Hepatitis B Virus and Liver Disease

Jia-Horng Kao
Editor

Second Edition

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To my mentors Professor Juei-Low Sung and Professor Ding-Shinn Chen

In memory of my father Shi-Yang Kao

To my mother Wen-Shu Ho

Foreword

Globally, hepatitis B virus (HBV) has been ubiquitous and continues to be responsible for significant morbidity and mortality through clinical consequences of chronic hepatitis, cirrhosis, liver failure, and hepatocellular carcinoma (HCC). Since the discovery of the virus in 1965 for which Dr. Baruch Blumberg was awarded the Nobel Prize, there have been tremendous advances in understanding the virology, the development of several serological markers in the diagnosis of the infection, and the development of effective vaccines and vaccination strategies to impact on the incidence and prevalence of HBV infection. Yet, there remains a sizable population with chronic HBV infection. Relatively, a major challenge has been in successfully "curing" this infection while developing effective and well-tolerated HBV suppressive therapies.

The second edition entitled *Hepatitis B Virus and Liver Disease* has compiled an outstanding group on internationally recognized "who's who" experts in addressing complex and currently relevant topics in HBV, and it serves as good reading and a reference for practicing physicians at all levels. The list of topics is well-thought-out by the editor and placed in proper order. In an era where there is abundant online material on a given topic, there is still a need for such products put together by experts. It particularly provides the novice reader an opportunity to quickly obtain precise information presented by an expert and thus not having to surf through plentiful and freely available material to get their information.

It begins with the fundamental and essential topic of molecular virology and life cycle of HBV and sequentially takes us through a series of topics that are essential in the day-to-day understanding of HBV infection. The book covers, extremely well, the gamut of important topics such as epidemiology, immunopathogenesis, noninvasive assessment of the degree of hepatic HBV-related fibrosis, unmet needs in basic science and clinical research, the therapeutic advances in chronic HBV infection, vaccines, and HBV reactivation. There has been a lot of interest in new and novel biomarkers that can have utility in assessing response to HBV therapy and the topic is covered well. It is up to date with little to no redundancy and ends up with a topic on the major unmet need of achieving a "cure" of this infection. This is the elusive and, at this stage, difficult to overcome barrier faced by all HBV investigators. Currently, there appears to be a consensus on the definition of what constitutes a cure based on the serologic status of HBV, which is the right step toward achieving the goal of cure. Eradication of cccDNA, while attempting to develop

serologic surrogates for this marker, appears to be the Achilles' heel for those intensely pursuing therapies to "cure" HBV. Our struggle with efforts at curing this infection continues and one hopes that we will see advances in this direction over the next few years. As Sir Winston Churchill aptly said "Success is not final, failure is not fatal: it is the courage to continue that counts."

Indeed, I have found this second edition entitled *Hepatitis B Virus and Liver Disease* to be comprehensive yet precise, covering the entire spectrum of relevant topics in HBV, and very readable with good tables and figures. It certainly is a book I would like to have on my shelf as ready reference material.

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Preface of the Second Edition

Hepatitis B virus (HBV) was identified in the early 1960s, and it was soon found that HBV infection is among the most frequent and important in humans. HBV causes a wide spectrum of liver diseases, spanning from acute/fulminant hepatitis to chronic hepatitis/cirrhosis and hepatocellular carcinoma. It is also related to certain extrahepatic manifestations. Over the past few decades, the understanding of HBV infection, especially the virology, immunopathogenesis, and management, has evolved dramatically. The pathogenesis of HBV infection is getting clearer after vigorous basic, clinical, and epidemiological studies. More constructively, acute HBV infection can now be prevented by effective vaccines, and chronic infection can be suppressed efficiently by antiviral agents, shedding light at the end of the tunnel toward HBV cure and even elimination of HBV infection.

The rapid progress prompted late Professor Ding-Shinn Chen and myself to edit the first edition of the monograph *Hepatitis B Virus and Liver Disease*, which was published by Springer Singapore Pte Ltd in 2018. We aimed to provide a comprehensive, state-of-the-art review of HBV infection and liver disease. Owing to the quick advance of HBV-related studies in these years, a new up-to-date book is thus urgently required. The new book updated the results of basic and translational medicine including hepatitis B viral life cycle, unmet needs of basic research, immunopathogenesis of HBV-induced chronic liver disease, pathology, molecular carcinogenesis, and viral and host genetic factors affecting disease progression. The clinical aspects of chronic HBV infection were elucidated by experts in epidemiology, natural history, hepatitis B vaccination, new biomarkers, noninvasive assessment of fibrosis, current treatment options, coinfection with hepatitis C or D viruses and human immunodeficiency virus, and management of special populations like children, pregnant women, and those under immunosuppressive therapy. The implications of occult HBV infection were also discussed. Finally, the advances and perspectives in the development of novel treatments for the cure of HBV infection and the possibility of HBV cure were included.

In June 2020, Professor Ding-Shinn Chen, the main driver and great helmsman of the nationwide hepatitis B vaccination program in Taiwan, passed away. Professor Chen's lifetime research has disclosed the causal relationship of HBV and HCV with chronic hepatitis, cirrhosis, and hepatocellular carcinoma. His important discovery and insightful perspective starting from Taiwan have successfully led to a global campaign of universal hepatitis B vaccination, which has saved millions of

lives across the world. Professor Chen is a role model for physician scientists. His research career originated from the curiosity for knowledge, persisted in pursuit of academic excellence, translated into clinical practices, and eventually extended to patient-centered care. His legacy will continue and guide us to eliminate HBV infection in the foreseeable future, not only in Taiwan but also in other parts of the world.

I hope this new edition of book covering all relevant aspects of HBV infection can serve as a useful resource for every reader who has interest in the management and study of patients with hepatitis B.

Taipei, Taiwan Jia-Horng Kao

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Molecular Virology and Life Cycle of Hepatitis B Virus

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Fleur Chapus, Maria Guadalupe Martinez, Barbara Testoni, and Fabien Zoulim

Abstract

Hepatitis B virus (HBV) is the prototypic member of *Orthohepadnaviridae*, hepadnaviruses that can lead to transient or persistent infection. When left untreated, chronic HBV infection leads to severe liver damage culminating in hepatocellular carcinoma (HCC). HCC represents the third cause of cancer-related death worldwide with more than 800,000 deaths every year, thus constituting a major health issue.

HBV genomic DNA is a relaxed-circular partially double-stranded DNA (rcDNA), which has to be converted into a covalently closed circular DNA (cccDNA) in hepatocytes nucleus to allow viral replication. cccDNA represents the viral genomic reservoir and the template for viral transcription of the viral pregenomic RNA (pgRNA) intermediate, which is then reverse-transcribed back to viral DNA. The persistence of cccDNA in infected hepatocytes accounts for chronicity of infection and the low rate of cure. Here, we review the current body of knowledge on HBV biology, with a particular focus on the complex jigsaw puzzle of HBV molecular mechanisms of replication.

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Keywords

HBV · Life cycle · Entry · cccDNA · Chromatin · pgRNA · Transcription · RNA maturation · Egress

Abbreviations

5’ RACE	5’ Rapid amplification cDNA end
AGL	Antigenic loop
AP-1	Activator protein-1
BCP	Basic core promoter
C/EBP	CCAAT/enhancer-binding proteins
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
CTD	Carboxy terminal domain
Cul4	Cullin 4
DDB1	DNA damage-binding protein 1
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
DR1/2	Direct repeat 1/2
dsl-DNA	Double-stranded linear DNA
DynLL1	Dynein light chain 1
EnhI/II	Enhancer I/II
ESCRT	Export and sorting complex required for transport
FEN1	Flap endonuclease 1
FXR	Farnesoid X receptor
H 2a/2b/3/4	Histone 2a/2b/3/4
HAT	Histone acetyl transferase
HBDSP	Hepatitis B double spliced protein
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B s antigen
HBSP	Hepatitis B spliced protein
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HDAC	Histone deacetylase
HGS/HRS	Hepatocyte growth factor-regulated tyrosine kinase substrate
HNF	Hepatocyte nuclear factor
HSPG	Heparan sulfate proteoglycan
kb	Kilobase

kDa	Kilodalton
m6A	N6 methyladenosine
METTL3/14	Methyltransferase-Like 3/14
miRNA	MicroRNA
mRNA	Messenger RNA
MVB	Multivesicular bodies
NES	Nuclear export signal
NF1	Nuclear factor 1
NF-κB	Nuclear factor-κ B
NPC	Nuclear pore complex
nt	Nucleotide
NTCP	Na+-taurocholate cotransporting polypeptide
NUC	Nucleos(t)ide analog
Nup153	Nucleoporin 153
ORF	Open riding frame
PAPD5/7	PolyA-RNA polymerase-associated domain containing protein 5/7
PAS	Polyadenylation signal
PCNA	Proliferating cell nuclear antigen
pgRNA	Pregenomic RNA
Pol	Polymerase
PolyA	Polyadenylation
PRE	Posttranscriptional regulatory element
P-S FP	Polymerase-surface fusion protein
PSF	PTB-associated splicing factor
PTM	Posttranslational modification
Rab5/7	Ras-Associated protein 5/7
rcDNA	Relaxed circular DNA
RER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
Smc5/6	Structural maintenance of chromosomes 5/6
SP1	Singly spliced product 1
SRE	Splicing regulatory element
SVP	Subviral particle
TBP	TATA-binding protein
TDP2	Tyrosyl-DNA phosphodiesterase 2
TP	Terminal protein
TREX	Transcription and export factor
TSS	Transcription start site
URR	Upper regulatory region
WHV	Woodchuck hepatitis virus
YTHDF	YTH domain-containing family protein

1 Viral Structure

1.1 Classification

Hepatotropism, genetic organization, morphology and replication mechanisms of hepatitis B virus (HBV) account for its classification into the Baltimore class VII *Hepadnaviridae* (Schaefer 2007). The family is subdivided into five *genera* according to their genomic sequence divergence, the size of their genome and their host range restriction. *Orthohepadnaviridae* infect mammals, *Avihepadnaviriade* infect birds, *Herpetohepadnaviridae* infect reptiles and frogs, and *Meta-* and *Parahepadnaviridae* infect teleost fishes (Magnius et al. 2020). Within *genera*, species are defined by approximately 20% divergence in their nucleotide sequence.

Common features of the *Hepadnaviridae* family members include (i) presence of an envelope surrounding the nucleocapsid, (ii) small size of approximately 42–50 nm in diameter, and (iii) a partially double-stranded circular DNA genome (rcDNA) of 3–3.4 kb in length. The replication of *Hepadnaviridae* requires the presence of an RNA intermediate (pre-genomic RNA, pgRNA) that is retrotranscribed inside a capsid shell in the cytoplasm of hepatocytes to form a new rcDNA. To be transcriptionally active, the rcDNA has to be completed to form a covalently closed circular DNA, or cccDNA, which encodes the viral RNAs. cccDNA is a viral minichromosome, which is associated to nucleosomes in the nucleus of the infected hepatocytes and contains regulatory elements embedded in the different viral Open Reading Frames (ORFs). Viral transcripts share a common 3′-end and polyadenylation site, while differ for their 5′-end transcription start site (TSS). They are generated from cccDNA by the cellular RNA polymerase II machinery and they are further translated into three sets of proteins, namely PreC/C, Polymerase, and PreS/S, except for *Orthohepadnaviridae*, which require a supplemental protein, called "x" protein to replicate (Magnius et al. 2020). Hepadnaviruses induce overproduction of surface proteins secreted into the blood as subviral particles devoided of viral genome (Magnius et al. 2020; Schaefer 2007). HBV is a prototypic member of *Orthohepadnaviridae*.

1.2 Viral Particle

The infectious particle, or Dane particle, is a 42-nm diameter particle whose envelope surrounds an icosahedral nucleocapsid containing a 3.2-kb rcDNA, covalently linked to the viral polymerase. The assembly of 90–120 HBV core protein (HBc) dimers forms the capsid shell. The capsid containing 90 HBc dimers harbors a $T = 3$ organization and measures 30 nm diameter, while capsid containing 120 HBc dimers harbors a $T = 4$ organization and measures 34 nm diameter. The $T = 4$ organization is preferentially used for envelopment in a cellular lipid bilayer containing the three viral envelope proteins S-, M-, and L-HBsAg in a ratio 4:1:1. In infected patients, the number of Dane particles can go up to 10^{10} genome copies per milliliter (Patient et al. 2009) (Fig. 1.1a).

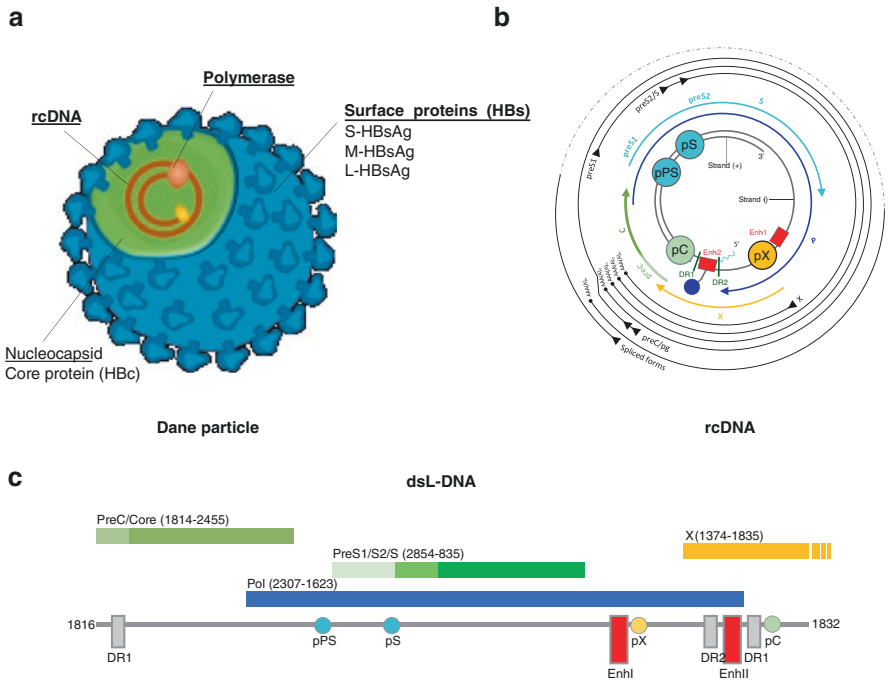

Fig. 1.1 HBV Dane particle and genome organization. Schematic representation of (**a**) the infectious HBV virion (Dane particle); (**b**) the structure of HBV genomic DNA, RNAs, ORFs, and regulatory elements. HBV genome encodes 6 viral mRNAs: the 2 longer than genome 3.5 kb precore and pregenomic RNAs, the 2.4-kb preS1 RNA, the 2.1-kb preS2 RNA, the 2.1 kb S RNA and the 0.7-kb X RNA. Several spliced isoforms have been identified (dotted line). The viral transcripts start at distinct TSS (black arrow) and end at a common polyA site (black dot—AAAA(A)n). Viral transcription is regulated by 4 promoters: promoter C (pC), promoter preS (pPS), promoter S (pS), and promoter X (pX); and by 2 enhancers: Enh-I and Enh-II; and (**c**) the ORFs of HBV dsl-DNA form. As a result of the generation of dsl-DNA forms by in situ priming, the X ORF is truncated at its C-terminus (by at least three amino acids) and the pre-core/core promoter is separated from its ORF. Numbering uses EcoR1 restriction site as +1 position, based on HBV DNA sequence X02763 (HBVdb.lyon.inserm.fr). *ORF* open reading frame; *TSS* transcription start site

1.2.1 Genome

Relaxed Circular DNA

HBV genome length varies between 3182 and 3221 bases according to the viral genotype. In Dane particles, the rcDNA is composed of a full length (−) strand having an 8–9 nt terminal redundancy covalently attached to the terminal protein domain of the viral polymerase which serves as a primer for reverse transcription (Summers et al. 1975) (Fig. 1.1b). The (+) strand contains a gap that can vary between 600 and 2100 nucleotides, creating a single-stranded DNA stretch accounting for up to 60% of the genome. The (+) strand is lengthened by a capped RNA oligomer derived from the pgRNA and which serves as a primer for the synthesis of the rcDNA (+) strand during the retro-transcription. The (−) and the (+) strands are

held together by an overlapping region of around 200 bp that confers the circular shape to the rcDNA (Gao and Hu 2007). This cohesive overlapping region contains the 11-bp direct repeat sequences DR1 and DR2 (5'-TTCACCTCTGC-3′), involved in rcDNA synthesis and found in HBV integrated forms (Bonilla Guerrero and Roberts 2005). Indeed, the RNA primer associated to the 5′ end of the negative strand has to be translocated onto the 3'end of the positive strand, and annealed to the DR2 sequence by sequence complementarity. Together with hairpin formation at DR1 and sequence identity between DR1 and DR2, these phenomena are mandatory for an efficient retrotranscription of pgRNA (Habig and Loeb 2006). Once translocated into the nucleus, the rcDNA has to be repaired and chromatinized to form the cccDNA, which is the unique genomic template that allows a complete viral transcription (see Sect. 2.3).

Double-Stranded Linear DNA

During rcDNA synthesis, RNA primer translocation can be altered, resulting in in situ priming from DR1 (Habig and Loeb 2006). This process leads to the formation of a double-stranded linear DNA (dsL-DNA) (Fig. 1.1c). dsL-DNA can then (i) form defective cccDNA, unable to support rcDNA synthesis due to insertion(s) or deletion(s) appearing during the ligation of dsL-DNA extremities; or (ii) be integrated into the host cell genome. In vitro, HBV dsL-DNA genomes are produced by reverse transcription within ~30% of mature nucleocapsids, while, for reasons yet to be determined, the mean in patient sera is ~7% (ranging from 3% to 36%) (Zhao et al. 2016b).

1.2.2 Proteins

The transcriptionally active cccDNA contains four ORFs oriented in the same direction. The PreC/C ORF harbors two in-phase start codons and generates the core protein HBc and the secreted HBeAg. The Polymerase ORF is the largest one, representing 80% of the genome. It encodes the viral polymerase/retro-transcriptase enzyme. The PreS1/PreS2/S ORF encodes the three envelope proteins (S-, M-, and L-HBsAg) starting from three in-phase start codons that are located within the Polymerase ORF. Finally, the shortest ORF is called X ORF and encodes the viral transactivator HBx. Importantly, the X protein is the only viral protein which is not present in the viral Dane particle (Nassal 2015).

Surface Proteins: L-, M-, and S-HBsAg

The Large surface protein or L-HBsAg is 42 kDa, the Medium, or M-HBsAg is 31 kDa, and the Small, or S-HBsAg, is 27 kDa. These three surface proteins share the same carboxyterminal domain (CTD) which contains four transmembrane domains and corresponds to the whole S-HBsAg sequence. L- and M-HBsAg share the PreS2 domain, while L-HBsAg is the only surface protein containing the PreS1 domain (Fig. 1.2a). HBs antigens are synthesized through the Rough Endoplasmic Reticulum (RER) and then undergo maturation in the Golgi Apparatus acquiring glycosylation (Prange 2012).

The S protein is the major component of both the viral and the subviral envelope. Beyond its scaffolding role, S contributes to HBV entry and egress assisting

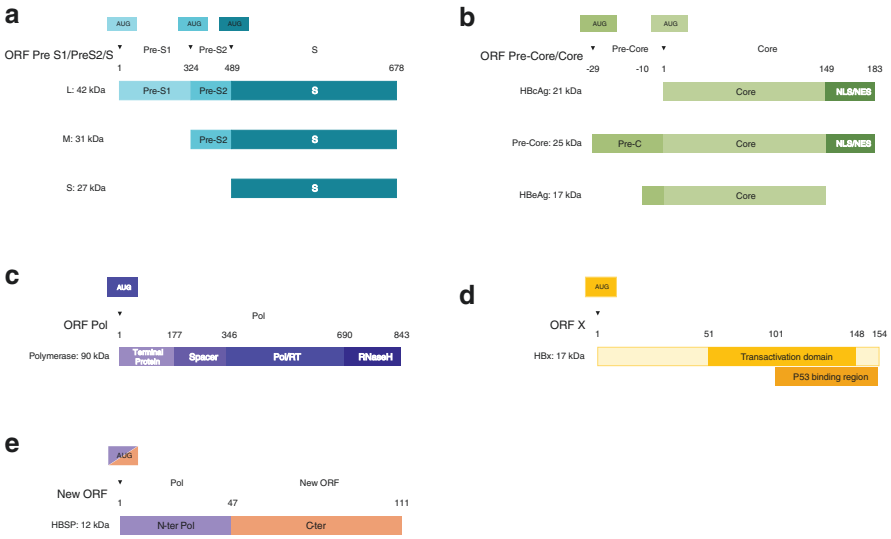

Fig. 1.2 Schematic representation of HBV proteins. (**a**) Large-, Medium-, and Small-S proteins constitute the viral envelope. These three proteins share the "S" domain, M-, and L- also share the "Pre-S2" domain. Only L-contains the "Pre-S1" domain. (**b**) HBcAg, Pre-core and HBeAg share the "core" domain, while only HBcAg and Pre-core proteins contain the NLS sequence. Pre-core is maturated through "Pre-core" and NLS domain cleavage to form the secreted HBeAg. (**c**) The viral polymerase is composed of a "TP" domain that mediates the covalent interaction with the rcDNA, a "Spacer" domain, a "Pol/RT" domain in charge of the (−) and the (+) DNA synthesis during pgRNA retro-transcription, and a "RNaseH" domain that degrades pgRNA during the (−) DNA strand synthesis. (**d**) HBx is a nonstructural viral transactivator. HBx is mandatory for the viral transcription and is implicated in HCC development. (**e**) The hepatitis B spliced protein (HBSP) is translated from the major form of pgRNA-derived spliced isoform SP1. This chimeric protein shares its N-ter with the viral polymerase/retro-transcriptase, and has its own C-ter encoded by a newly generated ORF originating from the splicing process

the L envelope protein in virus attachment to liver cells and nucleocapsid envelopment, respectively. The common S domain contains the antigenic loop (AGL) region that bears the so-called immunodominant a-determinant, the first HBV marker identified and conserved in all HBV strains (Julithe et al. 2014). The a-determinant is a conformational epitope that can attach Heparan Sulfate at the surface of hepatocytes. The close contact between the virion and the cell surface induces the myristoylation of the PreS1 domain on L-HBsAg and its interaction with the Na+/taurocholate cotransporting polypeptide (NTCP) receptor (Yan et al. 2012). Moreover, a stretch of 17 residues in the PreS1 domain mediates the interaction with the preformed cytosolic nucleocapsid for the envelopment process (Prange 2012).

Mutated or truncated HBV surface proteins can also be synthesized from HBV sequences integrated in the host genome (see Sect. 2.4). These mutant forms can accumulate in the cytoplasm and are associated to ER stress response that may increase the risk of HCC. Furthermore, mutated S proteins could confer proliferative advantages to hepatocytes, for example, by stimulating their expansion. Finally,

in HCC animal models, overexpression of HBs mutants drives precancerous liver damages (Tu et al. 2017).

Capsid Protein: HBc

The capsid protein, also called core protein, or HBc, is 183 to 185 amino acid long, depending on the genotype. This 21-kDa protein can assemble in dimers to form the capsid shell ($T = 3$ or $T = 4$ organization), via its "core" domain (residues 1–140). The secondary structure drives the ability of dimerization (Prange 2012). In particular, specific structures within the four-helix bundle that forms the intradimer interface regulate long range conformational changes required for capsid assembly (Zhao et al. 2020).

A 100-amino acid linker separates the "core" domain from the "protamine" domain (residues 150–183/185) located in the CTD. This latter is arginine-rich and drives the interaction between HBc and nucleic acids for proper pgRNA packaging and retro-transcription (Nassal 1992). The protamine domain contains a nuclear localization signal (NLS) and a nuclear export signal (NES) required for the shuttling of HBc between the nucleus and the cytoplasm (Fig. 1.2b). HBc CTD can be phosphorylated by different cellular kinases, thereby inducing conformational changes that loosen the contact with nucleic acids. The phosphorylation state of HBc CTD correlates with pgRNA packaging and rcDNA (+) strand synthesis, while a dephosphorylated state of HBc CTD is associated to capsid maturation, DNA synthesis, and subsequent nucleocapsid envelopment and release (Lee 1997; Zlotnick et al. 2015).

Pre-Core Protein: HBeAg

HBeAg is a soluble and non-particulate HBV antigen circulating in the serum of infected patients (Nassal 1992). With the exception of a 29 residues extension in its N-terminus (N-ter), HBeAg shares the same sequence as HBc (Fig. 1.2b). The N-ter domain of HBeAg is hydrophobic and drives the 25-kDa HBeAg precursor to the RER where it undergoes two successive cleavages to be fully mature. The first cleavage leads to a 22-kDa protein which can either be cleaved a second time to form the secreted HBeAg of 17 kDa (Messageot et al. 2003), or that can traffic to the cytosol where it forms the p22 viral protein (Dandri and Locarnini 2012) (Fig. 1.2b).

The secreted HBeAg is not required for the viral replication cycle, but exhibits immune-modulating properties which contribute to viral persistence (Dandri and Locarnini 2012).

Polymerase

The viral polymerase, or Pol, is a multifunctional 90-kDa protein and the only viral protein harboring enzymatic activities. HBV Pol contains four domains, from the N-ter: (i) the terminal protein (TP); (ii) the spacer domain; (iii) the reverse-transcriptase (RT) domain; and (iv) the RNase H domain (Fig. 1.2c).

The TP domain, from aa 1 to aa 180, connects the viral polymerase to the 5′ end of the rcDNA minus strand through its residue Y63 and confers the primase activity

required for the initiation of the pgRNA retro-transcription by synthesizing a 3–4 nucleotide primer. Moreover, the TP domain is also required for the pgRNA encapsidation by interacting with the pgRNA 5′ epsilon loop (Wang and Seeger 1992).

The spacer domain tethers the TP domain to the RT domain and can tolerate amino acid insertion or deletion. The role of this domain is unclear and appears to overlap with the PreS2 domain, suggesting a role in environmental adaptation and in providing flexibility in conformation changes (Chen et al. 2013).

The RT domain (residues 360–693) harbors a polymerase/retro-transcriptase activity responsible for pgRNA retro-transcription and subsequent rcDNA (+) strand synthesis.

The Pol CTD, from aa 694 to aa 845, carries an RNase H activity responsible for pgRNA digestion after retro-transcription, and generating the RNA primer required for the rcDNA (+) strand synthesis (Tavis and Lomonosova 2015).

X Protein: HBx

HBx is a nonstructural 17-kDa protein without any homology with other known cellular or viral proteins. Despite still unclear functions, two main roles have been proposed for HBx: (i) in viral replication; (ii) in hepatocellular carcinogenesis.

While not required for the establishment of HBV infection, HBx is essential for maintaining the viral replication. Indeed, HBx does not affect cccDNA formation, but it is required for its transcriptional activity. HBx ability to modulate HBV transcription is dependent on its CTD, aa 51 to aa 148, designated as transactivation domain (Fig. 1.2d). The essential role for maintaining the viral infection was firstly observed in Woochuck Hepatitis Virus (WHV) and then further demonstrated in HBV infection (Decorsière et al. 2016; Keasler et al. 2007; Lucifora et al. 2011) (see Sect. 2.3).

HBx has also been implicated in liver carcinogenesis, which is covered in other chapters of the book.

1.3 Other Viral Particles

Besides infectious particles, HBV also leads to the secretion of other noninfectious particles that are found in excess respect to Dane particles in the serum of infected patients.

1.3.1 Subviral Particles

HBV envelope proteins can assemble without any nucleocapsid and be further secreted as noninfectious empty particles called subviral particles (SVPs). SVPs can be released as octahedral spheres of 20 nm diameter, containing only S-HBsAg oligomerized in 48 homodimers, or as filaments of various lengths having the same composition in HBsAg as Dane particles. SVPs are found in 10^4–10^5 fold in excess compared to Dane particles in blood circulation (Ganem 2004) and are assumed to act as decoys by trapping the host's adaptive immune system (Patient et al. 2009) (Fig. 1.3).

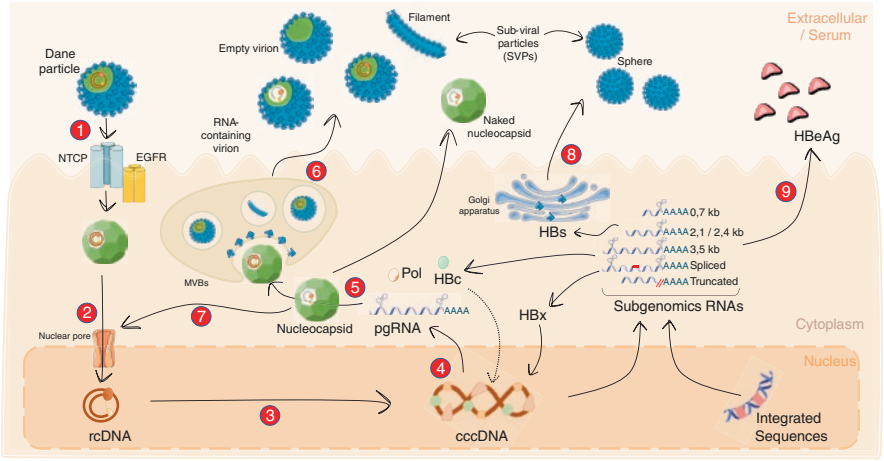

Fig. 1.3 Schematic representation of HBV life cycle. HBV enters differentiated hepatocytes via its receptor NTCP (1). At the nuclear pore, the capsid dissociates and rcDNA is released (2) and subsequently converted into cccDNA (3), which is the unique template for viral replication (4). The viral transcription generates an RNA intermediate called pregenomic RNA, which is encapsidated (5) while retro-transcribed in a (−) DNA strand. The (+) DNA strand is further synthesized to form the rcDNA. In 10% of cases, in situ priming of (+) DNA strand synthesis occurs and leads to the synthesis of a dsL-DNA, that can enter the nucleus to form a defective cccDNA or to be integrated in the host genome. rcDNA-containing nucleocapsids can be enveloped and secreted (6) to form new infectious particles, or recycled to the nucleus to replenish the cccDNA pool (7). HBV envelope proteins can assemble without any nucleocapsid and be further secreted as noninfectious empty particles called Subviral particles (SVPs) (8). In addition to SVPs, empty virions, RNA-containing virions and naked nucleocapsids have been identified in vitro or in patients' serum. HBeAg is a soluble and nonparticulate HBV antigen derived from cleavage of the pre-core protein (9)

1.3.2 Noncanonical Particles

In addition to SVPs, other viral particles have been identified in vitro or in patient's serum. While immature nucleocapsids have been demonstrated incompetent for envelopment and secretion, non-enveloped nucleocapsids have been identified in vitro in a hepatoma cell line transfected with the WT HBV genome (Watanabe et al. 2007). These nucleocapsids may contain the full viral genome, but also all the replication intermediates between the pgRNA and the rcDNA, including RNA:DNA hybrids (Hu and Liu 2017). Empty virions, or genome-free enveloped capsids, have also been identified in the blood of infected patients. These particles are called "light" virions as they appear empty under electron microscopy.

Finally, HBV RNA species can circulate in the serum of chronically infected patients and are under evaluation for the development of noninvasive biomarkers (Charre et al. 2019). The predominant form of circulating HBV RNA seems to be encapsidated pgRNA that can be present in the serum of HBV-chronic carriers within enveloped or naked nucleocapsids (Bai et al. 2018; Ning et al. 2011; Wang et al. 2016) (Fig. 1.3).

2 Viral Life Cycle

2.1 Entry

The first stage of infection is the attachment of the L- and S-HBsAg to the membrane of hepatocytes, which is crucial for viral entry. The a-determinant in the AGL of the three HBsAg constitutes a conformation epitope that can contact Heparan Sulfate Proteoglycans (HSPGs) at the cell surface (Le Duff et al. 2009; Leistner et al. 2007). HSPGs represent a low affinity HBV receptor abundantly expressed in the extracellular matrix of various cell types, including hepatocytes. Among HSPG family, Glypican 5 specifically mediates HBV entry into the hepatocyte (Verrier et al. 2016). Then, the myristoyl anchor domain of L-HBsAg undergoes myristoylation and interacts with the NA+/Taurocholate cotransporting polypeptide (NTCP) receptor to drive HBV entry into hepatocytes (Yan et al. 2012).

HBV internalization steps after NTCP binding are still poorly understood, but there is evidence for endosomal trafficking, notably via Rab5/7 (Macovei et al. 2013). Whether HBV particles are internalized through clathrin- (Huang et al. 2012) or caveolin- (Macovei et al. 2010) dependent mechanisms remains to be determined. A recent report described that epidermal growth factor receptor (EGFR)-associated machinery for endocytosis coordinates the transport of incoming hepatitis B virus to the late endosome as a critical step for successful infection (Iwamoto et al. 2020) (Fig. 1.3).

2.2 Nuclear Import

Internalized nucleocapsids are assumed to stay intact while trafficking in the cytoplasm, minimizing the recognition of the viral genome by host cell innate immune sensors. During retrograde transport toward the nucleus, nucleocapsids interact with the microtubular system, helped by the functional binding partner DynLL1 (Osseman et al. 2018).

Once located at the nuclear pore complex (NPC), HBV nucleocapsid interacts with the nucleoporin Nup153 (Schmitz et al. 2010) via the phosphorylated HBc CTD, exposed at the surface of the nucleocapsid. Via this interaction, the nucleocapsid reaches the nuclear basket, where only fully mature capsids can release the viral genome in the karyoplasm (Rabe et al. 2003) (Köck et al. 2010). In vitro data suggest that after capsid disassembly, HBc protein would dimerize to subsequently re-assemble into nuclear empty capsids (Gallucci and Kann 2017) and/or stably associate with cccDNA to ensure proper nucleosomal distribution (Bock et al. 2001).

2.3 cccDNA Biogenesis and Regulation

The cccDNA is the persistent form of HBV genome and is the crucial intermediate for viral replication. Harboring no origin of replication, cccDNA pool is maintained

through de novo infection or rcDNA-containing nucleocapsid recycling to the nucleus.

2.3.1 Formation

After being released in the nucleoplasm, rcDNA is converted into a transcriptionally active cccDNA through a multistep process that strongly relies on cellular proteins. The removal of the covalently bound viral polymerase from the 5′ end of the (−) strand is completed by a tyrosyl phosphodiesterase (TDP2) (Königer et al. 2014). The (+) strand completion is mediated by cellular polymerases, including the polymerase κ (Polκ), and to a lesser extent Polη and Polλ (Qi et al. 2016). The cleavage and the degradation of the RNA oligomer at the 5′ end of the plus strand and the removal of the redundant terminal region "r" are decisive events before ligation of both DNA strands. Cellular exo- and/or endonucleases and ligases appear to be fitting candidates for these processes (Long et al. 2017). Moreover, the "r" region located at the 5′ end of the negative strand is presumed to form a "flap" structure. Through its ability to cleave flap structure, the flap structure-specific endonuclease 1 (FEN1) constitutes a good candidate in cccDNA formation by cleaving the "r" region (Kitamura et al. 2018). Recently, besides FEN1 and Ligase 1, three other proteins have been identified as crucial for cccDNA formation in vitro: RFC, DNA Polδ and PCNA (Wei and Ploss 2020). Nonetheless, it is possible that cccDNA formation in vivo relies on more complex mechanisms and additional factors, thus further investigation is required to identify the chronological order and crucial proteins driving rcDNA to cccDNA conversion in vivo.

2.3.2 Chromatin Structure

The cccDNA harbors a nucleosomal organization resulting in a chromatin-like structure associated to the presence of H3, H4, H2A, and H2B canonical histones (Bock et al. 1994). Importantly, the nucleosome spacing is 10% reduced compared to the cellular chromatin (Bock et al. 1994; Newbold et al. 1995) and this seems to be due to the association of HBc to the chromatinized cccDNA (Bock et al. 2001). Once in the nucleoplasm, the HBV genome has been shown to position into the higher order architecture of human genome by contacting preferentially CpG islands associated with highly expressed genes (Moreau et al. 2018). This could favor the capacity of HBV to hijack the host cell transcriptional machinery for its transcription.

2.3.3 Regulatory Sequences

Since the only viral enzyme is represented by the polymerase/retro-transcriptase, HBV hijacks the host cellular RNA polymerase II machinery to ensure its efficient transcription, which is regulated by *cis*- and *trans*-regulatory elements embedded in viral ORFs (Rall et al. 1983).

Core Promoter

The core promoter regulates precore and pgRNA transcription. It is 232-bp long and divided into two elements: the upper regulatory region (URR) and the basic core

promoter (BCP). The core promoter is devoid of TATA-box motif, but harbors A/T-rich regions that binds the TATA-binding protein (TBP) (Kramvis and Kew 1999). The BCP is a weak promoter but sufficient to activate the precore and pgRNA transcription, preferentially in differentiated hepatocytes due to the presence of DNA binding sites for liver-specific transcription factors, such as HNF4-α (Zheng et al. 2004).

S Promoters

Two promoters regulate the transcription of the 2.4-kb PreS1 mRNA, encoding L-HBsAg, and the two 2.1-kb PreS2 and S transcripts encoding M- and S-HBsAg. The PreS1 promoter is the only TATA-box containing promoter in HBV genome. The PreS2 promoter regulates PreS2 and S transcription start sites and is regulated by seven regulatory elements that can act as positive or negative regulators according to their association with liver-specific and ubiquitous transcription factors (Moolla et al. 2002).

X Promoter

The X promoter is located 140 bp upstream of the X transcription start site and contains sites for liver-specific and ubiquitous transcription factors such as NF1; C/EBP, ATF; AP1/Jun-Fos and p53. Globally, X and Core promoters have a higher basal activity than PreS1 and PreS2 promoters, probably due to their association with several transcription factors and their high proximity to enhancers I and II (Moolla et al. 2002) (Fig. 1.1b).

Enhancer I and Enhancer II

The enhancer I favors the transcription under the control of X and core promoters to enhance the synthesis of the precore, pregenomic, and X transcripts. It is 120 bp long and organized in three domains that interact with several transcription factors. The 3′ region of EnhI overlaps the X promoter (Bock 2000). The enhancer II is 148 bp long and is located immediately upstream of the BCP. It controls the S, as well as the X and the Core promoters (Moolla et al. 2002).

Splicing and Export Signals

Mono- and multi-spliced transcript variants have been identified in HBV. Predicted constitutive and cryptic splicing sites vary in number and location according to HBV genotypes. To date, 17 spliced variants have been identified derived from the pgRNA, and four derived from S transcript (Candotti and Allain 2016; Chen et al. 2015). In HBV genome, some *cis*-acting elements participate to this process: two activators (position 2951–2970 and position 3051–3070) and one inhibitor (position 3138–3143) of splicing. Moreover, a splicing regulatory element (SRE) (position 1252–1288) stimulates HBV splicing via the binding of the splicing factor PSF (Heise 2006).

