spontaneous and provoked confabulations in patients with Korsakoff syndrome. The NVCL includes four factors: provoked confabulations, spontaneous confabulations, severity of spontaneous confabulations, and distorted sense of reality [31].

Clear diagnostic criteria for Korsakoff syndrome are lacking; it is often used interchangeably with alcohol-related dementia (ARD) [9, 28]. Arts et al. [1] suggested certain specific clinical criteria for Korsakoff syndrome including: (1) minimal severity of memory dysfunction, expressed in evidence-based cutoff scores for memory tests (such as the California Verbal Learning Test or the Rivermead Behavioral Memory Test); (2) in vivo evidence for Wernicke's encephalopathy pathology, either clinical (e.g., the operational criteria by Caine et al.), neuroradiological (i.e., MRI), or lab reports (very low serum thiamine) and (3) a set of exclusion criteria [1].

Neuroimaging

Current neuroimaging literature regarding Korsakoff syndrome clearly showed lesions to the thalamus, mammillary bodies and hippocampus [12, 20, 29]. In a population of patients with alcohol use disorders, comparing those with or without

Korsakoff syndrome, Segobin and Pitel [29] showed significantly greater bilateral grey matter loss in the thalami and mammillary bodies within the group of patients with Korsakoff syndrome. The fornix and the cingulum, both of which have direct connections to the thalamus and hippocampus, have been observed to have increased white matter bundles. Thus, impaired microstructural integrity seems to be more severe in Korsakoff syndrome [20, 29].

Treatment of Korsakoff Syndrome

Several authors argued that Korsakoff patients can improve through weeks, months, or years if adequately treated with thiamine, and if they abstain from alcohol use [1, 10, 12]. To date, no effective pharmacological treatment for Korsakoff syndrome, except thiamine, is available.

The treatment of Korsakoff syndrome is based on cognitive rehabilitation, including memory compensation techniques, and interventions based on errorless learning [1]. Memory compensation techniques such as using agendas, memory cards, smartphones and smartwatches are promising. Six studies on the use of traditional and digital assistive technologies provided evidence that these memory compensation techniques may be helpful in Korsakoff syndrome, improving autonomy in everyday life [20, 30–33]. Arts et al. [1] suggested that these techniques are helpful if (1) the formulated goals are restricted (2) sufficient time is available to guide the patient, and (3) the use of these technologies is holistically embedded or combined with elaborated learning techniques including memory compensation techniques and interventions based on errorless learning. Patients with Korsakoff syndrome and somatic or psychiatric comorbid conditions should receive integrated care based on accurate multidimensional and multidisciplinary diagnosis in which nurses play a prominent role.

Conclusions

Wernicke-Korsakoff syndrome is a complication of thiamine (vitamin B1) deficiency that requires urgent diagnosis and treatment to prevent serious and life-threatening complications. Recommended diagnosis is clinical, requiring two of the following: (1) dietary deficiency, (2) ocular symptoms, (3) cerebellar dysfunction and (4) either an altered mental state or mild memory impairment. Parenteral thiamine is a safe and effective treatment. Wernicke encephalopathy is readily reversible if treated within the first 48–72 h of symptom onset.

Untreated Wernicke's encephalopathy may lead to coma, or more rarely, death. It may also lead to Korsakoff syndrome which is chronic and may be irreversible. Characterized by anterograde and retrograde memory impairments, executive dysfunction, confabulation, apathy, affective and social-cognitive impairments, it is a highly debilitating syndrome. Cognitive rehabilitation, including memory

compensation techniques, as well as long-term thiamine supplementation, are currently recognized in the treatment of Korsakoff syndrome.

Conflicts of Interests AD received honoraria for lectures from Camurus, Indivior, and Lundbeck and have received travel support from Janssen and Otsuka; he has no conflicts of interests within the scope of this chapter.

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Part XII
Alcohol and Cancer

Chapter 73
Mechanisms of Alcohol-Mediated Cancer

Sebastian Mueller

Abstract Cancers of the upper digestive tract, including oral cavity, pharynx, larynx, esophagus squamous cell carcinoma), liver, colorectum, and female breast were causally related to alcohol consumption. This chapter introduces to the book part on cancer and alcohol consumption. According to a recent report from the International Agency for Research on Cancer (IARC), 4.1% of all new cases of cancer, are attributable to alcohol consumption. They could have been avoided if there had been no alcohol use. Mechanisms of cancer development during alcohol exposure are also briefly discussed. Of note, not ethanol itself but its mandatory oxidation to acetaldehyde causes carcinogenesis, in association with multiple other pathways that lead to the generation of reactive oxygen species (ROS) and accumulation of iron. Together, these metabolites are genotoxic, impair DNA repair, cause cell injury, enhanced cell regeneration and, hence, provide conditions for genomic instability, an optimal environment for cancer formation. In addition, a combination of immunosuppression and reduced tumor clearance through elimination pathways such as apoptosis further contribute to carcinogenesis. At the systemic level, enhanced red blood cell (RBC) turnover in combination with liver and bone marrow injury provide an additional novel loop that specifically challenges the immune system and provides enhanced toxic iron trafficking.

Keyword Alcohol intake · Mechanism · Breast cancer · Colorectal cancer · Liver cancer · Upper aero-digestive tract cancer · Acetaldehyde · ROS · Alcoholic liver cirrhosis · Haptoglobin · Hemopexin · Macrophage · Mortality · Adducts · Iron

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Introduction

Alcohol is responsible for up to 200 different diseases [1]. As is discussed in the subsequent Chaps. 74 and 75 by Pietro Ferrari and colleagues and Akira Yokoyama, the consumption of alcohol is one of the top-10 risk factors contributing to the worldwide burden of disease. Cancers of the upper aero digestive tract (UADT), including oral cavity, pharynx, larynx, esophagus and squamous cell carcinoma, as well as liver, colorectum, and female breast were causally related to the consumption of alcoholic beverages. The incidence of hepatocellular carcinoma (HCC) among patients with alcoholic cirrhosis ranges range from 7–16% [2] within 5 years to as much as 29% after 10 years. According to a recent report from the International Agency for Research on Cancer (IARC), 4.1% of all new cases of cancer, are attributable to alcohol consumption. They could have been avoided if there had been no alcohol use. Mechanisms of cancer development during alcohol exposure are also briefly discussed. Of note, not ethanol itself but its mandatory oxidation to acetaldehyde causes carcinogenesis, in association with multiple other pathways that lead to the generation of reactive oxygen species (ROS) and accumulation of iron. Together, these metabolites are genotoxic, impair DNA repair, cause cell injury, enhanced cell regeneration and, hence, provide conditions for genomic instability, an optimal environment for cancer formation. In addition, a combination of immunosuppression and reduced tumor clearance through elimination pathways such as apoptosis further contribute to carcinogenesis. At the systemic level, enhanced red blood cell (RBC) turnover in combination with liver and bone marrow injury provide an additional novel loop that specifically challenges the immune system and provides enhanced toxic iron trafficking.

Potential Mechanisms of Alcohol-Mediated Carcinogenesis

As shown in Fig. 73.1 not ethanol itself but its oxidation leads to formation of highly cancerogenic acetaldehyde and, through multiple pathways to the generation of ROS and accumulation of iron. Of note, cells cannot escape from ethanol metabolism and there are no negative feedback loops. Alcoholic beverages are group 1 carcinogens (known to be carcinogenic to humans) per classification by the IARC [3]. Thus, alcohol should be considered a procarcinogen that is converted to the primary carcinogenic metabolite, acetaldehyde. Individuals with the *ALDH2*2* (which encodes aldehyde dehydrogenase) loss- of-function mutation have an increased risk of esophageal cancer, which serves to convincingly link acetaldehyde to cancer [4, 5]. Acetaldehyde is electrophilic and, as mentioned previously, forms an adduct with DNA and interstrand crosslinks [6, 7]. Alcohol consumption also causes a gradual accumulation of cellular iron, mostly in the liver. Iron itself is considered a cancerogenic molecule, leading to highly reactive hydroxyl radicals through the Fenton chemistry [8]. As is also shown in Fig. 73.1, these toxic

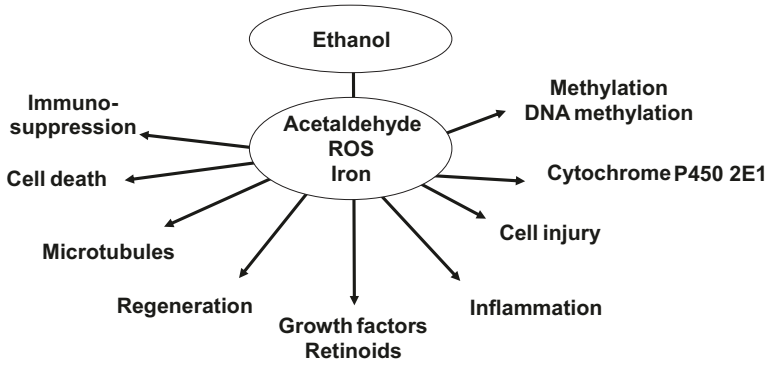

Fig. 73.1 Mechanisms of alcohol-mediated hepatocarcinogenesis

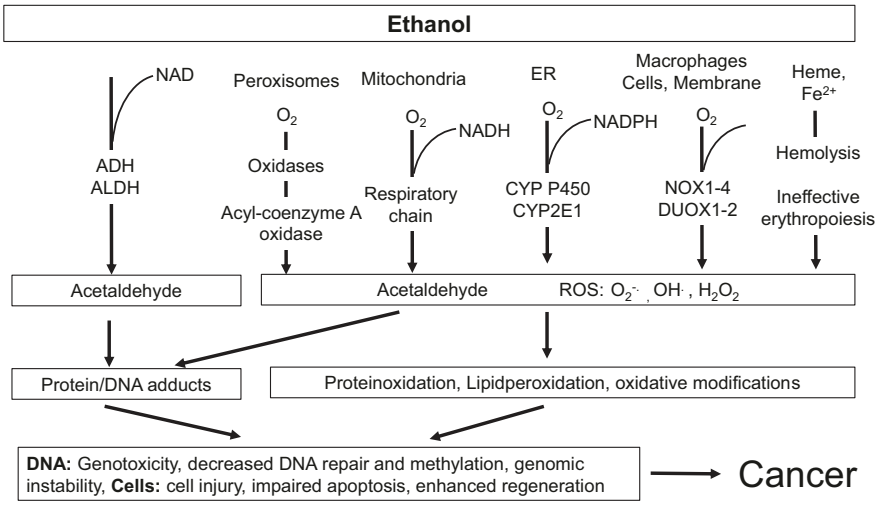

Fig. 73.2 Mechanisms of biochemical activation of oxygen to ROS in various organelles and the formation of the carcinogen call I acetaldehyde

metabolites can interfere with important physiological cellular functions known to be critical for cancer development.

Figure 73.2 shows at the cellular level how ethanol not only drives acetaldehyde formation but the formation of ROS through various oxygen-consuming pathways in mitochondria, peroxisomes, membrane-bound oxidases such as NADPH-dependent oxidases (NOXs) and, of course, the endoplasmic reticulum-localized cytochrome p450 system, namely the subtype CYP2E1. Together, these metabolites are genotoxic, impair DNA repair, cause cell injury, enhanced cell regeneration and, hence, provide conditions for genomic instability, an optimal environment for cancer formation. In addition, a combination of immunosuppression and reduced tumor clearance through elimination pathways such as apoptosis further contribute to

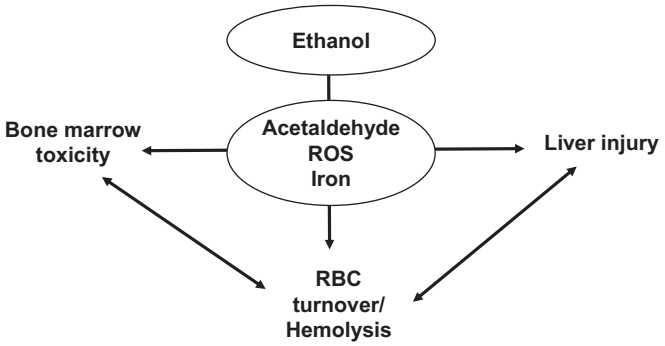

Fig. 73.3 Recent identification of a novel vicious loop between bone marrow, liver and blood. Ethanol impairs both RBCs and bone marrow leading to an enhanced RBC turnover and iron trafficking. The liver plays an important role in this cycle as the sole excretion organ of heme-degradation end products such as bilirubin, an intermediary storage of iron and the synthesis and release of important carrier proteins such as albumin, transferrin or haptoglobin

carcinogenesis. As is shown in Fig. 73.3, at the systemic level, enhanced RBC turnover in combination with liver and bone marrow injury provide an additional novel loop that specifically affects the immune system and provides enhanced iron trafficking. These findings are rather new and are described in more detail in the Chaps. 57 and 58 on iron and ALD and ethanol and bone marrow toxicity.

Carcinogenesis by Acetaldehyde

There are several biological mechanisms by which alcohol, as ethanol, induces cancer [9]. Once consumed, alcohol is metabolized to **acetaldehyde** by the enzymes alcohol dehydrogenase (ADH), cytochrome P-450 2E1 (CYP2E1) and bacterial catalase [10].

Acetaldehyde is electrophilic and highly reactive towards DNA and can bind directly to DNA to form DNA adducts which can block DNA synthesis and repair, induce point mutations, double-strand breaks, sister chromatid exchanges and structural changes to chromosomes [11, 12]. Another property of acetaldehyde is that it can also bind to proteins causing structural and functional changes to enzymes involved in DNA repair and DNA methylation. DNA methylation is often disrupted by both ethanol and acetaldehyde due to their ability to inhibit S-adenosyl-L-methionine synthesis and the DNA repair enzyme O6-methylguanine DNA methyltransferase (DNMT) activity as well as their ability to dysregulate one-carbon metabolism which has a downstream effect on DNA methylation [10, 13, 14]. Epigenetic changes induced by chronic heavy alcohol consumption can lead to chromosomal instability [15]. Hypomethylation of promoters for oncogenes (for

example, *SERPINB5* and *IGF2*) causes their aberrant activation and loss of imprinting (loss of the normal expression pattern), whereas hypermethylation of promoters of genes involved in cellular differentiation or DNA repair (for example, *MLH1* and *MGMT*) promotes transformation.

DNA mutations can result if DNA repair is insufficient, particularly for homologous recombination repair [7]. As mentioned above, ROS generated by CYP2E1 generates aldehydic lipid metabolites such as 4-HNE and MDA. The presence of MDA increases acetaldehyde adduct formation by ~10–30-fold, synergizing the formation of a highly reactive, hybrid MDA–acetaldehyde adduct [16]. These aldehydes modify proteins (generating neoantigens) and DNA (causing mutations) while depleting reduced glutathione, amplifying oxidant stress and cytotoxicity. Induced CYP2E1 also converts other procarcinogens to active carcinogens, including nitrosamines [17].

While acetaldehyde has many carcinogenic and genotoxic properties, it is not the final product of ethanol metabolism: non-toxic acetate is formed when aldehyde dehydrogenases (ALDH) oxidize acetaldehyde. The main ALDHs involved in acetaldehyde oxidation consist of ALDH1A1, ALDH2, and ALDH1B1, and ALDH2 is responsible for most of this processing in the liver [18]. The common polymorphism *ALDH2**2*, present among 28–45% of East-Asian populations, dramatically reduces ALDH2's ability to metabolize acetaldehyde and carriers have a substantially increased risk of UADT cancer when consuming alcohol [19].

Although gastric bacteria do not contribute much to overall alcohol metabolism, they are also capable of producing acetaldehyde from alcohol. Gastric acetaldehyde is thought to contribute to gastric mucosal damage and the pathogenesis of UADT cancer [20]. σ-ADH is also able to detoxify the dietary carcinogen nitrobenzaldehyde [21]. It has been shown that Japanese who lack this enzyme exhibit an increased risk for stomach cancer. In the colon, ADH isozymes may also activate dimethylhydrazine – a well-known colon carcinogen.

Generation of acetaldehyde also leads to damage of the microtubular system [22–24] with an altered secretion of proteins [25, 26], a decrease of the important antioxidant glutathione and an inhibition of the nuclear repair systems with an enhancement of carcinogenesis [27]. Although detailed studies on carcinogenesis are lacking these interactions are very likely to modulate the process of carcinogenesis.

Formation of Reactive Oxygen Species

Ethanol-mediated carcinogenesis can also result from the induction of oxidative stress by the increased activity of CYP2E1 which produces high quantities of **reactive oxygen species** (ROS) when oxidizing ethanol to acetaldehyde [28, 29]. Classical ROS are formed during the reduction of oxygen and may be released

during uncoupling of enzymatic reactions. The reduction cascade and the following ROS are shown in Appendix Figs. A.67 and A.68. Being highly reactive, ROS (especially superoxide anion and hydroxyl radicals) can induce lipid peroxidation resulting in the formation of aldehydes which can bind to DNA forming highly mutagenic etheno-DNA adducts [30, 31]. ROS can also induce metastasis and angiogenesis by interfering with signaling pathways to upregulate vascular endothelial growth factor and monocyte chemotactic protein 1 [32]. In addition to CYP2E1 activity, ROS can also be produced as a result of inflammation in the tumor microenvironment where monocytes and macrophages produce pro-inflammatory cytokines, such as tumor necrosis factor α and interleukins [32, 33], activating further ROS-generating enzymes [31]. ROS may also trigger the production of profibrotic cytokines and collagen in liver cells leading to liver cirrhosis which is another well-recognized intermediary step towards hepatocellular carcinoma development [34]. Up to 50% of ROS may be produced by the ER-localized p450 system, especially CYP2E1.

Major consequences are production of above mentioned ROS including hydroxylethyl radicals [11]. ROS results in lipid peroxidation with lipid peroxidation products such as 4-hydroxynonenal or malondialdehyde. 4-Hydroxynonenal binds to DNA, forming highly carcinogenic exocyclic etheno–DNA adducts [35]. Interaction of the microsomal ethanol metabolism with the metabolism of various drugs, leading to decreased drug blood levels and increased drug toxicity [36, 37]. Interaction of CYP2E1 ethanol metabolism with the metabolism of various xenobiotics and carcinogens, leading to increased toxicity and carcinogenesis [38]. Interaction of CYP2E1 ethanol metabolism with the metabolism of retinol and retinoic acid, leading to vitamin deficiency and increased toxicity, including enhanced carcinogenesis [11, 39].

Growth Factors

Another pathway through which alcohol is involved in carcinogenesis is through interference with **retinoid metabolism** and the oxidation of vitamin A to retinoic acid [11]. Retinoids are necessary for normal cell growth, cell differentiation, and apoptosis [32]. Alcohol also interferes with estrogen pathways by increasing estrone and estradiol levels and enhancing the activity of estrogen receptors which might be important in breast carcinogenesis [40]. Heavy use of alcohol has also been linked with increased circulating levels of estrone and estradiol as well as dehydroepiandrosterone sulphate which is subsequently metabolized to estrogen [41]. As is already discussed in Chap. 49 on the pathophysiology of alcohol, in general, ethanol blocks growth factors such as HGF1, IGF or EGF. The molecular reasons are still poorly understood. However, if physiological signals such as anemia or hepatocytes loss stimulate cell division, **DNA replication will be performed in a toxic environment despite regeneration blocking signals**.

Immune Dysfunction and Tumor Clearance

Alcohol reduces the normal function of the immune system by disrupting the production of **necessary proteins to target and destroy potentially cancerous cells** [33]. It has also been hypothesized that alcohol can activate natural killer T cells leading to liver injury and apoptosis of hepatocytes [33]. As is discussed in the chapter on iron and alcohol and bone marrow (Chaps. 57 and 58), the enhanced erythrophagocytosis may further "overcharge" the immune system and increase toxic iron trafficking.

Inflammation

Furthermore, chronic alcohol use can cause microbial dysbiosis and bacterial overgrowth in the intestine leading to "gut leakiness"; in this case, the intestinal lumen becomes so permeable that bacterial products including lipopolysaccharides and peptidoglycan move into the blood and reach the liver resulting in a state of chronic inflammation [10, 33, 42]. In the oral cavity, bacterial catalase metabolizes ethanol to acetaldehyde, but these bacteria have limited capacity to metabolize acetaldehyde to acetate, thus leaving the oral epithelia exposed to acetaldehyde and its carcinogenic properties for longer [11, 43]. There is further hypothesis that alcohol consumption might activate the pathways of other carcinogenic agents such as some pro-carcinogens in tobacco smoke and industrial chemicals (see UADT above) [11]. It is also postulated that ethanol might increase the absorption and penetration of these carcinogens in the mucosa of the UADT, [11, 44] where it has been reported that tobacco smoking and alcohol consumption have a synergistic effect on the risk of cancer [45, 46].

Alcohol-induced hepatic inflammation and the oxidative stress associated with such inflammation causes hepatocellular DNA damage and contributes to tumor initiation. Tumor-associated M2-polarized macrophages support tumor promotion, in part by activating hepatic stellate cells (HSCs). Ectopic expression of TLR4 in hepatocytes and its activation by LPS induces HCC [47] via generation of TLR4 and homeobox protein Nanog- dependent liver tumor-initiating stem-cell-like cells (TICs) [48]. In addition to promoting fibrosis, activated HSCs also promote HCC formation via production of matrix or soluble factors that support tumor cell survival and growth [49]. Activated HSCs also promote TIC-mediated liver tumorigenesis and liver tumor formation induced by a hepatotoxin, diethyl nitrosamine, and promoted by alcohol [50]. The two major drivers of alcohol-associated tumor initiation, CYP2E1 in hepatocytes [35, 51], and LPS from gut dysbiosis [52], also activate HSCs and promote tumor development. The role of the senescence- associated secretory phenotype of HSCs may be important in HCC promotion, as shown in obesity- associated HCC [53]. Alcohol- promoted hepatocarcinogenesis is associated with activation of the canonical WNT–β- catenin pathway [54], which may

allow β- catenin-dependent tumor growth and stimulate *CYP2E1* transcription [55]. Finally, alcohol consumption promotes HCC development via immunosuppression, with decreased numbers of antitumor $CD8^+$ cells [56], and by loss of miR-122, which upregulates HIF1α, a tumor- promoting transcription factor [57]. In summary, alcohol and its metabolite acetaldehyde are implicated in several interlinked pathways to carcinogenesis and thus demonstrate the complexity of alcohol-mediated carcinogenesis.

Genetic Associations and Liver Cancer

More recently, novel genes have been discovered that drive liver carcinogenesis independent of the cirrhosis status. More details are provided in the Chap. 52 by H. Innes and F. Stickel on the genetics of ALD. Briefly, In HCC tumors, the most frequently mutated genes are *TERT*, *CTNNB1* and *TP53* [58]. In their recent GWAS of ALD HCC, Buch et al. identified a new germline variant in *TERT* (rs2242652) [59] showing that both hereditary and somatic mutations in HCC driver genes are associated with hepatocarcinogenesis. Also, in the first French GWAS on HCC, it was reported that the lead variant (rs708113) was associated with somatic mutations in *CTNNB1* in HCC tumour cells [60]. It is hoped that these GWAS approaches will help to identify novel target genes and help to better understand the mechanisms that drive liver cancer during alcohol consumption.

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Chapter 74
Alcohol and Cancer: The Epidemiological Evidence

Pietro Ferrari, Harriet Rumgay, Elisabete Weiderpass, and Isabelle Soerjomataram

Abstract The consumption of alcohol is one of the top-10 risks contributing to the worldwide burden of disease. In 2012, the Monograph program at the International Agency for Research on Cancer (IARC) reviewed the epidemiological evidence on the possible association between alcoholic beverage consumption and cancer risk at 27 anatomical sites, and reported that **cancers of the upper digestive tract** (**UADT**: oral cavity, pharynx, larynx, esophagus squamous cell carcinoma), liver, colorectum, and female breast were causally related to the consumption of alcoholic beverages. In this chapter we review and summarize the most recent scientific evidence on the link between alcohol intake and cancer risk, including cancer sites with a non-established relationship with alcohol. Several candidate mechanisms of alcohol carcinogenesis are presented. An estimate of the global impact of alcohol on cancer burden is provided, using the population attributable fractions, according to a recent IARC study. Some 741,000 new cancer cases, equal to 4.1% of all new cases of cancer, globally in 2020 were estimated to be attributable to alcohol consumption and could have been avoided if there had been no alcohol use. Last, the future perspectives and challenges of the research on alcohol and cancer were comprehensively discussed.

Keywords Alcohol intake · Global burden · Mechanism · Breast cancer
Colorectal cancer · Liver cancer · Upper aero-digestive tract cancer · Acetaldehyde

Introduction

The consumption of alcohol is one of the top-10 risks contributing to the worldwide burden of disease [1]. In 2012, the Monograph program at the International Agency for Research on Cancer (IARC) reviewed the epidemiological evidence on the

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possible association between alcoholic beverage consumption and cancer risk at 27 anatomical sites, and reported that **cancers of the upper digestive tract** (**UADT**: oral cavity, pharynx, larynx, esophagus squamous cell carcinoma), liver, colorectum, and female breast were causally related to the consumption of alcoholic beverages [2, 3]. In parallel, the Continuous Update Project of the World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) concluded that there was convincing evidence that consumption of alcoholic beverages increased the risk of site-specific cancers mentioned above [4]. The WCRF/AICR also indicated that alcohol could be associated with stomach cancer, and reported suggestive evidence of a link with pancreatic, lung and skin cancers.

In 2016, alcohol consumption caused an estimated 3.0 million deaths from all causes worldwide, representing 5.3% of all deaths [5]. Cancer contributes a large proportion of the health burden caused by alcohol consumption. In 2016, alcohol caused an estimated 376,200 cancer deaths, representing 4.2% (95% uncertainty interval: 3.6–4.9%) of all cancer deaths, and an age standardized rate (ASR) of 4.8 deaths (95% confidence interval, CI: 4.2–5.7) per 100,000 people, as reported in Table 74.1 [6]. The proportion of alcohol-attributable cancers is thus defined by the proportion of cancers that would not have occurred if there had been no alcohol use. In parallel, a recent study conducted at IARC highlighted that globally, 741,300 new cancer cases in 2020 were attributable to alcohol consumption, the 4.1% of the total [7].

While alcohol intake is a risk factor for UADT, colorectum, liver, and female breast cancers, its impact on other cancers remains controversial. A comprehensive meta-analysis investigated the association between alcohol intake and 23 cancer

Table 74.1 Alcohol-attributable cancer deaths in 2016, by sex and cancer site

Outcome and cancer site	*ICD-10* code	Number of alcohol-attributable deaths/1000 95% uncertainty interval)			Percentage of deaths attributable to alcohol consumption (95% uncertainty interval)			Percentage of the total alcohol-attributable cancer deaths
		Men	Women	Both sexes	Men	Women	Both sexes	
Cancer	**C00–97**	**297.6 (246.9–346.1)**	**78.6 (66–115.4)**	**376.2 (324.9–439.7)**	**5.8 (4.8–6.8)**	**2.0 (1.7–3.0)**	**4.2 (3.6–4.9)**	**100.0**
Lip and oral cavity	C00–08	38.9 (30.4–46.0)	5.2 (3.8–7.3)	44.0 (35.3–52.3)	34.7 (27.1–41.0)	9.4 (7.0–13.3)	26.4 (21.2–31.4)	11.7
Other pharynx	**C09–10, C12–14**	**31.7 (24.9–37.7)**	**2.1 (1.5–3.0)**	**33.8 (27.0–39.9)**	**35.3 (27.8–42.1)**	**9.9 (7.3–14.2)**	**30.5 (24.4–36.1)**	**9.0**
Oesophagus	C15	66.9 (51.6–79.7)	5.8 (3.9–8.9)	72.7 (56.8–87.2)	21.7 (16.7–25.8)	4.8 (3.2–7.4)	16.9 (13.2–20.3)	19.3
Colorectum	**C18–21**	**75.9 (61.5–89.6)**	**13.8 (6.6–25.2)**	**89.8 (73.1–107.4)**	**17.6 (14.3–20.7)**	**3.8 (1.8–6.9)**	**11.3 (9.2–13.5)**	**23.9**
Liver	C22	65.1 (31.5–102.5)	18.9 (9.5–34.4)	84.0 (49.8–125.3)	11.1 (5.4–17.5)	7.8 (3.9–14.1)	10.1 (6.0–15.1)	22.3
Larynx	**C32**	**19.1 (14.8–23.1)**	**0.8 (0.6–1.0)**	**19.9 (15.6–24.0)**	**23.7 (18.4–28.6)**	**6.7 (5.2–9.2)**	**21.6 (16.9–26.1)**	**8.5**
Breast	C50	–	32.0 (26.8–51.1)	32.0 (26.8–51.1)	–	5.5 (4.6–8.8)	5.5 (4.6–8.7)	5.3
All causes	**A00–Z99**	**2307.3 (1929.7–2720.1)**	**681.0 (536.4–990.7)**	**2988.3 (2596.8–3523.9)**	**7.7 (6.4–9.0)**	**2.6 (2.0–3.8)**	**5.3 (4.6–6.2)**	–

ICD-10 International Statistical Classification of Diseases and Related Health Problems, 10th revision.

types through the examination of 572 studies and 486,538 cancer cases [8]. Results suggested that alcohol was positively related to the risk of other cancers, including pancreas and prostate cancers and melanoma. The relationship between alcohol and cancer is complex and characterized by different potential biological mechanisms. Several challenging aspects of alcohol intake, including drinking patterns, binge drinking, the existence of specific susceptibility exposure windows and the type of alcoholic beverages, may play a role on alcohol carcinogenicity and are far from being elucidated.

A recent comprehensive study by the Global Burden of Diseases (GBD) group evaluated the health risks associated with moderate alcohol consumption region, age, sex, and time [9]. Using updated systematic reviews, burden-weighted dose–response relative risk curves across 22 health outcomes, including cancer, were built to estimate the levels of alcohol consumption that minimise health loss through the theoretical minimum risk exposure level (TMREL) and non-drinker equivalence (NDE), the consumption level at which the health risk is equivalent to that of a non-drinker. The study confirmed that the level of alcohol consumption that minimises health loss varies significantly across populations and age groups and remains zero or very close to zero for several population groups, particularly young adults. Given these findings, the GBD group recommended a modification of existing policy guidelines to focus on emphasising differential optimal consumption levels by age, rather than the current practice of recommending different consumption levels by sex.

Colorectal Cancer

Colorectal cancer is the second most diagnosed cancer and impacted 1.9 million people in 2020 [10]. Alcohol intake has been consistently associated with ***colorectal cancer*** (CRC) risk [2, 11]. A comprehensive meta-analysis posterior to the Volume 100E of the IARC Monograph Program [3] was carried out by scientists of the WCRF/AICR Continuous Update Project (CUP) [12]. The study summarized the evidence on the link between alcohol intake and CRC based on data from 16 prospective studies reported in PubMed until May 2015. Each increase of 10 g/day of alcohol intake, as ethanol in alcoholic beverages, equivalent to a standard drink of wine (about 100 ml), beer (about 275 mL) or spirits (about 30 mL) was positively associated with CRC risk, with relative risk estimates (RR) equal to 1.07 (95% CI: 1.05, 1.09). Similar RR estimates were reported for colon cancer (1.07, 95% CI: 1.05, 1.09, based on 14 studies) and rectal cancer (1.08, 95% CI: 1.07, 1.10, based on 11 studies). Stratified analysis by sex showed positive associations in men and borderline significant relationships between alcohol intake and CRC risk in women. For colon and rectal cancer, alcohol intake was associated with a significant increase in women and men. Among five studies [13–16] with data on distal and proximal colon cancer, two observed a significant association with distal colon cancer: the Melbourne Cohort Study [16] and the European Prospective Investigation into

Cancer and Nutrition (EPIC) study [17]. Two studies in women observed a significant association with proximal cancer, the Iowa Women's Health Study (IWHS) [13] and the Netherlands Cohort Study (NLCS) [15].

Few studies have examined the association between CRC and other metrics of alcohol exposure, including average alcohol intake during the lifetime or during early adulthood, age at starting, duration. The limited evidence suggests that there is no strong association with duration of drinking in years or age at started drinking [17–19]. Associations with CRC risk were similar for baseline and lifetime alcohol intakes [17, 20]. The association of consumption of alcoholic beverages and CRC did not seem to differ by beverage type [13, 17, 20, 21]. No interaction was observed between alcohol drinking and smoking with respect to CRC risk [17]. Few studies have examined whether the association of alcohol with cancer of the colorectum varies by folate status; the European Prospective Investigation into Cancer and Nutrition (EPIC) found some evidence that the risk for colorectal cancer associated with alcohol intake was stronger in individuals with a low folate intake, but the interaction term was of marginal statistical significance [17], and two other studies found no evidence that the association of alcohol intake with risk differed according to intake of folate, or intake of related nutrients such as vitamin B6, vitamin B12 or methionine [22, 23].

Recent research also focused on early onset CRC, whose incidence has increased recently widely. A systematic literature review and meta-analysis of studies was conducted to examine demographic and lifestyle factors for early onset CRC, defined as CRC occurring before age 50 [24]. Based on two recent studies [25, 26] eligible for the review, alcohol intake was strongly associated to CRC risk, with RR estimates equal to 1.71 (95% CI: 1.62, 1.80), comparing high drinkers versus non-drinkers.

Breast Cancer

Breast cancer (BC) affects more than two million women each year around the world [27]. Although many risk factors for BC are not modifiable, understanding the role of the factors that can be altered, including alcohol intake, is critical. The IARC Monographs in 2010 [3] and 2012 [11] concluded that the occurrence of cancer of the ***female breast*** was causally associated with the intake of alcoholic beverages. This conclusion was based on data from more than 110 epidemiological studies, together with a pooled analysis of 53 studies on more than 58,000 women with BC, with a RR estimate equal to 1.07 (95% CI: 1.05, 1.09) for each 10 g/day of alcohol intake [28]. The Million Women Study in the United Kingdom, with over 28,000 incident cancers, is the largest single study to estimate the BC risk at low to moderate levels of alcohol consumption. A 10 g/day increase of alcohol intake was linearly associated with BC risk, with RR estimate equal to 1.12 (95%CI: 1.09, 1.14) [29]. Interestingly, variation over time was taken into account in the study by

repeating the alcohol intake assessments approximately 3 years after recruitment. Within the EPIC study, an evaluation that included over 11,000 incident BC cases, alcohol intake was significantly related to BC risk [30]. For each 10 g/day increase in alcohol intake the hazard ratio (HR) increased by 4.2% (95% CI: 2.7–5.8%). Taking 0.1–5 g/day as reference, alcohol intake between 5 and 15 g/day was related to a 5.9% increase in BC risk (95% CI: 1–11%). Significant increasing trends were also observed between alcohol intake and hormonal receptor status BC, notably in ER+/PR+, ER−/PR−, HER2− and ER-/PR−HER2− tumors. Associations were marginally stronger among women who started drinking prior to first full-time pregnancy.

A recent large meta-analysis of epidemiological studies until December 2018 provided a dose-response estimation between different aspects of alcohol intake and BC risk [31]. Dose-response analysis modeled the relationships between drinking type and BC risk. Sources of heterogeneity were explored, and sensitivity analyses were conducted to test the robustness of findings. In total, 22 cohort studies and 45,350 BC cases were included. Current drinkers for ER+ had an increased risk compared with never drinkers. In dose-response analysis, there was a statistically significant linear trend with BC risk increasing gradually by total alcohol and wine dose: for each 10 g per day of alcohol, RR estimates were 1.10 (95%CI: 1.08, 1.13) for total alcohol and 1.08 (95% CI: 1.04, 1.14) for wine intake. For postmenopausal women, the risk increased by 11.1% (RR = 1.11, 95% CI: 1.09, 1.13) with every 10 g of total alcohol per day.

Overall, there is consistent evidence that the risk for BC does not vary significantly by menopausal status [23, 30, 32–35] or beverage type [29, 35–38]. Noteworthily, while some evidence suggested that associations did not vary by folate intake [35, 37, 39], within the EPIC study the risk of BC per 10 g/day of alcohol intake was 1.06 (95% CI: 1.03, 1.08) for women with low intake of dietary fiber (<18.5 g/day), while among women with dietary fiber greater than 24.2 g/day the risk of BC was 1.02 (95% CI: 0.99, 1.05), with a statistically significant interaction (p-value for testing the homogeneity of associations was equal to 0.01) [40]. This modulating effect was stronger for dietary fiber from vegetables. These results suggested that dietary fiber intake may modulate the positive association of alcohol intake and BC.

Overall, evidence is strongest for North America and Europe, where more studies have been conducted, but other regions also show consistent associations [41]. Additional studies focusing on beverage type, the participant's age at the time of consumption provided less consistent findings. A better understanding of the roles of drinking pattern, by separating out drinking intensity and frequency, is needed. More studies of alcohol consumption and BC subtypes would help increase insights into this relationship. A clearer understanding of the effects of exposures in early life, including *in utero* exposure, is warranted. Examination of how other BC risk factors (e.g., physical activity, body mass index, smoking, reproductive history) interact with alcohol consumption in relation to BC incidence and prognosis is needed.

Liver and UADT Cancers

Alcohol intake has been consistently associated to ***liver cancer*** [3, 4], based on several case-control and cohort studies. The finding was also confirmed by a large meta-analysis of 36 studies and 8800 liver cancer cases [8]. The most recent IARC Monograph assessed a link for alcohol intake for both hepatocellular carcinoma and cholangiocarcinoma [3]. As cholangiocarcinoma is a rare cancer, the etiological link with alcohol was largely examined in case-control studies rather than in prospective cohorts. Chronic infection with hepatitis viruses B and C are the major causes of cancer of the liver, yet associations between alcohol and liver cancer were observed among individuals infected with hepatitis viruses and among uninfected individuals.

Quantification of the relationship between alcohol and cancer is challenging since cirrhosis and other liver disorders that often anticipate cancer onset tend to lead to a decrease or a cessation of consumption of alcoholic beverages many years before the occurrence of cancer of the liver (reverse causation). It has been estimated that at least 75% of ***UADT cancers*** (upper aerodigestive tract including cancers of the oral cavity, pharynx, larynx and esophagus) were attributable to a combination of cigarette smoking and alcohol drinking [42]. An open research question is if the only role of alcohol is through its synergistic association with tobacco or whether alcohol also has an independent effect in never-smokers. It was observed that alcohol acts as a solvent that enhances the penetration of carcinogenic compounds into the mucosa, thus facilitating the uptake of environmental carcinogens, especially from tobacco smoke [43]. The results of a pooled analysis of 15 case-control studies suggested that, in the absence of tobacco use, the association between alcohol consumption and risk of head and neck cancer was weak, and apparent only at high doses, mainly confined to pharyngeal and laryngeal cancers [44, 45].

Cancer Sites with a Suggestive Link with Alcohol

The link between alcohol use and the risk of cancer of several other sites is far from being established. It has been observed that alcohol use might be positively associated with risk of pancreatic cancer [46], stomach cancer [8], prostate cancer [8], and melanoma [47]. On the other hand, evidence is suggestive of a protective role of alcohol use in relation to the risk of cancer of the kidney [48] and thyroid [8], and Hodgkin [49] (HL) and Non-Hodgkin [50, 51] (NHL) lymphomas. The mechanisms for the inverse associations observed are unclear.

Prostate cancer is the most diagnosed cancer among men worldwide, with incidence rates displaying large variability between areas. The exact nature of the association between alcohol intake and prostate cancer risk remains unclear despite the large number of research studies [52]. Prostate cancer is a heterogenous disease and

risk factors may differ. In two recent studies [53, 54] that reported significant positive associations between alcohol use and the risk of prostate cancer [53, 54], associations were more apparent for lifetime alcohol use compared to alcohol use at recruitment.

Pancreatic cancer is characterized by the difficulty in detecting it at early stages and lack of effective treatments, leading to high fatality rates, necessitating the need to identify modifiable risk and preventive factors for this cancer. A large meta-analysis of 14 cohort and 15 case-control studies provided evidence for an increased risk of pancreatic cancer only for heavy alcohol drinking [55]. In a more recent study, it was estimated that heavy use of alcohol increased the risk of pancreatic cancer by 19% compared with nondrinkers or occasional drinkers, with homogenous associations across studies [8]. These findings of an association observed primarily for heavy alcohol use were also confirmed in several large pooling studies [46, 56–59], and in a recent EPIC study [59].

A role for alcohol use in the etiology of ***stomach cancer*** is plausible but the epidemiological evidence remains equivocal [8, 60]. Most evidence from prospective studies is based on consumption data that refer to the time of study recruitment, that is, alcohol intake at baseline, which might not be representative of participants' long-term consumption during earlier age periods, particularly for heavy drinkers who had reduced alcohol consumption. Within recent pooling study of EPIC and the Melbourne Collaborative Cohort Study (MCCS) including 1225 incident stomach cancers (78% noncardia), a weak association was observed between baseline alcohol intake and noncardia stomach cancer, but none for cardia cancer, nor using lifetime alcohol intake [61].

Evidence for a relationship between alcohol consumption and skin ***melanoma*** risk is inconsistent and findings from prospective investigations are so far very limited [29, 62, 63]. A recent meta-analysis [47] of 14 case-control and 2 cohort studies reported an increased risk of melanoma for increasing levels of alcohol intake, but the authors warned for caution in interpretation of their findings because of residual confounding by sun exposure, as alcohol increases sunburn severity, a major risk factor for melanoma. A recent study from the EPIC cohort reported that baseline alcohol intake was positively associated with risk of melanoma, and with of basal-cell and squamous-cell skin cancers [64].

The results on the relationship between alcohol consumption and risk of ***bladder cancer*** are controversial [8]. A recent meta-analysis of nine prospective studies a lack of association between alcohol intake and the risk of bladder cancer in the entire population. However, one alcoholic drink increments each day could elevate the risk of bladder cancer by 9% (RR = 1.09; 95%CI: 1.01–1.17), and an association was found for male drinkers [65].

Recent epidemiologic evidence reported that moderate alcohol consumption was inversely associated with the risk of ***kidney cancer***. This was observed in two comprehensive meta-analysis [8, 65], in a recent EPIC study on baseline and lifetime alcohol use [66], and in a systematic review of the literature about modifiable risk factors for kidney cancer [67]. The mechanism for the inverse association between alcohol consumption and renal cancer risk is not well understood. Moderate alcohol

consumption is associated with a lower risk for type-II diabetes and could be related to increased insulin sensitivity. Alcohol use would prevent insulin resistance and then indirectly renal cancer.

An inverse association of alcohol with both **Hodgkin lymphoma** ***(HL)*** [49] and **Non-Hodgkin lymphoma** ***(NHL)*** [51] has been reported in meta-analyses. The mechanisms accounting for a possible alcohol-induced decrease in the risk of lymphomas remain largely unknown but may in part be mediated by immune-related mechanisms, given that lymphomas appear to arise in a milieu characterized by immune activation or inflammation, and are believed to result from errors in cellular processes associated with normal lymphocyte maturation and differentiation [68]. If immune hyperactivation increases lymphoma risk and modest alcohol intake suppresses immune activation, this could plausibly explain reported modest inverse associations of alcohol with lymphoma [69, 70]. It has also been suggested that the inverse relationship observed could be partially attributable to reverse causation, whereby early symptoms of lymphomas may cause cancer cases to either quit or reduce their alcohol drinking [71].

The evidence for an association between alcohol use and ***thyroid cancer***, although mildly suggestive of an inverse relationship, is very sparse [8]. A recent evaluation based on 556 (90% women) thyroid cancer cases in the EPIC study provided some support to the hypothesis that moderate baseline and lifetime alcohol consumption may be associated with a lower risk of differentiated thyroid carcinoma [72].

Potential Mechanisms

There are several biological mechanisms by which alcohol, as ethanol, induces cancer [73]. Once consumed, alcohol is metabolized to acetaldehyde by the enzymes alcohol dehydrogenase (ADH), cytochrome P-450 2E1 (CYP2E1) and bacterial catalase [74]. Acetaldehyde is highly reactive towards DNA and can bind directly to DNA to form DNA adducts which can block DNA synthesis and repair, induce point mutations, double-strand breaks, sister chromatid exchanges and structural changes to chromosomes [43, 75]. Another property of acetaldehyde is that it can also bind to proteins causing structural and functional changes to enzymes involved in DNA repair and DNA methylation. DNA methylation is often disrupted by both ethanol and acetaldehyde due to their ability to inhibit S-adenosyl-L-methionine synthesis and DNA methyltransferase (DNMT) activity as well as their ability to dysregulate one-carbon metabolism which has a downstream effect on DNA methylation [74, 76].

While acetaldehyde has many carcinogenic and genotoxic properties, it is not the final product of ethanol metabolism: non-toxic acetate is formed when acetaldehyde dehydrogenases (ALDH) oxidize acetaldehyde. The main ALDHs involved in acetaldehyde oxidation consist of ALDH1A1, ALDH2, and ALDH1B1, and ALDH2 is

responsible for most of this processing in the liver [77]. The common polymorphism *ALDH2*2*, present among 28–45% of East-Asian populations, dramatically reduces ALDH2's ability to metabolize acetaldehyde and carriers have a substantially increased risk of UADT cancer when consuming alcohol [78].

Ethanol-mediated carcinogenesis can also result from the induction of oxidative stress by the increased activity of CYP2E1 which produces high quantities of reactive oxygen species (ROS) when oxidizing ethanol to acetaldehyde [79, 80]. Being highly reactive, ROS can induce lipid peroxidation resulting in the formation of aldehydes which can bind to DNA forming highly mutagenic etheno-DNA adducts [81, 82]. ROS can also induce metastasis and angiogenesis by interfering with signaling pathways to upregulate vascular endothelial growth factor and monocyte chemotactic protein 1 [83]. In addition to CYP2E1 activity, ROS can also be produced as a result of inflammation in the tumor microenvironment where monocytes and macrophages produce pro-inflammatory cytokines, such as tumor necrosis factor α and interleukins [83, 84], activating further ROS-generating enzymes [82]. ROS may also trigger the production of pro-fibrotic cytokines and collagen in liver cells leading to liver cirrhosis which is another well-recognized intermediary step towards hepatocellular carcinoma development [85].

Another pathway through which alcohol is involved in carcinogenesis is through interference with retinoid metabolism and the oxidation of vitamin A to retinoic acid [43], but retinoids are necessary for normal cell growth, cell differentiation, and apoptosis [83]. Alcohol might also interfere with estrogen pathways by increasing estrone and estradiol levels and enhancing the activity of estrogen receptors which might be important in breast carcinogenesis [86]. Heavy use of alcohol has also been linked with increased circulating levels of estrone and estradiol as well as dehydroepiandrosterone sulphate which is subsequently metabolized to estrogen [87].

Alcohol might reduce the normal function of the immune system by disrupting the production of necessary proteins to target and destroy potentially cancerous cells [84]. It has also been hypothesized that alcohol can activate natural killer T cells leading to liver injury and apoptosis of hepatocytes [84]. Furthermore, chronic alcohol use can cause microbial dysbiosis and bacterial overgrowth in the intestine leading to "gut leakiness"; in this case, the intestinal lumen becomes so permeable that bacterial products including lipopolysaccharides and peptidoglycan move into the blood and reach the liver resulting in a state of chronic inflammation [74, 84, 88]. In the oral cavity, bacterial catalase metabolizes ethanol to acetaldehyde, but these bacteria have limited capacity to metabolize acetaldehyde to acetate, thus leaving the oral epithelia exposed to acetaldehyde and its carcinogenic properties for longer [43, 89]. There is further hypothesis that alcohol consumption might activate the pathways of other carcinogenic agents such as some pro-carcinogens in tobacco smoke and industrial chemicals (see UADT above) [43]. It is also postulated that ethanol might increase the absorption and penetration of these carcinogens in the mucosa of the UADT [43, 90], where it has been reported that tobacco smoking and alcohol consumption have a synergistic effect on the risk of cancer [91, 92].

In summary, alcohol and its metabolite acetaldehyde are implicated in several interlinked pathways to carcinogenesis and thus demonstrate the complexity of alcohol-mediated carcinogenesis.

Global Impact of Alcohol on Cancer Burden

The impact of alcohol on cancer burden can be estimated using population attributable fractions (PAFs), which compare the current cancer burden to the expected burden if there had been no alcohol use. According to a study conducted at IARC which used the IARC Monograph and WCRF classifications of cancer risk factors to determine which cancer sites could have potentially alcohol-attributable cases, 741,300, or 4.1%, of all new cases of cancer globally in 2020 were attributable to alcohol consumption [7]. Men accounted for more than three-quarters (76.7%) of this total, with 568,700 alcohol-attributable cancer cases among men in 2020 and the remaining 172,600 alcohol-attributable cancer cases among women. As for the contributing cancer sites, the cancer sites with the highest PAFs attributable to alcohol at the global level were cancers of the esophagus (31.6%), pharynx (22.0%), and oral cavity (20.2%), although there were considerable differences by sex; for example, 39.2% of esophageal cancer cases among men were attributable to alcohol, versus 14.3% among women. But when considering the total burden by number of cases, the cancer sites that contributed the most alcohol-attributable cases were cancers of the esophagus (189,700 cases), liver (154,700 cases), and breast (98,300 cases, female only). Together, these results confirmed the higher burden of alcohol-attributable cancers among men, yet with breast cancer among the top three contributing cancer sites, this highlights that even though alcohol-attributable cancer is a predominantly male disease, in settings where the incidence of breast cancer among women is high, this female disease is placed among the top causes of alcohol-attributable cancers and causes a major burden of alcohol-attributable cancer. For example, in the United Kingdom, breast cancer contributed the most cases of cancer attributable to alcohol and represented nearly a quarter (24.2%) of total alcohol-attributable cases among both men and women.

The study conducted at IARC also presented the alcohol-attributable cancer burden according to three levels of alcohol intake: moderate (<20 g per day), risky (20–60 g per day), and heavy alcohol consumption (>60 g per day), which roughly corresponded to one or two alcoholic drinks per day (moderate), two to six alcoholic drinks per day (risky), and more than six alcoholic drinks per day (heavy). From this analysis, moderate drinking contributed 103,100 (13.9%) cases of alcohol-attributable cancer, risky drinking contributed 291,800 (39.4%) cases, and heavy drinking contributed 346,400 cases (46.7%). Moderate and risky drinking had a larger impact among women (32.3% and 50.3% of alcohol-attributable cancers among women, respectively) compared with men (8.3%, 36.1%, respectively).

The IARC study also presented PAFs for regions and countries of the world which uncovered further disparities (Fig. 74.1). The highest PAFs of cancer cases

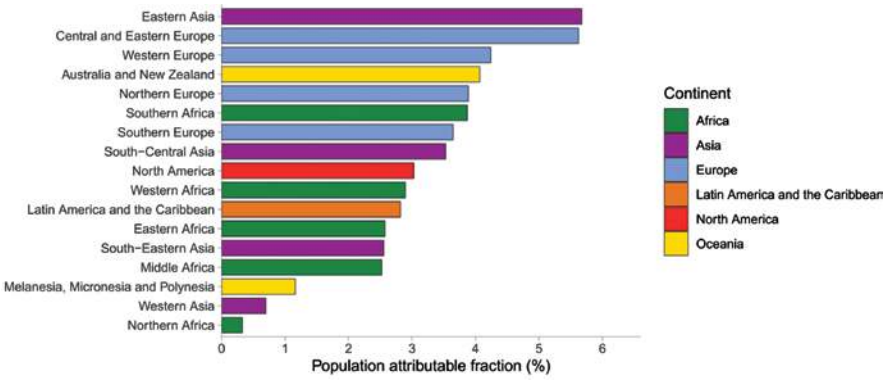

Fig. 74.1 Proportion of cancer cases in 2020 attributable to alcohol consumption, by world region. Chart created using results from Rumgay et al. [7]

attributable to alcohol were observed in eastern Asia and central and eastern Europe (5.7% and 5.6% of all cancer cases, respectively). These regions also held the countries with the highest PAF for alcohol-attributable cancers, namely Mongolia (9.8%), Moldova (7.9%), Romania (6.8%), Belarus (6.5%), and China (6.2%). The world regions with the lowest PAFs were northern Africa (0.3%) and western Asia (0.7%), which also included the countries with the lowest PAFs, namely Kuwait, Libya, and Saudi Arabia (all 0.1% of cancer cases attributable to alcohol). When exploring regional PAFs by sex, the patterns among men were of the same magnitude of the average for both sexes combined. Yet, differences were found for the patterns for women with the largest PAFs in central and eastern Europe (3.4%), Australia and New Zealand (3.3%), and western Europe (3.2%).

Future Perspectives

The assessment of the relationship with cancer sites that do not have an established link with alcohol intake presents several analytical challenges. First, any association with alcohol intake, if true, is likely to be weak or moderate. Thus, the statistical power to detect an association in a single study is likely very low. Second, cancer is a very heterogeneous disease, and some of the specific anatomical sites mentioned above are less common cancer sides, in particular pancreatic cancer, NHL in women and thyroid cancer in men. Accurate investigations on the role of alcohol on risk of less common cancers, as well the investigations of the alcohol and cancer relationship among specific subgroup of the study populations, e.g., among never smokers, requires the collection of information from a sizeable study population with a sufficiently large number of cancer cases.