Remarkably, in eukaryotic cells, only mature, spliced transcripts can be exported outside the nucleus, since the spliceosome itself recruits the required export factors. However, the majority of HBV RNAs are not spliced but still exported. This is

probably due to the presence of a posttranscriptional regulatory element (PRE) (position 1217–1582) within the 3′ sequence common to all HBV transcripts, where a 116-bp long sub-element, called SEP1, is responsible for the recruitment of the cellular transcription export complex (TREX) (Chi et al. 2014).

Polyadenylation Signals

All HBV RNAs terminate at an identical poly(A) signal having a non-perfect consensus sequence UAUAAA (position 1916–1921). Termination efficiency, therefore, depends on the participation of multiple upstream sequences that act to increase the efficiency of poly(A) signal recognition. This mechanism seems to be crucial for proper transcription of the overlength pgRNA, where the poly(A) signal is present in each terminally redundant segment (Russnak and Ganem 1990).

An additional poly(A) signal also having a non-perfect consensus sequence (CAUAAA, position 1788–1794) has been identified, but it appears to be active only under certain so far undefined conditions during HBV replication (Schutz et al. 1996). On the contrary, it seems to be used for the polyadenylation of truncated viral transcripts derived from integrated sequences lacking the canonical poly(A) signal (Kairat et al. 1999; Su et al. 2001; Wooddell et al. 2017).

2.3.4 Epigenetic Regulation

Chromatin structures undergo several epigenetic modifications that play key roles in gene expression regulation. The methylation profile of DNA wrapped around histones influences the strength of association between DNA and nucleosomes. Moreover, histone tails can experience posttranslational modifications (PTMs) modulating the transcriptional state of the chromatin. Similarly to cellular chromatin, HBV minichromosome is subjected to different epigenetic regulation processes.

DNA Methylation

HBV genotype D genome contains three CpG islands located at the following positions: CpG#1 (67–392) located near the preS2 ATG, CpG#2 (1033–1749) overlapping X ORF and EnhII and located 1 bp upstream of the BCP, and CpG#3 (2215–2490) near the polymerase ATG (Jain et al. 2015). Moreover, HBV infection modulates the expression of cellular DNA methyltransferases such as DNMT1, 2, 3a, and 3b that in turn lead to HBV DNA methylation and viral protein downregulation (Vivekanandan et al. 2010).

Histone PTMs

cccDNA-associated histones tails can be posttranslationally modified to alter the global chromatin structure. The most studied histone PTMs include H3/H4 acetylation or methylation (i.e., H3 lysine4 trimethylation), which serve as activation marks, facilitating chromatin accessibility and allowing gene transcription (Liu et al. 2013; Pollicino et al. 2006; Tropberger et al. 2015). Conversely, histones hypoacetylation or methylation (i.e., H3 lysine 9 or 27 trimethylation) leads to a more compact chromatin, silencing cccDNA transcription (Belloni et al. 2012; Lebossé et al. 2020; Rivière et al. 2015). Accordingly, hyperacetylation of

cccDNA-associated H3/H4 correlates with high viremia and higher intrahepatic cccDNA transcriptional activity in CHB patients (Flecken et al. 2019; Lebossé et al. 2020; Pollicino et al. 2006).

2.3.5 Transregulatory Factors

Viral Proteins

cccDNA is also associated to nonhistone proteins, notably to HBc and HBx viral proteins (Bock et al. 2001; Lucifora et al. 2011). The viral transactivator HBx is recruited onto the cccDNA and is required for its transcriptional activity (Cougot et al. 2012; Decorsière et al. 2016; Lucifora et al. 2011; Rivière et al. 2015). In particular, the direct binding of HBx to DDB1 is important to redirect the ubiquitin ligase activity of the CUL4-DDB1 E3 ligase (Li et al. 2010) to drive the degradation of the Smc5/6 complex, a restriction factor blocking cccDNA transcriptional activity (Decorsière et al. 2016).

The viral core protein HBc is also associated to cccDNA and influences the nucleosomal distribution along the HBV minichromosome (Bock et al. 2001). Even if a role for HBc has been described in maintaining the hypomethylated status of the cccDNA CpG islands (Guo et al. 2011), the need for HBc neosynthesis in cccDNA maintenance and transcription has been recently challenged in an in vitro model of HBV infection (Tu et al. 2021).

Host Proteins

A long list of cellular factors has been described as regulators of cccDNA transcriptional activity in different in vitro/in vivo models (Hong et al. 2017). It comprises both general (e.g., NF-kB, SP1, C/EBP) and liver-specific transcription factors (e.g., HNF1-4 and FXRα) (Mohd-Ismail et al. 2019), together with coactivators and corepressors. Histone deacetylases (HDACs) are a class of enzymes that remove acetyl groups from histones tails, allowing them to wrap the DNA more tightly, thus negatively regulating transcription, whereas histone acetyl transferases (HATs) acetylate histones, facilitating binding of transcription factors to DNA.

2.3.6 Mechanisms of cccDNA Persistence

Newly synthesized HBc forms the nucleocapsid that encapsidates pgRNA and allow RT to form rcDNA. Encapsidated rcDNA can be enveloped and released from the cells to form infectious Dane particles to infect new cells, or they may recycle back to the nucleus where the rcDNA is transformed into cccDNA, both phenomena leading to a replenishment of the nuclear pool of cccDNA. Nuclear levels of cccDNA are highly stable; however, how single cccDNA molecules are maintained remains controversial. A static model for cccDNA maintenance suggests that, as nuclear rcDNA import and rcDNA-to-cccDNA conversion begins to wane, cccDNA maintenance is achieved by repressing de novo cccDNA formation (Dandri et al. 2000; Lutgehetmann et al. 2010; Zhu et al. 2001). Consistent with this hypothesis, the use of NUCs, indirectly affecting cccDNA levels by inhibiting de novo production of rcDNA, only leads to minor effects on the nuclear pool. Observations in CHB patients under

Nucleo(s)tide Analogs (NUC) therapy proposed that complete cccDNA clearance could take decades (Lai et al. 2020; Werle-Lapostolle et al. 2004) suggesting that nuclear import of de novo synthesized rcDNA plays a minor role in the cccDNA pool maintenance and that nuclear cccDNA is highly stable (Moraleda et al. 1997).

Recent evidence suggests that residual levels of HBV replication persist during NUC treatment, which could imply the maintenance of the cccDNA pool despite treatment (Boyd et al. 2016; Gordon et al. 2013). Furthermore, serum of CHB patients with low viremia under NUCs treatment are still infectious in chimeric mice (Burdette et al. 2020). These results defy the static cccDNA model and imply that dynamic turnover could explain the maintenance of cccDNA levels (Huang et al. 2020; Zhu et al. 2001). In this dynamic model, constant degradation and de novo synthesis of cccDNA maintain stable nuclear copy numbers, which is compatible with the dependency of cccDNA pool maintenance on de novo infections (Ko et al. 2018). The underlying mechanism of cccDNA clearance and maintenance in such a model remains unclear: cccDNA destruction could occur dependently (Mason et al. 2005; Summers et al. 2003; Zhou et al. 2000) or independently of infected hepatocyte death (Guidotti 1999; Murray et al. 2005; Wieland et al. 2004).

Given the constraints of cccDNA quantification at the single cell level, the studies reported data as an average of cccDNA molecules per cell and not the actual cccDNA copy number per infected hepatocyte, or its temporal distribution. Thus, it remains debated if cccDNA replenishment is a rescue pathway for occasional cccDNA loss, or if it is indeed a strong driver counteracting ongoing degradation.

cccDNA does not follow a semiconservative replication pathway and is not tethered to chromosomes, thus its fate after cell division remains controversial. Studies in HBV-infected liver-humanized mice, in the context of increased hepatocyte proliferation and efficient inhibition of virus reinfection, showed a reduction of the cccDNA pool, suggesting cccDNA loss upon cell division (Allweiss et al. 2018). cccDNA labeling by fluorescence imaging in situ hybridization (FISH) in the presence of NUCs suggested that cccDNA is asymmetrically distributed to daughter cells, instead of being lost or duplicated during cell division (Li et al. 2017). Thus, in the presence of NUC, loss of cccDNA relies on the rate of infected hepatocyte death and further dilution by cell division. Consistent with these results, recent in vitro data suggest that cccDNA turnover is associated with the infected hepatocytes turnover (Tu et al. 2021).

Targeting cccDNA for its genetic or epigenetic silencing, or its degradation remains the holy grail of HBV research and drug discovery efforts to achieve a cure of the infection.

2.4 Integration

dsL-DNA is the presumed viral genome that integrates into the host, as virus–cell DNA junctions correspond to the termini of the dsL-DNA form (Fig. 1.1c) (Yang and Summers 1999). In the host, double-strand break containing regions represent the preferential target sites for HBV integration (Bill and Summers 2004).

HBV integrated sequences can support HBsAg and HBx synthesis, but no other viral protein production. Although the integration process seems to occur randomly,

recurrent insertion sites at the 3′ part of HBx disrupting the core promoter have been observed (Mason et al. 2010; Sung et al. 2012; Tu et al. 2018; Zhao et al. 2016a). Thus, while remaining intact, Pol and HBeAg ORFs are physically separated from their promoters (Fig. 1.1c). Hence, integrated genome neither supports pgRNA transcription nor production of viral particles. However, EnhI remains active in integrated forms and allows the transcription of a shorter X transcript that is further translated into a 3-aa truncated HBx protein (Tu et al. 2017).

dsL-DNA integration was assumed to be a causative process in tumorigenesis and associated to HCC initiation and progression. It is important to note that the mechanisms of HBV-induced HCC carcinogenesis are still poorly understood as they involve many other mechanisms besides viral integration (Levrero and Zucman-Rossi 2016).

2.5 Transcripts

All HBV RNAs are capped in their 5′ termini and polyadenylated at their 3′ end. Capping of pgRNA is essential for effective encapsidation process (Jeong et al. 2000). Recent results demonstrated that X mRNA can exist in the form of uncapped transcripts in viral particles secreted by HBV-producing HepAD38 cell line (Stadelmayer et al. 2020). Surprisingly, preC, pgRNA, and S RNA are not detected in 5' RACE experiments uncovering uncapped X transcripts (Stadelmayer et al. 2020). At present, the role of uncapped HBx transcripts remains unknown and raises the question about the fate of such RNA, notably concerning immune system recognition of uncapped RNA.

HBV transcript stability is also regulated by two noncanonical polyA polymerases, namely PAPD5 and PAPD7. These two proteins function in a redundant manner and their concomitant depletion leads to HBV RNA destabilization and degradation, without affecting the viral transcription (Mueller et al. 2019).

Finally, the viral transcripts are methylated in A residues belonging to ε-loop via the methylation writers (METTL3/14) and readers (YTHDF proteins) belonging to the cellular m^6A machinery (Imam et al. 2018). Interestingly, 5′ ε-loop and 3′ ε-loop methylation displays different functions in HBV life cycle, the first one being involved in pgRNA retro-transcription while the second mostly interfering with translation.

2.5.1 Pregenomic RNA

The pregenomic RNA is synthesized from the preCore/Core promoter and is 3.5-kb long (i.e., 1.1-fold longer than the viral genome). It contains the whole genomic information and is the RNA intermediate necessary to achieve the complete viral replication through reverse-transcription onto rcDNA. pgRNA is also a bicistronic RNA coding for HBc and Pol viral proteins.

2.5.2 Subgenomic RNAs

HBV genome also encodes five other subgenomic RNAs. Precore mRNA is also synthesized from the preC/Core promoter and is 3.5 kb long. The preS1 mRNA is 2.4 kb long and encodes the L-HBsAg. The preS2 and S mRNAs are 2.1 kb long and

encode the M- and the S-HBsAg. The shortest transcript encoded by HBV genome is called X mRNA and is 0.7 kb long. Importantly, each transcript starts at its own transcription start site and end at a common polyadenylation signal. Transcription start sites have been recently precisely mapped and, besides the six already well-described major TSS initiating the six majors HBV transcripts, additional 11 weaker TSS have been discovered, which could give rise to yet-to-be identified viral transcripts (Altinel et al. 2016; Stadelmayer et al. 2020).

2.5.3 Truncated RNAs

Truncated HBV surface proteins can be synthesized from integrated HBV genome, together with a 3 aa-truncated HBx RNA which is still functional (Tu et al. 2017). Transcription of integrated-deriving HBV transcripts ends at the cryptic polyadenylation site (Kairat et al. 1999; Su et al. 2001; Wooddell et al. 2017).

2.5.4 Spliced RNAs

Besides the abovementioned HBV transcripts, several spliced variants have been identified in vitro and in patients' samples. Seventeen spliced variants derive from the pgRNA and four spliced variants derive from the preS2 transcript and use donor and acceptor splicing sites which position can vary according to the viral genotype (Candotti and Allain 2016; Chen et al. 2015). Three spliced variants have been demonstrated to be protein-coding variants. The major spliced isoform SP1 measures approximately 2 kb and encodes the so-called hepatitis B spliced protein (HBSP) (Duriez et al. 2017; Soussan et al. 2003) (Fig. 1.2e). A doubly spliced variant called SP7 and measuring 2.2 kb long is translated into the doubly spliced protein HBDSP (Chen et al. 2010). Finally, a protein resulting in the fusion of the N-ter of the Polymerase and the C-ter of S, called Polymerase-Surface fusion protein (P-S FP), is encoded by the singly spliced isoform SP14 (Huang et al. 2000). If the spliced transcripts and derived proteins are not required for HBV replication, a role in HBV-derived pathogenesis has been proposed (Bayliss et al. 2013; Duriez et al. 2017).

2.6 Nucleocapsid Assembly and RT

HBV replication requires the reverse-transcription of the pgRNA into rcDNA while being encapsidated within the cytosol of infected hepatocytes. HBV reverse-transcription is initiated by the viral polymerase TP domain that specifically binds to the "bulge" region of pgRNA 5′ ε-loop and primes the synthesis of the (−) strand of rcDNA. The binding of the viral polymerase to the 5′ ε-loop of pgRNA triggers the encapsidation of pgRNA. While nucleocapsid assembles with HBc dimers, the reverse-transcription occurs.

Conformational changes of HBV Pol lead to its enzymatic activation and to the translocation of the Polymerase and the short oligonucleotide retro-transcribed from the 5′ ε bulge to the direct repeat (DR) 1 motif located in 3′ of the pgRNA. From this location, the minus strand synthesis continues until the 5′ of the pgRNA, generating

the terminal redundancy of 8–9 nucleotides. During the (−) strand DNA synthesis, the RNase H domain of the viral polymerase digests the pgRNA molecule. RNase H-mediated pgRNA degradation is not complete, leaving about 15–18 nucleotides in pgRNA 5'end, including the DR1 sequence. This 5′ capped RNA oligo serves as primer for the synthesis of the (+) rcDNA strand by translocating to a second DR sequence (DR2) located in 5′ of the newly synthesized (−) rcDNA strand allowing the circularization of the genome following (+) strand synthesis. The (+) strand is made using the (−) strand as template. The (+) strand synthesis is not complete, and only reaches 50–70% of the length of the (−) strand, forming the so-called "gap" leading to the partially double stranded rcDNA (Beck and Nassal 2007).

2.7 Assembly and Secretion

rcDNA-containing particles constitute mature nucleocapsids that have to acquire host-derived lipid bilayer in which viral envelop proteins are embedded. The viral envelop proteins are synthesized in the RER-Golgi axis and bud into its lumen. L-HBsAg directly interacts with the already assembled HBc and mediates the contact between S-HBsAg and HBc which is responsible for the envelopment of nucleocapsids (Pastor et al. 2019). Enveloped particles subsequently bud into the lumen of intracellular membrane compartments (Hu and Liu 2017) and are secreted out of the hepatocytes.

As many other enveloped viruses, HBV takes advantage of the endosomal sorting complex required for transport (ESCRT) important for the formation of multivesicular bodies (MVB). While spheres are secreted by the constitutive secretory pathway, filaments secretion is ESCRT/MVB-dependent (Hu and Liu 2017). In contrast to HBV viral particles, naked capsids budding is ESCRT-independent, but relies on the proteins Alix and HGS (HRS, hepatocyte growth factor-regulated tyrosine kinase substrate) (Jiang and Hildt 2020).

3 HBV Genomic Variability

3.1 Genotypes

The classification of HBV genotypes relies on a nucleotide sequence divergence higher than 8% for the entire genome and at least 4.1% in the surface genes (PreS1/PreS2/S). The viral strains have been divided into 10 genotypes, A–J, and 40 subgenotypes, identified mainly for genotypes A-D and F. Different genotypes show a distinct geographical distribution and affect disease severity, course and likelihood of complications, and response to treatment (Rajoriya et al. 2017; Sunbul 2014). Genotype A is mostly found in North America, Europe, South-East Africa and India; the genotypes B and C are present in Asia and Oceania; the genotype D is the most commonly found and is present in North America, North Africa, Europe, Middle-East and Oceania, the genotype E is present in West Africa; the genotype F

in South America and the genotypes G and H are mostly found in Central and South America. Patients infected with HBV genotype C2 are more susceptible to develop chronic infection than patients infected with HBV genotype B2. Genotype C is thus associated to an increased risk of HCC development compared to the genotypes A, B, and D (Rajoriya et al. 2017).

3.2 Mutants

With no proofreading activity, HBV Polymerase is highly prone to errors that can lead to genetic variability. Despite the genomic constraint induced by the four overlapping ORF, HBV genomic variability can be enhanced by several factors such as the route of infection, the host genetics and immune response, the genome structure and replication processes leading to recombination events (Rajoriya et al. 2017). Thus, HBV circulates as viral quasi-species that evolve over time depending on host selective pressure and the fitness of the variants.

The most commonly found mutation is a G to A substitution in position 1896 in the Precore region, leading to a premature stop codon and a loss of HBeAg production. Other common mutations are found in the BCP leading to decreased expression of HBeAg in serum. Both PreCore and BCP mutants can be selected during HBeAg seroconversion. BCP mutants have been associated with an increased risk of HCC development. Other HBV mutants exist, such as deletion mutants in PreS/S genes that are linked to higher risk on developing HCC. PreS gene deletion leads to accumulation of L-HBsAg in the ER and causes oxidative stress and subsequent DNA damages and mutagenesis in the host genome. Mutants in the "a" determinant of the S gene were shown to escape from vaccine induced anti-HBs response. Some of these mutants were shown to escape the historic HBsAg detection assay, but the newer version of assays combining different detection antibodies allowed to circumvent this issue. Drug-resistant mutants also exist and influence treatment outcomes. For example, PreCore and BCP mutants are less likely to respond to IFN therapy. Nucleo(s)tide analog therapies, especially with drugs having a low barrier to resistance, can select HBV species harboring mutations in the Pol gene with decreased susceptibility to the antiviral agents (Zoulim and Locarnini 2009). Importantly, drug-resistant mutants can pre-exist before treatments and generate quasi-species that are further selected by NUC therapies (Rajoriya et al. 2017). As the polymerase and surface genes overlap, mutants that can escape both NUC therapy and HBs antibodies have been described (Villet et al. 2006). The use of high barrier to resistance NUCs in the clinical management of patients has significantly reduced the issue of antiviral resistance with this class of drugs. With the development of new antivirals targeting the key steps of viral replication (Fanning et al. 2019), the emergence of drug-resistant mutants will have to be monitored carefully and prevented by combination of drugs with different modes of action.

Finally, mutations can also be specifically associated to certain genotypes. For example, the double mutation 1762T/1764T in the BCP is more frequent in genotype C than genotype B, whereas the precore 1896A mutation is more frequent in genotype B than genotype C (Rajoriya et al. 2017).

4 Perspectives

Despite continuous advances in understanding the HBV life cycle, several gaps in knowledge have to be addressed to completely dissect the complex mechanisms of HBV replication. In particular, a clearer understanding of the basic biology of cccDNA (biogenesis, turnover, epigenetic regulation) is required to help in the development of antiviral therapies able to eliminate or silence the HBV minichromosome and, thus, allow HBV cure. The study of HBV RNA transcription and maturation has risen much of attention recently, since the identification of RNA-containing circulating viral particles. A better understanding of how viral RNAs are produced, matured, and secreted could help in highlighting the still unclear role of these particles in HBV life cycle and pathogenesis.

Finally, the development of better model systems to further characterize the molecular mechanisms of the HBV replication cycle, particularly the formation and regulation of cccDNA, will be essential to help defining new targets for antiviral therapy to achieve an HBV cure.

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2 Unmet Needs in Basic Research of Hepatitis B Virus Infection: In Vitro and In Vivo Models

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Abstract

Chronic infection with hepatitis B virus (HBV) is a preventable but incurable disease that affects more than 250 million people. Current therapies are effective in controlling infection, but complete elimination of the virus will require targeting covalently closed circular DNA (cccDNA). HBV relies on numerous host factors, some of which are promising drug targets. However, HBV replicates efficiently only within differentiated human hepatocytes. This specificity has complicated the development of in vivo and in vitro experimental models and hindered drug discovery. The identification of NTCP as the HBV receptor explained the poor infectivity in hepatoma cell lines and small animal models, but a number of approaches not directly tied to NTCP have also been established. The pressing need for more effective HBV therapies coupled with the unique challenges in the development of HBV models has propelled advances in transgenic and chimeric

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mouse models, small primate models such as macaque and tree shrew, adenoviral and hydrodynamic delivery of infectious particles, stably transfected cell lines, induced pluripotent stem cells, and 2D and 3D microarchitecture models that blur the line between in vitro and in vivo models. An array of impressive tools is now available in the search for a cure for chronic HBV infection.

Keywords

Adeno-associated virus · Covalently closed circular DNA · Hepatitis B virus HepaRG · HepG2 · NTCP · Human hepatocyte chimeric mice · Induced pluripotent stem cells · Primary human hepatocytes

Abbreviations

AAV	Adeno-associated virus
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
cccDNA	Covalently closed circular DNA
CPD	Carboxypeptidase D
DMSO	Dimethyl sulfoxide
HBsAg	HBV surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
iPS	Induced pluripotent stem
LHBsAg	Large S antigen
NK cells	Natural killer
NOD	Nonobese diabetic
NTCP	Sodium taurocholate cotransporting polypeptide
uPA/SCID	Urokinase-type plasminogen activator/severe combined immunodeficiency

1 Introduction

Nearly 250 million people throughout the world currently suffer from chronic hepatitis B virus (HBV) infection. The introduction of an effective HBV vaccine in 1986 has greatly reduced the incidence of new infections, and the disease is now largely preventable due to improvements in public health and awareness. Even when exposed to the virus, most adults are able to successfully clear acute infection. Despite this progress, chronic infection is still difficult to treat, and long-term management of the infection rather than outright cure is usually the primary goal of treatment. Current therapies can successfully but temporarily suppress viral

replication, slowing progression of liver disease and reducing the risk of complications such as cirrhosis and hepatocellular carcinoma (Kwon and Lok 2011). Long-term or even life-long treatment with interferon or nucleoside analogues such as tenofovir disoproxil fumarate, tenofovir alafenamide, or entecavir is necessary to suppress HBV replication. Long-term therapy is expensive and inconvenient and poses a small risk of adverse events and eventual development of drug resistance. HBV reactivation may also occur if therapy is discontinued, especially in immunosuppressed individuals.

The main reason for this intractability is that current therapies target essential but late-acting stages in viral replication but fail to target the virus's failsafe in the form of covalently closed circular DNA (cccDNA) mini-chromosomes that are able to persist long-term within the nucleus.

HBV is most vulnerable to elimination during the early stages of infection. Although the virus is thought to largely avoid immune surveillance during initial establishment, most individuals eventually mount an effective immune response that clears the virus during the acute phase of the infection and does not progress to chronic HBV. However, if the initial response falls short, HBV becomes highly entrenched in the liver and infects nearly all hepatocytes. RNA viruses such as hepatitis C virus that must replicate continuously can be effectively targeted using direct acting antiviral (DAA) agents. Conversely, while peg-interferon and nucleos(t)ide analogs are able to suppress HBV replication, the presence of cccDNA in the nucleus allows HBV to persist even when active replication is suppressed and allows it to quickly reactivate if viral suppression is relieved. Therefore, a successful cure for chronic HBV must contend with this contingency and will likely involve direct elimination or silencing of cccDNA or host factors involved in its maintenance (Allweiss and Strick-Marchand 2020).

Although HBV replication can be suppressed, patients with chronic HBV infection have a much greater risk of developing hepatocellular carcinoma, and it is important to continue to strive for a true cure that completely eliminates the virus. Development of such a cure is daunting due to the complexity of the HBV life cycle and the virus's high specificity to human hepatocytes, which has long hindered the search for suitable in vitro and in vivo models. Although imperfect, several models have recently been developed that facilitate analysis of HBV replication and evaluation of potential drug candidates. However, antiviral therapy is likely to be only one aspect of a successful cure, and immunocompetent models are also needed to evaluate immunomodulation strategies to restore exhausted adaptive immune responses (Maini and Burton 2019). The models reviewed below have greatly expanded our knowledge of HBV.

1.1 Hepatitis B Virus

Hepatitis B virus is a member of the *Hepadnaviridae* family in the genus *Orthohepadnavirus*. The woodchuck hepatitis virus, the ground squirrel hepatitis virus, and the woolly monkey hepatitis B virus also belong to this genus. Other

viruses within the *Hepadnaviridae* infect birds (Avihepadnaviruses) and rodents (Orthohepadnaviruses). The virus that infects humans is thought to be specific to humans and chimpanzees and does not fully infect other animals (Wieland et al. 2004). This diverse host range suggests an ancient origin for HBV followed by a long period of adaptation to each host. Therefore, while these animals can provide insight into the HBV life cycle, key differences in required host factors and other species-specific adaptations must be considered.

1.2 Woodchuck Hepatitis Virus

Given the dearth of experimental models, woodchuck hepatitis virus (WHV) has often served as a useful system to investigate the HBV life cycle and interactions with immune effectors. As another member of the *Hepadnaviridae*, WHV shares similar morphology and genome organization with HBV but differs with respect to transcriptional regulation and pathogenesis. Laboratory maintained woodchuck colonies yield high rates of chronic infection and may develop hepatocellular carcinoma (HCC) (Tennant and Gerin 2001). The woodchuck (*Marmota monax*) is also not an ideal experimental organism due to a lack of information about its genome and immune system and practical experimental difficulties such as hibernation and lack of available reagents. Nonetheless, the *Marmota monax* genome sequence was recently published (Alioto et al. 2019), and the WHV model has been used to test antivirals and immunomodulatory drugs such as the TRL7 agonist GS-9620 (Menne et al. 2015) and has served as an important model for drug toxicity (Allweiss and Dandri 2016).

1.3 Duck Hepatitis B Virus

Duck hepatitis B virus is another useful infection model. The virus is a distant relative of human HBV and supports the full viral life cycle in duck hepatocyte tissue culture, including formation of cccDNA and production of DNA replication intermediates and HBc and HBs antigens (Tuttleman et al. 1986). However, key species-specific differences must be considered, including a critical role of carboxypeptidase D (CPD) for binding in duck but not in human (Spangenberg et al. 2001).

2 HBV In Vivo Experimental Models

Chronic HBV infection often requires life-long treatment with only a small likelihood of cure and a continuing risk of progressive liver disease. Although the need for novel therapies is clear, it is challenging to find animal models that adequately mimic the biochemical details of HBV infection in humans. HBV is indigenous to chimpanzees, gorillas, orangutans, gibbons, and other large primates, but these animals are impractical as experimental systems on ethical and logistical grounds.

Conversely, the lack of NTCP receptor prevents HBV infection in smaller primates. Nonetheless, despite the well-known species- and tissue-specificity of HBV, transduction of hNTCP in macaques, baboons, and pig hepatocytes confers support for HBV infection in vitro, suggesting that NTCP is largely responsible for this species barrier (Lempp et al. 2017). The advantages and disadvantages of several in vivo models are shown in Table 2.1.

2.1 Chimpanzee Model

Recently chimpanzees (*Pan troglodytes*) have received endangered species protection status, and their use in medical research has been expressly banned in the United States and other countries. However, chimpanzees have long played an important role in HBV research as the only primate that can fully support HBV infection (reviewed in (Wieland 2015)). After HBV was first characterized, HBsAg and anti-HBsAg antibodies were detected in blood drawn from chimpanzees (Hirschman et al. 1969; Lichter 1969; Maynard et al. 1971). It was shown that chimpanzees can become infected with as little as one to three genome equivalents of

Table 2.1 Advantages and disadvantages of in vivo HBV experimental models

Model	Advantages	Disadvantages
Chimpanzee	Fully infectious, immunocompetent, most similar to human infection	Banned in several countries, ethical and practical limitations, potential differences from human in innate immune response
Macaque	Smaller, readily available, fewer restrictions; naturally occurring HBV infection; transferable; recently updated reference genome	Not as well characterized as chimpanzee
Tree shrew	Can be infected with patient sera	Transient, self-limited infection; animal handling difficulties
Transgenic mice	Consistent and well-characterized inbred lines; can achieve high replication rates	Does not support full HBV life cycle and not useful for analysis of drug resistance
Human hepatocyte chimeric mice	Supports full HBV life cycle, can be used to compare different host and viral genotypes, avoids confounding effect of adaptive immune response	Does not reflect adaptive immune response; animals are delicate and expensive
BRGS-uPA mice	Immunocompetent; can be used to analyze NK cell, T cell, and antibody responses	Defects in NK cell maturation cause differences with respect to human response
Hydrodynamic injection	Immunocompetent; can be used to analyze viral mutants	Transient expression; technically difficult
Adenovirus-mediated delivery	Persistent viremia	Low-level viremia; vector induces immune response and inflammation
Adeno-associated virus delivery	Persistent viremia; useful for development of immune therapies	Suppresses immune response but can be overcome with agonist

HBV DNA isolated from human plasma (Barker et al. 1973; Komiya et al. 2008) and can develop chronic HBV infection similar to that of humans. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are elevated (Tabor et al. 1983), but symptoms are less severe than in humans (Barker et al. 1973). Chimpanzees also played an important role in the development of the first vaccines because the immune response is similar to that of humans (McAuliffe et al. 1980). A large overlap was observed in T-cell peptide-binding specificity (McKinney et al. 2000), but gene expression analysis revealed lower than expected induction of interferon-stimulated genes (ISGs), suggesting that HBV is able to avoid detection by innate immune defenses during early infection (Wieland et al. 2004; Wieland and Chisari 2005). However, later in vivo and in vitro studies appear to show that HBV plays a more active role in suppressing the innate immune response in humans (Shlomai et al. 2014; Luangsay et al. 2015; Yoneda et al. 2016) perhaps revealing an important distinction between the human and chimpanzee immune responses.

2.2 Macaque Model

Other primate models either present the same problems as chimpanzees or fail to support HBV infection or serial passage. One exception is macaques (*Macaca fascicularis*), small Old World monkeys that have been shown to harbor HBV DNA, HBsAg, and HBcAg from a strain of HBV genotype D that probably originated in humans (Bukh et al. 2013; Dupinay et al. 2013). Transduction of hNTCP allows macaques to support in vitro and in vivo infection for six or more weeks and demonstrate cellular and humoral immune responses that make them suitable for testing antiviral and immunomodulatory drugs (Burwitz et al. 2017). Several problems, possibly related to inadequate hNTCP delivery, may limit the usefulness of this model, including poor infection rates and undetectable levels of cccDNA. Nonetheless, macaques are the most widely used nonhuman primate model in biomedical research. A greatly improved *Macaca mulatta* reference genome was recently published in which nearly 100 million genetic variants were characterized (https://science.sciencemag.org/content/370/6523/eabc6617), providing an impetus to further develop this primate model of HBV infection.

2.3 Tupaia Model

Although no longer classified as a primate, the tree shrew (*Tupaia belangeri*) provides another potential animal model for HBV infection. These small squirrel-like mammals are closely related to primates and can support HBV infection from patient sera in vivo (Walter et al. 1996). Isolated *Tupaia* hepatocytes support infection with HBV or woolly monkey hepatitis B virus and produce HBsAg and HBeAg (Walter et al. 1996; Kock et al. 2001). This model is notable for its critical role in the identification of NTCP as the primary HBV receptor (Yan et al. 2012). Tree shrews also support infection with hepatitis C virus and herpes simplex virus 1 and

2 (Walter et al. 1996; Tsukiyama-Kohara and Kohara 2014). Although promising, problems with handling of the animals have limited wide adoption of tree shrew as an animal model (Tsukiyama-Kohara and Kohara 2014).

2.4 Transgenic Mice

Attempts to improve infection efficiency using NTCP transgenic animal models have been disappointing, underscoring the need for greater insight into host factors mediating HBV host specificity (Li and Urban 2016). Transgenic mouse models have been developed that can partially support HBV replication (HBVtg), but they cannot be used to investigate critical early steps in viral entry or cccDNA formation (Ortega-Prieto et al. 2019). Although the mice are immunocompetent, they are nonetheless immune-tolerant to viral proteins, limiting their usefulness in investigating the adaptive immune response (Allweiss and Strick-Marchand 2020).

Development of animal models based on outbreeding animals such as duck and woodchuck is hindered by the problem of genetic variability that makes it more difficult to unravel the underlying immunobiology. Inbred transgenic mice have long provided a controlled genetic background for investigating HBV proteins. Lineages have been developed that express HBV surface, core, precore, and X proteins either individually or together, but replication efficiency is low (Araki et al. 1989; Farza et al. 1988). Mice harboring 1.3X-genome length HBV sequences have been developed that support higher replication efficiency (10^7–10^8 copies per mL) without inducing cellular damage. However, while models such as HBVtg can be used to investigate parts of the HBV life cycle, they do not undergo early stages such as viral entry and formation of cccDNA that are of great interest for development of antiviral therapies (Ortega-Prieto et al. 2019). Even though the mice have a functional adaptive immune system, they do not mount an immune response to the transgenic products and so are also unsuitable for analysis of the immune response (Allweiss and Strick-Marchand 2020). Similarly, it is difficult to evaluate viral clearance due to the presence of integrated HBV DNA (Yang et al. 2014).

2.5 Human Hepatocyte Chimeric Mice

Humanized mouse models in which human hepatocytes are transplanted into immunodeficient mice have successfully been used to investigate the early stages of HBV infection and to evaluate antiviral drugs. In the early trimera mouse model, human hepatocytes were transplanted into mouse kidneys (Ilan et al. 1999), Even though most mice became infected, viremia was poor (10^5 IU/mL), in part because key liver architectural features were lacking. Replication rates were improved in other mouse models by changing the genetic background of the mice to induce liver damage and promote establishment of human hepatocytes.

Transgenic mice were established in which the urokinase gene is regulated by the human albumin promoter. These urokinase-type plasminogen activator (uPA) mice

were then mated with severe combined immunodeficiency (SCID) mice (Heckel et al. 1990). Hepatocyte death in the uPA/SCID offspring causes subacute liver failure that is compensated via transplantation of human hepatocytes (Rhim et al. 1995). This mouse model supports infection with both HBV (Dandri et al. 2001) and HCV (Mercer et al. 2001). A small number of native mouse hepatocytes remain in the liver, and a herpes simplex virus type-1 thymidine kinase (HSVtk)/ganciclovir (GCV) system was developed to remove residual mouse hepatocytes, but the approach was unsuccessful (Douglas et al. 2010). However, measuring the level of human albumin provides an estimate of the repopulation rate and can be used to monitor graft failure. HBV has been found to remain infective after passage in mice (Tsuge et al. 2005; Meuleman et al. 2005; Sugiyama et al. 2006), and the model has made it possible to examine early host and innate immune responses and to evaluate therapeutic agents over the full viral life cycle.

Nonetheless, the model is limited due to the lack of key components of the innate immune response and the complete lack of an adaptive immune response that is required to avoid rejection (Li and Di Santo 2019). Chimeric mice also require a source of donor hepatocytes, although this may also be seen as an advantage because the effects of donors with different genotypes can be compared. Explanted hepatocytes can also be used as a source of human hepatocytes for in vitro experiments (Michailidis et al. 2020). An alternative approach is to generate chimeric mice using hepatocytes derived from iPS or dHepaRG cells (Yuan et al. 2018a, b), and mouse models with inducible liver failure have been developed using fumaryl acetoacetate (FAH)−/− mice in which accumulation of a toxic metabolite kills hepatocytes unless the mouse is supplied with 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cycloh exanedione (NTBC). Notably, male FAH−/− mice have higher mortality (Michailidis et al. 2020).

2.6 Immune-Competent Mouse Models

A major shortcoming of chimeric mouse models is the lack of an adaptive immune response, a critical factor in determining whether or not HBV is able to establish chronic infection. One approach to establishing an immune-competent HBV infection animal model is to transplant both human hepatocytes and human immune cells (Tzeng et al. 2013). Transplantation of fetal hematopoietic stem cells and hepatoblasts resulted in low chimerism and modest replication (Kremsdorf and Strick-Marchand 2017; Douam and Ploss 2018), although the use of oncostatin M has been shown to improve chimerism (Billerbeck et al. 2016). Formed by transplanting both human hepatocytes and hepatic stellate cells, BALB/cRag2$^{-/-}$IL2rg$^{-/-}$SIRPaNOD-uPA (BRGS-uPA) mice demonstrate a number of attractive features, including good infectivity over several months, a high repopulation rate, presence of NK cells, Kupffer cells, PD-1Hi effector memory T cells, and development of IgG antibodies against the HBV surface and core proteins (Allweiss and Dandri 2016; Kremsdorf and Strick-Marchand 2017; Dusseaux et al. 2017; Lopez-Lastra and Di Santo 2017). Although the presence of NK cells is a chief advantage of this model, deficient cytokine production in BRGS-uPA mice due to the lack of human MHC results in

defects in NK cell functionality relative to human NK cells (Lopez-Lastra and Di Santo 2017).

2.7 Hydrodynamic Injection HBV Mouse Model

Efficient delivery of HBV into mice also poses a challenge with respect to immune competence. Hydrodynamic injection is a technically challenging method in which a large volume of DNA is injected rapidly into the mouse tail vein. This hydrodynamic effect increases the pressure in the inferior vena cava, causing the viral DNA to pass through the hepatic portal vein and through the liver fenestrae where it comes into direct contact with hepatocytes (Tzeng et al. 2013). Yang et al. injected greater than full-length HBV genomic DNA (pT-MCS-HBV1.3) into the tail vein of both immune-competent mice and nonobese diabetic (NOD)/SCID mice lacking T, B, and natural killer (NK) cells (Yang et al. 2002). While HBV gene expression and viremia were observed in both mice, the virus disappeared rapidly in immunocompetent mice following CD8+ T cell proliferation, whereas the virus remained detectable for several months in the immunocompromised mice.