For this purpose, this is likely achievable through international consortia to jointly analyze existing data from ongoing large prospective epidemiological

investigations. Third, most epidemiological investigations that evaluated the role of alcohol on risk of cancer focused primarily on total alcohol intake collected at baseline through self-reported dietary (or lifestyle) questionnaires. Several studies showed that exposure to alcohol in different life periods could be relevant for disease development [17, 93–96]. Recent studies have complemented analyses of baseline alcohol use with evaluations of alcohol use at different ages during participants' early and mid-adulthood, typically, at 20, 30, 40 and 50 years of age, a variable customarily referred to as 'lifetime alcohol intake' [17, 96]. Although more comprehensive, this approach still ignores potential within-person changes in alcohol intake during adulthood. To date, a few studies have evaluated the effect of changing alcohol intake during adulthood with respect to cancer risk using retrospective and/or prospective assessments in large epidemiological investigations [97, 98]. Fourth, drinking patterns may play a role in the carcinogenesis related to alcohol intake, and binge drinking has received increasing attention in cancer research [99]. Large amounts of alcohol consumed in a limited amount of time may trigger cancer risks that are more harmful than if the same alcohol quantities were consumed over a longer time span [87, 99]. Fifth, self-reported alcohol intake is, like other dietary factors, prone to exposure measurement errors, and more specifically underreporting [100]. Alcohol is a (culturally) sensitive exposure, rendering it susceptible to underreporting across self-reported assessments. However, the extent and distribution of measurement errors are unknown [101], and it is likely that observed associations between alcohol use and disease risk are biased. Objective assessments not relying on the capacity of study participants to recall their past exposure to alcohol would be useful [102]. Biomarkers of habitual alcohol use, including light-to-moderate drinking, would be necessary to more accurately assess alcohol exposure in epidemiological studies and to improve risk estimates for diseases including cancer where modest associations may exist. Throughout the present chapter, we presented an overview of the relationship between cancer and overall alcohol intake, without providing extensive details on the cancer site-specific risks associated with different types of alcoholic beverages (wine, beer, or spirits), although several studies attempted this type of examination [12, 17].

Conclusion

The carcinogenicity of alcohol drinking has been established and well documented over four decades [103], and in most recent assessment it has been linked to at least seven cancer sites, contributing to at least 740,000 new cancer cases annually worldwide. While studies have reported on causal relations of alcoholic beverages, drinking patterns and alcoholic types, questions regarding its associations to various cancer types such as stomach, bladder, prostate and pancreatic cancer, as well as inverse relations to HL, NHL, thyroid and kidney cancers remain. Mechanistic studies have gone a long way confirming the multiple, complex, pathways from alcohol drinking to carcinogenesis, yet a better understanding as to how other risk factors

such as smoking may modify the risk can prove extremely useful to design cancer prevention programs.

In this respect, it is noteworthy to mention the Pooling Project on Alcohol and Cancer (PPAC), an international collaborative project led by scientists at IARC (PI: Dr. Pietro Ferrari) and at the T'Chan Harvard School of Public Health (PI: Dr. Stephanie Smith-Warner), which aims at investigating the relationships between alcohol intake and risk with cancers of the prostate, pancreas, kidney, thyroid, and non-Hodgkin lymphoma, for which the evidence is still inconsistent or sparse. The PPAC will also focus on UADT cancer among non-smokers. The project gathered data from over 30 cohorts in Nort America, Europe, Asia and Australia, and was funded by the US National Institute of Alcohol Abuse and Alcoholism (NI-AAA). In addition, future research on alcohol might leverage available biomarker and -omics data to identify molecular correlates of alcohol drinking that may inform on mechanisms. Tools for causal inference create promising opportunities to quantify potential mediating role of sets of molecular data using individual features but also the concept of signatures, quantities that greatly summarize overwhelming amount of biological information into scores [104]. For this to happen, we will need flexible models for data sharing and resources for capacity building.

While alcohol consumption has decreased in countries where its prevalence was traditionally high, 2.34 billion adults worldwide consumed alcohol regularly in 2016. Studies that have examined public understanding of alcohol drinking and its consequences on health on cancer are lacking [41], but have showed low overall awareness e.g., in a study of women attending a breast screening clinic in the United Kingdom, only 19% were aware that alcohol consumption is a BC risk factor [105, 106]. The World Health Organization has therefore called for global commitments to reducing alcohol drinking in 2010 and renewed this call updating evidence-based actions through its best buys a set of most effective interventions including pricing policy through taxes on alcoholic beverages, bans or restrictions on advertising, restricting availability of retailed alcohol [107]. These interventions require modest investments from countries, yet they are expected to have a large impact on health including on reduction of cancer burden [108], and bring nations closer to better health for all.

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Chapter 75
Alcohol-Related Cancers of the Esophagus, Head and Neck, and Stomach in East Asians

Akira Yokoyama

Abstract Esophageal squamous cell carcinoma (SCC) in East Asians is characterized by field cancerization in the upper aerodigestive tract (UAT: esophagus and head and neck region) and stomach. In addition to alcohol consumption, smoking, and a low intake of fruit and vegetables, the combination of slow-metabolizing alcohol dehydrogenase-1B (ADH1B) and inactive aldehyde dehydrogenase-2 (ALDH2) with long and high exposure to ethanol and acetaldehyde increases the risk of SCC in the UAT. The combination of alcohol consumption and ALDH2 deficiency also increases the cancer risk in the stomach. Screening evaluations using chromoendoscopy or image-enhanced endoscopy and questionnaires that include alcohol flushing and drinking behaviors can provide predictors of both primary and secondary SCC of the UAT, and abstinence or a reduction in alcohol consumption has been reported to prevent second cancers in high-risk patients after UAT cancer treatment.

Keywords Alcohol · Alcohol dehydrogenase · Acetaldehyde · Aldehyde dehydrogenase · Esophageal cancer · Head and neck cancer · Stomach cancer

Introduction

The most common esophageal cancers in East Asians are squamous cell carcinomas (SCCs), which are strongly influenced by alcohol consumption and smoking and are characterized by multiple cancerization in the esophagus, head and neck, and stomach. The risk factors for esophageal SCC are clear, and treatment based on a risk assessment is useful for cancer prevention. Risk factors for secondary SCCs of the esophagus and head and neck (upper aerodigestive tract [UAT]) and the preventive effect of abstinence from alcohol have also been discussed.

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Carcinogenic Mechanism of SCC in the UAT

Synergistic Effects of Direct Alcoholic Beverage Exposure and Smoking

Alcohol beverages include acetaldehyde (AA), and many strong alcoholic beverages are strong AA beverages. The UAT is directly exposed to ethanol and AA. In Japan, there are reports of a higher SCC risk in the UAT from the consumption of strong beverages such as shochu (20%–25% v/v) and whiskey [1, 2]. While blood AA levels after drinking are on the order of 1–10 μM, beer contains an average of 100–200 μM, sake and wine 700 μM, and shochu and whiskey 1000 μM [3, 4], although there are large differences depending on the manufacturer, with some brands of shochu containing several μM only. Cigarette smoke also contains AA, and roughly 400 μM of AA is detected in saliva during smoking [5]. Ethanol is a solvent that allows tobacco smoke to penetrate the mucosa. The risks of esophageal SCC from drinking and smoking are synergistic, with odds ratios (ORs) reported to be 30–50 times higher in heavy drinking smokers [6, 7].

Ethanol Metabolism in Saliva and UAT Mucosa (Fig. 75.1)

The concentration of ethanol in saliva and blood is almost the same. Oral bacteria produce AA from ethanol, and salivary AA concentrations exceed 50–100 μM and are even higher in ALDH2-deficient individuals [8–11]. Although poor oral hygiene

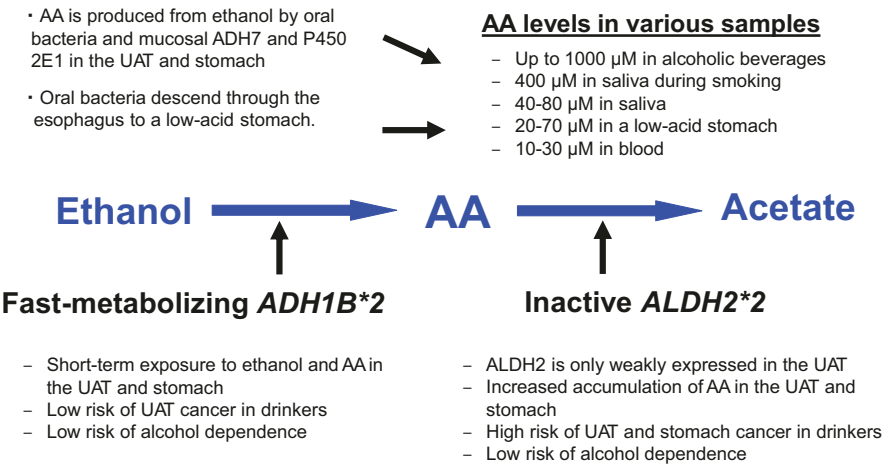

Fig. 75.1 Ethanol and acetaldehyde (AA) exposure and accumulation in the upper aerodigestive tract (UAT) and stomach and role of *alcohol dehydrogenase-1B*2* (*ADH1B*2*; rs1229984) and *aldehyde dehydrogenase-2*2* (*ALDH2*2*; rs671)

and dental care is a risk factor for UAT cancer [12], the quantity of bacteria in saliva is correlated with the ability to produce AA [8, 13]. In alcohol-dependent patients, the quantity of bacteria in saliva immediately before hospitalization is high; after hospitalization, the quantity of bacteria decreases with improvements in lifestyle, including teeth brushing, and the AA production capacity of saliva decreases accordingly [13].

Alcohol dehydrogenase-7 (ADH7), which has a relatively high Km value and is not found in the liver, is strongly expressed in the UAT, while aldehyde dehydrogenase-2 (ALDH2), which is strongly expressed throughout most of the body, is only slightly expressed in the UAT [14, 15]. Therefore, AA produced locally in the UAT mucosa tends to accumulate to high concentrations.

Genetic Polymorphisms of ALDH2 (rs671)

Ethanol is mainly metabolized in the liver, where it is converted to AA by ADHs and the microsomal ethanol oxidizing system (MEOS) and to acetate by ALDHs. ALDH2, which has a low Km value, plays a major role in AA metabolism. The Asian gene polymorphism of *ALDH2* is homozygous inactive (**2/*2* or *A/A*) in less than 10% of Japanese individuals, while a heterozygous inactive status (**1/*2* or *G/A*) is seen in about 40% and an active status (**1/*1* or *G/G*) is seen in more than 50%. The ALDH2 homozygous inactive form shows zero activity, and the heterozygous inactive form has a theoretical activity of 16% and a measured activity of 17% in liver tissue [16].

Many ALDH2-deficient individuals are flushers who experience facial flushing after drinking one glass (≈180 mL) of 5% beer, a typical Japanese beer glass, and they can develop hangovers after relatively small amounts of alcohol consumption [17]. Most homozygous ALDH2-deficient individuals are nondrinkers or occasional drinkers, but some heterozygous individuals are less prone to develop or lack alcohol flushing, and some can drink because they have developed tolerance, and more than 15% of alcohol-dependent individuals have an inactive heterozygous ALDH2 status [18]. The ALDH2-deficient genotype originated among the Han Chinese and spread to East and South Asia about 2000–3000 years ago. In Japan, the number of ALDH2-deficient individuals is relatively low in the southern and northern areas (e.g., less than 40%), while it is high in the central areas (e.g., around 50%), reflecting the history of immigration from the continent and racial mixing [19]. Thus, regional differences should be considered in ALDH2-related studies in Asians.

ALDH2 heterozygous deficiency increases the risk of UAT cancer arising from alcohol consumption. In Japan, 60%–70% of esophageal SCC patients are ALDH2 deficient [2, 20–25]. In a meta-analysis of 31 Asian studies of esophageal cancer, the OR for the heterozygous inactive form of ALDH2 per se was 6.50 (95% confidence interval, 5.34–7.92) among heavy drinkers and 3.79 (3.04–4.72) among light/moderate drinkers [26]. In a meta-analysis of 13 Asian studies of head and neck cancer, the OR for the inactive ALDH2 was 2.30 (1.11–4.77) among heavy drinkers

and 1.47 (1.16–1.86) among light/moderate drinkers [27]. Japanese [28] and Chinese [29] cohort studies in general populations have demonstrated that the ALDH2 genotype improves predictions of the development of cancer in the UAT. The risk of carcinogenesis arising from a heterozygous ALDH2 status is higher in Japan and Taiwan than in mainland China [30].

Genetic Polymorphisms of ADH1B (rs1229984)

Class I ADH in the liver consists of 1A, 1B, and 1C isozymes and is a major player in ethanol metabolism, with a low Km value and a high enzyme content. In non-Asian ethnic groups, more than 90% have the slow-metabolizing form of ADH1B (*ADH1B*1/*1* or *G/G*), while more than 90% of Asians have fast-metabolizing forms (**1/*2, *2/*2* or *G/A*, *A/A*) [31, 32]. In heavy drinkers with slow-metabolizing ADH1B, ethanol remains at a high concentration until the next day of drinking [11, 13, 33, 34]. Furthermore, some *ADH1B*1/*1* patients may start to drink again the next day when residual ethanol is still present in their system from their previous drink. The slow metabolizing form of ADH1B is a strong risk factor for alcohol dependence. Roughly 30% of patients with alcohol dependence in Japan have this form, and younger patients are more likely to have this form [18, 35]. In addition to heavy drinking, slow metabolizers of ADH1B are more likely to develop UAT cancer at the same dose. When higher levels of ethanol linger in alcohol-dependent *ADH1B*1/*1* carriers, the UAT is exposed to saliva containing higher levels of AA produced by oral microbes for longer periods of time [8, 13], especially in alcohol-dependent *ALDH2*1/*2* carriers [11]. In a meta-analysis of Asian esophageal cancer, the OR for the slow metabolizing form of ADH1B per se was 4.82 (3.50–6.64) among heavy drinkers, 2.01 (1.23–3.28) among moderate drinkers, and 1.67 (1.19–2.35) among other drinkers [36]. In a follow-up study of alcohol-dependent patients who were cancer-free at an initial endoscopic screening, 20% of patients had developed UAT cancer at 10 years, and the hazard ratio (HR) for UAT cancer was 11.55 (5.73–23.3) and 2.02 (1.02–4.02) for the heterozygous inactive ALDH2 type and the slow-metabolizing ADH1B type, respectively [37].

In heavy drinkers with slow-metabolizing ADH1B, UAT is exposed to high concentrations of ethanol for a longer time, resulting in longer exposure to AA that is locally produced by bacteria [11, 13, 33, 34]. The WHO's International Agency for Research on Cancer (IARC) concluded that alcoholic beverages, ethanol in alcoholic beverages, and AA associated with drinking are carcinogenic to humans (Group 1 carcinogen) [38]. There has been growing evidence that the effect of the *ALDH2* genotype is much stronger than that of the *ADH1B* genotype in esophageal SCC [2, 22, 23] and somewhat stronger in hypopharyngeal SCC [39–41], while the effects of the *ADH1B* genotype are equivalent to or somewhat stronger than that of ALDH2 in head and neck cancer overall [25, 39, 40, 42, 43].

Combination of Heterozygous Inactive ALDH2 Genotype and Slow-Metabolizing ADH1B Genotype

A large genome-wide association study (GWAS) in Japan reported the above genotype combination to account for 1.9% and 14.1%, respectively, in controls and esophageal SCC cases [22], and another large Japanese GWAS, 2.6% and 17.0%, respectively [23]. Many individuals with this genotype combination tend not to report alcohol flushing responses, partly because the initial AA production is slow due to the slow-metabolizing ADH1B, but the peak blood AA levels are high due to the heterozygous inactive ALDH2 [35, 44, 45]. This combination increases the OR of esophageal SCC by 29–56-fold [2, 22, 23]. When the genotype combination was combined with other risk factors, one Japanese GWAS showed a 189-fold (95–377) increase in the esophageal SCC risk for smokers who consumed more than 96.5 g of ethanol per week [22], while another Japanese GWAS showed a 357-fold (105–1210) increase in the esophageal SCC risk for alcohol consumption plus smoking [23], compared with a combination of low-risk factors. A Taiwanese case-control study of esophageal SCC also showed an OR of 382 (47–3085) for the genotype combination carriers who drink more than 30 g ethanol per day, compared with nondrinkers [46]. A low-cost *ADH1B*/*ALDH2* genetic analysis service is now available using mucosal smears; this service is expected to be used widely as well as clinically.

Genetic Damage by Acetaldehyde

In ALDH2 heterozygous drinkers, AA-DNA adducts [47, 48], sister chromatid exchanges [49] and micronucleus formation [50] occur more frequently than in ALDH2 active drinkers. Ethanol administration to *Aldh2*-knockout mice caused the formation of AA-DNA adducts in esophageal tissue [51], and in a 10% ethanol free drinking experiment, adducts in the esophageal tissues of *Aldh2*-deficient mice were markedly elevated for 1–3 days; the elevation persisted for 2 weeks and then quickly disappeared upon the discontinuation of ethanol administration [52]. *Aldh2* and *Fancd2* (a DNA repair pathway) double knockout mice showed chromosomal rearrangement from DNA double-strand breaks, and this damage also occurred in hematopoietic stem cells, further destabilizing the genome in the absence of p53 function [53].

Other ADH/ALDH Gene Polymorphisms

In a large scale GWAS in Europe, slow metabolizers of *ADH1B* (rs1229984) and *ADH1C* (rs1693482) and polymorphisms of unknown function in *ADH7* and *ALDH2* were associated with the UAT cancer risk [54]. The *ADH1B* variant has

consistently displayed pleiotropic UAT-cancer associations in GWASs with a European ancestry [55]. In Japan, a linkage disequilibrium exists between the slow metabolizing forms of ADH1B and ADH1C. The slow metabolizing form of ADH1C alone is a risk factor for esophageal and head and neck cancers, but the association disappears when corrected for the slow metabolizing form of ADH1B, reflecting the influence of the concomitant slow metabolizing form of ADH1B [2, 40]. In Japan, multiple polymorphisms of the *ADH4* and *ADH7* genes were also reported to be associated with UAT cancer, but the functions of the polymorphisms are unknown [56].

Induction of P450 2E1 by Alcohol Consumption

P450 2E1 in MEOS also oxidizes ethanol to AA, and habitual drinking increases the amount of enzyme [57]. This enzyme induction occurs not only in the liver, but also in the epithelium of the UAT, where P450 2E1 produces ROS and lipid peroxides as well as 4-hydroxynonenal and malondialdehyde, which form DNA adducts. In human esophageal biopsies, an increase in AA-DNA adducts was correlated with P450 2E1 induction [58]. P450 2E1 also activates carcinogenic precursors such as N-nitrosamines. P450 2E1 is induced in the esophagus of drinkers, promoting AA production and the activation of carcinogens.

Clinical Assessment for Cancer Risk in the UAT and Stomach

Endoscopic Screening of High-Risk Group

Since the burden arising from gastric cancer has been large among Asians, endoscopy has been commonly used in clinical practice and for cancer screening. In 2016, the Japanese government decided to introduce endoscopic screening for gastric cancer as a national program [59]. Endoscopic screening for gastric cancer also provides an opportunity for the early detection of asymptomatic esophageal and head and neck cancer. When the high-risk group for esophageal SCC was defined as men over 55 years old, heavy drinkers, and heavy smokers, esophageal SCC was diagnosed as a mainly superficial carcinoma in 0.4–0.7% of the high-risk group using Lugol chromoendoscopy with esophageal iodine staining screening [60]. We performed an initial screening of 2115 alcohol-dependent male patients (≥40 years) with no history of cancer in the UAT between 2004 and 2010 using Lugol chromoendoscopy with esophageal iodine staining [61]. Biopsy specimens of distinct iodine-unstained lesions in the esophagus showed low-grade intraepithelial neoplasia (LGIN) in 155 patients (7.3%), high-grade intraepithelial neoplasia (HGIN) including epithelial SCC in 57 patients (2.7%), and invasive SCC in 35 patients

(1.7%). However, the lesions were missed during the first passages of the endoscope before iodine staining in 97.4% of the subjects with LGIN, 77.2% of the subjects with HGIN, and 14.3% of the subjects with invasive SCC. A meta-analysis of 12 studies has shown that narrow-band imaging (NBI) and iodine staining can diagnose high-grade dysplasia and SCC in the esophagus with equal sensitivity in high-risk groups [62]. However, most of the studies were conducted in patients who had already been diagnosed as having UAT cancer. To evaluate whether image-enhanced endoscopy, such as NBI or Lugol chromoendoscopy, is more appropriate for esophageal cancer screening in high-risk groups, an evaluation of general screening in non-cancer-bearing high-risk groups is awaited.

Field Cancerization of UAT

In Japan, patients with esophageal SCC frequently have simultaneous and metachronous multiple cancers in the esophagus, the head and neck region, and the stomach [63]. Iodine staining examinations in 788 cases of head and neck SCC showed that esophageal SCC was diagnosed in 11.8% of the cases [64]. NBI examinations in 667 cases of esophageal SCC showed that head and neck SCC was diagnosed in 6.7%, and hypopharyngeal SCC was diagnosed in 5.4% [65].

Whether ALDH2 heterozygotes become heavy drinkers is influenced by social and cultural changes and associated peer pressure, and the number of ALDH2-deficient heavy drinkers is still progressively increasing. The frequency of the heterozygous form of ALDH2 deficiency in Japanese alcohol-dependent patients was 2.5% in 1972, increasing to 8.0% in 1986, 13.0% in 1993 [66], and up to 17.1% in 2013–2018 [67]. *ALDH2* genotyping of 8808 alcohol-dependent men from 1996–2019 has identified 4 inactive ALDH2 homozygotes since 2013 [68]. This change coincides with a dramatic increase in the proportion of multiple organ cancers among esophageal cancer patients in Japan over the years [63]. ALDH2 heterozygous deficiency is a risk factor for field cancerization of the UAT: 78%–95% of esophageal SCC Japanese patients with multiple SCCs in the UAT were ALDH2 deficient, and the ORs for multiple SCCs were 5.3–16.2 for ALDH2 deficiency [69, 70], while the HRs for second cancers were 2.25–3.97 [24, 25]. Simultaneous head and neck SCC occurred in 11% of alcohol-dependent Japanese males with esophageal SCC, and even in those without simultaneous head and neck SCC at an initial screening, head and neck SCC occurred in 39% at 5 years [71]. Multiple concurrent intraesophageal SCC lesions were also seen in 31% at an initial screening, and the ORs for heterozygous ALDH2 deficiency were 3.68 (1.17–11.57) for 2 SCC lesions and 4.73 (1.15–19.47) for three or more SCC lesions, compared with a solitary SCC lesion. Multiple esophageal SCCs and ALDH2 heterozygous deficiency resulted in HRs for the metachronous development of secondary SCCs of 3.09 (1.41–6.78) and 3.38 (1.45–7.85), respectively, in the esophagus after endoscopic treatment and 3.25 (1.41–7.47) and 4.27 (1.42–12.9), respectively, in the head and neck region [71]. In addition to inactive ALDH2, the slow-metabolizing ADH1B also increased the risk

of multiple UAT cancer in Japanese follow-up studies of esophageal SCC patients after mucosal resection [24, 25] and Taiwanese case-control studies of esophageal cancer patients [72] and head and neck cancer patients [73], but not in Japanese alcohol-dependent esophageal SCC patients [71]. In addition to cancer multiplicity, several studies have suggested that the presence of inactive ALDH2 is a biomarker associated with a poor prognosis in Asians with UAT cancer [74–76].

Iodine-Unstained Lesions (Lugol-Voiding Lesions [LVLs]) in the Esophagus (Fig. 75.2)

Morita et al. examined surgical esophageal SCC tissues and found that an increasing number of intraesophageal SCC lesions was associated with an increasing number of concurrent dysplasia lesions [77]. Shimizu et al. found that the cumulative incidence of metachronous SCC in the esophagus [78] and the head and neck region [79] after the endoscopic resection of superficial esophageal SCC was much higher in the group with numerous iodine-unstained lesions than in the group without such lesions. Muto et al. called iodine-unstained lesions LVLs and found that multiple LVLs (Fig. 75.2c) were a strong risk factor for multiple SCC in the UAT; furthermore, multiple LVLs were associated with alcohol consumption, smoking, and ALDH2 heterozygous deficiency [80, 81]. In the follow-up screening of Japanese alcohol-dependent patients, the 6-year cumulative incidences of the development of esophageal SCC and head and neck SCC were 31% and 20%, respectively, in cancer-free patients with biopsy-proven esophageal dysplasia from distinct LVLs ≥5 mm (Fig. 75.2b); 56% and 35%, respectively, in esophageal-SCC patients after endoscopic mucosectomy; and 4 and 4% in cancer-free patients without distinct LVLs ≥5 mm [82]. Thus, multiple LVLs and large dysplastic LVLs are strong predictors of field cancerization in the UAT.

In a prospective Japan esophageal cohort study (JEC study) of 331 cases with endoscopically resected esophageal SCC from 16 hospitals, subjects with esophageal LVLs visualized using Lugol chromoendoscopy were graded at the time of enrollment according to the maximum number of LVLs (i.e., A = no lesions; B = 1–9 lesions; C = ≥10 lesions in at least one endoscopic field of view) [83]. The 5-year cumulative incidence of secondary SCC in the UAT was particularly high in group C. For grades A, B, and C, the incidences of secondary SCC were 6.0%, 17.8%, and 47.1% for the esophagus and 0%, 4.3%, and 13.3% for the head and neck region, respectively [52]. A case-control study was conducted for the 331 patients in the JEC study and 1022 historical controls for whom information on drinking, smoking, eating habits, and alcohol flushing had been obtained using the same questionnaire [52]. Carcinogenic risk factors were alcohol consumption and the frequent drinking of strong alcoholic beverages, alcohol flushing, smoking, hot food, and the inadequate intake of green and yellow vegetables and fruit. The ORs for esophageal SCC with grade A, B, and C LVLs were 3.57, 45.2, and 241, respectively, for moderate

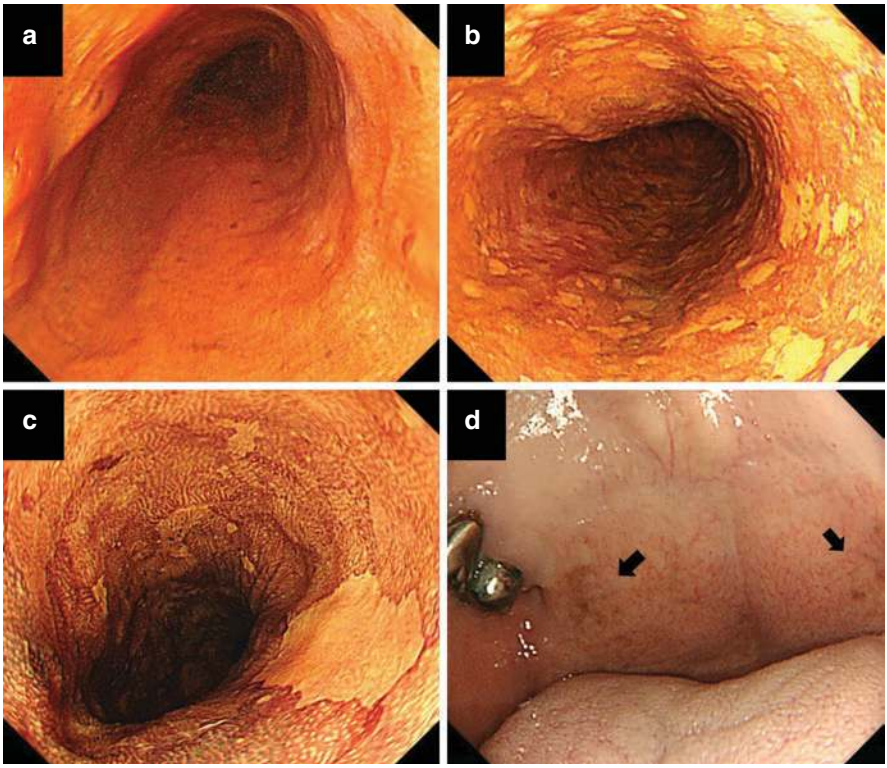

Fig. 75.2 Endoscopic screening for cancerogenic lesions in the upper digestive tract. (**a**) No Lugol voiding lesions (LVLs) in the esophagus. (**b**) The LVL ≥5 mm was diagnosed as mild dysplasia. (**c**) "Multiple LVLs" are present when 10 or more LVLs of any size are observed in at least one endoscopic field of view. (**d**) The endoscopic view of soft palatal melanosis shows multiple flat and pigmented (greenish-black) areas

drinkers (198–395 g ethanol/week) with current/former flushing but were only 1.17, 3.88, and 19.1, respectively, for moderate drinkers with never flushing, indicating that moderate drinking in flushers causes more aggressive esophageal SCC in terms of secondary cancer. A prospective follow-up study after the treatment of head and neck SCC in Taiwan also showed that the presence of multiple LVLs was a strong predictor of metachronous esophageal squamous neoplasia [84]. The presence of multiple LVLs was associated with ALDH2 deficiency in drinkers but was a predictor of second cancer in a manner that was independent from both ALDH2 deficiency and alcohol flushing [24, 52, 80, 81, 83, 85]. Multiple and large LVLs were associated with both heterozygous inactive ALDH2 and slow-metabolizing ADH1B in heavy drinkers [61], and abnormalities of P53 protein [86], TP53 gene mutations [83] and a shortened telomere length [87] were highly prevalent in the esophageal tissues with multiple and large LVLs.

Simple Flushing Questionnaire Method

When Japanese subjects were asked about current facial flushing after drinking alcohol without specifying the alcohol dose, half of those with active ALDH2 reported that they were sometimes or always flushers because they experienced facial flushing after drinking a substantial amount of alcohol [88]. When they were asked about current facial flushing after drinking a glass of beer, some ALDH2-deficient heavy drinkers answered "No" because they did not experience flushing due to AA tolerance, even though they were former flushers. So, "flushing" as determined using these two questionnaires was not useful for predicting ALDH2 deficiency or assessing UAT cancer risk [89, 90].

In the simple flushing questionnaire [44], the questionnaire asked (a) "Do you have a tendency to flush in the face immediately after drinking a glass of beer?" and (b) "Did you have a tendency to flush in the face immediately after drinking a glass of beer during the first 1–2 years after you started drinking?" A "glass" means about 180 mL, which is the volume of the most common size of Japanese beer glass. If either of these questions are answered affirmatively, the drinker is considered to be a flusher (current or former flusher), and ALDH2 deficiency can be determined with a sensitivity and specificity of about 90% in Japan [44, 91–93]. The sensitivity and specificity have been reported to be 79%–95% and 77%–82%, respectively, in Korea [94] and 89% and 81%, respectively, in Taiwan [95]. Drinking habits also affect the sensitivity of this method, with a reduced sensitivity seen for habitual drinkers, alcohol-dependent patients, and UAT cancer patients [44, 45, 95]. The risk of UAT cancer is significantly increased in drinkers with flushing [37, 39, 40, 44, 45, 52, 71, 83, 91, 92, 95–99]. The HR for second cancer of the UAT in 285 Japanese with head and neck cancers was 2.63 (1.25–5.52) in flushers identified using this method [97] and 1.70 (1.02–2.82) for second cancer of the esophagus in flushers of the JEC study [83]. Japanese people have a habit of toasting with a glass of beer of about 180 mL, so this question can be easily answered. If a smaller volume is used, the sensitivity decreases; if a larger volume is used, the specificity decreases. An esophageal examination of 815 cases of head and neck SCC in Taiwan showed that 15% had neoplasia, 47% of which were high-grade dysplasia or SCC, and the OR among the flushing drinkers was 8.6–9.6 [98]. In a cohort study in China, males who drank alcohol weekly were asked whether they had a flushing response "immediately after the first sip" [99]. Probably because this amount was too small, the sensitivity of discriminating ALDH2 deficiency was low at 57%, with a specificity of 88%. However, flushers had a drinking-adjusted HR of 1.45 (1.05–2.01) for esophageal cancer, compared with non-flushers, while the HR was 3.31 (1.94–5.67) for heterozygous ALDH2 deficiency. Although the discrimination of ALDH2 deficiency based on flushing is useful as a carcinogenesis risk assessment, highly accurate discrimination methods appropriate for populations with different drinking cultures are needed.

Health Risk Appraisal (HRA) Model for SCC in the UAT

Figure 75.3 shows the HRA model for SCC in the UAT based on a Japanese case-control study [100]. The results of the simple flushing questionnaire are combined with drinking, smoking, and dietary habits, and a score of 11 or more is considered to be a very high risk for SCC in the UAT. Fifty-eight percent of Japanese men in their 50s and 60s with esophageal SCC who completed the questionnaire [100] and 46% of men in their 60s and 70s with esophageal cancer who were enrolled in the JEC study [101] scored 11 or higher. In general, 5%–11% of middle-aged and older men scored 11 points or more [100, 102, 103]. A Lugol chromoendoscopic follow-up study of Japanese men showed that the detection rate of SCC in the UAT was 3.48 (1.28–7.58) per 100 person-years in subjects with 11 points or more, while it was 0.13 (0.02–0.46) in those with 10 points or less, resulting in a relative risk of 26.6 (51.5–134) for the high-risk group [102]. In a general endoscopic screening using iodine staining, the frequency of esophageal SCC among subjects with 11 points or more was 4.3%, which was six times higher than that in the group with less than 11 points (0.7%) [103]. The HRA model was also useful in assessing the risk of second cancer of the esophagus; when male patients in the JEC study were divided into those with an HRA score of 12 or more and those with an HRA score

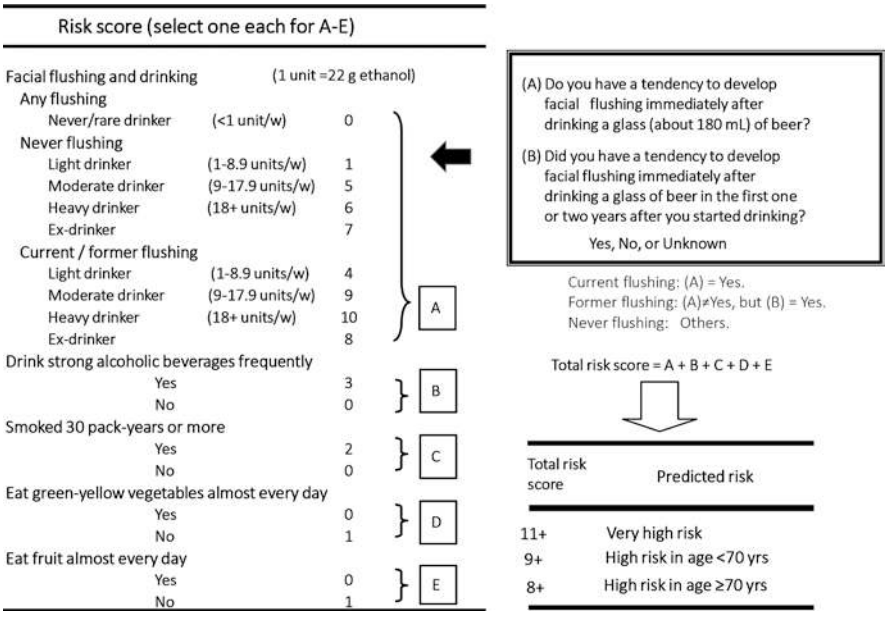

Fig. 75.3 Simple flushing questionnaire used to identify inactive ALDH2 and a health risk appraisal model for SCC in the UAT

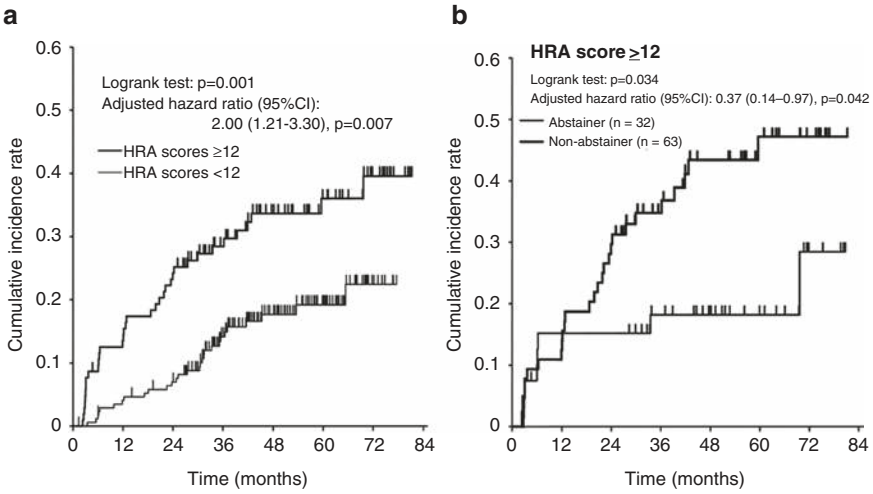

Fig. 75.4 Cumulative incidences of metachronous esophageal SCC after endoscopic mucosectomy of superficial esophageal SCC according to HRA score (**a**) and drinking status in the high-HRA-score drinker group (**b**). Hazard ratio was adjusted for age and LVL grades. PLoS One 2017; 12: e0175182. https://doi.org/10.1371/journal.pone.0175182

of less than 12, the detection rates for second esophageal SCC were 9.8 (6.8–13.7) and 4.5 (3.0–6.5) per 100 person-years, respectively, with a 2.00-fold (1.21–3.30) HR for those with an HRA score of 12 or more (Fig. 75.4a) [101].

Alcohol Use Disorders Identification Test (AUDIT) in UAT Cancer Patients

The AUDIT is a screening test developed by WHO to identify "suspected alcohol dependence" and "hazardous drinking" for early intervention and the prevention of alcohol problems [104]. The cutoff values are uniquely set according to the situation in each country. In Japan, an AUDIT score of 8 or higher is considered "hazardous drinking," while an AUDIT score of 15 or higher is considered "suspicious of alcohol dependence"; in the general adult male population, 26% have an AUDIT score of 8 or higher and about 5% have an AUDIT score of 15 or higher [105]. However, 62.1% of the esophageal SCC patients had an AUDIT score of 8 or higher and 25.4% had an AUDIT score of 15 or higher at the time of enrollment in the JEC study [105]. The AUDIT score was positively associated with the grade of multiple LVLs. In the grade C group, 37.7% had an AUDIT score of 15 or higher. Furthermore, the HR for the development of second cancer in the head and neck region was 6.98 (1.31–37.1) in the group with an AUDIT score of 15 or more, "suspected alcohol

dependence", compared with the group with a score of 0–7, and the HR was 3.19 (1.19–8.54) in the grade C group, compared with the grade A group. Secondary cancers of the head and neck after esophageal cancer treatment are markedly more frequent among Japanese alcohol-dependent patients than esophageal cancer patients in general [71], and the "suspected alcohol-dependency" group in the AUDIT may be indicative of this feature. A comprehensive assessment using AUDIT for the detection of alcohol problems is desirable once alcohol-related cancer, not just esophageal cancer, is diagnosed.

Melanosis, Macrocytosis, Macrocytic Anemia, Leukopenia

Melanosis of the soft palate, pharynx, and esophagus looks like a small greenish-black stain (Fig. 75.2d). This overexpression of melanin is highly comorbid with UAT neoplasia [106–108]. Melanosis is particularly common in heavy drinkers with ALDH2 heterozygous deficiency and increases with smoking and aging. Soft palate melanosis can be observed with a penlight and is a high-risk finding that is instantly apparent upon endoscopic insertion.

The mean corpuscular volume (MCV) of red blood cells increases with heavy drinking, but in alcohol-dependent individuals with ALDH2 heterozygous deficiency, the MCV is particularly large and is associated with macrocytic anemia [109, 110] (see also Chaps. 37, 57 and 58). The combination of heterozygous inactive ALDH2 and fast-metabolizing ADH1B causes a particularly pronounced macrocytic anemia [110]. Alcohol-dependent patients with this genotype combination had the highest levels of AA-DNA adducts in leukocytes [48] and the highest blood AA/ethanol ratio [33]. While the precise mechanisms of red cell enlargement are not fully known, high AA exposure is probably a major cause of this finding. Increased MCV and macrocytic anemia are also associated with smoking, aging, folic acid deficiency, and emaciation in alcohol-dependent patients. The ORs for SCC detection in the esophagus [109] and the head and neck region [39] were 3.68 (1.96–6.93) and 2.71 (1.42–5.16), respectively, in Japanese alcohol-dependent men with an MCV ≥106 fl, compared with those with an MCV <106 fl. In alcohol-dependent patients with an MCV ≥106 fl, SCC in the UAT occurred in more than 20% of patients at 5 years, with a HR of 2.91 (1.63–5.21) [37]. Furthermore, the OR of UAT neoplasia increased to 1.49, 3.14, 4.80, and 7.80 for each additional one of the four risk factors of 55 years of age or older, flusher, MCV ≥106 fl, and soft palate melanosis [107].

The association between leukocytopenia and alcohol dependence has long been known, and monocytes, granulocytes, and lymphocytes are all decreased in patients with inactive heterozygous ALDH2; when inactive ALDH2 is combined with the fast-metabolizing forms of ADH1B, the reductions in the monocyte and granulocyte counts are particularly prominent [111]. This phenomenon can also be explained by high AA exposure in the blood of alcohol-dependent patients with this ALDH2/

ADH1B combination [33, 48]. This change is considerably reversed after 4 weeks of abstinence from alcohol [112]. Recent studies have shown that in non-abstinent alcohol-dependent patients, not only the number of monocytes is reduced, but also the ability to produce LPS-stimulated inflammatory cytokines is suppressed in this genotype combination, with a partial recovery observed after sobriety [113]. Similar findings were observed using ethanol feeding in *Aldh2*2* transgenic mice [113]. About 60% of men with esophageal SCC in Japan have this genotype combination [2, 22, 23], and anemia and a decreased immune response can interfere with cancer treatment; abstinence from alcohol before treatment should be considered from the viewpoint of bone marrow recovery. The immune deficiency caused by high AA exposure may be another possible cause of alcohol-related carcinogenesis.

Esophageal SCC in Female Asian Drinkers

A case-control study in Japanese women showed a markedly high risk of esophageal SCC in women with the inactive heterozygous *ALDH2*1/*2* genotype who were heavy drinkers [91]; this result was comparable to the results of a male study conducted simultaneously [2]. A Japanese GWAS also showed similar ORs for esophageal SCC for the *ADH1B*1/*1* genotype and the *ALDH2*1/*2* genotype between men and women [22]. Meta-analyses of Asian case-control studies showed that female drinkers with the *ALDH2*1/*2* genotype had an increased risk of esophageal SCC [26, 30]. The drinking culture in Japan is much more restrictive for women than for men, and the male-to-female ratio for alcohol-dependence is approximately 10:1 according to the latest nationwide survey [114]. The age-adjusted mortality rate for esophageal cancer per 100,000 Japanese men in 2019 was estimated to be 7.1, whereas that for Japanese women was 1.2 [115]. However, alcohol-dependent women and men share several common risk factors of esophageal neoplasia and multiple LVLs, but with considerably different magnitudes. These factors include the slow-metabolizing *ADH1B*1/*1* genotype, the inactive heterozygous *ALDH2*1/*2* genotype, a low BMI, and a large MCV [96].

Gastric Cancer and Alcohol

In a pooled analysis of 20 studies, drinking more than 48 g/day increased the risk of gastric cancer by 26%, and drinking more than 72 g/day increased the risk by 48% [116]. In 2018, the World Cancer Research Fund International concluded that a greater consumption of alcoholic drinks was probably a cause of stomach cancer. This was based on evidence obtained from individuals with intakes of greater than

45 grams per day [117]. Gastric cancer has been frequently detected in esophageal SCC patients in Japan [63, 64, 67, 118, 119]. One reason is the very high incidence of *H. pylori*-associated atrophic gastritis in Japan. In a study by the Aichi Cancer Center (1375 cases, 2050 controls), the OR of gastric cancer increased with increasing alcohol consumption only in ALDH2-deficient individuals, compared with non-drinkers with active ALDH2, reaching 3.57 (2.04–6.27) at 230 g/week or more, suggesting the direct involvement of AA [120]. In addition to *H. pylori*-associated atrophic gastritis, ALDH2 heterozygous deficiency, MCV enlargement, and concurrent UAT cancer were risk factors for gastric cancer diagnosed by screening in Japanese alcohol-dependent men, suggesting the involvement of ethanol and AA in gastric carcinogenesis [121]. The first meta-analysis of 7 Asian studies showed that ALDH2 deficiency increased the OR of gastric cancer (1.42 [1.21–1.67]) in drinkers, but not in non-drinkers [122]. Another meta-analysis that included 3251 cases and 4943 controls showed that ALDH2 deficiency increased the risk of gastric cancer in moderate to heavy drinkers (OR = 1.85 [1.52–2.25]) more than in non−/mild drinkers (OR = 1.19 [1.05–1.36]) [123]. A genomic-scale trans-ethnic analysis of gastric cancers (319 Asians and 212 non-Asians) also showed one distinct gastric cancer subclass with a clear alcohol-associated mutation signature and strong Asian specificity, almost all of which were attributable to alcohol intake behavior, smoking habit, and inactive ALDH2 [124].

In a meta-analysis of seven studies, the presence of atrophic gastritis was associated with an esophageal SCC OR of 1.94 (1.48–2.55) [125]. *H. pylori*-associated atrophic gastritis was at more advanced stages in Japanese alcohol-dependent men with esophageal SCC than in those without esophageal SCC [119]. In a follow-up study of Japanese alcohol-dependent men after treatment for esophageal SCC, the frequency of metachronous gastric cancer was 15% at 5 years [119]. This result is comparable to the high rate of metachronous gastric cancer after endoscopic treatment for gastric cancer in the Japanese general population.

In heavy drinkers, smoking, a salt preference, and a low intake of fruit and vegetables are also likely to coexist, which is a common risk for esophageal SCC, atrophic gastritis, and gastric cancer. On the other hand, the progression of atrophic gastritis may increase the risk of esophageal SCC. A major component of the esophageal microbiota is oral *Streptococcus* species [126]. It has been experimentally shown that oral bacteria descend and multiply in a low-acid stomach environment, producing AA from ethanol and increasing the concentration of AA in gastric juice [127]. Asian studies have demonstrated positive associations between excessive drinking and gastroesophageal reflux disease or short segmental Barrett's esophagus [128, 129]. Antiseptic acid reflux alters the esophageal microbiota in high-acid subjects without gastric atrophy [127]. The frequent occurrence of gastric cancer in esophageal SCC patients might be partly attributable to the low-acid stomach environment and the subsequent alterations of microbiota and AA exposure in the esophagus and stomach.

Detection Rates of Esophageal SCC and Gastric Cancer Have Decreased in Japanese Alcohol-Dependent Men in Parallel with the Decreasing Rate of Infection with *H. Pylori*

The author has been conducting esophageal iodine staining screening for men with alcohol dependence since 1993. We performed an initial screening of 7582 patients (40–79 years old) with no history of cancer until 2018 and diagnosed head and neck SCC in 1.1%, esophageal SCC in 3.5%, and gastric cancer in 1.1%; at 5-year intervals over a 26-year period, esophageal SCC was diagnosed at rates of 3.7, 3.9, 4.3, 3.0, and 2.1%; thus, the rate of esophageal SCC detection has decreased over the last 10 years [67]. Furthermore, the detection of not only SCC, but also of large LVLs and multiple LVLs in the esophagus has decreased over the past decade [130]. As a result of the decrease in *H. pylori* infection, the rates of gastric cancer also decreased during the above-mentioned periods to 1.5%, 1.4%, 1.3%, 0.3%, and 0.7%, respectively [67]. The combined effect of a decrease in *H. pylori*-associated gastric atrophy, which is associated with the risk of esophageal SCC, a decrease in smoking, an improved nutritional status in terms of BMI, and a change in preferences for alcoholic beverage types may be responsible for the decrease in esophageal SCC and LVLs. However, even after adjusting for these confounding factors, the cause of the decrease in esophageal squamous neoplasia has not been determined and will continue to be a subject of future research [67, 130]. This outcome might be linked to global trends showing a decreasing incidence of male esophageal SCC during recent decades [131].

Effects of Abstinence or Drinking Moderation on SCC in the UAT

Inactive ALDH2 has two opposing effects on the digestive tract: direct carcinogenic effects in response to drinking, and indirect protective effects arising from less or no drinking because of aversive alcohol flushing responses [132]. In a Japanese male case-control study, the OR of esophageal SCC among ALDH2 heterozygotes who drank less than 198 g ethanol per week was 6.8, compared with non-drinkers, but the OR was 65.3 for those who drank 198–396 g ethanol per week. It was estimated that 53% of male esophageal SCC cases would not have occurred if ALDH2-heterozygous moderate drinkers had not drunk or drunk less than 198 g per week from the beginning [92]. Another Japanese case-control study estimated that 12% of heterozygous drinkers who drink more than 46 g ethanol on each occasion and on 5 days a week or more are likely to have esophageal SCC by the age of 64 years and 20% by the age of 79 years, while among those who drank less, only 5% are expected

to have esophageal SCC by the age of 79 years [133]. Although many studies have shown that there is no safe level of alcohol consumption for alcohol-related cancer [38, 134, 135], a steep increase in the risk of esophageal SCC occurs in ALDH2-deficient individuals with a daily alcohol consumption of around 30 g or more.

In a meta-analysis of nine studies of esophageal cancer, the risk decreased significantly early in abstinence and disappeared at 16.5 years [136]. In a meta-analysis of 4 studies of laryngeal cancer and 8 studies of pharyngeal cancer, 5 years of drinking cessation was associated with a reduction of around 15% in the alcohol-related elevated risk, but a long time period was required to eliminate the elevated alcohol-related risk of laryngeal (36 years) and pharyngeal cancers (39 years) completely [137].

In the JEC study, after a median of 80.7 months of prospective follow-up, abstinence from alcohol after enrollment was associated with an HR of 0.47 (0.26–0.85) for secondary esophageal SCC, compared with continued alcohol consumption [52]. Moreover, the preventive effects of abstinence on the secondary esophageal SCC were more pronounced in the high-risk groups, with an HR of 0.30 (0.13–0.67) for abstinence (vs. continued drinking) in the group with grade C LVLs [52]. In a subanalysis of men in the JEC study, abstinence from alcohol reduced the HR for the secondary esophageal SCC by 63% in the group with an HRA score of 12 or more (Fig. 75.4b) [101]. In a Hokkaido University study of 158 patients with endoscopically resected esophageal SCCs followed for a median of 80 months, the HR increased by 2.25-to-4.39-fold for the ALDH2 heterozygous deficient group for a second, third and fourth SCC of the UAT [25]. Drinking cessation or reducing alcohol consumption to less than 198 g ethanol per week significantly reduced the HRs to 0.35 (0.20–0.63) and 0.22 (0.07–0.56), respectively, for a second and third SCC [25]. In a retrospective multicenter study of 198 patients with head and neck SCC (mainly hypopharyngeal) who were treated with transoral surgery, the HRs for a second and third SCC of the head and neck region were significantly reduced to 0.54 (80.31–0.92) and 0.19 (0.03–0.65), respectively, when those who drank at least 198 g ethanol per week abstained from alcohol or reduced their alcohol intake to less than 198 g ethanol per week after treatment [138]. Quitting or reducing alcohol to less than about 30 g per day may significantly reduce the risk of esophageal and head and neck SCCs, including second SCCs.

Conclusion

Knowledge of alcohol-related cancers in the UAT and stomach has been accumulating, but it still remains challenge to translate this knowledge into prevention and clinical practice.

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Part XIII
Center Experiences for the Interdisciplinary Management of Alcohol Use Disorders

Chapter 76
Model for Secondary Health Care Alcohol Services: Optimising the Response to Alcohol Use Disorders in Acute Hospitals

Omar Elshaarawy, Lynn Owens, Edward Britton, and Paul Richardson

Abstract Optimising the care of patients attending acute hospitals with an alcohol use disorder requires health organizations to deliver (a) consistent and accurate screening processes, (b) develop locally appropriate integrated care packages, and (c) identify co-existing physical and psychological comorbidities. It has been shown that Alcohol Care Teams (ACT) are best placed to respond to this challenge, affording the opportunity to develop a highly skilled workforce with specific emphasis on development of skills and competence that support and enable delivery of a wide range of clinical pathways. In this chapter, we will explore the core service components of an Alcohol Care Team and the potential pathways required to optimize care and improve clinical outcomes.

Keywords Alcohol care team · Alcohol use disorder · Patient pathways Screening · Pharmacotherapy

Introduction

Optimising the care of patients attending acute hospitals with an alcohol use disorder (AUD) requires health organizations to deliver (a) consistent and accurate screening processes, (b) develop locally appropriate integrated care packages, and (c) identify co-existing physical and psychological comorbidities. It has been shown that Alcohol Care Teams (ACT) are best placed to respond to this challenge,

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affording the opportunity to develop a highly skilled workforce with specific emphasis on development of skills and competence that support and enable delivery of a wide range of clinical pathways. In this chapter, we will explore the core service components of an Alcohol Care Team and the potential pathways required to optimize care and improve clinical outcomes.

Background, Burden and Magnitude of the Problem

More than 60 medical conditions have been linked to alcohol consumption, including circulatory and digestive diseases, liver disease, a variety of malignancies, and depression. In England, there were 337,113 hospital admissions with an alcohol-related diagnosis as the primary diagnosis between 2016 and 2017 [1, 2]. Heavy drinkers are at a higher risk of developing alcohol-related chronic diseases. Importantly, this burden is set to rise as the incidence of alcohol-related disease is increasing year on year across all age groups. Worryingly, there is an increasing incidence in younger age groups. The burden of alcohol-related disease to acute care accounts for 78% of the total estimated £3.5 billion per year cost of alcohol to the NHS [3].

Data indicate that around 5% of inpatients in acute hospitals are alcohol dependent compared to 1.4% in the general population. The average length of stay for alcohol related admissions is 5.69 days compared to 2.25 days for non-alcohol admissions for the same diagnosis. Alcohol-related attendances comprise 12–15% of accident and emergency (A&E) visits. There were 12,600 emergency admissions for alcohol-related liver disease (ALD) alone in 2016–2017 in Royal Liverpool University Hospital [4]. 12% of patients admitted with an alcohol-specific disease are re-admitted to the hospital within 30 days of discharge [4, 5].