2.8 Adenovirus-Mediated Delivery

Another way to deliver viral DNA to the hepatocytes is via delivery with another virus. Infection with HBV DNA cloned into adenovirus or adeno-associated virus (AAV) yielded low-level per persistent viremia (Tzeng et al. 2013; Bramson et al. 1995; Huang et al. 2006). However, AAV both elicits an immune response, including the release of cytokines and chemokines, while also suppressing immune responses (Tzeng et al. 2013). Recent innovations, such as the less strongly immunogenic AAV2/8, have been used to establish persistent infections that last several months (Paulk et al. 2018). AAV can also be used to compare virus–host interactions and response to treatment with different HBV genotypes (Liu and Kao 2013), including genotypes A, B, and C (Huang et al. 2006; Li et al. 2013, 2016). While an advantage of AAV mouse models is that immunocompetent mice can be used, the full viral life cycle is not represented, as the virus is not able to reinfect hepatocytes, and it is not a suitable model to evaluate cccDNA (Lucifora et al. 2017). Furthermore, the murine immune response may not adequately reflect the human response.

3 HBV In Vitro Experimental Systems

Animal models are indispensable for elucidating complex host–virus interactions and evaluating antiviral therapies, but identification and testing of drug targets requires an efficient and reproducible in vitro model. Nonetheless, the development of a suitable in vitro model that supports viral entry and the complete viral life cycle has proven problematic. Each in vitro model has specific use cases as well as limitations, and no single model has so far proven superior for all applications (Table 2.2).

Table 2.2 Advantages and disadvantages of in vitro HBV experimental models

Model	Advantages	Disadvantages
Primary human hepatocytes	Gold standard for in vitro analysis of HBV infection and analysis of drug toxicity	Difficult to obtain, limited genetic variability, and rapid loss of infectivity
HepG2 cells	Produces HBV virions, polarized cells	Morphological and chromosomal differences from primary hepatocytes; does not support HBV entry
HepaRG cells	Supports replication of HBV and HDV; supports HBV entry	Requires time-consuming differentiation step; chromosomal differences
NTCP-expressing cells	Supports HBV entry and replication	Yield of HBs, HBe, and HBV DNA is low
Human hepatocytes isolated from chimeric mice	Has most advantages of human hepatocytes with few of the disadvantages, improved infectivity	Expensive, complex, requires proliferation in chimeric mice
2D/3D/microfluidic culture	More accurately reflect liver architecture and interactions among cell types	Complex; still shows differences compared to in vivo
Induced pluripotent stem cells	Supports HBV infection and analysis of innate immune response	Complex, heterogeneous, difficult to establish in culture

3.1 Primary Human Hepatocytes

Fresh primary human hepatocytes (PHHs) probably best recapitulate conditions in the liver and are a natural choice for in vitro analysis (Shimizu et al. 1986; Gripon et al. 1988, 1993; Ochiya et al. 1989; Galle et al. 1994), but there are a number of drawbacks in relying on them as an in vitro model of HBV infection. Not only are donor cells difficult to obtain and heterogeneous genetic backgrounds may introduce confounding, but the cells quickly begin to de-differentiate and lose the ability to support HBV infection due to changes in gene expression resulting in loss of hepatocyte-specific factors (Guillouzo et al. 2007; Wilkening and Bader 2003; Wilkening et al. 2003; Birkus et al. 2019). The strong tissue tropism exhibited by HBV is driven in part by liver-specific expression of NTCP as well as nuclear factors required for efficient transcription of the HBV genome. Treatment with dimethyl sulfoxide (DMSO) and dexamethasone and hydrocortisone helps to maintain hepatocyte differentiation and prolongs infectivity (Evripioti et al. 2019). Similarly, the use of polyethylene glycol and a high MOI helps to improve infection efficiency (Verrier et al. 2016a). Co-culture with mouse embryonic fibroblasts and establishment of 3D microfluidic liver culture also facilitates long-term infection by helping to recreate the functional architecture of the liver (Winer et al. 2017, 2020; Ortega-Prieto et al. 2018). However, availability remains a key limitation of PHHs. One solution is to further drive de-differentiation of PHHs to form liver progenitor cells then induce them to proliferate and re-differentiate into PHHs (Fu et al. 2019). Primary hepatocytes derived from chimpanzees can also support HBV infection but face many of the same issues as PHHs. Fortunately, tree shrew primary hepatocytes

also transiently support HBV infection and were instrumental in identifying NTCP as primary HBV receptor (Walter et al. 1996; Yan et al. 2014).

3.2 Hepatoma Cell Lines

Although PHHs provide an attractive HBV infection model, they are not ideal. Aside from being difficult to acquire, fresh PHHs rapidly de-differentiate and no longer support HBV infection. To overcome these limitations, hepatoma cell lines have been widely used to investigate mechanisms of viral replication, identify host factors, and evaluate drug candidates. Unfortunately, HepG2 and Huh7 cell lines do not support HBV entry due to lack of hNTCP expression, and instead HBV production is made possible by transfecting or integrating HBV genomes (Verrier et al. 2016a).

3.3 HepG2 Cells

Given the limited availability and short window of infectivity of PHHs, hepatoma cell lines offer a number of potential advantages. Derived from a hepatocellular carcinoma in an adolescent male, HepG2 cell lines such as HepG2.2.15 support HBV production using transfected HBV DNA (Sells et al. 1987; Ladner et al. 1997). HepG2 cells do not support HBV entry but retain important aspects of liver microarchitecture, including polarization into basolateral and apical domains (Glebe and Urban 2007). Nonetheless, HepG2 cells differ with respect to morphology, chromosome number, and the number of nuclei (Wilkening et al. 2003; Natarajan and Darroudi 1991). While housekeeping genes and some liver-specific genes are expressed at comparable levels to PHHs, several key transcription factors and enzymes, such as C/EBP-α and CYP3A, are expressed poorly in HepG2 cells (Wilkening et al. 2003; Knowles et al. 1980; Rodriguez-Antona et al. 2002; Jover et al. 2001). Such differences complicate drug development and identification of essential host factors.

3.4 HepaRG Cells

The HepaRG cell line was derived from an HCV-associated hepatocellular carcinoma (Guillouzo et al. 2007) and is characterized by an additional chromosome 7 and a translocation between chromosomes 12 and 22 that resulted in a deletion of the 12p region (Gripon et al. 2002). HepaRG cells support HBV and HDV infection and (Gripon et al. 2002; Hantz et al. 2009) but first require a time-consuming differentiation process. Addition of DMSO and hydrocortisone hemisuccinate induces differentiation into hepatocytes and biliary cells. Hepatocytes maintain stable expression of liver-specific factors for over 6 weeks, but albumin levels are variable, and CYP3A4 and CYP7A1 levels are strongly upregulated relative to PHHs (Kanebratt and Andersson 2008a, b).

3.5 HepCHLine-4 Cell

The key to improving HBV infectivity in vitro may be to combine the infectivity of primary human hepatocytes with the advantages of hepatoma cell lines. To this end, Jiang et al. fused primary human hepatocytes with HepG2 cells to create the HepCHLine-4 (Jiang et al. 2009). These cells remained susceptible to HBV infection even after a year of subculturing and produce cccDNA and viral particles.

3.6 Recombinant cccDNA

Given the importance of cccDNA as a key target in ongoing drug development, reliance on HBV plasmids is not ideal. Instead site-specific DNA recombination and minicircle technology have made it possible to deliver recombinant cccDNA molecules into hepatoma cells (Yan et al. 2017). rcccDNA support stable HBV production, and use of a luciferase reporter system facilitates development of compounds that target cccDNA, but the model cannot be used to examine viral entry (Allweiss and Strick-Marchand 2020) and delivery of rcccDNA into mice through hydrodynamic injection or adenovirus is technically challenging and may induce inflammation in the liver (Yan et al. 2017; Li et al. 2018).

3.7 NTCP Expression as a Limiting Factor for HBV Infection

The revelation that NTCP serves as the primary HBV receptor went far to help understand the species- and tissue-specificity of HBV infection and revealed a potential approach to develop new infection models (Watashi et al. 2014). The lack of robust NTCP expression in hepatoma cell lines prevents HBV from binding and entering HepG2 cells, and the rapid decrease in NTCP expression in PHH cell culture limits the ability of cultured cells to maintain long-term infection (Chen and Ye 2012). While HepaRG cells do express NTCP, the orientation of the basolateral membrane limits physical access by the virus (Schulze et al. 2012).

3.8 NTCP-Expressing Cell Lines.

Once the identity of the HBV receptor was known, it became possible to modify existing hepatoma cells to support HBV entry and spread among cells (Li and Urban 2016), leading to the development of NTCP-expressing cell lines such as hNTCP-HepaRG, hNTCP-Huh, hNTCP-HepG2, and hNTCP-HEK293 (Yan et al. 2012; Iwamoto et al. 2014). For example, Iwamoto et al. transfected an NTCP expression plasmid into HepG2 (Iwamoto et al. 2014). The resulting HepG2-NTCP-C4 cells could be infected with serum-derived HBV with an infection rate close to 50%. Yan et al. transfected the pcDNA6-NTCP plasmid into HepG2 cells to produce the HepG2-NTCP12 line and improved the initially low infection rate using

centrifugation (Yan et al. 2015). Although NTCP expressing cell lines are promising, production of HBs antigen, HBe antigen, and HBV DNA remain low even with the use of a high viral titer (6000–18,000 GEq/cell) (Yan et al. 2012; Iwamoto et al. 2013; Ni et al. 2014). While NTCP is the primary receptor for HBV, co-receptors or other host factors that are deficient in hepatoma cells probably assist in viral entry or replication. For example, RNA silencing of glypican 5 (GPC5) hindered HBV binding and suppressed HBsAg and HBV pgRNA levels, suggesting that GPC5 plays an accessory role in HBV entry (Verrier et al. 2016b). Similarly, Iwamoto et al. recently showed that epidermal growth factor receptor (EGFR) is critical for internalization of bound virions (Iwamoto et al. 2019). It is likely to be necessary to induce expression of additional host factors in order to achieve efficient HBV replication in hepatoma cells (Tnani and Bayard 1999).

3.9 Human Hepatocytes Isolated from Humanized Mice

Given the variety of challenges of restoring hepatocyte-specific gene expression in hepatoma-derived cell lines, improving the cell culture properties of primary hepatocytes remains an important goal. While PHHs are known to rapidly lose infectivity in culture, Ishida et al. noted that human hepatocytes explanted from human hepatocyte chimeric mice tended to remain infective longer (Ishida et al. 2015). They proposed the humanized mouse model as a source of primary human hepatocytes (Fig. 2.1). Cryopreserved hepatocytes from a single donor are transplanted into uPA/SCID mice (Tateno et al. 2004), allowed to proliferate, and then isolated using a two-step collagenase perfusion method and cultured in hepatocyte clonal growth medium. While the need for an animal model as a first step in establishing an in vitro model is expensive and complex, this approach offers several advantages, including a 500–1000 fold increase in yield in the number of cells derived from the

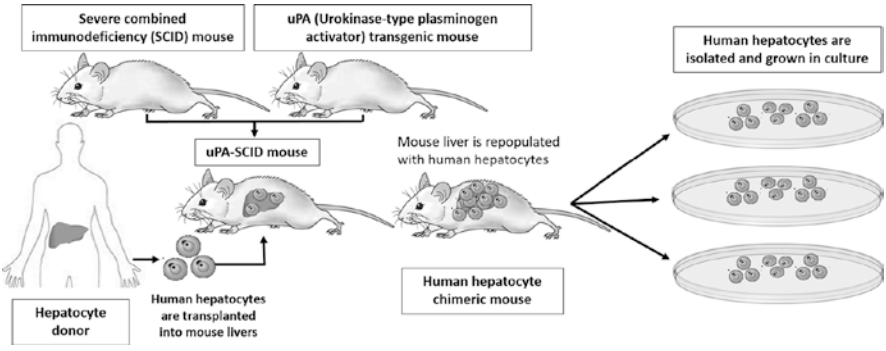

Fig. 2.1 Human hepatocyte chimeric mice as a source of primary human hepatocytes. Human hepatocyte chimeric mice are prepared by transplanting cryopreserved human hepatocytes from a donor into urokinase-type plasminogen activator-transgenic/severely combined immunodeficient (uPA/SCID) mice. Hepatocytes divide several times and repopulate the mouse liver. Human hepatocytes are then isolated from the mouse livers and grown in culture

same donor, as well as better homogeneity and higher rates of infection and virus production than PHHs, HepaRG, and NTCP-HepG2 cell lines.

3.10 Non-Cancer-Derived Immortalized Human Hepatocytes

The use of cancer cells has made it possible to analyze the HBV life cycle in detail, but cancer cell lines differ from primary hepatocytes in a number of ways. E/NtG8 cells are immortalized NTCP expressing human hepatocytes that do not derive from a cancer cell line (Akahori et al. 2020). When cultured under three-dimensional conditions, the cells support infection with HBV from blood as well as from recombinant HBV from culture, suggesting that E/NtG8 cells may support investigation of the HBV life cycle under conditions that better replicate the liver environment.

3.11 Improvements to Primary Hepatocyte Culturing

One disadvantage of cell culture is that normal interactions among cells of the same and different types cannot be fully recreated. Hepatocytes are by far the most common cells in the liver (80%), but the remaining 20% of cells are also important in establishing the functional architecture of the sinusoid. A number of approaches have been made to more accurately model the liver microenvironment, including 2D, 3D, or microfluidic culture, and co-culture with non-parenchymal cells (Shlomai et al. 2014; Godoy et al. 2013; Petropolis et al. 2016). Although complex, these approaches might yield insight into early steps in infection that are difficult to examine by other means, such as passing through the sinusoidal endothelial barrier and interaction with Kupffer cells and hepatic stellate cells (Petropolis et al. 2016).

3.12 Hepatic Cell Lines Derived from Human Induced Pluripotent Stem Cells

Unraveling the formation and maintenance of cccDNA represents a key goal in the development of HBV therapies. While PHHs recapitulate some aspects of the cellular environment within the liver, PHHs are in limited supply and lose infectivity rapidly. An alternative approach is to induce pluripotency in somatic cells and then drive hepatocyte-specific cell differentiation. For example, stem cell-derived hepatocyte-like cells (HLCs) possess characteristics of mature hepatocytes and can be used for drug testing (Xia et al. 2017). Kaneko et al. developed two induced pluripotent stem cell (iPS) models of HBV infection: immature proliferating progenitor-like cells (iPS-HPCs), and differentiated hepatocyte-like cells (iPS-Heps) (Kaneko et al. 2016). HBV replicates successfully and induces a primary hepatocyte-like innate immune response in both cell lines. While iPS-Hep cells supported higher infection efficiency, iPS-HPC cells were more homogeneous and easier to culture. Overexpression of NTCP helped to improve infection efficiency in iPS-HPC cells. cccDNA is detectable in these cells

and is maintained by inhibition of the Janus-kinase pathway. Therefore, iPS cells can serve as a suitable substitute for primary human hepatocytes for large-scale applications such as drug screening (Kaneko et al. 2016).

4 Conclusions

In hindsight, it is remarkable how much progress has been made in unraveling the HBV life cycle and developing antiviral therapies given the lack of animal models and limitations in the ability to infect hepatocyte-derived cells in vitro. Nonetheless, infection with related but distinct viruses or delivery methods that omit part of the viral life cycle, as well as the use of immunocompromised non-primate animal models, has in some cases led to confusion in interpreting the role of host factors and identifying drug targets, suggesting that candidate drugs must be evaluated using more than one model (Allweiss and Strick-Marchand 2020). Most notably, this ambiguity long delayed the discovery of the primary HBV receptor. The lack of NTCP in hepatoma cell lines and small animal models explained much of the species and tissue specificity of HBV, and it became possible to modify existing cell lines to gain or improve infectivity. While overexpression of NTCP can allow HBV to enter previously non-susceptible cells, infection rates are typically far below maximum levels observed in vivo, suggesting that additional factors may be involved that are lacking in cultured cells. Improvements in primary hepatocyte cell culture have also helped to overcome problems due to use of immortalized cell lines. Recent attempts to recreate the microarchitecture and cell-to-cell interactions of the liver help to bridge the gap between in vivo and in vitro experiments and provide insight into aspects of HBV binding and viral spread that are difficult to address by other methods. While the chimpanzee is no longer a viable option as an animal model in many countries, several other primates and small animal models have been established, including tree shrews and macaques. Similarly, human hepatocyte chimeric mice, as well as immunocompetent mouse models, can be used to examine different aspects of the immune response in a mouse model. Each experimental system has advantages and disadvantages, and no single system is currently useful for all purposes, but the current arsenal of models and delivery methods are suitable for a wide range of research questions. The stage is finally set for a new era in research into the treatment of chronic HBV infection.

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Unmet Needs in Clinical Research Hepatitis B

3

Geoffrey Dusheiko

Abstract

Several unmet clinical needs are required to improve access to diagnosis and current therapies. Although a test for HBsAg has long been available, only 30 million individuals (10%) are believed to have been diagnosed. A relatively small proportion of persons worldwide receive treatment. Deaths will increase in infected adults unless large increases in screening and a nexus to care are implemented. The age-specific disease burden is incompletely understood in many geographical regions. Clinical research to dissect the effect of prolonged suppression on cccDNA copy number will consolidate treatment and management. Accurate data, to establish appropriate treatment criteria for chronic HBV in different regions is required. It would be invaluable to improve signature phenotyping of the disease to stratify risk, prognosis and treatment indications. New, standalone, easy-to-use point of care and affordable HBV DNA tests will overcome the inability to test more widely and facilitate treatment decisions. More precise molecular and immunological data would further identify risk and treatment indications. Timing of therapy in patients with chronic hepatitis B requires re-evaluation. Further studies are required to predict outcomes after cessation of nucleoside analogue to ensure immunological control without severe aftermath. Newer compounds interfering with translation or HBsAg assembly offer the possibility of a direct reduction of HBsAg in serum. The clinical effect of deepening inhibition or a shutdown of HBV replication could be achieved with a combination of nucleoside analogues and capsid inhibitors. Data suggest that detectable pgRNA and HBcrAg together reflect residual cccDNA and transcription.

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1 Introduction

Chronic hepatitis B will remain a cause of substantial morbidity and mortality for several decades, despite effective vaccination programmes. Despite the accumulated knowledge of the biology of hepatitis B virus (HBV), and the pathogenesis of the resultant hepatic and extrahepatic disease, current therapies do not cure the disease. Eradication of HBV from the host is not possible in the majority. The variable course of the infection is imperfectly understood. Patients with chronic HBV infection are candidates for maintenance suppressive treatment with nucleoside analogues. Several unmet clinical needs are required to further the aim of cure, but also to improve access to diagnosis and current therapies.

2 Hepatitis B Lifecycle

After viral entry, partially double-stranded relaxed circular (rcDNA) is transported to the hepatocyte nucleus and converted to a covalently closed circular minichromosome (cccDNA). The stable episomal cccDNA minichromosome is the transcriptional template for HBV mRNAs, and pregenomic RNA (pgRNA) to initiate viral replication. cccDNA is thought to be synthesized from rcDNA derived from incoming virions but is also replenished from intracellular nucleocapsids via an intracellular cccDNA shuttle amplification pathway, and thus is maintained as a stable minichromosome in the nucleus of hepatocytes. cccDNA is not rapidly degraded by current nucleoside analogue therapy, as only minus strand and plus strand DNA synthesis is inhibited. Clinical research to dissect the effect of prolonged suppression on cccDNA copy number will consolidate treatment and management.

The goal of curative therapies is to clear HBsAg. Random integration of the HBV genome and continued production of HBsAg thwarts cure: HBsAg in serum is derived from a large excess of subviral particles, as well as from mature infectious virion. Transcription of HBsAg occurs from integrated viral genomes in both HBeAg-positive and -negative patients. In turn, the high HBsAg protein antigen concentrations may drive antigen-specific immune dysfunction and T and B cell exhaustion. Studies are in progress examining long reading frames and transcription from HBV integrations, which are discernably scattered randomly throughout human chromosomes (Wooddell et al. 2013). There is an important clinical need to accurately disaggregate HBsAg derived from episomal versus integrated HBV DNA to understand the variable contribution of circulating HBsAg from these sources (Fig. 3.1). HBx encoded by the X gene functions as a regulatory protein.

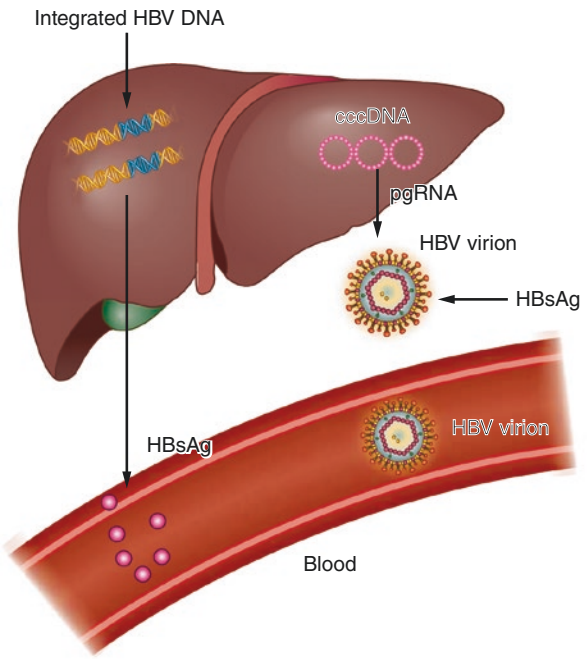

Fig. 3.1 Sources of HBsAg Particles in blood

HBx enhances cccDNA transcriptional activation and is an attractive viral target to potentially silence cccDNA (Lucifora and Baumert 2020; Minor et al. 2020). The protein is highly conserved but the difficulty detecting X protein or HBx mRNA limits clinical utility.

3 Improved Screening, Diagnostic Testing and Linkage to Care

The growing realization of the global health threat from chronic hepatitis B (HBV) prompted the publication of the World Health Assembly targets to control and eliminate HBV. The aims include 90% complete coverage of HBV vaccination and vaccination at birth, a reduction in prevalence of HBsAg amongst 5-year olds to 0.1% and finally, to improve treatment rates to 80%, by 2030. (WHO 2017) Two age-dependent interventions are therefore required to reduce the incidence of adverse outcomes: Effective prevention of neonatal and childhood infection by vaccination and secondly, prevention of cirrhosis and hepatocellular carcinoma (HCC) in adults, by appropriate treatment.

Although a diagnostic test for HBsAg has long been available, only 30 million individuals (10%) are believed to have been diagnosed and of these, perhaps approximately 5 million are currently receiving antiviral treatment. Without action, new chronic HBV infections will accrue unless appropriate prevention at birth is applied. Deaths will increase in infected adults unless large increases in screening and a

nexus to care are implemented. Improved seroprevalence testing will inform interventions. The age-specific disease burden is incompletely understood in many geographical regions. Barriers to expanding therapy for hepatitis B in resource-limited settings include a limited understanding of the prevalence of the disease and its distribution, particularly in low-income countries and limited resources to perform appropriate diagnostic and monitoring assays. However large-scale structural changes may not be required if existing HIV services are utilized.

As noted by the WHO, surveillance for HBV varies widely in its methods and completeness. International guidelines recommend screening for high-risk groups, including household and sexual contacts of persons with CHB, HIV-infected individuals, people injecting drugs (PWID), men who have sex with men and sex workers. Those at risk who lack immunity must be offered hepatitis B vaccination. Other groups that may be considered for screening include indigenous peoples, the incarcerated and transgender persons. Screening programmes have been implemented in some first nation peoples. Many countries offer routine antenatal screening to detect HBsAg and levels of viraemia in the mother, but the utilization of universal birth dose vaccination obviates maternal screening for hepatitis B, missing infected women of childbearing age, and highly viraemic mothers.

Few major efforts to identify persons with progressive disease are in progress, except in some industrialized countries. These policies need extension to socially marginalized immigrant populations. There is an urgent need to link programmes to employment and national insurance, and in high prevalence countries, and to widespread population-based screening in urban and rural citizens. Screening for HIV in HBsAg-positive individuals is mandatory, but the reverse does not occur—thus again impairing the diagnosis of hepatitis B.

4 Improved Strategies to Control Hepatitis B

The burden of disease associated with untreated hepatitis B is substantial: Accurate prevalence data, and establishing appropriate treatment criteria for chronic HBV in different regions and appropriate ascertainment of the stage of disease would more precisely determine the number of HBV-infected individuals eligible for treatment worldwide. Despite their relatively low costs, generic nucleoside analogue treatments do not reach the majority of HBV mono-infected individuals at minimum target prices. HBV DNA diagnostic testing remains the fundamental means of identifying levels of viremia that specify treatment, linkage to care and consideration of antiviral treatment in pregnancy to prevent mother-to-child transmission (MTCT), and treatment to prevent progressive disease (WHO 2020). National policies can be tethered to modeled benchmarks to enable governments and health agencies to gauge and mount the effort necessary to control HBV (Collaborators 2018; Hecht et al. 2018).

It remains imperative to break down barriers that exist because of political indifference, poverty, and poor infrastructure. Clearly, resource requirements are critical considerations, but the investment in infrastructure will provide returns in health gain.

5 Antiviral Treatment

It is estimated that around 650,000 people die each year from chronic hepatitis B infection, and overall, HBV accounts for around 45% of cases of HCC and 30% of cirrhosis worldwide. Whilst interferon-alpha therapy may be applicable in some, maintenance suppressive nucleoside analogue treatment is favoured for the majority requiring therapy.

Only a small proportion of people worldwide receive treatment. Barriers to expanding therapy in resource-poor countries include a limited grasp of the prevalence of the disease, and limited resources to perform diagnostic and monitoring assays. Antiviral treatments for hepatitis B have become affordable through generic manufacture. However, in many high prevalence countries health care costs are met by individuals; the ability to treat HBV infection will require investment or nongovernmental programmes. Long acting formulations could assist adherence and practicality for rural populations.

5.1 Indications for Treatment

The aims of current treatment are sustained suppression of HBV replication and disease remission. There is currently incomplete consensus on which patients should be treated. In general, treatment of chronic HBV infection is targeted at patients with active disease and viral replication, but preferably at a stage before significant liver injury has developed. Since chronic HBV infection is a dynamic process within individuals, longitudinal assessment of disease activity and viral replication is usually necessary to identify the most appropriate patients and optimal timing for treatment.

The important REVEAL studies showed that serum HBV DNA concentrations are associated with prospective incidence of HCC (Chen et al. 2006). Not all patients with chronic hepatitis B are candidates for long-term antiviral treatment. It would be invaluable to improve phenotyping of the disease to stratify risk, prognosis and treatment indications. Current assessment of the disease phase is based on longitudinal assessments of HBV serology (the presence or absence of HBeAg), serum aminotransferase levels and, HBV DNA measurements. Either liver biopsy or non-invasive markers are utilized to stage and grade the degree of fibrosis and inflammation. Since HBV liver disease is a dynamic process, repeated assessment of these parameters over time are often required in order to accurately establish disease activity. Markers to accurately signify prospective risk are required.

Recent studies have suggested that in HBeAg-negative patients serum HBV DNA concentrations are linearly associated with HCC risk: the risk is higher for patients with HBV DNA concentrations >5 $\log_{10}$ IU/mL. However amongst HBeAg positive patients, HCC risk is highest in those with serum DNA >6–7 $\log_{10}$ IU/ml, but paradoxically lowest in those with >8 $\log_{10}$ IU/ml. There is a key clinical distinction between HBeAg positive patients with high levels of HBV DNA but normal serum ALT and minimal hepatitis (in the “immunotolerant” phase of chronic

hepatitis B), and those in the "immunoactive" phase with raised serum ALT and active hepatitis histologically. Because antiviral drug therapy rarely results in sustained HBsAg clearance and the disease in this phase is only slowly progressive, antiviral therapy is currently not advocated during the "immunotolerant" phase. Immunological tolerance may be a constituent of neonatal hepatitis B infection and subsequent chronicity, but tolerance is imprecisely defined in later stages of the evolving disease, and there are no clinically applicable diagnostic tests to verify immunological tolerance (Kennedy et al. 2012).

However, timing of therapy in patients with HBeAg-positive chronic hepatitis B is a critical consideration that requires re-evaluation. Patients with HBV DNA concentrations >8 $\log_{10}$ IU/ml may not exhibit severe hepatic necro-inflammation but those with somewhat lower HBV DNA concentrations may be traversing a biological gradient and transitioning to a different stage of the natural history characterized by reducing HBV DNA concentrations, and an altered immune response (Choi et al. 2019; Mason et al. 2016). To avoid the hepatic injury associated with the transition, consideration may be given to extend treatment to chronically infected patient with replicative hepatitis B (Kim et al. 2020). Unfortunately long-term controlled studies would not be ethically permissible, therefore increasing the need for new signature biomarkers and immunological phenotyping.

The recent WHO HBV guidelines are primarily targeted at LMICs. These guidelines suggest that treatment be targeted to patients at high risk of disease progression, and are based on the detection of raised ALT and HBV DNA levels >20,000 IU/mL regardless of HBeAg status (Dusheiko and Wang 2019). A diagnosis of advanced fibrosis includes clinical criteria or testing (APRI >2 or Fibroscan). Thus, although these guidelines are a useful benchmark, they underestimate the need for treatment (Sonderup et al. 2020; Shimakawa et al. 2018; Dusheiko and Lemoine 2019; Aberra et al. 2019).

The utilization of single-agent nucleoside analogues including tenofovir or entecavir could theoretically increase the risks of antiviral resistance (Park et al. 2019). The set point of suppression in patients with such high levels of replication also requires determination, as current evidence may favour complete suppression versus incomplete suppression. Tenofovir alafenamide fumarate (TAF), is an orally bioavailable prodrug of tenofovir; the compound reduces known toxicities of tenofovir. Unfortunately costs savings are not being realized.

6 Therapy with Current Drugs

Suppression of HBV replication has an important beneficial effect on progression of liver disease. Nucleoside analogue anti-viral drugs can alter the natural history of hepatitis B disease (and reduce the risk of cirrhosis, hepatic decompensation and hepatocellular carcinoma) but are given as maintenance suppressive therapies. Nucleoside analogues act as chain terminators to block reverse transcription of pgRNA to rcDNA, and minus and plus strand synthesis. Spontaneous loss of HBsAg is rare and only occurs in a small fraction of patients, perhaps 1–2% of patients

per annum (Yeo et al. 2019). Therefore, HBsAg remains detectable for a prolonged, indefinite period during long-term nucleoside analogue therapy, albeit that cccDNA concentrations may show a slow decline (Lai et al. 2016). PEG IFN exerts pleiotropic antiviral molecular and immunological effects that are still imperfectly understood: PEG IFN decreases cccDNA transcription via epigenetic modification in experimental systems. Interferon-alpha and lymphotoxin-beta receptor agonists lead to upregulation of APOBEC3A cytidine deaminases, in infected cells, to degrade cccDNA (Lucifora and Protzer 2016).

PEG IFN or nucleoside analogues may lead to HBsAg loss in approximately 5–10% of patients after one year of treatment. Response to interferon-alpha requires a yet poorly defined immunological primed state, but other than manifestly elevated serum aminotransferase, the likelihood of response remains imprecisely delineated. It is not determined to what extent HBeAg loss, or HBsAg loss or prolonged HBV DNA suppression to below the level of detection (<20 IU/ml) represents a reduction in hepatocytes harbouring HBV cccDNA minichromosomes or a reduction, inactivation, or silencing of cccDNA (Dusheiko and Wang 2019).

6.1 Nucleoside Analogues and PEG IFN

Add-on or switch therapies may amplify treatment responses, to result in HBsAg seroclearance, but the probability of HBsAg loss remains somewhat unpredictable in both HBeAg-positive and -negative patients. The addition or switching to PEG IFN provides a synergism that may yet require exploitation with new investigational agents (Vigano et al. 2016). However, PEG IFN as a primary treatment of hepatitis B is being phased out. The compound remains for testing proof of principle experimental steps to cure in clinical trials, as an adjunct or additive to other therapies.

7 Cessation of Nucleoside Analogues After Long-Term Suppression

Discontinuation of antiviral therapy carries the risk severe acute-on-chronic liver injury or progressive disease. However, recent evidence suggests that NAs can be discontinued, in anti-HBe positive patients after prolonged suppression. An increasing number of studies have examined curtailing treatment after long-term nucleoside analogue maintenance suppressive therapy (Berg et al. 2017). The aims are twofold: to achieve cessation of treatment but maintained suppression, or to trigger HBsAg loss. Paradoxically, HBsAg loss can be induced in a proportion of patients following cessation of treatment. However, such a therapeutic strategy holds for relatively few patients. Further studies are required to predict outcomes after cessation of nucleoside analogue and importantly to ensure immunological control without a severe aftermath. Severe post-cessation exacerbations are detrimental and injurious to the liver.

Recent studies of nucleotide analogue cessation have varied in design, and ethnic composition. The mechanism of the differential timing of onset of biochemical and virological flares between tenofovir and entecavir has not been explained. Off-treatment follow-up indicates rates of HBsAg loss varying from 4% to 10% within 48 weeks. Lower concentrations of HBsAg favour HBsAg loss, but predictors of HBsAg vary from <1000 U/L to <10 U/L. The positive predictive value of HBsAg loss in patients with defined, low concentrations of HBsAg remains relatively low. However, high negative predictive values (98–100%) have been reported for HBsAg concentrations above 10 U/L (for Asian patients) and >1000 U/ml for Caucasian patients at the time of stopping the nucleoside analogue (Seto et al. 2015). Thus, HBsAg quantitation is an imprecise measure of the risk of reactivation after NA withdrawal, and confers predictive value only at low concentrations, which are not observed in the majority of patients (Lucifora and Protzer 2016; Dusheiko and Wang 2019; Vigano et al. 2016; Berg et al. 2017; Li et al. 2019). Other parameters incorporating HBcrAg, pgRNA and HBsAg concentrations may prove more useful (Hsu et al. 2019a).

8 Reactivation of Hepatitis B

Reactivation of HBV may occur in HBsAg positive persons, as well as HBsAg negative, anti-HBc positive individuals, under different circumstances. It can occur spontaneously but usually results from immunosuppressive therapies and chemotherapy. Reactivation is frequent after the cessation of nucleoside analogues and may be severe. The outcomes can be severe if unchecked, or in patients with advanced fibrosis or cirrhosis. The residual persistence of cccDNA serves as a marker of the risk of reactivation. However, it is not a practical diagnostic test (Sastre et al. 2019; Bath et al. 2019).

Determining who is at risk in patients receiving chemotherapy for haematological or solid tumours or patients receiving immunosuppressive treatment for autoimmune disorders, or more recently for HBV-HCV positive patients receiving direct-acting antiviral therapy for hepatitis C remains a challenge. The high risk associated with B cell depleting agents is unexplained but points to an important role of B cell immunity (Ciccullo et al. 2019). There is an urgent need for better markers to stratify the risk in patients before receiving immunosuppressive, chemo or direct-acting antiviral therapy to better tailor prophylactic strategies. Currently, patients receiving these therapies should be screened for HBsAg, anti-HBc and if anti-HBc positive, HBV DNA (Myint et al. 2020). All HBsAg positive patients are better served by receiving pre-emptive prophylaxis. The risk is lower in HBsAg negative, anti-HBc positive persons. The intensity and nature of the immunosuppressive therapy should be taken into account. Because of the unpredictability, erring on the side or prevention is safer even if the risk is considered low. Nonetheless, there is an urgent need to utilize improved markers to define the risk and pre-emptive strategies (Huang et al. 2020a). Reactivation of hepatitis B generally requires the urgent administration of a nucleoside analogue but the outcome (including possible

HBsAg loss) cannot be predicted with certainty, emphasizing the potential clinical difficulty. Although reactivation may presage HBsAg loss the low predictive value of current tests (serum aminotransferase, and HBV DNA, and possibly anti-HBc) and the risks of acute liver failure generally require early intervention to prevent adverse events (Lee et al. 2019).

9 Scaling Up Treatment

Nucleoside analogue costs are low: generic tenofovir or entecavir should be widely available at less than US$ 50 per year of treatment. Unfortunately, many countries face significant challenges in implementing antiviral therapy for viral hepatitis. Despite a precipitous decline in drug costs, costs linked to Gross National Product are still high in areas where most patients pay out of pocket. There is an imperative need to identify those with advancing disease, to target treatment to prevent cirrhosis. Routine assessment of hepatitis B is required to stage the disease and to assess levels of replication. The ability to assess predictors of disease progression, especially HBV DNA concentrations, is severely constrained in LMICs and unfortunately HBV DNA quantitation is not widely available as a point of care test. The cost of medicines and costs of diagnostics remains a key barrier to HBV treatment. [1]Chronic viral hepatitis needs to be finally recognized as a priority by governments where the disease is common.

10 Monitoring and Low-Intensity Monitoring

An assessment of ALT, AST (for APRI), HBsAg, HBeAg and serum HBV DNA levels should be performed. It is feasible to perform annual monitoring. Societal and anthropological studies are required (World Health Organisation 2017; WHO 2016). The need for more frequent monitoring for some categories of patient should be established: for example, persons with more advanced disease; persons not yet on treatment to identify a change in clinical status which may indicate progression to active disease requiring treatment; or during the first year of treatment where the adherence is a concern.

11 Risk Factor Analysis and Assessment of the Stage of Disease

The natural history of HBV is complex, and unpredictable. A spectrum of relatively benign to life-threatening diseases exists. The disease can be broadly sub-divided into four major phases: A high replicative, low inflammatory phase, immune

[1] Shirin Demma, Emmanouil Tsochatzis, Geoffrey Dusheiko. Expansion of access to HBV treatment. Current Hepatology Reports (2015) 14: 195–202. https://doi.org/10.1007/s11901-015-0272-8

reactive, low replicative inactive HBsAg carrier stage and reactivation phase. Treatment indications are based on the pattern of viral replication and the degree of necroinflammation and fibrosis. CHB-related liver disease can range from minimal fibrosis to cirrhosis, leading to a number of potentially life-threatening complications. Accurate and validated non-invasive tests can help the prioritization of persons with CHB for antiviral therapy (Desalegn et al. 2017).

Fibroscan (transient elastography) or aspartate aminotransferase (AST)-to-platelet ratio index (APRI) scores [APRI = *(AST/ULN) × 100)/platelet count(109/L)] or FIB-4 for estimating hepatic fibrosis (FIB-4 = (age (year) × AST(IU/L))/(platelet count(109/L × [ALT(IU/L])) are useful. The positive predictive value for ascertainment of cirrhosis is low for all non-invasive metrics, including APRI. Fibroscan (Echosens, Paris), 2-D acoustic radiation force impulse imaging (ARFI) and shear-wave elastography are not widely used in low income regions due to their cost.

Molecular diagnostic automated PCR-based assays for HBV DNA, as for HIV viral load, are transferable technologies, as demonstrated during the COVID-19 pandemic and Ebola epidemic in West Africa. More precise molecular and immunological data would further identify risk and treatment indications. Genotype A1 infected Africans are predisposed to chronic hepatitis and fibrosis and are at an elevated risk of HCC despite a lower viral load (Kramvis 2018). Variations at position 1809–1812 in the Kozak sequence of the precore-core open reading frame in genotype A1 decrease translation of HBeAg. Sub-genotype A1 acquires unique mutations in the basal core promoter and precore regions affecting transcription of precore mRNA, and thus reducing HBeAg expression—but the double mutation is an important risk factor for HCC. These molecular virological suggest an important functional oncogenic influence of genotype A1 despite lower levels of HBV viral loads and HBeAg-negativity and a need to consider treatment in patients with the prevalent HBeAg-negative phenotype with relatively low HBV DNA concentrations. Clinical differences between genotypes B and C are apparent but not explained (Tseng et al. 2015). There remains considerable interest on the impact of HBV genotype and molecular characteristics of hepatitis B but careful clinical, in vitro molecular and immunological correlations are required (Atsama et al. 2019; Wong et al. 2018; Kuhnhenn et al. 2018).

It remains uncertain what proportion of HBeAg-negative patients have significant fibrosis despite relatively low levels of HBV replication, and in whom molecular virological changes, clonal lineages and necro-inflammatory change nonetheless presage a risk of advanced liver disease or oncogenesis and ultimate development of HCC. A lack of resources underpins a relative paucity of clinical studies and estimates of HBV replication in those with HBeAg-negative hepatitis B from some regions of sub-Saharan Africa. Guidelines require substantiation for African patients as theoretically, the same thresholds may not be applicable.

HBsAg quantitation can predict progression of disease (McMahon 2010; Liu et al. 2014). HBsAg concentrations below 1000 IU/mL (or lower) together with HBV DNA levels below 2000 IU/mL and normal serum aminotransferases predict a stable inactive phase. Serum HBV DNA has been correlated with disease

progression and is useful to discriminate active HBeAg-negative disease from inactive disease. A rise in HBV DNA concentrations during treatment usually signify the development of resistance. New, standalone, easy-to-use point of care and affordable HBV DNA tests to allow same-day point-of-care testing and monitoring will overcome the inability to test more widely and facilitate treatment decisions.

The outcome of disease associated with complete suppression of viraemia (HBV DNA target not detectable) requires comparison to outcomes, particularly in HBeAg negative patients, associated with detectable but unquantifiable HBV DNA.

12 Functional Cure of Hepatitis B

The concept of a functional cure of hepatitis B has been accepted: A functional cure is defined as sustained loss of HBsAg, with or without acquisition of anti-HBs, and undetectable HBV DNA six months after completing treatment. Finite treatment rather than continued long-term treatment is implied (Cornberg et al. 2020). A finite cure of hepatitis B is not a complete cure or eradication of HBV infection from the host: a complete sterilizing cure for most is not considered attainable at this point in time, as the latter would require eradication of all hepatocytes harbouring both episomal cccDNA and integrated viral genomes from the host. Nonetheless, a finite cure with loss of HBsAg from serum, using a test with a sensitivity of at least 0.05 U/L allows cessation of antiviral treatment.

Cure, even if measured solely by HBsAg seroclearance, remains a major hurdle: Seroclearance of HBsAg improves the prognosis of hepatitis B, but to guarantee an improved outlook and survival, HBsAg loss should ideally occur early in the course of the disease, at a comparatively young age, and before the onset of advanced fibrosis to minimize the risk of subsequent hepatic failure and hepatocellular carcinoma (HCC). It may be possible to define applicable molecular characteristics accompanying HBsAg seroclearance that presage a benign outcome. In our current state of knowledge, the advantages of HBsAg loss can be inferred from either spontaneous loss of HBsAg, or a treatment-induced HBsAg seroclearance. Maintained DNA suppression reduces the risk of HCC (Papatheodoridis et al. 2020). However, HBsAg loss versus suppression of HBV DNA further reduces the risk, despite the likely persistence of integrated viral genomes (Yip et al. 2019).

A partial functional cure has been more tentatively defined as a decline in HBsAg concentrations to lower levels after finite treatment duration: these patients remain HBsAg positive with low concentrations of serum HBV DNA and normal serum aminotransferases. Such a low replicative state is recognized in chronic hepatitis B. The outcome is considered relatively favourable. However, it is not clear whether regulatory authorities will accept the conversion to a partial functional cure, given the risk of reactivation and the necessity for continued long-term monitoring. The question also arises whether more profound suppression of hepatitis B replication—achievable, for example, with the addition of capsid inhibitors to nucleoside analogues—will confer an improved prognosis and sustained of treatment response. Only future trials can answer the latter inquiry.

13 Cure of Hepatitis B

New investigational treatments to provide a hepatitis B cure are being examined. The life cycle of HBV involves several steps that are targets for potential new curative treatments, including viral entry, viral un-coating, HBV DNA transport to the nucleus, cccDNA transcription, nucleocapsid assembly, pregenomic RNA (pgRNA) incorporation and reverse transcription and subviral HBsAg particle assembly and secretion from hepatocytes. In broad terms, two major strategies are currently considered: (1) deepening inhibition of HBV replication to effect a cure or (2) a modulation in HBsAg presentation to result in ultimate HBsAg seroclearance. We are beginning to see improved on-treatment reductions or seroclearance of HBsAg in phase 2 studies not seen previously with chain terminators. Unfortunately, it is difficult to envisage a means of eradicating integrated HBV genomes without hepatocyte lysis.

14 HBsAg Seroclearance Strategies

Although HBsAg derived from integrated viral genomes is relatively inaccessible, newer compounds interfering with translation or HBsAg assembly offer the possibility of a direct reduction of HBsAg in serum. Specific, directed strategies to promote HBsAg loss, to expressly decrease HBsAg translation by RNA interference or interference with intracellular chaperoning and assembly of HBsAg are being investigated in current trials. These compounds inhibit sub-viral particle production and possibly virion assembly but their effect on HBV replication and cccDNA remains less well characterized. A reduction in HBsAg presentation may directly result in enhancement and recovery of dysfunctional T and B cell responses. The rapid 2–4 $\log_{10}$ reduction in HBsAg will provide an opportunity to examine the potential and necessary immunomodulatory response and appropriate immunomodulatory therapies. Whilst no immunomodulatory trial has been effective (other than interferon alfa treatment), an immunomodulatory strategy may be required to effect immunological control. The sensitivity of detection of HBsAg will require standardization.

Interestingly the HBsAg reductions have been frequently accompanied by serum aminotransferase flares following HBsAg reduction. Longer phase II studies have commenced. Hepatocyte cytolysis or apoptosis may be required to effect sustained declines in HBsAg concentrations or HBsAg seroclearance.

Other routes to decrease particulate HBsAg are being intensely evaluated. Nucleic acid polymers (NAPs) and STOPS (S antigen traffic inhibiting oligonucleotide polymers) are a class of amphipathic phosphorothioate oligonucleotides (Boulon et al. 2020). Their mechanism of action to reduce serum HBsAg concentrations has been recently elucidated. The host target for the class has been identified: The endoplasmic reticulum Golgi intermediate compartment—endoplasmic reticulum (ER/ERGIC) resident HSP40 chaperone DNAJB12 (Vaillant A personal communication).

Small molecule substrates of sodium taurocholate cotransporting peptide and NTCP inhibitors, for example, myrcludex B (bulevertide) block the entry of HBV (and hence HDV); . Inhibition of HBV entry reduces the spreading cycle. To date diminutions in HBsAg have been observed in a proportion of patients. The effect on integrated viral genomes is uncertain (Wedemeyer et al. 2019).

15 Inhibitors of HBV Replication

Deepening inhibition or a shutdown of HBV replication could be achieved with a combination of nucleoside analogues and capsid inhibitors. An additive block to HBV replication has been demonstrated by ultra-sensitive tests for HBV DNA (Huang et al. 2020b; Sulkowski et al. 2019) and the turnover of cccDNA is being analyzed. Interference with capsid assembly and inhibition of pgRNA encapsidation implies that the "primary" mechanism of action predominates. A reduction in pgRNA encapsidation is evident by a reduction in serum HBV RNA containing particles. The combination of a nucleoside analogue and capsid inhibitor leads to a relatively low decline (of the order of 0.5_{10} log change) in HBsAg concentrations in HBeAg-positive nucleoside naïve patients after 24 weeks of treatment. A lower reduction of HBsAg has been observed in HBeAg-positive nucleoside analogue suppressed or HBeAg-negative naive or nucleoside analogue suppressed patients. PgRNA reductions from baseline have been most profound in HBeAg-positive nucleoside analogue naive patients. The different population-dependent reductions of HBsAg, may be the result of a primary reduction of HBsAg stemming from Dane particles. The effect of longer administration in phase II studies requires ongoing assessment. However, these markers do not specify whether secondary mechanisms—preventing capsid disassembly—are operative or prove an action on HBsAg transcription from integrated viral genomes (Berke et al. 2020).

16 Augmentation and Restoration of both T and B Cell Host Immunity

Immunomodulatory therapy has lagged behind direct antiviral therapy. Studies of immunomodulatory agents including Toll-like receptor agonists, immune checkpoint inhibitors, therapeutic vaccines, immunological engineered cells to enhance T and B cell recognition, cytokine stimulation as well as pathogen receptor agonists have begun—with disappointing outcomes to date. The data have been limited to in vitro and woodchuck efficacy. Target engagement is demonstrable—utilizing agonists of compounds such as selgantolimod (GS 9688, an oral TLR8 agonist). Immune cell subset activation, and dose-dependent cytokine responses have been observed, and (in relatively small studies) 5% of patients lost HBsAg after 24 weeks of treatment in virally suppressed patients (Gane 2020).

An augmentation of the multilayered dysfunctional immune response in hepatitis B is perhaps more feasible after a reduction in host antigen burden: An important

proof of principle has been established in studies utilizing male C57BL/6 mice that persistently replicate HBV. After siRNAs knockdown of HBsAg expression, the mice were immunized with an adjuvanted HBV S and core antigen construct, followed by modified vaccinia virus Ankara vector to induce antigen-specific T and B cell responses. Vaccination induced the production of neutralizing antibodies and increased the number and functionality of HBV-specific, CD8T cells in mice with low levels of HBsAg, eliminating HBV (Michler et al. 2020). A deeper understanding of strategies to successfully restore T cell function may provide a scaffold to eliminate HBV in a wider array of patients.

17 Newer Biomarkers

A dichotomous separation of hepatitis B DNA and HBsAg concentrations in serum is observed during both the natural history of hepatitis B and treatment with nucleoside analogues. Recent studies have examined the role of HBV RNA and prRNA as surrogates either of cccDNA or cccDNA transcription and silencing. These markers are relatively sensitive biomarkers of continued transcription of cccDNA in HBeAg negative patients despite the marked HBV DNA inhibition by nucleoside analogues. These newer markers auger worse outcomes during follow-up, and predict reactivation or severe flares of treatment cessation (Tseng et al. 2019). Their measurement could assist new drug development and disease management, and identification of "grey zone" patients for whom treatment is indicated (Testoni et al. 2019). Novel serum biomarkers are needed to replace liver biopsies, which are invasive and cannot be repeatedly performed (Zhou et al. 2019).

A chemiluminescent enzyme assay has been developed for HBcrAg, which tests antigenic reactivity of HBeAg, HBcAg and a putative 22-kd precore protein, all products derived from the precore and core gene. HBcrAg may be an aberrantly processed protein; it is uncertain how the protein assembles into capsids but the marker nonetheless is ostensibly a surrogate serum marker of the HBV cccDNA pool. A cut off of 10 KU/ml has been used to rank the risk of HCC. The quantitation of anti-HBc seems somewhat indirect to accurately predict clinical outcomes (Hsu et al. 2019b).

Although HBV is an enveloped DNA virus, serum is now known to contain virion-like particles containing HBV RNA, and empty viral envelopes containing capsid without genomes. HBV RNA can be quantified in serum via high throughput HBV RNA research tests (Kock et al. 1996; Butler et al. 2018). We have observed that both pgRNA and HBcrAg remain detectable for years after profound HBV DNA by chain suppressing nucleoside analogues in HBeAg negative patients, in whom HBsAg concentrations do not decline with treatment (Carey et al. 2019). Current findings do not establish whether the residual detection of pgRNA in patients after long-term suppression is any measure of silencing, depletion, or damage to the copy number and pool of cccDNA molecules in treated patients, or explain differences in the rates of disappearance. The apparent differences in silencing of cccDNA transcription between patients will require further study but could

be due to differences in cell turnover, HBx activation, or epigenetic modification of cccDNA (Liu et al. 2020; Liao et al. 2019).

The data may suggest that detectable pgRNA and HBcrAg together reflect residual cccDNA and transcription that could contraindicate NA withdrawal, but prospectively designed studies are necessary to prove this supposition. The clinical utility of HBcrAg, pgRNA and quantitative HBsAg will require standardized assays, for extraction and measurement of pgRNA with units linked to WHO standards, and careful correlative longitudinal studies (Mak et al. 2018).

18 Prevention

18.1 Prevention of Mother-to-Child Transmission

Mother-to-child transmission (MTCT) from highly viraemic (HBV > 200,000 IU/mL) pregnant women occurs, creating an important reservoir of infected infants and children: Infection in the neonate and childhood remains the leading residual source of new chronic hepatitis B. The WHO has recently published new guidelines advocating nucleoside analogue prophylaxis in the third trimester for highly viraemic mothers (WHO 2020). However, the number of highly viraemic mothers receiving antiviral prophylaxis to reduce MTCT is not well documented and is likely to be extremely low in sub-Saharan African women. Antiviral therapy for pregnant women in the third trimester is effective in reducing vaccine and immunoglobulin failure in children born to highly viraemic mothers, but HBV DNA cannot be ascertained in many low-income countries. Thus, the prevalence of HBV DNA concentrations of greater than 200,000 IU/ml in HBeAg negative women across different regions of the world has not been fully assessed. Universal birth dose vaccination now means that mothers are not tested for HBsAg and therefore HBV DNA levels. Consequently, HBV mono-infected women in many low-income countries with higher levels of HBV replication will not be offered prophylactic nucleoside analogue therapy in the third trimester of pregnancy unless their infectivity is ascertained. Infants born to these mothers are neither given hyperimmune globulin and HBV birth dose vaccine at the time of delivery, and are at risk of acquiring incident chronic hepatitis B.

Timely birth dose vaccination intriguingly, may offset the necessity for antiviral prophylaxis to reduce viral load in highly viraemic mothers (Jourdain et al. 2018; Dusheiko 2018). Of countries with a high prevalence, only a limited number have scaled up timely birth dose coverage to 90%. HBIG is simply not feasible (for many countries) and may indeed not be required for infants born to HBeAg negative mothers—the majority in SSA. Pregnant women are routinely screened for HIV and initiated on antiretroviral therapy, and therefore because antiviral prophylaxis is more widely applied, the risk of transmission from HBV-HIV positive mothers (in whom HBV DNA levels are typically higher), is lower from identified mothers. Other factors that lead to transmission including intrauterine transmission, will be more difficult to control but do not account for the majority of infected neonates.

18.2 Vaccination

Universal hepatitis B immunization programmes for infants, with the first dose at birth, will reduce the incidence and the prevalence of Hepatitis B. Vaccination is succeeding and has had a great impact in many countries. However, vaccination, whilst critical will not impact on the rates of end-stage liver disease for HCC decades. Less satisfactory progress has been made in implementing birth dose vaccination.

19 Awareness and communication

There is a need to raise awareness of HBV to the same level as that of HIV and a pressing prerequisite for inexpensive, innovative point-of-care nucleic acid testing for HBV DNA, paired with HCV RNA and HIV RNA assays.

The acceptance and awareness of basic social measures to contain COVID-19 have been remarkable. The current societal awareness of severe viral infection leverages an opportunity to amplify awareness of viral hepatitis to the same level as that of HIV and COVID-19 (and in Sub-Saharan Africa, Ebola) to improve health.

20 Conclusions

Progress in treatment is being observed (Table 3.1). A considerable commitment to improve rates of hepatitis B cure is manifest. New compounds offer some promise. The right combinations and sequential utilization will require painstaking empirical research.

New biomarkers Including HBV RNA, and HBcrAg reflect transcriptional activity of cccDNA and will be utilized to comprehend checkpoints of HBV replication. We lack tools to identify the reduction in the pool of infected hepatocytes; biopsy studies to confirm measurement of intrahepatic cccDNA will be technically and ethically difficult. The place of immune modulators after reducing HBsAg antigen concentrations—or even amplification by PEG IFN—needs ongoing assessment.

Hepatitis B remains a major public health problem. The disease is common in endemic regions and in low-income countries of sub-Saharan Africa, Asia and the America's. The question of affordable cures, however, portends further inequalities in health care for the disease. The WHO has set priorities for achieving global goals for HIV treatment, and there have been remarkable achievements: Treatment for HIV now needs to encompass treatment of hepatitis B (and C). An important first step would be to stop mother-to-child transmission with the use of nucleoside analogues for highly viraemic mothers, but prophylaxis would require a quantitative assessment of viraemia in pregnant mothers.

Targeted screening of individuals would likely enhance the outcomes of screening. There are challenges in finding those unaware of their hepatitis B

Table 3.1 Unmet clinical needs hepatitis B

Improved screening
• Scaling up of diagnosis and linkage to care in endemic and non endemic regions
• Development of rapid point of care tests for HBV DNA to stage disease
• Improved linkage to care for HBsAg positive persons
• Improved understanding of distribution of disease phases in anti-HBe positive individuals
• Extension of screening in socially marginalized immigrant populations
New biomarkers
• Ability to disaggregate HBsAg derived from episomal versus integrated HBV DNA
• Ability to measure HBx transcription and effects of shut down.
• Analyse place of newer biomarkers and risk factor analysis including HBV RNA and HBcrAg with current and newer treatments
Improved strategies for control and antiviral treatment
• Establish accurate and appropriate treatment criteria for HBeAg positive and negative persons
• Ascertain risk in HBeAg positive patients with high levels of HBV DNA but normal serum ALT.
Therapy with current drugs
• Establish which patients are most likely to benefit from
• Add on or switch to pegylated interferon
• Improved predictability of nucleoside analogue cessation
• Improve understanding of risk of reactivation
New therapeutic frameworks
• Identification of patients likely to clear HBsAg with RNA interference combination regimens
• Determine benefit of complete shutdown of HBV replication with new combination treatments
• Understand role decrease in cccDNA transcription, (epigenetic change) vs hepatocyte turnover and loss
• Characterize and define immunological phenotypes associated with ALT flares
• Determine effectiveness of immune modulatory therapies including interferons after HBsAg knockdown
• Explain pgRNA reductions from baseline in HBeAg-positive versus negative and nucleoside analogue naive versus experienced patient populations treated with capsid inhibitors
Prevention
• MTCT: the prevalence of HBV DNA concentrations of greater than 200,000 IU/ml in HBeAg negative women across different regions of the world requires accurate assessment
• The role of nucleoside analogue prophylaxis in anti-HBe positive mothers versus timely birth dose vaccination
Awareness and communication
• A need to raise awareness of HBV to the same level of other viruses including HIV, SARS-Cov2

status. Scaling up efforts require national programmes. The fight against AIDS changed with the Global Fund to fight AIDS, TB and Malaria, and the US Presidents Emergency Plan for AIDS relief (PEPFAR). A stronger collaboration between WHO and UNAIDS co-sponsors is required to improve the outlook for hepatitis B.

The resources poured into AIDS have not been invested in chronic viral hepatitis and policy makers, and global health organizations must overcome the myopic view that vaccination makes it unnecessary to deliver treatment for chronic hepatitis. Simple-to-use, low-cost point-of-care tests for measuring HBV DNA are required. Task shifting with for low-intensity laboratory monitoring influence and adequate supplies of antiviral therapy will be a substantial step forward.

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Immunopathogenesis of Hepatitis B Virus Infection

4

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Abstract

The immune response plays conflicting roles in the outcome of Hepatitis B virus (HBV) infection. It simultaneously controls HBV in the liver and drives liver damage responsible for progression of disease. During acute HBV infection, the balance leans toward effective viral control with robust T and B cell immunity, limiting the duration of liver damage inflicted by the immune system and providing long-term immunity to the virus. During chronic infection, dysregulated innate and adaptive immunity fails to effectively clear the virus, resulting in lifelong infection and persistent liver damage that can lead to fibrosis and cirrhosis. This chapter will cover the current state of knowledge of HBV immunity and what we know about the immune-pathogenic mechanisms causing liver damage. We have incorporated new knowledge, made possible by technological advancements, that provide the most detailed picture of the immune response in chroni-

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cally infected patients to date. These advances continue to open opportunities for immune targeting immunotherapeutic strategies to achieve cure in chronic hepatitis B.

Keywords

T cell · B cell · Innate immunity · Pathogenesis · Inflammation · Immunomodulation · Acute hepatitis B · Chronic hepatitis B

1 Introduction

Investigating the immune response has always posed unique challenges for HBV infection because of the difficulty capturing patients with acute HBV infection, the complexity of chronic hepatitis B and the extremely low frequencies of HBV-specific immune cells. However, technological advancement and patient-oriented research have dramatically refined our understanding of how both the liver environment, and HBV itself, interacts with the immune system. The field has moved beyond comparing the frequency and magnitude of immunity between acute and chronic hepatitis B and it is now possible to define, down to the epitope-specific level ex vivo, phenotypic, and functional characteristics of both HBV-specific T cells and B cells. This knowledge is expanding targets for host-targeted immunotherapies to promote the goal of HBV cure. While great advancements have been made in unraveling adaptive immune responses, the areas of innate immunity and immunopathogenesis remain areas for further development due to either conflicting literature (innate immunity) or a lack of patient-derived data (immunopathogenesis). This chapter will address each of these topics to provide a comprehensive review of the current state of knowledge on the immune response during acute and chronic HBV infection and mechanisms of immunopathogenesis.

2 Acute HBV Infection

2.1 Innate Immunity in Acute HBV Infection

Due to the fact that patients are rarely captured during the incubation phase of HBV infection, before liver damage induces clinical symptoms to seek medical attention, very little is known about the innate response. Few studies have identified patients with known exposure events and followed them longitudinally to measure the activation of innate immunity. In these human studies, no induction of type I or type III interferons (IFN) was observed in the periphery (Dunn et al. 2009). NK cells showed early signatures of activation but impaired functionality during the viremic phase of acute HBV infection compared to NK cells from healthy donors. Impaired NK cell function coincided with IL-10, which correlated with HBV DNA (Dunn et al. 2009;

Fisicaro et al. 2009). Even animal models pose challenges to studying the early events of acute HBV infection because of species specificity of HBV. Studies in chimpanzees and woodchucks confirm the lack of type I IFN induction upon HBV infection or during the viremic phase (Wieland et al. 2004; Suresh et al. 2019). In addition, similar to what was observed in acute HBV patients, NK cell activation was detectable at the peak of woodchuck hepatitis virus (WHV) DNA in experimentally infected animals, but functional studies were not reported and likely limited by available reagents (Suresh et al. 2019). Therefore, due to the lack of data, our understanding of the innate immune response during acute HBV infection remains incomplete. However, the innate immune response plays a critical role in the outcome of HBV infection, which can be observed in susceptibility to chronic infection based on the age of exposure, suggesting maturation of innate immunity is required to induce the protective T and B cell responses discussed below (Prendergast et al. 2012).

2.2 HBV-Specific T Cells in Acute HBV Infection

The characteristics of the HBV-specific T cell response associated with viral control have been well characterized in acute and resolved hepatitis B patients and multiple animal models. Data from acute HBV-infected chimpanzees demonstrate that HBV-specific T cell responses develop 2–3 months post-infection (Thimme et al. 2003; Asabe et al. 2009; Shin et al. 2016). HBV-specific T cell responses from self-limiting acute HBV infections are reported to be multi-specific against HBsAg, HBcAg, and HBV polymerase (Dunn et al. 2009; Rehermann et al. 1995; Maini et al. 1999; Webster et al. 2000, 2004). More importantly, these are polyfunctional responses, with readily detectable virus-specific T cells secreting IFN-γ and IL-2 (Fisicaro et al. 2009). Longitudinal tracking of HBV-specific T cell immunity shows that declines in serum HBV DNA are observed following the emergence of peripheral and intrahepatic HBV-specific T cell responses in acutely infected humans and infected chimpanzees, respectively (Dunn et al. 2009; Fisicaro et al. 2009; Wieland et al. 2004; Thimme et al. 2003; Urbani et al. 2005). HBV-specific T cells display high proliferative capacity in vitro and readily produce antiviral cytokines in response to stimulation. This highly proliferative and functional HBV-specific T cell profile wanes (but remains detectable) over the course of acute infection, mirroring serum ALT levels as it also recedes back to normal steady state (Dunn et al. 2009; Fisicaro et al. 2009; Wieland et al. 2004; Webster et al. 2004; Urbani et al. 2005).

The importance of T cells in mediating viral control is highlighted by HBV-infected chimpanzee studies when global depletion of CD4+ or CD8+ T cell populations prevented HBV clearance and severely delayed clinical recovery (Thimme et al. 2003; Asabe et al. 2009). While HBV-specific CD8 T cells are necessary for viral clearance, these studies demonstrate that CD4+ helper T cells are critical in priming and facilitating functional HBV-specific CTL T cell responses (Urbani et al. 2005). The emergence of HBV-specific CTL responses precedes peaks in liver enzyme ALT, which is a clinical indicator of liver damage.

HBV is a non-cytopathic virus, meaning liver injury and pathogenesis are mediated by the host's immune system. Viral clearance was initially thought to occur only upon lysis of infected hepatocytes, however, decrease in HBV DNA is observed before clinical hepatitis, indicative of non-cytopathic viral clearance during acute infection. Indeed, transgenic mouse models (Moriyama et al. 1990; Guidotti et al. 2000) and chimpanzee studies (Guidotti et al. 1999; Wieland 2015) have shown that proinflammatory type I and type II cytokines induce non-cytolytic clearance of HBV DNA from infected hepatocytes. Of note, IFN-γ and TNF-α, which are secreted by functional HBV-specific CD8+ T cells, are potent cytokines that act synergistically to inhibit HBV replication (Xia et al. 2016; Pasquetto et al. 2002; Biermer et al. 2003; Phillips et al. 2009). A proposed mechanism for this non-cytolytic inhibition by IFN-y involves the expression of inducible nitric oxide synthase (iNOS) in Kupffer cells and hepatocytes. Nitric oxide is a pleiotropic free radical known to exert antimicrobial and antiviral effects (Suri et al. 2001; Akaike and Maeda 2000). Its importance is further highlighted with the abolishment of non-cytolytic HBV clearance in iNOS-deficient mice by HBV-specific T cells (Guidotti et al. 2000). IFN also activates indoleamine 2,3-dioxygenase (IDO1), which acts to deprive tryptophan in host cells and preferentially inhibits viral protein translation without significantly affecting global protein synthesis (Mao et al. 2011; Yoshio et al. 2016). Lastly, IFN-γ and TNF-α are also known to target the cccDNA minichromosome by inducing the expression of nuclear deaminases APOBEC3A and APOBEC3B (Xia et al. 2016). Upon deamination, cccDNA is susceptible to degradation by IFN-regulated nucleases and further studies show that other cytokines such as lymphotoxin β, IFN-α, IFN-λ, and TGF-β are also capable of triggering cccDNA deamination and its subsequent clearance from infected primary human hepatocytes, but to a lesser extent (Lucifora et al. 2014; Bockmann et al. 2015; Qiao et al. 2016; McClary et al. 2000; Kakimi et al. 2000).

Previous in vitro T cell studies have shown that cytotoxic T cells specific for HBV polymerase, envelope or core epitopes are present during acute infection, but whether a certain specificity is more predominantly cytotoxic or confers more protection against progression to chronic disease is yet to be determined. Ex vivo phenotypic analysis of HBV-specific T cells from acutely-infected patients show that T cell phenotypes differ between HBV epitopes (Hoogeveen et al. 2018; Cheng et al. 2019). This antigen-dependent heterogeneity is thought to arise from differences in relative viral antigen quantities. A unique quality of HBV is its ability to secrete "decoy" subviral particles, which wholly consist of HBV envelope proteins (HBsAg) and are thus incapable of causing new infections. These HBsAg particles greatly outnumber mature virus particles and are speculated to be the main mechanism by which the adaptive immune system is overwhelmed and rendered tolerant toward HBV infection. It is further speculated that the propensity of certain antigens to be presented by different cell types (hepatocytes or professional APCs) can also influence the elicited immune response (Murata et al. 2018). To that point, much controversy surrounds the role of hepatocytes in priming T cell responses, and how this overall contributes to viral persistence and progression to chronic infection (Murata et al. 2018; Bertoletti and Kennedy 2019). Investigating how HBV antigens

ultimately affect T cell responses and how these differ in patients with self-limiting acute HBV infection can provide insight for clinical interventions aiming to restore T cell functionality among chronically-infected patients.

2.3 B Cells in Acute HBV Infection

B cells encounter their antigens and subsequently differentiate into antibody producing plasmablast/plasma cells and memory cells. Induction of neutralizing anti-HBs upon recombinant HBsAg vaccination confers protection against HBV infection (Shouval 2003). Anti-HBs prevents infection (or re-infection) of hepatocytes by blocking the interaction between the "a" determinant of the HBsAg with the low-affinity cell receptor heparan sulfate proteoglycan (HSPG) and the interaction between the binding site of Pre-S1 and NTCP (Shouval 2003; Wands et al. 1984; Rendi-Wagner et al. 2006). Anti-HBs contained in hepatitis B immunoglobulin (HBIG) is also highly effective in preventing perinatal transmission of HBV (combined with vaccination at early birth), post-exposure prophylaxis following needle-stick injuries, and preventing transplanted liver against HBV reinfection in previously infected recipients (Xu et al. 2013; Roche and Samuel 2014). Preclinical studies in chimpanzee models have also confirmed neutralization activity of anti-HBs to prevent acute HBV infection (Hong et al. 2004; Wi et al. 2017). Anti-HBs may also bind to HBsAg presented on the surface of HBV infected hepatocytes and, through contribution of NK cells, macrophages, other myeloid cells (e.g., neutrophils), and antibody/complement-dependent cytotoxicity, mediate their lysis (Eren et al. 1998; Ray and Desmet 1976). Anti-HBs via interaction with FcRn receptor may also be transported into the hepatocytes, and block the release of HBV subviral particles and virions (Rendi-Wagner et al. 2006; Eren et al. 1998). In addition to antibody production, B cells are also responsible for antigen presentation and cytokine production. Several reports in HBV resolved patients with hematological malignancies have demonstrated that depletion of B cells with drugs, such as Rituximab, are associated with HBV reactivation, sometimes leading to liver failure and death (Yeo et al. 2009; Dervite et al. 2001; Niscola et al. 2005; Westhoff et al. 2003). These data suggest an important role of B cells in controlling HBV infection. Results of utilizing HBsAg-specific monoclonal Abs in preclinical and clinical trial studies in CHB patients and animal models have shown some short-term direct HBsAg suppression (Corti et al. 2018; Heijtink et al. 2001; Neumann et al. 2010; Galun et al. 2002; Wang et al. 2020). It has been suggested that such neutralizing mAbs may be used as adjuvant treatment to reduce HBsAg load, thus rescuing adaptive immunity and aiming a better effect of new antiviral drugs.

Generally, measurements of specific Abs against different HBV antigens (including HBcAg, HBeAg, and HBsAg) are used to distinguish different clinical phases of HBV infection (Dunn et al. 2009; Wieland et al. 2004; Davison and Strasser 2014; Maruyama et al. 1993, 1994). In acute HBV infection, anti-HBc IgM is the earliest detectable antibody that can be seen in the serum shortly before the symptom onset. Anti-HBc IgM coexist with a high level of HBV replication (Hoofnagle

et al. 1973), whereas anti-HBc IgG prevails thereafter, and persists lifelong after clinical recovery. In contrast to anti-HBc, antibodies specific for HBeAg and HBsAg appear much later. In acute hepatitis, anti-HBs are detected only when HBsAg declines. The emergence of anti-HBs usually takes several weeks or even months after the disappearance of HBsAg (Gerlich and The 2007). Results of a study by Wieland et al. (2004) on gene expression profile in the liver of three acutely HBV-infected chimpanzees were in line with this phenomenon. Unlike a large number of T cell-derived IFN-gamma-regulated genes, which were induced in the liver during viral clearance, Ig heavy and light chains were the only two clearance-associated genes that reached peak induction late in infection. This data reflects the expansion of a functional B cell infiltrate, when viral DNA was disappearing from the liver. The newly recruited B cells likely secret Abs that neutralize any remaining viral particles to inhibit reinfection of hepatocytes.

The absence of anti-HBs during the early acute phase of HBV infection is not due to a lack of HBsAg-specific B cells. Newly developed protocols using direct ex vivo visualization of HBV-specific B cells showed that acute HBV patients (HBsAg+, IgM anti-core+, high ALT) have HBsAg-specific B cells in circulation, with frequencies similar to healthy vaccinated individuals (Salimzadeh et al. 2018). Phenotypic characterization of the cells did not show induction of plasmablasts ($CD19^{+}$, $CD10^{-}$, $CD21^{low/-}$, $CD27^{hi}$, $CD38^{hi}$) among global or HBsAg-specific B cells. Only after resolution of infection, and anti-HBs positivity in serum, were HBV-specific B cells able to mature to anti-HBs producing B cells. The late induction of antibody-secreting HBsAg-specific B cells could be interpreted as a sign that B cells have a limited role in the clearance of the virus (at least in the initial phase of clearance when HBsAg is present) and function to maintain protection upon HBV clearance.

2.4 Immunopathology During Acute HBV Infection

Defining the earliest mechanisms initiating liver damage have been challenging in humans due to the fact that patients seek medical attention at the onset of symptoms, which often occurs as HBV DNA is declining and triggers for inflammation have passed. Investigations into known exposure events have failed to find significant innate immune activation in the peripheral blood during the early viremic phase of acute HBV infection (Dunn et al. 2009). Therefore, animal models of HBV infection have served as the primary resource to unravel the kinetics of HBV-related liver damage. In almost all cases, animal model data resembles liver damage associated with acute HBV infection, which is illustrated by natural infection in chimpanzees with HBV, woodchucks with woodchuck hepatitis virus (WHV) or the adoptive transfer of functional HBV-specific T cells to HBV transgenic mice. In chimpanzees infected with HBV and woodchucks with WHV, innate immunity is weakly activated and viral control and liver damage were linked to CD8 T cells (Wieland et al. 2004; Suresh et al. 2019; Thimme et al. 2003). HBV-specific CD8 T cells recognize and kill infected hepatocytes and produce IFN-γ, which induces the production of CXCL-9 and CXCL-10 (Isogawa et al. 2005). These two chemokines recruit

inflammatory immune cells and have been correlated with liver damage in both mice and patients (Kakimi et al. 2001). In addition to chemokines, recruitment of the inflammatory infiltrate appears dependent on neutrophil and platelet activation in the liver (Sitia et al. 2002, 2012). Neutrophil extracellular traps (NETs) are known to activate platelets (McDonald et al. 2017), which leads to an arrest of CD8 T cells in the liver (Iannacone et al. 2005; Guidotti et al. 2015). Subsequent to arresting immune cells in the liver, the matrix degrading enzymes produced by neutrophils facilitate immune cell infiltration (Sitia et al. 2004) (Fig. 4.1a, b).

Because of overlap in the antiviral response (HBV DNA decline) with tissue injury (ALT increase), it has been difficult to separate the amount of liver damage attributable to direct lysis of infected hepatocytes by HBV-specific CD8 T cells to hepatocyte lysis by the nonspecific inflammatory infiltrate. While it is possible for patients to clear acute HBV infection in the absence of liver inflammation (Fisicaro et al. 2009), CD8 T cells are believed to be the primary mediators of liver damage. Depletion of CD8 T cells in HBV infected chimpanzees highlighted the role of HBV-specific CD8 T cells in viral clearance and demonstrated their contribution to nonspecific liver damage (Thimme et al. 2003). Early depletion of CD4 T cells in chimpanzees impaired functional maturation of HBV-specific CD8 T cells (Asabe et al. 2009). However, liver damage still correlated with the infiltration of total CD8

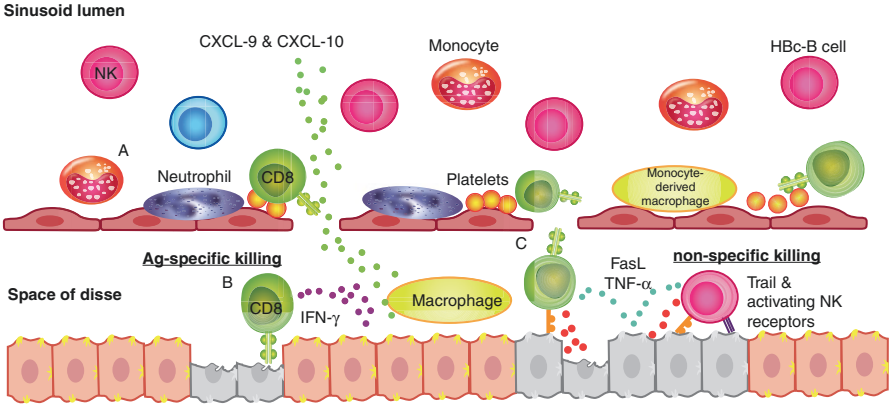

Fig. 4.1 Mechanism of liver damage during acute and chronic HBV infection. While the initial triggers of inflammation and liver damage remain unclear in natural HBV infection, the kinetics have been well described in animal models. (**a**) Neutrophils and monocytes appear to be among the first infiltrating immune cells. Neutrophil activation can lead to neutrophil extracellular traps (NETs) that digest the extracellular matrix and induce platelet activation. CD8 T cells recognize the activated platelets and begin rolling on the endothelium. (**b**) In the case of HBV-specific CD8 T cells, they sample the hepatocyte microvilli protruding through the fenestrated endothelial layer. Antigen recognition stimulates IFN-γ production, which induces both macrophage activation and the chemokines primarily responsible for mononuclear cell infiltration, CXCL-9 and CXCL-10. (**c**) Infiltrating immune cells respond to the inflammatory environment of the liver, upregulating pathways of nonspecific hepatocyte damage such as Fas ligand, TRAIL, and activating NK cell receptors. The mechanisms responsible for terminating liver inflammation and damage in acute or chronic HBV infection have not been described

T cells to the liver, suggesting HBV-specific CD8 T cells are not required for tissue damage (Asabe et al. 2009). Overall, this indicates that a majority of liver damage during acute HBV infection is nonspecific but these data still rely on relatively few reports in patients or natural infection models.