To satisfy the requirements of these vulnerable group with alcohol use disorder, effective models of care and clinical pathways are required and they need to be implemented in all hospitals by a highly trained workforce. This model of care we are presenting in this chapter has been explicitly identified milestone toward achieving the second aim of the alcohol harm reduction strategy by NHS England [4, 6]. This has targeted early identification and treatment of adults with AUD in either acute or secondary care setting.

Aims of the Optimal Alcohol Care Team

ACTs are teams in acute hospitals that provide specialist support services to patients with a suspected or established AUD [7]. They aim to:

- Improve identification of AUD as a contributing factor in hospital attendance
- Reduce avoidable alcohol-related hospital admissions

- Reduce rates of re-admission
- Reduce the length of stay for inpatients
- Improve the management of acute alcohol withdrawal (AAW)
- Provide timely and meaningful psychosocial interventions
- Facilitate integrated alcohol related care between secondary, primary and community care providers
- Provide evidence-based treatments to support recovery (pharmacotherapy and psychological treatments)
- Support the wider workforce on AUDs identification and management
- Improve data collection and opportunities for audit and clinical research

Core Service Components of the Alcohol Care Team

A multi-faceted ACT should be able to provide packages of care that include [7, 8]:

- Case identification/alcohol identification and delivery of brief advice (IBA)
- Comprehensive alcohol assessment
- Specialist nursing and medical care planning
- Management of medically-assisted alcohol withdrawal (MAW)
- Provision of psychosocial interventions
- Planning safe discharge, including referral to community services
- Clinical leadership by a senior clinician with dedicated time for the team
- Provision of hospital wide education and training in relation to alcohol

Roles of the Alcohol Care Team

Emergency Department (ED) Presentations

The ACT will use well validated screening tools to assess patients presenting to the ED to accurately identify the presence of an AUD and stratify the severity. Patients in acute withdrawal will undergo a comprehensive assessment to ensure that the most appropriate and safe care can be planned. This may require admission or an ambulatory care plan to monitor and manage mild alcohol withdrawal symptoms. Patients presenting with other alcohol-related complications will be assessed by the ACT, who will contribute to their care plan [9].

This is where you need to discuss screening for co-morbidities using liver elastography such as Fibroscan to screen for liver fibrosis, MoCA (Montreal Cognitive Assessment), etc. and appropriate referral to specialist clinicians.

Patients who present to A&E will be assessed to determine the appropriate pathway according to the risk of AUD. Screening using a validated tool as AUDIT-C which is a brief version of AUDIT (see also App. Fig. A20) is usually more feasible

to use in screening. Assessment determines the nature and extent of alcohol abuse, a person's level of need, and necessary interventions. Assessment depth and detail vary by purpose and expected outcome. This is best achieved by acquiring a full history of alcohol intake, which can be limited due to underreporting. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) suggests a single-question screening that asks, "How many times in the last year have you drunk 5 or more drinks in a day (for men) or 4 or more drinks in a day (for women)?" [10]. If one or more episodes are reported, additional questioning with the Alcohol Use Disorders Identification Test (AUDIT) is advised. This comprises 10 questions about drinking habits, dependent symptoms, and any alcohol-related concerns. The AUDIT has been demonstrated to detect harmful alcohol consumption in individuals with a score of eight or higher, or moderate or severe AUD in patients with a score of 15 or higher. According to the scoring, patients are classified according to risk of AUD into "no risk", "moderate risk" or "high risk" [9, 10]. Most patients who will present to A&E with alcohol related medical issues will require at least a brief intervention as moderate risk group or will even require alcohol detox either ambulatory or as in patient as high risk group (see Fig. 76.1).

Inpatients

Inpatients with an AUD admitted for any condition will undergo a comprehensive assessment by the ACT. Based on the assessment outcome, the ACT will contribute to the overall care plan with alcohol specific treatments. This will include plans for ongoing care post hospital discharge. The initial care plan could be used to focus on a patient's engagement in the treatment system, ensure their immediate needs are met, build a therapeutic relationship with patients, and establish adequate interim support if they are waiting for other specialist treatments (see Fig. 76.1) [11].

The ACT usually assesses the patient to see if there are underlying problems triggering alcohol use disorder and would do comprehensive assessment. Comprehensive assessment is for problem drinkers with complex needs who may need structured treatment. The assessment aims to determine the patient's alcohol and other substance misuse problems and co-existing health, social functioning, offending, and legal issues which might require risk assessment. The goal of risk assessment is to determine whether the individual has or has had in the past certain experiences or displayed certain behaviours that could lead to harm to themselves or others [12]. The main areas of risk that must be assessed are as follows:

- Risks associated with alcohol use or other substance use (such as physical damage, alcohol poisoning)
- Risk of self-harm or suicide
- Risk of harm to others (including risks of harm to children and other domestic violence, harm to treatment staff and risks of driving while intoxicated

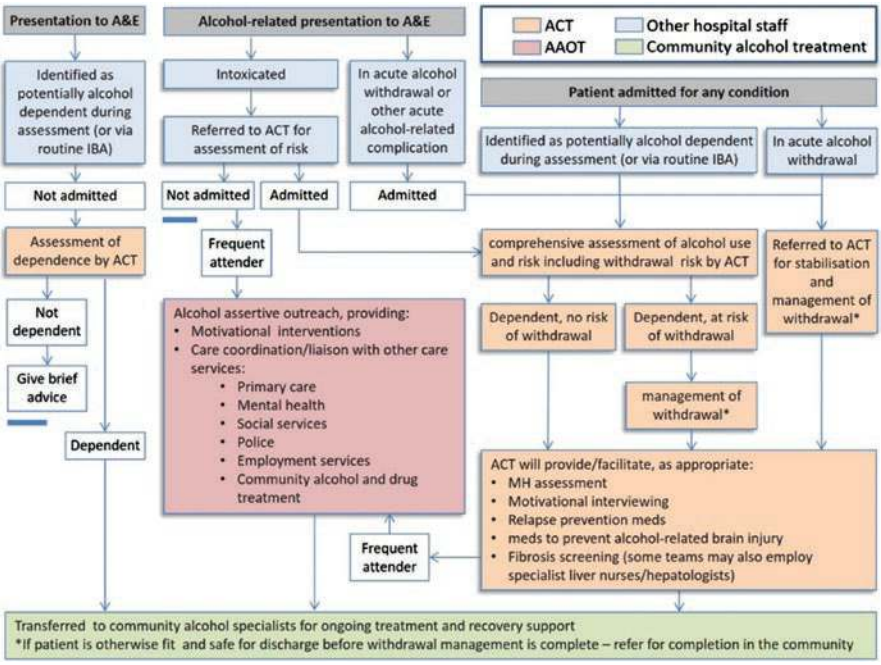

Fig. 76.1 Role of Alcohol Care Teams in the pathway for alcohol dependent patients. Adopted from NHS England Long term Plan https://www.longtermplan.nhs.uk) [11]. *AAOT* alcohol assertive outreach treatment; *ACT* alcohol care teams

- Risk of harm from others (including being a victim of domestic abuse)
- Risk of self-neglect.

Risk management plans must be developed and implemented once risks have been identified in order to mitigate immediate risk. Risk assessment, like comprehensive assessment, is an ongoing process that must be integrated into care planning. Local protocols should be followed if ACT is concerned about the risk of significant harm, social services should be involved in the risk assessment process [11].

According to the outcomes of the assessment process, referring patients to specialist mental health assessment could be a necessary step to initiate psychosocial interventions to support engagement with community alcohol treatment and to identify and mange co-existent metal heath diagnosis. All alcohol-dependent patients will be referred to specialist alcohol support in the community for continuation of alcohol treatment on discharge. When patients are medically fit for discharge before their alcohol detoxification is complete, the ACT will advise on the appropriateness of completion of detoxification in the community on a case by case basis, based on comprehensive assessment (see Fig. 76.1) [11, 12]. An alcohol care plan may include a medication regimen to support management of symptoms of AAW and /or a medication to support sustained abstinence or consumption reduction, and should be in accordance with national guidelines (NICE CG100).

Management of Patient with AUD

The ACT aims toward providing access to pharmacotherapy and screening for end organ damage such as alcohol associated cognitive impairment and liver fibrosis.

Access to Pharmacotherapy

The main outpatient task of the ACT is providing access to pharmacotherapy promoting abstinence. Pharmacological therapies are most effective when used in conjunction with psychosocial therapies as part of a comprehensive care plan. Pharmacotherapy includes medications to promote abstinence or prevent relapse, including sensitising agents [13].

ACT follow up patients in telephone and face to face clinics after prescribing medications to promote abstinence such as acamprosate and baclofen. Nurse led phone clinics by the ACT provide the required support for patients on pharmacotherapy regarding management of side effects, mild withdrawal symptoms, checking blood tests for patients after being on ambulatory detoxification or being discharged from hospital [13, 14].

Screening for End Organ Damage

Detection of Chronic Liver Disease

Only 35% of patients with high-risk alcohol consumption develop steatohepatitis and alcoholic hepatitis (AH), and 10–20% develop cirrhosis, indicating the existence of associated risk factors [10]. Women have been found to be more at risk for ALD, with higher risk related with daily alcohol use of 20–40 g compared to 60–80 g for males [15]. Finally, various concurrent liver illnesses, notably nonalcoholic fatty liver disease, hepatitis C, and hemochromatosis, can exacerbate ALD and encourage the rapid development of severe fibrosis and cirrhosis.

Transient elastography has been well established to assess the degree of fibrosis without dedicated ultrasound knowledge. Transient elastography has a sensitivity of 86% and a specificity of 95% for diagnosing advanced fibrosis or cirrhosis using a threshold value of 15 kilopascals in patients with hazardous alcohol use [10]. However, it is critical to screen for active alcohol consumption prior to undergoing these tests, as liver stiffness can improve after 2 weeks of abstinence [16]. Furthermore, the fibrosis detection threshold may need to be changed based on AST and bilirubin levels, as elevations signal hepatic inflammation and may exaggerate the degree of fibrosis present. In UK, Commissioning for Quality and Innovation

(CQUIN) promotes fibrosis assessment for all patients presenting with AUD [10]. More details are provided in the respective chapter on liver elastography in this book.

Detection of Alcohol-Related Cognitive Impairment

Studies show that about 35% of patients with AUD suffer from some form of Alcohol-related cognitive impairment (ARBI). Many patients present with multiple types of symptoms of ARBI, and about 25% also show evidence of other brain trauma. This can render an accurate diagnosis quite difficult. Many of these conditions are treatable. For example, about 75% of people with Wernicke-Korsakoff syndrome recover to some degree after appropriate treatment (see also chapter on Wernicke-Korsakoff syndrome in this book). Therefore, an early diagnosis is important [15, 17].

ACT flags patients with alcohol-related cognitive impairment and request professional mental and cognitive capacity assessment usually through Montreal Cognitive Assessment test (MoCA). Clinicians usually perform full neurological examination and exclude any neurological abnormality via proper brain imaging. Risk factors for ARBI include history of heavy drinking (5+ years of 35+ units per week), poor nutrition, particularly thiamine deficiency and history of withdrawal/poorly controlled alcohol detoxification [18]. Alcohol-related liver disease has been identified as a risk factor of ARBI as well as low socio-economic status. ACT is not clinically involved with the alcohol-related cognitive impairment clinical pathway, they just screen and flag patients with risk.

Patients with alcohol-related cognitive impairment may present with one of the following: Confusion, confabulations, slurring of speech, personality changes, poor impulse control, memory deficits, difficulties in decision-making, executive function difficulties, unsteady gait and problems with walking and other movement.

Confounding factors include the following: Coexisting physical and/or mental health conditions, hard to assess cognitive impairment whilst intoxicated, clinical lack of knowledge of conditions, misdiagnosis as dementia, shame and stigma affecting help-seeking behaviours of carers, reluctance of patients to seek help, belief that nothing can be done [17, 18].

Training and Competencies for ACT

The changing nature and aspects of medical care require nurses to ensure that they have the appropriate skills and knowledge to practice. Champs Public Health collaborative in collaboration with Chairs of the Cheshire and Merseyside ‘Reduction of Harm from Alcohol’ Programme launched PROACT Competencies Framework: **PRO**gram for **A**lcohol **C**are **T**eams Competencies which is a competency

framework and development plan for nurses working in an ACT [19]. In the detailed framework, each competency was discussed in detail and how to gather information and evidence to support the competencies as outlined. Each individual nurse is expected to develop an action plan that is specific to their own learning needs and style, with support of learning aids. Some examples of learning aids which can be useful within this process are the following: R.

1. Reflective diaries
2. Attending clinical ward rounds
3. Critical incident analysis
4. Profiling of populations
5. Caseload/individual case analysis
6. Shadowing of other disciplines
7. Clinical supervision/group supervision
8. Peer review
9. Mandatory training
10. Critiquing research articles
11. Dissemination of findings.

Assessment of competencies should be undertaken by an appropriate assessor, which could be a senior medical consultant and pharmacist. Some skills can be measured using the objective structured clinical examination (OSCE) style tick lists [19]. Competencies highlighted by the framework to be achieved before assessment include the following [19]:

1. The nurse/patient relationship - communicating with families and multidisciplinary teams (mdt).
2. Equality and diversity
3. Alcohol screening and detection of alcohol use disorders
4. Assessment and management of the alcohol dependent patient and alcohol withdrawal syndrome
5. Brief interventions
6. Non-medical prescribing
7. Detection and management of Wernicke's encephalopathy and alcohol related brain injury (cognitive impairment)
8. Detection and management of alcohol related liver disease
9. Detection and management of dual diagnosis patients
10. Leadership
11. Service development

The aim of this framework is to define and describe the knowledge and skills which ACTs need in order to deliver quality services. It will support the nurse candidate in identifying their learning needs and plan the candidate development. Matching learning to domains within the Knowledge and Skills Framework (KSF), they can identify the knowledge, skills, learning and development that they need to do their job well. This approach can also support a fair and consistent approach to Personal Development Planning and Review (PDP&R) [19].

Evaluating Effectiveness of Care Pathways

At the Royal Liverpool University Hospital, for many years, we have routinely collected data on all referrals to our ACT. The data is based on standard operating procedures and include:

1. Patient experience and perceptions
2. Alcohol screening
3. Psychosocial interventions
4. Screening to detect co-morbid disease (Liver, Brain)
5. Provision of ambulatory care and prescribing for alcohol withdrawal management
6. Prescribing in relapse prevention
7. Provision of bespoke care at end of life

Over time, bespoke databases have been designed to enable service evaluation and map patient outcomes. Within this routine data set, unique patient outcome measures can be matched to alcohol phenotype and co-morbidities. This provides confidence for wider implementation of any recommendations that emerge from our findings. It also has potential to benefit clinical practice, and subsequently and most importantly provide improvements in access to and quality of alcohol care for patients in general hospitals [20]. After establishing these databases, we are currently starting analytics to drive the services toward a better patient care which includes the implementation of alcohol care pathways using routinely collected data and evaluation of staff, patient experience and perceptions.

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Chapter 77
An Interdisciplinary Approach to Alcohol Use Disorders: What Hepatologists Can Do in a Resource-Limited Setting

Andreea Fodor, Andra Nicoara, Madalina Taru, Vlad Taru, Andreea Bumbu, and Horia Stefanescu

Abstract The prevalence of alcohol use disorders and alcohol-related liver disease is high in Romania, due to multiple socioeconomic, demographic, and cultural factors. Their management is also precarious, mainly because there is a lack of understanding towards addiction issues and an increased level of stigma. The hepatologist is often in the middle of this landscape, as many of the alcoholics seek medical care when liver disease is overt, or advanced, or having a complication. Consequently, the liver specialist is subjected to a considerable burden, which he/she copes with by creating a multidisciplinary team, by getting involved in research and development projects, or by education and training. The AddictHelp Project describes a local hepatologist driven initiative towards the establishment of a multidisciplinary team, and also towards providing education and raising awareness.

Keywords Alcohol use disorder · Alcohol-related liver disease · Resource limited setting · Hepatologist · Multidisciplinary team · Addiction

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Background

Alcohol misuse remains worldwide the only addiction to a psychoactive agent that is not actively regulated by a control body [1]. It represents, together with tobacco smoking and high salt/sugar food, the main driver of increasing incidence of non-communicable diseases [2]. Although the so-called "social drinking" is not considered harmful, recent data demonstrates the opposite [3], especially among young and middle-aged populations (15–49 years), where it represents the main cause of mortality (approximatively 12% among men, and 4% among women). Not only that alcohol represents a risk factor for 23 diseases, its misuse is responsible for a significant number of domestic and social violence incidents and also for a decreased workforce productivity.

Alcohol-related liver diseases (ALD) represent a particular facet of alcohol use disorders (AUD), because alcohol is associated (at least in the western world) with an increasing risk of liver cirrhosis and hepatocellular carcinoma, conditions that are diagnosed in up to 75% of cases in a late or advanced stage. About 1.25 million individuals die annually because of a liver disease, representing 2.3% of total deaths (>50% higher than 30 years ago) [4]. More details on the epidemiology of AUD and ALD are discussed in part I of this book.

The Romanian Case: A Resource Limited Country in the Western World

Romania has significantly changed and improved in the last 30 years since the end of the communist dictatorship and the Cold War in terms of economic growth and quality of life (GDP/capita PPP increased from ~11,000 USD in 1992 to >35,000 USD in 2021, as World Bank data suggest). Romania became full member in NATO (2004) and EU (2007), which established its way towards development and recovering the disparities from the Western countries. However, there are specific challenges that characterize and impact the country's transition.

Demographics and Socioeconomics

The majority of communist industrial and agricultural plants and factories closed due to their unsustainable business model, leaving a huge number of persons unemployed and many small towns without any perspective. As a consequence, more than 3.5 million individuals left Romania. Nowadays (2017), the unemployment rate in Romania is 6.8%, at a population of 19.815.000 [5]. Almost half of this

population still lives in rural areas [6], which is an important issue, because the disparities between rural and urban areas increased, leading to a situation where 46.6% of rural workforce is either unemployed, or working in subsistence agriculture [7]. This situation leads to a paradox: although the national unemployment rate is lower than the EU average (9.4%), the relative poverty rate is double (19.8%) [5].

It is not a surprise that under these circumstances, the alcohol intake in Romania is high and continues to grow, both in terms of volume of pure alcohol (11.7 L/capita—higher than the EU average—8.7 L/capita, ranking ninth among the EU countries) and in episodes of heavy drinking (first for males and second overall), as recent (2019) report on the prevalence of heavy episodic drinking in European countries, at least once a month among alcohol drinkers shows [8]. Apart from thus, there is a significant problem of illicit and domestic alcohol production, which continues to be high and is virtually not accessible to objective quantitation.

The amount of alcohol intake is important because since it is directly related to ALD prevalence and progression. Thus, it has been demonstrated that most patients with advanced liver disease due to alcohol are abusive drinkers (>120 units/week for men and >80 units/week for women) [9] [10]. Apart from the quantity, the drinking pattern (moderate vs binge; constant vs. occasional), the type of beverage (beer, wine, or spirit) and their quality influence the progression of ALD. For instance, it has been shown that, in countries with low GDP, the high prevalence of advanced liver diseases and increased mortality is associated with the intake of low-quality spirits [11].

Healthcare System

The healthcare system in Romania is shifting from being inpatient oriented to community and primary care, but still bears unsolved problems:

- Low health care expenditure: lowest in EU, both in EUR/capita (814), or as GDP share (4.9%, as compared with the EU average of 9,9%);
- Shortage of medical staff, affecting both doctors (2.8/1000 population vs. 3.5/1000 in EU) and nurses (6.4/1000 population vs. 8.4/1000 in EU), ranking among the three most affected countries in EU. This is a consequence of a major brain drain affecting healthcare professionals in the 2000s, especially after 2007 as a response to EU accession and lowering wages due to the economic crisis;
- Underdeveloped and outdated infrastructure, especially in rural areas [5].

All these factors led to an increased number of amendable or avoidable deaths, the highest in the EU [5]. This is also mirrored by the number of liver diseases in Romania being the country with the highest prevalence of chronic liver diseases (~1200/100000), but also the country with the highest mortality due to cirrhosis and liver cancer (40/100000) [12].

Cultural Issues

Despite its progress, Romania remains a traditional society, being less inclusive and tolerant. Therefore, stigmatization is frequent, especially in poorly educated communities. Education is an important factor in this regard, since the functional illiteracy is the second worst in EU (reaching 42%), despite the high rate of adult literacy (~99%) [13]. A systematic review investigating stigma in various mental illnesses, found that AUD patients are less frequently regarded as mentally ill, but ranked highest for every aspect of stigma: blame, dangerousness, social rejection, negative emotions, and they are at particular risk for structural discrimination [14]. This creates the premises for another Romanian paradox: a high AUD stigma in a country with an increased alcohol misuse. Not surprisingly, in the Romanian Code of Occupations there is no equivalent for the "drug and alcohol addiction counsellor" (ESCO 2635.1.2).

The Role of the (Romanian) Hepatologist

All these socioeconomic and cultural factors, together with a precarious healthcare system render healthcare professionals working in liver clinics the first who get in direct touch with AUD patients, when they seek medical assistance e.g. because of gastrointestinal bleeding, decompensated liver cirrhosis, episodes of alcoholic hepatitis (AH) or acute on chronic liver failure (ACLF). Therefore, the skills and knowledge, used by the hepatologist in everyday practice, shifted from clinical—specific to each spectrum of ALD (see Table 77.1) to more complex ones, needed to diagnose and quantify alcohol misuse and addiction and to stratify risk of chronic liver disease (CLD) (see Table 77.2). Table 77.3 shows how to characterize alcohol misuse according to DSM-V.

Whatever the clinical situation is in a resource limited settings,, the hepatologist faces all the socio-economic, demographic and cultural issues mentioned before. All this translates into poorer care and bad outcomes for patients, and burnout for caregivers. All in one, an increased burden on the medical staff that deals with ALD is observed, as depicted in Fig. 77.1. However, the hepatologist "under siege" has several solutions in hand to overcome the situation: education, research, development and coagulation around him/her of all the other actors involved in delivering better care for ALD patients.

Table 77.1 Traditional assessment of patients with ALD by the hepatologist

Spectrum of ALD	Clinical signs and symptoms[a] [15]	Diagnostic tests	Therapeutic measures
Alcohol abuse	Alcoholic halena Stellate angiomas on the chest and face; palmar erythema, leukonychia, ecchymosis, Dupuytren's contracture, bilateral parotid gland hypertrophy, rhinophyma; overweight/obesity	Increased AST and ALT; AST/ALT >1; increased GGT [16] [17]	Refer to psychologist/psychiatrist
Alcoholic steatosis		Abdominal ultrasound LSM (+ CAP) by liver elastography	Silymarin Phospholipids
Alcoholic cirrhosis	Jaundice, minimal/overt hepatic encephalopathy Weight loss-due to muscle atrophy or weight gain-due to ascites and oedema	MELD & CPT scores Ultrasound & CT scan (HCC screening) EGDS—variceal screening	NSBB, diuretics Rifaximin, lactulose EBL
Alcoholic hepatitis (AH)	Jaundice, fever, nausea, ascites, SIRS/infection	Maddrey, ABIC, GAHS & MELD scores Lille model ± liver biopsy (neutrophil infiltration, Mallory-Denk bodies, ballooning, cholestasis, steatosis	Prednisolone, ACC, antibiotics, nutrition, ICU admission, vasoactive drugs, mechanical ventilation, dialysis; early liver transplantation or palliative care
Decompensated liver disease	Liver, kidney, and other organ failures	SOFA score	

Abbreviations: *ABIC* age-bilirubin-INR-creatinine score, *ACC* acetyl cysteine, *CAP* continuous attenuation parameter, *CPT* child-pugh-turcotte, *EBL* endoscopic band ligation, *GAHS* glasgow alcoholic hepatitis score, *ICU* intensive care unit, *LSM* liver stiffness measurement, *MELD* model for end-stage liver disease, *NSBB* non selective beta blockers, *VCTE* vibration controlled transient elastography;

[a]At any time, alcohol withdrawal syndrome (which is a psychiatric disorder caused by the cessation of a heavy, prolonged alcohol consumption) may occur, and it could involve: autonomic hyperactivity, tremor, insomnia, nausea, hallucinations, psychomotor agitation, anxiety and seizures. It is not necessarily associated with distress or impairment [18]

Table 77.2 Integrative multidisciplinary assessment of patients with ALD, typically performed by the hepatologist

	Assessment of liver involvement		Assessment of alcohol misuse		
Patient category	Method	Intervention	Type	Methods	Comments/intervention
AUD	Screening of fibrosis (serum test)—most frequently used: Fib4 score		Biomarkers [15]	**CDT** (serum) **EtG** (urine, hair) **PEth** (serum) (cutoff value: 20 ng/ml)	Low sensitivity and specificity False + Limited availability High sensitivity and NPV
ALD	Assess risk for compensated advanced chronic liver disease (LSM by VCTE) or CSPH (add platelets count) [19] (Adjust LSM values according to ALT/AST [20]) Use ultrasound and/CT to screen for HCC Address frailty and sarcopenia by nutritional assessment	Start NSBB if LSM >25, or LSM > 20& PLT <150.000 Monitor annually Biannual Adjust diet; promote exercise Treat complications if they occur	Psychologic	**AUDIT** [21]—most used; 10 q; screening; evaluates: 1. Alcohol intake, 2. Potential dependence 3. Experience of alcohol-related harm **CAGE** [22]—4q; more inaccurate **ASQ** [23]—15 y/n q; can be self-administered; proved to be a reliable predictor for various subjectively perceived effects of alcohol use.	Brief intervention (BI) according to AUDIT score [24]: **0–3**: Positive health message; **4–9**: BI aimed to reduce use (advise) **10–13**: BI aimed to reduce or abstain + appoint follow-up visit; **14+**: BI aimed to accept referral for full assessment and therapy Educational booklet Information about support groups (alcoholics anonymous) Pharmacologic treatment of WS Pharmacologic prevention of relapse [15] (*baclofen—the only compound studied in the setting of ALD!*) [25]
Decompensated ALD	See table 1 for AH and ACLF				

All patients should be characterized for alcohol misuse according to DSM-V* as shown in Table 77.3

Abbreviations: *ASQ* alcohol sensitivity questionnaire, *AUDIT* alcohol use disorders identification test, *CAGE* cut down, annoyed, guilty, eye-opener test, *CDT* carbohydrate deficient transferrin, *EtG* ethyl glucuronide, *NPV* negative predictive value, *PEth* phosphatidylethanol, *Q* question, *se* sensitivity, *Sp* specificity, *Y/N* yes/no

Table 77.3 Characterization of alcohol misuse according to DSM-V

1. Alcohol is often taken in larger amounts or over a longer period than was intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control alcohol use.
3. A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects.
4. Craving or strong desire, or urge to use alcohol.
5. Recurring alcohol use resulting in a failure to fulfil major role obligations at work, school or home.
6. Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of alcohol.
7. Important social, occupational, or recreational activities are given up/reduced because of alcohol use.
8. Recurrent alcohol use in situations in which it is physically hazardous.
9. Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol.
10. Tolerance, as defined by either of the following:
    a. Need for markedly increased amounts of alcohol to achieve intoxication or desired effect;
    b. Markedly diminished effect with continued use of the same amount of alcohol.
11. Withdrawal or taking alcohol to relieve withdrawal.

For AUD to be present, at least 2 of the 11 depicted criteria have to be met in the span of 12 months If the criteria are met for AUD, the severity can then be ascertained as follows: (i) Mild: between 2–3 symptoms in the past year; (ii) Moderate: between 4–5 symptoms in the past year; (iii) Severe: 6 symptoms or more in the past year [18]

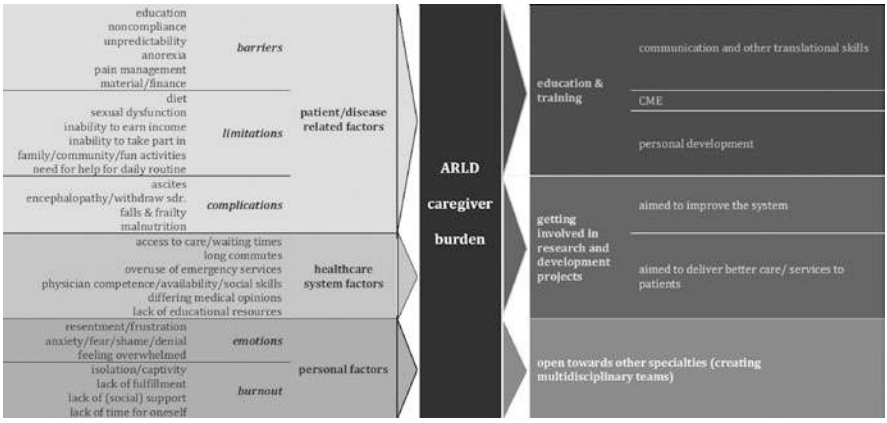

Fig. 77.1 Factors that increase the burden/burnout of caregivers involved in alcohol related chronic liver disease (ARLD) and generate change in practice and care (adapted from [26])

The AddictHelp Project: A Possible Solution for Many Problems

The AddictHelp Project is implemented by Liver Research Club in close partnership with Regional Institute of Gastroenterology and Hepatology from Cluj-Napoca, Romania and is financed through the Active Citizens Fund (an EEA Grants Program). The projects aim at increasing the social inclusion and mobilization of alcohol users by:

(i) Increasing the access to medical and psychological services;
(ii) Educating healthcare professionals and social workers towards increasing compliance to and reducing stigma of alcoholics;
(iii) Increasing awareness of general population regarding these issues.

These objectives are being implemented by:

1. Creating the first outpatient clinic (offering both medical assessment and psychological counselling) for individuals with alcohol misuse;
2. Creating an online educational platform;
3. Generating a set of guidelines, resources, and good practices for the management of addiction

The AddictHelp Outpatient Clinic

The center offers integrated care, both psychological and medical and offers a personalized advice (from educational resources, brief intervention to linkage to care—either medical, or support groups), as shown in Fig. 77.2a. The protocol used to assess every client combines the AUDIT questionnaire with simple non-invasive tests (Fib4, liver elastography such as Fibroscan and abdominal ultrasound), as detailed in Fig. 77.2b. The implementation of the project was superimposed over the COVID-19 pandemic. Of course, this led to certain delays e.g. the launch of the outpatient clinic had to be delayed. On the other hand, this situation offered the opportunity to develop an internet-based resource that offers (i) an educational resource—the AddictHelp Guide (available https://addicthelp.ro/cursuri/), and also (ii) a self-evaluation tool, consisting of the AUDIT questionnaire—to assess the individual alcohol misuse, and the Medical Condition Regard Scale (MCRS)—to evaluate the stigma towards alcoholics.

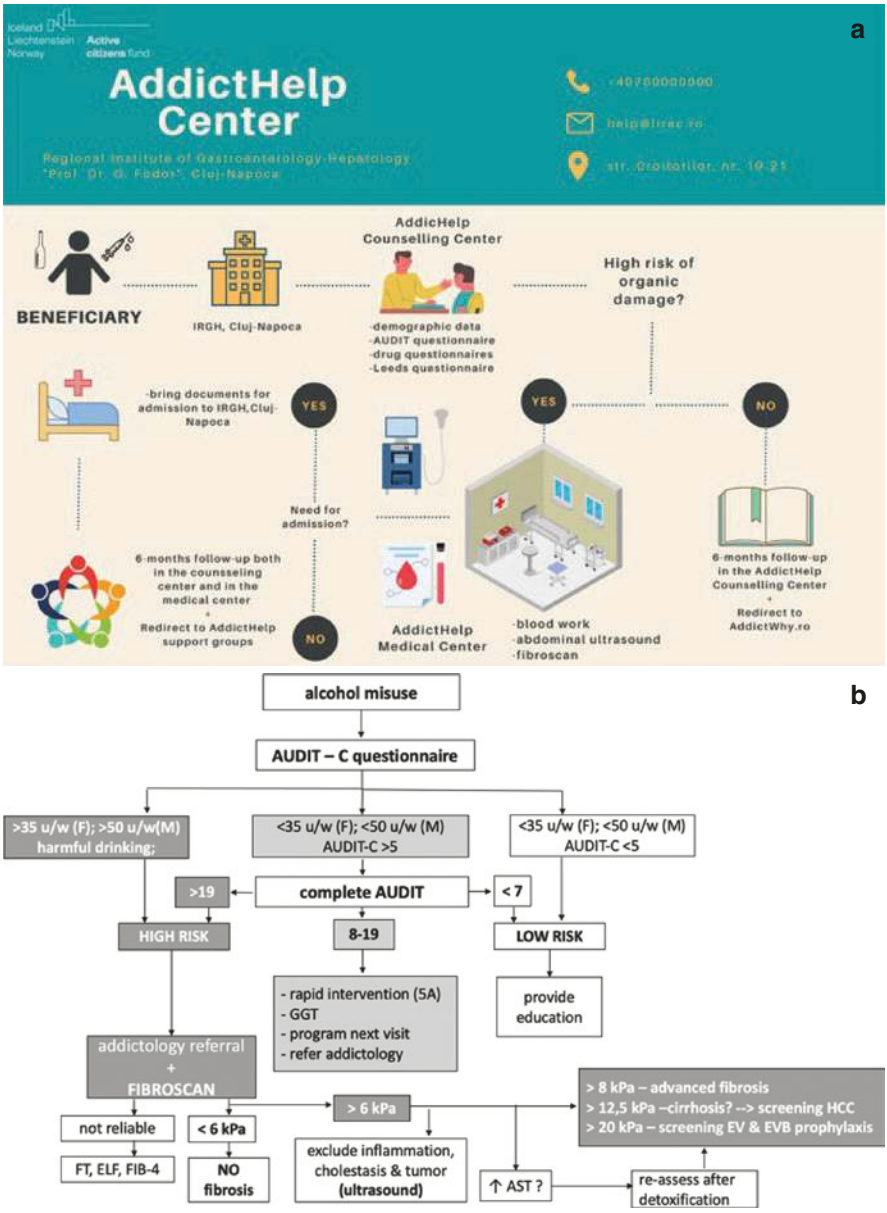

Fig. 77.2 The functional diagram of the AddictHelp outpatient clinic. (**a**) General view of the patient's flow. (**b**) Detailed examination protocol combining addiction assessment and clinical (focused on liver disease) evaluation

Conclusion

The alcohol consumption in Romania is high, because of socioeconomic, demographic, and cultural factors. This is also generating an important number of liver diseases and deaths. On the other hand, the management of alcohol misuse and ALD is precarious, mainly because there is a lack of understanding towards addiction issues and an increased level of stigma. The hepatologist is often in the middle of this landscape, as many of the alcoholics seek medical care when liver disease is overt, or advanced, or having a complication. Consequently, the liver specialist is subjected to a considerable burden, which he/she copes with by creating a multidisciplinary team, by getting involved in research and development projects, or by education and training. All these solutions are viable, as they are aimed to improve the care (medical and psychological) addressed to patients with alcohol addiction. Apart from these local initiatives, comprehensive Governmental measures are needed, especially targeted towards community care, education, and prevention.

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Appendix A: Figures/Biochemical Schemes and a Clinical Case

Sebastian Mueller

Abstract This appendix of the book "Alcohol and Alcohol-Related Diseases" is aimed to provide the reader with easy accessible general data on ethanol and alcohol-related diseases. Data encompass basic knowledge about physics, chemistry and biochemistry of ethanol, general data on addiction, epidemiology and natural course of alcohol-related diseases. A major body consists of important biochemical pathways that are relevant for ethanol metabolism. In addition, important histology scores are listed for the diagnosis of alcohol-related liver disease (ALD), alcoholic hepatitis, general prognosis scores, diagnostic work-ups using liver elastography and mortality data. This appendix also provides various images from light microscopy and electron microscopy of liver samples from ALD patients. Finally, a clinical cases is presented with questions/answers to actively recapitulate and apply the knowledge obtained from the book.

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General Information on Alcohol

See Figs. A.1, A.2, and A.3.

Ethanol conentration				Energy	Comments/ Effects in biological systems
volume %	g/L	M	mM	kJ/L	
Potential beverage ethanol concentrations					
100%	800	17.4	17391.3	23200	
90%	720	15.7	15652.2	20880	
80%	640	13.9	13913.0	18560	
70%	560	12.2	12173.9	16240	
60%	480	10.4	10434.8	13920	
50%	400	8.7	8695.7	11600	
40%	320	7.0	6956.5	9280	
30%	240	5.2	5217.4	6960	
20%	160	3.5	3478.3	4640	
15%	120	2.6	2608.7	3480	
10%	80	1.7	1739.1	2320	Cell lysis
5%	40	0.9	869.6	1160	K_M of ADH5
3%	24	0.5	521.7	696	
2%	16	0.3	347.8	464	LD50 in rats
1%	8	0.2	173.9	232	K_M range of ALDH1 and 2
Potential blood ethanol concentrations					
5‰	4	8.7E-02	87.0	116	Lethal in humans
4‰	3.2	7.0E-02	69.6	92.8	
3‰	2.4	5.2E-02	52.2	69.6	K_M values for some ADH2,5,7
2‰	1.6	3.5E-02	34.8	46.4	
1‰	0.8	1.7E-02	17.4	23.2	
0.5‰	0.4	8.7E-03	8.7	11.6	

Fig. A.1 Ethanol concentrations (in volume%, g/L, M and mM) corresponding energies and important biological thresholds

Properties	Ethanol	Acetaldehyde	Acetic acid (conjugate base: acetate)
Chemical formula	C_2H_6O	C_2H_4O	CH_3COOH
Molar mass in g·mol^{-1}	46.069	44.053	60.052
Appearance	Colourless liquid	Colourless gas or liquid	Colourless liquid
Odor	Methanol-like	Ethereal	Heavily vinegar-like
Density in g/cm^3 (at 20 °C)	0.789	0.784	1.049 (liquid) 1.27 (solid)
Melting point in °C	−114.14	−123.37	16.5
Boiling point in °C	78.2	20.2	118.5
Solubility in water	Miscible	Miscible	Miscible
Solubility in fat	Miscible Amphiphile	Miscible	Miscible
log P	−0.18	-0.34	-0.28
Vapor pressure mmHg 20°C	44.6	740	11.6
Acidity (pKa) water	15.9	13.6	4.7
Viscosity mPa·s (at 20 °C)	1.2	0.21	1.22
Heat capacity C J/(mol K)	111.4	89.0	123.1
LD50 (median dose) g/kg (rat, oral)	0.73	1.9	3.31

Fig. A.2 Chemical and physical properties of ethanol, acetaldehyde, and acetic acid

Beverage	Ethanol content				Energy content		
	Vol%	g/100 mL	Vol% (calc)	g/L	kJ/L	kcal/L	Liter to reach 10 kJ/day*
Alcohol-free beer	<0.5	0.4	0.2	2	57.4	13.9	174.2
Low-strength beer	2	1.6	2.2	22	631.4	152.5	15.8
Standard beer (e.g. lager)	3–4	2.4–3	4	40	1148.0	277.3	8.7
Pilsner	4–5.7	3.1–4.5	5	50	1435.0	346.6	7.0
Bock beer	7–8	5.5–6.4	7	70	2009.0	485.3	5.0
Fruit wine	8–14	6–11.5	10	100	2870.0	693.2	3.5
White wine, red wine, sparkling wine	8–15	6.3–12	14	140	4018.0	970.5	2.5
Liqueur	25–45	2.0–3.5	30	300	8610.0	2079.7	1.2
Spirits (brandy. cognac)	30–40	2.3–3.2	30	300	8610.0	2079.7	1.2
Whiskey. gin	35–45	2.7–3.6	35	350	10045.0	2426.3	1.0
Fruit brandy, slivovitz, vodka	40–50	3.1–4.0	40	400	11480.0	2772.9	0.9
Spirit of Melissa	60–70	4.7–5.5	60	600	17220.0	4159.4	0.6
Rum	40–70	3.1–5.5	60	600	17220.0	4159.4	0.6
Vodka	40	4.0	40	400	11480	2772.9	0.9

Fig. A.3 Alcoholic beverages and corresponding ethanol and energy content. *Liter of alcoholic beverage required to cover the daily caloric need of a standard person estimated with 2500 kcal/day or 10,000 kJ/day. Note that caloric needs strongly depends on age, gender, weight, and physical activity. Also note that only ethanol is considered but not other energy carriers

Epidemiology of Alcohol Consumption

See Figs. A.4, A.5, and A.6.

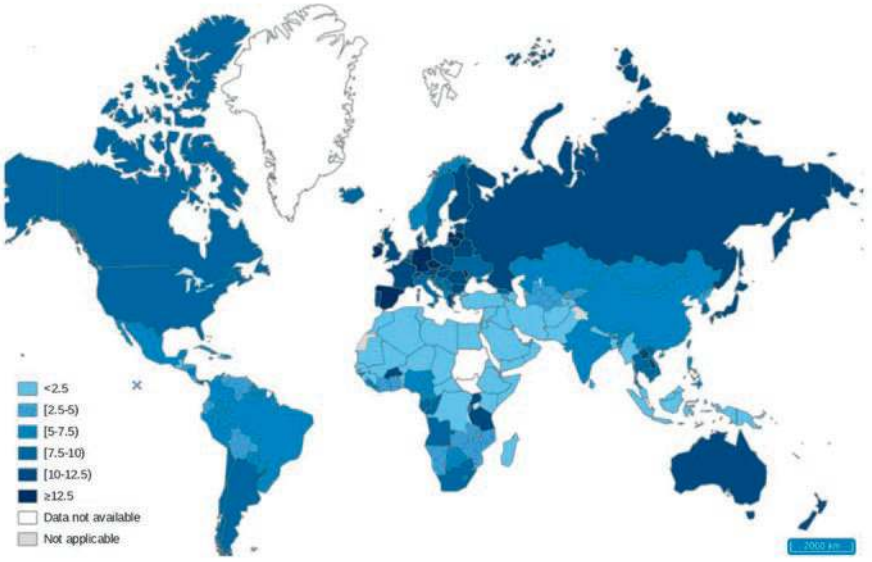


Fig. A.4 Adult per capita consumption of alcohol in litres ethanol for 2019. (Source: World Health Organization, World Health Statistics 2022, see also chapter on Epidemiology by J. Rehm/Part I)

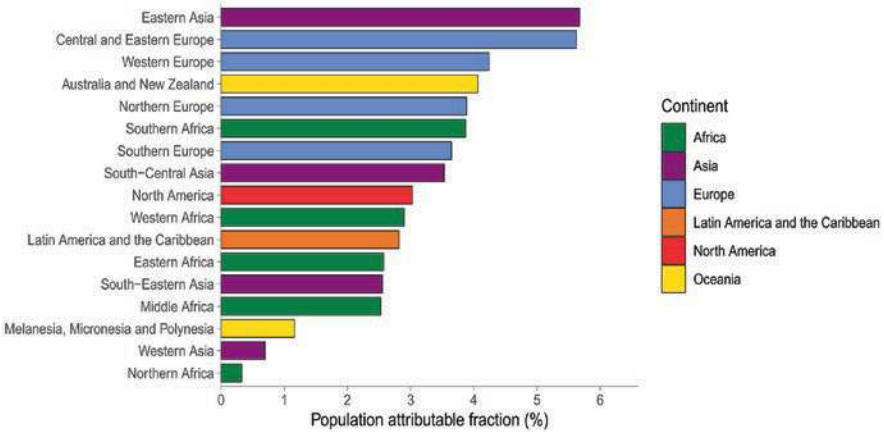


Fig. A.5 Proportion of cancer cases in 2020 attributable to alcohol consumption, by world region. (Chart created using results from *Rumgay H et al. Lancet Oncol. 2021;22(8):1071–80*. See also chapter on Alcohol and Cancer: The Epidemiological Evidence by P. Ferrari)

Alcohol-related disease	Number of death	Percentage
Cirrhosis of the liver	588,100	19.8%
Road injury	370,800	12.5%
Tuberculosis	236,300	8.0%
Haemorrhagic stroke	287,000	9.7%
Ischaemic heart disease	250,800	8.5%
Self-harm	147,000	5.0%
Alcohol use disorders	145,600	4.9%
Liver cancer	101,400	3.4%
Lower respiratory infections	95,200	3.2%
Colon and rectum cancers	92,600	3.1%
Interpersonal violence	86,800	2.9%
Oesophagus cancer	82,900	2.8%
All alcohol-attributable deaths	2,967,800	100.0%

Fig. A.6 Number of alcohol-attributable deaths by single cause of death categories with globally more than 80,000 alcohol-attributable deaths in 2016. (See also chapter on Epidemiology of Alcohol and Opioids by J. Rehm. Compilation based on Shield K et al. The Lancet Public Health. 2020;5(1):e51–e61)

Natural Course of Alcohol-Related Liver Disease (ALD)

See Figs. A.7 and A.8.

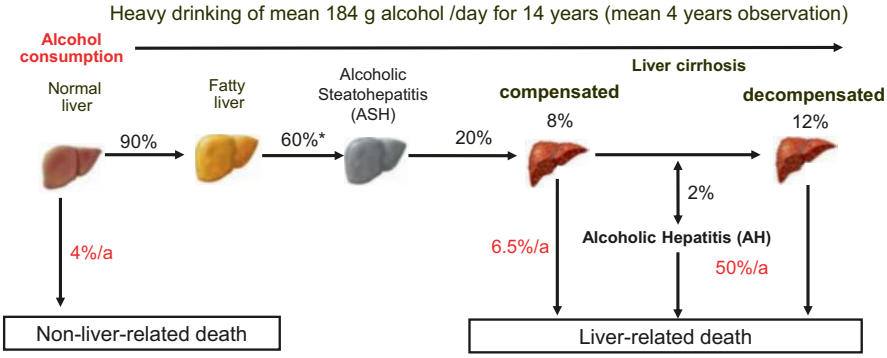


Fig. A.7 Progression of liver disease from the Heidelberg cohort of heavy drinkers ($n = 1078$). Percentage number are related to 100% of initial drinking cohort. See also Tables B.1–B.9 and Chap. 7 on Mortality in Part I. Signs of inflammation are seen in 78% in the liver biopsy cohort, and in 42–62% using biochemical markers in necrosis/inflammation/damage

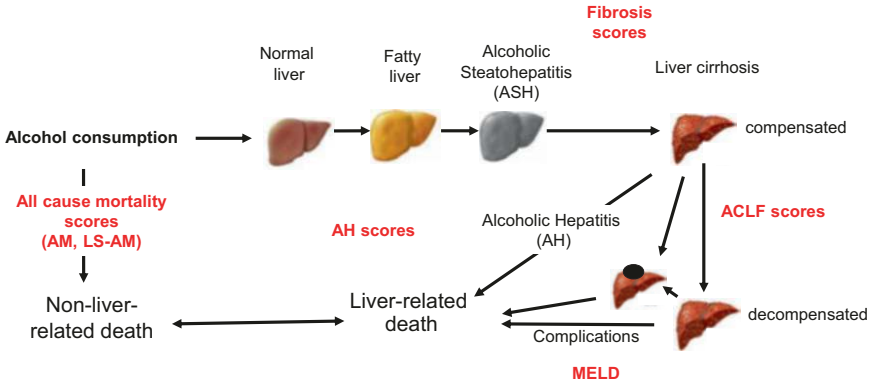


Fig. A.8 Natural course of alcohol consumption, endpoints and various scores. See also following figures for more information about scores. *ACLF* acute on chronic liver failure, *AH* alcoholic hepatitis, *AM* alcohol mortality score, *LS-AM* liver stiffness alcohol mortality score. See also Mortality Chap. 7

Scores for ALD, ACLF and AH

See Figs. A.9, A.10, A.11, A.12, A.13, A.14, A.15, A.16, A.17, A.18, A.19, A.20, A.21.