One of the limitations to understanding liver damage in HBV infection is a lack of understanding of the mechanisms actually killing hepatocytes. The data described above identify key cell types involved in recruitment and infiltration but the mechanisms used by CD8 T cells to kill hepatocytes are less clear. A mouse HBV hydrodynamic transfection model suggested a minor role of perforin-mediated hepatocyte lysis and a significant role for Fas ligand (FasL)-mediated killing (Yang et al. 2009). Supporting this mechanism, FasL is elevated in the serum of patients experiencing liver damage (Lapinski et al. 2004; Kondo et al. 1997). Similarly, mouse models indicated that TNF-α can preferentially kill infected hepatocytes (Lampl et al. 2020). These mechanisms, FasL and TNF-α, are likely primary contributors to the extent of liver damage during acute hepatitis, where the majority of infected hepatocytes outnumber HBV-specific T cells (Fig. 4.1c). However, more data, particularly in the liver of hepatitis B patients, is required to validate and expand these mechanisms of tissue damage.

3 Chronic Hepatitis B

3.1 Innate Immune Response in Chronic HBV Infection

Innate immunity did not evolve to control persistent infections. It evolved to respond rapidly through activation of pattern recognition receptors and inflammatory cytokine production to instruct maturation of an adaptive immune response (Brubaker et al. 2015). It is this perspective, instruction of adaptive immunity, that has largely been used to investigate innate immunity in chronic hepatitis B. Numerous studies have tried to investigate the impact of virions and viral antigens on the ability of professional antigen-presenting cells, such as myeloid dendritic cells (DC), plasmacytoid DC, monocytes, and macrophages, to support T and B cell activation through cytokine production and T cell stimulation. The use of recombinant antigens, in vitro systems and heterogeneous patient populations have resulted in a substantial amount of conflicting data, which we argued is a consequence of the short-lived nature of myeloid cells in the peripheral blood—where most experiments were performed (Kuipery et al. 2020). Furthermore, clinical observation of CHB patients does not suggest significant, persistent, impairment of innate immunity as they are not more susceptible to opportunistic infection.

Rather than support T/B cell immunity in CHB patients, myeloid cells appear more likely to regulate the inflammatory process that leads to tissue damage. Monocytes are among the first cells recruited in different models of liver inflammation and damage (Mossanen et al. 2016; Blériot et al. 2015; Liaskou et al. 2012) and found in CHB patients with liver damage (Zhang et al. 2011). The role of monocytes/macrophages in liver inflammation and damage is further supported by data showing that the scavenger receptor, CD163, is elevated in the serum of CHB

patients during liver damage (Kazankov et al. 2014; Dultz et al. 2014). CD163 is shed from the myeloid cell surface upon pattern recognition receptor activation (Weaver et al. 2006; Hintz et al. 2002).

The trigger of myeloid recruitment and activation during chronic HBV-mediated liver inflammation and damage remains undefined. HBV itself does not robustly activate innate immunity in vivo (Suslov et al. 2018). However, CHB patients are heterogeneous (Lampertico et al. 2017) and the hepatocyte–HBV interaction is dynamic, with modulation of metabolic function and hepatocyte turnover that can potentially induce endogenous danger signals capable of activating immune cells. This has been observed in the mouse model, where HBV infection alters the lipid profile of hepatocytes, making them targets for invariant NK T cells (Baron et al. 2002; Zeissig et al. 2012). The same phenomenon has not been demonstrated in the CHB patient liver but defines the concept that alterations in endogenous host molecules can lead to innate immune activation.

Aside from the role of myeloid cells in liver inflammation, NK cells have been reported to display altered function during chronic HBV infection, in particular their ability to produce IFN-γ (de Groen et al. 2017). Whether NK cells can contribute to the antiviral response in HBV infections remains to be conclusively demonstrated (Kakimi et al. 2000; Yang et al. 2009) but inhibition of IFN-γ would significantly impact their role in antiviral responses. Impairment of IFN-γ production was linked to IL-10 present in the serum which, similar to the data from acute HBV infection, correlated to the degree of liver damage. The greatest recovery of NK cell function upon neutralizing IL-10 was observed in patients with high ALT levels (Peppa et al. 2010). These data represent another example of how the inflammatory environment within CHB patients can dynamically change innate immune cell function.

3.2 HBV-Specific T Cells in Chronic HBV Infection

In direct contrast to patients resolving acute infection, HBV-specific T cells during chronic infection are often undetectable and defined as functionally exhausted. T cell exhaustion is a hallmark of chronic viral infection and commonly develops in conditions with persistently high viral antigen levels. It is characterized by loss of functionality, loss of proliferative capacity, and eventually leads to T cell anergy and death (Wherry 2011). Such parallels can be drawn from other T cell exhaustion models such as chronic LCMV mouse models, and from HIV- and HCV-infected chronic patients (Wherry 2011; Day et al. 2006; Kahan et al. 2015). Exhausted T cells are commonly found to express high levels of co-inhibitory receptors and demonstrate a $Tbet^{lo}Eomes^{hi}$ transcriptional profile similar to memory T cells despite lacking the ability to mount an effective cytokine response (Wherry and Kurachi 2015; Buggert et al. 2014). Thanks to recent HBV-specific T cell studies, it is now known that beyond their decreased functionality, exhausted HBV-specific T cells harbor further phenotypic, transcriptional, and metabolic changes that distinguish them from the functional T cells found among resolved or acutely-infected patients (Hoogeveen et al. 2018; Schuch et al. 2019; Schurich et al. 2016; Fisicaro et al. 2017) (Fig. 4.2).

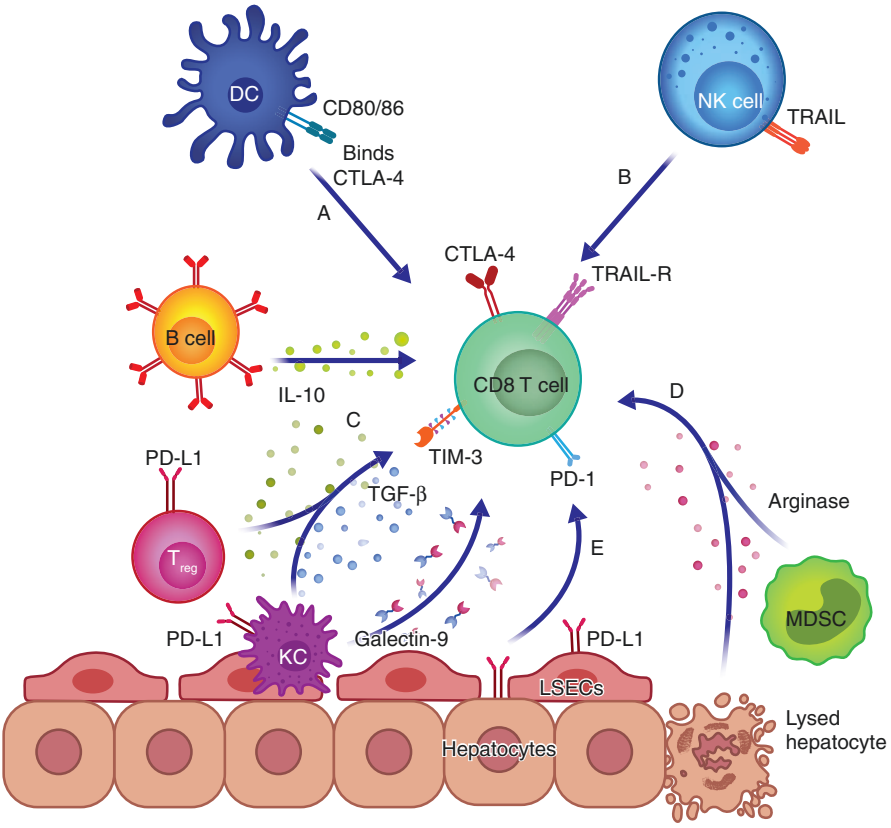

Fig. 4.2 Multiple intrahepatic factors contribute to T cell exhaustion during chronic HBV infection. (**a**) Upregulation of immune checkpoint markers is a hallmark of T cell exhaustion. While professional APCs traditionally provide stimulatory effects through CD80/86, the upregulation of co-inhibitory receptor CTLA-4 leads to T cell inhibition instead upon receptor-ligand binding. (**b**) HBV-specific T cells can be eliminated by intrahepatic NK cells through TRAIL-mediated killing, further decreasing virus-specific T cell frequencies. (**c**) Soluble anti-inflammatory cytokines IL-10, TGF-β, and Gal-9 are highly enriched in the liver and heavily inhibit T cell functionality. These factors are secreted by multiple intrahepatic cell populations including B cells, T regulatory cells, Kupffer cells, and hepatic stellate cells. (**d**) Another critical soluble factor is arginase, secreted by myeloid-derived suppressor cells and lysed hepatocytes. Arginase deprives the liver microenvironment of arginine, which is critical for T cell activity and its absence further restricts an already exhausted T cell population. (**e**) Upregulation of PD-1 on exhausted HBV-specific T cells binds to PD-L1 expressed on numerous intrahepatic cell populations including T regulatory cells, hepatocytes, Kupffer cells, and liver sinusoidal endothelial cells

Chronic LCMV mouse models dictate that loss of functional capabilities during T cell exhaustion occurs in a stepwise, hierarchical manner as chronic disease progresses, beginning with the loss of IL-2 production, followed by TNF-α, and lastly IFN-γ (Wherry and Kurachi 2015; Yi et al. 2010). Studies in HBV transgenic mice and from longitudinal studies of chronic patients also demonstrate this marked loss of functionality among HBV-specific T cells when compared to acute or resolved infections (Webster et al. 2004; Ferrari et al. 1991; Kakimi et al. 2002; Boni et al. 2007). This loss of cytokine production is also associated with decreased proliferative capacity and is known to be induced by the PD-1/PD-L1 axis.

The PD-1/PD-L1 axis evolved as a means to limit immunopathogenic damage to the host (Francisco et al. 2010). In response to persistent antigen stimulation, exhausted virus-specific T cells upregulate PD-1 expression, along with a myriad of other co-inhibitory receptors such as TIM-3, LAG-3, and CTLA-4 (Wherry and Kurachi 2015). Indeed this is observed among HBV-specific T cells in chronic patients (Heim et al. 2019; Das et al. 2008; Park et al. 2016; Raziorrouh et al. 2010) (Fig. 4.2a, c, e). Recent studies using tetramer enrichment show that core- and polymerase-specific T cells possess distinct levels of inhibitory marker expression (Hoogeveen et al. 2018; Schuch et al. 2019). Core-specific T cells expressed higher levels of CD160, PD-1, and 2B4 compared to polymerase-specific T cells, but despite this, polymerase-specific T cells were found to be less capable of cytokine production and clonal expansion following peptide stimulation (Hoogeveen et al. 2018; Schuch et al. 2019). KLRG1 expression was higher among polymerase-specific T cells; demonstrating that relative levels of PD-1 expression alone may not be indicative of the degree of T cell exhaustion, and that further in-depth analysis should be conducted. These studies demonstrate that exhausted HBV-specific T cell populations are not homogenous, but instead consist of heterogenous epitope-specific subsets with differing degrees of functional exhaustion. Immune checkpoint inhibitors are widely used in cancer therapies and their potential to induce lymphocyte activation also garnered interest as to whether HBV-specific T cell exhaustion can be overcome by immune checkpoint blockades (Boni et al. 2007; Fisicaro et al. 2010; Bengsch et al. 2014). Initial HBV transgenic mouse models show that administering anti-PD-1 antibodies abolished immune dysfunction and HBV viral persistence (Maier et al. 2007; Tzeng et al. 2012). Current research in chronic patient PBMCs show that PD-1 blocking may promote HBV-specific T cell proliferation and function (Boni et al. 2007; Fisicaro et al. 2010; Bengsch et al. 2014) but its efficacy across patients in other clinical phases is yet to be extensively investigated.

Further phenotypic analyses on exhausted HBV-specific T cells show that the IL-7 receptor, CD127, is found to be downregulated among chronic patients (Boettler et al. 2006; Radziewicz et al. 2007). IL-7 signalling is essential for T cell proliferation and maintenance following memory establishment (Ahmadzadeh et al. 2009). Indeed the presence of CD127 is strongly associated with a protective and functional HBV-specific memory T cell response among individuals resolving acute HBV infection (Boettler et al. 2006). Phenotypic changes are also associated with upregulation of CD127, including the downregulation of PD-1 and induced

expression of lymphoid organ homing signal, CCR7, to establish a protective memory cell population in secondary lymphoid structures (Fisicaro et al. 2010; Boettler et al. 2006; Radziewicz et al. 2007). CD127 expression is also higher among core-specific T cells compared to the more functionally exhausted polymerase-specific T cell population (Hoogeveen et al. 2018; Schuch et al. 2019).

Well-defined transcription factors of T cell development and lineage differentiation, T-bet and Eomesodermin (Eomes) are widely associated with distinct T cell subsets (Knox et al. 2014; Intlekofer et al. 2005; Pearce et al. 2003) and its balance is highly critical in the context of chronic infections. High T-bet expression is associated with Th1 CD4 T cell differentiation (Zhu et al. 2010) and short-lived effector CD8 T cells (Intlekofer et al. 2005; Sullivan et al. 2003) as it promotes T cell mobilization (CXCR3), cell signalling (IL-12), and effector cytokine production (IFN-γ, perforin, and Granzyme B); whereas Eomes expression promotes long-lived memory cell maintenance. $Eomes^{hi}PD\text{-}1^{hi}$ exhausted T cells have been correlated with severity of chronic HIV and HCV (Buggert et al. 2014; Paley et al. 2012). While HBV-specific T cells are $T\text{-}bet^{hi}$ during acute HBV infection (Kurktschiev et al. 2014), in the context of chronic HBV infection, exhausted peripheral HBV-specific T cells are observed to be $T\text{-}bet^{lo}Eomes^{hi}$ [33], a transcriptional profile which is highly reminiscent of memory T cells. However, unlike memory T cells, exhausted $T\text{-}bet^{lo}Eomes^{hi}$ HBV-specific T cells are less capable of clonal expansion and lack robust CD127 expression (Schuch et al. 2019; Heim et al. 2019). How these expression patterns impact HBV-specific T cell functionality across different clinical phases has yet to be determined. Additional studies highlight the role of transcriptional regulator Thymocyte Selection-Associated High Mobility Group Box (TOX) in identifying and maintaining exhausted virus-specific T cells (Heim et al. 2020; Alfei et al. 2019; Khan et al. 2019). In chronic HBV infection, TOX expression among HBV-specific T cells has been associated with higher levels of PD-1, CD57, Eomes, and Helios, along with impaired proliferative capacity and poor cytokine production (Heim et al. 2020). Indeed TOX is found to be more highly expressed in functionally exhausted $CD127^{-}PD\text{-}1^{+}$ HBV-specific T cells in contrast to memory-like $CD127^{+}PD\text{-}1^{+}$ HBV-specific T cells (Heim et al. 2019, 2020; Maini and Burton 2019).

Compartmentalization of HBV-specific T cell responses, between the blood and liver, has also been defined in CHB patients (Pallett et al. 2017). Tissue-resident ($CD69^{+}$ $CD103^{+}$) T cells are found to be enriched in the liver (11%) while being virtually absent from the periphery (Pallett et al. 2017). Comparisons among inactive carriers revealed distinct functional and phenotypic differences between these populations. While intrahepatic HBV-specific T cells were found to have higher expression levels of PD-1 (Fisicaro et al. 2010; Pallett et al. 2017), they were found to produce equivalent levels of IFN-γ when compared to peripheral T cells (Pallett et al. 2017). Furthermore, intrahepatic T cells produced more IL-2 and displayed higher levels of HLA-DR expression. These highly functional T cells also exhibited higher levels of autophagy, a cellular process that eliminates unwanted cytoplasmic content and catabolizes them to provide biomolecules necessary for optimal cellular metabolism (Swadling et al. 2020). These high levels of autophagy led to healthier mitochondrial fitness and conferred a stronger immune response from liver-resident

T cells, highlighting induction of autophagy as a target to restore HBV-specific T cell function. While these studies collectively establish that tissue-resident T cells can be highly functional, they are found to lack in cytolytic capacity in comparison to their peripheral counterparts (Pallett et al. 2017; Stelma et al. 2017). How these unique cellular characteristics are impacted throughout the clinical phases of chronic HBV remain to be studied.

It is now understood that T cell exhaustion entails a complex relationship of various cellular characteristics. Even at normal physiological conditions, the hepatic microenvironment is highly immunotolerogenic due to the liver's physiological function of being exposed to toxic substances, and subsequently metabolizing and clearing them. In particular, MDSCs (Pallett et al. 2015), Tregs (Franzese et al. 2005), LSECs (Diehl et al. 2008; Knolle et al. 1998), and Kupffer cells (Nebbia et al. 2012; Li et al. 2012) are documented to highly express PD-L1 and other co-inhibitory receptors to regulate adaptive immune responses (Francisco et al. 2010; Mühlbauer et al. 2006; Horst et al. 2016). Furthermore, these cell populations secrete immunosuppressive cytokines such as TGF-β and IL-10 that act to promote anti-inflammatory effects and regulate immune responses in the liver (Pallett et al. 2017; Knolle et al. 1998; Horst et al. 2016; Das et al. 2012; Knolle and Thimme 2014), further impeding HBV clearance (Fig. 4.2c). Upon hepatocyte lysis, arginase is released and acts to deprive the hepatic microenvironment of arginine, an amino acid essential for T cell function and proliferation (Das et al. 2008; Munder et al. 2006). Indeed MDSCs are also found to suppress HBV-specific T cell functions in an arginase-dependent manner (Pallett et al. 2015) (Fig. 4.2d). Thus, immunotherapeutic strategies aiming to restore exhausted HBV-specific T cells also need to account for the various immunoregulatory functions present in the liver.

Currently, chronic HBV patients undergo nucleoside analogue (NUC) treatment indefinitely, which presents a huge global health burden. Discontinuation of NUCs is being tested to limit the duration of antiviral therapy but is often associated with high rates of virological relapse and potentially severe hepatic inflammation. Three studies have been published that sought to determine if the presence or functionality of HBV-specific T cell immunity correlates with off-treatment outcome. Results suggest that T cell frequency after in vitro expansion from baseline samples correlates with off-treatment response but the data are not clearly distinguishable enough establish frequency as a robust biomarker (Rivino et al. 2018; Rinker et al. 2018; García-López et al. 2020). In addition, these studies used in vitro expansion to monitor peripheral T cell functionality whereas ex vivo and intrahepatic data is likely to be more informative. Therefore, initial data appear positive but require additional validation.

3.3 B Cells in Chronic HBV Infection

Compared to HBV-specific T cells, there is little known about B cell phenotype and functionality in CHB patients. However, distinct features have been identified in total B cells during chronic HBV infection. There is an increased proportion of both

memory and naïve B cell subsets expressing CD69 and CD71 activation markers in CHB patients compared to healthy individuals. Bulk B cells from patients with CHB display enhanced differentiation to Ab-producing B cells, but lower proliferative capacity compared to healthy controls (Oliviero et al. 2011). Phenotypic and gene expression profiling have defined B cell signatures associated with different stages of disease, in particular, B cells in the immune active phase of CHB (Vanwolleghem et al. 2015; Xu et al. 2015). Transcriptional profiling in Immune Tolerant patients also identified alterations in gene expression of global B cells, which was not observed in the T cell compartment (Salimzadeh et al. 2018). Furthermore, expansion of regulatory B cells producing IL-10, which can impair CD8 T cell function, and reduction in TLR-9 expression have been observed in B cells from CHB patients (Das et al. 2012; Tout et al. 2018). However, available data suggest that antibody secretory capability appears intact in total B cells, which is in line with the fact that patients with CHB maintain the ability to produce antibodies to recall antigens such as soluble protein vaccines.

In patients with CHB, unlike HBV vaccinated healthy individuals, anti-HBs producing B cells were either completely absent (Barnaba et al. 1985, 1987; Dusheiko et al. 1983) or were detected in very low numbers using functional, antibody secreting, assays (Xu et al. 2015; Tian et al. 2018). However, HBsAg-anti-HBs immune complexes were identified in patients with CHB (Madalinski et al. 1991; Rath and Devey 1988), suggesting that HBsAg-specific B cells are present in CHB patients but anti-HBs antibodies are sequestered by the large quantities of HBsAg in the circulation (Fig. 4.3a, b). Only recently have new techniques for direct visualization of HBV-specific B cells, using fluorescently labelled HBV-antigen baits, been developed to quantify and phenotype HBV-specific B cells. This technology demonstrated that HBsAg-specific B cells are present in the circulation of CHB patients at frequencies comparable with those of acute-resolving infection and HBV-vaccinated healthy individuals. The frequency of HBsAg-specific B cells showed no correlation with HBsAg quantity, HBV DNA, or ALT in the serum of CHB patients (Salimzadeh et al. 2018; Burton et al. 2018). HBsAg-specific B cells from different clinical phases of chronic HBV infection were unable to mature into IgG anti-HBs producing cells (Salimzadeh et al. 2018; Burton et al. 2018) and required additional help from CD40 ligand overexpressing feeder cells. However, compared to healthy vaccinated subjects, anti-HBs response in CHB patients remained much lower (Salimzadeh et al. 2018). This patient-derived data is consistent with the importance of the OX40/OX40-ligand axis for HBsAg seroconversion in a transgenic mouse model of chronic HBV infection, via boosting the T helper and T follicular helper cell responses (Publicover et al. 2011) (Fig. 4.3c). Deeper characterization of HBsAg-specific B cells showed expansion of a functionally defective B cell subset described as atypical memory B cells (AtM) or tissue-like memory B cells (TLMs). AtMs have been reported in HIV, HCV, and *Plasmodium falciparum* infection (Moir et al. 2008; Kardava et al. 2014; Knox et al. 2017; Sullivan et al. 2015) as well as some autoimmune diseases (Jacobi et al. 2008; Adlowitz et al. 2015). AtMs ($CD19^{+}$, $CD21^{low/-}$, $CD27^{-}$) express inhibitory receptors, including FcRL5 and PD-1, and are characterized by attenuated BCR stimulation, impaired cytokine production,

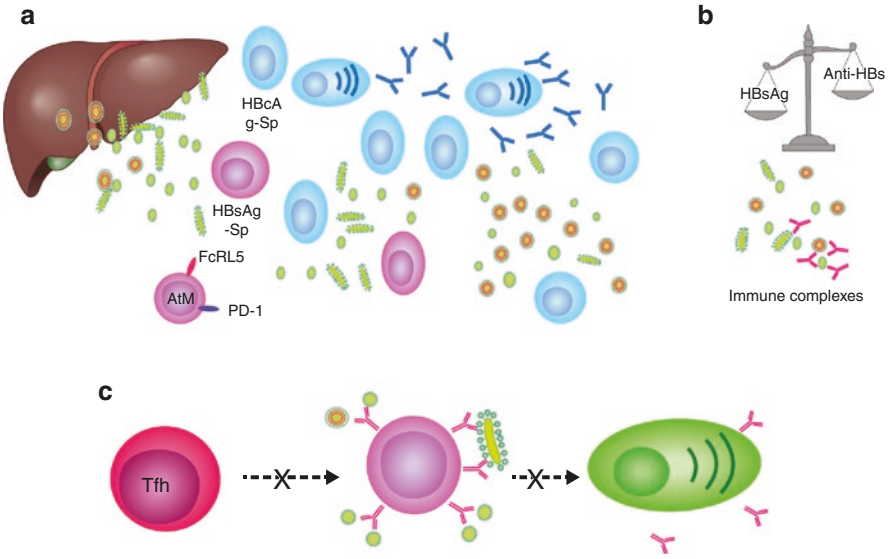

Fig. 4.3 Dysfunctional HBsAg-specific B cell in chronic HBV infection. (**a**) HBsAg-specific B cells are present in circulation and liver of CHB patients with frequencies lower than HBcAg-specific B cells. HBsAg-specific B cells show expansion of an exhausted B cell subset called Atypical memory (AtM) B cells expressing PD-1 and FcRL5 and they have defects in differentiation to anti-HBs secreting plasma cells/plasmablasts. (**b**) Any available anti-HBs are sequestered with HBsAg and form immune complexes in circulation of CHB patients. (**c**) While the mechanisms of dysfunctionality of HBsAg-specific B cells in CHB is unclear, it is likely that persistent stimulation of B cells by circulating HBV virions and subviral particles, plus lack of help via T follicular helper cells, contribute to this process

susceptibility to cell death, and defects in differentiation into plasma cells. PD-1 blockade of cultured HBsAg-specific B cells or global AtMs in combination with CD40-L stimulation could partially restore their functionality to produce anti-HBs and antiviral cytokines respectively (Salimzadeh et al. 2018; Burton et al. 2018). HBsAg-specific B cells were also found in liver of CHB patients and showed accumulation of AtM B cells (Burton et al. 2018). Of note, the expansion AtM B cells within the HBsAg-specific B cell compartment (median ~ 15%) cannot wholly explain their defective anti-HBs production, since the majority of HBsAg-specific B cells were classical resting-memory B cells.

Using the same technology, HBcAg-specific B cells were found at higher frequencies than HBsAg-specific B cells in the peripheral blood of CHB patients and matured efficiently into anti-HBc IgG producing cells in vitro (Le Bert et al. 2020; Vanwolleghem et al. 2020) (Fig. 4.3a). HBc-specific B cells did not display the same atypical phenotype as HBs-specific B cells. Differential regulation of HBsAg- vs. HBcAg-specific B cells in CHB is not yet clear. Perhaps persistent interaction of circulating HBsAg with specific B cells results in overstimulation, induction of their terminal differentiation and exhaustion, analogous to that described in the case of exhausted HBV-specific T cells (Bertoletti and Ferrari 2016) and cancer (Zhang

et al. 2020). In contrast, the intact functional capacity of HBc-specific B cells is likely related to the fact that HBcAg is not present in circulation with frequency as high as HBsAg. In addition, the potent immune stimulatory properties of HBcAg can induce development of anti-HBc secreting plasma cells in a T cell-independent manner (Milich and McLachlan 1986).

3.4 Immunopathology in Chronic HBV Infection

Liver damage is used to discriminate between the clinical stages of chronic hepatitis B (Lampertico et al. 2017; Yuen et al. 2018). Liver damage presents as spontaneous hepatitis flares, which are transient, or chronic active hepatitis that requires initiating antiviral therapy. Because of the unpredictable nature of flares or the duration of chronic hepatitis, the trigger of inflammation and damage during chronic hepatitis B also remains undefined. In contrast, acute HBV infection, where HBV-specific CD8 T cells display the full spectrum of functionality (Maini et al. 1999; Webster et al. 2000; Penna et al. 1996; Ferrari et al. 1990), capable of inducing chemokines associated with liver damage, virus-specific T cells are profoundly exhausted in CHB patients (Hoogeveen et al. 2018; Boni et al. 2007; Fisicaro et al. 2010; Bengsch et al. 2014). However, similar to acute HBV infection, the majority of liver damage is likely not the result of HBV-specific CD8 T cells but rather dysregulated activation of intrahepatic immunity leading to nonspecific hepatocyte killing (Maini et al. 2000).

Chronic liver damage is associated with inflammatory immune markers in patient serum that overlap with those observed with liver damage during acute infection. These include type I interferons, chemokines, particularly CXCL-8, CXCL-9 and CXCL-10, and FasL (Lapinski et al. 2004; Dunn et al. 2007; Tan et al. 2010). Markers of myeloid (CD163) and lymphocyte (soluble PD-1) activation correlate with liver damage during chronic hepatitis B and may be present during acute infection but have not been investigated (Kazankov et al. 2014; Dultz et al. 2014; Zhou et al. 2019). An increased frequency of HBcAg-specific B has been found during periods of liver damage in chronic HBV infection (Vanwolleghem et al. 2015; Le Bert et al. 2020). In particular, plasma cells secreting IgG and IgM against HBcAg, along with complement deposition in liver, are associated with fulminant hepatitis (Chen et al. 2018, 2020).

Unlike acute infection where a majority of liver damage is attributed to CD8 T cells, the specific cell types responsible for nonspecific hepatocyte killing during chronic HBV infection are less clearly defined. The cytotoxic effector molecules FasL and TNF-related apoptosis-inducing ligand (TRAIL) correlate with liver damage. FasL can be produced by both T cells and NK cells (Kondo et al. 1997; Zou et al. 2009), the involvement of TRAIL in chronic hepatitis B implicates a significant role for NK cells (Dunn et al. 2007). The relative contribution of T cells and NK cells to liver damage remains to be determined. There has not been a mechanism defined for nonspecific, contact-mediated killing of hepatocytes. However, the upregulation of activating NK receptors has been identified in the chronic hepatitis B liver and CD8 T cells can upregulate NKG2D and kill cells in a TCR-independent

manner (Kennedy et al. 2008; Schulthess et al. 2012) (Fig. 4.1c). The challenge, however, has been demonstrating such pathways in situ due to limitations of chronic HBV infection models and measuring these dynamic processes in the liver of hepatitis B patients.

Beyond the mechanisms responsible for hepatocyte killing are the secondary consequences of hepatocyte lysis. It was described 40 years ago that factors released upon hepatocyte killing have immune-suppressive effects (Chisari 1978; Chisari et al. 1985). The primary factor was identified as arginase. As mentioned above, arginase impairs T cell function (Das et al. 2008). Similar impairment of T cell functionality was observed during acute hepatitis B where HBV-specific T cells displayed a stunned phenotype (Sandalova et al. 2012). Therefore, careful consideration has to be made when assessing the function of immunity in patients with liver inflammation. These data further demonstrate how the dynamic inflammatory environment in CHB patients can complicate analysis, similar to what has been seen with innate immune responses (Kuipery et al. 2020).

Currently, the best scenario to study mechanisms inducing liver inflammation and damage in CHB patients is in patients stopping NUC therapy. Upon therapy withdrawal, viral DNA rebounds rapidly and the majority of patients will develop an inflammatory response that coincides with liver injury (Berg et al. 2017; Liem et al. 2019). The inflammatory response appears 8–12 weeks post-stopping therapy, after HBV DNA rebound, indicating that liver damage is not a consequence of HBV DNA alone. There are minimal induction cytokines upon HBV DNA rebound and no changes in NK cell phenotype; however, NK cell cytotoxic potential correlated with liver damage after therapy discontinuation (Zimmer et al. 2018; Siederdissen et al. 2016). Intrahepatic analysis at the earliest stages of HBV reactivation after stopping therapy will likely be necessary to identify the mechanisms inducing HBV-mediated liver damage in chronic patients.

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Pathology of Hepatitis B Virus (HBV) Infection and HBV-Related Hepatocellular Carcinoma

5

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Abstract

Hepatitis B infection can cause a wide range of histopathological changes in the liver with different presentations during the acute and chronic infection phases. These findings can often overlap with other viral and nonviral causes of hepatitis. Therefore, clinical information and serological findings are important in confirming the diagnosis of hepatitis B. Acute hepatitis B is not routinely biopsied as its histological findings are often nonspecific with hepatocyte injury, regeneration, inflammation, and necrosis. However, when acute hepatitis B progresses to chronic hepatitis B, histological examination of the liver biopsies plays a key role in guiding treatment decisions and surveillance for sequelae. Liver biopsy is currently the gold standard for evaluating the degree of fibrosis (stage) and necroinflammatory activity (grade) in chronic hepatitis B. In addition, monitoring for sequelae of chronic hepatitis B is important as chronic infection can lead to the development of cirrhosis and liver malignancies. The hepatitis B virus has been found to be causally linked to the development of hepatocellular carcinoma (HCC) and possibly associated with the development of cholangiocarcinoma. Active surveillance with imaging and liver biopsies can help with early detection of liver cancer to improve outcomes.

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Acute hepatitis B · Chronic hepatitis B · Grade · Stage · Ground glass hepatocytes · Cirrhosis · HBV-associated hepatocellular carcinoma · Cholangiocarcinoma

1 Pathology of Hepatitis from HBV Infection

Since the discovery of the hepatitis B virus (HBV) in the 1960s, there have been numerous studies describing various pathological changes that occur in the liver following HBV infections. However, challenges remain using microscopic findings alone to diagnose specific viral hepatitis. The liver only has a limited number of ways to respond to a wide range of injury, therefore, the pattern of injury along with pertinent clinical information is invaluable to pathologists in making the most accurate diagnosis. Since HBV hepatitis shares many features with other viral and non-viral hepatitis, the diagnosis of HBV hepatitis requires serological, virological, or immunohistochemical correlation in addition to the histological findings.

To best understand the histopathologic features, HBV hepatitis can be categorized based on acute and chronic infection phases. Biopsies from chronic HBV hepatitis are more often seen by the pathologists than biopsies from acute HBV hepatitis. HBV infection that persists beyond 6 months is deemed chronic since it is less likely to spontaneously resolve. Liver biopsies with chronic hepatitis B provide critical information on disease severity and determine the occurrence of complications such as portal hypertensions, cirrhosis, or hepatocellular carcinoma.

In this chapter, we review the histopathological features of HBV hepatitis and discuss HBV-related hepatocellular carcinoma and its precursor lesions.

1.1 Acute Hepatitis B

Acute hepatitis B shares many common histopathologic features with acute hepatitis of other causes and there is significant morphological overlap among the various hepatotropic viruses without distinct criteria. Clinical information and virological/serological testing are needed to yield the correct diagnosis in acute hepatitis because the liver responds to a wide range of injury nonspecifically in a limited number of manners (Ferrell 2000). Hence, liver biopsies for acute hepatitis B are not routinely performed; however, when there is clinical suspicion for alternative diagnosis, question about disease severity and stage, or the need to distinguish between rejection or viral infection or reinfection in posttransplant patients, understanding the histopathologic changes in acute viral hepatitis is critical (Lefkowitch 2007).

Generally, the main pathologic features of acute viral hepatitis include hepatocellular damage and regeneration, and various patterns of inflammation. Other features such as cholestasis and bile duct damage can also be present. The histopathologic changes are a result of the immune response to the viral antigen displayed on hepatocytes. HBV itself can replicate in hepatocytes without causing direct cell damage

(Tan et al. 2015). Macroscopically, the liver becomes red and swollen with capsular tension and edema. Focal depressions and wrinkling can be seen in the capsule from necrosis and collapsed liver parenchyma (Butler et al. 2018). The histopathologic patterns of acute hepatitis B can be best described as the classic form and its variations.

In the classic form of acute hepatitis B (Fig. 5.1a, b), the liver parenchyma shows hepatocyte damage and death in the form of spotty necrosis which is often more severe in the centrilobular region near the terminal hepatic venule (Kobayashi et al. 1993). There is associated reticulin framework condensation and distortion secondary to hepatocyte death, however, no significant structural alteration is typically seen with minimal fibrosis (Thung and Gerber 1982; Kanta 2016). The injured hepatocytes can show ballooning and acidophilic changes which represent different stages of cellular degeneration. The ballooning hepatocytes have swollen cytoplasm, rounding, swollen nuclei, and prominent nucleoli from protein accumulation (Ranek 1976). Ballooning degeneration itself is a nonspecific finding that can be seen in other types of hepatic injury such as alcoholic/nonalcoholic steatohepatitis or toxin-induced hepatitis. The formation of acidophil (Councilman) bodies is likely a later stage of hepatocyte apoptosis where the cytoplasm becomes acidophilic and the nuclei become pyknotic (Saxena et al. 2002). In addition to hepatocyte degeneration and death, there is also hepatocyte regeneration. This can be seen as hepatocytes with mitotic figures, variable sizes, rosette formation, and distortion of hepatocytic plates (Nagore et al. 1989). Overall, the hepatocyte necrosis, cellular regeneration changes, and consequential lobular disarray seen on biopsy specimens as reticulin framework distortion help with diagnosis of acute hepatitis.

On top of the liver parenchyma disarray, inflammatory infiltrates predominately composed of lymphocytes, plasma cells, and activated macrophages are another feature that is present in acute hepatitis B (Volpes et al. 1991; Mietkiewski and Scheuer 1985). The most prominent inflammation is typically found in the liver

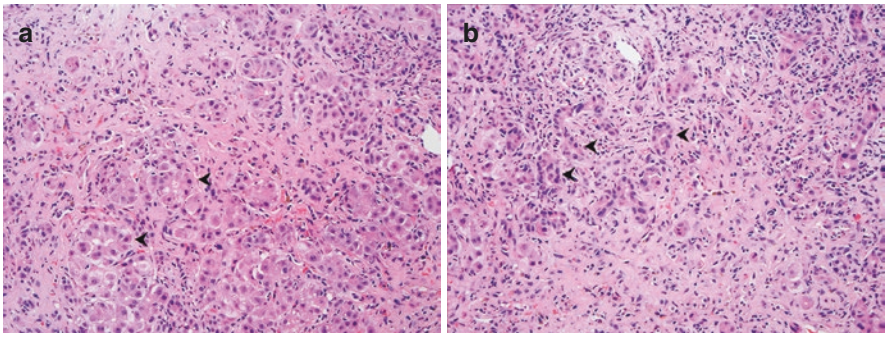

Fig. 5.1 Changes in acute hepatitis B, hematoxylin and eosin (H&E), magnification 200X. (**a**) This section shows lymphocytic and histiocytic inflammation, hepatocyte swelling, and pseudorosette formation (➤) with confluent necrosis in the left upper corner. (**b**) This section shows ductular reaction (➤), bile duct injury, and mixed inflammation with bridging and confluent necrosis in the lower half

parenchyma where it is typically associated with hepatocyte injury and death. This is often seen in the centrilobular and perivenular regions where the hepatocyte damage is the most severe (Theise et al. 2018a). The lymphocyte predominate inflammatory infiltrates can also be seen at portal tracts with or without periportal involvement. Although portal tract inflammation can often be present in acute viral hepatitis, the amount of inflammation and distribution may vary. It is important to note that when the portal inflammation is focal, it can be missed on needle biopsies.

Cholestasis and bile duct injury are additional features that can be seen in the classic form of acute viral hepatitis (Sciot et al. 1986). Cholestasis presents as either bile plugs in the canaliculi in the lobules or as intracellular bile deposits (Postnikova et al. 2012). Intracanalicular cholestasis can range from scant fragments of bile to large bile plugs that cause canalicular dilatation. Intracellular cholestasis is rare and difficult to be identified on light microscopy. Bile duct injury is often mild and infrequent in acute hepatitis B. When present, the injured interlobular bile ducts show epithelial cell irregularities and crowding. Clinicians and pathologists should keep in mind that the degree of bile duct injury does not always correlate with clinical presentation of cholestasis because the bile duct damage is typically contained and non-progressive.

Histopathologic variations often occur in the classic form of acute hepatitis. In acute HBV hepatitis, severe necrosis can often be observed on top of the classic histopathology described (Liang et al. 1991; Omata et al. 1991). This can present as confluent necrosis, bridging necrosis, or the more severe panacinar necrosis. Bridging necrosis can be subdivided into central venule to central venule (central–central) or central venule to portal tract (central–portal) bridging necrosis. There has been some evidence suggesting that central–portal necrosis is more indicative of progression to chronic hepatitis than central–central necrosis (Boyer and Klatskin 1970; Ware et al. 1975). However, other studies suggest bridging necrosis is not a useful indicator for chronicity (Spitz et al. 1978; Wiener et al. 1984). Regardless of the prognostic nature of bridging necrosis, it is still an indicator of a more severe form of acute hepatitis. In panacinar or panlobular necrosis, there is almost complete destruction of all hepatocytes within a lobule. This variation is also known as massive hepatic necrosis when there is diffuse panacinar necrosis across the entire liver parenchyma; this is frequently the morphologic counterpart of fulminant liver failure (Craig et al. 2004). However, studies have shown that confluent necrosis is not a homogenous process across the liver (Hanau et al. 1995). With that in mind, using needle biopsy specimen alone for assessing the extent of necrosis can be misleading.

Despite the varying amount of necrosis and liver damage that can occur in acute viral hepatitis, there is also a significant amount of regeneration that happens, allowing the liver to return to its normal function in surviving patients (Gove and Hughes 1991). The regeneration as mentioned above, occurs throughout the acute phase. As the hepatocyte injury decreases and the necrosis ceases, there is an increase in phagocytic activity marking the regression stage. In hepatitis B, following the acute phase there can be occult disease with mild nonspecific histopathologic changes lasting for over a decade before the liver returns to normal (Yuki et al. 2003). The

histological outcomes following acute hepatitis B include complete resolution, scar formation, progression to chronic hepatitis and cirrhosis, or even death.

1.2 Chronic Hepatitis B

Chronic hepatitis B (CHB) is defined by the presence of hepatitis B surface antigen (HBsAg) for at least 6 months (Terrault et al. 2018). Histological examination of the liver through biopsy specimens in CHB plays an important role in guiding treatment decisions and surveillance for sequelae (Terrault et al. 2018; Zeng et al. 2016; Huang and Lim 2020). Liver biopsy is currently the gold standard and the only means to evaluate the degree of fibrosis and inflammation present. A good understanding of the histopathologic findings in CHB is essential for pathologists and clinicians.

Macroscopically, changes in CHB livers can be variable, subtle, and nonspecific; it may range from near normal to increased redness in appearance. The liver may appear yellow if there is concurrent steatosis and green-tinged if there is concurrent cholestasis. The presence of nodularity suggests cirrhosis. In CHB, there is typically diffuse macronodularity, however, there can also be a mixture of macro- and micronodularity (Sinniah 1972). Definitive conclusions should not be drawn until the liver is examined microscopically.

The basic histologic findings in CHB liver parenchyma include inflammation, hepatocyte injury and death, atrophy, regeneration, and fibrosis (Fig. 5.2a–c) (Theise et al. 2018a). These findings are not unique to CHB as it is also seen in variable amounts and distribution in other types of viral and nonviral hepatitis. Luckily, the diagnosis of CHB is typically established and the purpose of the liver biopsy is to evaluate the severity of disease and exclude complications of CHB or other concomitant diseases.

In evaluating the severity of disease, an assessment of the disease activity should be made by looking at hepatocyte injury and inflammation to provide a grade for the activity. The amount and distribution of fibrosis should also be evaluated so a stage can be assigned.

One of the identifying lesions of all chronic viral hepatitis is portal inflammation with predominately lymphocytes and plasma cells (Kasper et al. 2009; Walewska-Zielecka et al. 2008). Macrophages near the portal tract can also be seen and typically contains diastase-resistant material on Periodic acid-Schiff-diastase (PAS-D) stain. The inflammatory infiltrates are denser than in acute viral hepatitis and can involve some or all portal tracts. The inflammatory filtrates can cause portal tract widening by filling the fibrous stroma surrounding the tracts and pushing into adjacent structures. Bile ducts may be injured by the inflammation and there may be bile ductular reaction in some cases (Shah et al. 1995). In addition, interface hepatitis with apoptotic hepatocytes and predominately lymphocytic infiltrates can be present at the portal stromal and liver parenchymal border (Eddleston and Mondelli 1986; Kerr et al. 1979; Chen et al. 2004). Emperipolesis, where lymphocytes are seen within hepatocytes, can occur. Some study has suggested that the presence of

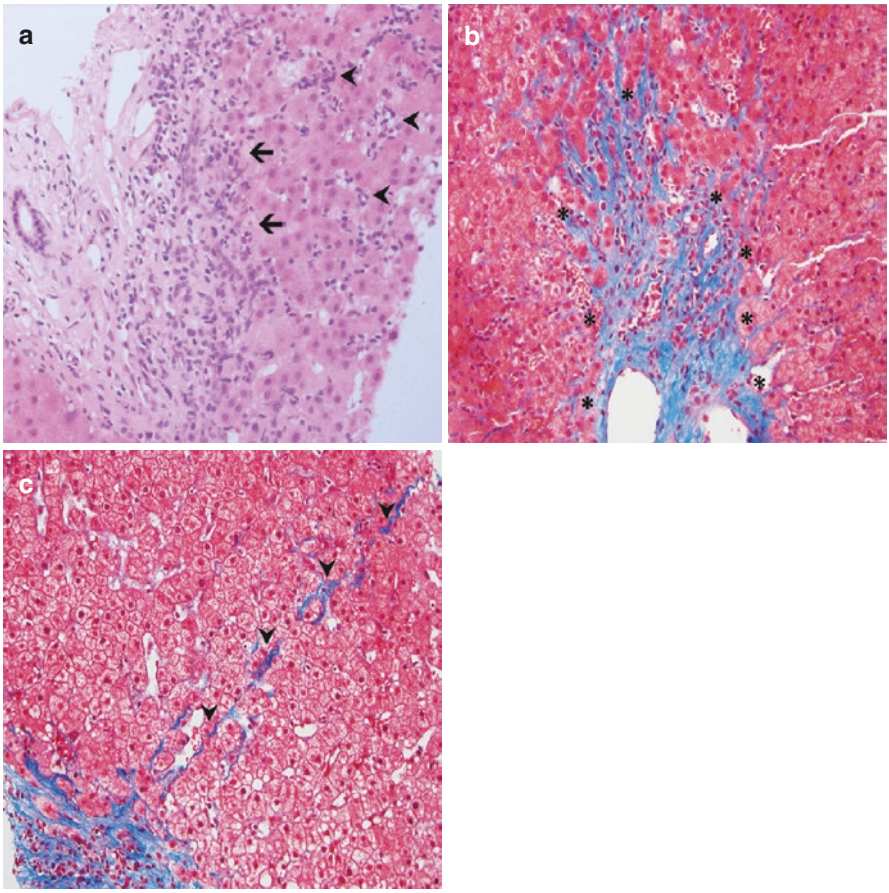

Fig. 5.2 (**a**) The H&E stained section shows chronic hepatitis B with moderate activity including interface inflammation between the portal structures and parenchyma (←) and scattered foci of lobular inflammation (➤) within the parenchyma, magnification 200X. (**b**) The Masson Trichrome stain highlights periportal fibrosis (*) without significant septal fibrosis which is consistent with stage 2 of the Batts-Ludwig system, magnification 200X. (**c**) The Masson Trichrome stain highlights portal fibrosis with perforated delicate fibrous septa (➤) representing regression of bridging fibrosis, magnification 200X

emperipolesis in CHB may be an indicator of active liver inflammation (Hu et al. 2015).

Lobular hepatitis, where the hepatic lobules show activity in the form of necrosis and inflammation, is another component to consider in assessing CHB (Liaw et al. 1982; Chen and Liaw 1988). The necrosis that is present is typically focal with the presence of acidophil bodies, debris, and distorted reticulin framework; however, confluent or bridging necrosis (central–central or central–portal) may occur as well. It has been suggested that bridging necrosis in CHB can be a prognostic factor for progression to cirrhosis, however, studies also suggest it may be paradoxical and

indicate healing (Chen and Liaw 1988; Cooksley et al. 1986). In severe cases, panlobular activity may be present when the liver parenchyma is collapsed due to necrosis, the portal tracts become abnormally close to each other, and there are elastic fiber depositions (Scheuer and Maggi 1980). The presence of elastic fibers, which can be highlighted by orcein staining, suggests there is associated fibrosis and favors chronic over acute hepatitis (Scheuer and Maggi 1980). Mononuclear inflammatory infiltrates including lymphocytes, macrophages, and plasma cells often accompany the necrosis in liver parenchyma.

The hallmark histopathologic findings in CHB are ground glass hepatocytes (GGH) and sanded nuclei (Fig. 5.3a). The GGHs are liver cells whose cytoplasm appear eosinophilic, granular, and glassy, containing abundant smooth endoplasmic reticulum where HBsAg accumulate (Popper 1975; Hadziyannis et al. 1973). Studies have found that there are two major types of GGHs, type I and II, with distinct morphology and distribution (Su et al. 1985; Wang et al. 2003). Type I GGHs are seen scattered throughout the liver lobules during the viral replicative phases and they contain deletion mutations over the pre-S1 region. These hepatocytes have more eccentric nuclei and accumulation of ground glass material and HBsAg in their cytoplasm (Fig. 5.3b) (Wang et al. 2003). Type II GGHs are seen in late nonreplicative stage or in cirrhotic livers and contain deletion mutations over the pre-S2 region. They have less HBsAg expression in their cytoplasm and are found in large clusters (Fig. 5.3c) (Wang et al. 2003). The two types of GGHs contain different HBsAg mutants and have different biological activities. Type II GGHs contain immune escape HBsAg mutants that remain undetected by immune surveillance and can persist in cirrhotic lesions in CHB (Wang et al. 2003). It has been found that type II GGHs are more likely to be present in hepatocellular carcinoma; current data suggest that mutant HBsAg plays a role in the pathogenesis of HBV-related hepatocellular carcinoma (Mathai et al. 2013). Sanded nuclei can also be seen in some hepatocytes in persistent HBV infection; it appears as finely granular eosinophilic nuclear inclusions due to the accumulation of excess hepatitis B core antigens (HBcAg) (Huang et al. 1972; Bianchi and Gudat 1976). This finding can be subtle on light microscopy.

In addition to portal and lobular activity, CHB livers also contain variable degrees of fibrosis and hepatocyte regeneration. Fibrosis is not a mandatory feature for chronic viral hepatitis as it may or may not be present in all patients. However, assessment of fibrosis is relevant for both prognosis and treatment initiation. Prior to antiviral therapy era, fibrosis was thought to be an irreversible process (Wanless et al. 2000; Hytiroglou and Theise 2018; Ohkoshi et al. 2016; Fong et al. 1993; Bortolotti et al. 2005). Fibrosis typically starts and extends from the portal stroma, but it can also be perivenular and pericellular as a result of continuous inflammation and injury which induces the deposition of extracellular matrix (Theise et al. 2018a; Ohkoshi et al. 2016). Portal or periportal fibrosis can progress, linking other portal tracts and forming septal fibrosis with inflammation and associated interface hepatitis. This typically stains dark blue on the Masson Trichrome stain since it contains abundant collagen content and elastic fibers. Perivenular fibrosis resulting from confluent necrosis is typically bland and acellular without associated inflammation.

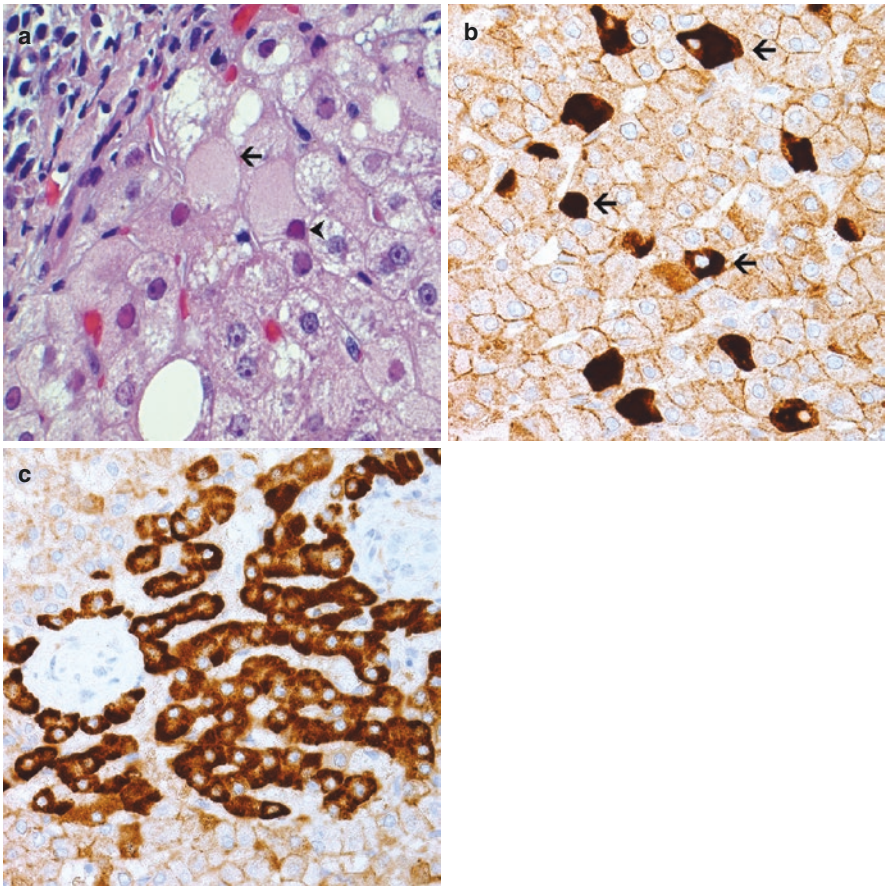

Fig. 5.3 (**a**) The H&E stained section shows ground glass hepatocytes (←) with eosinophilic granular and glassy cytoplasm containing abundant smooth endoplasmic reticulum where HBsAg accumulates. Sanded nuclei (➤) with finely granular eosinophilic nuclear inclusions from the accumulation of HBcAg can also be appreciated here, magnification 600X. (**b**) Immunohistochemical stain for HBsAg highlights the scattered type I ground glass hepatocytes (←), magnification 400X. (**c**) Immunohistochemical stain for HBsAg highlights a large cluster of type II ground glass hepatocytes, magnification 400X

Perivenular fibrosis may progress with linking to other central veins or portal tracts. Regeneration can occur concurrently as fibrosis progress. Regeneration of hepatocytes can be seen as thickened liver plates that are two to three cells in thickness and may have variable nuclear size (Duncan and Soto-Gutierrez 2013; Michalopoulos 2007).

In CHB, a dynamic process occurs with fibrosis progression and regression along with hepatocyte regeneration and injury. Cirrhosis can occur with continuous infection and chronic liver injury. Histopathologically, it is defined as the formation of

regenerative nodules divided by fibrous septae and clinically, cirrhosis leads to portal hypertension and end-stage liver disease (Schuppan and Afdhal 2008). It was traditionally believed that cirrhosis is irreversible. In late stage cirrhosis, there is significantly less hepatocyte regeneration while there is an increase in ductular reaction (Falkowski et al. 2003). With current advancements in antiviral therapy for hepatitis B, elimination of infection and a significant decrease in viral activity can be achieved (Hytiroglou and Theise 2018; Halegoua-De Marzio and Hann 2014). When patients are successfully treated, reparative changes become predominant, leading to regression of fibrosis and even regression of cirrhosis where the fibrous septae becomes thinned and perforated over time leading to disappearance of parenchymal nodularity (Halegoua-De Marzio and Hann 2014). In general, when evaluating liver biopsies in patients with suspected cirrhosis, pathologists and clinicians should keep in mind that cirrhotic areas of the liver may be separated by normal appearing liver. Thus, cirrhosis may not always be captured on core biopsies and non-cirrhotic appearing liver does not necessarily mean complete regression of fibrosis.

Although current data shows that cirrhosis can be reversed with successful antiviral treatment, it may not be completely reversible (Hytiroglou and Theise 2018; Valeer and Desmet 2004). In addition to fibrosis, vascular changes also occur in cirrhosis and it plays an important role in the development of portal hypertension (Bedossa et al. 2018). These changes include thrombosis, arteriovenous and portovenous shunt development, obstruction, and recanalization (Hytiroglou and Theise 2018). The combination of vascular changes and fibrosis can significantly change the liver's microvasculature and compromise hepatocyte function and survival. It has been suggested that the vascular changes may not be reversible. This may explain the persistence of portal hypertension despite regression of fibrosis in patients with cirrhosis (Hytiroglou and Theise 2018). New studies in vascular biology suggest the pathogenesis of portal hypertension may have targetable molecular pathways in the future (Yasuko Iwakiri and Don 2014).

1.3 Post-viral Eradication

In patients who become serum HBsAg negative either through spontaneous remission or as a result of antiviral treatment, it has been found that a mild degree of hepatitis persists despite normal serum aminotransferase activity (Fong et al. 1993). There is usually mild portal inflammation, residual fibrosis, and rare foci of parenchymal necrosis. It was demonstrated in several studies that there was persistence of low-level HBV DNA in the liver despite loss of serum HBsAg and HBV DNA (Kuhns et al. 1992; Loriot et al. 1992; Bréchot et al. 1985). Some studies suggest that the low levels of HBV DNA in the liver represent active viruses rather than just nonreplicative forms of the viral genome (Fong et al. 1993). This raises the concern of developing long-term complications of CHB in patients who appear to have completely recovered.

1.4 Immunohistochemical Stains

Immunohistochemical stains for confirming the presence of hepatitis B virus are not routinely performed on liver biopsy specimens for assessing chronic viral hepatitis today. Historically, a modification of the orcein staining for elastic tissue was described by Shikata et al. to highlight HBsAg in the cytoplasm of hepatocytes (Henwood 1983; Shikata et al. 1974). Currently, immunohistochemical stains for the HBsAg and HBcAg are available for clinical use. It has been described that the staining distribution of HBsAg and HBcAg reflects various histologic patterns of hepatitis (Ray et al. 1976a, b). For example, the predominate cytoplasmic staining pattern of HBsAg correlates with near-normal liver with persistent chronic hepatitis without much activity while predominate membrane staining pattern of HBsAg correlates with chronic hepatitis with more activity (Ray et al. 1976a). HBcAg staining can be seen in the cytoplasm, membrane and nuclei of the hepatocyte, and its positivity is more prevalent in more aggressive forms of CHB or CHB in immunosuppressed patients (Ray et al. 1976a). However, more recent studies did not demonstrate a significant correlation between the pattern of HBsAg and HBcAg staining with CHB activity (Sharma et al. 2002). In general, these immunohistochemical stains may be best reserved for cases of suspected hepatitis B based on hematoxylin and eosin (H&E) staining patterns but no clinically confirmed diagnosis of hepatitis B.

1.5 Grading and Staging in Chronic Hepatitis B

The current practice in assessing liver biopsies in chronic hepatitis is for pathologists to report the grade of necroinflammatory activity and the stage of fibrosis as separate statements to best guide treatment and evaluate prognosis (Brunt 2000). In order to reduce inter- and intraobserver variabilities, many scoring systems exist to allow semiquantitative, objective, and reproducible descriptions of chronic hepatitis lesions. The Knodell score or histology activity index, published in 1981, is the benchmark and first of such system (Knodell et al. 1981). Since the Knodell score, many other systems have been created such as the Ishak score (modified Knodell score), the METAVIR score, the Scheuer system, and the Batts-Ludwig system (Knodell et al. 1981; Batts and Ludwig 1995; The French METAVIR Cooperative Study Group 1994; Bedossa and Poynard 1996; Ishak et al. 1995; Scheuer 1991).

The scoring systems can be grouped into complex and simple systems. For individual patient care, a simple scoring system such as the METAVIR score, Scheuer system, or Batts-Ludwig system is preferred as there are fewer categories, thus, more user-friendly and supports more inter- and intraobserver agreements (Batts and Ludwig 1995; The French METAVIR Cooperative Study Group 1994; Bedossa and Poynard 1996; Scheuer 1991; Goodman 2007). The trade-offs for these simple systems are that there is a larger range of lesional presentation within each category and mild changes between biopsies may not be detected (Goodman 2007). Table 5.1 summarizes and compares the simple systems.

Table 5.1 Simple scoring system

METAVIR score			Scheuer system			Batts-Ludwig system		
Grading system for necroinflammatory activity								
Interface inflammation	Lobular necrosis	**Activity score**[a]	Periportal/portal activity	Lobular activity	**Grade**[b]	Lymphocytic interface hepatitis	Lobular inflammation and necrosis	**Grade and description**[b]
0	0	A0—None	None	None	0	None	None	0—No activity
0 1	1 0, 1	A1—Mild	Minimal portal inflammation	Inflammation without necrosis	1	Minimal to patchy	Minimal or occasional spotty necrosis	1—Minimal activity
0 1 2	2 2 0, 1	A2—Moderate	Mild interface hepatitis	Focal necrosis or acidophil bodies	2	Involving some or all portal tracts	Mild hepatocellular damage	2—Mild activity
2 3	2 0, 1, 2	A3—severe	Moderate interface hepatitis	Severe focal hepatocyte damage	3	Involving all portal tracts	Noticeable hepatocellular damage	3—Moderate activity
			Severe interface hepatitis	Injury including bridging necrosis	4	May have bridging necrosis	Prominent and diffuse hepatocellular damage	4—Severe activity
Staging system for fibrosis								
No fibrosis		F0	No fibrosis		0	Normal		0—No fibrosis
Portal fibrosis without septa		F1	Enlarged fibrotic portal tracts		1	Fibrous portal expansion		1—Portal fibrosis
Portal fibrosis with few septa		F2	Periportal or portal-portal septa Architecture intact		2	Periportal or rare portal-portal septa		2—Periportal fibrosis
Portal fibrosis with numerous septa No obvious cirrhosis		F3	Fibrosis with architectural distortion No obvious cirrhosis		3	Fibrous septa with architectural distortion. No obvious cirrhosis		3—Septal fibrosis
Cirrhosis		F4	Probable or definite cirrhosis		4	Cirrhosis		4 - cirrhosis

Comparison of grading and staging for the METAVIR score (The French METAVIR Cooperative Study Group 1994; Bedossa and Poynard 1996), Scheuer system (Scheuer 1991), and Batts-Ludwig system (Batts and Ludwig 1995)

[a]METAVIR activity score is calculated by combining interface inflammation score (0 - none, 1 - mild, 2 - moderate, 3 - severe) and lobular necrosis score (0 - none or mild, 1 - moderate, 2 - severe)

[b]Scheuer and Batts-Ludwig systems both assess portal/periportal/interface activity and lobular activity separately then give an overall activity score based on the more severe grade of the two

The more complex numerical systems such as the Knodell score and Ishak score are frequently used in research as they provide more information and can detect minor changes in both necroinflammatory activity and the extent of fibrosis (Knodell et al. 1981; Ishak et al. 1995). The disadvantages of the complex systems are that they are more tedious, and less user friendly and less reproducible. For these reasons, the complex scoring systems are less commonly used clinically. Table 5.2 summarizes and compares the Knodell score and Ishak score.

One disadvantage of both the simple and complex scoring systems discussed above is that neither address the variable degrees of advanced fibrosis and cirrhosis. To address this deficiency, the Laennec staging system and the Beijing classification system have been proposed recently (Kutami et al. 2000; Sun et al. 2017). The Laennec staging system is a modification of the METAVIR fibrosis score for cirrhosis (F4); it was created to report the variable range of cirrhosis and to help correlate the severity of cirrhosis to liver-related events (Kutami et al. 2000; Kim et al. 2012). Studies have found that more severe Laennec cirrhosis stage are associated with higher risk of liver-related events such as hepatocellular carcinoma or liver decompensation (Kim et al. 2012). The Beijing classification system was developed in response to the era of successful hepatitis B therapy (Sun et al. 2017). The liver biopsy in patients undergoing antiviral treatment demonstrates both progression and regression as current therapy can reverse fibrosis and even early cirrhosis in patients with hepatitis B (Sun et al. 2017; Theise et al. 2018b). This classification system allows the reflection of the dynamic changes that occur in fibrosis of treated CHB liver biopsies. Table 5.3 summarizes the Laennec system and the Beijing classification system for cirrhosis.

2 Pathologic Differential Diagnosis with Other Liver Diseases

When evaluating biopsy specimens for both acute and chronic hepatitis B, a list of other causes needs to enter the possible differential diagnosis. In the case of acute hepatitis B, the differential remains fairly wide based on histological grounds alone. This includes other viral infections, drug reactions, toxin exposure, or autoimmune diseases. Clinical history and serology/virology are often key in such cases. Subtle histological findings such as eosinophilia in drug reaction or fatty changes with Mallory-Denk bodies in steatohepatitis may sway the diagnosis in some cases. However, multiple etiologies for causing acute hepatitis may be present in the same specimen. Similarly, hepatitis B is among many etiologies that can cause chronic hepatitis. The differential diagnosis includes hepatitis C, autoimmune hepatitis, metabolic disease, Wilson disease, alpha1-antitrypsin deficiency, and drug-induced hepatitis. Primary biliary cholangitis, primary sclerosing cholangitis, and chronic biliary diseases often manifest with portal accentuated inflammation and can also mimic CHB. However, they should present with positive anti-mitochondrial antibody or ERCP result, respectively.

Table 5.2 Complex scoring system

Knodell score[a]		Ishak score[b]	
I. Periportal ± bridging necrosis	Score	A. Periportal or periseptal interface hepatitis	Score
None	0	Absent	0
Mild interface hepatitis	1	Mild	1
Moderate interface hepatitis	3	Mild/moderate	2
Marked interface hepatitis	4	Moderate	3
Moderate interface hepatitis with bridging necrosis	5	Severe	4
Marked interface hepatitis with bridging necrosis	6		
Multilobular necrosis	10		
		B. Confluent necrosis	Score
		Absent	0
		Focal confluent necrosis Zone 3 necrosis in some areas	1 2
		Zone 3 necrosis in most areas	3
		Zone 3 necrosis and occasional portal-central bridging	4
		Zone 3 necrosis and multiple portal-central bridging	5
		Panacinar or multiacinar necrosis	6
II. Intralobular degeneration and focal necrosis	Score	C. Focal lytic necrosis apoptosis, and focal inflammation	Score
None	0	Absent	0
Mild	1	≤1 focus per 10X field	1
Moderate	3	2–4 foci per 10X field	2
Marked	4	5–10 foci per 10X field	3
		>10 foci per 10 X field	4
III. Portal inflammation	Score	D. Portal inflammation	Score
None	0	Absent	0
Mild	1	Mild	1
Moderate	3	Moderate	2
Marked	4	Moderate/marked	3
		Marked	4
IV. Fibrosis	Score	Fibrosis, architectural changes, and cirrhosis	Score
None	0	None	0
Portal fibrosis	1	Some portal fibrosis	1
Bridging fibrosis	3	Most portal with fibrosis	2
Cirrhosis	4	Portal fibrosis and occasional bridging fibrosis	3
		Portal fibrosis and marked bridging fibrosis	4
		Incomplete cirrhosis	5
		Cirrhosis	6

Comparison of grading and staging for the Knodell score (Knodell et al. 1981) and Ishak (modified Knodell) score (Ishak et al. 1995)

[a]The Knodell score is a composite score based on inflammation and fibrosis ranging from 0 to 22

[b]The Ishak score reports necroinflammation and fibrosis as separate scores

Table 5.3 The Laennec staging system and the Beijing Classification System for fibrosis quality

Laennec Staging System		Beijing Classification System: P-I-R score[a]
4A	Mild cirrhosis with cirrhotic nodules enclosed by thin fibrous septa	Regressive predominate
4B	Moderate cirrhosis with cirrhotic nodules enclosed by broad fibrous septa	Indeterminate
4C	Severe cirrhosis with very broad fibrous septa and more than ½ of the biopsy with micronodules	Progressive predominate[b]

Comparison of staging fibrosis quality for the Laennec Staging system and the Beijing Classification system (Kim et al. 2012; Kutami et al. 2000; Sun et al. 2017).

[a]The Beijing Classification System is not applicable to biopsies without bridging fibrosis (i.e. early stage fibrosis).

[b]The progressive predominate classification can appear as Laennec 4A, 4B, or 4C, whereas indeterminate classification correlates with 4B and regression predominate correlates with 4A

In addition, diseases like lymphoma or leukemia may sometimes mimic the inflammatory infiltrate seen in CHB. Pathologists should be vigilant in such cases and look for monomorphism and atypia in the inflammatory infiltrates.

3 Hepatitis B and Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy with high mortality (Petrick et al. 2020). CHB infection is a major cause of HCC globally, accounting for at least 50% of cases and it is the first virus to be causally linked to a human malignancy (Arbuthnot and Kew 2001; Xie and Hepatitis 2017; Parkin 2006; Beasley et al. 1981; Chang et al. 1997). Other etiologies linked to the development of HCC include hepatitis C, chronic liver disease, and cirrhosis (Ferrell et al. 2018) (Fattovich et al. 2004). The carcinogenesis of HBV-related HCC is well studied, yet it remains widely debated. No dominant HBV oncogene has yet been identified. The development of HCC in patients with CHB is thought to be a result of direct viral mechanisms and host factors (Chen et al. 1991; Kao et al. 2000, 2003; Arbuthnot and Kew 2001).

Although the majority of HCC develops in cirrhotic livers, there is a significant portion of HBV-related HCC that occurs in patients with CHB without cirrhosis. This observation supports the direct role of HBV in tumorigenesis. Studies found that chronic infection with HBV triggers specific signaling pathways such as the pro-apoptotic and inflammatory pathways which may contribute to tumor development (Neuveut et al. 2010). There is evidence that HBV-related HCC tends to be more moderate to poorly differentiated with genetic instability and higher rates of chromosomal alterations compared to HCC related to other risk factors (Marchio et al. 2000; Hoshida et al. 2009; Laurent-Puig et al. 2001). It has been found that the HBx protein expression has a strong link to chromosomal instability in HBV-associated tumorigenesis despite the yet to be determined mechanisms (Neuveut et al. 2010). Between

asymptomatic CHB to the development of HCC, the hepatitis B viral genome can accumulate mutations and integrate itself into the host chromosome. Several mutations in the HBV genome, such as T1762/A1764 in the basal core promoter and preS region mutations at C1653T in enhancer II and at T1753V, have been identified to be associated with increased risk of HCC development (Kao et al. 2003; Liu et al. 2009). Although HBV replication does not require viral DNA integration into the host's chromosome, HBV genome integrates into the host DNA in almost all cases. It was previously thought that the viral integrations are randomly inserted into the human chromosome, more recent studies have found that HBV integration tends to target gene families involved in cell survival, proliferation, and immortalization (Neuveut et al. 2010). This may be the first genetic hit in tumorigenesis. In addition to direct viral mechanism in tumorigenesis, CHB results in chronic necroinflammation which increases hepatocyte turnover rate. Normal hepatocytes divide infrequently, and the increased hepatocyte proliferation provides an opportunity for spontaneous replication errors and less time for mutation rectification before the next cell division. Over time, the accumulation of mutations can contribute to tumor formation.

HCC is often clinically silent until there is advanced disease. Patients may present with nonspecific symptoms such as vague abdominal pain, fatigue, and weight loss (Ferrell et al. 2018). Active surveillance is crucial in high risks patients like those with CHB to improve survival. As imaging modalities advance, active surveillance facilitates early detection of HCC. Although HCC can often be diagnosed by imaging alone, liver biopsies can be diagnostically helpful when lesions are suspicious but not definite for HCC on imaging or suspicious for non-HCC malignancy. Liver biopsy also provides information on histologic grading and molecular characteristics of the tumor.

Several hepatic lesions have been studied as potential precursors to HCC in the setting of cirrhosis. No definitive precursor lesions have been identified in non-cirrhotic CHB liver and of the precursor lesions identified in cirrhotic liver, none are specific to HBV-related HCC. The first precursor lesions described are large cell dysplasia, now known as large cell change (LCC) (Anthony et al. 1973). LCC can be best recognized at low magnification as clusters of enlarged cells with slight nuclear pleomorphism and occasional multinucleated hepatocytes (Torbenson et al. 2018). It is debatable whether these are truly related to hepatocarcinogenesis as they are heterogeneous lesions with most cases showing senescent and degenerative changes (Torbenson et al. 2018; Park 2011; Kojiro 2000). LCC is often seen in CHB which led to some suggesting that it may be a pre-cancerous lesion in HBV-related HCC (Niu et al. 2016). However, despite the frequent detection of LCC in CHB with advanced histologic stage and HCC, the large cells often express senescent features (Ikeda et al. 2009). Perhaps the senescent changes in the large cells develop as a safeguard against malignant transformation rather than being a precursor lesion (Ikeda et al. 2009). Small cell change (SCC), previously known as small cell dysplasia, is another suggested HCC precursor lesion. This lesion is also best seen at low magnification as discrete groups of hepatocytes with reduced cytoplasm, increased nuclear to cytoplasm ratio, and minimal cellular atypia (Torbenson et al. 2018; Kojiro 2000). SCC can be clonal, and it has been shown to have chromosomal

damage and DNA changes that support it being a precursor lesion to HCC (Park 2011; Marchio et al. 2001). Lastly, the most widely accepted precursor HCC lesion is dysplastic nodule. These nodular lesions are seen in cirrhotic liver with increased cellularity, architectural abnormality, and cytologic atypia (Torbenson et al. 2018). They can be subdivided into low- and high-grade dysplastic nodules where low-grade contains more cytologic atypia and high-grade contains more architectural abnormalities. Compared to HCC, dysplastic nodules have no loss of reticulin, minimal mitosis, and at least some portal structure. Overall, dysplastic nodules are associated with an increased risk of developing HCC, particularly the high-grade dysplastic nodules (Iavarone et al. 2013; Seki et al. 2000; Sato et al. 2015).

The macroscopic and microscopic findings of HBV-related HCC are not unique from HCC of other etiologies. Grossly, there is a wide range of presentation, varying from tan-white to yellow or green in color, depending on the amount of steatosis and bile content present. It can also be variegated as it often contains necrosis and hemorrhage. These lesions tend to be soft and bulges from the liver's cut surface in resection specimens due to the loss of reticulin and normal architecture. HCC can present as a discrete solitary nodule, a dominant nodule with multiple associated discrete nodules within 2 cm, multiple distant discrete nodules, or less commonly as numerous small nodules mimicking cirrhotic nodules (cirrhotomimetic) that may evade detection (Torbenson et al. 2018; Jakate et al. 2010). In some patients, HCC can also present as a pedunculated nodule from the liver surface. Although debatable, some studies have found that this presentation may have a better prognosis (Anthony et al. 1973; Yeh et al. 2002; Horie et al. 1999). It is important that the pathologist sample any nodule or lesion that appears different from the background cirrhotic nodules in liver resection specimens.

HCC can be a challenging histological diagnosis to make, especially in a biopsy specimen. The key to diagnosis is still heavily dependent on the histological findings despite available ancillary immunostains. The general histopathological appearance of HCC can be categorized as cytological atypia and architectural abnormalities, both of which contain plenty of room for variation. The architectural abnormalities include loss of normal portal tracts, presence of fibrous septae, thickening of hepatocellular plate, and pseudoacinar formation. There can also be abnormal arterialization of the hepatic lobules with small unpaired arterioles (Fig. 5.4a) (Schlageter et al. 2014). Cytologically, HCC may have nuclear hyperchromasia, irregular nuclear membranes, multinucleation, prominent nucleoli, size variation, and increased nuclear-to-cytoplasmic ratio. The cytoplasm may be more basophilic or eosinophilic than background nonneoplastic, containing increased lipofuscin deposition, or inclusions (Torbenson et al. 2018). In addition, four main growth patterns exist for HCC with the most common being trabecular growth patterns accounting for 70% of cases (Shah et al. 1995). Other patterns include solid, pseudoglandular or pseudoacinar, and the least common macrotrabecular pattern. Some studies have found that macrotrabecular pattern may carry the worst prognosis (Lauwers et al. 2002). Pathologists and clinicians should be aware that multiple growth patterns are often present within the same specimen and the distinction between patterns such as trabecular and solid are not always easy. Currently, most

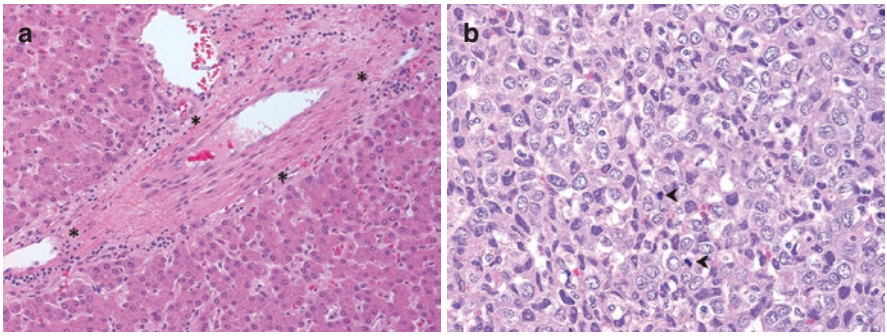

Fig. 5.4 (**a**) The H&E stained sections show a well-differentiated hepatocellular carcinoma with minimal nuclear atypia and hepatocellular plates no more than two layers think that resembles non-neoplastic liver parenchyma. However, there is abnormal arterialization with an unpaired arteriole (*) and loss of normal portal tracts that occurs in hepatocellular carcinoma, magnification 200X. (**b**) The H&E stained sections show a poorly differentiated hepatocellular carcinoma with loss of normal architecture, significant nuclear pleomorphism, enlarged nuclei, and multiple mitotic figures (➤), magnification 400X

HCCs lesions are chemoembolized or ablated prior to surgery, this can further complicate histological assessment as it can change the gross and histological findings. These specimens often contain necrosis, increased inflammation, and increased fibrosis (Torbenson et al. 2018). Pathologists should specify the amount of necrosis present in these cases to provide information to correlate with imaging-based downstaging (Yao et al. 2008).