	APRI	ELF	Fibrometer	MELD	FIB4	Forns index	ABIC	AM-LS	CHILD	GASH	Lille	Hepascore	AM	Sum
Aim of score	fibrosis	fibrosis	fibrosis	mortality fibrosis	fibrosis	fibrosis	AH		mortality fibrosis	AH	AH	fibrosis		
Sum	2	3	3	3	4	4	4	4	5	5	6	6	6	
Age					1	1	1	1		1	1	1	1	8
Bili				1			1	1	1	1	1	1	1	8
INR				1			1		1	1	1			5
Platelets	1				1	1							1	4
Crea				1			1				1			3
AST	1				1									2
GGT						1						1		2
Hyaluronic acid		1										1		2
Albumin									1		1			2
α2-Macroglobulin			1									1		2
Cholesterol						1							1	2
Alkaline phosphatase								1					1	2
Erythrocytes													1	1
ALT					1									1
TIMP1		1												1
PIIINP		1												1
Ascites									1					1
Encephalopythy									1					1
Haptoglobin			1											1
Apolipoprotein A1			1											1
Urea										1				1
Leukocytes										1				1
Bili decrease											1			1
Gender												1		1
Liver stiffness								1						1

Fig. A.9 Different scoring systems related to alcohol-related liver diseases and their parameters. Different scores aim at predicting different endpoints such as fibrosis stage, mortality by fibrosis, mortality by alcoholic hepatitis, or all-cause mortality. Parameters are sorted in descending order depending on their used frequency in the various scores

a **Diagnosis of alcoholic hepatitis (AH)**

- Clinical diagnosis!
- Previous heavy alcohol consumption
- Elevated AST/GOT und ALT/GPT (< 400 U/L)
- AST/ALT>1.5
- Bilirubin > 3 mg/dL
- (Elevated INR/PT)
- (Elevated leukocytes (Neutrophiles))
- In case of comorbidities consider liver biopsy

b

Definite AH	Probable AH	Possible AH
clinically diagnosed		
biopsy-proven	without confounding factors	with potential confounding factors

Fig. A.10 Clinical definition and diagnosis of alcoholic hepatitis (AH). (**a**) Clinical and laboratory criteria for AH. (**b**) Criteria for diagnosis of AH in clinical trials used by the NIAAA. (See also Chaps. 64, 65, 66 and 67. See also Louvet A et al. *Liver Int*. 2022;42(6):1330–1343; Singal et al., Journal of Hepatology 2018, vol. 69: 534–543 and Crabb DW et al. Gastroenterology. 2016;150:785–790)

Author	Parameter/ Survival	Maddrey et al (1), 1978	Dunn et al (2), 2005	Forrest et al (3), 2005	Louvet et al (4), 2007	Dominguez et al (5), 2008
Score		MDF	MELD	GAHS	Lille Model	ABIC
n		55	73	241/195	295/115	103/80
AUROC	30 d		0.83	0.81		
	90 d		0.86	0.78		0.82
	6 mo				0.89	
Parameters	INR	+	+	+	+	+
	Bilirubin	+	+	+	+	+
	Creatinine		+		+	+
	Age			+	+	+
	Leukocytes			+		
	Urea			+		
	Albumin				+	
	Bili decrease				+	

Fig. A.11 Clinical scores for the prognosis of alcoholic hepatitis. AUROCs for survival prediction, parameters and patient numbers are given (modified from Mueller S et al. World J Gastroenterol. 2014;20(40):14626–14641. References: (1) Maddrey WC et al. Gastroenterology. 1978;75(2):193–9. (2) Dunn W et al. Hepatology. 2005;41(2):353–8. (3) Forrest EH et al. Gut. 2005;54(8):1174–9. (4) Louvet A et al. Hepatology. 2007;45(6):1348–54. (5) Dominguez M et al., Rincon D, Abraldes JG, Miquel R, Colmenero J, Bellot P, et al. A new scoring system for prognostic stratification of patients with alcoholic hepatitis. Am J Gastroenterol. 2008;103(11):2747–56). *ABIC* age, serum bilirubin, INR, and serum creatinine score, *GAHS* Glasgow alcoholic hepatitis score, *MDF* Maddrey's discrimination function, *MELD* model for end-stage liver disease

a Original of MDF formula with PT by Maddrey et al. Gastroenterology 75,193:1978

MDF = 4.6 x (PT Patient – PT control) + Bilirubin (mg/dL)

b Example of MDF: DF > 32 = 1-month mortality 50 %

c Estimated MDF using INR (not PT):

MDF = ca. 46 x (INR -1) + Bilirubin (mg/dL)

d Example with estimated INR formula:

INR=1.5 und Bili=24 mg/dL → MDF= 47 → 1-month mortality >50 %

Fig. A.12 Maddrey's discrimination function (MDF) to calculate short-term mortality in patients with alcoholic hepatitis (**a**). (**b**) Severe disease ≥32 with 1-month mortality of 50%. (**c**) and (**d**) Provide an estimated alternative (**c**) and an example (**d**) using the widely available INR, since standardized PT values are not available in many laboratories

Author	Forrest et al (1), 2005	Mookerjee et al (2), 2011	Altamirano et al (3), 2013
Score	GAHS	ASH Grade	ASH score
N	241/195 (137)	68	121+205
AUROC	0.65-0.71	0.8	0.74
Day Survival	28 and 84 d	?	90 d
Histological Parameters	Steatohepatitis	Fibrosis Cholestasis Cholangiolitis Steatosis Ballooning Steatosis	Fibrosis Bilirubinostasis Megamitochondria PMN infiltration
INR		1.7	1.6
Bilirubin	9	13.3	9.7
Age		51	49
Albumin		25	
Urea		13.5	
Leuko		13.3	
MELD		12.5	18
DF (Maddrey)	41	38	

Fig. A.13 Histological scores for alcoholic hepatitis. Note that AUROCs are comparable to clinical scores (modified from Mueller S et al. World J Gastroenterol. 2014;20(40):14626–14641 References: (1) Forrest EH et al. Gut. 2005;54(8):1174–9. (2) Mookerjee RP et al. Current opinion in critical care. 2011;17(2):170–6. (3) Affo S et al. GUT. 2013;62(3):452–60). *ASH* alcoholic steatohepatitis, *GAHS* Glasgow alcoholic hepatitis score

Chevallier (SSS) Score		**Points**
A. Central lobular vein (CLV)		
1. Normal vein or absence of vein		0
2. Moderately thickened (stellate aspect of vein wall)		1
3. Markedly thickened (annular aspect of vein wall with numerous fibrous intercellular extensions)		2
B. Portal tract (PT)	***C. Perisinusoidal space (PS)***	
1. Normal	1. Normal	0
2. Enlarged without septa	2. Localized fibrosis	1
3. Enlarged with septa	3. Diffuse fibrosis	2
4. Cirrhosis		3
D. Number of septa (NS)	***E. Width of Septa (WS)***	
1. Absence	1. Thin and or incomplete	0
2. $\leq$ 6 septa /10 mm	2. Thick and loose connective matrix	1
3. > 6 septa / 10 mm	3. Very thick and dense connective matrix	2
4. Nodular organization	4. >2/3 of biopsy area	3
Score expression : SSS = CLV + PS + PT + 2(WS x NS)		

Fig. A.14 Chevallier semiquantitative scoring system (SSS) for liver fibrosis. (See also Chevallier M et al. Hepatology. 1994;20(2):349–355)

NASH Clinical Research Network Scoring System Definitions			
A. Steatosis grade	***B. Location (predominant)***	***C. Lobular inflammation***	**Points**
1. < 5%	1. Zone 3	1. No foci	0
2. 5%-33%	2. Zone 1	2. < 2 foci per 200 x field	1
3. > 33%-66%	3. Azonal	3. 2-4 foci per 200 x field	2
4. > 66%	4. Panacinar	4. > 4 foci per 200 x field	3
D. Micro vesicular steatosis	**E. Micro-granulomas**	**F. Large lipogranuloma**	
1. Absent	1. Absent	1. Absent	0
2. Present	2. Present	2. Present	1
G. Fibrosis stages			
1. None			0
2. Perisinusoidal or periportal			1
3. Mild, zone 3, perisinusoidal			1A
4. Moderate, zone 3, perisinusoidal			1B
5. Portal/periportal			1C
6. Perisinusoidal and portal/periportal			2
7. Bridging fibrosis			3
8. Cirrhosis			4
H. Portal inflammation	***I. Pigmented macrophages***	***J. Mega mitochondria***	
1. None to minimal	1. None to rare	1. None to rare	0
2. More than minimal	2. Many	2. Many	1
K. Ballooning	***L. Glycogenated nuclei***	***M. Acidophil bodies***	
1. None	1. None to rare	1. None to rare	0
2. Few ballooned cells	2. Many	2. Many	1
3. Many cells / Prominent ballooning			2

Fig. A.15 Histological NASH clinical research network score. *NASH* – non-alcoholic steatohepatitis. This score is also frequently used for ALD studies. (Kleiner DE et al. Hepatology. 2005;41(6):1313–21)

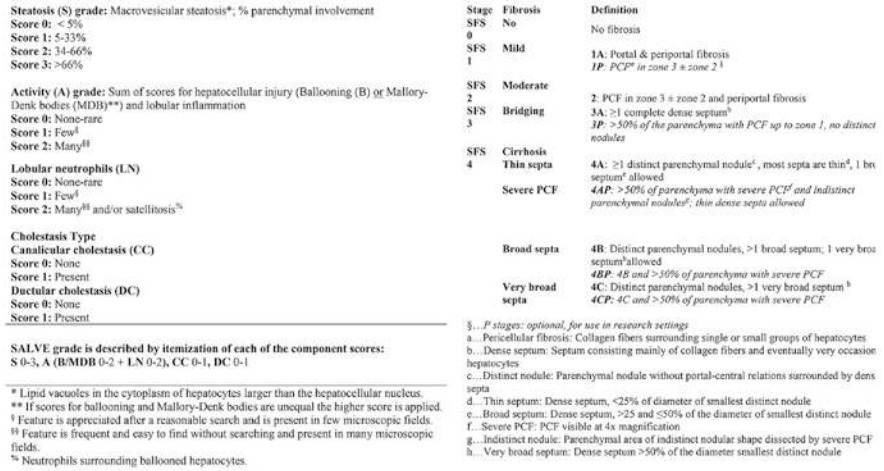


Fig. A.16 Histological SALVE grading for ALD. (See also Lackner C et al. J Hepatol 2021; 75:810–819)

Organ/System	Subscore = 1	Subscore = 2	Subscore = 3
Liver	Bilirubin < 6 mg/dl	Bilirubin ≥6 mg/dl and < 12 mg/dl	Bilirubin ≥12 mg/dl
Kidney	Creatinine < 1.5 mg/dl Creatinine 1.5–1.9 mg/dl	Creatinine ≥2 mg/dl and < 3.5 mg/dl	Creatinine ≥3.5 mg/dl or renal replacement
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3–4
Coagulation	INR <2.0	INR 2.0–2.4	INR ≥2.5
Circulatory	MAP ≥70 mm Hg	MAP < 70 mm Hg	Vasopressor requirement
Respiratory	PaO2/FIO2 >300 SpO2/FIO2 >357	PaO2/FIO2 201–300 SpO2/FIO2 215–357	PaO2/FIO2 ≤200

Fig. A.17 Clinical criteria for the diagnosis of acute-on-chronic liver failure (ACLF) (adapted from Jalan, R. et al. J. Hep. 2014. 61(5): p. 1038–1047). *FiO_2* fraction of inspired oxygen, *INR* international normalized ratio, *MAP* mean arterial pressure, *PaO_2* partial pressure of arterial oxygen, *SpO_2* oxygen saturation as measured by pulse oximetry

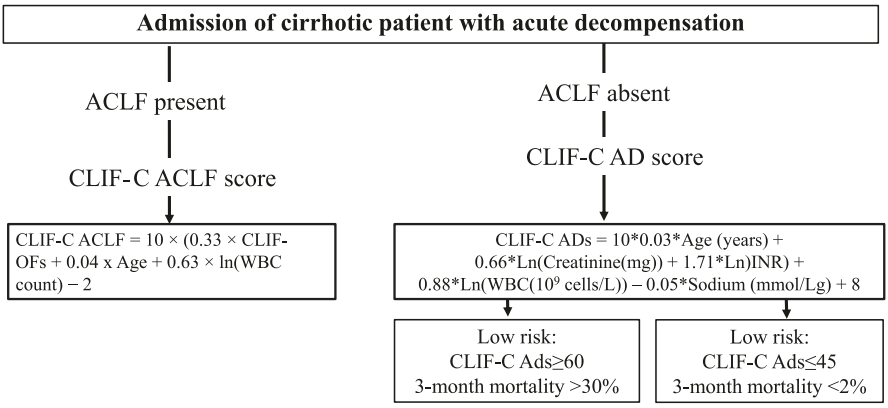


Fig. A.18 Proposed algorithm for the use of EASL-CLIF Consortium predictive scores for ACLF and non-ACLF patients (adapted from Jalan R et al. *J Hepatol*. 2015;62(4):831–840. See also chapter by R. Jalan). *AD* acute ecompensation, *ACLF* acute-on-chronic liver failure, *EASL-CLIF* The European Association for the Study of the Liver–Chronic Liver Failure

Patient group	Prevalence over 1287 patients (%)	28-day Mortality (%)	Assigned grade
Absence of organ failure	68.3	4.4	Absence of ACLF
Single non-Kidney organ failure without KD or BD	9.9	6.3	
Single KF	6.7	18.6	ACLF-1
Single non-Kidney organ failure with KD or BD	4.2	27.8	ACLF-1
Two organ failures	7.5	32	ACLF-2
Three organ failures	1.9	68	ACLF-3
Four to six organ failures	1.4	88.9	ACLF-3

Fig. A.19 Mortality of ACLF patients according to its severity (adapted from Moreau, R., et al., Gastroenterology, 2013. 144 (7): p. 1426–1437. e9). *ACLF* acute-on-chronic liver failure, *BD* brain dysfunction, *KD* kidney dysfunction, *KF* kidney failure. See also chapter on ACLF by R. Jalan

	Score	0	1	2	3	4
Central nervous system	**Glasgow coma scale**	15	13–1	10–12	6–9	< 6
Cardiovascular system	**Mean arterial pressure OR administration of vasopressors required**	MAP ≥ 70 mmHg	MAP < 70 mmHg	dopamine≤ 5 µg/kg/min or dobutamine (any dose)	dopamine > 5 µg/kg/min OR epinephrine ≤ 0.1 µg/kg/min OR norepinephrine ≤0.1µg/kg/min	dopamine > 15 µg/kg/min OR epinephrine > 0.1 µg/kg/min OR norepinephrine >0.1µg/kg/min
Respiratory system	**PaO2/FiO2 [mmHg (kPa)]**	≥ 400 (53.3)	< 400 (53.3)	< 300 (40)	< 200 (26.7) and mechanically ventilated including CPAP	< 100 (13.3) and mechanically ventilated including CPAP
Coagulation	**Platelets×103/µl**	≥ 150	< 150	< 100	< 50	< 20
Liver	**Bilirubin (mg/dl) [µmol/L]**	< 1.2 [< 20]	1.2–1.9 [20-32]	2.0–5.9 [33-101]	6.0–11.9 [102-204]	> 12.0 [> 204]
Renal function	**Creatinine (mg/dl) [µmol/L] (or urine output)**	< 1.2 [< 110]	1.2–1.9 [110-170]	2.0–3.4 [171-299]	3.5–4.9 [300-440] (or < 500 ml/day)	> 5.0 [> 440] (or < 200 ml/day)

Fig. A.20 The sequential organ failure assessment score (SOFA score) for liver failure. It is typically used to track a person's status during the stay in an intensive care unit (ICU) to determine the extent of a person's organ function or rate of failure (adapted from Weng C-H, et al. (2012) PLoS ONE 7(12): e51743). *CPAP* continuous positive airway pressure, *MAP* mean arterial pressure

New definition of Acute Kidney Injury – AKIN score
serum creatinine increase of ≥ 0.3 mg/dL in 48 h or > 50% of reference serum creatinine or sustainable in the last 7 days

AKIN stages	Criteria	3-months survival
AKI 1A	Increase of serum creatinine >0.3 mg/dL (26.4 µmol/L) or more than 1.5 times of reference serum creatinine	70%
AKI 1A	Serum creatinine < 1.5 mg/dL (132.6 µmol/L)	82%
AKI 1B	Serum creatinine ≥ 1.5 mg/dL (132.6 µmol/L)	55%
AKI2	Increase more than 2-3 times than reference serum creatinine	42%
AKI3	Increase >3 times from reference serum creatinine or Serum creatinine ≥ 4 mg/dL (353.6 µmol/L) or Renal replacement therapy	31%

Fig. A.21 Diagnosis criteria for acute kidney injury (AKI) also applied to hepato-renal syndrome in ALD patients. Substages of AKI are based on serum creatinine. AKIN is also used assess hepatorenal syndrome (HRS). See also chapter by A. Trifan on "Hepato-Renal Syndrome in Patients with Alcohol-Related Liver Disease". *AKIN* acute kidney injury network

Alcohol Dependence and Brain Research

See Figs. A.22, A.23, A.24, A.25, A.26, A.27, A.28, and A.29.

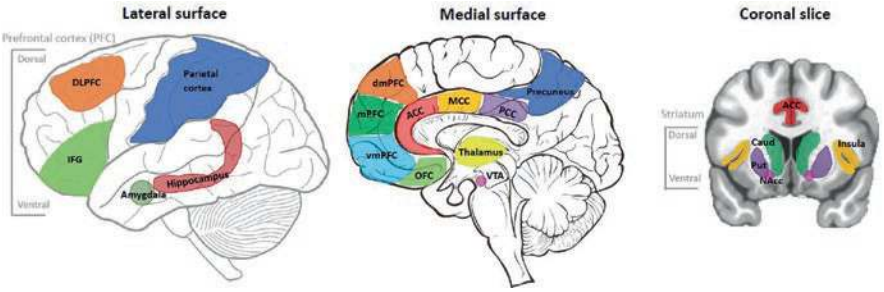


Fig. A.22 Brain areas referenced in this book. *DLPFC* dorsolateral PFC, *IFG* inferior frontal gyrus, *dmPFC* dorsomedial PFC, *mPFC* medial PFC, *vmPFC* ventromedial PFC, *OFC* orbitofrontal cortex, *ACC* anterior cingulate cortex, *MCC* middle cingulate cortex, *PCC* posterior cingulate cortex. Note that the amygdala and hippocampus are located in the medial temporal lobe but are depicted on the lateral surface image. On the coronal slice, the striatum comprises the caudate (Caud), putamen (Put), and nucleus accumbens (NAcc). For details see Chap. 26 by J. Schacht entitled "Structural and Functional Imaging of Alcohol's Effects on the Brain"

Questions	0	1	2	3	4
1. How often do you have a drink containing alcohol?	Never	Monthly or less	2 to 4 times a month	2 to 3 times a week	4 or more times a week
2. How many drinks containing alcohol do you have on a typical day when you are drinking?	1 or 2	3 or 4	5 or 6	7 to 9	10 or more
3. How often do you have 5 or more drinks on one occasion?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
4. How often during the last year you found that you were not able to stop drinking once you had started?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
5. How often during the last year have you failed to do what was normally expected of you because of drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
7. how often during the last year have you had a feeling of guilt or remorse after drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
8. How often during the last year have you been unable to remember what happened the night before because of your drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
9. Have you or someone else been injured because of your drinking?	No		Yes, but not in the last year		Yes, during the last year
10. Has a relative, friend, doctor or other health care worker been concerned about your drinking or suggested you cut down?	No		Yes, but not in the last year		Yes, during the last year

Fig. A.23 AUDIT questionnaire to identify patients with alcohol use disorder. To score the AUDIT questionnaire, sum the scores for each of the 10 questions. A total ≥ 8 for men up to age 60, or ≥ 4 for women, adolescents, or men over age 60 is considered a positive screening test. *AUDIT* alcohol use disorders identification test

Medication (daily dose) *	Site of action	FDA Approval	Efficacy	Common side effects
Medications approved for treatment of AUD				
Disulfiram (125-500 mg)	Inhibition of alcohol metabolism (acetaldehyde dehydrogenase enzyme)	AUD	Tachycardia, headache, nausea, and vomiting after alcohol consumption. Medium effect in open-label trials, no effect in blind designs.	disulfiram-alcohol interaction
Acamprosate calcium (666 mg TID)	Putative antagonism of NMDA receptors	AUD	Improves probability of maintaining abstinence. Reduces the number of drinking days.	diarrhea
Naltrexone (50 mg)	Antagonism of μ-, κ-, and δ-opioid receptors	AUD Opioid use disorder (OUD)	Reduces craving, number of heavy drinking days, risk of return to drinking, risk of binge drinking, and improves quality of life.	nausea, headache, dizziness, and sleep problems
Naltrexone LAI (380 mg / 4 week) i.m.	Antagonism of μ-, κ-, and δ-opioid receptors	AUD OUD	Reduces number of drinking days and number of heavy drinking days. Compared to oral naltrexone, time to relapse is longer.	injection site related inflammation, infection
Nalmefene (18 mg)	Antagonism of μ- and δ-opioid receptors; partial agonism of κ-opioid receptor	Reversal of opioid overdose. Approved for AUD in Europe	Reduces number of heavy drinking days and total alcohol consumption.	nausea, vomiting, fatigue, insomnia, and dizziness

Fig. A.24 Approved, effective and promising new medication for AUD. See also Chap. 16. *Oral administration, unless otherwise specified

Medication (daily dose) *	Site of action	FDA Approval	Efficacy		Common side effects
Additional effective medications based on clinical trials					
Topiramate (100–300 mg)	Antagonizes glutamatergic AMPA and kainate receptors, and facilitates GABA activity; blocks L-type calcium channels, reduces voltage-dependent sodium channel activity, inhibits carbonic anhydrase	Adjunct for partial and tonic-clonic seizures and migraines. Topiramate ER in combination with phentermine for weight management in obesity	Reduces craving, percentage of heavy drinking days and drinks per drinking days.	paresthesia, taste abnormalities, anorexia, difficulties with concentration	
Gabapentin (600–1800 mg)	Blocks voltage-gated calcium channels and enhances permeability of voltage-gated potassium channels. Indirectly modulates GABA receptors.	Adjunct for partial seizure and postherpetic neuralgia	Reduces the number of drinks and percentage of heavy drinking days, especially in patients with history of alcohol withdrawal symptoms.	dizziness, somnolence	
Gabapentin enacarbil extended-release (GE-XR) (1200 mg)	Blocks voltage-gated calcium channels and enhances permeability of voltage-gated potassium channels. Indirectly modulates GABA receptors.	Restless leg syndrome and postherpetic neuralgia	Reduces the number of drinks and drinks per heavy drinking days in patient subgroup with higher baseline heavy-drinking days per week, impulsivity, and lower levels of anxiety, depression, and mood disturbances.	fatigue, dizziness, somnolence	
Baclofen (30-80 mg)	Agonism of $GABA_B$ receptors	Muscle spasticity	Improves probability of maintaining abstinence; decreases craving. More effective in patients with alcohol-associated liver disease.	sedation, drowsiness, dizziness, headache	
Varenicline (2 mg)	Partial agonism of α4β2 nicotinic acetylcholine receptors	Smoking cessation	Decreases craving in smokers and non-smokers. Reduces mean number of drinks in smokers. Better response in less-severe AUD, depressive symptoms, males.	nausea, abnormal dreams, constipation	
Ondansetron (8 μg/kg)	Antagonism of 5-HT3 serotonin receptor	Nausea and vomiting	Reduces amount and frequency of drinking in early-onset AUD.	headache, constipation	
Prazosin / Doxazosin (16 mg)	Antagonism of α1 receptors	Hypertension and benign prostatic hyperplasia	Reduces number of heavy drinking days and drinks per week. Better response in patients with higher blood pressure and positive AUD family history.	drowsiness, dizziness, fatigue	

Fig. A.25 Approved, effective and promising new medication for AUD (continued). See also Chap. 16. *Oral administration, unless otherwise specified

Medication (daily dose) *	Site of action	FDA Approval	Efficacy	Common side effects
Promising new medications				
Zonisamide (500 mg)	Blocks voltage-sensitive sodium channels and T-type calcium channels, enhances synaptic inhibition by facilitating GABAergic, dopaminergic, and serotonergic transmission. Indirectly attenuates glutamatergic transmission.	Adjunct treatment for epilepsy	Reduces number of heavy drinking days, drinks per week, and alcohol craving	Drowsiness, difficulties with concentration, decreased appetite, abdominal pain
Aripiprazole (15-30 mg)	Partial agonism of D2 dopaminergic and 5HT1A serotonergic receptors, antagonism of 5HT2A receptors	Schizophrenia, bipolar disorder	Reduces number of drinks per drinking days. Better response in patients with higher impulsivity. Genetic polymorphisms of dopamine transporter (DAT), dopamine receptor and catechol-O-methyltransferase (COMT) genes moderate the effects.	insomnia, anxiety, restlessness, and disturbances in attention
Mifepristone (600 mg)	Antagonism of glucocorticoid receptors	Pregnancy termination	Reduces alcohol craving.	nausea, vomiting, diarrhea
N-acetylcysteine (2400 mg)	Antioxidant, affecting glutamatergic neurotransmission	Antioxidant (over the counter)	Decreases alcohol drinking in cannabis dependent patients.	dry mouth, nausea, vomiting, diarrhea
Oxytocin (24 IU) intranasal	Agonism of oxytocin receptors	Labor induction, termination of pregnancy (not the intranasal form)	Reduces alcohol cue-elicited brain activity, craving and symptoms of alcohol withdrawal.	runny nose, nasal discomfort, headache
Promising new compounds				
ABT-436 (800 mg)	Antagonism of V1b vasopressin receptors	Not approved	Increases percentage of abstinent days.	diarrhea, anxiety, and nausea
LY 2940094 (40 mg)	Antagonism of nociceptin (NOP) receptors	Not approved	Decreases percentage of heavy drinking days and increases percentage of abstinent days.	insomnia, anxiety, vomiting
LY2196044 (250 mg)	Antagonism of μ, κ, and δ-opioid receptors	Not approved	Increases percentage of abstinent days per month and decreases mean number of drinks per day. Better response in patients with dopamine receptor type 4 (DRD4) gene polymorphism.	diarrhea, nausea, abdominal pain, and flatulence
Samidorphan (1-10 mg)	Antagonism of μ-opioid receptor, mixed agonism-antagonism of κ, and δ-opioid receptors	Not approved	Reduces craving and average daily alcohol consumption.	nausea, vomiting, somnolence, dry mouth, dizziness
Ibudilast (100 mg)	Inhibits phosphodiesterase-4 (PDE4) and -10 (PDE10), and macrophage migration inhibitory factor (MMIF). Reduces neuroinflammation and supports neurotrophin expression	Not approved	Reduces craving, percentage of heavy drinking days and alcohol-cue elicited activation in brain. Better response in patients with depressive symptoms.	headache, nausea
Ifenprodil (60 mg)	Inhibits G protein-activated inwardly rectifying potassium (GIRK) channel	Not approved	Reduces frequency of alcohol drinking and presence of heavy drinking.	nausea, dry mouth, dizziness, headache

Fig. A.26 Approved, effective and promising new medication for AUD (continued). See also Chap. 16. *Oral administration, unless otherwise specified

Country	Standard drink (g)	Guidelines			
		Women		Men	
		(g)/day	(g)/week	(g)/day	(g)/week
Australia [15]	10	20	–	20	–
Austria [10]	20	16	112	24	168
Bosnia and Herzegovina [27]	10	10	–	20	–
Bulgaria[a]	13	–	–	–	–
Canada [16]	~13.6	~27	~136	~40.7	~204
Chile [23]	14	42	98	56	196
China[a]	10	–	–	50	100
Croatia[a]	10	10	–	20	–
Denmark [17]	12	–	84	–	168
Estonia[a]	10	20	–	40	–
Fiji [18]	10	20	100	30	150
France [19]	10	20	140	30	210
Germany [24]	12	12	–	24	–
Grenada [6]	~14	~14	–	~14	–
Iceland [7]	8	–	112	–	168
India [28,45]	10	10	–	20	–
Ireland [31]	10	–	110	–	170
Italy [43]	12	20	–	36	–
Japan [8]	–	20	–	40	–
Latvia [44]	12	12–16	96	24	156
Luxembourg [14]	12.8	12.8	–	25.6	–
Malta[a]	8–10	–	112–140	–	168–210
Malaysia[a]	10	–	–	–	–
Mexico [9]	14	14	126	28	168
New Zealand [20,25]	10	20	100	30	150
Philippines [42]	14	14	–	28	–
Poland [21]	10	20	140	40	280
Portugal[a]	10–12	10–24	–	10–24	–
Singapore [37]	10	10	–	20	–
Slovenia[a]	10	10	–	20	–
South Africa [11]	~11–12	24	–	24	–
Spain [39]	10	–	110	–	170
Sweden [30]	10	10	–	20	–
Switzerland [13]	10–12	20–24	–	30–36	–
United Kingdom [22]	8	16–24	–	24–32	–
United States [12,46]	14	42	98	56	196
Vietnam [38]	10	20	140	40	280

Fig. A.27 Governmental standard drink definitions and low-risk consumption guidelines in grams of pure ethanol. (Kalinowski & Humphreys, Addiction 2016 111(7):1293–8. More details are provided in Chap. 12

Alcohol Dependence	Harmful Pattern of Use of Alcohol
A disorder of regulation of substance use arising from repeated or continuous use of substance. The characteristic feature is a strong internal drive to use substance, which is manifested by impaired ability to control use, increasing priority given to use over other activities and persistence of use despite harm or negative consequences The diagnosis requires two or more of the following three central features to be evident over a period of at least 12 months, but the diagnosis may be made if alcohol use is continuous for at least 1 month. 1. Impaired control over alcohol use – in terms of the onset, level, circumstances or termination of use, often but not necessarily accompanied by a subjective sensation of urge or craving to use alcohol. 2. Alcohol use becomes an increasing priority in life – takes precedence over other interests or enjoyments, daily activities, responsibilities, or health or personal care. Alcohol use takes an increasingly central role in the person's life and relegates other areas of life to the periphery; continues despite the occurrence of problems. 3. Physiological features (indicative of neuroadaptation to the alcohol) as manifested By; (i) tolerance, (ii) withdrawal symptoms following cessation or reduction in use of that substance or (iii) repeated use of the substance (or pharmacologically similar substance) to prevent or alleviate withdrawal symptoms. Withdrawal symptoms must be characteristic for the withdrawal syndrome for alcohol and must not simply reflect a hangover effect.	A pattern of alcohol use that has caused damage to a per-son's physical or mental health or has resulted in behaviour leading to harm to the health of others The pattern of alcohol use is evident over a period of at least 12 months if substance use is episodic or at least 1 month if use is continuous. Harm to health of the individual occurs due to one or more of the following: 1. Behaviour related to intoxication 2. Direct or secondary toxic effects on body organs and systems 3. A harmful route of administration. Harm to health of others includes any form of physical harm, including trauma, or mental disorder that is directly attributable to behaviour related to alcohol intoxication on the part of the person to whom the diagnosis of Harmful Pattern of Use of Alcohol applies.

Fig. A.28 Alcohol dependence and harmful pattern of use of alcohol as described by ICD-11. For more details see also Chap. 12

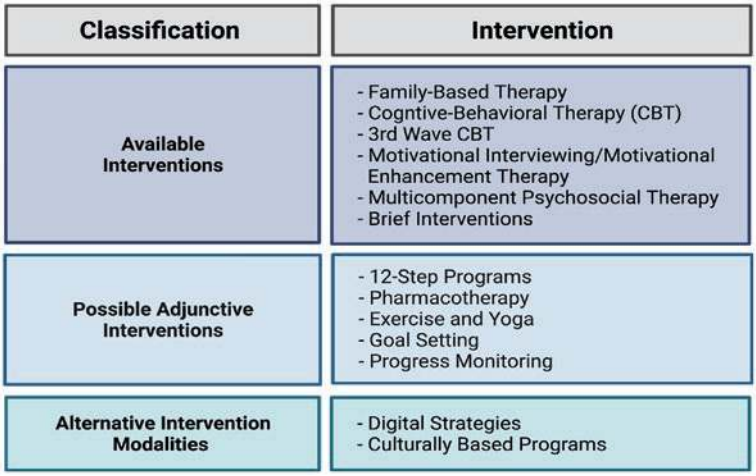


Fig. A.29 Overview of adolescent AUD treatments covered in this chapter. Interventions range from available, possible adjunctive, and alternative modalities. For more details see Chap. 18

Macro- and Microscopic Images of Alcohol-related Liver Disease (ALD)

See Figs. A.30, A.31, A.32, A.33, A.34, A.35, and A.36.

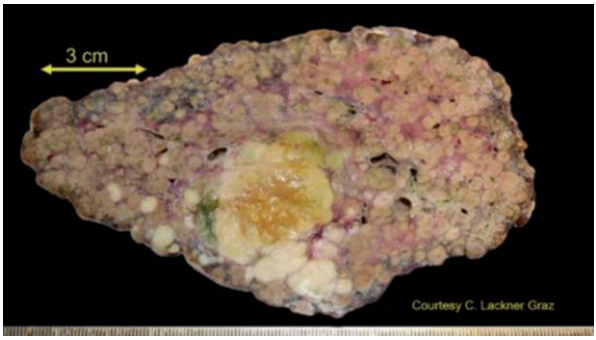


Fig. A.30 Liver explant from a male heavy drinker (53 years) with alcohol-related liver cirrhosis. (Courtesy C. Lackner Graz)

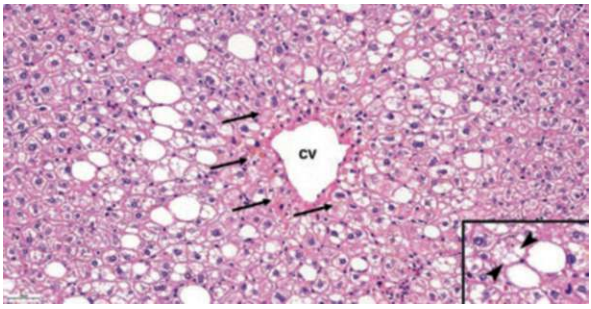


Fig. A.31 Alcohol-related macrovesicular steatosis: hepatocytes in intermediate and central portions of the hepatic lobules contain large lipid vesicles taking up most of the hepatocellular cytoplasm and dislocating the nucleus to the periphery of the cell (inset). The large macrovesicles may result from confluence of several smaller ones (marked by arrow head in inset). Also note that bilirubin-stain seems to appear in the pericentral hepatocytes in cytoplasm (arrows). It needs to be resolved whether this is related to the hemolytic anemia seen in heavy drinkers that also drives mortality. See also Chaps. 38, 57 and 58 on Histology, Iron, and Bone Marrow Toxicity. Image: H&E; *CV* central vein. (Courtesy of C. Lackner, Graz. See also chapter by C. Lackner on "Histology and ALD")

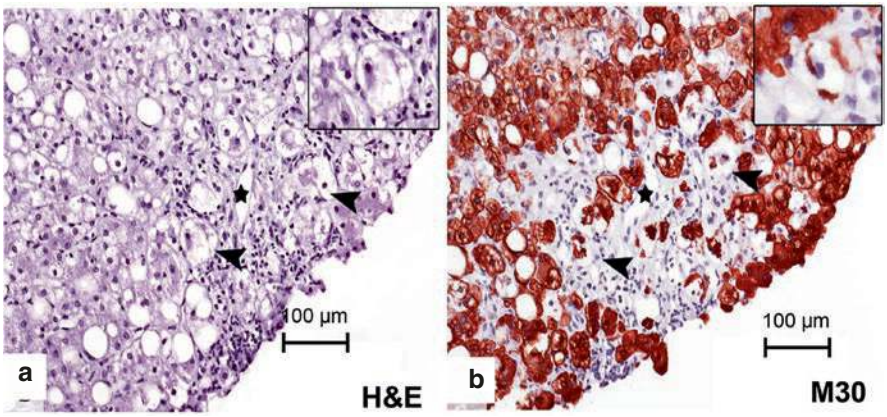


Fig. A.32 Morphological and immunohistochemical features of steatohepatitis and M30 expression in a case of alcoholic steatohepatitis (**a**, **b**). Note that serum M30 levels correlate excellently with the hepatic expression of M30 in liver stains. M30 allows to diagnose liver apoptosis in the serum and is more sensitive than transaminases. (**a**) Centrilobular area (asterisks marks central vein) with ballooned hepatocytes (enlargement shown in inset) and hepatocytes with fatty change are surrounded by inflammatory infiltrates (H&E, 200×). (**b**) Consecutive section of centrilobular area shown in (**a**) (asterisks marks central vein). The ballooned hepatocytes show loss of cytoplasmic staining while Mallory Denk bodies are decorated with antibodies against keratin (K)8 and 18 (enlargement shown in inset). Regular sized hepatocytes and hepatocytes with fatty change retain a K8/18 (M30) positive cytoplasm (200×). Serum level of M30 in this patient was 4753.0 U/L. (Modified from Mueller S et al. *Hepatology*. 2017;66(1):96–107)

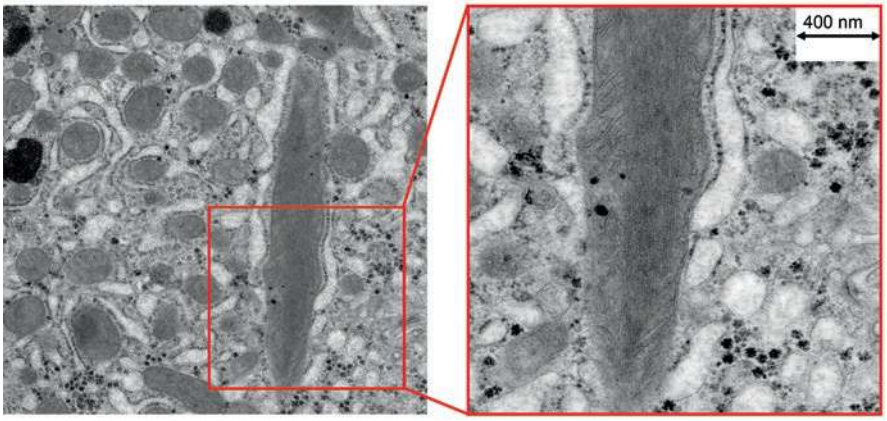


Fig. A.33 Electron micrograph of a liver specimen from a patients with alcoholic hepatitis (female, 53 years). Mitochondrial inclusions show para-crystalline ("parking lot") inclusions that are classically seen with electron transport chain defects. In light microscopy, this mitochondrion would resemble a megamitochondrium

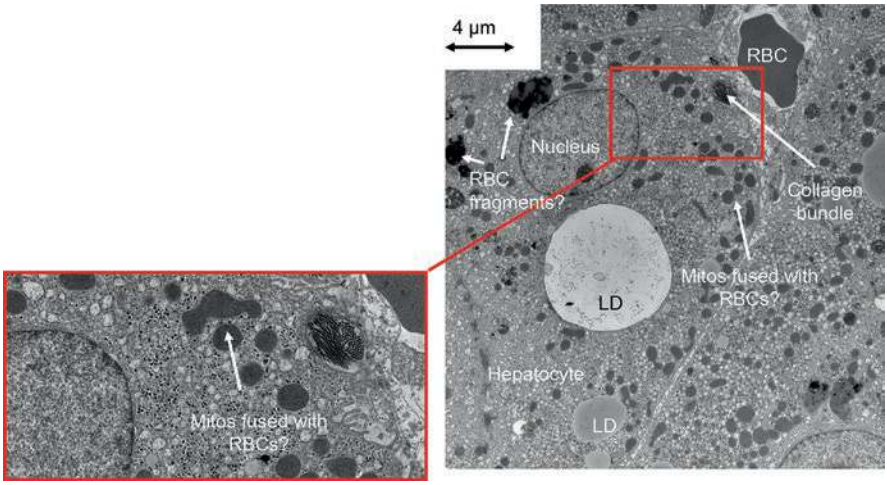


Fig. A.34 Electron micrograph of a liver specimen from a patients with alcoholic hepatitis. In the lower magnification on the right, an RBC is seen in the liver sinus (top right). It remains to be studied why the small mitochondria structures resemble RBC texture. It could well be that RBCs are efferocytosed and fuse with mitochondria. More studies are needed (see also Electron micrographs Figs. A.33, A.35, and A.36) and Chap. 57 on Iron and ALD

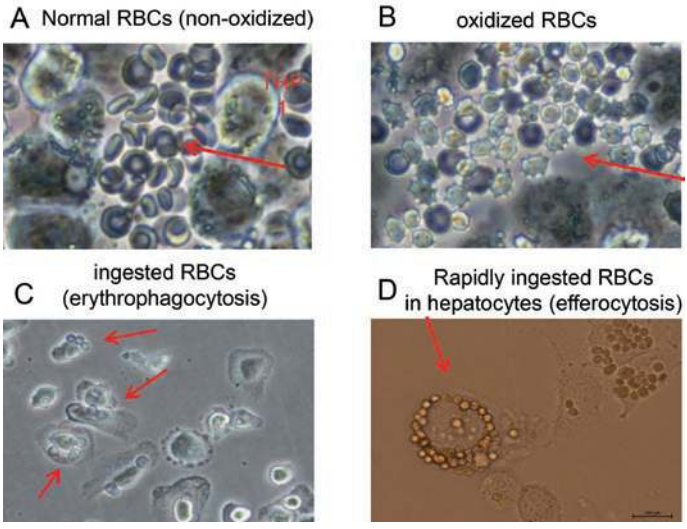


Fig. A.35 Phagocytosis and efferocytosis of red blood cells (RBC) by human macrophages (THP1) and hepatocytes (Huh7). Mortality, clinical and biochemical data suggest that hepatocytes contribute to RBC turnover. More studies are needed to better understand potential RBC uptake and digestion (see also Electron micrographs Figs. A.33, A.34, and A.36) and Chap. 57 on Iron and ALD. (**a**) Control RBCs (red arrow) and co-cultured human THP1 macrophages. (**b**) Morphological changes (spur cells, red arrow) of RBCs in the presence of copper sulfate-induced oxidative stress after 120 min. (**c**) Erythrophagocytosis of oxidized human erythrocytes (red arrow, oxidized by copper sulfate) by THP-1 cells. (**d**) Efferocytosis of oxidized RBCs by hepatocytes. Huh7 cells were exposed for 60 min to oxidized human RBCs. RBCs are also rapidly ingested by hepatocytes which is demonstrated by aligning around the cell nucleus (see also Zheng, C. et al, 2023, Front. Imm. in press)

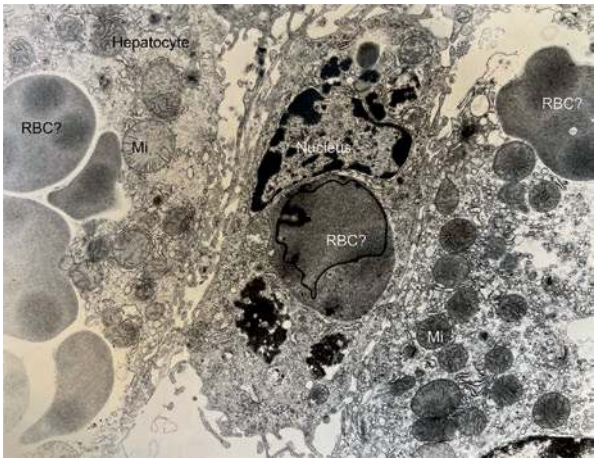


Fig. A.36 Electron micrograph of a rat liver specimen exposed to mild hemolysis using the glucose oxidase (GOX) system (for details see also Rost D et al. *J Hepatol*. 2007;46(3):482–491). Note a phagocytosed RBC in a macrophage in the middle. In remains to be studied whether the RBCs on the left are RBCs and fragments efferrocytosed by a hepatocyte. *GOX* glucose oxidase, *Mi* mitochondria, *RBC* red blood cell

Biochemical Pathways Relevant for Ethanol Metabolism

See Figs. A.37, A.38, A.39, A.40, A.41, A.42, A.43, A.44, A.45, A.46, A.47, A.48, A.49, A.50, A.51, and A.52.

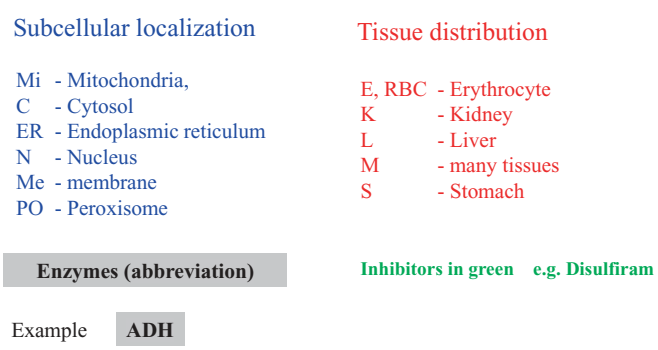


Fig. A.37 Abbreviations and colours used for the biochemical pathways and enzyme reactions (Figs. A.38–A.76)

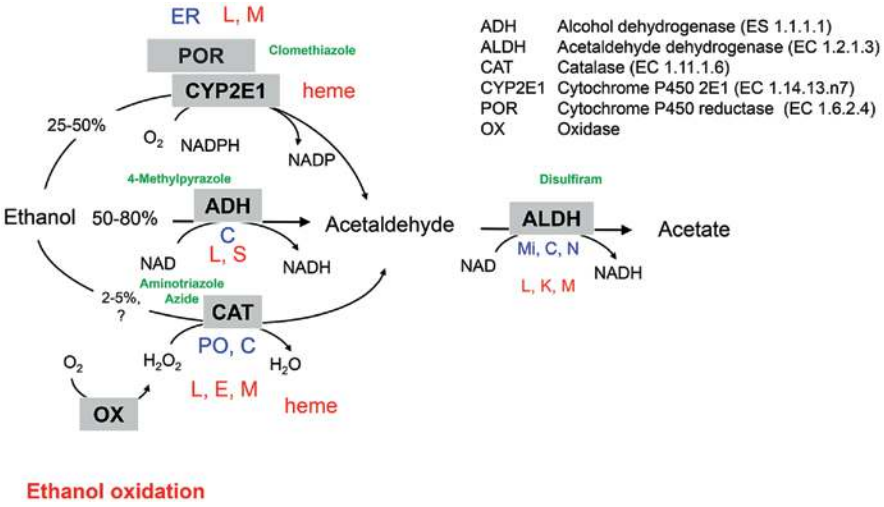


Fig. A.38 Major enzymatic ethanol oxidation pathways. ADH and ALDH convert ethanol to acetic acid by transforming NAD to NADH. This causes an important shift of the redox potential and is responsible for many biochemical consequences including enhanced lipogenesis. Also note that CYPs require NADPH. They directly use oxygen which can lead to ROS formation through uncoupling. The proximity to carbon monoxide releasing HO1 in the endoplasmic reticulum has less well studied but suggests a potential inhibitory interaction between CYPs and HO1. See also Chap. 57. For abbreviations: see previous page

Gene[a]	Class	Alias[a]/Isoforms	Synonyms[b]	Subunit encoded[c]	K_M (mM)	V_{max} $(min)^{-1}$	Subc.[d]	Tissue[e]	Comments
ADH1A	I	aclohol dehydrogenase 1A (class I), alpha polypeptide	ADH1	α-ADH, ADH1	4	30	C	S, L	Metabolizes a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. major role in ethanol catabolism.
ADH1B	I	aclohol dehydrogenase 1B (class I), beta polypeptide	ADH2	β-ADH, ADH2			C	S, L	Single nucleotide polymorphism rs1229984, that changes arginine to histidine at residue 48. The 'typical' variant of this has been referred to as ADH2(1) or ADH2*1 while the 'atypical' has been referred to as, e.g., ADH2(2), ADH2*2, ADH1B*48His. This SNP is associated with the risk for alcohol dependence, alcohol use disorders and alcohol consumption, with the atypical genotype having reduced risk of alcoholism.
		ADH1B*1; ADH1B1[Arg48/Arg370]	AHD2*1	β1-ADH, ADH2*1	0,0013	5,2	C		Metabolizes ethanol at the slowest rate among the 3 isoforms. It is the most common isoform globally except in much of East Asia, and it is the form to which others are compared. (Edenberg 2018)
		ADH1B*2; ADH1B[His48/Arg370]	ADH2*2	β2-ADH, ADH2*2	1,8	190	C		Differs from ADH1B*1 only due to rs1229984. In vitro, ADH1B*2 oxidizes ethanol much faster than ADH1B*1. High frequency in China and Japan. Protective. (Edenberg 2018)
		ADH1B*3; ADH1B[Arg48/Cys370]	ADH2*3	β3-ADH, ADH2*3	61	140	C		Differs from ADH1B*1 only due to rs2066702. The turnover number for ADH1B*3 is more than 60-fold that of ADH1B*1 in vitro. Almost only found in individuals of african origin (Edenberg 2018)
ADH1C	I	Alcohol dehydrogenase 1C (class I), gamma polypeptide	ADH3	γ-ADH, ADH3			C	S, L	Class I alcohol dehydrogenase, consisting of several homo- and heterodimers of alpha, beta, and gamma subunits, exhibit high activity for ethanol oxidation and play a major role in ethanol catabolism. Association of ADH1C with alcohol dependence is less robust than that of ADH1B (Edenberg 2018)
		ADH1C*1; ADH1C[Arg272/Ile350]	ADH1C*1	γ1-ADH, ADH1C*1	0,1	32	C		Encoded at position 350 by rs698.
		ADH1C*2; ADH1C[Gln272/Val350]	ADH1C*2	γ2-ADH, ADH1C*2	0,14	20	C		Encoded at position 272 by rs1693482
ADH4	II	Alcohol dehydrogenase 4 (class II), pi polypeptide	class II ADH, ADH2	π-ADH, ADH4	11	9	C	S, L	Metabolize a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. high activity for oxidation of long-chain aliphatic alcohols and aromatic alcohols and is less sensitive to pyrazole.
ADH5	III	Alcohol dehydrogenase 5 (class III), chi poypoptide	class III ADH ADH 3	χ-ADH, ADH5	>1000	100	C	S, L	Glutathione-dependent formaldehyde dehydrogenase, has virtually no activity for ethanol oxidation, but exhibits high activity for oxidation of long-chain primary alcohols and for oxidation of S-hydroxymethyl-glutathione, important for the elimination of formaldehyde
ADH6[d]	V	Alcohol dehydrogenase 6 (class V)	class V ADH, ADH5	-	-	-	?	L	Metabolizes a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products but may have a distinct physiologic function
ADH7	IV	Alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide	class IV ADH, ADH4	μ-ADH, σ-ADH, ADH7	30	1800	C	S, L	Metabolizes a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. inefficient in ethanol oxidation, but highly active as a retinol dehydrogenase

[a] HUGO Gene Nomenclature Committee.
[b] Position in precursor protein; aa487 in the mature protein
[c] Protein subunits have traditionally been named with Greek symbols, but can also be named based upon the gene encoding them; genes are in italics, proteins in roman font.
[d] Subcellular localization: C - cytosol
[e] tissue localization: L - liver, S - Stomach

Fig. A.39 Alcohol dehydrogenases. (Modified from Edenberg HJ et al. ACER. 2018;42(12):2281–97; Marchitti SA et al. Expert Opinion on Drug Metabolism & Toxicology. 2008;4(6):697–720)

Gene[a]	Chromos. location	Synonyms[b]	K_M (µM)	V_{max} $(min)^{-1}$	preferred substrate	Subc.[c]	Tissue[d]	Comments
ALDH1A1	9q21.12	ALDH1; ALDH-E1; ALDH11; RALDH1; ALDC	180	380	Retinal, Acetaldehyde	C	L, D, K, T, B, Lu, RBC, lens	Belongs to corneal crystallins.
ALDH1A2	15q21.3	RALDH2			Retinal	C	U, T	Catalyzes the synthesis of retinoic acid (RA) from retinaldehyde.
ALDH1A3	15q26.3				Retinal	C	T, U, prostate, bladder, skeletal muscle	
ALDH1B1	9p13.1	ALDH5; ALDHX	55	655	Acetaldehyde	M	L, K	May protect the cornea from UV-light.
ALDH1L1	3q21.3	10-formyltetrahydrofolate dehydrogenase			10-Formyltetra-hydrofolate	C	L, K	Catalyzes the conversion of 10-formyltetrahydrofolate to tetrahydrofolate, formate oxidation in vivo. Deficiencies result in methanol poisoning.
ALDH1L2	12q23.3	10-formyltetrahydrofolate dehydrogenase			10-Formyltetra-hydrofolate	M	Pancreas, salivary gland	Converts 10-formyltetrahydrofolate to tetrahydrofolate.
ALDH2	12q24.12	ALDHI; ALDH-E2; ALDM	-	-	Acetaldehyde, 4-hydroxy-2-nonenal (a-HNE), Malondialdehyde (MDA)	M	Fat, L	Major encyme in ethanol metabolism.
ALDH2 Isoform		ALDH2*1; ALDH2[Glu504][b]	0,2	280				Most common isoform.
ALDH2 Isoform		ALDH2*2; ALDH2[Lys504][b] variant rs671		in-active				Present in more than 50% of East Asians, makes ALDH2 inactive.
ALDH3A1	17p11.2				Aromatic and aliphatic aldehydes	C, N	Cornea, Esophagus, stomach, skin	Conversion of aldehydes from lipid peroxidation to their corresponding carboxylic acids in mammalian cornea and saliva e.g. 4-Hydroxynonenal (4HNE).
ALDH3A2	17p11.2	Fatty aldehyde dehydrogenase (or Long-chain-aldehyde dehydrogenase)			Fatty aldehydes, Hexaldehyde	Mic, Per, ER	L, Skin, adrenal glands	Associated with Sjögren-Larsson-Syndrome.
ALDH3B1	11q13.2				Octaldehyde, Benzaldehyde and others	C, PM	L, K, Lu, bone marrow	
ALDH3B2	11q13.2				Medium-chain to long-chain aldehyde	LD	Skin, esophagus	

[a] HUGO Gene Nomenclature Committee.
[b] Position in precursor protein; aa487 in the mature protein
[c] Subcellular localization: Mi- mitochondria, C - cytosol/cytoplasm, N - nucleus, ER - endoplasmatic reticulum, Mi - microsome, PO - peroxisome, PM - plasma membrane, LD - lipid droplet, Mem - membrane
[d] tissue localization: L - liver, K - kidney, D - duodenum, T - testis, Lu - lung, RBC - red blood cells, U - uterus, MP - macrophages

Fig. A.40 Acetaldehyde dehydrogenases. Important ALDH2 in bold. (Modified from Edenberg HJ et al. ACER. 2018;42(12):2281–97; Marchitti SA et al. EODMT. 2008;4(6):697–720; Jackson B et al. Human genomics. 2011;5(4):283–303)

Gene[a]	Chromos. location	Synonyms[b]	K_M (μM)	V_{max} $(min)^{-1}$	preferred substrate	Subc.[c]	Tissue[d]	Comments
ALDH4A1	1p36.13	Delta-1-pyrroline-5-carboxylate dehydrogenase			Glutamate γ-semialdehyde	M	L, K	Catalyzes proline degradation pathway, converting pyrroline-5-carboxylate to glutamate.
ALDH5A1	6p22.3	Succinate-semialdehyde dehydrogenase			Succinate semialdehyde	M	L, Brain	Deficiency causes 4-hydroxybutyricaciduria, inborn error in the metabolism of the neurotransmitter γ-aminobutyric acid (GABA)
ALDH6A1	14q24.3	Methylmalonate-semialdehyde dehydrogenase			Malonate semialdehyde	M	L, K, others	Role in the valine and pyrimidine catabolic pathways. Catalyzes the irreversible oxidative decarboxylation of malonate and methylmalonate semialdehydes to acetyl- and propionyl-CoA.
ALDH7A1	5q23.2	antiquitin			α-Aminoadipic semialdehyde	C, M, N	L, K	Involved in lysine catabolism of mitochondrial matrix. Mutations are associated with pyridoxine-dependent epilepsy.
ALDH8A1	6q23.3				Retinal	C	L, K, others	Aldehyde dehydrogenase of the kynurenine pathway, oxidizing 2-aminomuconate semialdehyde to 2-aminomuconic acid.
ALDH9A1	1q24.1	4-trimethylamino-butyraldehyde dehydrogenase			γ-Aminobutyr-aldehyde	C	L, K, Fat, others	High activity for oxidation of gamma-aminobutyraldehyde and other amino aldehydes
ALDH16A1	19q13.33				Unknown	Mem	Spleen, D, MP	
ALDH18A1	10q24.1				Glutamatic γ-semialdehyde	M	D, small intestine	Reduction of glutamate to delta1-pyrroline-5-carboxylate, a critical step in the de novo biosynthesis of proline, ornithine and arginine.

[a] HUGO Gene Nomenclature Committee.
[b] Position in precursor protein; aa487 in the mature protein
[c] Subcellular localization: Mi- mitochondria, C - cytosol/cytoplasm, N - nucleus, ER - endoplasmatic reticulum, Mi - microsome, PO - peroxisome, PM - plasma membrane, LD - lipid droplet, Mem - membrane
[d] tissue localization: L - liver, K - kidney, D - duodenum, T - testis, Lu - lung, RBC - red blood cells, U - uterus, MP - macrophages

Fig. A.41 Acetaldehyde dehydrogenases. (Modified from Edenberg HJ et al. ACER. 2018;42(12):2281–97; Marchitti SA et al. Expert Opinion on Drug Metabolism & Toxicology. 2008;4(6):697–720; Jackson B et al. Human genomics. 2011;5(4):283–303)

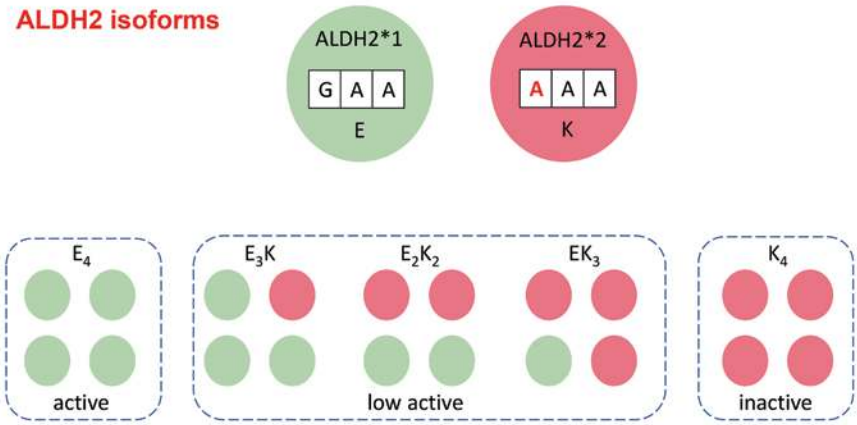


Fig. A.42 Acetaldehyde dehydrogenases 2 (ALDH2) is composed as a tetramer. In East Asians, due to the inactive ALDH2*2 isoform, various enzyme variants are formed with reduced activity. (Adapted from Gao J, et al., Int J Mol Sci 2022;23)

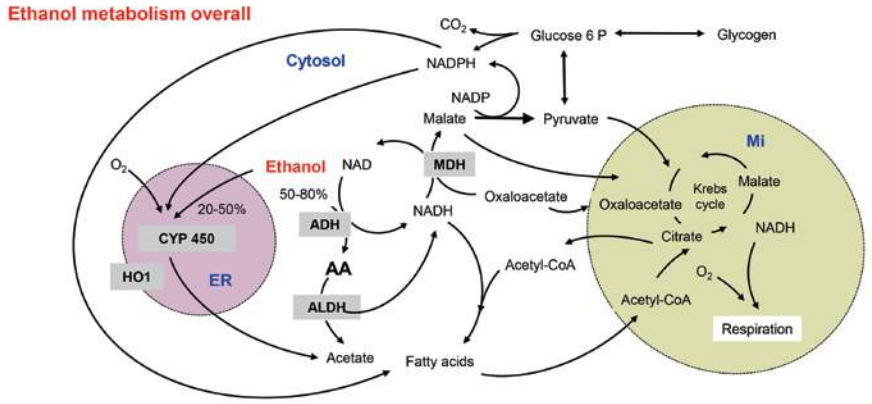


Fig. A.43 Simplified scheme of ethanol metabolism in hepatocytes. Due to abundance of NADH, mitochondria are overloaded with hydrogen donors which contribute to mitochondrial damage. *AA* acedaldehyde, *ADH* alcohol dehydrogenase, *ALDH* acetaldydedehydrogenase, *CYP450* cytochromes p4502E1, *HO1* hemo oxygenase 1, *MDH* malate dehydrogenase

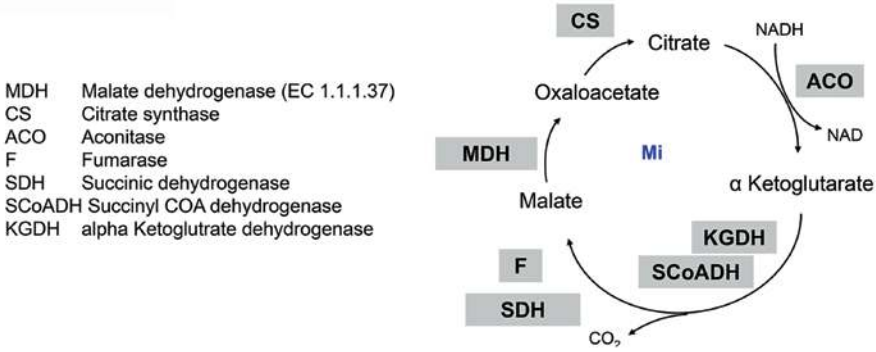


Fig. A.44 The citric acid cycle. (Krebs cycle or tricarboxylic acid cycle) is a series of chemical reactions to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins. The Krebs cycle is used in respirating organisms to generate energy, either anaerobically or aerobically. In ethanol metabolism, all potential substrates such as oxaloacetate, pyruvate and acetyl Co A as well as NADH are abundant which determines the balance of reversible enzyme reactions

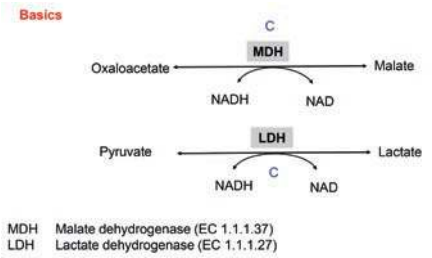


Fig. A.45 Important dehydrogenases. Through the Malate Aspartate Shuttle, cytosolic NADH can be transferred to the mitochondrial compartment

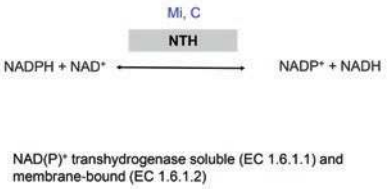


Fig. A.46 NAD(P)$^+$ transhydrogenase

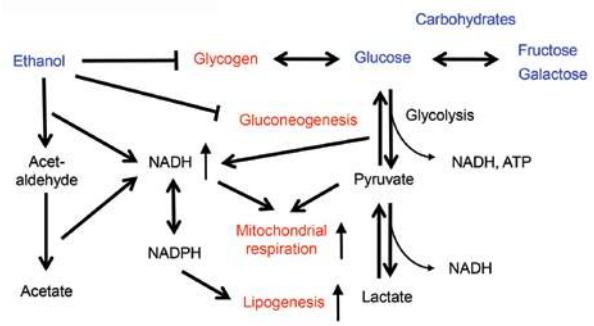
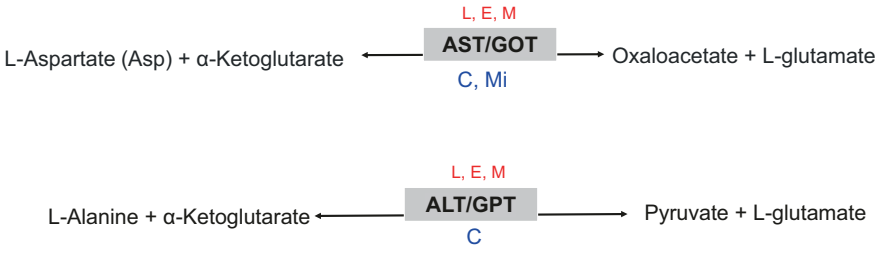
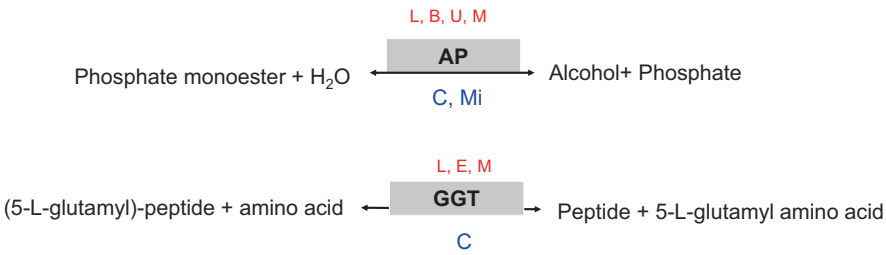


Fig. A.47 Carbohydrate and ethanol metabolism. Some similarities may explain why ethanol, obesity and diabetes mellitus all cause a similar hepatic steatohepatitis whether alcohol-associated or non-alcoholic. Both glycolysis and ethanol oxidation lead to formation of NADH which drives mitochondrial respiration and lipogenesis and may be the joint key feature in causing mitochondrial and organ damage. In difference to carbohydrates, however, ethanol oxidation ultimately blocks gluconeogenesis causing rapid glycogen depletion. Hence, glucose, which is essential for brain, red blood cells or muscles, becomes limiting. Sugars and ethanol also share the fate of having almost no evolutionary evolved negative feedback loops except by elimination through lipogenesis or oxidation. Not by chance, vital energy metabolism is also closely related to dependence (alcohol and food dependence)



AST =GOT	Aspartate aminotransferase/Glutamic oxaloacetic transaminase (EC 2.6.1.1)
ALT=GPT	Alanine aminotransferase/Glutamic-pyruvic transaminase (EC 2.6.1.2)

Fig. A.48 Transaminases



AP	Alkaline phosphatase (EC 3.1.3.1)
GGT	Gamma-glutamyltransferase/ γ-glutamyltransferase, gamma-glutamyl transpeptidase (EC 2.3.2.2)

Fig. A.49 AP and GGT

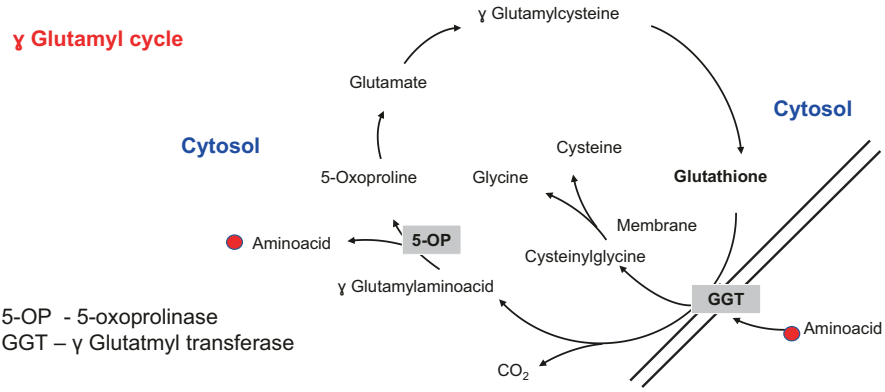


Fig. A.50 γ Glutamyl cycle. The metabolism of glutathione (GSH) is closely connected to Meister's γ-glutamyl cycle, in which a pivotal role is played by membrane GGT. GGT participates in the salvage pathway of extracellular GSH by catalyzing its hydrolysis to amino acid components of cysteine, which is used for intracellular GSH biosynthesis. Consequently, the importance of the γ-glutamyl cycle lies in recovering and delivering cysteine. In most biological systems, glutathione serves as the γ-glutamyl donor. GGT is strongly induced in many heavy drinkers. It is highly expressed in cholangiocytes and supports intracellular GSH synthesis with substrates from bile

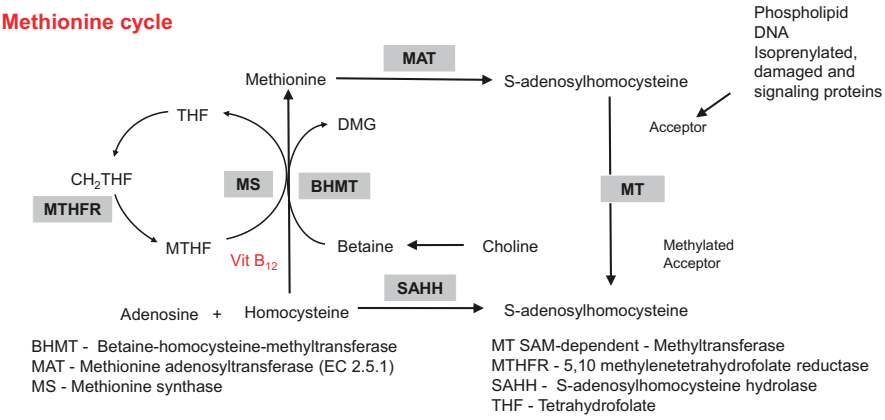


Fig. A.51 Methionine adenosyltransferase (MAT) is a methyl donor for transmethylation. It is also the propylamino donor in polyamine biosynthesis and the rate rate-limiting step of the methionine cycle. As a methyl donor MAT allows DNA methylation. It is also involved in gene transcription, cell proliferation, and production of secondary metabolites. More details are provided in the Chap. 55

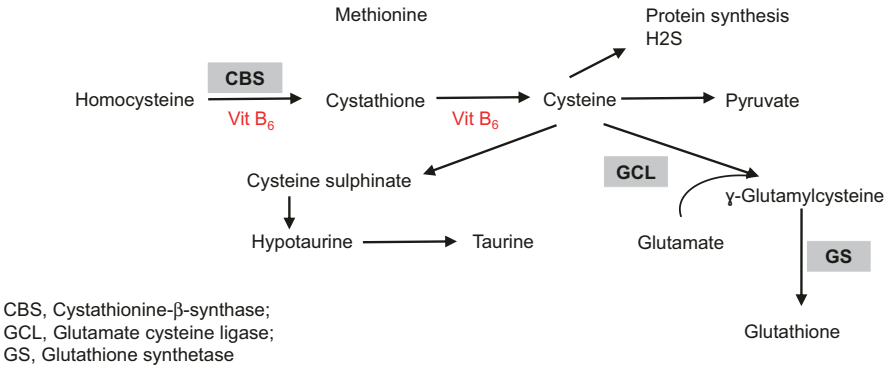


Fig. A.52 The transsulfuration pathway. The transsulfuration pathway is a metabolic pathway involving the interconversion of cysteine and homocysteine through the intermediate cystathionine. All transsulfuration enzymes require vitamin B6 in its active form (pyridoxal phosphate) which is critically limited in heavy drinkers

Pathways of Inflammation and Lipid Signaling

See Figs. A.53, A.54, A.55, A.56, and A.57.

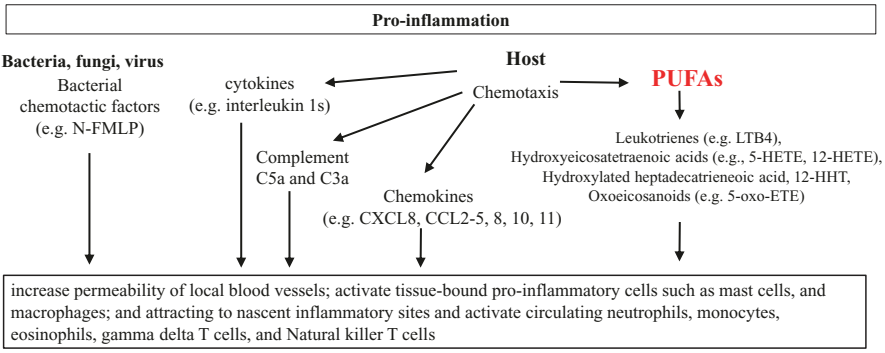


Fig. A.53 Pro-inflammatory molecules/pathways that are involved in ethanol-mediated metabolism. Especially with PUFA involvement, ethanol-metabolism is directly biochemically interacting with these signaling pathways. Important ethanol-metabolizing enzymes such as cytochromes p450 are also involved in SPM synthesis. *PUFA* poly unsaturated fatty acids, *SPM* specialized pro-resolving mediators. (See also Serhan CN et al. BBRC 118 (3): 943–9)

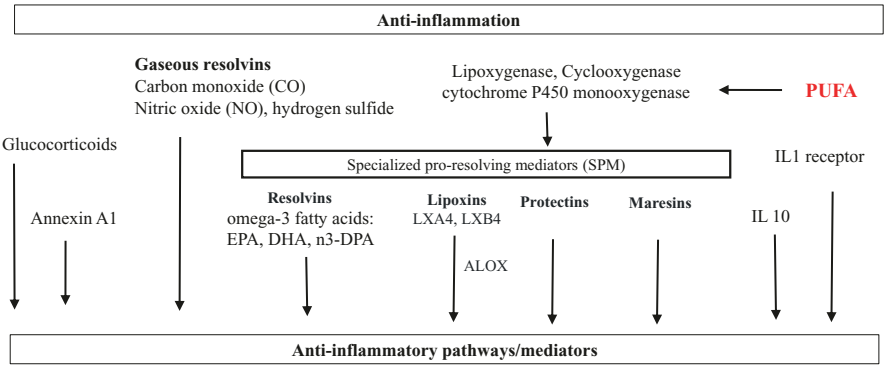


Fig. A.54 Anti-inflammatory molecules/pathways that are involved in ethanol-mediated metabolism. The specialized pro-resolving mediators (SPM) are only recently gaining attention. SPMs can modulate leukocyte migration and function, alter cytokine/chemokine release, modify autophagy, among other immune-related activities. As shown in Tables B.10–B.20 and following, preliminary data indicate that SPMs play an important role (lipidomics). Moreover, important ethanol-metabolizing enzymes such as cytochromes p450 are also involved in SPM synthesis

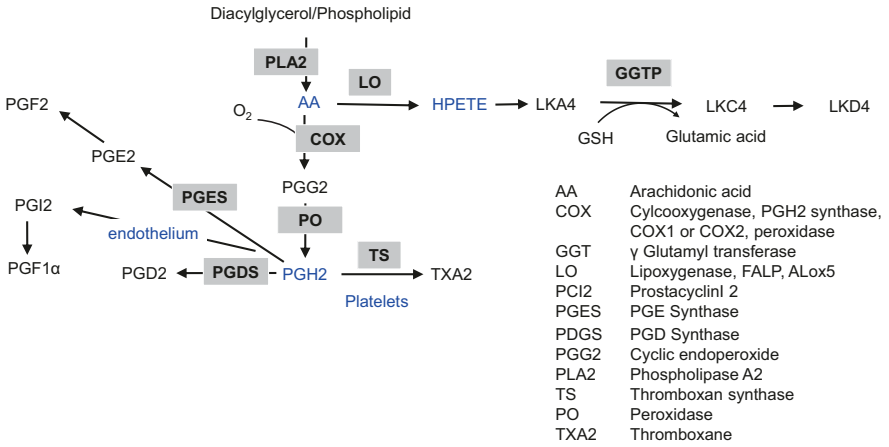


Fig. A.55 Arachidonic acid related synthesis of prostaglandins. Important cellular and systemic mediators are generated that can both have anti- or pro-inflammatory functions. Ethanol strongly interferes with this metabolism since P450 cytochromes are also involved in the synthesis. PGI2, for instance is a prostaglandin member of the eicosanoid family of lipid molecules that inhibits platelet activation and is also an effective vasodilator

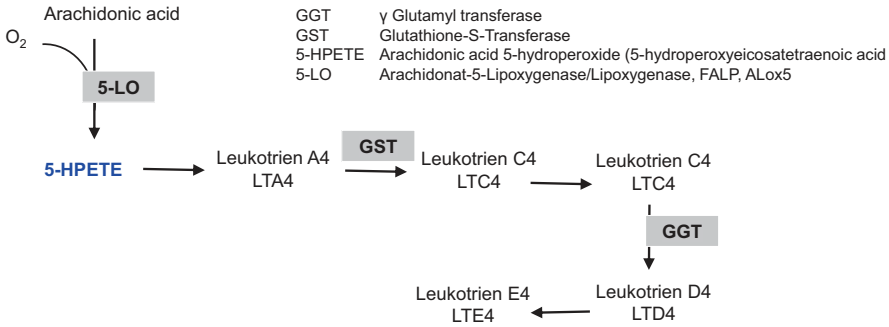


Fig. A.56 Leukotriene metabolism. Leukotrienes are a family of eicosanoid inflammatory mediators produced in leukocytes by the oxidation of arachidonic acid and the essential fatty acid eicosapentaenoic acid (EPA) by the enzyme arachidonate 5-lipoxygenase. Leukotrienes use lipid signaling to convey information to either the cell producing them (autocrine signaling) or neighboring cells (paracrine signaling) in order to regulate immune responses. The production of leukotrienes is usually accompanied by the production of histamine and prostaglandins, which also act as inflammatory mediators. One of their roles (specifically, leukotriene D4) is to trigger contractions in the smooth muscles. Their overproduction is a major cause of inflammation. Ethanol strongly interferes with lipid metabolism but more studies are needed to better understand its molecular interactions

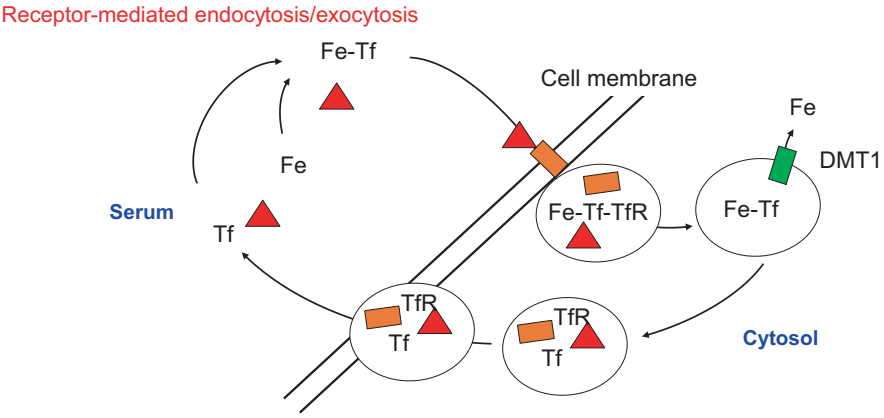


Fig. A.57 Example of receptor-mediated endocytosis: iron uptake by transferrin-bound iron through the Transferrin-transferrin receptor 1 (TfR1). Receptor-mediated endocytosis plays and important role in the liver and ALD and are both not fully understood well. Iron is typically transported in the serum bound to transferrin, bound to transferrin receptor 1 (TfR1) and internalized in vesicles. Iron is released insight through acidification and pumped into the cytosol through metal transporter DMT1. Other examples include uptake of Apo Lipoproteins, Haptoglobin and Albumin, the latter has been shown to transcytose through endothelial cells within 15 s. Alcohol seems to block endocytosis. It remains to be studied whether this is merely due to toxic modifications or is an adaptive response. *DMT1* dimetal transporter 1, *Tf* transferrin

Types of Cell Death and Hepatic Excretion

See Figs. A.58, A.59, and A.60.

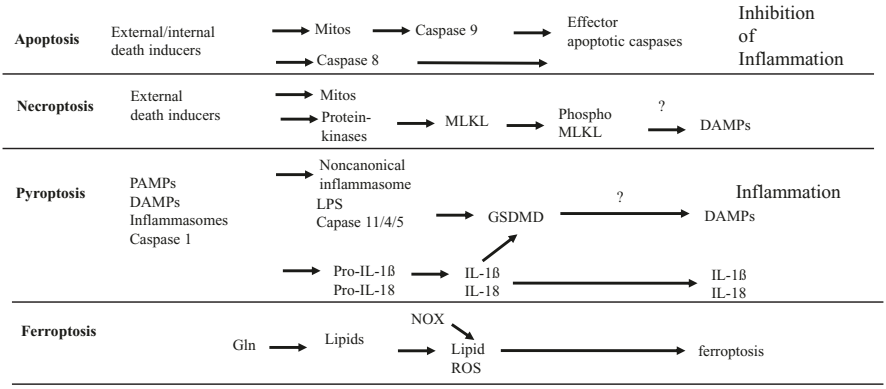


Fig. A.58 Cell death pathways with important pathways are shown for apoptosis, necroptosis, pyroptosis and ferroptosis. Pathways are not yet fully understood. As discussed in Chap. 64 on AH, first preliminary data suggest that ferroptosis is playing an important role in ALD, most likely related to RBC degradation and turnover. *MLKL* mixed lineage kinase domain-like; *GSDMD* Gasdermin-D, for other abbreviations see front matter

Phases of excretion of organic compounds and bile acids

Phase 0 Uptake (NTCP, OATPs, OATs, OCT1)

Phase I (Cytochrome P450 3A1)
hydrophobic potentially toxic secondary bile salts (lithocholate and deoxycholate), and other lipid soluble organic anions like bilirubin are hydroxylated

Phase II (Uridine glucuronyl transferase and sulfatase)
metabolism to form water soluble glucuronide and sulfate conjugates. These important metabolic processes enable these compounds to be more readily

Phase III (MRP2, 3, and 4) export pumps.
Unconjugated bile salts are processed in peroxisomes where they are conjugated with taurine or glycine through the action of the enzyme, bile acid acyl co-A transferase. See reference (160) for details of the role of peroxisomes in bile salt metabolism. For a detailed account of the enzymes, regulation, and genetics of bile acid synthesis, see reference (473).

Fig. A.59 The hepatic clearance of bile salts and other organic solutes is determined by four phases. Expression of these key genes is regulated by nuclear receptors such as the retinoid X receptor (RXR), fetal transcription factor (FTF), and hepatocyte nuclear factor 1 (HNF-1). Enhanced turnover of red blood cells could be an important physiological stimulus, in contrast to xenobiotics, for these phases, and drastically be enhanced during ethanol metabolism. See also Chaps. 49 and 57

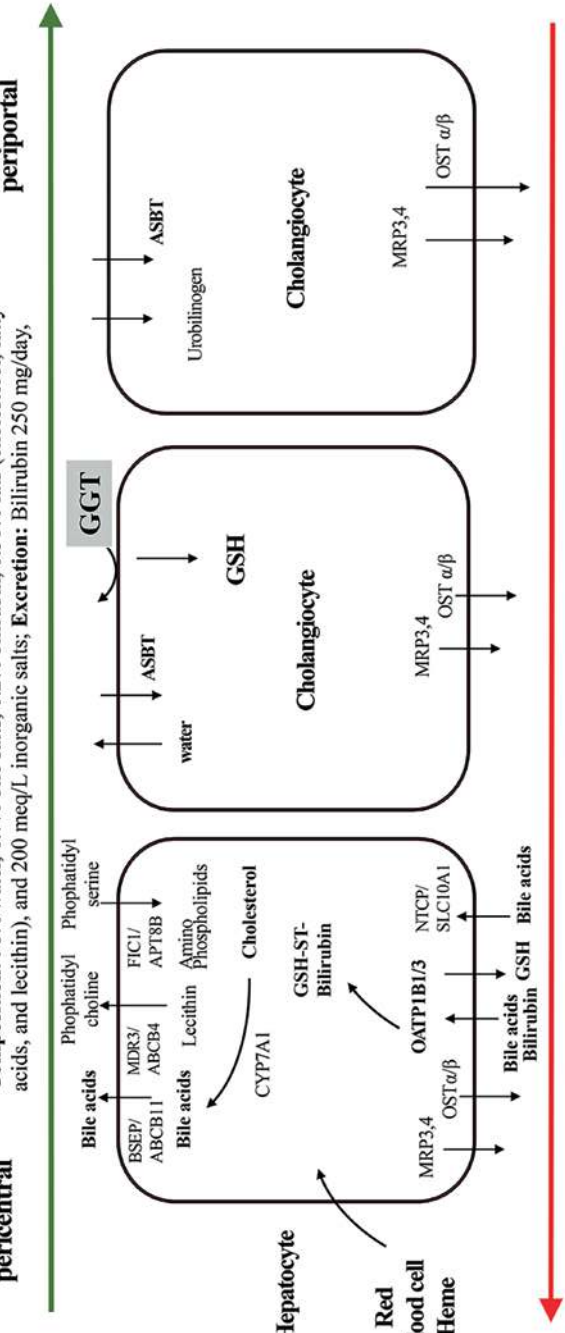


Fig. A.60 Bile acid production. In phase 0, bile acids and other organic products are transported through the basolateral membrane of the hepatocyte. Phase I and II take place in the cytoplasm through activation and metabolization of different CYPs. In phase III the bile acids are secreted into the biliary canalicus by the transmembrane transporters. *ASBT* apical sodium dependent bile acid transporter (SLC10A2), *ABCG5/8* ATP-binding cassette sub-family G member 5/8, *BSEP* bile salt export pump, *BRCP* breast cancer resistance protein, *CYP7A1* cytochrome P450 family 7 subfamily A member 1, *GGT* glutamyltransferase, *GSH* glutathione, *MRPs* multidrug resistance-associated proteins, *MDRs* multidrug resistance glycoproteins, *FIC1* familial intrahepatic cholestasis 1, *NTCP* Na+-taurocholate cotransporting polypeptide, *OATPs* organic anion transporting polypeptides, *OST α/β* organic solute transporter. (Further reading: Boyer JL. Compr Physiol. 2013;3(3):1035–1078 and Boyer JL et al. J Hepatol. 2021;75(1):190–201)

Reactive Oxygen Species, CYP2E1 and Redox Regulation

See Figs. A.61, A.62, A.63, A.64, A.65, A.66, A.67, A.68, A.69, A.70, A.71, and A.72.

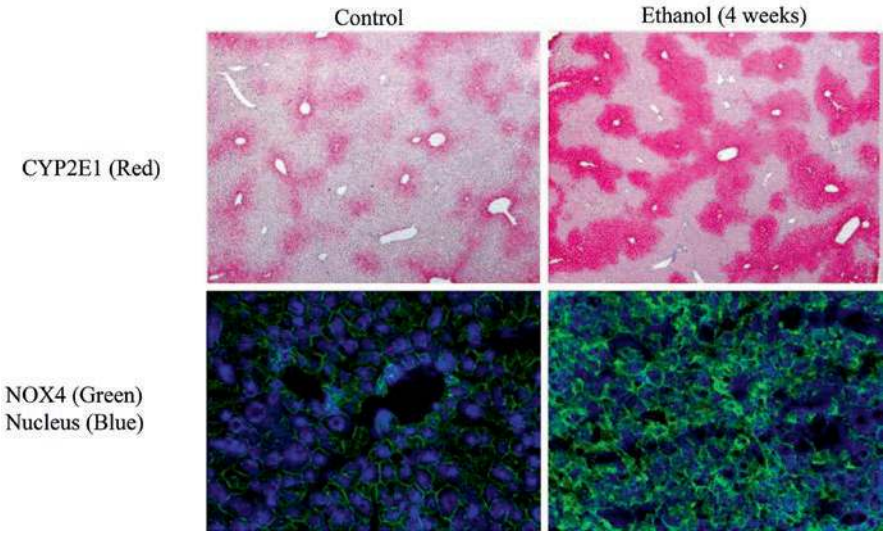


Fig. A.61 Typical hepatic, pericentral expression of CYP2E1 in mice that is strongly induced after 4 weeks of ethanol exposure (drinking model, 14%). In contrast, NADPH dependent oxidase 4 (NOX4) shows a membrane expression pattern with no zonal preference. According to Fig. A.76, CYP2E1 could be expressed to compensate for lower oxygen levels in the pericentral region while HO1-released carbon monoxide increases oxygen availability evenly in the cells to induce NOX4. (C. Chen and S. Mueller, Heidelberg)

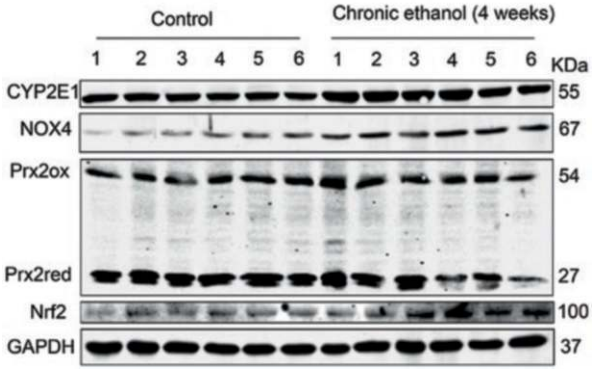


Fig. A.62 Expression of redox-related proteins in mice exposed to ethanol for 4 weeks in drinking water (15%). As can be seen in this Western blot, without further densitometry, CYPE1 is induced and reduced peroxiredoxin 2 decreases. The transcription factor Nrf2 is also upregulated (also see Fig. A.75). (unpublished data from C. Chen and S. Mueller, Heidelberg)

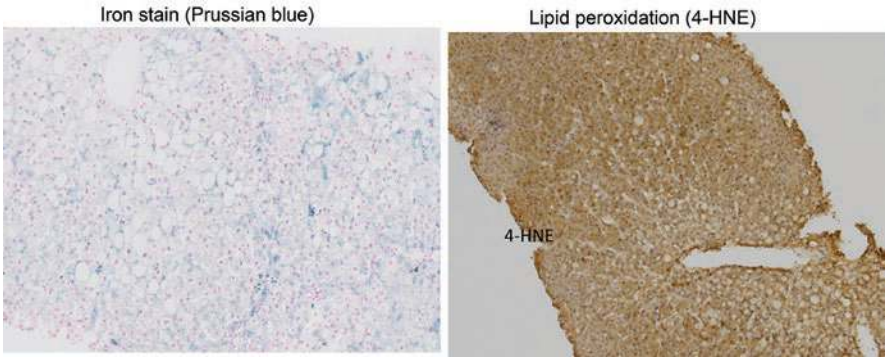


Fig. A.63 Prussian blue stain for iron (left) and immunostaining for the lipid peroxidation products 4-hydroxynonenal (right) in a human liver biopsy from a heavy drinker. Chronic alcohol consumption leads to hepatic iron accumulation and lipid peroxidation (see also Chap. 57). As shown in both images, no zonation is observed and both iron and lipid peroxidation are evenly distributed in patients with ALD. (unpublished from S. Mueller, Heidelberg)

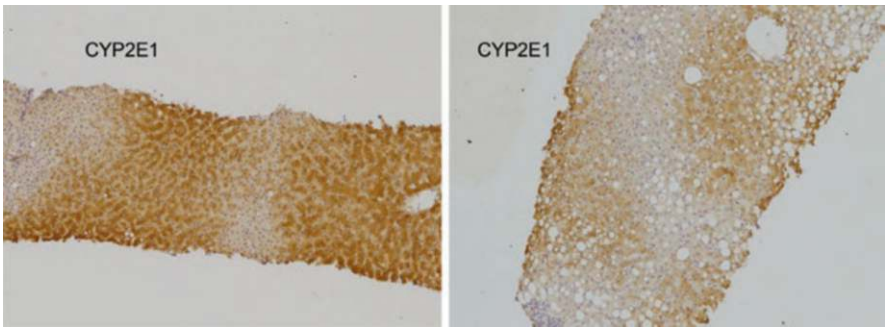


Fig. A.64 Typical zonal expression pattern of CYP2E1 in two human liver samples from heavy drinkers. Note that CYP2E1 is predominantly expressed in the pericentral region most likely due to reduced oxygen levels in the pericentral region (8% vs 16% oxygen). Since CYP2E1 is an oxygen-consuming enzyme, increased expression could allow for same turnover rates despite lower oxygen levels. (unpublished, S. Mueller, Heidelberg)

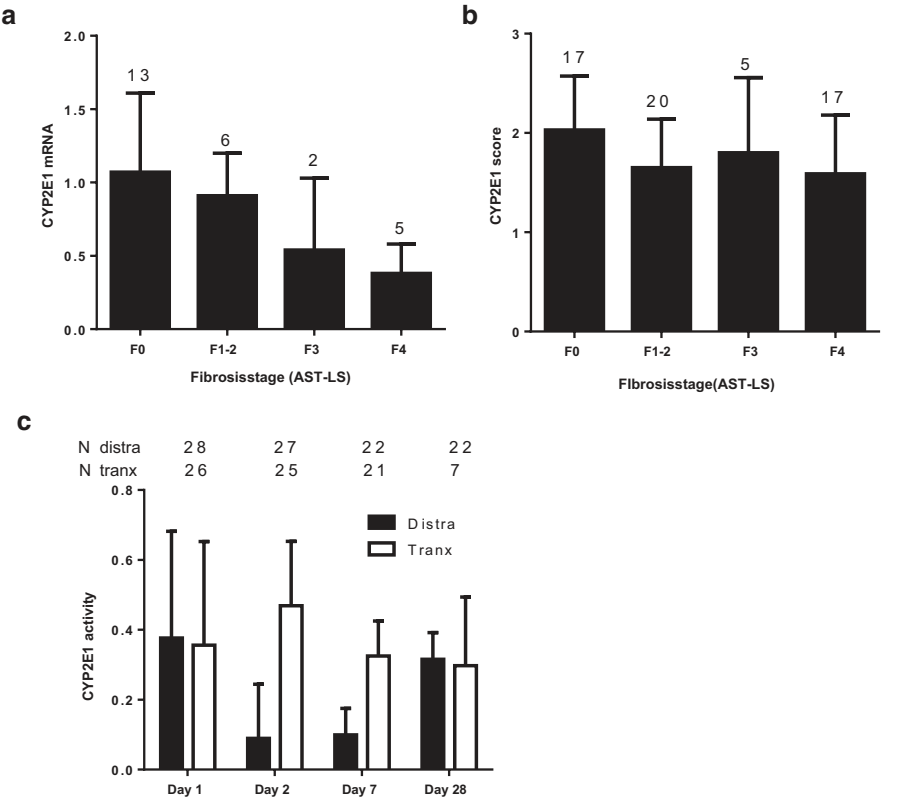


Fig. A.65 CYP2E1 mRNA, protein and activity in heavy drinkers. (**a**) Both CYP2E1 mRNA and (**b**) protein expression decrease with higher fibrosis stages. The most likely explanation is the fact that, with advanced liver fibrosis, the liver is increasingly supplied through the hepatic artery. As a consequence, nutritional ethanol uptake will not directly reach the liver through the portal vein but it will first distributed systemically. (**c**) Efficient inhibition of CYP2E1 in vivo activity by the CYP2E1 inhibitor chlormethiazole (black bars) in heavy drinkers after 5 days of treatment during alcohol detoxication therapy. No inhibition is seen in patients treated with the benzodiazepine chlorazepate (white bars). Of note, after 28 days of abstinence, CYP2E1 activity is only marginally reduced. This raises the question whether CYP2E1 is induced only by alcohol or whether additional factors such as enhanced red blood cell (RBC) turnover contribute to it. CYP2E1 activity was measured using the chlorzoxazone method. (From Lucas D et al. in: Johnson EF, Waterman MR, eds. Methods in Enzymology. Vol. 272: Academic Press; 1996:115–123. Modified from Hohmann N et al. *Gut*. 2022;71(4):842–844)

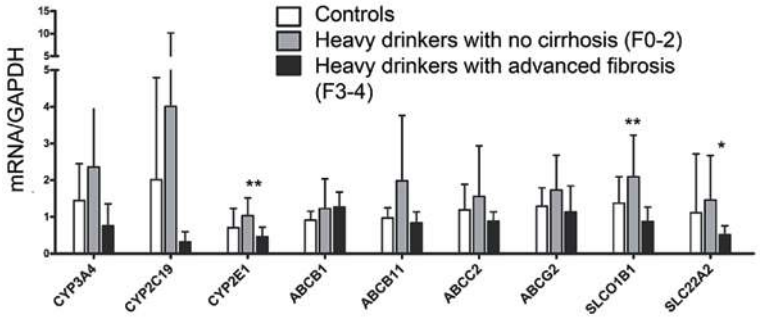


Fig. A.66 Differences in expression levels of CYPS and bile transporters between controls (white bars, $n = 5$), heavy drinkers with no/moderate increased liver stiffness (LS) (<8.0 kPa; gray bars, $n = 18$), and heavy drinkers with increased LS and F3/4 fibrosis (>8.0 kPa; black bars, $n = 8$). Depicted are mean ± SD. Statistical significance was evaluated using unpaired, two-sided Student's *t*-test for comparison of all groups with each other within the particular gene. See also Theile D et al. Alcohol Clin Exp Res 2013;37 Suppl 1:E17–22

ROS/RNS	Antioxidative defense systems	
	Enzymatic defenses	Non-enzymatic defenses
Superoxide anion (O_2·-) Hydrogen peroxide (H_2O_2)	SODs (against O_2·-) GSH (against H_2O_2)	Vitamin A/C/E Ubiquinone
Hydroxyl radical (.OH)	Glutathione peroxidases (GPx 1-8)	Uric acid
Peroxyl radical (ROO·)	Catalase (CAT)	
Nitrix oxide (NO)	Peroxiredoxins	
Nitrogen dioxide (NO_2·)	Heme oxygenases (HO)	
Peroxynitrite (ONOO)	Thioredoxin (Trx) system	
	Ferritin	
	Ceruloplasmin	

Fig. A.67 Reactive oxygen species (ROS), reactive nitrogen species (RNS) and important cellular antioxidative defense systems. ROS and RNS are heavily involved in the pathology of ethanol

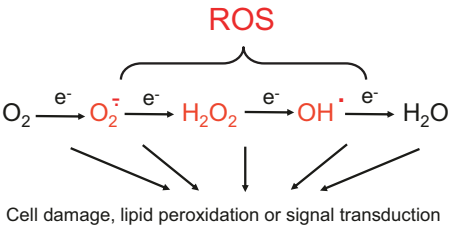


Fig. A.68 Reduction cascade of oxygen leading to so-called reactive oxygen species (ROS). Superoxide, hydrogen peroxide and hydroxyl radicals are considered the classical ROS. Note, by physico-chemical definition (at least one unpaired spin of outer orbital electron/valence electron), however, hydrogen peroxide is not a radical, while oxygen is a bi-radical

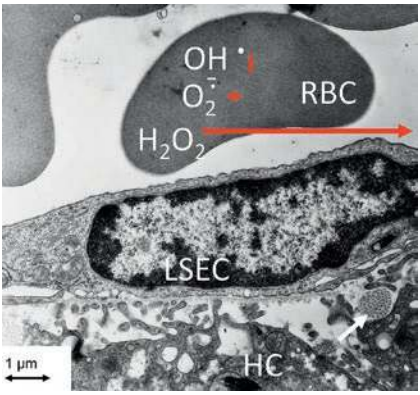


Fig. A.69 Mean diffusion distance of reactive oxygen species (ROS). Due to reactivity and diffusion, ROS have different mean diffusion distances (red arrows). Especially H_2O_2 has the lowest reactivity with a half-time of milliseconds. It thus can cross membranes and, participate in intercellular and interorganelle communication. Electron micrograph 7500×. *RBC* red blood cell, *LSEC* liver sinusoidal endothelial cell, *HC* hepatocytes, *white arrow* collagen bundle in the Disse space. (Further reading: Radi, R. Biochemistry115 (23) 5839–5848). Electron micrograph from Mueller, S. unpublished

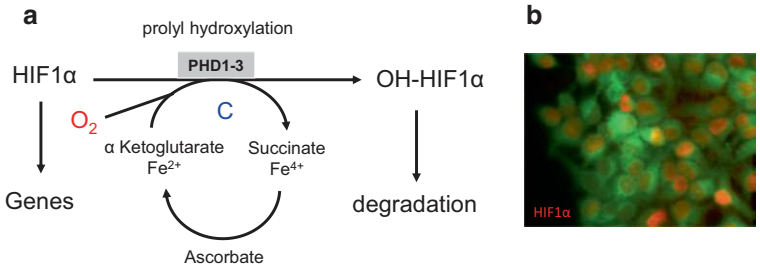


Fig. A.70 Relation of hypoxia signaling with energy and iron metabolism. (**a**) Through ethanol metabolism, oxygen is consumed potentially leading to hypoxia. Hypoxia inducible factor 1 alpha (HIF1α) is an important transcription factor induced in response to hypoxia that controls more than 5% of the genome, including many metabolic and energy pathways. Mechanistically, HIF1α is marked by prolyl hydroxylases (PHD1–3) mark HIF1α for degradation under normoxia. As demonstrated, the reaction is also linked to iron and energy metabolism (modified from Jaakkola *et al.*, *Science* 2001; R. K. Bruick, S. L. McKnight, *Science* 2001). (**b**) Nuclear and heterogenous HIF1α expression is shown in cultured hepatocytes after enzymatic induction of hypoxia. The hypoxia dye pimonidazole is used as counterstain (green) (modified from Millonig G et al. FRBM 2009;46(2):182–191)

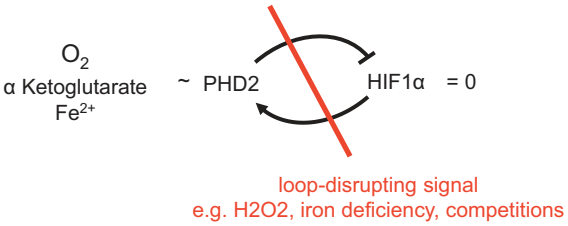


Fig. A.71 HIF1α causes its own downregulation by inducing its target gene PHD2. Consequently, hypoxia only causes a transient upregulation of HIF1α while loop disruption signals such as iron chelators, hydrogen peroxide or metabolic changes affect or compete with α-ketoglutarate or succinate and will strongly affect HIF1α expression. HIF1α should thus be better called a **metabolic control**. (Modified from Millonig G et al. FRBM 2009;46(2):182–191)

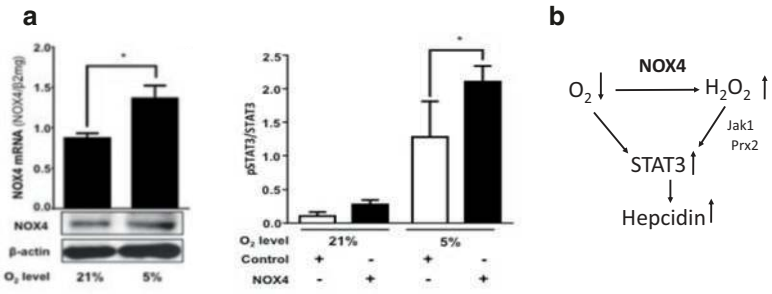


Fig. A.72 Redox regulation of the systemic iron masterswitch hepcidin by an oxidase (NOX4). Note that ethanol induces hepatic NOX4 in mice (see Fig. A.61 and A.62). NOX4 uses oxygen (hypoxia) to produce H_2O_2. Since both hypoxia and H_2O_2 are able to induce hepcidin, NOX4 could be an important upstream regulator of hepcidin in alcohol-related liver disease. (**a**) In vitro, hypoxia also increases NOX4 mRNA and protein levels in Huh7 cells and overexpression of NOX4 increases expression of the redoxsensitive iron hormone hepcidin and enhances hypoxia-mediated hepcidin induction. (**b**) These data point to an important role of oxidases such as NOX4 for the regulation of iron homeostasis. (Further reading: Silva I et al. Redox Biol. 2018;16:1–10. Millonig G et al. J Biol Chem. 2012;287(44):37472–37482. To independently control oxygen and peroxide levels in cell culture, enzymatic systems such as the GOX/CAT system can be explored (see also Mueller S et al. Adv Med Sci. 2009:1–15). NOX4- NADPH-dependent oxidase 4

Pathways of Heme Degradation and Signaling (HO1, Nrf2)

See Figs. A.73, A.74, and A.75.

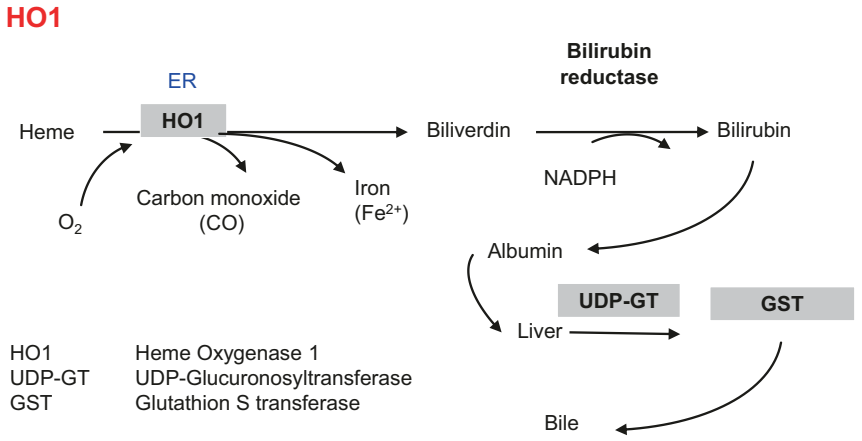


Fig. A.73 Heme degradation by heme oxygenase 1

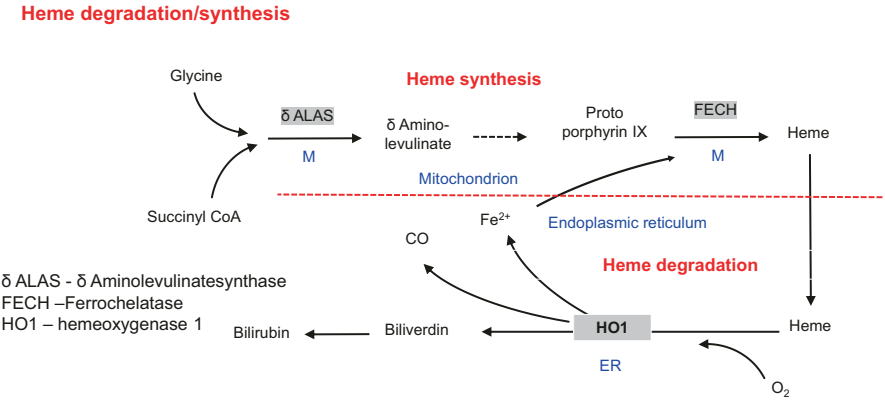


Fig. A.74 Heme degradation versus synthesis. Note that both pathways are placed in different compartments (Endoplasmic reticulum versus mitochondria). Cellular iron and heme trafficking is not yet fully understood. Iron-rich, originally bacteria-derived mitochondria could play an important role in the aquisition of efferocytosed red blood cells (see also Figs. A.33–A.36)

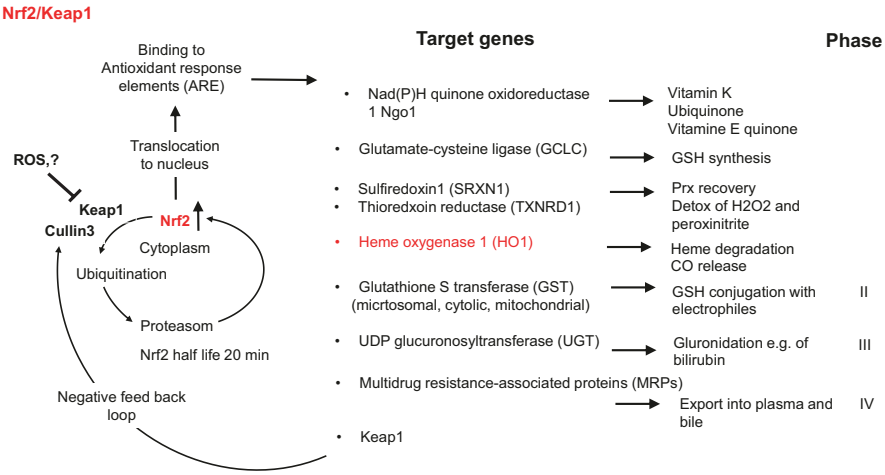


Fig. A.75 Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling. Nrf2 is a transcription factor that regulates the expression of antioxidant proteins by binding to antioxidant response elements (AREs) in the promoter regions of genes encoding cytoprotective proteins. Nrf2 is redox-sensitive and also induces the expression of heme oxygenase 1. In ALD, during hemolysis and enhanced red blood cell (RBC) turnover, it could play an important role in cellular signaling. See also Fig. A.63

Diagnosis of ALD: Laboratory and Liver Elastography

See Figs. A.76, A.77, A.78, A.79, A.80, A.81, A.82, A.83, A.84, and A.85.