Several immunohistochemical and special stains are available to help confirm hepatocytic differentiation and distinguish malignant from benign hepatocellular lesions, particularly in poorly- and well-differentiated HCCs, respectively. In both cases, it is important to interpret the immunostains in the appropriate microscopic and clinical contexts. No known molecular methods are currently available clinically to diagnose conventional or HBV-related HCC. Hepatocytic differentiation markers including HepPar1, Glypican 3, Arginase 1, alpha-fetoprotein, and albumin in situ hybridization are useful to support the diagnosis of HCC. None of these stains are perfect. In general, multiple stains should be used together as not all will be positive in every tumor. Glypican 3, which is positive in only HCC and not in benign hepatocytes, has been found to be more likely positive in HBV-related HCC and in poorly differentiated HCC (Yan et al. 2011). It is important to note that both HepPar1 and Arginase 1 are positive in both benign and malignant hepatocytes, therefore, positive staining alone does not prove malignancy. Well-differentiated HCC can be difficult to distinguish from background liver and benign liver lesions, particularly in regions with high prevalence of hepatitis B infection. A reticulin stain can be helpful in such cases to highlight the thickness of hepatocytic trabeculae and determine if there is focal reticulin loss (Nørredam 1979; Koelma et al. 1986; Bergman et al. 1997). A Ki-67 immunostain can also be helpful by highlighting the proliferation rate. A high proliferation index will favor malignancy since nonneoplastic liver tends to not be very mitotically active (Yeh et al. 2007). Lastly, a CD34

Table 5.4 Hepatocellular carcinoma histological grading (Torbenson et al. 2018)

Grade	Definition
Well-differentiated	Definite hepatocellular differentiation. Immunostains and/or special stains are needed to support malignancy.
Moderately differentiated	Definite malignant morphology and strongly suggestive of HCC. Immunostains and/or special stains may be helpful to confirm HCC.
Poorly differentiated	Clearly malignant. Needs immunostains to confirm hepatocellular origin.
Undifferentiated	Clearly malignant. No morphological features of hepatocellular differentiation. Lacks immunohistochemical markers for HCC. Immunostain needed to confirm epithelial origin.

immunostain which is normally positive in endothelial cells lining the sinusoids can become diffusely positive along the sinusoids in HCC (Ruck et al. 1995). However, this staining pattern is not always sensitive or specific to HCC. It is always important to consider the results of special stains and immunostains in conjunction with histological findings as a whole.

Once the diagnosis of HCC is established, the next step is to determine the grade of the tumor as it is important for predicting prognosis (Benckert et al. 2005; Hyder et al. 2014; Zhou et al. 2007; Lang et al. 2007). Histological grade has also been shown to correlate with tumor size and metastatic rate (Lauwers et al. 2002). Many grading systems exist such as the Edmondson-Steiner system and the WHO 2010 scheme, however, clinically it is most practically based on a 4-tier system of well-differentiated, moderately differentiated, poorly differentiated (Fig. 5.4b), and undifferentiated HCC (Table 5.4).

The differential diagnosis of HCC in HBV depends on whether the presence or absence of cirrhosis in the background liver and the grade of HCC. In well-differentiated HCC, consider focal nodular hyperplasia and hepatocellular adenoma in non-cirrhotic liver and macroregenerative nodule and dysplastic nodule in cirrhotic liver. In more poorly differentiated HCC, cholangiocarcinoma, and metastatic neoplasm should be considered. Cholangiocarcinoma should especially be considered in cirrhotic liver. It has been found that both hepatitis B infection and cirrhosis are associated with cholangiocarcinoma (Shaib and El-Serag 2004). Lastly, combined hepatocellular cholangiocarcinoma, should also be considered as it can occur in CHB. This entity can pose diagnostic challenges with its unique biphenotyic tumor morphology. Further investigation is still needed to determine its unclear biology (Brunt et al. 2018; Komuta and Yeh 2020).

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Molecular Carcinogenesis of Hepatitis B Virus-Related Hepatocellular Carcinoma

6

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Abstract

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer. Hepatitis B virus (HBV) is a major risk factor for the development of HCC. HBV oncogenic mechanisms are both indirect and direct. Indirectly, repetitive liver injury is induced by consistent inflammation and oxidative stress due to chronic infection of HBV. Over time this causes genomic instability, telomere shortening, and malignant transformation. The HBx protein encoded by HBV can also directly contribute to oncogenesis by deregulating the cell cycle, causing mitochondrial dysfunction and activating proliferation pathways. In addition, HBV genome integration also contributes to malignant transformation through both direct and indirect mechanisms. Random insertions lead to genomic instability. Insertional mutagenesis can also cause the constitutive activation of oncogenes such as *TERT* at the integration site. Particular characteristics of the HBV genome, including genotypes and mutations, have been reported to increase the risk of developing HCC. Comprehensive genomic profiling of hundreds of HBV-related HCC has determined recurrent alterations and integration sites. Molecular classification schema characterizes a majority of HBV-related HCC tumors as Proliferative and associated with a poorer prognosis.

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Prevention, including vaccination programs, remains the best method to decrease the incidence of HBV-related HCC. Promising immunotherapies specifically targeting HBV epitopes are being developed. In this chapter, we will review the current knowledge regarding the impact of HBV on development of HCC.

Keywords

Hepatocellular carcinoma · Viral integration · Malignant transformation · HBx protein · *TERT* · Oxidative stress

1 Introduction

Primary liver cancer (PLC) is the sixth leading malignancy diagnosis and the fourth leading cause of cancer-related death worldwide. In contrast to most other cancer types, which have declining incidence and death rates, PLC is actually increasing, especially in the United States at annual rates around 2–3% (Jemal et al. 2017). The most common form of PLC is hepatocellular carcinoma (HCC), accounting for ~90% of cases. Cholangiocarcinoma (CCA), or bile duct cancer, accounts for a majority of the other cases of PLC. There are consistent subsets of the population that are more often diagnoses with PLC. Geographically, over 70% of cases occur in Asia. The male-to-female ratio is about 3:1. This may be in part due to differences in risk factors (Global Burden of Disease Liver Cancer Collaboration 2017). Several risk factors exist for the development of HCC including alcohol abuse, non-alcoholic fatty liver disease (NAFLD), chemical carcinogens such as aflatoxin B1 and chronic viral hepatitis B and C (Villanueva 2019). All of these can culminate in cirrhosis, end-stage liver disease, as evident by accumulation of scar tissue over years of injury (Friedman 2008). Overwhelmingly, over 90% of HCC develops in the background of cirrhosis.

Hepatitis B is responsible for a huge burden of HCC development. Approximately 257 million people worldwide have been diagnosed with hepatitis B virus (HBV) (Custer et al. 2004). More than 50% of HCC cases can be attributed to chronic HBV infection (CHB) (Parkin 2006). A high viral load, hepatitis B e antigen (HBeAg) positivity, and certain HBV genotypes all increase the risk of developing HCC. Currently, there is no treatment specifically designed for HBV-associated HCC, although molecular classification has identified certain characteristics associated with these tumors, which may lead to more targeted therapies (Ye et al. 2003; Roessler et al. 2010; Lee et al. 2004). Regardless of etiology, early-stage HCC can be curatively treated with surgical resection (Villanueva 2019). Non-curative treatments for intermediate and advanced HCC include chemoembolization and systemic therapy such as the multi-kinase inhibitors sorafenib or lenvatinib (Llovet et al. 2008, 2018; Kudo et al. 2018). Most recently immunotherapeutic approaches including the checkpoint inhibitors nivolumab, pembrolizumab, and combination therapy atezolizumab/bevacizumab have shown promise for the next generation of

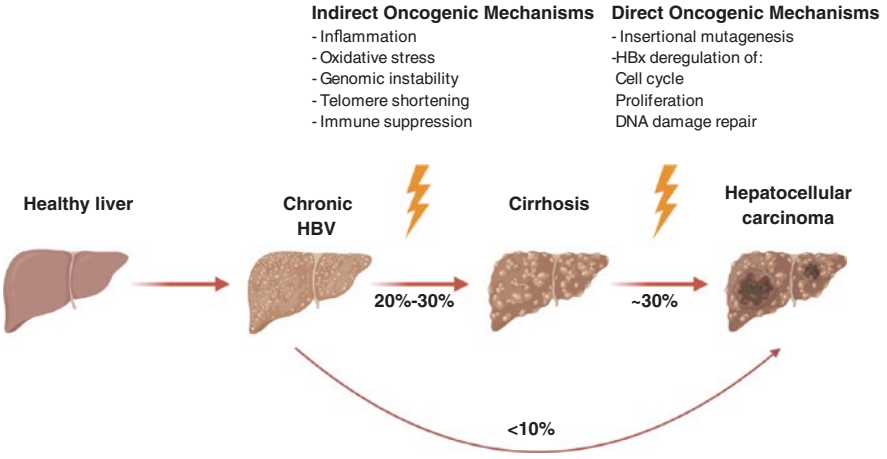

Fig. 6.1 Stages of HCC development induced by HBV infection. 20–30% of patients with chronic hepatitis B (CHB) will go on to develop end-stage liver disease known as cirrhosis. About 30% of patients with cirrhosis will further go on to develop HCC. 10% of patients with CHB will develop HCC without first developing cirrhosis. CHB promotes development of HCC through both indirect and direct methods. Indirect methods include inflammation and oxidative stress, whereas direct methods include insertional mutagenesis and deregulation of oncogenic pathways by the HBx protein

HCC treatment (El-Khoueiry et al. 2017; Zhu et al. 2018; Finn et al. 2020). HBV vaccination programs as a preventative approach have also been shown to dramatically lower HBV-related HCC diagnoses (Chang et al. 2009).

There are several oncogenic mechanisms by which HBV leads to HCC. The HBV genome encodes 4 proteins: viral surface envelope proteins (HBsAg), viral nucleocapsid (HBcAg), HBeAg, and the HBV X protein (HBx) (Liang 2009). HBx, necessary for the viral replication cycle, has been shown to be directly oncogenic by altering cancer signaling, cell cycle, and mitochondrial function. Additionally, CHB infection also initiates hepatocarcinogenesis by increasing genomic instability after integration into the host genome. The chronic inflammation and hypoxic environment created by HBV infection lead to DNA damage and transformation. In this chapter, we will review the direct and indirect molecular mechanisms of HBV-associated HCC (Fig. 6.1).

2 HBV-Derived Liver Injury and Inflammation

The majority of HBV-associated HCCs (upwards of 80%) arise in the background of cirrhosis, scarring of the liver which can eventually lead to liver failure (Yang et al. 2011). HBV is a common source of chronic liver disease that leads to cirrhosis. 20–30% of patients with CHB will go on to develop cirrhosis (Poh et al. 2015; World Health Organisation 2017). There is a cyclical pattern of hepatic tissue damage, inflammation, repair, and regeneration driven by HBV infection that leads to

the development of cirrhosis. Throughout this process, hepatocytes acquire molecular alterations that lead to their transformation into cancer cells (D'souza et al. 2020).

HBV has an excess of mechanisms by which it causes DNA damage and cell death. First, DNA damage can be caused by the generation of reactive oxygen species (ROS). ROS are highly reactive intermediates of O_2 reduction that are capable of modifying biomolecules, including DNA (Ivanov et al. 2017; Hussain et al. 2003). HBx causes the loss of mitochondrial membrane potential needed for oxidative phosphorylation by binding to the outer membrane of the mitochondria (Rahmani et al. 2000). Disruption of the respiratory complex leads to the generation of ROS and oxidative damage (Zou et al. 2015). ROS formation can also be triggered by HBsAg (Lee et al. 2015). HBsAg is secreted from the cell and thus is processed through the ER to the cell membrane. However, several variants of HBsAg have a propensity to accumulate in the ER, leading to activation of the Unfolded Protein Response (UPR). UPR signaling triggered by ER stress results in ROS production and apoptosis. DNA damage caused by HBV infection has been empirically measured. Experimental mouse models of chronic HBV infection have an accumulation of 8-oxo-2'deoxyguanosine, a DNA adduct caused by oxidative damage, within their livers (Hagen et al. 1994). A longitudinal study also observed extensive oxidative damage in patients with diagnosed HBV-associated HCC over a 20-year follow-up of disease progression (Fujita et al. 2008). Second, HBx inhibits DNA repair pathways, allowing for the accumulation of mutations and genomic instability (Qadri and Fatima 2011). HBx interferes with nucleotide excision repair (NER) by binding to XAP-1, which initiates NER through binding damaged DNA (Becker et al. 1998). HBx also directly inhibits p53, a tumor suppressor key in orchestrating the DNA damage response (Wang et al. 1994).

Cellular stress and cell death cause the release of signaling molecules called disease-associated molecular patterns (DAMPs) that recruit immune cells and promote inflammation (Villanueva and Luedde 2016). DAMPs include HMGB1, S100, ATP, and fragmented DNA. DAMPs activate Kupffer cells (liver-resident macrophages), which release cytokines such as TNFα, IL1-ß, and IL-6, attracting circulating immune cells to the site of liver damage induced by HBV and triggering compensatory proliferation of hepatocytes through activation of JAK-STAT signaling. Hepatocytes themselves can also release pro-inflammatory cytokines through ROS-mediated NF-κB signaling (D'souza et al. 2020). Recruited Natural Killer cells (NK) and HBV antigen-specific CD8+ T-cells kill infected or damaged hepatocytes. Unfortunately, this immune response is not always strong enough to eliminate every infected cell, allowing for chronic infections to perpetuate (Kubes and Mehal 2012). In fact, an immunosuppressive environment develops as CD8+ T-cells become dysfunctional. Regulatory T-cells (T-regs) accumulate in correlation with HBV viral load, abrogating the cytotoxic function of CD8+ T-cells (Miroux et al. 2010). This consistent necro-inflammation acts as an extrinsic source of oxidative stress exposure causing further cellular damage of hepatocytes (D'souza et al. 2020).

Typically for an acute hepatic injury, the liver is repaired or regenerated and inflammation necessary for the healing process is resolved. This is not the case during consistent inflammation caused by CHB. With chronic infection, not all

damaged hepatocytes will be eliminated by an active immune response, leading to the continuous cycle of injury, repair, and regeneration, sometimes over decades. DAMPs released by damaged or dying hepatocytes that trigger an active immune response also activate stellate cells, which deposit collagen, creating the scarring associated with cirrhosis and end-stage liver disease (Friedman 2008; An et al. 2020). At the same time, hepatocytes are under oxidative stress from both the hypoxic microenvironment within the cirrhotic liver and intrinsic ROS created by the above-mentioned HBV-induced mechanisms. Oxidative stress leads to the accumulation of molecular alterations within hepatocytes, which leads to clonal expansions. Further accumulation of driver alterations will initiate hepatocarcinogenesis (Villanueva and Luedde 2016). One of the earliest initiating events for transformation is telomere stabilization. Telomeres, repetitive stretches of DNA at the end of chromosomes, shorten with every cell replication. At a terminal short length, cells will typically senescence. Studies show that telomere length gradually shortens through increasing degrees of liver disease due to the high proliferation and turnover of infected hepatocytes (Miura et al. 1997). Transformed cells immortalize by overcoming this problem through the activation of telomerase (*TERT*), the enzyme that lengthens chromosomal ends by adding nucleotide base pairs. *TERT* activation is a major initiating event, occurring in >90% of HCC (Totoki et al. 2014). A majority of HBV-related HCCs activate telomerase by acquiring mutations in its promoter (~60%), leading to constitutive activation (Nault et al. 2013). Others acquire HBV integration within the telomerase promoter, discussed in the next section.

3 Genome Integration of HBV DNA

HBV DNA can integrate into the genomes of hepatocytes. While not necessary for viral replication, it nevertheless is an early event in the viral life cycle and is detected in the majority of HBV-related HCC cases (~80%) (Minami et al. 2005). HBV integration is an early event in hepatocarcinogenesis, as it is observed in premalignant lesions and can even be found in the acute stages of infection (Nault et al. 2014; Yang and Summers 1999). Additionally, multiregional and single-cell sequencing of HBV-related HCCs shows consistent HBV integration sites across all regions or cells, also supporting these events as clonal early events (Zhai et al. 2017; Duan et al. 2018). HBV DNA integration contributes to the initiation of hepatocarcinogenesis through two main mechanisms: genomic instability and direct insertional mutagenesis. HBV integration is associated with genomic instability, often co-occurring with copy number alterations (CNAs) and translocations. Indeed, CNAs have been observed directly at HBV-induced breakpoint locations (Sung et al. 2012). Due to the breakpoints created by integration, HBV-associated HCCs are often more genomically unstable compared to HCCs of other etiologies (Lee et al. 2008). While integration occurs randomly throughout the genome, several recurrent locations have been found in HCCs, suggesting that these sites provide a survival advantage. For example, HBV integration into coding and promoter regions occurs at a higher frequency in HCCs than non-tumoral

tissue and integration sites are enriched on chromosome 17 (Yan et al. 2015; Zhao et al. 2016).

Integration at certain genomic locations creates a growth advantage for the affected cell compared to neighboring cells, leading to clonal expansions and subsequent malignant transformation. The most prominent documented location is the *TERT* promoter, which occurs in 10–15% of cases (Sung et al. 2012). This causes the constitutive activation of *TERT*, which lengthens telomeres causing cells to be able to avoid senescence. Other recurrent integration sites include coding regions or promoters of the genes *RARβ, CCNA1, CCNE1, MLL4, SENP5, ROCK1, FN1, ARHGEF12, CYP2C8, PHACTR4, PLXNA4, RBFOX1,* and *SMAD5* (Levrero and Zucman-Rossi 2016). Oncogenic mechanisms have been established for several of these sites. *MLL4* is a histone methyltransferase and a critical epigenetic regulator tumor suppressor. HBV integrations lead to loss of function of *MLL4* (Cleary et al. 2013). *CCNA1* (cyclin A) and *CCNE1* (cyclin E) are both cyclins that have a regulatory role in the cell cycle. Cyclin A is expressed through S and G_2 phases of the cell cycle, while cyclin E is expressed through G_1 to S phase. HBV integration of *CCNA1* and *CCNE1* leads to the transactivation of these cyclins (Bisteau et al. 2014). Proliferating cells undergo well-regulated sequences of events controlled by cyclin-dependent kinases (CDKs). Transactivation of *CCNA1* and *CCNE1* disrupts this sequential process, leading to bypasses of cell cycle checkpoints. This further causes DNA damage and genomic instability to accumulate. Chimeric proteins produced by the fusion of HBV genes with host genes can also be oncogenic. This is the method by which *CCNA1* becomes transactivated. Another well-described example is the HBx-LINE fusion (Lau et al. 2014). Long interspersed nuclear elements (LINEs) are repetitive elements of which thousands of copies exist in the genome and are especially amenable to integration by HBV. The HBx-LINE chimeric transcript has been observed in 20–40% of HBV-related HCC cases (Lau et al. 2014; Liang et al. 2016). The HBx-LINE transcript acts as an oncogenic long non-coding RNAs (lncRNA), which sequesters micro-RNAs (miRNAs) including miR-122. miR-122 is important for the regulation of liver homeostasis. Overexpression of HBx-LINE experimentally drives migration and invasiveness by activating the epithelial-mesenchymal transition (EMT) (Liang et al. 2016).

4 Oncogenesis of the HBx Protein

The HBx protein is critical for viral replication and modifies several cellular functions necessary to maintain homeostasis. Because of this, HBx is a key player in the transformation of hepatocytes. In previous sections, we discussed how HBx contributes to DNA damage and genomic instability. Here we will review how HBx influences proliferation, cell cycle, epigenetic regulation, non-coding RNAs, and "stem-like" characteristics (Fig. 6.2).

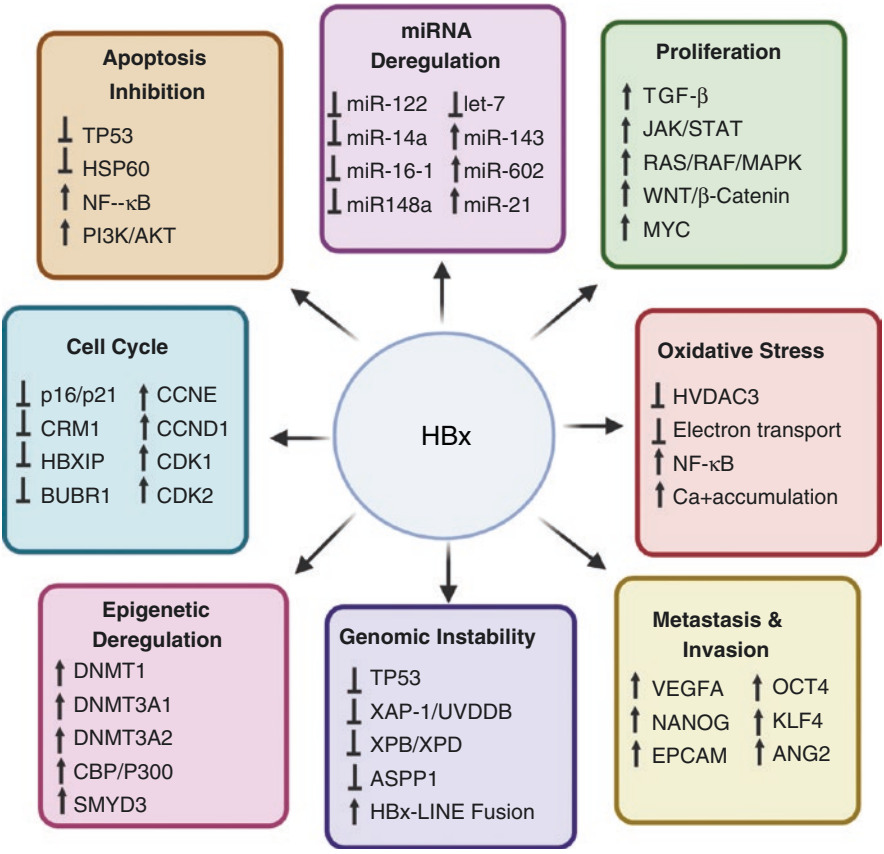

Fig. 6.2 Oncogenic mechanisms of the HBx protein. HBx protein has a wide range of both direct and indirect interactions that deregulate cellular functions, leading to malignant transformation. These interactions mostly fall into the categories of apoptosis inhibition, cell cycle, epigenetic deregulation, genomic instability, metastasis and invasion, oxidative stress, proliferation, and miRNA deregulation

4.1 Cancer-Related Signaling Pathways

Years of research have shown HBx drives proliferation through the activation of cancer-related signaling pathways. These pathways include TGF- β, JAK-STAT, RAS/RAF/MAPK, NF-κB, and WNT/β-catenin (Murata et al. 2009; Lee and Yun 1998; Benn and Schneider 1994; Liu et al. 2010; Hsieh et al. 2011). HBx can induce TGF-β to switch from a tumor suppressive function to pro-oncogenic (Murata et al. 2009). By activating upstream Src family nonreceptor tyrosine kinases, HBx stimulates RAS/RAF/MAPK, and JAK-STAT signaling. HBx also leads to the accumulation of β-catenin, which then localizes to the nucleus and upregulates transcription of target genes including *VEGFA*, *MYC*, and *CCND1,* driving tumorigenesis. While β-catenin is typically degraded by the destruction complex, HBx interferes with the

complex by competitively binding to one of the main components, APC (Hsieh et al. 2011).

4.2 Cell Cycle and Division

HBx deregulates the cell cycle and causes centrosome amplification. The centrosome is an organelle important during cell division. It is the main microtubule organizing center. During the cell cycle, the centrosome duplicates and mitotic spindle forms between them. The centrosomes then move to opposite sides of the cell, separating duplicated chromosomes into two daughter cells. Centrosome duplication is a well-orchestrated process that corresponds to the G_1 to S phases of the cell cycle. The CRM1 nuclear export protein complexes with Ran-GTPase in order to assemble mitotic spindle and maintain centrosome assembly. HBx disrupts the function of this complex by binding and sequestering CRM1 (Budhu and Wang 2005). This leads to centrosome amplification and mitotic defects. HBx also directly interacts with HBXIP, an important protein for bipolar spindle formation and centrosome duplication (Wen et al. 2008). Interference with HBXIP leads to defective spindle formation and abnormal chromosome segregation. Asides from interfering with centrosome duplication, HBx also inactivates BubR1, a part of the mitotic checkpoint complex, that specifies where correct microtubule attachments are located (Kim et al. 2008). This also leads to abnormal chromosome segregation. Abnormal chromosome segregation and centrosome amplification lead to the accumulation of genomic instability and the development of HCC.

Besides deregulation of centrosome duplication, HBx also impacts the function of CDK proteins important for progression through the various stages of the cell cycle and traversing cell cycle checkpoints. HBx has been shown to shorten the amount of time a cell stays in the quiescent G_0 stage of the cell cycle and further drives progression through entry into S phase and checkpoint controls to G_2/M stages faster than uninfected cells. Exit from G_0 is caused by HBx decreasing levels of *p15/p16* and increasing levels of G_1 phase proteins including cyclin D and cyclin E. Further progression through cell cycle checkpoints is dependent on the activity of CDKs. HBx is capable of activating *CDK1* and *CDK2* (Benn and Schneider 1995). On the other hand, studies have shown that HBx is also capable of inhibiting *CDK2* causing cells to stall in G_1 phase (Gearhart and Bouchard 2010). These results suggest that the G_1 phase is the most efficient phase for viral replication. Even so, deregulation of the cell cycle caused by HBx may lead cells to be substantially more susceptible to malignant transformation.

4.3 Non-coding RNAs

HBx influences the function of multiple non-coding RNAs crucial for transcription regulation. Classes of non-coding RNAs particularly affected by HBx include miRNAs and lncRNAs. As alluded to in their names, non-coding RNAs are classified by

their length, whereas non-coding RNAs below 200 nucleotides are considered miRNAs and those longer than 200 nucleotides are considered lncRNAs (Ghidini and Braconi 2015). The function of many miRNAs is posttranscriptional gene expression regulation. miRNAs bind to mRNAs with complementary sequences, resulting in those transcripts being either silenced or degraded. The HBx transcript binds and downregulates several tumor suppressive functioning miRNAs, including miR-15a and miR-16-1 (Wang et al. 2013). HBx also indirectly deregulates miRNAs including miR-143, miR-29a, miR-602, let-7 family members, miR-148a, miR-21, miR-122, miR-132, and miR-152, leading to pro-oncogenic signaling (Zhang et al. 2017). As previously mentioned, HBx-LINE fusions suppress miR-122 expression, an anti-tumorigenic miRNA found specifically in the liver (Lau et al. 2014). The other major class of non-coding RNAs affected by HBx are lncRNAs, which regulate gene expression through a variety of mechanisms. LncRNAs can provide scaffolds for proper transcription factor binding, sequester miRNAs, and regulate splicing (Marchese et al. 2017). HBx can upregulate lncRNAs *HULK*, *UCA1*, and *DBH-AS1* (Zhang et al. 2017). *UCA1* and *HULC* inhibit the tumor suppressors p18, whereas *DBH-AS1* increases proliferative MAPK signaling.

4.4 Epigenetic Regulation

Epigenetics includes processes that affect gene activity without altering the DNA sequence. These processes include DNA methylation, histone modifications, and chromatin remodeling. DNA methylation regulates gene expression. Gene expression is repressed when CpG islands located in promoter regions are methylated. In HCC, oncogenes can be overexpressed by demethylating their promoters and tumor suppressors can be silenced by increased methylation of their promoters. Additionally, global hypomethylation is common in cancer, possibly as a consequence of cell cycle deregulation and chromatin restructuring. The DNA methyltransferase (DNMT) family catalyzes the transfer of methyl groups to cytosine bases of DNA. HBx upregulates *DNMT1, DNMT3A1*, and *DNMT3A2*, which hypermethylate tumor suppressors such as *P16/INK4A, RAR*-β, and *CDH1* (Zheng et al. 2009). Repression of *P16/INK4A* causes cell cycle deregulation. HBx also alters histone acetylation, which alters expression of cancer-related genes (Cougot et al. 2007).

4.5 Stemness

HCC is a notoriously heterogeneous tumor type. This heterogeneity can be driven by cancer stem cells (CSCs) (Zheng et al. 2018). CSCs possess hepatic progenitor-like features and self-renewal capabilities (Yamashita and Wang 2013). HCCs that express stem markers or contain CSCs have a poor prognosis and more aggressive disease (Yamashita et al. 2009; Ma et al. 2007). HBx induces stem-like characteristics in HCC. HBx induces *EPCAM* expression, an established CSC marker. This

subsequently is associated with increased cell migration (Arzumanyan et al. 2011). Further evidence that HBx drives *EPCAM* expression is when analyzing CSCs isolated from human resected HCCs. *EPCAM*+ CSCs were most likely to be found in HBV-associated HCCs than any other etiology (Yamashita et al. 2009). Additional stem cell markers that HBx causes an increase in expression include *NANOG*, *OCT4*, *MYC*, and *KLF4* (Levrero and Zucman-Rossi 2016). HBx further can downregulate E-cadherin, causing regression to a more pluripotent state and activation of EMT in tumor cells (Arzumanyan et al. 2012). Tumor cells that have undergone EMT become more invasive and metastasize more readily.

5 HBV Genotypes and Mutations

The HBV viral polymerase has a relatively high error rate, due to the lack of proofreading abilities. This coupled with the high rate of replication for HBV leads to accumulation of mutations or variants (Levrero and Zucman-Rossi 2016). Over time, this has created as many as 10 documented genotypes of HBV, with several including multiple subtypes (Lin and Kao 2015). Genotypes are regionally separated and correlate with clinical outcomes, including development and prognosis of HCC. Genotypes A and D are mainly found in Sub-Saharan Africa and India, whereas genotypes B and C are mainly found in East Asia. Overall, patients infected with genotype C HBV develop worse liver disease (Lin and Kao 2015). A higher risk of developing HCC is associated with infection with genotype C (Chan et al. 2004). The serum viral load and liver inflammation are typically higher with genotype C, which may play a role in the association with HCC development.

Aside from genotypes, other specific mutations in the HBV genome are associated with HCC development and severity. The HBx protein is often found in a truncated form in HCC tissues (Ma et al. 2008). This is caused by a 3′ end deletion of the HBx gene in the HBV genome. Truncated HBx is associated with metastasis and invasive HCCs (Sze et al. 2013). Other alterations of HBx that are frequently found in HCC include the point mutations K130M and V131I (An et al. 2018). Due to the overlapping nature of the HBV genome, these two alterations correspond to A1762T/G1764A in the basal core promoter. The A1762T/G1764A variants are an independent predictor for the development of cirrhosis and are significantly associated with the development and progression of HCC (Kao et al. 2003). Finally, several alterations have been identified in the PreS/S and PreC/C regions of the HBV genome that are frequently found in HCC. Deletions in the pre-S gene create misfolded proteins that are more likely to aggregate in the ER, producing oxidative stress and DNA damage (Lee et al. 2015).

6 HBV-Related Molecular Subtypes of HCC

Several comprehensive large-scale genomic profiling studies have characterized the main driver genes and signaling pathways most important in HCC. A major realization from these studies is the incredible amount of diversity between tumors of

different patients. Because HBV is a key background liver disease etiology of HCC, most of these studies include a significant number of patients who have HBV-related HCC. In fact, some studies have focused specifically on HBV-related HCC. One study in particular by Zhao et al. profiled 426 patients using high-throughput viral integration and RNA sequencing (Zhao et al. 2016). This study confirmed known HBV DNA integration sties and discovered novel sites near *TP53* and *MLL4*. The authors also described an enrichment of HBV DNA integration in CpG islands and chromosome 17.

After molecular profiling of hundreds of HCC tumors across dozens of studies, consensus driver genes and molecular alterations have been agreed upon. As previously mentioned, telomere maintenance is an early initiating event in a majority of HCCs. Telomerase expression is upregulated in 90% of HCCS by *TERT* promoter mutations, HBV insertion into the *TERT* promoter or *TERT* amplification (Nault et al. 2013). The p53 cell cycle pathway is the next most frequently altered in HCC. *TP53* mutations are found in 12%–48% of HCCs. *TP53* is the most frequently altered gene in HBV-related HCC (Amaddeo et al. 2015). Crucial for regulation from G_1 to S phase, *RB1* is mutated 3%–18% of the time and *CDKN2A* is deleted in 2–12% of HCCs. As discussed, HBV also deregulates this pathway through insertions of *CCNE1* and HBx mediated upregulation of *CCND1, CDK1*, and *CDK2* (Benn and Schneider 1995). The WNT/β-catenin proliferation pathway is activated in a subset of HCCs. Activating mutations of *CTNNB1* are found in 11%–37% of HCCs (de La Coste et al. 1998). *AXIN1* and *APC,* both part of the β-catenin destruction complex, are frequently mutated in HCC. This leads to the accumulation of β-catenin, which traverses to the nucleus and activates transcription of pro-oncogenic targets. The epigenetic modifiers *ARID1A* and *ARID2* have inactivating mutations in 3%–18% of HCCs (Guichard et al. 2012). The histone methylation writers *MLL, MLL2, MLL3*, and *MLL4* are frequently altered by mutations. HBV insertions are specifically found in *MLL4 (*Cleary et al. 2013*). NFE2L2* mutations are found in 3%–6% of HCCs, interfering with the activation of detoxification transcriptional programs when oxidative stress is present. Finally, the AKT/MTOR oncogenic pathway is activated in HCC by mutations in *PIK3CA, TSC1*, and *TSC2* (Totoki et al. 2014).

Albeit HCC being a heterogenous disease, many studies have been undertaken to classify patients into groups whereas the molecular characteristics of the tumors are similar. This could serve as a way to select patients for particular targeted therapies that they would most benefit from. Several groups have established molecular classifications based on transcriptomic or methylation data that are able to identify patients with a poorer prognosis. Overall, the findings from various studies have provided consistent results upon which a refined molecular classification could be built. Further meta-analysis established two molecular classifications: Proliferation and Non-Proliferation classes (Zucman-Rossi et al. 2015). The Proliferation class comprises approximately half of the patients with HCC. These patients have a poorer prognosis and worse survival than those in the Non-Proliferation class. As acknowledged in the name, these tumors are more proliferative and aggressive. They comprise a number of activated signaling pathways including RAS/MAPK and AKT/MTOR (Zucman-Rossi et al. 2015). The

Proliferation class can further be broken down into tumors with progenitor-like or hepatocyte-like features. Progenitor-like tumors also activate NOTCH1 and IGF2 signaling, whereas hepatocyte-like tumors activate TGF-β and non-canonical WNT signaling. Clinically, HCCs of the Proliferation class have higher AFP levels, are poorly differentiated and are more likely to have vascular invasion. On the other hand, the Non-Proliferation class is mostly enriched for canonical WNT signaling, accounting for 25% of cases (Lachenmayer et al. 2012). These tumors are mostly hepatocyte-like regarding cell lineage features and are enriched for immune infiltrate or inflammation related gene signatures (Sia et al. 2017). These patients have a better prognosis and overall survival. Clinically, they have lower levels of AFP, either well or moderately differentiated tumors and are less likely to have vascular invasion. HBV-related HCCs are more likely to be a part of the Proliferation class, associated with a poorer prognosis (Levrero and Zucman-Rossi 2016). HCV and alcohol-related HCCs are mainly classified in the Non-Proliferation class.

7 HBV-Related Cholangiocarcinoma

While HCC is the most common form of PLC, CCA makes up approximately 10–15% of PLC cases worldwide. CCA is most prevalent in Asia and the Middle East. Developing from bile duct cells, CCA can be found within the liver (intrahepatic, iCCA) or in bile ducts outside of the liver (extrahepatic, eCCA). While approximately half of CCAs are idiopathic, several risk factors exist for the development of CCA, most prominently liver fluke infections endemic to Southeast Asia. Several epidemiological studies have established a strong relationship between CCA and HBV infection (Ralphs and Khan 2013). Specifically, HBV has been identified as a risk factor for iCCA, but not eCCA. There is a positive association between HBsAg and iCCA; however, no association was detected for eCCA (Ralphs and Khan 2013). HBV DNA has been detected in up to 70% of Chinese CCA patients (Li et al. 2020). HBV-associated CCA is characterized by different pathological features than non-HBV-associated CCA. HBV DNA integration was identified in HBV-associated iCCA. Interestingly, recurrent integration sites, including the *TERT* promoter, determined in HCC were also discovered in iCCA (Li et al. 2020). This suggests that CHB infection induction of genomic instability and unresolved inflammation may play a similar oncogenic role in the development of iCCA as it does in HCC. Regarding prognosis of HBV-related iCCA, one study found negative HBsAg was associated with early recurrence and high HBV DNA levels were associated with late recurrence (Wang et al. 2019). Similar to HCC, a recent study from the TIGER-LC consortium revealed that iCCA may contain common molecular subtypes shared among Asian populations associated with various different etiologies including HBV (Chaisaingmongkol et al. 2017). These studies suggest that iCCA-related etiologies are complex, and a combination of different etiologies may be necessary to drive cholangiocarcinogenesis.

8 Prevention and Treatment

HCC is notoriously resistant to treatment, despite recent approvals of several new drugs. Systemic therapy is given to patients with advanced HCC typically only extend life by a matter of months. Early HCC can be treated with curative resection, though most HCCs are not caught early enough to receive this treatment (Heimbach et al. 2017). Research is underway to develop more sensitive tests for detection of early HCC than the gold standard of MRI/ultrasound scans, as biopsies are not necessary for the diagnosis of HCC. Pre-clinical studies have described liquid biopsies as a viable test in the future after further refinement, Liquid biopsies detect tumor by-products such as DNA or cells into the circulation. Liquid biopsy may be the new noninvasive means of HCC detection (von Felden et al. 2018). Second, surveillance is key for the detection of early HCC. Since there are clear risk factors for the development of HCC, most importantly cirrhosis, there is a clear population of patients who benefit from surveillance programs. Currently, guidelines recommend surveillance by ultrasound scans and AFP every 6 months (Kanwal and Singal 2019). Increasing adherence in surveillance programs is a crucial area of ongoing research. The current treatment strategy for CHB is nucleos(t)ide analogues (NUC). NUC therapy restricts viral replication by interfering with the HBV polymerase. HCC incidence is reduced in patients undergoing NUC therapy (Udompap and Kim 2019). A recent study demonstrates a proof of principle to use a viral exposure history to define early onset of HCC that includes HBV-related tumors (Liu et al. 2020). This approach may help define HCC prior to its clinical presentation and may be useful for HCC surveillance. Still, the best method we have for decreasing the rates of HBV-related HCC is prevention. High-performing HBV vaccines have been available for 40 years. Several studies have shown widespread vaccine campaigns strongly reduce the rates of HCC development, especially in Asia where HBV infections are endemic (Chang et al. 2009).