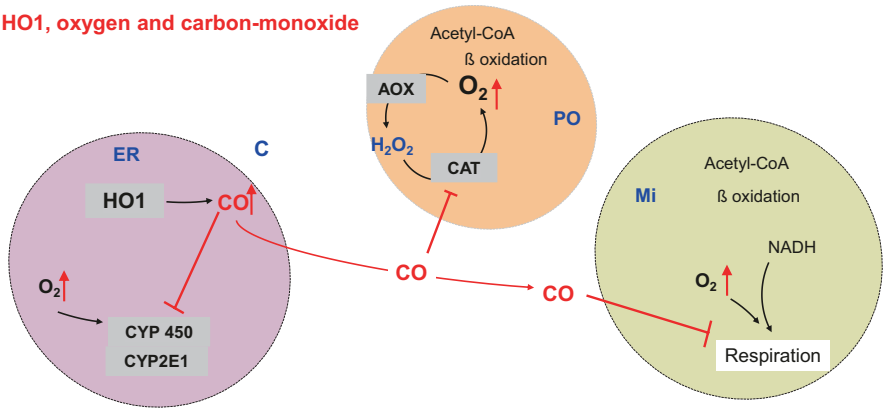


Fig. A.76 Potential intra- and inter-organelle role of the gaseous resolving carbon monoxide (CO) in ethanol-metabolism. Enhanced hemolysis in ALD causes CO-release through HO1 which could directly block CYP2E1 in the ER. It could also block mitochondrial respiration and peroxisomal catalase. In summary, CO-release would ultimately decrease oxygen consumption, e.g. as adaptive response to hypoxia

Duration of alcohol consumption	Amount of alcohol consumption	
	> 1g/d	> 40-60g/d
< 1 day	serum, urine: EtOH,EtG, EtS	Serum and urine: EtOH, EtG, EtS PEth in whole blood and dried blood spots (LC-MS/MS)
> 1 day	serum, urine: EtOH,EtG, EtS	Serum and urine: EtOH, EtG, EtS PEth in whole blood and dried blood spots (LC-MS/MS)
> 14 days	serum, urine: EtOH,EtG, EtS	Serum and urine: EtOH, EtG, EtS PEth in whole blood and dried blood spots (LC-MS/MS)
Weeks to months	serum, urine: EtOH, EtG, EtS	Serum and urine: EtOH, EtG, EtS PEth in whole blood and dried blood spots (LC-MS/MS), EtG and FAEE in hair

Abbreviations: EtOH,Ethanol; EtG, Ethyl glucuronide; EtS, Ethyl sulfate; PEth, Phosphatidylethanol; FAEE, Fatty-acid ethyl esters

Fig. A.77 Clinically relevant options for the determination of direct biomarkers, concerning the amount and duration of alcohol intake. (For more details see also Chap. 13. Modified according to Thon N et al. Fortschr Neurol Psychiatr 81:493–502)

Biomarkers	Amount of alcohol consumption	Cut-off values
EtG in hair	Abstinence and low intake (< 10g alcohol /day)	< 7 pg/mg
	Social consumption (20-40g/d)	7 – 30 pg/mg
	Excessive drinking (>60g/d)	> 30 pg/mg
FAEE in hair	Repeated alcohol intake	≥ 200 pg/mg
	Excessive intake	≥ 500 pg/mg
EtG in urine	Total abstinence	0.1mg/L
	- unintentional intake - recent alcohol use - longer back-dated alcohol intake in larger amounts	0.1mg/L – 0.5 mg/L
	unintentional intake unlikely, but possible, active alcohol intake probable	0.5-1 mg/L
EtS in urine	Total abstinence	0.05mg/L
PEth	>40g/d, more than 2 weeks alcohol intake at least once with 1 detectable	HPLC: 0.22µM, LC/MS-MS: 20/30ng/ml

Abbreviations: EtOH,Ethanol; EtG, Ethyl glucuronide; EtS, Ethyl sulfate; PEth, Phosphatidylethanol; FAEE, Fatty-acid ethyl esters

Fig. A.78 Clinically relevant options for the determination of direct biomarkers, concerning the amount and duration of alcohol intake. (For more details see also Chap. 13. Modified according to Thon N et al. Fortschr Neurol Psychiatr 81:493–502)

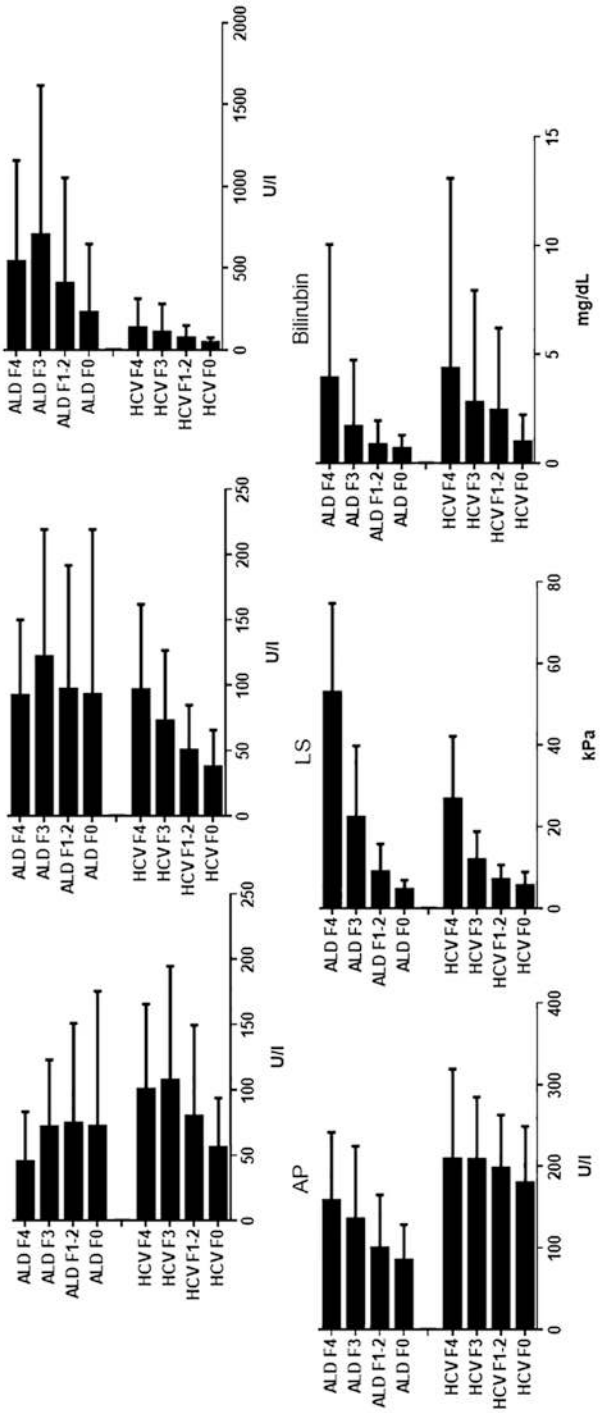


Fig. A.79 Comparison of important liver parameters between alcohol-related liver disease (ALD, lobular liver disease) and HCV (hepatitis C infection, portal disease) according to biopsy-proven fibrosis stage F0–F4. GGT is higher throughout all fibrosis stages in ALD. While AST is almost constant in ALD, it increases with fibrosis stage in HCV. In contrast, AP is generally higher in HCV and constant, while it increases with fibrosis stage in ALD. As is discussed in Chap. 7 and 41 on AST and Mortality, in ALD, AST is mostly derived from red blood cells. In addition, AP is an important prognostic parameter in ALD. Finally, as discussed in Chap. 42 on Elastography and Fig. A.81, liver stiffness (LS) values are generally higher in ALD as compared to HCV

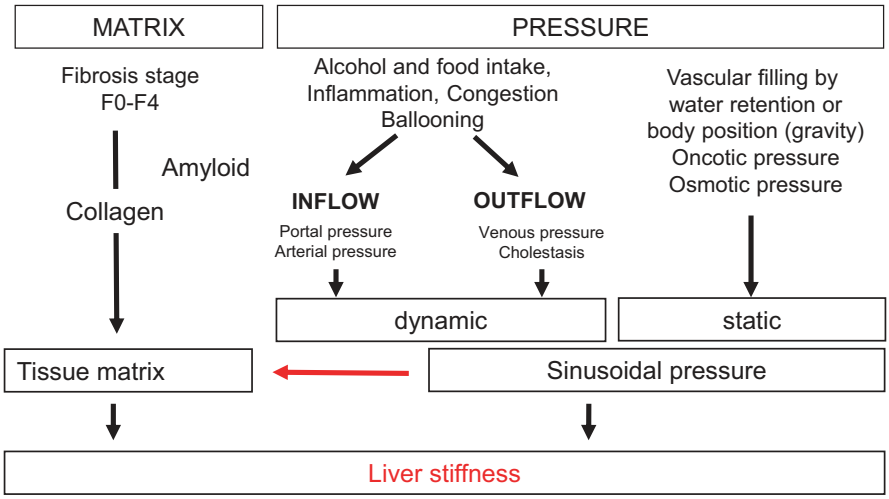


Fig. A.80 Established confounders of liver stiffness. Irrespective of fibrosis (left), many important and pressure-related confounders cause liver stiffness elevation through an increased sinusoidal pressure. Thus, in normal livers, liver stiffness reflects the sinusoidal pressure. According to the **sinusoidal pressure hypothesis**, this pressure drives fibrosis (red arrow and responsible e.g. for the typical macroscopic changes in the cirrhotic liver). (Modified from Mueller S. World J Gastroenterol. 2016;22(48):10482–10501). See also Chaps. 42 and 49, and Fig. A.92

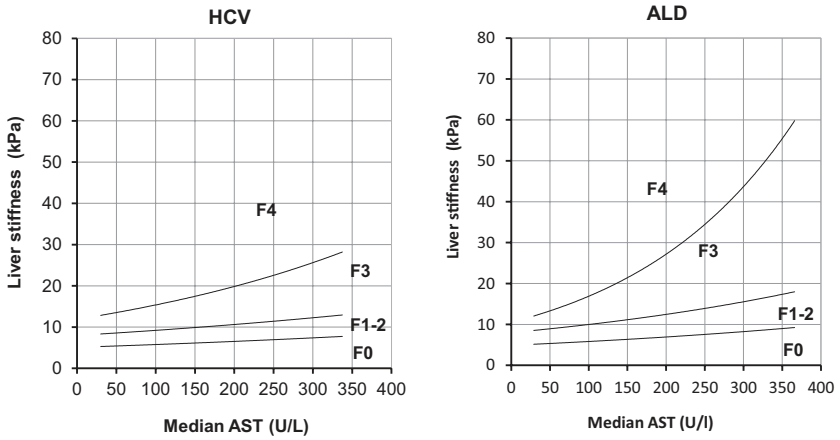


Fig. A.81 AST-adapted cut-off values for alcohol-related liver disease (ALD) and hepatitis C virus infection (HCV). Note that identical AST levels cause higher LS elevation in ALD as compared to HCV. These graphs allow for instant fibrosis stage reading based on liver stiffness measurements and laboratory markers. A more precise assessment of fibrosis stage requires treatment interventions to remove the inflammatory component such as alcohol detoxification or antiviral therapy. (Modified from Mueller S et al. *Liver Int*. 2015;35(12):2514–2521). See also Chap. 42

Fibrosis stage	Cut-off for Liver Stiffness (kPa) in HCV	Cut-off for Liver Stiffness (kPa) in ALD
F0 vs F1-2	≥ 5.1 x exp (0.0018 x AST)	≥ 4.9 x exp (0.0022 x AST)
F1-2 vs F3	≥ 9.0 x exp (0.0023 x AST)	≥ 8.1 x exp (0.0046 x AST)
F3 vs F4	≥ 11.9 x exp (0.0035 x AST)	≥ 10.5 x exp (0.0069 x AST)

Fig. A.82 Calculation of AST-adapted cut-off values for alcohol-related liver disease (ALD) and chronic hepatitis C virus infection (HCV). These formulas are useful to calculate fibrosis stages based on transient elastography data and AST levels. (Modified from Mueller S et al. *Liver Int*. 2015;35(12):2514–2521)

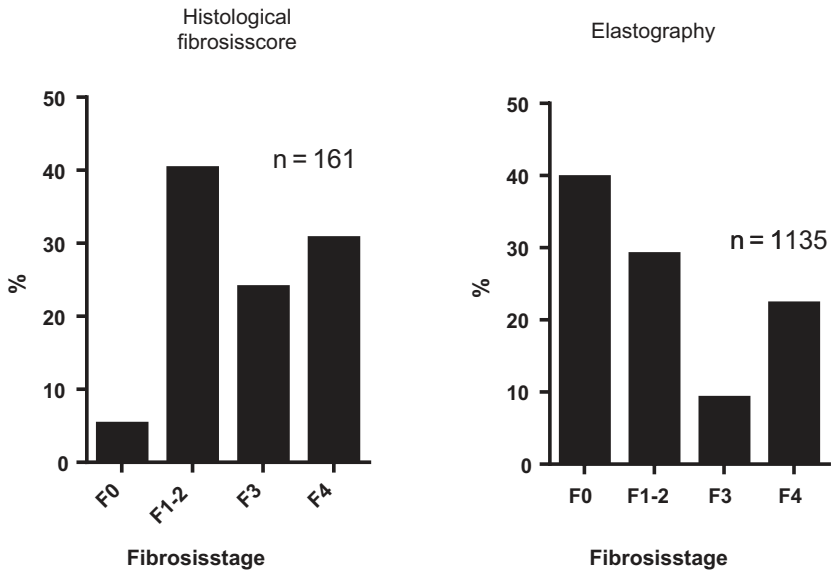


Fig. A.83 Fibrosis distribution of heavy drinkers based on liver biopsy (left) and transient elastography (right). Note that the non-invasively characterized cohort contains 10 times more patients with normal livers as patients with a healthy liver are not frequently biopsied. AST-adapted cut-off values were used (see Figs. A.81 and A.82) for instant fibrosis stage reading (Mueller S et al. *Liver Int*. 2015;35(12):2514–2521). Another important conclusion from these data is, that biopsy-proven study cohorts tend to have higher fibrosis stages. This may negatively impact upon search strategies for disease mechanisms, as "non-diseased" patients are also required for such strategies

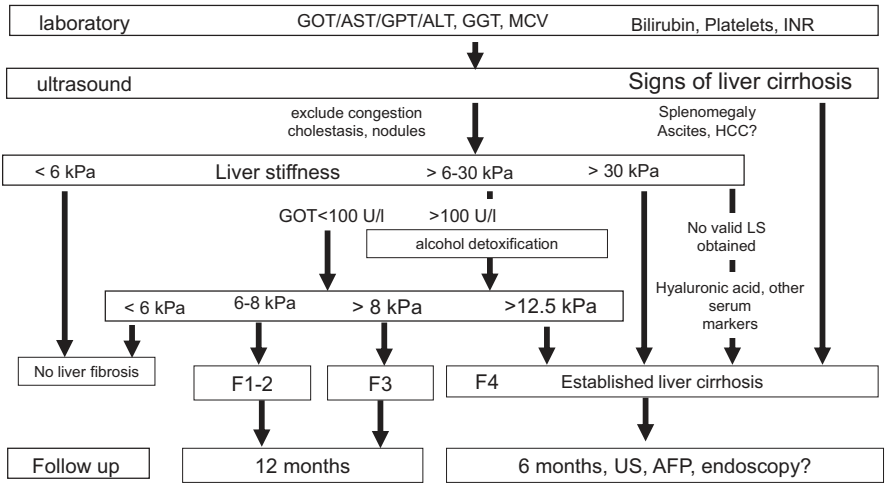


Fig. A.84 Example of a diagnostic flow chart for the diagnosis of alcohol-related liver disease using liver stiffness measurements. (Modified from Mueller S et al. *World J Gastroenterol*. 2014;20(40):14626–14641). See also Chaps. 40 and 42

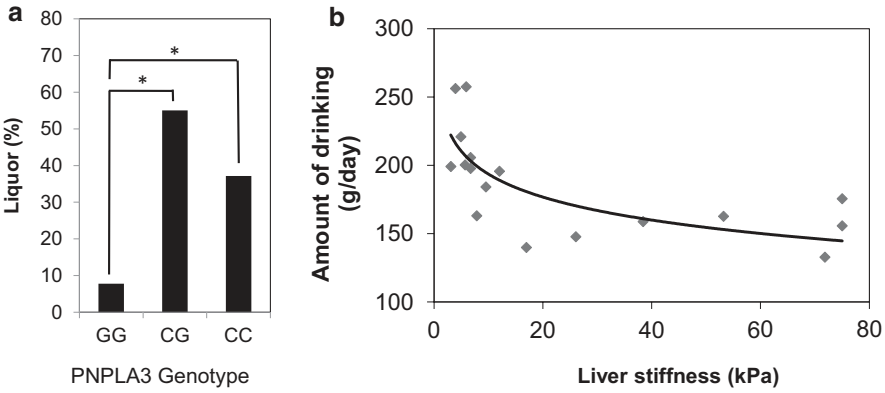


Fig. A.85 Effect of liver gene variants or fibrosis stage on daily drinking behavior in heavy drinkers. (**a**) Homozygote carriers of the GG genotype of adiponutrin/PNPLA3 drink significantly less high-percentage spirits (n = 503, unpublished). (**b**) The amount of consumed alcohol decreases with increasing liver stiffness (n = 1041, not published) (Mueller S et al. *Suchtmedizin*. 2018;20(1):1–9). Data are from the Heidelberg cohort of heavy drinkers (see also Tables B.1–B.30)

Kaplan-Meier Survival Curves of Various Parameters in Drinkers

See Figs. A.86, A.87, A.88, A.89, and A.90.

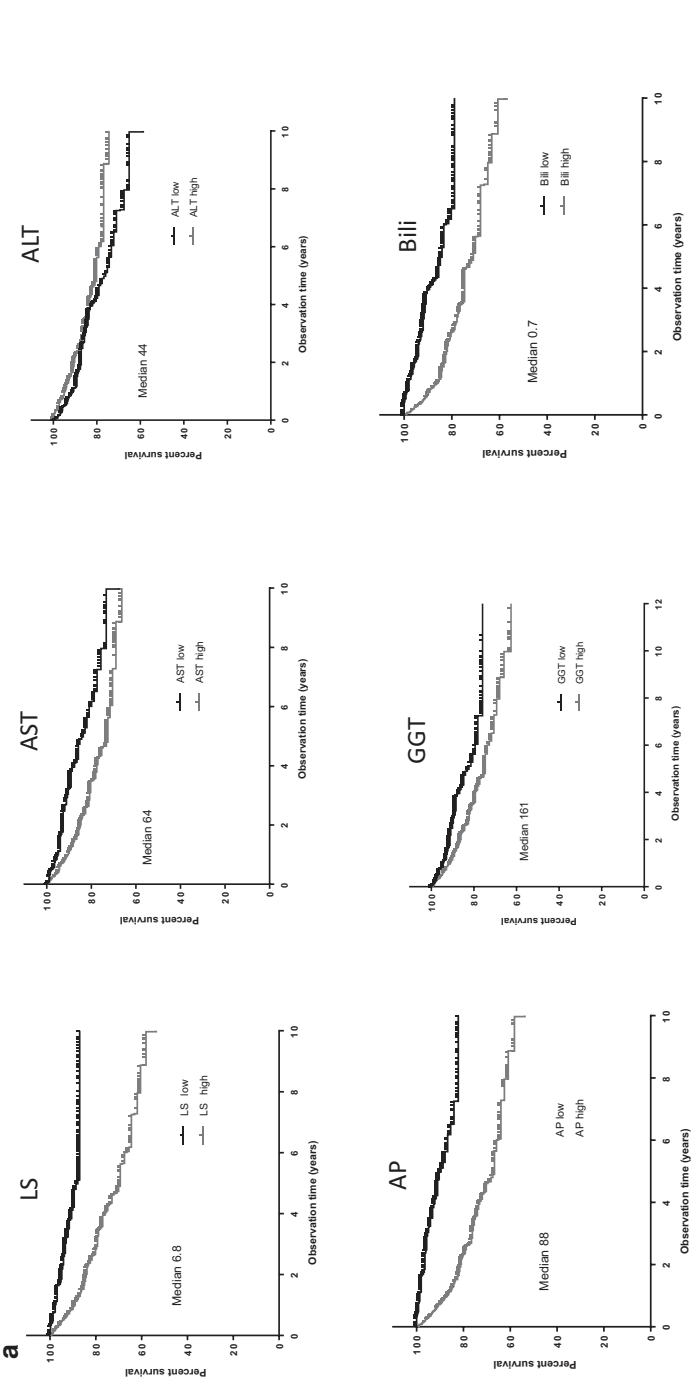


Fig. A.86 Routine laboratory parameters and Kaplan Meier survival curves. Kaplan Meier curves are shown for upper and lower median of various routine parameters. Please also see chapter on Mortality in Part I. Note that RBC count, AP and liver stiffness have the highest prognostic value for long-term survival

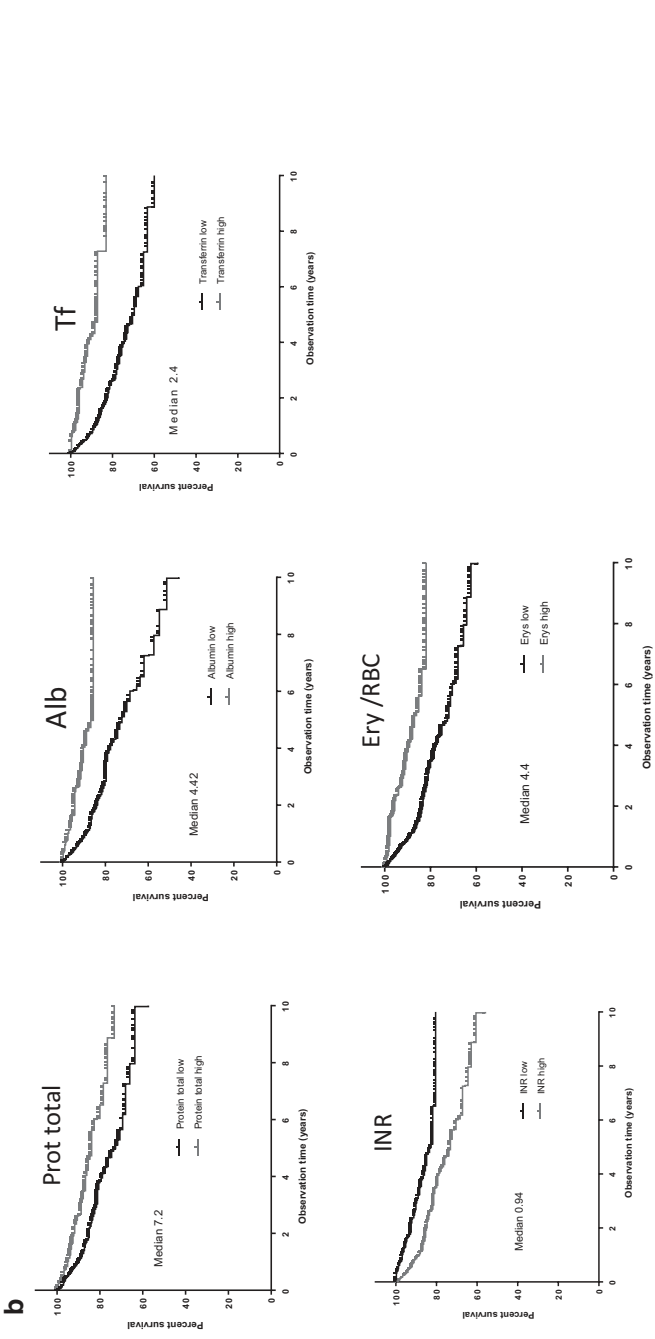


Fig. A.87 (continued) **Routine laboratory parameters and Kaplan Meier survival curves.** Kaplan Meier curves are shown for upper and lower median of various routine parameters. Please also see Chap. 7 on Mortality in Part I. Note that RBC count, AP and liver stiffness have the highest prognostic value for long-term survival

AM score (alcohol mortality score without LS):

AM Score = 0.03*Age + 0.0027 *AP + 0.053 *Bili + -0.549 *Erys -0.003 * Platelets - 0.0034 * Cholesterol;

AM-LS score (alcohol mortality score with LS)

AM-LS score =0.0593 * Bili+-0.5385 * Erys+0.0028 * AP+0.0074 * LS+0.0316 * Age

Logarithmized AM-LS score:

AM-LS LN score: = 17.1 * LN(Age) + 7.8 * LN(AP) + -18.9 * LN(Erys) + 2.1 * LN(LS)-79.15
(for conventient calculation multiplied by 10 and subtracted by 79.15)

Fig. A.88 Developed alcohol mortality scores for predicting of mid and long-term survival in alcohol consumers. See also Chap. 7 on Mortality in Part I

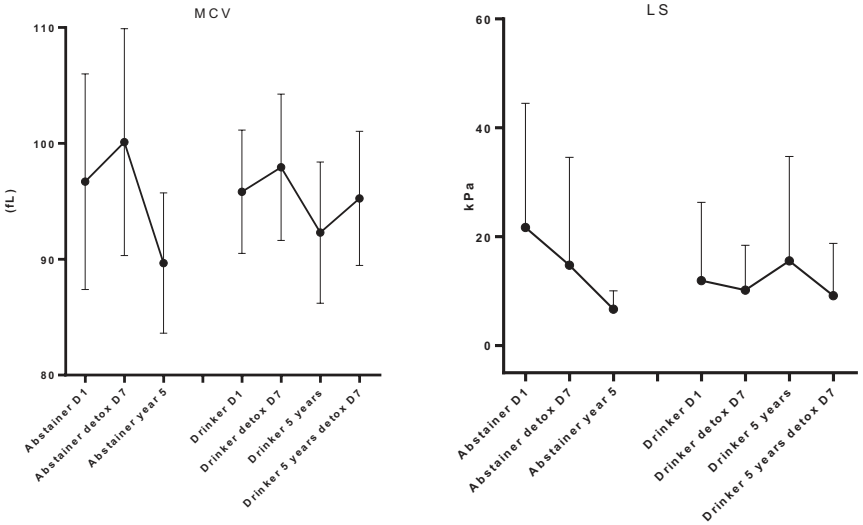


Fig. A.89 Effect of 5 years abstaining from alcohol versus continued drinking in heavy drinkers on various parameters. Data are shown for initial admission (D1), 7 days after alcohol detoxification (D7) and 5 years later. For continued drinkers, an additional time point 7 days after detox can be shown. In every figure, abstainers are shown on the left, drinkers on the right. Mean corpuscular volume (MCV) always increased during alcohol detoxification but normalized after long-term abstinence. Note that consequent abstainers have significantly higher initial liver stiffness in contrast to continued drinkers. After 5 years of abstaining from alcohol, liver stiffness was lower as compared to continued drinkers

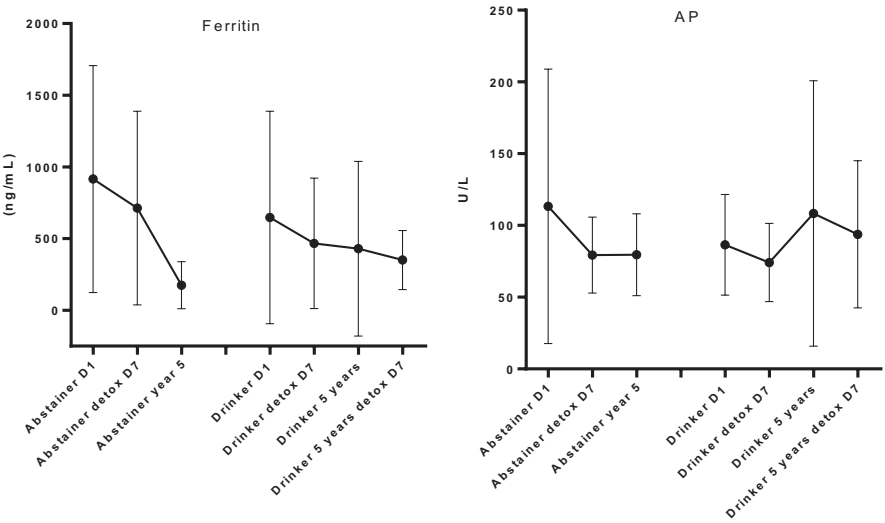


Fig. A.90 Effect of 5 years abstaining from alcohol versus continued drinking in heavy drinkers on various parameters. Data are shown for initial admission (D1), 7 days after alcohol detoxification (D7) and 5 years later. For continued drinkers, an additional time point 7 days after detox can be shown. In every figure, abstainers are shown on the left, drinkers on the right. Ferritin always decreases during alcohol detoxification but was finally higher in drinkers. In contrast, prognostically relevant AP increased during long-term drinking. Note that consequent abstainers have significantly higher initial liver stiffness in contrast to continued drinkers (see also Fig. A.89)

Pathological, Mechanical and Hemodynamical Aspects of Cirrhosis

See Figs. A.91, A.92, A.93, and A.94.

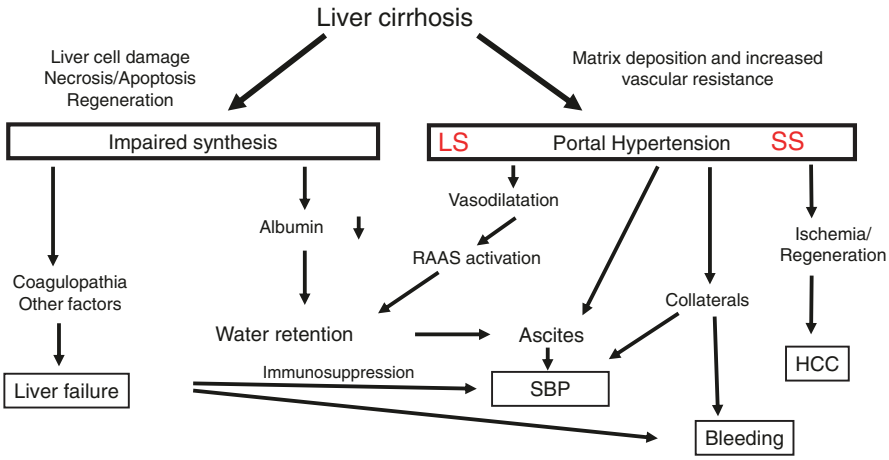


Fig. A.91 Clinical significance of synthesis impairment and portal hypertension in cirrhotics. Both factors are independently and individually occurring in cirrhotic patients and determine the individual risk of severe complications (framed). While liver synthesis is easily assessed by lab tests (e.g. INR, albumin), elastographic techniques are the methods of choice to identify patients with portal hypertension through measurement of liver stiffness (LS) and spleen stiffness (SS)

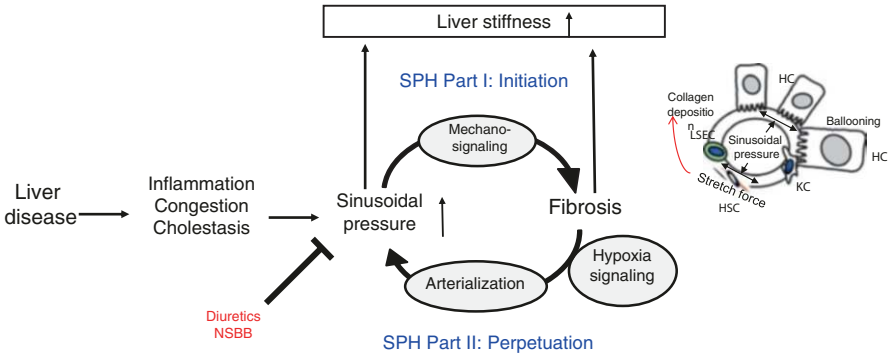


Fig. A.92 Sinusoidal pressure hypothesis (SPH) at the whole organ level and therapeutic target sides (red). Sinusoidal pressure (SP) is the driving force of matrix deposition. Irrespective of the etiology, all liver pathologies (shown in the left) increase the SP that initiates matrix deposition via specific inter- and intracellular biomechanic signaling pathways (SPH Part I, Initiation). LS should be regarded as the combined read-out of elevated pressure and fibrosis. Both SP elevation and matrix deposition increase vascular resistance that ultimately lead to elevated hepatic arterial flow and finally complete arterial blood supply of the liver (without portal blood supply). Depending on dosage (>12 mmHg) and time (>4 weeks), this vicious cycle will ultimately cause a complete arterialization of the liver leading to irreversible cirrhosis by exposing the low pressure organ liver to permanent high pressure (SPH Part II, Perpetuation). According to SPH, non-selective beta blockers (NSBB) and diuretics are not only symptomatic therapies but interrupt the vicious cycle of pressure-driven fibrosis progression. The right panel demonstrates how elevated sinusoidal pressure causes stretching of perisinusoidal aligned cells to induce mechano-signaling. (Modified from Mueller S. World J Gastroenterol. 2016;22(48):10482–10501)

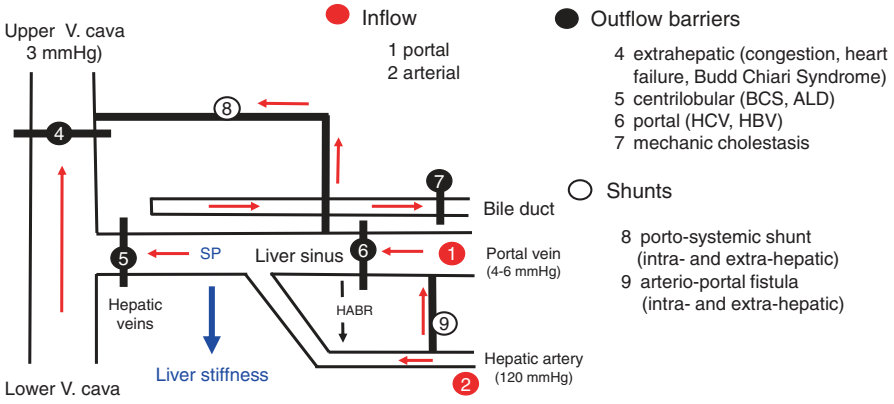


Fig. A.93 Hemodynamics of the low-pressure organ liver in the context of systemic circulation. Cirrhosis causes an increased vascular resistance, collateral formation and increased hepatic arterial flow to maintain hepatic perfusion. Elevated hepatic arterial flow can be observed already before the onset of fibrosis. It eventually leads to a complete arterialization of the cirrhotic liver, sometimes even with hepatofugal flow through the portal vein (modified from Mueller S. World J Gastroenterol. 2016;22(48):10482–10501). *HABR* hepatic arterial buffer response

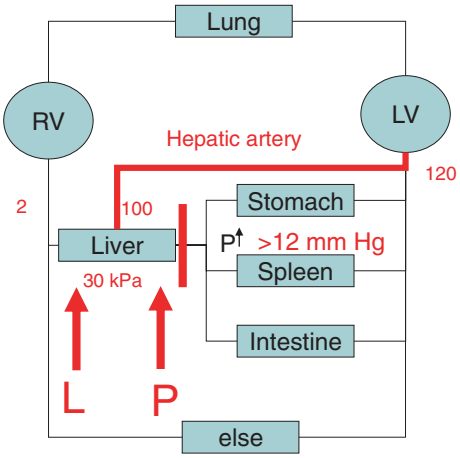


Fig. A.94 Systemic circulation in a patient with liver cirrhosis. Note that portal pressure is increased and the hepatic artery takes over the majority of hepatic blood supply. This not only increases the cardial work (heartwork) but seems also to increase shear stress on red blood cells (RBC), potentially one reason for pericentral RBC uptake by macrophages/hepatocytes but also for the elevation of transaminases such as AST (see also Chaps. 7, 41 and 57). Depending on the location of the liver inflammation (portal versus pericentral,) the sinusoidal pressure is first elevated in the indicated areas (arrow P = portal and L = lobular) (see also Elshaarawy O et al. JHEP Reports. 2019;1(2):99–106). As a consequence, in portal disease, spleen stiffness increases earlier, while in lobular disease (e.g. ALD), liver stiffness is higher. The ratio of both (SS/LS) can help to dissect the location of liver disease. Complete arterialization also determines the irreversibility of the liver disease. (See also Mueller S. Does pressure cause liver cirrhosis? The sinusoidal pressure hypothesis. World J Gastroenterol. 2016;22(48):10482–10501)

Clinical Case with Questions (Mild Alcoholic Hepatitis)

See Figs. A.95, A.96, A.97, A.98, A.99, A.100, A.101, A.102, A.103, A.104, A.105, A.106, A.107, A.108, A.109, A.110, A.111, A.112, A.113, and A.114.

Fig. A.95 Elshaarawy, O./ Mueller, S. Clinical case presented in Grand Round at EASL Vienna 2019

(1) Clinical case - Personal history

- 51 year old female patient
- Presenting again for alcohol withdrawal after relapse
- Ca. 180 g alcohol per day, mostly white wine
- 15 years of heavy drinking
- Obesity with a BMI of 34.6 kg/m^2
- Diabetes mellitus II for 10 years
- Otherwise, almost no symptoms at admission and no history of any chronic illness

Fig. A.96 Physical examination: see also Chap. 37

(2) Physical examination

- Fully conscious and oriented with a tinge of jaundice
- Normal blood pressure
- Tachycardia
- Hepatosplenomegaly
- No detectable abnormalities in cardiac, respiratory or nervous system examination

(3) Laboratory investigations at admission

Blood count

- Hemoglobin 11.8 g/dL
- MCV 110 /fL
- RBC 3.1 /pL
- WBC 6.1 /nL
- Platelets 134 /nL

Liver function tests

- Total bilirubin 5.9 mg/dL
- AST 233 U/L
- ALT 91 U/L
- AP 410 U/L
- gGT 3359 U/L
- INR 0.84

Other parameters

- Serum Ferritin 2420 ng/mL
- All markers for HCV, HBV and AIH were negative
- B12 and folic acid were normal

Fig. A.97 Laboratory examinations: see also Chaps. 37, 39, 40 and 41. Elshaarawy, O./ Mueller, S. Presented in Grand Round at EASL Vienna 2019

(4) Imaging

Abdominal ultrasound

- Hepatomegaly 19 cm
- Hepatic steatosis grade III
- Splenomegaly 13.5 cm
- No ascites

Fibroscan

- XL probe
- Liver stiffness 55.1 kPa
- IQR 21.6 kPa

- CAP 247 dB/M
- IQR 37 dB/M
- S2

Fig. A.98 Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019

(5) Question 1: Does the patient have cirrhosis with respect to elastography?

1- Yes

2- No

Fig. A.99 For elastography, see also Chap. 42. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019

(6) Manifest liver cirrhosis according to inflammation-adapted cut-off values of elastography.

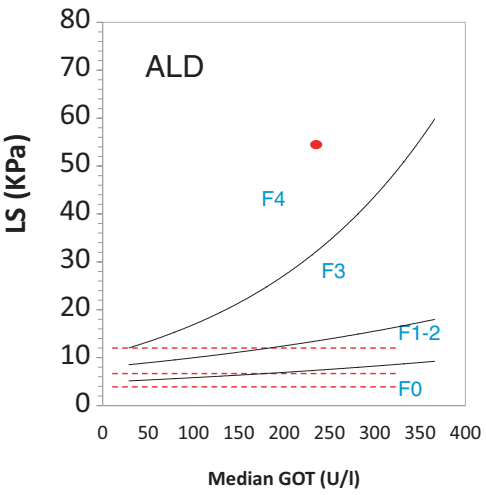


Fig. A.100 Elastography: see also Chap. 42. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. For more details see Chaps. 37, 38, 39, 40, 41 and 42 on the Diagnosis of ALD in part VII of this book. (Adapted from Mueller et al., 2015, Liver International; 35:2514–2521)

Fig. A.101 Prognostic score. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. For more details about AH and ACLF scores also see Figs. A.9–A.21 and respective Chaps. 64, 65, 66, 67 and 68 in part X

(7) Prognostic scores: day of admission

- Maddrey score 16
  (PT - Control PT – Total bilirubin)
- Glasgow AH score 6
  (Age, BUN, WBCs, PT, PT lab normal, Bilirubin)
- CLIF-C OF score 8 (grade 0)
  (Bilirubin, Creatinine, INR, Encephalopathy, MAP, SPO2)
- CLIF-C AD score 38
  (Age, Creatinine, WBCs, INR, Na)

(8) Queston2: What is your preliminary diagnosis?

1- Acute on chronic liver failure (ACLF)

2- Alcoholic hepatitis (AH)

Fig. A.102 Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. Answer: Mild AH. See also Figs. A.7–A.21 and Chap. 67 on AH and ACLF

(9) Question 3: Would you do a liver biopsy?

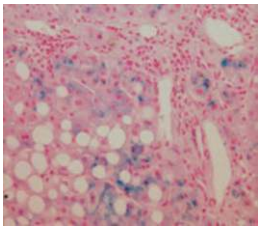
1- Yes
2- No

Fig. A.103 Liver biopsy. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. Answer: Not mandatory. See also Figs. A.7–A.21 and Chaps. 38, 64, 67 and 68 on the pros and concs for performing a liver biopsy in these patients

Fig. A.104 Liver histology in ALD. See also Chap. 38. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. For iron markers, see also Chap. 57 and Fig. B.7. ALD patients do have iron overload, low transferrin and elevated ferritin levels

(10) Liver biopsy

- Severe steatohepatitis
- Concomitant hepatocellular siderosis
- In addition to portal, peri-cellular, perisinusoidal and septal fibrosis
- Findings consistent with alcoholic hepatitis



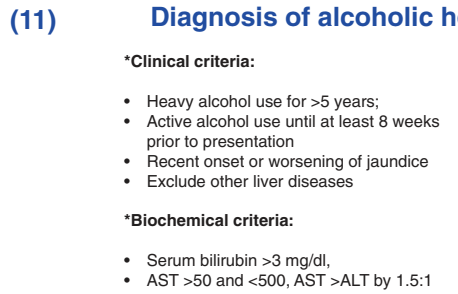


Fig. A.105 Alcoholic hepatitis. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna. See also Figs. A.7–A.21 and Chaps. 64, 65, 66, 67 and 68 on AH

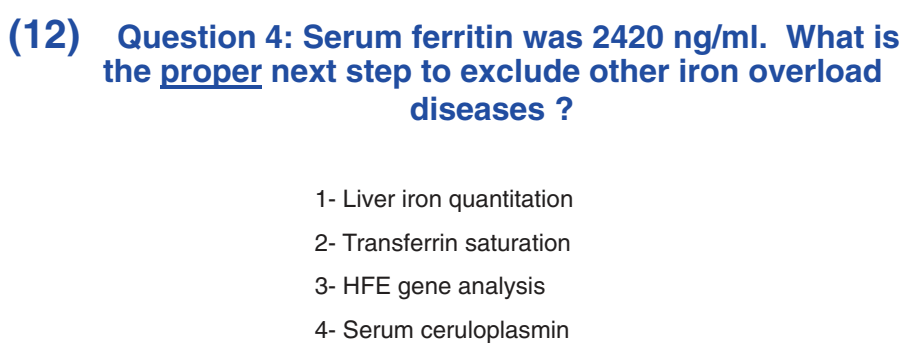


Fig. A.106 Iron parameters in patients with ALD. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. See also Chaps. 37 and 57

Fig. A.107 Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. (For more details see Table B.7 and Chap. 57. (see also Mueller, S., Seitz, H. K., and Rausch, V. (2014) *World J Gastroenterol* 20, 14626–14641, Makker, J et al., Case Rep Gastroenterol 2015;9:7–14)

(14) After 1 week of alcohol withdrawal

Score	Admission	1 week
Maddrey	16	24
Glasgow AH	6	8

- Thiamin substitution
- Detox with chlormethiazole, tapering scheme
- Serum bilirubin started to increase from 5.9 to 17.7 mg/dl in the first week while INR was within normal range.
- LS increased to 75 kPa.

Fig. A.108 Treatment of ALD: see also Chaps. 22, 48, 66, and 67. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. (EASL CPG ALD. J Hepatol 2018;69:154–81)

(15) Question5: How would you manage the case?

1-Prescribe prednisolone

2-Refer for liver transplant

3-Supportive nutritional care

Fig. A.109 Treatment of ALD. See also Chaps. 66 and 67. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. See also Figs. A.7–A.21 and chapters on AH

(16) Follow up of laboratory parameters

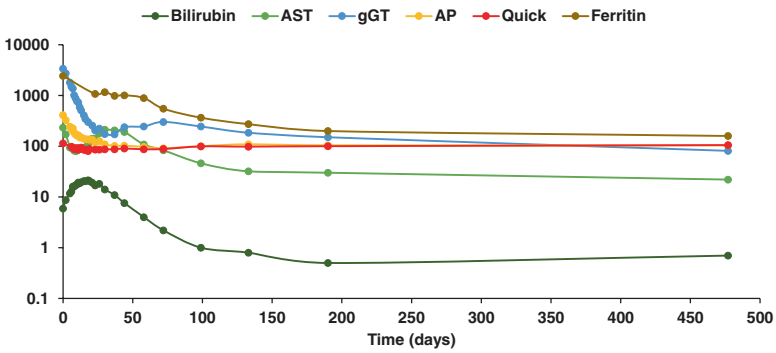


Fig. A.110 Laboratory markers in ALD. See also Chap. 37. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. Note how liver markers improve over more than 12 months of abstaining from alcohol

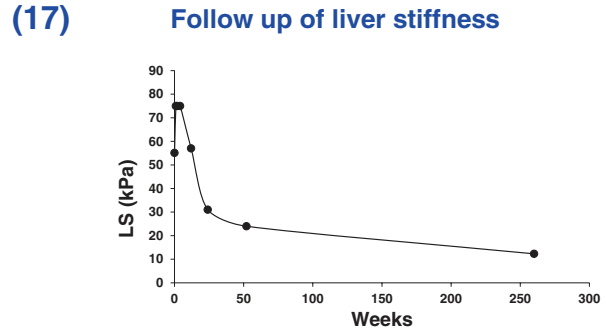


Fig. A.111 Follow-up of liver stiffness measurement: See also Chaps. 7 and 42. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. Note how liver stiffness improves over more than 12 months of abstaining from alcohol

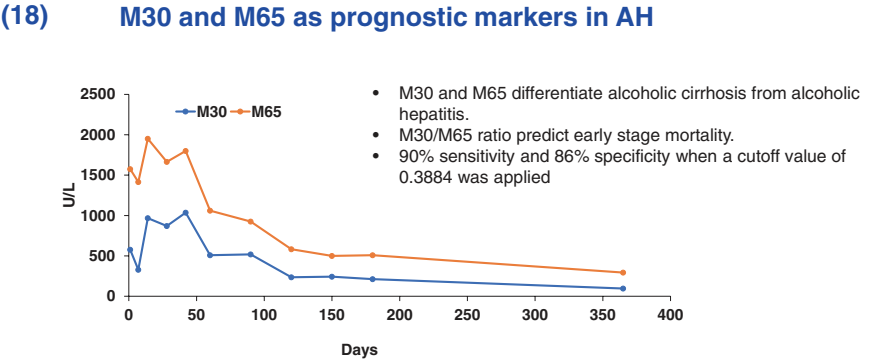


Fig. A.112 Apoptosis marker: See also Fig. A. 31 and A.32. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019. Note how markers of apoptosis and necrosis (M30 and M65) improve over more than 12 months of abstaining from alcohol. (Woolbright BL et al., *Gene Expr*. 2017. Mueller S et al. *Hepatology*. 2017;66(1):96–107)

(19) Long term management

- Referral to addiction center
- Absolute abstinence and future liver transplant
- Screening gastroscopy to exclude esophageal varices

Fig. A.113 Managment of ALD: see especially part III and X of the book. Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019

(20) Current challenges and future directions

For better management, there is a strong need for the following issues to be solved:

- Selection criteria for early liver transplantation
- The need for systematic screening for sub-clinical infection before starting prednisolone
- Alternative treatment protocols
- See chapters on AH and ACLF and book parts 8 and 10.

Fig. A.114 Elshaarawy, O./Mueller, S. Presented in Grand Round at EASL Vienna 2019

Appendix B: Patient Data from a Heavy Drinking Cohort[1]

Sebastian Mueller

Abstract This appendix provides tables with preliminary data from a heavy drinking cohort (Heidelberg) which has been collected prospectively for the last 15 years. These data are aimed at stimulating the interdisciplinary discussions in the area of alcohol-related diseases and providing non-clinicians with "real-life" data from such patients. Besides basic patient characteristics, mortality data and comparison between specific cohort such as alcohol-related liver disease versus non-alcoholic fatty liver (ALD) disease (NAFLD) are provided. Additional tables compare ALD cohorts with and without anemia or with and without advanced fibrosis. Moreover, complete, unfiltered results from specific correlation analyses are shown such as correlation with status of mortality, status of severe alcoholic hepatitis, degree of erythrophagocytosis, transaminase elevation and ferroptosis. Of note, the data not only include medical history, broad clinical characterization, ultrasound and elastography data but also hepatic immunostaining, mRNA expression, specific protein data from the serum, genetics and hepatic lipidomics. The data will hopefully also help to identify future scientific projects and to compare data from in vitro and animal models with data from human drinkers.

Keywords Alcohol, Alcohol-related diseases, Mortality, Laboratory, Lipidomics, Alcohol-related diseases, Anemia, Alcohol-related liver disease, Liver cirrhosis, Patient characteristics, PNPLA3, MCV

[1] Due to space limitations, not all abbreviations could be listed in the abbreviation list of the front matter, but they should be identifiable in the internet. To facilitate the search, parameters are associated in some tables with categories to better allocate their source, e.g. from histological, serum, ultrasound or lipidomics studies.

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https://doi.org/10.1007/978-3-031-32483-3

Patient Characteristics from Prospective Heidelberg Cohort of Heavy Drinkers

See Tables B.1, B.2, and B.3.

Table B.1 Patient characteristics from prospective Heidelberg cohort of heavy drinkers (total, alive and deceased after a mean observation of 3.8 years). For more details (study designs) see Chap. 7. on Mortality by S. Mueller et al.

	Total ($N = 763$)			Alive ($N = 624$)			Dead ($N = 139$)			*T*-test, Chi²-test (a) or Mann-Whitney-*U* test (b)	
Parameter	Mean ± SD or %			Mean ± SD or %			Mean ± SD or %			*P*	
Basic information											
Sex (1:male)	71.4%			70.7%			74.8%			3.3E−01	a
Age (years)	52.6	±	11.2	51.6	±	11.2	57.3	±	10.2	4.4E−08	
BMI (kg/m²)	25.60	±	4.95	25.58	±	4.82	25.74	±	5.52	7.4E−01	
Diabetes (0 or 1)	12.3%			9.7%			24.4%			8.9E−06	a
Hypertension (0 or 1)	38.1%			38.1%			38.1%			1.0E+00	a
Smoker (0 or 1)	66.6%			66.6%			66.7%			9.9E−01	a
Pack years (0 or 1)	24.4	±	23.1	22.8	±	21.7	31.8	±	28.1	3.1E−04	
Encephalopathy (0 or 1)	2.7%			1.5%			8.6%			9.7E−05	a
Alcohol consumption (g/day)	184.6	±	122.7	193.1	±	128.7	145.2	±	79.2	6.2E−05	
Duration of heavy alcohol drinking (years)	14.4	±	9.8	13.7	±	9.5	17.8	±	10.3	2.2E−03	
Routine laboratory											
AST (U/L)	94	±	97	90	±	96	110	±	98	2.7E−02	
ALT (U/L)	63	±	67	63	±	65	63	±	77	9.3E−01	
GGT (U/L)	420	±	637	386	±	608	574	±	737	1.9E−03	
AP (U/L)	110	±	72	101	±	62	151	±	97	2.6E−14	
Bilirubin total (mg/dL)	1.48	±	2.89	1.16	±	2.20	2.95	±	4.66	3.0E−11	
Bilirubin indirect (mg/dL)	0.48	±	0.72	0.40	±	0.55	0.87	±	1.18	2.7E−03	
Quick (%)	99.64	±	22.51	101.92	±	20.46	89.32	±	27.96	2.4E−09	
INR	1.03	±	0.34	1.00	±	0.30	1.15	±	0.44	1.2E−06	
Urea (mg/dL)	23.6	±	16.6	23.1	±	12.4	25.8	±	28.7	7.9E−02	
Creatinine (mg/dL)	0.73	±	0.32	0.73	±	0.31	0.74	±	0.37	9.6E−01	
Lipase (U/L)	57.3	±	68.4	57.0	±	69.5	58.5	±	63.2	8.3E−01	
PTT (s)	33.62	±	7.84	32.90	±	7.34	36.84	±	9.13	4.3E−07	

Table B.2 Patient characteristics (routine laboratory) from prospective Heidelberg cohort of heavy drinkers (total, alive and deceased after a mean observation of 3.8 years)

	Total ($N = 763$)			Alive ($N = 624$)			Dead ($N = 139$)			T-test, Chi^2-test (a) or Mann-Whitney-U test (b)	
Parameter	Mean ± SD or %			Mean ± SD or %			Mean ± SD or %			P	
Routine laboratory											
Hemoglobin (g/dL)	14.02	±	2.05	14.26	±	1.85	12.92	±	2.50	1.6E−12	
Hematocrit (%)	39.8	±	5.6	40.6	±	5.0	36.6	±	6.7	1.9E−14	
MCV (fL)	93.6	±	10.8	93.0	±	10.6	96.6	±	11.3	1.7E−03	
Erythrocytes (/pL)	4.28	±	0.72	4.38	±	0.66	3.84	±	0.82	4.7E−16	
Leukocytes (/nL)	7.83	±	4.07	7.83	±	4.24	7.84	±	3.22	9.7E−01	
Sodium (mmol/L)	137.4	±	4.7	137.7	±	4.4	135.9	±	5.5	3.8E−04	
Potassium (mmol/L)	3.90	±	0.48	3.90	±	0.47	3.90	±	0.51	8.9E−01	
Platelets (/nL)	202.4	±	84.9	209.0	±	82.7	172.7	±	88.8	4.8E−06	
Ferritin (ng/mL)	604.7	±	629.1	579.6	±	626.5	720.3	±	630.6	2.0E−02	
CRP (mg/L)	7.26	±	16.29	5.76	±	13.16	14.00	±	25.09	6.6E−08	
Glucose (mg/dL)	111.2	±	39.3	108.7	±	37.4	122.2	±	45.3	4.1E−04	
HbA1C (%)	5.51	±	0.85	5.50	±	0.82	5.59	±	0.99	3.4E−01	
Protein total (g/dL)	7.29	±	2.65	7.37	±	2.90	6.95	±	0.82	1.1E−01	
Serum iron (µg/dL)	123.9	±	61.3	124.5	±	60.3	121.4	±	65.4	6.1E−01	
Triglycerides (mg/dL)	190.8	±	200.1	202.6	±	212.0	136.9	±	119.3	1.1E−03	
Cholesterol (mg/dL)	213.2	±	63.7	218.5	±	62.2	189.4	±	65.4	4.4E−06	
HDL cholesterol (mg/dL)	72.05	±	37.74	73.16	±	36.33	66.70	±	43.72	1.2E−01	
LDL cholesterol (mg/dL)	109.62	±	48.66	113.61	±	49.12	90.24	±	41.44	1.3E−05	
LDH (U/L)	237.80	±	116.94	225.86	±	72.34	295.55	±	225.95	2.8E−06	

Table B.3 Patient characteristics (ultrasound and liver elastography) from prospective Heidelberg cohort of heavy drinkers (total, alive and deceased after a mean observation of 3.8 years)

Parameter	Total ($N = 763$)			Alive ($N = 624$)			Dead ($N = 139$)			T-test, Chi^2-test (a) or Mann-Whitney-U test (b)	
	Mean ± SD or %			Mean ± SD or %			Mean ± SD or %			P	
Ultrasound and TE											
Liver size (cm)	16.07	±	2.71	16.12	±	2.73	15.78	±	2.64	2.2E−01	
Hepatic steatosis (US) (0–3)	1.87	±	0.89	1.86	±	0.90	1.96	±	0.82	3.5E−01	b
Spleen size (cm)	10.16	±	2.28	10.09	±	2.22	10.46	±	2.55	1.2E−01	
Ascites (0 or 1)	10.9%			7.1%			28.1%			2.1E−12	a
Signs of cirrhosis (US) (0 or 1)	18.1%			13.6%			39.2%			7.9E−12	a
Liver stiffness (kPa)	17.5	±	22.2	14.5	±	19.3	31.8	±	28.8	3.6E−16	
CAP (dB/m)	292.4	±	55.3	291.7	±	55.0	296.6	±	57.6	5.0E−01	

Mortality, Medical History and Blood Count from the Heidelberg Cohort of Heavy Drinkers Stratified According to Fibrosis Stage

See Tables B.4, B.5, B.6, B.7, B.8, and B.9.

Table B.4 Mortality, medical history and blood count from the Heidelberg cohort of heavy drinkers stratified according to fibrosis stage. Apart from mean values, the percentage of pathological parameters are shown. Decreased values are underlined. $n = 1185$

Parameter	Units	Normal value/range	All fibrosis stages (F0–4)				No advanced fibrosis stages (F0–2)		Advanced fibrosis stages (F3–4)	
			Mean		SD	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)
Demographics and drinking history										
Male gender			70.8%				71.3%		68.2%	
Duration of heavy alcohol drinking	Years	0	13.7	±	10.1					
Last alcohol consumption	g/day	<30	186.0	±	126.7					
Fibrosis stage	1–4	0	1.738	±	1.588	57.0%	0.833	41.0%	3.710	100.0%
Alcoholic hepatitis (AH)	Yes = 1	0	2.3%			2.1%	0.1%	0.1%	7.6%	6.8%
Medical history										
Diabetes	Yes = 1	0	11.4%			11.4%	7.0%	7.0%	20.6%	20.6%
Smoker	Yes = 1	0	64.3%			64.3%	69.1%	69.1%	55.4%	55.4%
BMI	kg/m^2	18.5–24.9	25.6	±	4.9	43.7%	25.0	41.2%	26.6	50.3%
BMI for obesity	kg/m^2	>30	25.6	±	4.9	13.9%	25.0	10.6%	26.6	19.6%
Mortality										
Status death final	Yes = 1		20.2%				12.5%		34.9%	
Observation time	days		1390.0	±	1023.8		1472.4		1249.2	
Liver related death			41.7%				16.2%		56.8%	

(continued)

Table B.4 (continued)

Parameter	Units	Normal value/range	All fibrosis stages (F0–4)				No advanced fibrosis stages (F0–2)		Advanced fibrosis stages (F3–4)	
			Mean		SD	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)
Blood count										
Erythrocytes	/pL	4.5–5.9	4.5	±	8.3	55.9%	4.8	47.5%	3.9	76.5%
Hemoglobin	g/dL	12–16	13.9	±	2.0	12.0%	14.5	6.1%	12.8	33.0%
Hemoglobin <10 (anemia)	g/dL	12–16	13.9	±	2.0	5.1%	14.5	0.8%	12.8	12.9%
Hematocrit	%	40–53	39.7	±	5.6	39.2%	41.2	0.0%	36.8	60.5%
MCV	fL	80–96	93.4	±	9.5	31.7%	92.3	23.6%	96.2	50.9%
Leukocytes	/nL	3.5–10.0	7.8	±	3.8	15.7%	7.7	14.5%	8.1	18.3%
Platelets	/nL	140–360	206.4	±	87.4	24.1%	223.3	14.8%	172.7	42.4%

Table B.5 Liver parameters from the Heidelberg prospective cohort of heavy drinkers, stratified according to fibrosis stage. Apart from mean values, the percentage of pathological parameters are shown. Decreased values are underlined. $n = 1185$

Parameter	Units	Normal value/range	All fibrosis stages (F0–4)				No advanced fibrosis stages (F0–2)		Advanced fibrosis stages (F3–4)	
			Mean		SD	Elevated/<u>decreased</u> (in %)	Mean	Elevated/<u>decreased</u> (in %)	Mean	Elevated/<u>decreased</u> (in %)
Liver parameters										
AST	U/L	<50	93.6	±	100.8	57.6%	91.3	52.2%	99.1	69.6%
ALT	U/L	<50	64.4	±	78.1	42.1%	71.3	44.6%	50.7	37.0%
GGT	U/L	<60	406.9	±	656.5	74.0%	301.8	68.0%	648.2	87.3%
AP	U/L	40–130	111.7	±	75.1	23.6%	91.7	10.9%	154.4	49.9%
Bilirubin (total)	mg/dL	<1.2	1.6	±	3.3	23.8%	0.7	11.1%	3.3	50.3%
Bilirubin (total) >4 (jaundice)	mg/dL	<1.2	1.6	±	3.3	7.6%	0.7	1.3%	3.3	21.0%
Bilirubin (indirect)	mg/dL	<0.3	0.5	±	0.8	43.3%	0.3	33.7%	0.7	55.2%
Bile acids (total)	µM	2–5	19.5	±	23.8	68.9%	11.0	60.0%	43.5	93.8%
M30	U/L	<200	603.0	±	976.0	64.2%	453.4	57.4%	1000.8	83.3%
M65	U/L	<400	1108.7	±	2105.4	63.2%	820.6	55.2%	1871.4	84.5%
Quick	%	>70	98.7	±	22.7	13.2%	107.9	2.6%	79.4	34.8%
INR		0.85–1.15	1.0	±	0.3	17.5%	0.9	3.2%	1.2	46.7%
PTT	s	25–35	33.5	±	7.6	27.4%	31.5	13.2%	37.4	55.5%

Table B.6 Routine laboratory parameters from the Heidelberg prospective cohort of heavy drinkers, stratified according to fibrosis stage. Apart from mean values, the percentage of pathological parameters are shown. Decreased values are underlined. $n = 1185$

Parameter	Units	Normal value/ range	All fibrosis stages (F0–4)				No advanced fibrosis stages (F0–2)		Advanced fibrosis stages (F3–4)	
			Mean		SD	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)
Routine laboratory										
Creatinine	mg/dL	0.7–1.2	0.7	±	0.3	4.2%	0.7	1.8%	0.8	8.4%
Urea	mg/dL	<50	23.9	±	16.7	4.4%	22.4	1.4%	26.9	10.1%
Uric acid	mg/dL	3.5–7.0	6.5	±	2.1	38.4%	6.6	37.3%	6.6	42.9%
Lipase	U/L	13–60	55.2	±	59.8	27.0%	51.2	24.1%	65.8	34.9%
CRP	mg/L	<5	7.1	±	16.4	26.5%	4.2	15.6%	12.9	48.1%
CRP >30	mg/L	<5	7.1	±	16.4	5.9%	4.2	2.1%	12.9	13.3%
CRP >60	mg/L	<5	7.1	±	16.4	1.8%	4.2	1.0%	12.9	3.2%
Glucose	mg/dL	60–100	112.0	±	42.2	54.4%	108.4	49.4%	119.8	64.3%
HbA1C (%)	%	4.8–5.9	5.5	±	0.9	13.8%	5.6	10.9%	5.5	19.9%
Albumin	g/dL	3.4–5.4	4.3	±	0.7	9.5%	4.5	1.8%	3.9	24.3%
Protein (total)	g/dL	6.6–8.3	7.2	±	2.2	15.8%	7.2	10.9%	7.3	26.0%
APO A1	mg/dL	90–170	184.6	±	70.6	9.4%	203.1	2.0%	134.9	27.8%

Parameter	Units	Normal value/ range	All fibrosis stages (F0–4)				No advanced fibrosis stages (F0–2)		Advanced fibrosis stages (F3–4)	
			Mean		SD	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)
CK	U/L	<190	180.4	±	231.0	27.1%	189.7	29.9%	167.4	22.8%
Triglycerides	mg/ dL	<150	193.5	±	236.8	40.2%	209.4	43.1%	163.7	35.2%
Cholesterol	mg/ dL	<200	212.5	±	67.4	57.1%	225.4	65.9%	187.3	39.4%
HDL cholesterol	mg/ dL	>40	69.9	±	36.6	20.7%	79.0	9.7%	50.0	45.0%
LDL cholesterol	mg/ dL	<160	112.5	±	47.8	14.4%	118.7	15.9%	101.1	12.5%
Potassium	mM	3.5–4.6	4.1	±	4.5	15.4%	4.1	12.5%	3.9	19.9%
Sodium	mM	136–145	137.5	±	5.6	23.9%	138.3	18.4%	135.8	35.2%

Table B.7 Parameters of hemolysis, iron, vitamins and alcohol biomarkers from the Heidelberg prospective cohort of heavy drinkers, stratified according to fibrosis stage. Apart from mean values, the percentage of pathological parameters are shown. Decreased values are underlined. $n = 1185$

Parameter	Units	Normal value/range	All fibrosis stages (F0–4)				No advanced fibrosis stages (F0–2)		Advanced fibrosis stages (F3–4)	
			Mean		SD	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)
Hemolysis parameters										
LDH	U/L	<250	235.8	±	112.0	31.1%	223.6	26.3%	260.5	41.8%
Haptoglobin	g/L	0.3–2.0	1.4	±	0.8	6.4%	1.5	2.9%	1.2	15.2%
CD163	ng/mL	<800	1571.0	±	1032.7	75.7%	1118.0	63.0%	2218.8	94.4%
Iron-related parameters										
Ferritin >150	ng/mL	50–150/400	594.8	±	656.1	75.3%	567.0	75.8%	674.4	75.9%
Ferritin >400	ng/mL	50–150/400	594.8	±	656.1	44.3%	567.0	41.7%	674.4	50.3%
Ferritin >1000	ng/mL	50–150/400	594.8	±	656.1	19.5%	567.0	17.2%	674.4	25.6%
Serum iron	µg/dL	95–158	125.5	±	63.1	25.3%	129.2	25.6%	117.9	25.1%
Transferrin	g/dL	2.0–3.6	2.3	±	0.6	24.7%	2.5	2.3%	2.0	44.8%
Transferrin saturation	%	16–45	42.9	±	24.6	35.0%	40.7	31.1%	48.3	44.5%
Iron stain macrophages	0–2	0	0.609	±	0.792	44.2%	0.600	43.0%	0.632	46.1%
Iron stain hepatocytes	0–2	0	0.551	±	0.789	38.5%	0.575	40.5%	0.526	36.8%
Liver iron concentration (AAS)	mg/g dry weight	<0.8	1.3	±	1.3	58.9%	1.4	83.3%	1.3	47.2%

Parameter	Units	Normal value/range	All fibrosis stages (F0–4)				No advanced fibrosis stages (F0–2)		Advanced fibrosis stages (F3–4)	
			Mean		SD	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)
Vitamins										
Vitamin B12	pM	145–596	527.2	±	353.7	3.8%	442.8	4.4%	658.7	3.4%
Folic acid	nmol/L	>7.1	15.4	±	10.5	20.5%	19.2	7.7%	11.0	33.3%
Alcohol levels and biomarkers										
Serum alcohol level	g/L	<0.1	1.0	±	1.2	49.9%	1.0	51.7%	0.9	47.5%
EtG	ng/mL	0	1254.9	±	1727.6	83.9%	1191.6	85.5%	1436.9	79.2%
EtS	ng/mL	0	474.1	±	570.5	74.2%	446.6	75.4%	553.4	70.8%
PEth	ng/mL	0	1695.3	±	1304.5	100.0%	1733.2	100.0%	1586.4	100.0%

Table B.8 Parameters of abdominal ultrasound and liver elastography (Fibroscan) from the Heidelberg prospective cohort of heavy drinkers, stratified according to fibrosis stage. Apart from mean values, the percentage of pathological parameters are shown. Decreased values are underlined. $n = 1185$

			All fibrosis stages (F0–4)				No advanced fibrosis stages (F0–2)		Advanced fibrosis stages (F3–4)	
Parameter	Units	Normal value/ range	Mean		SD	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)
Ultrasound parameter										
Liver size	cm	<16	16.1	±	2.7	5.9%	15.7	40.1%	16.8	52.8%
Hepatic steatosis (US)	0–3	0	1.8	±	0.9	91.2%	1.8	90.4%	2.1	93.2%
Spleen size	cm	<11.5	10.2	±	2.2	21.5%	9.5	8.7%	11.6	47.6%
Ascites	0–1	0	0.1	±	0.3	10.7%	0.0	0.3%	0.3	31.6%
Signs of cirrhosis (US)	0–1	0	0.2	±	0.4	19.6%	0.0	2.2%	0.6	57.3%
Liver elastography										
Liver stiffness	kPa	<6	17.9	±	22.3	57.9%	6.4	38.7%	43.2	100.0%
CAP	dB/m	<240	288.6	±	57.1	77.8%	286.6	79.1%	293.1	75.0%

Table B.9 Histological parameters and their frequency from the Heidelberg prospective cohort of heavy drinkers, stratified according to fibrosis stage. Apart from mean values, the percentage of pathological parameters are shown. Decreased values are underlined. $n = 1185$. For histological Kleiner score, see also Fig. A.15

Parameter	Units	Normal value/ range	All fibrosis stages (F0–4)				No advanced fibrosis stages (F0–2)		Advanced fibrosis stages (F3–4)	
			Mean		SD	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)	Mean	Elevated/decreased (in %)
Kleiner score										
Steatosis	0–3	0	1.850	±	0.966	90.6%	1.788	87.3%	1.913	93.8%
Microvesicular steatosis	0–1	0	0.925	±	0.264	92.5%	0.913	91.1%	0.938	93.8%
Lobular inflammation	0–3	0	1.484	±	0.810	90.6%	1.241	83.5%	1.713	97.5%
Microgranulomas	0–1	0	0.331	±	0.472	33.1%	0.213	21.5%	0.438	43.8%
Large lipogranulomas	0–1	0	0.125	±	0.332	12.5%	0.075	7.6%	0.175	17.5%
Portal inflammation	0–1	0	0.406	±	0.493	40.6%	0.250	25.3%	0.550	55.0%
Ballooning	0–2	0	0.975	±	0.752	70.6%	0.650	51.9%	1.288	88.8%
Acidophil bodies	0–1	0	0.200	±	0.417	19.4%	0.113	10.1%	0.275	27.5%
Pigmented macrophages	0–1	0	0.369	±	0.484	36.9%	0.388	39.2%	0.338	33.8%
Megamitochondria	0–1	0	0.050	±	0.219	5.0%	0.000	0,0%	0.100	10.0%
Mallory hyaline	0–1	0	0.388	±	0.489	38.8%	0.213	20.3%	0.563	56.3%
Glycogenated nuclei	0–1	0	0.113	±	0.317	11.3%	0.025	2.5%	0.200	20.0%
Steatohepatitis	0–2	0	1.318	±	0.781	78.8%	1.079	67.1%	1.551	90.0%
Fibrosis score (Kleiner)	0–4	0	2.733	±	1.171	95.1%	1.925	91.1%	3.519	98.8%
Chevallier fibrosis score										
Central lobular vein	0–2	0	0.589	±	0.698	46.8%	0.479	42.9%	0.686	50.0%
Portal septa	0–2	0	1.340	±	0.596	93.6%	1.014	87.1%	1.657	100.0%
Portal tract	0–3	0	1.801	±	0.912	92.9%	1.225	87.1%	2.371	98.6%
Septa (number)	0–3	0	1.305	±	1.224	62.4%	0.394	31.4%	2.200	92.9%
Septa (width)	0–3	0	0.950	±	0.995	54.6%	0.254	20.0%	1.629	88.6%
Chevallier-score	0–25	0	8.213	±	6.119	97.2%	3.563	94.3%	12.743	100.0%

Univariate Correlation (Spearman Rho) with All-Cause Death in Heavy Drinkers (Complete)

See Tables B.10 and B.11.

Table B.10 Univariate correlation (Spearman Rho) with all-cause death in heavy drinkers. Parameters for analysis included routine and special laboratory, general information, medical history, comorbidities and morphometric data. A number of $n = 777$ was included with 127 deceased patients. Parameters are shown in descending order of the absolute correlation coefficient r (ignoring plus/minus signs). Please note that, in contrast to univariate Cox regression analysis, the observation time is not considered

Spearman rho correlation with status dead (1 or 0)			
Parameter	Category	*r*	*P*
Liver stiffness (kPa)	Ultrasound	0.299	**6.0E−17**
Erythrocytes (/pL)	Routine laboratory	−0.281	**1.6E−15**
Signs of cirrhosis (1 or 0)	Ultrasound	0.275	**4.1E−14**
AP (U/L)	Routine laboratory	0.269	**2.4E−14**
Bilirubin indirect (mg/dL)	Special laboratory	0.258	**4.9E−03**
Transferrin (g/L)	Special laboratory	−0.257	**6.2E−11**
CD163 (ng/mL)	Special laboratory	0.256	**6.8E−04**
Hematocrite (%)	Routine laboratory	−0.252	**1.2E−12**
LDH (U/L)	Routine laboratory	0.244	**4.6E−07**
Bilirubin total (mg/dL)	Routine laboratory	0.242	**9.4E−12**
Ascites (1 or 0)	Ultrasound	0.233	**1.3E−10**
Hemoglobin (g/dL)	Routine laboratory	−0.232	**6.5E−11**
Albumin (g/dL)	Special laboratory	−0.229	**1.2E−08**
PTT (s)	Routine laboratory	0.219	**7.7E−09**
INR	Routine laboratory	0.210	**3.6E−09**
Quick (%)	Routine laboratory	−0.208	**5.9E−09**
Age (years)	general information	0.204	**1.0E−08**
Platelets (/nL)	Routine laboratory	−0.192	**6.8E−08**
MCV (fL)	Routine laboratory	0.192	**1.4E−06**
CRP (mg/L)	Routine laboratory	0.175	**1.0E−06**
LDL cholesterol (mg/dL)	Routine laboratory	−0.170	**3.3E−05**
Cholesterol (mg/dL)	Routine laboratory	−0.168	**7.9E−06**
Glucose (mg/dL)	Routine laboratory	0.168	**6.3E−06**
Duration of heavy alcohol drinking (years)	Alcohol	0.161	**1.8E−03**
Triglycerides (mg/dL)	Routine laboratory	−0.152	**5.4E−05**
Sodium (mmol/L)	Routine laboratory	−0.131	**1.2E−03**
GGT (U/L)	Routine laboratory	0.121	**7.6E−04**
Spleen size (cm)	Ultrasound	0.117	**3.0E−03**
AST (U/L)	Routine laboratory	0.111	**1.9E−03**
HDL cholesterol (mg/dL)	Routine laboratory	−0.103	**1.2E−02**
Hepcidin (ng/mL)	Special laboratory	−0.094	1.7E−01

Table B.10 (continued)

Spearman rho correlation with status dead (1 or 0)			
Parameter	Category	*r*	*P*
Protein total (g/dL)	Routine laboratory	−0.093	**1.6E−02**
CK	Routine laboratory	−0.091	1.0E−01
Ferritin (ng/mL)	Routine laboratory	0.076	**3.7E−02**
Haptoglobin (g/L)	Special laboratory	−0.070	1.4E−01
CAP (dB/m)	Liver elastography	0.060	1.9E−01
Hepatic steatosis (US) (0–3)	ultrasound	0.035	3.9E−01

Table B.11 Spearman rho correlations with death in the Heidelberg cohort. Complete list. Sorted in descending order according to absolute *r* value. $P < 0.05$ in bold, <0.1 in cursive. Since the clarification of all abbreviations would exceed the space limit of the book, they are assigned to categories, e.g. lipidomics, histology or routine laboratory. Additional information is provided about whether the sample was measured prior (1) to or 1 week after (2) alcohol detoxification

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
9,10-EpOME (ng/g)		Lipidomics	−0.644	**7.1E−03**	16
12,13-EpOME (ng/g)		Lipidomics	−0.644	**7.1E−03**	16
9-HEPE (ng/g)		Lipidomics	−0.588	**1.7E−02**	16
CYP3A4		Liver mRNA	−0.545	**1.9E−02**	18
FSP1/VCP (Rep)		Liver western blotting	0.535	**2.2E−02**	18
16,17-EDP (ng/g)		Lipidomics	−0.532	**3.4E−02**	16
FMO3/b2mg		Liver mRNA	−0.520	**1.9E−02**	20
FMO3/GADH		Liver mRNA	−0.520	**1.9E−02**	20
FTL/GAPDH		Liver mRNA	−0.520	**1.9E−02**	20
ALAS1/GAPDH		Liver mRNA	−0.520	**1.9E−02**	20
CAT/b2mg		Liver mRNA	−0.520	**1.9E−02**	20
CAT/GAPDH		Liver mRNA	−0.520	**1.9E−02**	20
β-actin		Liver western blotting	−0.512	**3.0E−02**	18
NOX1 intensity (0–5)		Liver immunostaining	−0.512	**3.6E−02**	17
8-HEPE (ng/g)		Lipidomics	−0.504	**4.6E−02**	16
CYP2C19		Liver mRNA	−0.495	**3.7E−02**	18
NOX1 cytoplasma (0–3)		Liver immunostaining	−0.479	5.2E−02	17
GPX4 (Rep)		Liver western blotting	−0.478	**4.5E−02**	18
CYP2E1		Liver mRNA	−0.477	**4.5E−02**	18
Total protein (μg/μL)		Liver western blotting	−0.477	**4.5E−02**	18

(continued)

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
11,12-DiHETE (ng/g)		Lipidomics	−0.476	6.2E−02	16
10,11-EDP (ng/g)		Lipidomics	−0.476	6.2E−02	16
13,14-EDP (ng/g)		Lipidomics	−0.476	6.2E−02	16
4-HDHA (ng/g)		Lipidomics	−0.476	6.2E−02	16
RDW-CV (%)	2	Special laboratory	0.468	**2.4E−02**	23
Hepcidin/b2mg		Liver mRNA	−0.462	**4.0E−02**	20
SLCO1B1		Liver mRNA	−0.460	5.5E−02	18
5-HEPE (ng/g)		Lipidomics	−0.448	8.2E−02	16
7-HDHA (ng/g)		Lipidomics	−0.448	8.2E−02	16
IGF1 (ng/mL)	1	Special laboratory	−0.448	**4.8E−03**	38
ACSL4/VCP (Rep)		Liver western blotting	0.445	6.4E−02	18
TBL/b2mg		Liver mRNA	−0.443	6.6E−02	18
Hepcidin/GADH		Liver mRNA	−0.434	5.6E−02	20
ALAS1/b2mg		Liver mRNA	−0.434	5.6E−02	20
7,8-EDP (ng/g)		Lipidomics	−0.420	1.1E−01	16
15-HEPE (ng/g)		Lipidomics	−0.420	1.1E−01	16
Spleen stiffness (kPa)	2	Ultrasound	0.414	2.7E−01	9
Spleen stiffness (kPa)	1	Ultrasound	−0.411	2.7E−01	9
FSP (Rep)		Liver western blotting	0.410	9.1E−02	18
ABCG2		Liver mRNA	−0.409	9.2E−02	18
GPX4		Liver western blotting	−0.409	9.2E−02	18
IL-6 (pg/mL)	1	Special laboratory	0.409	**4.3E−03**	47
MT1F/b2mg		Liver mRNA	−0.405	7.7E−02	20
MT1FGAPDH		Liver mRNA	−0.405	7.7E−02	20
FMO2/b2mg		Liver mRNA	0.405	7.7E−02	20
C18:3 n-3 α (µg/g)		Lipidomics	−0.394	1.6E−01	14
5,6-EEQ (ng/g)		Lipidomics	−0.392	1.3E−01	16
RDW-SD (/fL)	2	Special laboratory	0.389	6.6E−02	23
Transferrin (g/L)	2	Special laboratory	−0.385	**7.0E−05**	101
IGF1 (ng/mL)	2	Special laboratory	−0.383	**2.1E−02**	36
Perilipin/b2mg		Liver mRNA	−0.364	1.7E−01	16
ADRP/b2mg		Liver mRNA	0.364	1.7E−01	16
11,12-DHET (ng/g)		Lipidomics	−0.364	1.7E−01	16
20-HEPE (ng/g)		Lipidomics	−0.364	1.7E−01	16
8-HDHA (ng/g)		Lipidomics	−0.364	1.7E−01	16
RelB hepatocyte-cytosol Score		Liver immunostaining	0.364	1.1E−01	20
C18:3 n-6 γ (µg/g)		Lipidomics	−0.358	2.1E−01	14

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
C20:5 n-3 (µg/g)		Lipidomics	−0.358	2.1E−01	14
ABCC2		Liver mRNA	−0.358	1.4E−01	18
HEPASCORE		Score	0.353	**1.2E−02**	50
Bcl-xL hepatocyte2 quality		Liver immunostaining	−0.351	1.4E−01	19
FTL/b2mg		Liver mRNA	−0.347	1.3E−01	20
Fibrosis stage (LS) (0–3)	1	Score	0.345	**1.7E−16**	537
Apoptosis M30 (0–3)		Histology	−0.344	6.2E−02	30
GPX4/VCP (Rep)		Liver western blotting	−0.341	1.7E−01	18
19,20-EDP (ng/g)		Lipidomics	−0.336	2.0E−01	16
9-HETE (ng/g)		Lipidomics	−0.336	2.0E−01	16
20-HETE (ng/g)		Lipidomics	−0.336	2.0E−01	16
Apoptosis aC3 (0–3)		Histology	−0.334	9.5E−02	26
Kleiner fibrosis score (0–4)		Histology	0.333	**3.4E−04**	112
C24:1 n-9 (µg/g)		Lipidomics	−0.318	3.1E−01	12
TF/GADH		Liver mRNA	−0.318	1.7E−01	20
AHR/b2mg		Liver mRNA	−0.318	1.7E−01	20
MRI Fat content (%)		Fat	−0.316	4.9E−01	7
IL-6 (pg/mL)	2	Special laboratory	0.314	**1.3E−02**	62
Forns index	1	Score	0.312	**4.0E−17**	693
Mcl-1 hepatocyte 2 quality		Liver immunostaining	0.309	2.0E−01	19
Mcl-1 hepatocyte 3 Score		Liver immunostaining	0.309	2.0E−01	19
8,9-DHET (ng/g)		Lipidomics	0.308	2.5E−01	16
11,12-EEQ (ng/g)		Lipidomics	−0.308	2.5E−01	16
18-HEPE (ng/g)		Lipidomics	−0.308	2.5E−01	16
17-HDHA (ng/g)		Lipidomics	−0.308	2.5E−01	16
ACSL4 (Rep)		Liver western blotting	0.307	2.1E−01	18
EGF (pg/mL)	2	Special laboratory	−0.307	8.7E−02	32
RelB hepatocyte-cytosol quality		Liver immunostaining	0.305	1.9E−01	20
Morphometry (%)		Morphometry	0.301	**3.2E−02**	51
Liver stiffness (kPa)	1	Ultrasound	0.299	**6.0E−17**	749
PAI 1 (ng/mL)		Special laboratory	0.295	1.0E−01	32
ALBI Fib4	1	Score	0.292	**2.1E−13**	608
EtG (ng/mL)	1	Alcohol	0.292	**9.6E−03**	78

(continued)

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
TF/b2mg		Liver mRNA	−0.289	2.2E−01	20
AHR/GAPDH		Liver mRNA	−0.289	2.2E−01	20
Steatosis (parenchymal involvement) (0–3)		Histology	−0.285	1.0E−01	34
C24:0 (µg/g)		Lipidomics	0.282	5.0E−01	8
Erythrocytes (/pL)	1	Routine laboratory	−0.281	**1.6E−15**	776
8,9-EEQ (ng/g)		Lipidomics	−0.280	2.9E−01	16
17,18-EEQ (ng/g)		Lipidomics	−0.280	2.9E−01	16
Fib4	1	Score	0.280	**2.3E−15**	773
Bcl-xL hepatocyte 3 Score		Liver immunostaining	−0.278	2.5E−01	19
EtG (ng/mL)	1	Alcohol	0.276	**1.4E−02**	78
B2MG (µg/mL)	1	ELISA	0.275	6.1E−02	47
Signs of cirrhosis (1 or 0)		Ultrasound	0.275	**4.1E−14**	727
Bilirubin direct (mg/dL)	1	Special laboratory	0.274	6.2E−02	47
AP (U/L)	1	Routine laboratory	0.269	**2.4E−14**	777
HGF (pg/mL)	1	Special laboratory	0.266	1.4E−01	32
C20:2 n-6 (µg/g)		Lipidomics	−0.266	4.6E−01	10
ABIC	1	Score	0.260	**2.7E−13**	764
NDRG1&/GAPDH		Liver mRNA	−0.260	2.7E−01	20
RelB hepatocyte-nucleus score		Liver immunostaining	0.259	2.7E−01	20
RelB cholangiocyte1 quantity		Liver immunostaining	−0.259	2.7E−01	20
MELD	1	Score	0.258	**3.9E−13**	766
Bilirubin indirect (mg/dL)	1	Special laboratory	0.258	**4.9E−03**	118
Transferrin (g/L)	1	Special laboratory	−0.257	**6.2E−11**	626
Lobular inflammation (0–3)		Histology	−0.256	1.5E−01	33
Fib4	2	Score	0.256	**2.6E−09**	526
CD163 (ng/mL)	1	Special laboratory	0.256	**6.8E−04**	173
Reticulocytes (‰)	2	Special laboratory	0.253	2.4E−01	23
Fibrosis stage (LS) (0–3)	2	Score	0.253	**5.5E−06**	315
14,15-EEQ (ng/g)		Lipidomics	−0.252	3.5E−01	16
5-HETE (ng/g)		Lipidomics	−0.252	3.5E−01	16
12-HEPE (ng/g)		Lipidomics	−0.252	3.5E−01	16
10-HDHA (ng/g)		Lipidomics	−0.252	3.5E−01	16
16-HDHA (ng/g)		Lipidomics	−0.252	3.5E−01	16
20-HDHA (ng/g)		Lipidomics	−0.252	3.5E−01	16

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
Hematocrit (%)	1	Routine laboratory	−0.252	**1.2E−12**	775
TNF alpha (pg/mL)	2	Special laboratory	0.251	**4.7E−02**	63
CLIF-C AD	2	Score	0.248	**8.3E−06**	316
LDH (U/L)	1	Routine laboratory	0.244	**4.6E−07**	416
MELD-Na	2	Score	0.242	**1.2E−05**	318
Bilirubin total (mg/dL)	1	Routine laboratory	0.242	**9.4E−12**	773
Hyaloronan (ng/mL)	2	Special laboratory	0.242	**2.3E−02**	88
Erythrocytes (/pL)	2	Routine laboratory	−0.241	*1.7E−08*	533
ACSL4		Liver western blotting	−0.240	3.4E−01	18
ABIC	2	Score	0.239	*1.2E−07*	481
ABCB11		Liver mRNA	−0.239	3.4E−01	18
GPX4/β-actin		Liver western blotting	−0.239	3.4E−01	18
Quantimeter_ALD	1	Score	0.238	*7.7E−07*	422
ALBI		Score	0.237	*3.4E−09*	609
Septa (0–3)		Histology	0.236	*1.9E−02*	99
RelB cholangiocyte2 quality		Liver immunostaining	0.235	3.2E−01	20
Microves S score (1 or 0)		Histology	0.234	1.9E−01	33
Ascites (1 or 0)		Ultrasound	0.233	*1.3E−10*	741
AP (U/L)	2	Routine laboratory	0.233	*5.3E−08*	533
Hemoglobin (g/dL)	1	Routine laboratory	−0.232	*6.5E−11*	775
GDF15-2	2	Special laboratory	0.231	*3.0E−02*	88
AST/ALT	1	Score	0.231	*7.0E−11*	776
Albumin (g/dL)	1	Special laboratory	−0.229	*1.2E−08*	608
22-HDHA (ng/g)		Lipidomics	−0.224	4.0E−01	16
Maddrey	1	Score	0.222	*4.8E−10*	768
NOX4 Nucleus (0–2)		Liver immunostaining	−0.220	4.0E−01	17
PTT (s)	1	Routine laboratory	0.219	*7.7E−09*	678
Liver stiffness (kPa)	2	Ultrasound	0.219	*1.6E−06*	472
CirrhosisScore_ Virus3G	1	Score	0.218	*7.3E−06*	417
Sodium (mmol/L)	2	Routine laboratory	−0.218	*4.8E−05*	343
FibrosisScore.1_ Virus3G	1	Score	0.217	*7.7E−06*	417
TNF alpha (pg/mL)	1	Special laboratory	0.216	*2.5E−02*	108
MELD-Na	1	Score	0.215	*1.0E−07*	603
C18:1 n-9 c oleic acid (μg/g)		Lipidomics	−0.215	4.6E−01	14

(continued)

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
Kleiner fibrosis score (0–4)		Histology	0.213	2.8E−01	28
INR	1	Routine laboratory	0.210	*3.6E−09*	772
PIIINP1 (ng/mL)	1	Special laboratory	0.210	*5.8E−06*	460
Hematocrit (%)	2	Routine laboratory	−0.210	*1.1E−06*	533
AST/ALT	2	Score	0.209	*1.0E−06*	535
RDW-CV (%)	1	Special laboratory	0.209	2.1E−01	37
WS (1–5)		Histology	0.208	*3.9E−02*	99
Quick (%)	1	Routine laboratory	−0.208	*5.9E−09*	770
Bilirubin total (mg/dL)	2	Routine laboratory	0.207	*1.5E−06*	531
Epo (mIU/mL)	1	Special laboratory	0.207	1.0E−01	63
Histological diagnosis k8/18 ASH (1 or 0)		Histology	0.206	2.7E−01	31
CLIF-C AD	1	Score	0.205	*3.6E−07*	605
Chevallier-fibrosis score (1–15)		Histology	0.204	*4.3E−02*	99
Age (years)		General information	0.204	*1.0E−08*	776
IL-6 (pg/mL)	1	Special laboratory	0.202	*2.2E−02*	128
IL-8 (pg/mL)	2	Special laboratory	−0.201	2.5E−01	35
ELF	2	Score	0.197	*6.6E−02*	88
CHILD POINTS		Score	0.197	*5.5E−08*	751
13-HODE (ng/g)		Lipidomics	−0.196	4.7E−01	16
14,15-DHET (ng/g)		Lipidomics	−0.196	4.7E−01	16
10,11-DiHDPA (ng/g)		Lipidomics	−0.196	4.7E−01	16
13,14-DiHDPA (ng/g)		Lipidomics	−0.196	4.7E−01	16
12-HETE (ng/g)		Lipidomics	0.196	4.7E−01	16
11-HDHA (ng/g)		Lipidomics	−0.196	4.7E−01	16
13-HDHA (ng/g)		Lipidomics	−0.196	4.7E−01	16
NPDx (ng/g)		Lipidomics	−0.196	4.7E−01	16
GPX8/VCP (Rep)		Liver western blotting	0.194	4.4E−01	18
PAPP-A (ng/mL)	1	Special laboratory	0.193	1.6E−01	54
Platelets (/nL)	1	Routine laboratory	−0.192	*6.8E−08*	776
RelB hepatocyte-nucleus quality		Liver immunostaining	0.192	4.2E−01	20
CD163 µg/mL	1	ELISA	0.192	2.0E−01	47
MCV (fL)	1	Routine laboratory	0.192	*1.4E−06*	625
Hyaloronan1 (ng/mL)	1	Special laboratory	0.190	*1.5E−05*	513
CRP (mg/L)	2	Routine laboratory	0.188	*1.7E−05*	519
HCC (1 or 0)		Mortality	0.187	*6.8E−07*	698
ELF	1	Score	0.187	*5.6E−05*	460

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
HGF (pg/mL)	2	Special laboratory	0.184	3.1E−01	32
E′		Echo	−0.184	*5.7E−03*	225
M65 (U/L)	1	Special laboratory	0.184	*2.8E−05*	515
Microgranulomas (1 or 0)		Histology	−0.183	*5.3E−02*	112
C20:0 (µg/g)		Lipidomics	0.183	6.4E−01	9
PT (0–3)		Histology	0.181	*7.3E−02*	99
NOX1 nucleus (0–2)		Liver immunostaining	−0.180	4.9E−01	17
C14:1 n-5 (µg/g)		Lipidomics	−0.179	5.4E−01	14
C20:1 n-9 (µg/g)		Lipidomics	−0.179	5.4E−01	14
C22:5 n-6 (µg/g)		Lipidomics	0.179	5.4E−01	14
MELD	2	Score	0.178	*8.5E−05*	481
Vitamin B12 (pmol/L)	1	Special laboratory	0.178	3.2E−01	33
FibrosisScore_ALD	1	Score	0.177	*2.7E−04*	422
Hemoglobin (g/dL)	2	Routine laboratory	−0.175	*5.1E−05*	530
CRP (mg/L)	1	Routine laboratory	0.175	*1.0E−06*	773
LDH (U/L)	2	Routine laboratory	0.174	*8.1E−02*	101
Highest degree		Family history	0.174	*8.0E−02*	102
APO A1 (mg/dL)	2	Special laboratory	−0.174	*8.2E−02*	101
HIF2a/b2mg		Liver mRNA	0.173	4.6E−01	20
TfR1/b2mg		Liver mRNA	0.173	4.6E−01	20
PALBI	1	Score	−0.173	*1.8E−05*	609
TIMP1 (ng/mL)	1	Special laboratory	0.172	*2.0E−04*	460
LDL cholesterol (mg/dL)	1	Routine laboratory	−0.170	*3.3E−05*	593
TIMP1 (ng/mL)	2	Special laboratory	0.169	1.2E−01	88
Bile acids (µmol/L)	2	Special laboratory	0.168	4.0E−01	27
Cholesterol (mg/dL)	1	Routine laboratory	−0.168	*7.9E−06*	699
14,15-DiHETE (ng/g)		Lipidomics	−0.168	5.3E−01	16
Glucose (mg/dL)	1	Routine laboratory	0.168	*6.3E−06*	715
Glasgow ASH	2	Score	0.167	*2.4E−04*	480
Weight with 20 years (kg)		Family history	0.166	1.1E−01	93
Glasgow ASH	1	Score	0.164	*5.4E−06*	761
Marihuana/Cannabis		Nutrition	0.163	1.0E−01	102
PIIINP2 (ng/mL)	2	Special laboratory	0.161	1.3E−01	88
Transferrin saturation (%)	1	Special laboratory	0.161	*6.8E−05*	606

(continued)

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
Duration of heavy alcohol drinking (years)		Alcohol	0.161	*1.8E−03*	373
Bile acids (μmol/L)	1	Special laboratory	0.160	3.0E−01	44
Encephalopathy (1 or 0)		Medical history	0.160	*1.2E−04*	569
MRI R2 (1/s)		Iron	−0.158	7.3E−01	7
Alcohol consumption (drinks per day)		Alcohol	−0.157	*4.0E−05*	680
EGF (pg/mL)	1	Special laboratory	−0.154	4.0E−01	32
Triglycerides (mg/dL)	1	Routine laboratory	−0.152	*5.4E−05*	700
edA score (0–3)		Liver immunostaining	−0.151	5.4E−01	19
MCV (fL)	2	Routine laboratory	0.149	*4.4E−03*	366
Chlormethiazole (1 or 0)		Medication	−0.148	*2.7E−04*	601
Location (0–3)		Histology	−0.147	1.3E−01	110
Alcohol consumption (g/day)		Alcohol	−0.147	*6.1E−05*	738
RelB cholangiocyte3 score		Liver immunostaining	0.145	5.4E−01	20
AST (U/L)	2	Routine laboratory	0.145	*7.8E−04*	535
NDRG1/b2mg		Liver mRNA	−0.145	5.4E−01	20
FMO2/GAPDH		Liver mRNA	0.145	5.4E−01	20
C12:0 (μg/g)		Lipidomics	−0.143	6.3E−01	14
C20:4 n-6 (μg/g)		Lipidomics	0.143	6.3E−01	14
MR-pro-ANP (pmol/L))	2	Special laboratory	0.143	1.8E−01	88
Diabetes (1 or 0)		Medical history	0.143	*1.7E−04*	686
Platelets (/nL)	2	Routine laboratory	−0.143	*9.5E−04*	533
M65 (U/L)	2	Special laboratory	0.142	*2.2E−02*	261
M30 (U/L)	1	Special laboratory	0.140	*1.4E−03*	515
8,9-DiHETE (ng/g)		Lipidomics	−0.140	6.0E−01	16
16,17-DiHDPA (ng/g)		Lipidomics	−0.140	6.0E−01	16
11-HETE (ng/g)		Lipidomics	−0.140	6.0E−01	16
14-HDHA (ng/g)		Lipidomics	−0.140	6.0E−01	16
RDW-SD (/fL)	1	Special laboratory	0.139	4.1E−01	37
Pack years		Medical history	0.137	*5.1E−04*	635
ADH Activity (U/L)			0.136	5.1E−01	26
SLC22A2		Liver mRNA	−0.136	5.9E−01	18
GGT (U/L)	2	Routine laboratory	0.134	*1.9E−03*	532

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
Ferritin (ng/mL)	2	Routine laboratory	0.132	*3.7E−03*	485
Sodium (mmol/L)	1	Routine laboratory	−0.131	*1.2E−03*	611
Ballooning k8/18 (0–2)		Histology	0.129	4.9E−01	31
Reticulocytes (‰)	1	Special laboratory	0.125	4.6E−01	38
TGF (ng/mL)	2	Special laboratory	−0.124	4.8E−01	35
Kleiner steatosis (0–3)		Histology	−0.124	1.9E−01	112
Bcl-xL hepatocyte 1 quantity		Liver immunostaining	0.122	6.2E−01	19
NOX4 Intensity (0–5)		Liver immunostaining	−0.122	6.4E−01	17
GGT (U/L)	1	Routine laboratory	0.121	*7.6E−04*	769
edA (PxS)		Liver immunostaining	−0.120	6.3E−01	19
M30 (U/L)	2	Special laboratory	0.117	*5.9E−02*	261
Spleen size (cm)		Ultrasound	0.117	*3.0E−03*	645
EGR1/b2mg		Liver mRNA	0.116	6.3E−01	20
HIF2a/GAPDH		Liver mRNA	−0.116	6.3E−01	20
S (Echo)		Echo	−0.115	*8.4E−02*	225
Maddrey	2	Score	0.115	*9.6E−03*	504
18-HETE (ng/g)		Lipidomics	−0.112	6.8E−01	16
AST (U/L)	1	Routine laboratory	0.111	*1.9E−03*	776
PINP (ng/mL)		Special laboratory	0.110	4.3E−01	53
Quick (%)	2	Routine laboratory	−0.110	*1.3E−02*	505
INR	2	Routine laboratory	0.110	*1.3E−02*	507
Glycogenated nuclei (1 or 0)		Histology	0.108	2.6E−01	112
CTRP1 (ng/mL)	1	Special laboratory	0.108	3.2E−01	88
Education		Family history	0.108	2.8E−01	102
C16:1 n-7 (µg/g)		Lipidomics	−0.107	7.1E−01	14
C18:0 (µg/g)		Lipidomics	−0.107	7.1E−01	14
C18:2 n-6 c (µg/g)		Lipidomics	−0.107	7.1E−01	14
C22:1 n-9 (µg/g)		Lipidomics	0.107	7.1E−01	14
C22:5 n-3 (µg/g)		Lipidomics	−0.107	7.1E−01	14
Copeptin (pmol/L)	2	Special laboratory	0.107	3.2E−01	88
TNF (pg/mL)	1	Special laboratory	0.106	3.4E−01	82
Marriage status		Family history	−0.106	2.9E−01	102
PEth (ng/mL)	1	Alcohol	0.105	3.6E−01	78
HDL cholesterol (mg/dL)	1	Routine laboratory	−0.103	*1.2E−02*	590
CTRP1 (ng/mL)	2	Special laboratory	0.100	3.5E−01	88

(continued)

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
Microvesicular (1 or 0)		Histology	−0.100	2.9E−01	112
Iron-macrophages (0–3)		Histology	−0.100	3.0E−01	109
Apoptosis HE score (0–3)		Histology	−0.099	6.1E−01	29
NOX4 cytoplasma (0–3)		Liver immunostaining	−0.097	7.1E−01	17
MR-pro-ANP (pmol/L)	1	Special laboratory	0.097	3.7E−01	88
NAFLD activity score (1–5)		Histology	−0.097	3.1E−01	112
Black tea		Nutrition	−0.095	3.4E−01	102
Hepcidin (ng/mL)	1	Special laboratory	−0.094	1.7E−01	214
Waist (cm)		Morphometric data	0.094	*1.4E−02*	685
Protein total (g/dL)	1	Routine laboratory	−0.093	*1.6E−02*	679
LDL cholesterol (mg/dL)	1	Routine laboratory	−0.091	1.0E−01	316
Pigmented macrophages (1 or 0)		Histology	−0.090	3.5E−01	112
Portal inflammation (1 or 0)		Histology	0.090	6.3E−01	32
IL-8 (pg/mL)	1	Special laboratory	0.089	4.3E−01	82
Hip (cm)		Morphometric data	0.088	*2.1E−02*	685
MT2a/GAPDH		Liver mRNA	0.087	7.2E−01	20
Co/b2mg		Liver mRNA	0.087	7.2E−01	20
Co/GAPDH		Liver mRNA	−0.087	7.2E−01	20
HO1/b2mg		Liver mRNA	0.087	7.2E−01	20
HO1/GAPDH		Liver mRNA	−0.087	7.2E−01	20
K19/CYFRA	1	Special laboratory	0.085	6.1E−01	38
Portal inflammation (1 or 0)		Histology	0.084	3.8E−01	112
TIP47/b2mg		Liver mRNA	−0.084	7.6E−01	16
MLDP/b2mg		Liver mRNA	−0.084	7.6E−01	16
12,13-DiHOME (ng/g)		Lipidomics	−0.084	7.6E−01	16
14,15-EET (ng/g)		Lipidomics	−0.084	7.6E−01	16
5,6-DiHETE (ng/g)		Lipidomics	−0.084	7.6E−01	16
17,18-DiHETE (ng/g)		Lipidomics	−0.084	7.6E−01	16
19-HETE (ng/g)		Lipidomics	0.084	7.6E−01	16
12-HpETE (ng/g)		Lipidomics	−0.084	7.6E−01	16
4-HNE score (0–3)		Liver immunostaining	−0.083	7.4E−01	18

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
GDF15	1	Special laboratory	0.083	4.4E−01	88
Lobular inflammation (0–3)		Histology	−0.082	3.9E−01	112
Serum alcohol level (g/L)	1	Alcohol	0.082	1.8E−01	265
Hemopexin (mg/mL)	1	ELISA	0.081	5.9E−01	47
IL-1b (pg/mL)	1	Special laboratory	0.081	5.9E−01	47
APO A1 (mg/dL)	1	Special laboratory	−0.081	*9.5E−02*	424
a2-Makroglobulin (mg/dL)	1	Special laboratory	0.079	1.0E−01	425
Ferritin (ng/mL)	1	Routine laboratory	0.076	*3.7E−02*	753
TDI-s		Echo	0.075	2.6E−01	224
Hepcidin (ng/mL)	2	Special laboratory	−0.075	4.1E−01	126
RelB hepatocyte-nucleus quantity		Liver immunostaining	0.074	7.6E−01	20
Coffee w/o coffein (cups/day)		Nutrition	−0.074	4.6E−01	102
E/A		Echo	−0.074	2.7E−01	224
Pericellular fibrosis (0–3)		Histology	0.073	6.9E−01	32
Systolic pressure (mmHg)		RR	−0.072	*7.3E−02*	616
PAP		Echo	−0.072	4.5E−01	113
C16:0 (µg/g)		Lipidomics	−0.072	8.1E−01	14
RA		Echo	0.070	2.9E−01	232
Haptoglobin (g/L)	1	Special laboratory	−0.070	1.4E−01	456
PTT (sec)	2	Routine laboratory	0.069	1.2E−01	489
Nitrate (µM)			−0.069	7.1E−01	32
TNF (pg/mL)	2	Special laboratory	−0.069	6.9E−01	35
LIC (RTS) (µg/g wet weight)		Iron	−0.069	3.7E−01	170
Mallory hyaline (1 or 0)		Histology	−0.069	4.7E−01	112
TM6SF2 TT (1 or 0)		Genes	0.067	*9.8E−02*	605
Cumulative alcohol quantity (kg per life)		Alcohol	0.065	2.1E−01	369
LV mass		Echo	0.064	7.2E−01	33
Red wine (1 or 0)		Alcohol	−0.062	2.3E−01	377
Size (m)		Morphometric data	−0.062	1.0E−01	686
HBs-antigen (1 or 0)	1	medical History	0.061	1.2E−01	651

(continued)

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
CAP (dB/m)	1	Ultrasound	0.060	1.9E–01	480
Hip/waist ratio		Morphometric data	–0.059	1.2E–01	672
EGR1/GAPDH		Liver mRNA	0.058	8.1E–01	20
MT2a/b2mg		Liver mRNA	0.058	8.1E–01	20
TfR1/GAPDH		Liver mRNA	–0.058	8.1E–01	20
Strain		Echo	–0.058	4.0E–01	218
PS (0–2)		Histology	0.057	5.7E–01	99
5,6-EET (ng/g)		Lipidomics	–0.056	8.4E–01	16
11,12-EET (ng/g)		Lipidomics	–0.056	8.4E–01	16
7,8-DiHDPA (ng/g)		Lipidomics	0.056	8.4E–01	16
8-HETE (ng/g)		Lipidomics	0.056	8.4E–01	16
15-HETE (ng/g)		Lipidomics	–0.056	8.4E–01	16
Wine (1 or 0)		Alcohol	0.055	1.6E–01	644
HbA1C (%)	1	Routine laboratory	0.054	2.0E–01	545
Septum		Echo	0.053	4.1E–01	238
K19/CYFRA	2	Special laboratory	–0.052	7.6E–01	36
Creatinine (mg/dL)	2	Routine laboratory	–0.051	2.5E–01	509
Back wall		Echo	0.050	4.4E–01	237
Leukocytes (/nL)	2	Routine laboratory	0.050	2.5E–01	534
Weight at 40 years (kg)		Family history	0.050	6.4E–01	90
LIC (AAS) (mg/g dry weight)		Iron	0.050	7.6E–01	41
Histological diagnosis HE ASH (1 or 0)		Histology	0.050	7.8E–01	33
Liver size (cm)		Ultrasound	–0.049	2.1E–01	647
a2-Makroglobulin (mg/dL)	2	Special laboratory	–0.048	6.3E–01	101
Classification steatohepatitis (0–2)		Histology	–0.048	6.2E–01	109
Diastolic pressure (mmHg)		RR	–0.046	2.5E–01	616
CYP2E1 score (0–3)		Liver immunostaining	–0.045	7.8E–01	39
CK (U/L)	2	Routine laboratory	–0.045	6.6E–01	99
RV		Echo	0.044	5.1E–01	232
TM6SF2 CC (1 or 0)		Genes	–0.044	2.8E–01	605
Liquor (1 or 0)		Alcohol	0.043	2.8E–01	636
DT-E		Echo	0.042	5.3E–01	220
Ballooning HE score (0–2)		Histology	0.041	8.2E–01	33

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
VEGF (pg/mL)	2	Special laboratory	−0.041	8.2E−01	35
LVESD		Echo	−0.040	5.5E−01	227
White wine (1 or 0)		Alcohol	0.040	4.4E−01	382
GPX8 (Rep)		Liver western blotting	0.039	8.8E−01	18
Weight discharge (kg)		Morphometric data	−0.038	8.1E−01	41
Vitamin D3 (ng/mL)	1	Special laboratory	−0.037	7.9E−01	53
VCI		Echo	−0.036	5.9E−01	219
EF		Echo	−0.036	6.0E−01	215
C14:0 (µg/g)		Lipidomics	−0.036	9.0E−01	14
C20:3 Σ (µg/g)		Lipidomics	−0.036	9.0E−01	14
Hepatic steatosis (0–3)		Ultrasound	0.035	3.9E−01	585
CT-pro-ET1 (pmol/L)	1	Special laboratory	−0.035	7.5E−01	88
A′		Echo	0.034	6.1E−01	221
ACSL4/β-actin		Liver western blotting	−0.034	8.9E−01	18
ABCB1		Liver mRNA	0.034	8.9E−01	18
MBOAT7 GC (1 or 0)		Genes	0.034	4.0E−01	618
TGF (ng/mL)	1	Special laboratory	0.034	7.6E−01	82
CD91 (µg/mL)	1	ELISA	0.033	8.2E−01	47
Catapresan (1 or 0)		Medication	−0.033	4.2E−01	594
ALT (U/L)	1	Routine laboratory	−0.032	3.7E−01	777
VEGF (pg/mL)	1	Special laboratory	−0.032	7.8E−01	82
Folic acid (nmol/L)	1	Special laboratory	−0.031	8.7E−01	32
TM6SF2 CT (1 or 0)		Genes	0.029	4.8E−01	605
Copeptin (pmol/L)	1	Special laboratory	−0.028	7.9E−01	88
MBOAT7 CC (1 or 0)		Genes	−0.028	4.8E−01	618
HSD17B13 TT (1 or 0)		Genes	0.028	5.6E−01	440
9,10-DiHOME (ng/g)		Lipidomics	−0.028	9.2E−01	16
5,6-DHET (ng/g)		Lipidomics	0.028	9.2E−01	16
19,20-DiHDPA (ng/g)		Lipidomics	−0.028	9.2E−01	16
16-HETE (ng/g)		Lipidomics	−0.028	9.2E−01	16
17-HETE (ng/g)		Lipidomics	0.028	9.2E−01	16
Beer (1 or 0)		Alcohol	0.028	4.9E−01	628
TAPSE		Echo	0.026	7.0E−01	224
Acidophil bodies (1 or 0)		Histology	0.026	7.9E−01	112
Sex (male: 1)		General information	0.025	4.8E−01	779
HSD17B13 TTA (1 or 0)		Genes	−0.025	6.1E−01	440

(continued)

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
PEth (ng/mL)	2	Alcohol	−0.024	8.4E−01	74
RelB hepatocyte-cytosol quantity		Liver immunostaining	0.023	9.2E−01	20
Hepatitis C antibody (1 or 0)	1	medical History	0.023	5.7E−01	640
LA		Echo	0.023	7.3E−01	237
Coronary heart disease (1 or 0)		Medical history	0.022	6.0E−01	563
Benzodiazipine (1 or 0)		Medication	0.022	5.9E−01	594
PNPLA3 GG (1 or 0)		Genes	−0.022	5.8E−01	632
ALT (U/L)	2	Routine laboratory	−0.022	6.1E−01	535
Creatinine (mg/dL)	1	Routine laboratory	−0.021	5.6E−01	775
edA (% pos. nuclei)		Liver immunostaining	0.020	9.4E−01	19
Lipase (U/L)	2	Routine laboratory	0.019	6.7E−01	470
Coffee black (cups/day)		Nutrition	0.019	8.5E−01	102
LVDD		Echo	0.019	7.7E−01	237
CLV (0–2)		Histology	0.017	8.7E−01	99
ERFE (ng/mL)	1	ELISA	0.017	9.1E−01	47
Urea (mg/dL)	2	Routine laboratory	−0.016	7.2E−01	508
BMI (kg/m^2)		Morphometric data	0.015	7.0E−01	682
Megamitochondria (1 or 0)		Histology	0.014	8.8E−01	111
Surface (m^2)		Morphometric data	−0.014	7.4E−01	573
MBOAT7 GG (1 or 0)		Genes	−0.014	7.4E−01	618
Smoker (1 or 0)		Medical history	−0.014	7.2E−01	687
IVRT		Echo	0.013	8.5E−01	216
Epo (mIU/mL)	2	Special laboratory	0.012	9.2E−01	63
Potassium (mmol/L)	1	Routine laboratory	−0.012	7.6E−01	611
CT-pro-ET1 (pmol/L)	2	Special laboratory	−0.012	9.1E−01	87
Hypertension (1 or 0)		Medical history	0.012	7.6E−01	686
MAPSE		Echo	−0.011	8.7E−01	224
Serum iron (µg/dL)	1	Routine laboratory	−0.011	7.8E−01	702
PNPLA3 CC (1 or 0)		Genes	0.009	8.2E−01	632
Iron hepatocytes (0–3)		Histology	−0.009	9.3E−01	109
BMP6 ng/mL	1	ELISA	−0.008	9.6E−01	47

Table B.11 (continued)

Parameter	Prior (1) or after (2) detox	Category	*r*	*P*	*N*
Large lipogranulomas (1 or 0)		Histology	−0.008	9.3E−01	112
Weight admission (kg)		Morphometric data	−0.008	8.3E−01	685
Leukocytes (/nL)	1	Routine laboratory	−0.008	8.2E−01	776
ADH act./ADH1 int.			0.008	9.7E−01	26
HSD17B13 TATA (1 or 0)		Genes	−0.007	8.8E−01	440
Ballooning (0–2)		Histology	0.007	9.4E−01	112
Urea (mg/dL)	1	Routine laboratory	−0.005	8.9E−01	771
EtOH (μmol/μL)			−0.005	9.8E−01	30
Aortic root		Echo	0.005	9.4E−01	240
Green tea		Nutrition	0.004	9.7E−01	97
Lipase (U/L)	1	Routine laboratory	0.003	9.3E−01	736
PNPLA3 CG (1 or 0)		Genes	0.003	9.5E−01	632
Coffee green (cups/day)		Nutrition	−0.003	9.8E−01	97
Coffee		Nutrition	−0.001	9.9E−01	102
CAP (dB/m)	2	Ultrasound	0.001	9.9E−01	338
Potassium (mmol/L)	2	Routine laboratory	0.000	1.0E+00	342
TBLR1/b2mg		Liver mRNA	0.000	1.0E+00	18
8,9-EET (ng/g)		Lipidomics	0.000	1.0E+00	16
C22:2 n-6 (μg/g)		Lipidomics	0.000	1.0E+00	8
C22:6 n-3 (μg/g)		Lipidomics	0.000	1.0E+00	14

Spearman Rho Correlation of Alcoholic Hepatitis (AH) Status

See Tables B.12 and B.13.

Table B.12 Spearman Rho correlation of alcoholic hepatitis (AH) status (n = 25) with total cohort of heavy drinkers (n = 1063). Note that parameters were first sorted according to P value and then according to absolute r value in descending order. Number of available parameters are shown in right column. Importantly, markers of hemolysis are significantly associated with AH but also deficiency in folic acid and lipidomics parameters

	AH		
Spearman Rho correlation with AH status	*r*	*P*	*N*
14.15-epoxyeicosatrienoic acid (ng/g)	−0.465	**2.5E−02**	23
5-HEPE (ng/g)	0.465	**2.5E−02**	23
Apoptosis aC3 0–1	0.424	**6.4E−03**	40
Megamitochondria 0–1	0.371	**1.9E−06**	156
Ascites 0/1	0.366	**7.7E−36**	1087
RDW-SD (/fL)	0.357	**1.9E−02**	43
Folic acid (nmol/L)	−0.316	**2.0E−02**	54
Vitamin B12 (pmol/L)	0.294	**1.9E−02**	63
CYP2E1 score (immunostain)	−0.277	**3.9E−02**	56
Mallory hyaline 0–1	0.275	**5.0E−04**	157
Bilirubin indirect (mg/dL)	0.270	**1.8E−05**	246
Signs of cirrhosis (US)	0.262	**3.2E−18**	1070
Bilirubin total (mg/dL)	0.245	**2.4E−17**	1163
INR	0.237	**2.8E−16**	1163
Liver stiffness (kPa)	0.235	**1.7E−15**	1116
HDL cholesterol	−0.231	**3.7E−12**	881
Transferrin (g/L)	−0.221	**4.1E−11**	872
Leukocytes (/nL)	0.220	**3.3E−14**	1163
AST/ALT	0.220	**3.5E−14**	1163
CD163 (ng/mL)	0.217	**6.9E−04**	241
Albumin (g/dL)	−0.216	**6.3E−11**	897
CRP (mg/L)	0.215	**1.3E−13**	1159
Erythrocytes (/pL)	−0.213	**2.3E−13**	1163
Hematocrit (%)	−0.199	**8.5E−12**	1162
M65 (U/L)	0.194	**3.7E−07**	675
M30 (U/L)	0.191	**5.7E−07**	675
Hemoglobin (g/dL)	−0.190	**7.1E−11**	1161
Cholesterol (mg/dL)	−0.188	**1.3E−09**	1027
APO A1 (mg/dL)	−0.186	6.4E−02	100
PTT (s)	0.185	**2.8E−09**	1013
AP (U/L)	0.185	**1.9E−10**	1162
Ballooning 0–2	0.173	**3.0E−02**	157

Table B.12 (continued)

	AH		
Spearman Rho correlation with AH status	*r*	*P*	*N*
HbA1C (%)	−0.172	**8.0E−07**	814
Kleiner fibrosis score 0–4	0.171	**3.3E−02**	156
Liver size (cm)	0.141	**1.2E−05**	955
MCV (fL)	0.141	**1.2E−05**	959
AST/GOT (U/L)	0.140	**1.5E−06**	1163
Spleen size (cm)	0.129	**7.0E−05**	945
Status dead (1/0)	0.102	**4.6E−03**	768
Hepatic steatosis (US)	0.066	5.7E−02	843
Transferrin saturation (%)	0.060	8.4E−02	844

Table B.13 Comparison between alcoholic hepatitis (AH) and alcoholic cirrhosis matched for histological fibrosis. Twenty-five patients with AH were histologically matched with patients with alcoholic liver cirrhosis. Only 7/25 (28%) had a liver biopsy, 2 with F3 fibrosis and 5 with F4 cirrhosis. All patients in the cirrhosis group had histologically proven liver cirrhosis F4. Also note that AH scores, leukocyte count and signs of hemolysis are higher in the AH group

		Alcoholic hepatitis (AH)	*T*-test	Alcoholic cirrhosis
Parameter	Units	Mean	*P*	Mean
MELD		20.1	**2.3E−06**	12.4
Quick	%	52.2	**2.5E−06**	74.4
Leukocytes	/nL	13.6	**1.5E−05**	8.5
Ascites	0 or 1	0.9	**2.8E−05**	0.3
Maddrey DF		40.6	**5.2E−05**	13.2
AH criteria		0.4	**5.4E−05**	0.0
Transferrin	g/L	1.2	**1.8E−04**	1.9
Bilirubin (total)	mg/dL	9.7	**2.4E−04**	2.9
PNPLA3 CC	0 or 1	0.6	**3.0E−04**	0.1
INR1		1.7	**3.1E−04**	1.2
Liver stiffness	kPa	69.1	**4.8E−04**	52.2
Kleiner-fibrosis 0–4	0–4	3.7	**6.3E−04**	4.0
CRP	mg/dL	30.0	**6.4E−04**	12.3
Bilirubin indirect	mg/dL	1.9	**2.2E−03**	0.4
Sodium	mmol/L	131.2	**3.6E−03**	135.4
Erythrocytes	/pL	3.1	**4.0E−03**	3.6
Cholesterol	mg/dL	137.5	**4.3E−03**	187.3
HDL cholesterol	mg/dL	18.3	**4.5E−03**	43.9
Hematocrit (%)	%	31.1	**5.2E−03**	36.2
Hyaloronan	ng/mL	1304.9	**5.8E−03**	458.6
Diabetes	1 or 0	0.0	**7.3E−03**	0.3
Platelets	/nL	196.1	**8.0E−03**	144.3
Megamitochondria	0–1	0.4	**1.1E−02**	0.1

(continued)

Table B.13 (continued)

Parameter	Units	Alcoholic hepatitis (AH) Mean	*T*-test *P*	Alcoholic cirrhosis Mean
Hemoglobin	g/dL	10.9	**1.4E−02**	12.5
Mallory hyaline	0–1	1.0	**2.1E−02**	0.5
PNPLA3 GG	0 or 1	0.0	**3.1E−02**	0.3
14.15-EET	ng/g	3244.5	**3.4E−02**	8040.7
Protein (total)	g/dL	6.5	**3.7E−02**	7.1
13.14-EDP	ng/g	929.3	**3.8E−02**	1896.4
ABIC 1	rel units	7.7	**4.9E−02**	7.0
10.11-EDP	ng/g	1147.2	5.3E−02	1869.0
5-HEPE	ng/g	583.9	5.4E−02	252.3
16.17-EDP	ng/g	771.1	5.7E−02	1453.7
CD163	ng/mL	3567.7	6.2E−02	2424.2
TM6SF2 CC	1 or 0	0.9	6.5E−02	0.7
Albumin	g/dL	3.3	7.5E−02	3.7
APO A1	mg/dL	57.6	9.4E−02	101.0

Characteristics and Significant Differences Between ALD and NAFLD

See Table B.14.

Table B.14 Characteristics and significant differences between ALD and NAFLD patients matched for gender, fibrosis and age. Parameters are sorted in ascending order according to their level of significance of the difference between ALD and NALFD. Significant differences between both groups are indicated in bold letters. ALD, alcohol-related liver disease; NAFLD, non-alcoholic fatty liver disease. For Kleiner score see Fig. A.14

Parameter	Method	*T* test matched NAFLD and ALD	Mean ALD	Mean NAFLD
Alcohol (g/d)	Reporting	**4.0E−13**	182.9	0.9
Urea	Laboratory	**3.3E−07**	20.2	5.5
Erythrocytes (/pL)	Laboratory	**1.0E−06**	4.1	4.9
Glycogenated nuclei	Histology (Kleiner)	**4.7E−05**	0.0	0.4
Pigmented macrophages	Histology (Kleiner)	**8.9E−05**	0.4	0.9
Creatinine	Laboratory	**3.1E−04**	0.7	0.9
AST (U/L)	Laboratory	**1.1E−03**	113.9	57.5
Hb (g/dL)	Laboratory	**3.0E−03**	13.6	14.9
GGT (U/L)	Laboratory	**4.7E−03**	544.1	172.7
Ferritin (ng/mL)	Laboratory	**1.1E−02**	736.9	298.3
PTT (s)	Laboratory	**2.0E−02**	33.4	30.6
CRP (mg/dL)	Laboratory	**3.9E−02**	2.2	0.4
BMI (kg/m^2)	Morphometry	6.3E−02	25.0	26.8
Glucose (mg/dL)	Laboratory	9.2E−02	97.6	108.8
Bilirubin (mg/dL)	Laboratory	9.6E−02	1.4	0.8
AP (U/L)	Laboratory	1.0E−01	133.6	89.7
Liver stiffness (kPa)	Laboratory	1.2E−01	13.5	7.1

Spearman Rho Correlation of Parameters with the Erythrophagocytosis Marker CD163

See Table B.15.

Table B.15 Spearman Rho correlation of parameters with the erythrophagocytosis marker CD163. Positive correlations are shown on the left (descending order) and negative on the right. Note that carrier proteins such as APO A1, transferrin or albumin are negatively associated with hemolysis, while markers of hemolysis and liver damage are positively correlated

	CD163			CD163	
Positive Spearman Rho	*r*	*P*	Negative Spearman Rho	*r*	*P*
Bile acids (µmol/L)	0.757	3.4E−07	APO A1 after detox (mg/dL)	**−0.772**	5.9E−07
Liver stiffness (kPa)	0.670	2.5E−33	APO A1 (mg/dL)	**−0.639**	1.6E−13
Reticulocytes after detox (‰)	0.647	8.3E−02	Albumin (g/dL)	−0.497	3.4E−12
Bilirubin indirect (mg/dL)	0.626	2.5E−07	Transferrin (g/L)	−0.455	3.8E−11
Maddrey	0.580	7.9E−23	Hemoglobin (g/dL)	−0.254	5.6E−05
Bilirubin total (mg/dL)	0.562	8.2E−22	Hemopexin (mg/mL)	−0.236	4.0E−02
M30 (U/L)	0.547	1.8E−20	Serum iron (µg/dL)	−0.067	3.0E−01
AST (U/L)	0.533	1.5E−19			
Reticulocytes (‰)	0.451	1.2E−01			
ERFE (ng/mL)	0.436	1.0E−04			
MCV (fL)	0.345	9.1E−08			
CRP (mg/L)	0.323	2.3E−07			
Ferritin (ng/mL)	0.289	4.2E−06			
GPT (U/L)	0.255	5.1E−05			

ALD Patients with Ineffective Erythropoiesis (Elevated Ferritin, 52.7%) with Anemia vs No Anemia

See Tables B.16 and B.17.

Table B.16 Routine parameters in heavy drinkers with ineffective erythropoiesis that best discriminate those with anemia from those without anemia. Ineffective erythropoiesis was considered when ferritin was elevated (52.7% of total group). Note that bilirubin carrier albumin and iron carrier transferrin are downregulated in the anemia group

			Anemia		No anemia		*T* test
Parameter	Units	Normal range	Mean	*N*	Mean	*N*	*P*
Hemoglobin	g/dL	13.5–17.5	11.36	*133*	14.98	*362*	**3.8E−101**
Erythrocytes	/pL	4.5–5.9	3.28	*133*	4.47	*362*	**5.5E−76**
Albumin	g/dL	3.82–5.92	3.64	*94*	4.45	*283*	**1.0E−26**
Transferrin	g/L	2–3.6	1.58	*97*	2.27	*299*	**8.5E−23**
Ascites	0 or 1		0.36	*125*	0.05	*337*	**3.4E−20**
Liver stiffness	kPa	<6	36.01	*128*	16.59	*349*	**3.4E−16**
AP	U/L	40–130	169.59	*132*	111.27	*361*	**1.6E−13**
Bilirubin total	mg/dL	<1.3	4.43	*133*	1.41	*359*	**2.8E−13**
INR		0.85–1.15	1.21	*132*	1.00	*360*	**1.7E−12**
Serum iron	µg/dL	59–158	111.26	*120*	148.51	*323*	**4.9E−09**
Urea	mg/dL	< 50	29.62	*132*	21.30	*360*	**5.0E−07**
APO A1	mg/dL		139.39	*48*	199.02	*146*	**5.7E−07**
HDL cholesterol	mg/dL	>40	49.93	*95*	72.58	*278*	**8.7E−07**
CRP	mg/dL	<0.5	18.59	*133*	7.24	*361*	**1.0E−06**
Status death	0 or 1		0.40	*96*	0.18	*249*	**1.2E−05**
Spleen size	cm		11.14	*109*	10.04	*292*	**1.6E−05**
Leukocytes	/nL	3.7–10.0	8.65	*133*	7.36	*362*	**1.1E−04**
MCV	fL	80–96	99.07	*120*	94.79	*300*	**1.6E−04**
Bilirubin indirect	mg/dL	<0.8	1.01	*30*	0.40	*82*	**3.1E−04**
ALT	U/L	<50	70.19	*133*	99.92	*362*	**2.0E−03**
Cholesterol	mg/dL	<200	201.21	*106*	226.30	*323*	**4.2E−03**
LDH	U/L	<250	304.36	*90*	253.74	*200*	**4.6E−03**
Transferrin saturation	%	16–45	59.07	*93*	50.71	*284*	**5.5E−03**
Transferrin	g/L	2–3.6	1.89	*6*	2.64	*20*	**9.8E−03**
Ferritin	ng/mL	30–400	1248.31	*133*	1075.76	*362*	**1.0E−02**
Age	years		55.20	*133*	52.54	*361*	**1.0E−02**
CAP	(dB/m)	<240	289.19	*84*	305.63	*249*	**1.5E−02**
LDL cholesterol	mg/dL	<160	100.87	*95*	113.50	*278*	**3.4E−02**
a2-Makroglobulin 2	mg/dL	480–940	219.92	*13*	183.98	*44*	**4.0E−02**
CD163	ng/mL	<800	2041.76	*44*	1675.11	*80*	**4.8E−02**
M65	U/M	<400	1694.48	*69*	1313.29	*227*	6.1E−02
GGT	U/L	<60	733.50	*133*	596.94	*359*	7.6E−02

(continued)

Table B.16 (continued)

Parameter	Units	Normal range	Anemia		No anemia		*T* test
			Mean	*N*	Mean	*N*	*P*
Triglycerides	mg/dL	<200	186.02	*107*	242.54	*325*	8.8E−02
M30	U/L	<200	940.59	*69*	745.35	*227*	1.2E−01
Platelets	/nL	150–360	181.05	*133*	188.91	*362*	3.6E−01
AST	U/L	<50	132.73	*133*	143.41	*362*	4.0E−01
Uric acid	mg/dL	2.6–6.4	6.74	*8*	6.42	*27*	7.4E−01
Folic acid	nmol/L		11.59	*12*	12.02	*21*	8.8E−01
Vitamin B12	pmol/L		653.15	*13*	663.96	*22*	9.4E−01

Table B.17 Special iron-related parameters, marker of erythropoiesis and cytokines in heavy drinkers with ineffective erythropoiesis with and without anemia. Iron-related parameters include important upstream regulators of hepcidin. Reticulocytes boost after alcohol detox and under elevated erythropoietin (EPO). Hepcidin is non-significantly suppressed in the anemia group. Data on upstream regulators are non-conclusive: BMP6, IL1ß and IL6 are suppressed, but ERFE is also suppressed (negative regulator). These patient data underline that, in addition to specific regulation of hepcidin, iron homeostasis is controlled by other mechanisms, e.g. erythrophagocytosis and most likely efferocytosis. Numbers (1 or 2) after each parameter refer time before or after alcohol detoxification

Parameter	Units	Normal range	Anemia		No anemia		*T* test
			Mean	*N*	Mean	*N*	*P*
Hepcidin levels							
Hepcidin	ng/mL		16.90	*49*	20.58	*95*	1.2E−01
Hepcidin mRNA	mRNA		0.90	*2*	1.20	*11*	3.5E−01
Iron compartment							
Hemoglobin	g/dL	13.5–17.5	11.36	*133*	14.98	*362*	**3.8E−101**
Serum iron	µg/dL	59–158	111.26	*120*	148.51	*323*	**4.9E−09**
Ferritin (ng/mL)1	ng/mL	30–400	1248.31	*133*	1075.76	*362*	**1.0E−02**
Intracellular iron							
Pigmented macrophages 0–1	0–1		0.59	*27*	0.39	*52*	8.5E−02
Iron stain Kupffer cells	0–4		1.07	*27*	0.75	*51*	1.1E−01
Iron stain hepatocytes (0–4)	0–4		0.96	*27*	0.70	*51*	2.0E−01
Quantitative iron detection							
Liver iron concentration (RTS)	µg/g ww		321.6	*41*	333.4	*93*	8.9E−01
Liver iron concentration (AAS)	mg/g dw	<2	1.95	*20*	1.34	*26*	1.6E−01
Important hepcidin regulators							
ERFE	ng/mL		0.39	*15*	1.30	*29*	3.0E−01
BMP6	ng/mL		0.10	*15*	0.35	*30*	2.8E−01
TNF alpha	pg/mL		5.72	*6*	3.03	*16*	1.2E−01
GDF15-1	U/L		3474.9	*7*	2910.6	*33*	5.8E−01
IL-8	pg/mL		104.3	*18*	66.0	*34*	1.3E−01
IL-6	pg/mL		28.4	*16*	125.8	*30*	1.3E−01

Table B.17 (continued)

Parameter	Units	Normal range	Anemia		No anemia		*T* test
			Mean	*N*	Mean	*N*	*P*
IL-1b	pg/mL		20.8	*16*	103.3	*30*	1.8E−01
PRX2 ox/red	rel units		0.86	*5*	2.69	*5*	2.8E−01
NOX4	rel units		1.00	*6*	2.20	*5*	1.3E−01
Erythropoiesis							
EPO1	mIU/mL		13.2	*6*	7.4	*27*	1.0E−01
Reticulocytes 1	‰	8–25	28.5	*7*	18.0	*12*	1.1E−01
Reticulocytes 2	‰	8–25	41.5	*4*	18.0	*6*	**2.0E−02**
Erythrocytes 1	/pL	4.5–5.9	3.28	*133*	4.47	*362*	**5.5E−76**

Correlation with Levels of AST/GOT in Heavy Drinkers

See Tables B.18 and B.19.

Table B.18 Correlation with levels of AST/GOT in heavy drinkers. AST correlates highly with markers of necrosis and apoptosis (M65/M30), but also markers of RBC/heme turnover such as LDH, ferritin and CD163. AST levels are also highly associated with lipidomics parameters. As is discussed in the chapter on AST, most of serum AST elevation is due AST derived from red blood cells

	AST/GOT (U/L)	
Spearman	*r*	*P*
ALT (U/L)	0.814	1.6E−295
M65	0.775	3.4E−138
C24:1 n-9	0.730	1.3E−03
C16:1 n-7	0.729	4.0E−04
GGT (U/L)	0.708	7.0E−188
C14:1 n-5	0.706	7.4E−04
M30	0.697	5.0E−101
C18:1 n-9 c (oleic acid)	0.670	1.7E−03
C20:2 n-6	0.669	8.9E−03
C20:1 n-9	0.649	2.6E−03
C18:2 n-6 c	0.635	3.5E−03
C16:0	0.630	3.8E−03
C12:0	0.624	4.3E−03
C18:0	0.622	4.4E−03
LDH (U/L)	0.617	3.8E−77
ABCG2	−0.598	1.3E−03
C14:0	0.590	7.9E−03
8-HETE	−0.554	5.0E−03
C20:3 Σ	0.549	1.5E−02
C18:3 n-3 α	0.545	1.6E−02
Ferritin (ng/mL)	0.540	9.5E−92
CD163 1 (ng/mL)	0.533	1.5E−19
C18:3 n-6 γ	0.533	1.9E−02
8,9-EET	−0.514	1.0E−02
C22:6 n-3	0.499	3.0E−02
MLDP/b2mg	−0.493	1.4E−02
Vitamin B12 (pmol/L)	0.491	1.9E−06
11-HETE	−0.480	1.8E−02
SLCO1B1	−0.478	1.4E−02
ERFE (ng/mL)	0.459	4.0E−05
11,12-EET	−0.448	2.8E−02

Table B.19 Correlation with AST/GOT levels (continued)

	AST (U/L)	
Spearman	*r*	*P*
AP (U/L)	0.433	5.5E−58
ABCC2	−0.429	2.9E−02
Bile acids (μmol/L)	0.426	4.4E−04
Bilirubin total (mg/dL)	0.424	3.6E−55
5,6-EET	−0.422	4.0E−02
Liver stiffness (kPa)	0.411	4.1E−50
CAT/b2mg	−0.377	4.8E−02
Reticulocytes (‰)	0.353	1.7E−02
EtG (ng/mL)	0.343	1.3E−02
Platelets (/nL)	0.340	6.3E−26
Ballooning (0–2)	0.333	1.5E−05
Liver size (cm)	0.316	3.3E−25
Bilirubin indirect (mg/dL)	0.307	9.1E−08
VEGF (pg/mL)	−0.307	9.0E−04
Actual drinking (1 or 0)	0.306	1.1E−05
Transferrin saturation (%)	0.297	2.3E−19
MCV (fL)	0.286	7.0E−21
Transferin (g/L)	−0.284	4.6E−02
CAP (dB/m)	0.278	4.7E−16
Erythrocytes (/pL)	−0.252	2.2E−19
Haptoglobin (g/L)	−0.210	9.0E−06
Megamitochondria (0–1)	0.157	4.7E−02
Signs of liver cirrhosis	0.147	1.4E−05
LIC-RTS (μg/g wet weight)	−0.123	4.3E−02
HSD17B13 TT (1 or 0)	0.121	3.2E−03
Status dead (1 or 0)	0.112	1.7E−03
Alcohol consumption (drinks per day)	0.098	1.4E−03
Benzodiazepin (tranxilium)	0.096	3.8E−03
Alcohol consumption (g/day)	0.087	3.2E−03
Hemoglobin (g/dL)	−0.079	5.2E−03
Wine (1 or 0)	0.077	1.5E−02
Glucose (mg/dL)	0.067	2.2E−02
Spleen size (cm)	0.063	4.4E−02

Differences Between High and Normal MCV in Heavy Drinkers

See Tables B.20, B.21, B.22, B.23, B.24, and B.25.

Table B.20 Various parameters (mortality, hematopoiesis, iron) in heavy drinkers grouped according to red blood cell size (MCV). Note, that drinkers with signs of hemolytic anemia (high MCV, signs of hemolysis, decreased hemoglobin) have a three-times increased mortality. Hemolytic anemia is also not due to the lack of folic acid and vitamin B12. Potential other causes are related either directly to RBC or bone marrow toxicity

Groups	Units	Normal range	*P**	High MCV >96	Normal MCV 80–96	Low MCV <80	All
MCV	fl	80–96	***	101.7	90.4	65.2	93.4
Percentage	%			31.8%	65.7%	2.5%	100.0%
Hemoglobin	g/dL	>12.5	***	13.4	14.4	12.1	14
Anemia fraction				28.4%	13.0%	33.7%	19.7%
Erythrocytes	/pL	4.5–5.9	***	3.7	4.5	4.7	4.3
Hematocrit	%	40–53	***	37.8	40.8	35.8	39.8
All-cause mortality			***	31.1%	11.6%	20.0%	
Parameters of hematopoiesis							
Vitamin B12	pmol/L	145–596		616.2	494.4	341.0	524.5
Folic acid	nmol/L	>7.1	**	10.7	17.3	8.6	15.3
Erythropoietin (EPO)	IU/mL	6–15	**	11.7	6.2	0.5	8.0
Reticulocytes	‰	8–25	***	27.0	15.4	16.8	19.5
Parameters of iron metabolism							
Ferritin	ng/mL	>400/150	***	853.4	484.1	272.1	594
Elevated ferritin fraction				62.9%	38.0%	20.0%	44.9%
Transferrin	g/L	2–3.6	***	2.0	2.5	2.6	2.4
Serum iron	µg/dL	95–158		129.1	122.2	103.7	123.9
Transferrin saturation	%	16–45	***	49.5	38.9	32.6	42.1
Hepcidin	ng/mL	1–55	**	13.9	17.1	24.4	15.8

*P** comparison between high and normal MCV * <0.05, ** <0.01, *** <0.005

Table B.21 Various parameters (hemolysis, liver) in heavy drinkers grouped according to red blood cell size (MCV). Hemolytic anemia is also tightly linked to liver damage, as shown by elevated transaminases, bilirubin and liver stiffness. Although sigs of hemolysis occur already prior to the onset of liver damage, progressing cirrhosis further deteriorates RBC turnover. CD163, the hemoglobin-haptoglobin scavenging receptor is also highest in the high MCV group

Groups	Units	Normal	*P**	High MCV >96	Normal MCV 80–96	Low MCV <80	All
MCV	fL	80–96	***	101.7	90.4	65.2	93.4
Percentage	%			31.8%	65.7%	2.5%	100.0%
Parameters of erythrophagocytosis/hemolysis							
CD163	ng/mL	<1500	***	1945.0	1325.8	1149.8	1686.3
Elevated CD163 fraction	ng/mL		***	59.6%	32.5%	38.5%	44.7%
Bilirubin indirect	mg/dL	0.2–0.8	*	0.57	0.40	0.37	0.46
LDH	U/L	<250	***	268.9	223.8	210.6	238.5
Haptoglobin	g/L	0.3–2.0	*	1.3	1.5	1.7	1.4
Liver parameters							
Liver stiffness (fibrosis)	kPa	< 6 kPa	***	27.8	12.8	17.1	17.7
CAP (steatosis)	dB/m	<240	*	283.7	268.4	286.2	294.1
GOT	U/L	<50	***	118.9	84.8	81.3	95.6
GPT	U/L	<50	ns	66.9	68.2	60.3	67.1
GGT	U/L	<60	***	601.5	304.9	348.3	400.3
AP	U/L	40–130	***	134.3	101.9	123.9	112.8
Bilirubin total	mg/dL	<1.3	***	2.5	1.1	0.8	1.6
Albumin	g/dL	3.4–5.4	***	4.1	4.4	4.1	4.3

*P** comparison between high and normal MCV. * <0.05, ** <0.01, *** <0.005

Table B.22 Differences between high and normal MCV in heavy drinkers (complete list). The data may help to identify the underlying mechanisms that lead to increased mean corpuscular volume (MCV) in heavy drinkers. Each parameter is allocated to a category. Numbers 1 and 2 after each parameter refer to the time either prior or after alcohol detoxification

Category	Parameter	*T* test high vs normal	Ratio	Mean: High MCV >96	Mean: Normal MCV 80–96	Mean: Low MCV <80
Routine laboratory	MCV/Ery (/fL)	**3.2E−78**	1.40	28.5	20.4	15.2
Routine laboratory	Erythrocytes (/pL)1	**5.8E−69**	0.83	3.7	4.5	4.5
Routine laboratory	Erythrocytes (/pL)2	**5.6E−53**	0.82	3.6	4.4	4.5

(continued)

Table B.22 (continued)

Category	Parameter	*T* test high vs normal	Ratio	Mean		
				High MCV	Normal MCV	Low MCV
				>96	80–96	<80
Liver elastography	Liver stiffness (kPa)1	**2.2E−24**	2.16	27.8	12.8	17.1
Special laboratory	Transferrin 1 (g/L)	**5.9E−23**	0.80	2.0	2.5	2.6
Routine laboratory	Ferritin (ng/mL)1	**7.2E−17**	1.76	853.4	484.1	272.1
Routine laboratory	Hematocrit (%)1	**8.5E−17**	0.93	37.8	40.8	35.8
Ultrasound	Signs of cirrhosis (US) (1 or 0)	**5.4E−13**	2.48	0.3	0.1	0.2
Liver elastography	Liver stiffness (kPa)2	**1.1E−12**	2.19	23.7	10.8	11.0
Routine laboratory	Hemoglobin (g/dL)1	**3.2E−11**	0.93	13.4	14.4	12.1
Special laboratory	Albumin (g/dL)1	**6.4E−11**	0.92	4.1	4.4	4.1
Clinics	Ascites (1 or 0)	**6.4E−11**	1.13	1.2	1.0	1.1
AH score	Maddrey1	**1.9E−10**	72.94	7.3	0.1	1.2
Routine laboratory	Bilirubin total (mg/dL)1	**8.4E−10**	2.24	2.5	1.1	0.8
Clinics	Status death (1 or 0 –yes or no)	**2.1E−09**	2.68	0.3	0.1	0.2
Routine laboratory	Transferrin saturation 1	**5.7E−08**	1.27	49.5	38.9	32.6
Routine laboratory	INR1	**1.1E−07**	1.10	1.2	1.1	1.1
Special laboratory	M30-1	**2.3E−07**	1.95	870.6	447.5	247.1
Routine laboratory	LDH (U/L)1	**4.5E−07**	1.20	268.9	223.8	210.6
Routine laboratory	AST (U/L)1	**8.2E−07**	1.40	118.9	84.8	81.3
Routine laboratory	Bilirubin total (mg/dL)2	**1.1E−06**	2.38	2.0	0.9	0.4
AH score	Glasgow ASH 1	**8.7E−06**	1.05	6.2	5.9	5.6
Ultrasound	Hepatic steatosis (US)	**9.8E−06**	1.19	2.0	1.7	1.5
Special laboratory	CD163 1 all (ng/mL)	**1.4E−05**	1.47	1945.0	1325.8	1149.8
Routine laboratory	AST (U/L)2	**1.6E−05**	1.38	74.4	53.7	33.6

Table B.22 (continued)

Category	Parameter	*T* test high vs normal	Ratio	Mean		
				High MCV	Normal MCV	Low MCV
				>96	80–96	<80
Special laboratory	M30 (U/L)2	**1.4E−04**	1.60	664.6	414.6	141.7
Special laboratory	Transferrin (g/L)2	**2.4E−04**	0.78	1.7	2.2	
Clinics	Alcohol consumption (g/day)	**5.7E−04**	0.79	169.8	215.9	188.2
Histology	Kleiner steatosis 0–3	**7.4E−04**	1.36	2.1	1.5	1.8
Routine laboratory	Reticulocytes1 (‰)	**1.0E−03**	1.75	27.0	15.4	24.5
Special laboratory	APO A1 2 (mg/dL)	**1.3E−03**	0.76	96.6	127.6	
Ultrasound	Liver size (cm)	**1.7E−03**	1.04	16.5	15.9	16.1
Routine laboratory	LDL cholesterol (mg/dL)	**1.8E−03**	0.90	103.8	115.8	91.8
Special laboratory	Epo (mIU/mL)1	**2.0E−03**	1.88	11.7	6.2	0.5
Histology	Ballooning 0–2	**4.1E−03**	1.50	1.1	0.7	0.3
Lipidomics	C18:0 (µg/g)	**5.1E−03**	2.14	30825.6	14402.0	
Routine laboratory	Reticulocytes2 (‰)	**6.8E−03**	1.94	31.4	16.2	34.0

Table B.23 Differences between high and normal MCV in heavy drinkers (continued)

Category	Parameter	*T* test high vs normal	Ratio	Mean		
				High MCV	Normal MCV	Low MCV
				>96	80–96	<80
Special laboratory	GDF15 (U/L)2	**6.9E−03**	1.82	1882.7	1035.5	953.5
Special laboratory	Folic acid (nmol/L)	**9.3E−03**	0.62	10.7	17.3	8.6
Special laboratory	Bile acids (µmol/L)2	**1.2E−02**	6.52	37.4	5.7	3.4
Routine laboratory	HDL cholesterol (mg/dL)	**1.6E−02**	0.90	65.3	72.3	65.4
Lipidomics	C18:1 n-9 c	**1.7E−02**	2.36	145131.0	61604.7	
Lipidomics	C16:0	**1.7E−02**	2.41	98847.2	41020.4	
Special laboratory	APO A1 1 (mg/dL)	**2.0E−02**	0.90	171.9	191.0	153.8
Special laboratory	Hemopexin mg/mL	**2.5E−02**	0.66	0.3	0.5	0.2
Liver elastography	CAP1(dB/m)	**2.7E−02**	1.04	294.1	283.7	268.4

Table B.23 (continued)

Category	Parameter	*T* test high vs normal	Ratio	Mean		
				High MCV	Normal MCV	Low MCV
				>96	80–96	<80
Special laboratory	Hepcidin (µg/mL)1	**2.8E−02**	0.81	13.9	17.1	24.4
Special laboratory	Bile acids (µmol/L)1	**3.0E−02**	2.18	31.9	14.6	6.0
Special laboratory	Haptoglobin (g/L)1	**3.0E−02**	0.86	1.3	1.5	1.7
Special laboratory	Transferrin (g/L)1	**3.1E−02**	0.83	2.3	2.8	
Heme	Bilirubin indirect (mg/dL)	6.2E−02	1.43	0.6	0.4	0.3
Routine laboratory	Protein total (g/dL)1	6.8E−02	0.96	7.0	7.3	7.2
Histology	Ballooning HE score	6.9E−02	3.75	0.5	0.1	1.0
mRNA	Catalase/GAPDH	8.2E−02	0.77	1.1	1.4	
Routine laboratory	LDH (U/L)2	8.4E−02	1.22	193.3	158.4	
mRNA	NDRG1/GAPDH	8.8E−02	0.75	1.0	1.3	
Lipidomics	5,6-EET	1.1E−01	0.74	2701.2	3663.2	
Special laboratory	GDF15 (U/L)1	1.1E−01	1.42	2540.0	1791.5	1337.4
Special laboratory	IGF1 1	1.2E−01	0.86	89.9	104.0	
AH score	Glasgow AH score	1.2E−01	0.95	6.2	6.6	7.0
Histology	Glycogenated nuclei 0–1	1.3E−01	0.51	0.1	0.2	0.0
Routine laboratory	Serum iron (µg/dL)	1.3E−01	1.06	129.1	122.2	103.7
Histology	Lobular inflammation 0–3	1.3E−01	1.15	1.6	1.4	1.0
Special laboratory	HGF (pg/mL)1	1.4E−01	1.43	921.5	644.1	
Special laboratory	Vitamin B12 (pmol/L)	1.6E−01	1.25	616.2	494.4	341.0
Western blotting	GPX8 (Rep)	1.7E−01	3.61	0.4	0.1	0.0
Lipidomics	Cholesterol (mg/dL)	1.8E−01	0.97	206.2	212.9	196.7
Western blotting	GPX8/VCP (Rep)	1.8E−01	5.66	0.8	0.1	0.0
Special laboratory	CD91 (µg/mL)	1.9E−01	0.80	5.1	6.4	4.6
mRNA	FTL/GAPDH	2.0E−01	0.89	1.4	1.6	

Table B.23 (continued)

Category	Parameter	*T* test high vs normal	Ratio	Mean		
				High MCV	Normal MCV	Low MCV
				>96	80–96	<80
Special laboratory	Vitamin D3 (ng/mL)	2.2E−01	0.79	10.3	13.0	7.2
mRNA	MT1FGAPDH	2.2E−01	0.70	0.9	1.2	
mRNA	FMO2/GAPDH	2.6E−01	1.18	0.8	0.7	
Special laboratory	PEth (ng/mL)2	2.7E−01	0.82	691.1	841.7	1545.0
mRNA	Coeruloplasmin/ b2mg	2.7E−01	1.19	1.1	1.0	

Table B.24 Differences between high and normal MCV in heavy drinkers (continued)

Category	Parameter	*T* test high vs normal	Ratio	Mean		
				High MCV	Normal MCV	Low MCV
				>96	80–96	<80
mRNA	HO1/b2mg	2.7E−01	1.19	0.0	0.0	
Lipidomics	5-HEPE	3.1E−01	1.38	253.1	183.9	
Western blotting	GPX4	3.2E−01	0.68	0.3	0.5	0.6
Special laboratory	VEGF 1	3.4E−01	0.88	71.2	80.5	133.3
Immunostaining	NOX1 Nucleus	3.4E−01	1.56	1.3	0.8	
mRNA	ALAS1/GAPDH	3.6E−01	0.87	0.6	0.7	
mRNA	EGR1/GAPDH	3.7E−01	1.73	0.5	0.3	
Routine laboratory	GPT (U/L)2	3.8E−01	0.92	54.4	59.1	41.5
mRNA	MLDP/b2mg	3.9E−01	0.87	3.8	4.4	
Special laboratory	Hepcidin (µg/mL)2	4.1E−01	0.90	18.0	19.9	24.7
Routine laboratory	Triglycerides (mg/dL)	4.1E−01	0.93	182.2	196.6	189.8
mRNA	Coeruloplasmin/ GAPDH	4.3E−01	1.13	1.2	1.0	
mRNA	ADRP/b2mg	4.4E−01	1.24	0.8	0.6	
mRNA	AHR/GAPDH	4.8E−01	0.92	1.0	1.1	
Special laboratory	Ethylglucuronid1 (mg/L)	4.9E−01	0.22	8.6	39.6	
Immunostaining	TfR1	4.9E−01	0.90	1.6	1.8	
Western blotting	ACSL4/VCP (Rep)	4.9E−01	1.71	0.7	0.4	0.1
Iron	LIC-AAS (mg/g dry weight)	5.1E−01	1.19	1.5	1.2	0.2
Immunostaining	NOX4 Cytoplasm	5.2E−01	0.71	1.0	1.4	
Blood count	delta Reticulocytes (‰)	5.6E−01	6.25	−4.2	−0.7	−1.0
Special laboratory	a2-Makroglobulin 2 (mg/dL)	5.8E−01	1.04	200.5	192.9	

(continued)

Table B.24 (continued)

Category	Parameter	*T* test high vs normal	Ratio	Mean		
				High MCV	Normal MCV	Low MCV
				>96	80–96	<80
mRNA	HIF2a/b2mg	5.9E−01	1.05	0.8	0.8	
mRNA	hepcidin/GADH	6.0E−01	0.89	1.1	1.2	
Special laboratory	PEth1 (ng/mL)	6.1E−01	0.91	1588.0	1739.6	2750.0
mRNA	HO1/GAPDH	6.2E−01	1.11	0.0	0.0	
mRNA	AHR/b2mg	6.2E−01	0.94	1.0	1.0	
Immunostaining	NOX4 Nucleus	6.3E−01	1.25	1.5	1.2	
Special laboratory	a2-Makroglobulin 1 (mg/dL)	6.4E−01	1.02	274.8	269.6	271.9
Immunostaining	NOX1 Intensity	6.5E−01	0.93	2.4	2.6	
Special laboratory	EGF-1	6.5E−01	1.18	7.6	6.5	
Special laboratory	IGF1-2	6.5E−01	0.94	133.0	140.9	
mRNA	CYP3A4	6.7E−01	0.85	1.8	2.1	
mRNA	ACSL4/β-actin	7.0E−01	0.78	0.4	0.5	0.0
Special laboratory	VEGF 2	7.2E−01	1.07	62.3	58.1	86.0
mRNA	FMO3/b2mg	7.3E−01	0.97	1.0	1.1	
Special laboratory	EGF-2	7.4E−01	1.08	16.7	15.5	
mRNA	TfR1/b2mg	7.5E−01	1.06	1.3	1.2	

Table B.25 Differences between high and normal MCV in heavy drinkers (continued)

Category	Parameter	*T* test high vs normal	Ratio	Mean		
				High MCV	Normal MCV	Low MCV
				>96	80–96	<80
mRNA	FMO3/GADH	7.6E−01	0.96	1.1	1.1	
Immunostaining	ACSL4 (Rep)	7.7E−01	1.25	0.2	0.2	0.0
Immunostaining	GPX4 (Rep)	8.0E−01	1.10	0.5	0.5	0.7
Special laboratory	Epo (mIU/mL)2	8.0E−01	1.06	6.7	6.3	0.5
mRNA	ABCB11	8.1E−01	0.91	1.6	1.8	
mRNA	MT2a/GAPDH	8.3E−01	0.88	4.2	4.7	
Iron	LIC-RTS (µg/g wet weight)	8.3E−01	1.05	299.6	286.6	637.0
mRNA	MT2a/b2mg	8.4E−01	0.89	4.0	4.5	
mRNA	HIF2a/GAPDH	8.5E−01	1.02	0.8	0.8	
Immunostaining	ACSL4	8.5E−01	1.14	0.3	0.2	0.0
Histology	Portal inflammation 0–1	8.5E−01	0.97	0.4	0.5	0.5

Table B.25 (continued)

Category	Parameter	*T* test high vs normal	Ratio	Mean		
				High MCV	Normal MCV	Low MCV
				>96	80–96	<80
Routine laboratory	ALT (U/L)1	9.0E−01	0.99	66.9	67.6	55.3
Lipidomics	19,20-DiHDPA	9.3E−01	1.02	334.9	328.4	
mRNA	Transferrin/GADH	9.4E−01	1.01	1.1	1.1	
Immunostaining	NOX1 cytoplasma	9.4E−01	1.02	2.3	2.2	
Immunostaining	FSP1/VCP (Rep)	9.5E−01	1.06	0.7	0.7	0.0
mRNA	ABCB1	9.6E−01	1.01	1.2	1.2	
Special laboratory	Haptoglobin1 (g/L)	9.6E−01	1.00	1.4	1.4	1.6
mRNA	TfR1/GAPDH	9.7E−01	0.99	1.3	1.3	
mRNA	Transferrin/b2mg	9.9E−01	1.00	1.0	1.0	

Parameters in Heavy Drinkers That Discriminate Between Low and High Hemoglobin

See Tables B.26, B.27, B.28, and B.29.

Table B.26 Parameters (demographics, ultrasound, scores) in heavy drinkers that discriminate between low and high hemoglobin. A hemoglobin of 14 g/dL was used as cut-off value. Parameters are grouped into categories and sorted according to the *P* value. Only patients with ineffective erythropoiesis (elevated ferritin) were used which represent 55.7% of the total cohort. Number of patients used for *T*-test are shown in cursive letters. A *P* value <0.05 is depicted in bold

Parameter	Units	Normal range	Hb < 14 g/L		Hb > 14 g/L		*P*
			Mean	*N*	Mean	*N*	
Demographics							
Gender	Male =1		0.64	*202*	0.84	*293*	**4.5E−08**
Status death	0 or 1		0.35	*137*	0.17	*208*	**1.0E−04**
Age	years		55.4	*202*	51.8	*292*	**1.1E−04**
Weight (admission)	Kg/m^2		76.5	*182*	81.9	*267*	**7.7E−04**
Smoker	1 or 0		0.55	*180*	0.70	*264*	**1.7E−03**
BMI	kg/m^2	18–25	25.4	*172*	26.2	*259*	6.5E−02
Coronary heart disease	1 or 0		0.03	*117*	0.04	*210*	8.3E−01
Liver related death (1 or 0)	1 or 0		0.39	*24*	0.37	*19*	8.7E−01
Ultrasound/elastography							
Ascites	1 or 0		0.27	*190*	0.04	*272*	**1.9E−14**
Liver stiffness	kPa	<6	31.1	*195*	15.2	*282*	**1.4E−13**
Signs of cirrhosis (US)	1 or 0		0.38	*185*	0.12	*270*	**2.3E−11**
Spleen size	cm		10.7	*167*	10.1	*234*	**9.1E−03**
CAP	(dB/m)	<240	292.9	*131*	307.3	*202*	**1.6E−02**
Liver Size	cm	<16.5	17.0	*26*	16.8	*42*	7.7E−01
Scores							
MELD-Na			14.3	*154*	9.3	*220*	**2.3E−15**
Maddrey score			10.1	*183*	0.2	*274*	**1.0E−10**
CLIF-C AD1			45.7	*154*	40.3	*223*	**6.9E−10**
AST/ALT			2.1	*185*	1.6	*281*	**1.5E−07**
Fib4 score			7.5	*183*	4.7	*279*	**2.4E−06**
Hepa score			0.62	*71*	0.49	*124*	**1.2E−02**
APRI score			0.03	*183*	0.02	*279*	**2.6E−02**
Alcoholic hepatitis	1 or 0		0.53	*14*	0.30	*10*	2.7E−01
Glasgow AH score			6.5	*15*	6.6	*14*	8.8E−01
CHILD points			5.9	*162*	5.0	*245*	**2.3E−11**

Table B.27 Parameters (routine laboratory) in heavy drinkers that discriminate between low and high hemoglobin. A hemoglobin of 14 g/dL was used as cut-off value. Parameters are grouped into categories and sorted according to the *P* value. Only patients with ineffective erythropoiesis (elevated ferritin) were used which represent 55.7% of the total cohort. Number of patients used for *T*-test are shown in cursive letters. A *P* value <0.05 is depicted in bold

			Hb < 14 g/L		Hb > 14 g/L		
Parameter	Units	Normal range	Mean	*N*	Mean	*N*	*P*
Routine laboratory							
Hemoglobin	g/dL	13.5–17.5	12.0	*202*	15.4	*293*	**1.0E−110**
Erythrocytes	/pL	4.5–5.9	3.5	*202*	4.6	*293*	**1.1E−86**
Albumin	g/dL	3.82–5.92	3.89	*146*	4.47	*231*	**1.9E−17**
Protein total	g/dL	6.6–8.3	6.8	*176*	7.4	*261*	**6.2E−17**
AP	U/L	40–130	154.0	*201*	107.7	*292*	**9.3E−11**
Bilirubin total	mg/dL	<1.3	3.6	*202*	1.2	*290*	**9.9E−11**
INR		0.85–1.15	1.16	*201*	0.99	*291*	**7.4E−10**
Serum iron	µg/dL	59–158	118.9	*182*	152.6	*261*	**3.4E−09**
CRP	mg/dL	<0.5	16.9	*202*	5.6	*292*	**5.4E−08**
MCV	fL	80–96	98.5	*177*	94.1	*243*	**2.2E−05**
HbA1C	%	4.4–6.1	5.3	*132*	5.6	*214*	**2.4E−04**
LDH	U/L	<250	297.9	*131*	244.8	*159*	**1.3E−03**
Triglycerides	mg/dL	<200	172.2	*166*	264.8	*266*	**1.5E−03**
Leukocytes	/nL	3.7–10.0	8.2	*202*	7.4	*293*	**5.5E−03**
Ferritin	ng/mL	30–400	1209	*202*	1060	*293*	**1.3E−02**
Cholesterol	mg/dL	<200	208.8	*165*	227.4	*264*	**1.6E−02**
HDL cholesterol	mg/dL	>40	60.9	*143*	70.7	*230*	**1.8E−02**
LDL cholesterol	mg/dL	<160	103.0	*143*	115.0	*230*	**2.3E−02**
Platelets	/nL	150–360	178.1	*202*	192.9	*293*	5.3E−02
CK 2	U/L	<170	62.0	*30*	93.0	*38*	8.7E−02
Transferrin saturation	%	16–45	55.3	*147*	51.1	*230*	1.2E−01
GGT	U/L	<60	681.3	*202*	599.7	*290*	2.4E−01
CK 1	U/L	<170	164.5	*97*	195.5	*129*	3.9E−01
Glucose	mg/dL	60–100	112.8	*186*	115.6	*272*	4.1E−01
Uric acid	mg/dL	2.6–6.4	6.4	*11*	6.5	*24*	8.6E−01
AST	U/L	<50	141.2	*202*	140.1	*293*	9.3E−01

Table B.28 Parameters (hemolysis, blood count) in heavy drinkers that discriminate between low and high hemoglobin. For details see Tables B.26 and B.27 hemoglobin of 14 g/dL was used as cut-off value. Parameters are grouped into categories and sorted according to the *P* value. Only patients with ineffective erythropoiesis (elevated ferritin) were used which represent 55.7% of the total cohort. Number of patients used for *T*-test are shown in cursive letters. A *P* value <0.05 is depicted in bold

Parameter	Units	Normal range	Hb < 14 g/L		Hb > 14 g/L		*P*
			Mean	*N*	Mean	*N*	
Electrophoresis							
Gamma	g/dL		1.67	*9*	1.24	*11*	1.7E−01
Beta	g/dL		0.66	*9*	0.77	*11*	2.2E−01
Alpha2	g/dL		0.54	*9*	0.61	*11*	2.8E−01
Alpha1	g/dL		0.28	*9*	0.27	*11*	5.4E−01
Erythropoiesis							
Reticulocytes 2	‰	8–25	35.2	*6*	15.8	*4*	7.3E−02
Epo 2	mIU/mL		9.6	*12*	5.6	*21*	9.0E−02
Epo 1	mIU/mL		10.6	*12*	7.4	*21*	2.9E−01
Reticulocytes 1	‰	8–25	23.6	*10*	19.3	*9*	5.0E−01
Hemolysis							
Bilirubin indirect	mg/dL	<0.8	0.80	*50*	0.36	*62*	**4.2E−03**
CD163	ng/mL		1982	*64*	1603	*60*	**3.1E−02**
Haptoglobin	g/L	0.3–2.0	1.3	*93*	1.5	*136*	1.1E−01
CD91	µg/mL		5.6	*25*	7.0	*19*	2.3E−01
Hemopexin	mg/mL		0.37	*25*	0.45	*19*	4.3E−01

Table B.29 Parameters (genes) in heavy drinkers that discriminate between low and high hemoglobin. For details see Tables B.26 and B.27 hemoglobin of 14 g/dL was used as cut-off value. Parameters are grouped into categories and sorted according to the *P* value. Only patients with ineffective erythropoiesis (elevated ferritin) were used which represent 55.7% of the total cohort. Number of patients used for *T*-test are shown in cursive letters. A *P* value <0.05 is depicted in bold. ALD patients were genotyped for four polymorphisms known to affect liver disease progression (MBOAT7, PNPLA3, HSD17B13, TM6SF2)

Parameter	Units	Normal range	Hb < 14 g/L		Hb > 14 g/L		*P*
			Mean	*N*	Mean	*N*	
Genes							
MBOAT7 GG			0.23	*131*	0.33	*210*	**4.5E−02**
MBOAT7 GC			0.56	*131*	0.48	*210*	1.2E−01
TM6SF2 TT			0.00	*126*	0.01	*207*	2.6E−01
HSD17B13 TA/TA			0.10	*77*	0.06	*166*	3.4E−01
PNPLA3 CG			0.37	*135*	0.40	*221*	5.7E−01
TM6SF2 CC			0.87	*126*	0.85	*207*	6.2E−01
PNPLA3 CC			0.53	*135*	0.52	*221*	7.2E−01
PNPLA3 GG			0.10	*135*	0.09	*221*	7.4E−01
MBOAT7 CC			0.20	*131*	0.19	*210*	7.4E−01
HSD17B13 TTA			0.30	*77*	0.32	*166*	7.6E−01
TM6SF2 CT			0.13	*126*	0.14	*207*	7.9E−01
HSD17B13 TT			0.60	*77*	0.62	*166*	8.2E−01

Correlation of Ferroptosis Marker ACSL4 (Long-Chain-Fatty-Acid—CoA Ligase 4) with Various Clinical and Laboratory Markers from a Heavy Drinking Cohort

See Table B.30.

Table B.30 Correlation of ferroptosis marker ACSL4 (long-chain-fatty-acid—CoA ligase 4) with various clinical and laboratory markers from a heavy drinking cohort. ACSL4 mRNA was assessed in liver biopsies from heavy drinkers using Western blotting and subsequent densitometry. Parameters were first sorted according to *P* value and then, in descending order, according to the absolute correlation coefficient (Spearman Rho correlation). Numbers of samples are indicated in the far-right column. ACSL4 converts free long-chain fatty acids into fatty acyl-CoA esters, preferentially arachidonate. Note that this ferroptosis markers is highly correlated with markers of hemolysis or iron (CD163, liver iron, haptoglobin, ERFE), liver damage (Mallory hyaline, lobular inflammation) and directly with the Maddrey score. Maximum 21 samples were available for analysis. *P* is given in bold if smaller than 0.05

		ACSL4/β-actin		
Parameter	Category	*r*	*P*	*N*
ERFE (ng/mL)	Special laboratory	0.943	**4.8E−03**	6
CD163 (µg/mL)	Special laboratory	0.886	**1.9E−02**	6
Liver iron conc. (RTS, µg/g ww)	Iron	0.729	**2.1E−03**	15
Gender	General information	−0.567	**7.3E−03**	21
Haptoglobin (g/L)	Special laboratory	−0.516	5.9E−02	14
APRI	AST/platelets	0.600	**1.4E−02**	16
Mallory hyaline 0–1	Histology	0.517	**1.6E−02**	21
Bilirubin (µmol/L)	Laboratory	0.502	**4.0E−02**	17
Lobular inflammation 0–3	Histology	0.452	**4.0E−02**	21
MBOAT7 CC	Genes	0.451	**4.6E−02**	20
Alcohol consumption (g/day)	Alcohol	−0.442	5.8E−02	19
Maddrey	Score	0.433	6.4E−02	19
Ballooning 0–2	Histology	0.428	5.3E−02	21

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