The new frontier of treatment for HCC is immunotherapy. Checkpoint inhibitors have been successful in a subset of HCC patients. Nivolumab and pembrolizumab, PD-1 inhibitors, were given accelerated FDA approval after phase 2 studies (El-Khoueiry et al. 2017; Zhu et al. 2018). Albeit not meeting endpoints in the phase 3 trial, approximately 20% of patients had durable responses. Most recently, the FDA has approved 2 immunotherapy combination treatments. Atezolizumab/bevacizumab is a combination of a PD-L1 inhibitor and a multi-tyrosine kinase inhibitor (Finn et al. 2020). The second combination therapy is nivolumab/ipilimumab, PD-1, and CTLA4 inhibitors. Being virally induced, several unique immunotherapeutic approaches may be feasible for treatment of HBV-related HCC. For example, clinical trials for adoptive T-cell therapy specifically targeting HBV epitopes are ongoing (Tan and Schreiber 2020). To engineer T-cells for immunotherapy, they must first be isolated and activated in vitro. Activated T-cells are next genetically modified to express an HBV-specific receptor. This receptor could be a mostly unmodified human T-cell receptor (TCR) that recognizes an HBV epitope, or it could be an engineered chimeric antigen receptor (CAR). CAR T-cells contain an antigen recognition domain that can bind epitopes without up-front processing through the

MHC pathway (Tan and Schreiber 2020). Because of this, CAR T-cells are only able to recognize cell surface proteins. Once the T-cells have been genetically engineered, they are expanded and reintroduced back into the patient. Upon recognition of HBV antigens presented by HBV-related HCC cells, engineered T-cells release cytolytic molecules that kill the identified cells. CAR T-cells have been designed to target HBsAg and TCRs have been engineered to recognize both HBsAg and HBcAg (Tan and Schreiber 2020). One drawback of this technique is that engineered T-cells cannot distinguish between infected hepatocytes and infected HCC cells, which could induce fulminant hepatitis. Techniques are being developed to allow only transient expression of engineered receptors to avoid this.

9 Concluding Remarks

Worldwide, HBV remains one of the major etiologies of liver disease that leads to the development of HCC. The means by which HBV contributes to hepatocarcinogenesis are both direct and indirect. The HBV HBx protein has direct oncogenic capabilities through numerous mechanisms, including deregulation of the cell cycle, disrupting mitochondrial function, and activating proliferation pathways. Insertional mutagenesis is another direct oncogenic mechanism of HBV. For example, HBV integration into the *TERT* promoter is a major method ensuring telomere stability. Indirectly, HBV causes inflammation of the liver, leading to the development of fibrosis and cirrhosis. The hypoxic and immunosuppressive environment within the cirrhotic liver is primed for malignant transformation. Additionally, the oxidative stress created by HBV within hepatocytes generates DNA damage and the accumulation of oncogenic driver events.

A dramatic amount of knowledge regarding HBV-related HCC has been acquired over the last 30 years. Several HBV risk factors including viral load and genotypes allow clinicians to estimate the severity of HBV-related HCC cases. Mechanistically, we now understand the numerous roles that the HBx protein plays in hepatocarcinogenesis. New immunotherapy treatments are on the horizon. But much work remains to be done. Mechanistic data needs to be further developed into therapeutics. Further, understanding of how molecular classification of HCC can influence response to therapy is needed followed by the development of drugs for enriched patient populations. HBV-related HCC incidence can be greatly reduced through national vaccine programs and precision medicine.

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7

Epidemiology and Natural History of Hepatitis B Virus Infection: Time-Dependent Driving Factors of Chronic Hepatitis B Progression

Hwai-I Yang and Chien-Jen Chen

Abstract

This chapter reviews studies on the epidemiology and natural history of hepatitis B virus (HBV) infection. Most data in this chapter was derived from community-based cohort (such as REVEAL-HBV) studies and hospital-based cohort (such as ERADICATE-B) studies. They are considered to be the best choice for elucidating the natural history of the disease because of their long-term prospectiveness, wide range of disease severity, and comprehensive repeated follow-up examinations. Although study participants from the community-based study are usually asymptomatic or only have mild chronic hepatitis B compared to individuals recruited from hospitals, both studies are important for investigating the full spectrum of the natural history of HBV infection. The transition rates of various serological milestones (including spontaneous seroclearance of hepatitis B e antigen, HBV DNA and hepatitis B surface antigen) and end-stage liver diseases (including hepatitis B e antigen-negative chronic hepatitis, cirrhosis, and hepatocellular carcinoma) and the major driving factors of these outcomes of chronic hepatitis B progression were reviewed and summarized.

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Natural history · Hepatitis B virus · Cohort studies · Risk factors · Transition rate · Cirrhosis · Hepatocellular carcinoma · HBeAg · HBV DNA · HBsAg

1 Epidemiology of Hepatitis B Virus Infection

Hepatitis B virus (HBV) infection is a serious global health problem because it is geographically widespread and may cause advanced liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). More than 2 billion people are infected with HBV, of which 257 million have chronic HBV infection as estimated by the World Health Organization in 2015 (WHO 2020). Chronic HBV infection accounts for approximately 30% of all cirrhosis and 53% of all HCC cases globally (Perz et al. 2006), and 15–25% of patients with chronic hepatitis B (CHB) eventually die from these two advanced diseases (Schweitzer et al. 2015), In addition, it was estimated that 600,000 deaths can attribute to HBV infection each year (GBD Mortality and Causes of Death Collaborators 2015).

The prevalence of hepatitis B surface antigen (HBsAg)-positivity worldwide was estimated to be 3.9% (Polaris Observatory Collaboratory 2018). However, the prevalence varies greatly from one WHO region to another. In most African (especially sub-Saharan Africa), Western Pacific (including China, Taiwan, and most Pacific Islands), and South-Eastern Asian, the prevalence is the highest, reaching 8–15%. About 45% of HBV-infected individuals reside in these regions, and their lifetime risk of infection exceeds 60%. Most infections in these regions are acquired from perinatal (China, South Korea and Taiwan) and child-to-child (sub-Saharan Africa) transmission, which leads to the greatest risk of becoming chronic infection. In the Eastern Mediterranean region (including South-Central and Southwestern Asia), Europe (Southern and Eastern regions), and Americas (Central and Southern) regions, the prevalence of HBsAg-positivity is moderate, ranging from 2% to 7%. Forty-three percent of HBV-infected people live in these areas, and their lifetime risk of infection is between 20% and 60%. In these areas, HBV is transmitted in infants, early childhood, adolescence, and adulthood as a mixed pattern. The remaining 12% of HBV-infected people reside in low endemic areas, including the United States, Western Europe, and Australia, where the prevalence is less than 2%, and the lifetime risk of infection is less than 20%. Most HBV infection in this area occur in adolescents and young adults through injecting the drug, men having sex with men, medical practices, and blood transfusion or hemodialysis (Schweitzer et al. 2015; Nelson et al. 2016; Te and Jensen 2010; Liaw et al. 2010).

The prevalence of HBsAg-positivity has generally declined in most WHO regions and countries (Nelson et al. 2016). The prevalence in the Eastern Mediterranean region has dropped sharply, while the prevalence in Eastern and Western Europe has remained stable. Meanwhile, the decline in prevalence in South-Eastern Asian and the Western Pacific regions ranges from moderate to low, with the most significant decline occurring in China and Malaysia (Schweitzer et al.

2015; Ott et al. 2016). However, the prevalence in African and Eastern European regions has increased significantly (Schweitzer et al. 2015).

According to the phylogenic analysis of HBV genomes, 10 HBV genotypes (A to J), which are dispersed across different geographical regions, have been identified on the basis of more than 8% difference in their genome sequences (Sunbul 2014). Genotype A is widespread in Western and sub-Saharan Africa, Northern and Northwestern Europe, North America, and India. Genotypes B and C are predominant in Asia. Genotype D is commonly found in Africa, Eastern Europe, Mediterranean countries, the Middle East, Central Asia, and India. Genotype E is prevalent in Western Africa, while genotypes F and H are common in South and Central America. Genotype G is most predominant in France, Germany, Mexico, and the United States. Recently, genotype I was identified in Vietnam and Laos, and genotype J was reported on Japan's Ryukyu Island (Te and Jensen 2010; Liaw et al. 2010; Sunbul 2014; Allain 2006; Liu and Kao 2013; McMahon 2009; Schaefer 2007a, b).

2 A Brief View of the Natural History of HBV Infection

Some studies have investigated the natural history of HBV infection (Beasley et al. 1981; Chen and Yang 2011; Ganem and Prince 2004; Liaw and Chu 2009; Lok and McMahon 2009; Kao and Chen 2002). Although the transition rates of milestones, determinants of disease progression, and risk prediction models between these studies are diverse, all the evidence point to that most infections are acute and self-limited. The rate of persistent HBV infection is between <5% and 90%, depending on the age of infection, HBsAg and e antigen (HBeAg) serostatus of mother, and the immune competence of the infected individual. Among individuals chronically infected with HBV, chronic hepatitis, fibrosis, cirrhosis, and liver cancer may gradually develop after long-term subclinical infection.

Before 1980, HBV infection was highly prevalent in the general population in Taiwan. An early epidemiological study from Taiwan demonstrated the probability of becoming a chronic carrier in neonates born to mothers who were seropositive for HBsAg and HBeAg was as high as 30% and 90%, respectively (Stevens et al. 1975). Vertical/perinatal transmission plays an important role in maintaining the high prevalence of HBV infection in infants. Although transmission of HBV through iatrogenic exposure during childhood can also lead to chronic HBV infection, the possibility of chronicity is lower than that of vertical transmission. Acute and chronic HBV infections in infants and preschool children are usually asymptomatic and self-limited. However, few of them may develop into fulminant hepatitis, chronic hepatitis, liver cirrhosis and HCC. In Taiwan, the national hepatitis B vaccination program launched in 1984 has significantly reduced the risk of fulminant hepatitis, cirrhosis, and HCC (Chang et al. 1997; Chien et al. 2006; Chiang et al. 2013).

A survey in Taiwan showed that the HBV infection rate in young children among the unvaccinated population was as high as 80% and peaked as 90% in early

adulthood, while the HBsAg seroprevalence was around 20% at ages 10–14 years old (Chung et al. 1988). Most children with chronic HBV infection were HBeAg-seropositive with high serum HBV DNA and HBsAg levels. The rate of HBeAg seroclearance among children with chronic HBV infection was around 70% and 50%, at ages 5–9 and 10–14 years old, respectively (Chang et al. 1989). In a study on the natural history of chronic HBV infection in Taiwanese children, the annual HBeAg seroclearance rate was as low as <2% during the first 3 years of life and then increased with age (Chang et al. 1997). For children whose mothers were HBsAg-seropositive and had elevated serum levels of alanine aminotransferase (ALT), the seroclearance of HBeAg in children increased significantly. Information on the incidence and determinants of HBV DNA seroclearance in children or adolescents is still lacking.

According to the age-specific data of the community-based REVEAL-HBV study established in 1991, the seroprevalence of HBsAg steadily declined from 21% for 30–34 years old to 13% for 60–64 years old. In addition, the proportion of HBeAg-seropositivity in chronic HBsAg carriers dropped from 26% at the age of 30–34 to 6% at the age of 60–64. The levels of serum HBV DNA and HBsAg at the time of enrollment also decreased significantly with age. The REVEAL-HBV study also estimated the cumulative lifetime risk of active hepatitis (as indicated by elevated serum ALT levels), cirrhosis, and HCC between 30 and 75 years of age as 67%, 41%, and 19%, respectively (Chen et al. 2016).

3 Milestones of Progression in Chronic HBV Infection

Chronic HBV infection consists of dynamic interactions between HBV, hepatocytes, and the host immune system. As shown in Fig. 7.1, patients with CHB may achieve several milestones during the natural history of chronic HBV infection, which can be divided into two groups; one involves clinical phases that patients may go through, and the other involves substantial disease progressions such as the development of cirrhosis and HCC.

The natural course of perinatal-acquired chronic HBV infection was classified as three chronological phases traditionally; the immune tolerance phase, the immune clearance phase, and the low-replicative residual integrated phase (Liaw and Chu 2009). The three phases were later refined as “immune-tolerant,” “immune-active,” “immune-escape,” and “immune-control.” The immune tolerance phase is characterized by HBeAg-seropositivity, high HBV DNA levels, normal ALT levels and no sign of liver injury. Most liver damage occurs during the immune clearance phase as the host immune system tries to clear infected hepatocytes, which may lead to the development of cirrhosis and HCC. This phase features inflammation of the liver, elevated serum ALT levels, gradual reduction of circulating HBV DNA levels, and seroconversion of HBeAg to its antibody (anti-HBe).

After HBeAg seroconversion, some patients remain viremic and continue to have active liver disease, which is characterized by HBeAg-seronegative with serum HBV DNA levels ≥2000 IU/mL with persistently or intermittently abnormal ALT and considered to have HBeAg-negative chronic hepatitis B (European Association

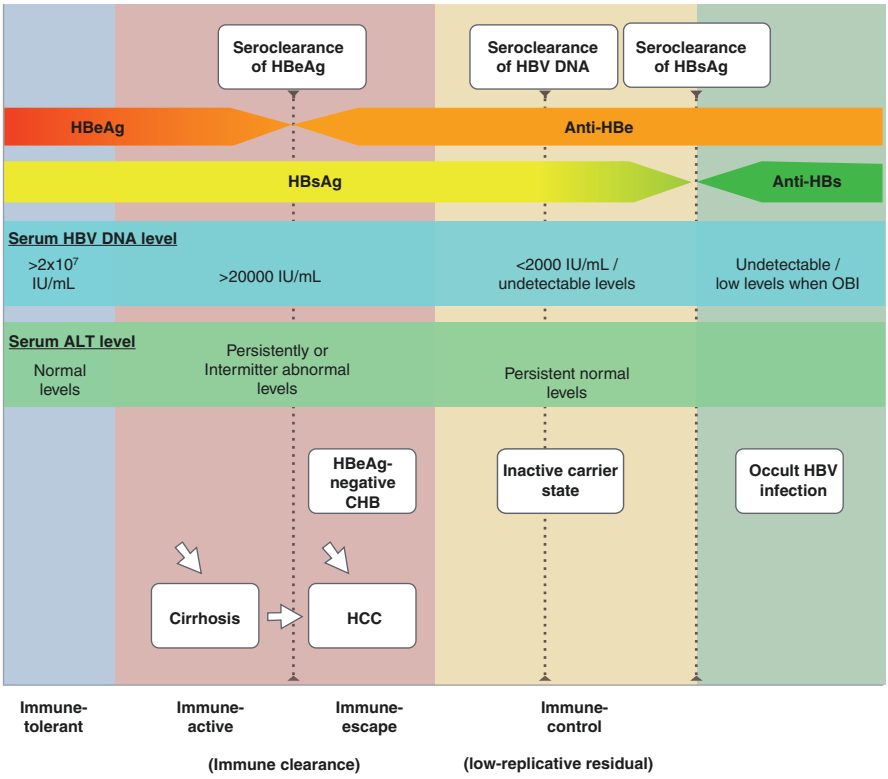

Fig. 7.1 Milestones of chronic hepatitis B progression

for the Study of the Liver 2012). On the contrary, some patients can eventually inactivate the replication of HBV and enter the immune-control low-replicative residual (inactive carrier) state, where HBeAg is seronegative, serum HBsAg remains detectable, but serum HBV DNA levels are lower than 2000 IU/mL with repeatedly normal (or minimally raised) ALT levels. Few infected persons are then able to spontaneously clear HBsAg and make the infection under control. However, a small proportion of patients may also be identified as occult HBV infection (OBI) after HBsAg seroclearance, in whom low levels of HBV DNA can be detected by sensitive polymerase chain reaction (PCR) assays in the serum and/or liver samples, despite HBsAg seronegativity.

The aforementioned classification of natural history is based on the reciprocal relationship between age, viral replication, and histological activity (Chu et al. 1985a, b), and its segmentation is based on the immune response of the host. However, the boundaries between phases are poorly defined because of the lacks of robust immunologic markers. Another classification approach for the clinical phases of CHB is based on transitions of seromarkers such as HBeAg, HBV DNA, and HBsAg (Yang et al. 2012; Liu et al. 2010, 2014a, b). These milestones can be accurately detected through repeated measurements.

The chronological order of the serological milestones in the natural history of CHB has been demonstrated by the REVEAL-HBV study that HBeAg seroclearance occurs first, followed by the seroclearance of HBV DNA, and then the seroclearance of HBsAg (Yang et al. 2012). Through repeated measurements of HBeAg, HBV DNA levels, and HBsAg serostatus, it has been shown that HBeAg seroclearance occurs first, followed by the seroclearance of HBV DNA, and then the seroclearance of HBsAg (Yang et al. 2012; Liu et al. 2010). This time sequence is partly different from the occurrence sequence under antiviral therapy, during which patients' HBV DNA levels are quickly suppressed to undetectable levels in HBeAg-seropositive patients.

4 The Serological Milestone of HBeAg Seroclearance

The presence of HBeAg in the serum is an indicator of active viral replication of HBV in hepatocytes. HBeAg seroclearance together with the emergence of anti-HBe have been key endpoints for antiviral treatment. It has been shown that among CHB carriers, the incidence rate and cumulative incidence of HCC were significantly higher among those who were HBeAg-seropositive than those who were HBeAg-seronegative ($P < 0.001$). Compared to patients with HBsAg-seronegative, the relative risk of HCC was higher in those with HBeAg-seropositivity (60.2, 95% CI = 35.5–102.1) than in those with HBeAg seronegativity (9.6, 95% CI = 6.0–15.2) (Yang et al. 2002). The spontaneous or interferon alpha-induced anti-HBe seroconversion leads to improvement in clinical outcomes (Niederau et al. 1996; Lin et al. 1999), suggesting that HBeAg is a useful marker for predicting end-stage liver diseases.

In children under 15 years of age who infected with HBV, the rate of HBeAg-seropositivity was 80–85% (Chang 2008). In most instances, spontaneous HBeAg seroclearance occurs during adolescence or early adulthood and rarely occurs before the age of 3 (Chang 2008; Chang et al. 1995). Age of infection and maternal HBsAg status are determinants for HBeAg seroclearance in children (Table 7.1) (Chang et al. 1989, 1995; Marx et al. 2002). In addition, several viral factors may influence HBeAg-seropositivity in children. A long-term follow-up study of 460 Taiwanese children with chronic HBV infection showed that the positive rate of HBeAg after 20 years of follow-up was 70% in genotype C and 40% in genotype B carriers (Ni et al. 2004a). A Taiwanese study showed that serum level of hepatitis B core antibody (anti-HBc) titer >500 IU/mL, HBV genotype B and B + C, and a baseline HBsAg titer of ≤4.8 log10 IU/ mL were predictive of spontaneous HBeAg seroconversion in HBeAg-positive children with a normal ALT level (Chen et al. 2019). Genetic features of HBV are also predictors of HBsAg seroclearance in adult CHB patients. An Alaska Native cohort study found that individuals with genotype C achieved HBeAg seroclearance at an older age, and genotypes C and F were associated with HBeAg reversion after HBeAg seroclearance (Livingston et al. 2007a). Some studies found the precore G1896A and basal core promoter (BCP) A1762T/G1764A mutants were associated with HBeAg seroconversion in both adults and

Table 7.1 Determinants of spontaneous HBeAg seroclearance in the natural history of chronic hepatitis B

For children
Age older than 3 years (vs. under 3 years)
Maternal HBsAg-negative (vs. HBsAg-positive)
Horizontal infection (vs. perinatal infection)
HBV genotype B (vs. C)
qAnti-HBc > 500 IU/mL
qHBsAg ≤ 4.8 log10 IU/ mL
Precore G1896A and BCP A1762T/G1764A mutants
For adults
Female (vs. male)
Elevated serum ALT
Precore G1896A mutant
Genotype B (vs. C)
Low HBV DNA levels
qAnti-HBc ≥3.25 log10 IU/mL

children (Lok et al. 1995; Chang et al. 1998; Chan et al. 1999; Yuen et al. 2002; Ni et al. 2004b; Nie et al. 2012).

The REVEAL-HBV study has estimated the annual incidence rate of spontaneous HBeAg seroclearance as 61.6 per 1000 person-years (Liu et al. 2014a). Among individuals with serum HBV DNA levels $\geq 10^4$ copies/mL at study entry of REVEAL-HBV cohort, the cumulative lifetime incidence of spontaneous HBeAg seroclearance increased from 38.8% at 40 years of age to 95.5% at 74 years (Yang et al. 2012). Serum HBV DNA level was a significant determinant of HBeAg seroclearance in the multivariate analysis. The multivariate-adjusted rate ratio (95% CI) of HBeAg seroclearance was 1.89 (1.28–2.78) and 3.27 (2.01–5.32) for those with HBV DNA levels of 10^6–$<10^8$, and 10^4–$<10^6$ copies/mL, respectively, compared to individuals with HBV DNA levels $\geq 10^8$ copies/mL (Liu et al. 2014b). In addition to serum HBV DNA levels, gender, serum ALT levels, precore mutation, and HBV genotype were also significantly associated with HBeAg seroclearance after multivariate adjustment (Yang et al. 2012; Liu et al. 2014b). The multivariate-adjusted rate ratio (95% CI) of spontaneous HBeAg seroclearance was 1.92 (1.29–2.85) for women compared to men; 2.11 (1.40–3.18) for baseline serum ALT levels of 45 or more compared to less than 45 U/L; 1.66 (1.03–2.68) for the precore 1896 G/A mutant compared to wild-type, and 3.06 (2.11–4.44) for HBV genotype B or B and C compared to genotype C (Liu et al. 2014b). The effect of HBV genotype on HBeAg seroclearance/seroconversion has been intensively investigated in other studies. In a Taiwanese hospital-based cohort study of 272 CHB patients, genotype C infection was associated with lower rates of spontaneous HBeAg seroconversion than genotype B (27% vs. 47%, $P < 0.025$) during the follow-up (Kao et al. 2002). In Spanish CHB patients, genotypes A and D HBV infection did not show a difference in the incidence of HBeAg seroconversion. Nevertheless, the rate of sustained remission after HBeAg seroconversion was

higher in genotype A patients (55% vs. 32%, $P < 0.01$) (Sanchez-Tapias et al. 2002). The subsequent REVEAL-HBV study found that a novel quantitative serum marker, anti-HBc level, was an independent predictor of spontaneous HBeAg seroclearance among untreated HBeAg-positive individuals in Taiwan, whereas hepatitis B core-related antigen (HBcrAg), which simultaneously measures the levels of hepatitis B core antigen, HBeAg and a 22-kDa precore protein without C-terminal arginine-rich domain (p22cr) , was not a significant predictor (Liu et al. 2019). Compared to patients with anti-HBc levels <Q1 (<3.25 log10 IU/mL), those with anti-HBc levels Q2 (3.25–4.09 log10 IU/mL), Q3 (4.09–4.33 log10 IU/mL), and ≥Q4 (≥4.33 log10 IU/mL) had an adjusted rate ratio of 3.49 (95% CI 1.80–6.75) for HBeAg seroclearance, after adjustment of HBcrAg level, sex, age, levels of ALT, HBV DNA and HBsAg (qHBsAg), HBV genotype and precure G1896A mutant (Liu et al. 2019).

A prediction nomogram for HBeAg seroclearance was developed by integrating the aforementioned significant determinants in the REVEAL-HBV study with the prediction scores ranged from 0 to 7 (Liu et al. 2014b). The score can predict the 5- and 10-year probabilities of HBeAg seroclearance from 0.08 to 0.72 and from 0.23 to 0.98, respectively. The area under the receiver operating characteristic (AUROC; 95% CI) for predicting the 5- and 10-year probability of HBeAg seroclearance was 0.85 (0.80–0.90) and 0.78 (0.73–0.83), respectively.

5 The Serological Milestone of HBV DNA Seroclearance

Serum HBV DNA level is a marker of viral replication and efficacy for antiviral treatment in CHB patients (Mommeja-Marin et al. 2003). The findings from the REVEAL-HBV study suggested that among CHB carriers, elevated serum HBV DNA levels are a crucial risk predictor of HCC independent of HBeAg status, serum ALT levels, and the presence of liver cirrhosis and that progression to liver cirrhosis is strongly correlated with increasing serum HBV DNA levels, independent of HBeAg status and serum ALT level (Chen et al. 2006a; Iloeje et al. 2006). Thus, the seroclearance of HBV DNA is an important milestone, marking an improvement in prognosis and a reduction in the incidence of advanced liver disease. Some determinants may help in predicting this serological milestone (Table 7.2).

An annual incidence rate of 30.1 per 1000 person-years for spontaneous HBV DNA seroclearance was estimated by the REVEAL-HBV study (Liu et al. 2014b).

Table 7.2 Determinants of spontaneous seroclearance of HBV DNA in the natural history of chronic hepatitis B

Age greater than 60 years (vs. <60 years)
Female (vs. male)
Low qHBsAg levels (dose-dependent)
Precore wild-type (G allele; in patients with serum HBV DNA levels (≥10^4 copies/mL)
Lower HBV DNA levels (in patients with serum HBV DNA levels (≥10^4 copies/mL)
qAnti-HBc < 3 log10 IU/mL

Among individuals with serum HBV DNA levels $\geq 10^4$ copies/mL at study entry, the cumulative lifetime incidence of spontaneous HBV DNA seroclearance increased from 10.1% at 40 years of age to 82.8% at 77 years of age (Yang et al. 2012). In the multivariate analysis, serum HBsAg level was a significant determinant of HBV DNA seroclearance. The multivariate-adjusted rate ratio (95% CI) of HBV DNA seroclearance was 1.18 (0.61–2.27), 2.49 (1.31–4.74), and 6.18 (3.24–11.79) for carriers with serum HBsAg levels of 10^3–$< 10^4$, 10^2–$< 10^3$, and $<10^2$ IU/mL, respectively, compared to individuals with serum HBsAg levels $\geq 10^4$ IU/mL at study entry (Liu et al. 2014b). In addition to serum HBsAg levels, age and gender were also significantly associated with HBV DNA seroclearance after multivariate adjustment (Liu et al. 2014b). The multivariate-adjusted rate ratio (95% CI) for HBV DNA seroclearance was 1.35 (1.00–1.82) for those ≥60 years compared to <60 years, and 1.37 (1.10–1.72) for women compared to men (Liu et al. 2014b). In REVEAL-HBV participants with high serum HBV DNA levels ($\geq 10^4$ copies/mL) at study entry, serum HBV DNA level and precore wild-type (G allele) were also significant predictors of HBV DNA seroclearance (Yang et al. 2012).

As the predictability for spontaneous HBV DNA seroclearance was not improved by the addition of HBV genotype, serum HBV DNA level, and precore mutation, a score-based prediction model and nomogram for HBV DNA seroclearance was created by integrating only age, gender, and serum HBsAg levels (Liu et al. 2014b). The total score of the prediction model for HBV DNA seroclearance ranged from 0 to 8, which predict the 5- and 10-year probabilities of HBV DNA seroclearance from 0.04 to 0.36 and from 0.14 to 0.80, respectively (Liu et al. 2014b). The AUROCs (95% CI) for predicting the 5- and 10-year probability of HBV DNA seroclearance were 0.77 (0.72–0.82) and 0.73 (0.70–0.76), respectively.

Although serum HBV DNA levels play a critical role during the transition between milestones of CHB progression, the updated analysis has shown that serum HBV DNA level was no longer a significant predictor for HBV DNA seroclearance after taking serum HBsAg levels into consideration (Liu et al. 2014b). Moreover, a recent finding from the REVEAL-HBV study showed that quantitative anti-HBc may be a useful biomarker for more accurate prediction. The incidence rate of undetectability of HBV DNA increased with decreasing serum anti-HBc levels and was highest in those with anti-HBc levels <3 log10 IU/mL. In the multivariate analysis, compared with anti-HBc levels ≥4 log IU/mL, anti-HBc levels <3 log IU/mL were significantly associated with undetectable HBV DNA (adjusted rate ratio = 2.69; 95% CI 1.94–3.73). The value of this novel marker might be that it could further stratify patients' probabilities of achieving HBV DNA undetectability in patients with very low HBsAg levels (<100 IU/mL). When compared with individuals who had anti-HBc levels ≥3 log IU/mL and HBsAg levels ≥1000 IU/mL, those with anti-HBc levels <3 log IU/mL and HBsAg levels <100 IU/mL had an adjusted rate ratio of HBsAg seroclearance of 16.45 (95% CI 11.15–24.28) (Hu et al. 2019).

6 The Serological Milestone of HBsAg Seroclearance

For HBeAg-seronegative patients, HBsAg seroclearance is considered as the resolution or a functional cure of hepatitis B and the most important clinical and therapeutic endpoint as it indicates an improved prognosis and lower rates of HCC and other clinical consequences (European Association for the Study of the Liver 2012; Liu et al. 2014a; Sorrell et al. 2009; Simonetti et al. 2010). In a community-based study among Alaskan natives, the incidence rates of HCC were significantly decreased in those with HBsAg seroclearance when compared to those who remained HBsAg-positive (36.8 vs. 195.7 per 100,000 person-years) (Simonetti et al. 2010). In the REVEAL-HBV study from Taiwan using repeated measurements of seromarkers, reaching HBsAg seroclearance during follow-up was indicative of a significantly decreased risk for developing HCC in the future (Liu et al. 2014a).

In the REVEAL-HBV study, the annual incidence rate of spontaneous HBsAg seroclearance among untreated Taiwanese individuals was quite rare, only 2.26% per year (Liu et al. 2010). This study also showed that female gender, increasing age, increasing body mass index (BMI), ethnicity of mainland Chinese (versus Fukienese), and decreasing serum HBV DNA levels were associated with increasing rate of HBsAg seroclearance (Table 7.3) (Liu et al. 2010).

After the introduction of qHBsAg as a potential marker for immune response, the determinants of HBsAg seroclearance were updated (Liu et al. 2013). The cumulative lifetime incidence of spontaneous HBsAg seroclearance among HBeAg-seronegative patients with detectable serum HBV DNA (≥57 IU/ml) increased from 3.0% at 40 years of age to 62.1% at 77 years of age (Chen et al. 2016). The cumulative lifetime incidence of HBsAg seroclearance among those with undetectable HBV DNA (<57 IU/ml) was higher, which was 31.5% at 40 years of age and 98.8% at 77 years of age, respectively (Chen et al. 2016). Serum HBsAg levels were shown to be the strongest predictor of spontaneous HBsAg seroclearance in the multivariate analysis. The multivariate-adjusted rate ratio (95% CI) of spontaneous HBsAg seroclearance was 3.55 (2.51–5.02) and 10.96 (7.92–15.16), respectively, for those with serum HBsAg levels of 100–999 and <100 IU/ml, compared to serum HBsAg levels ≥1000 IU/ml. The effect of serum HBV DNA level on HBsAg seroclearance decreased after adjustment for serum HBsAg levels but was still statistically significant. These results suggested that both serum HBsAg and HBV DNA levels should be considered when monitoring CHB patients.

Table 7.3 Determinants of spontaneous HBsAg seroclearance in the natural history of chronic hepatitis B

Increasing age
BMI ≥30 kg/m^2
Decreasing qHBsAg levels (dose-dependent)
Decreasing HBV DNA levels (dose-dependent)
Maternal HBsAg and HBeAg serostatus (in children)
Non-GG genotype of rs9277535 (near *HLA-DPB1*)
qAnti-HBc titer <3 log IU/mL

Similar results were observed in the hospital-based SEARCH-B study of 390 Taiwanese HBeAg-positive CHB patients who newly and spontaneously cleared HBeAg during follow-up (Tseng et al. 2011). The average annual rate of HBsAg loss was 0.62% during a mean follow-up of 7.4 years. Serum levels of HBsAg and HBV DNA measured 1 year after HBeAg seroconversion were inversely associated with HBsAg loss in a dose-dependent manner, with the hazard ratios of HBsAg loss for those with HBsAg levels of 100 to 999 and <100 IU/mL 4.4 (95% CI 1.1–17.0) and 24.3 (95% CI 8.7–67.5), respectively, compared with patients with HBsAg levels ≥1000 IU/mL. This study also showed that serum HBsAg levels were a better predictor than HBV DNA levels by receiver operating characteristic curve analysis for those who underwent HBsAg loss within 6 years of follow-up. The subsequent study of the same group showed that HBsAg level was the strongest predictor of HBsAg loss in HBeAg-negative patients with serum HBV DNA levels <2000 IU/mL (Tseng et al. 2012a).

A cohort study of children also found that children with serum HBsAg levels <1000 IU/mL had a much greater chance of clearing HBsAg (HR [95% CI] = 5.23 [2.77–9.85]) (Chiu et al. 2014). In addition, HBsAg seroclearance was significant association with maternal serostatus of HBsAg and HBeAg. Furthermore, it has been shown that the non-GG genotype of the single nucleotide polymorphism rs9277535, which is near HLA-DPB1 region, was associated with a higher likelihood of spontaneous HBsAg seroclearance in CHB patients (Cheng et al. 2013).

A prediction score/nomogram was developed, integrating predictors including age, BMI, HBV DNA levels and HBsAg levels for the prediction of spontaneous HBsAg seroclearance (Liu et al. 2013). This 30-point scoring system was able to predict 5- and 10-year probabilities of spontaneous HBsAg seroclearance with AUROC's of 0.89 and 0.84, respectively. This model showed that the addition of serum HBsAg levels to the original HBV DNA-based models significantly improves the predictability of HBsAg seroclearance among HBeAg-seronegative patients (Liu et al. 2013). These results have been externally validated in a hospital-based cohort of 1934 untreated patients, which had more severe disease (Liu et al. 2014c).

Quantitative anti-HBc has been considered as a useful biomarker for spontaneous HBsAg seroclearance. As shown in a report from the REVEAL-HBV study, the incidence rate of HBsAg seroclearance increased with decreasing serum anti-HBc levels and was highest in those with anti-HBc levels <3 log10 IU/mL. In the multivariate analysis, compared with anti-HBc levels ≥4 log IU/mL, anti-HBc levels <3 log IU/mL were significantly associated with HBsAg seroclearance (adjusted rate ratio = 1.94; 95% CI 1.46–2.60). Serum anti-HBc level could further stratify patients' probabilities of achieving HBsAg seroclearance, especially in patients with very low HBsAg levels (<100 IU/mL). When compared with individuals who had anti-HBc levels ≥3 log IU/mL and HBsAg levels ≥1000 IU/mL, those with anti-HBc levels <3 log IU/mL and HBsAg levels <100 IU/mL had an adjusted rate ratio of HBsAg seroclearance of 17.95 (95% CI 12.49–25.81) (Hu et al. 2019).

7 HBeAg-Negative Chronic Hepatitis B

In the natural course of CHB infection, the majority of infected individuals are HBeAg-seronegative. The severity of the disease of HBeAg-seronegative individuals is heterogeneous and can be either inactive or active (Chen and Yang 2011; European Association for the Study of the Liver 2012). Previous studies have shown that the survival rate of inactive carriers can even be compared with that of non-infected individuals (de Franchis et al. 1993). In addition, the REVEAL-HBV also showed that inactive carriers have significantly decreased risk for clinical endpoints such as cirrhosis and HCC (Chen et al. 2006a, 2010; Iloeje et al. 2006). Thus, differentiating between inactive carriers and active CHB carriers is clinically important. It may help the identification of a lower-risk population who need less stringent follow-up. On the other hand, the earlier diagnosis could lead to earlier initiation of antiviral therapy for patients with active hepatitis. Nevertheless, the traditional identification of active carriers is difficult and costly, as ALT and HBV DNA levels tend to fluctuate during the natural course of the disease. Because of this limitation, rare studies have examined inactive or active hepatitis among the community, and further researches on factors that can accurately differentiate the two are warranted (Table 7.4).

A community-based cohort of 414 Alaskan Native Persons (with HBV genotype A, B, C, D, and F) who already had inactive hepatitis showed that the annual incidence rate of reactivation (defined as HBV DNA ≥2000 IU/mL and ALT ≥40 U/L) was 12 per 1000 person-years (Tohme et al. 2013). In the multivariate analysis, individuals who were 30–39 or 40–49 years old had adjusted hazard ratios (95% CI) of reactivation of 0.34 (0.12–90) and 0.20 (0.05–0.70), respectively, compared to individuals between 18–29 years old. In addition, males, those with HBV DNA levels of 1000–1999 IU/mL (compared to HBV DNA <29 IU/mL), and genotype B (compared to genotype non-B) were significant predictors of hepatitis B reactivation (Tohme et al. 2013).

Another community-based study in Taiwan followed 113 asymptomatic HBeAg-seronegative individuals to elucidate factors predicting reactivation of hepatitis B (Chu and Liaw 2007). In this cohort consisting of genotype B and C individuals, reactivation occurred with an annual incidence rate of 33 per 1000 person-years. Males (vs. females), genotype C (vs. genotype B), ALT levels >5x upper limit of

Table 7.4 Determinants of HBeAg-negative chronic hepatitis B in the natural history of chronic hepatitis B

Young age
Male
HBV DNA levels 1000–1999 IU/mL (vs. <29 IU/mL)
ALT levels >5 × ULN (vs. <2 × ULN)
HBV genotype B (vs. non-B in Alaskan natives); genotype C (vs. B)
HBeAg seroconversion at ≥40 years old
HBV DNA levels ≥2000 IU/ml at 1 year post-HBeAg seroconversion
HBV DNA <2000 (or <200) IU/mL and qHBsAg <1000 IU/mL (predicting inactive carriers)
HBcrAg >3.14 log IU/mL

normal (ULN) (vs. ALT levels <2xULN), and HBeAg seroconversion at ≥40 years old (vs. HBeAg seroconversion at <40 years) had a higher risk of reactivation (Chu and Liaw 2007). The hospital-based SEARCH-B study examined the impact of viral load on long-term outcomes after spontaneous HBeAg seroconversion. Their findings suggested that serum HBV DNA levels ≥2000 IU/ml at 1 year post-HBeAg seroconversion were associated to an increased risk of HBeAg-negative hepatitis (Tseng et al. 2012b).

Some studies have investigated the role of qHBsAg in differentiating inactive and active hepatitis B carriers. In a hospital-based Italian study of 209 genotype D carriers, a one-time measurement of HBV DNA <2000 IU/mL and qHBsAg <1000 IU/mL could accurately differentiate inactive from active carriers with a sensitivity, specificity, and diagnostic accuracy of 91.1%, 95.4%, and 94.3%, respectively (Brunetto et al. 2010). In the other clinic-based study of 129 patients with genotypes A-E, a one-time measurement of HBV DNA >200 IU/mL and qHBsAg >1000 IU/mL can differentiate HBeAg-negative CHB from inactive carriers with a sensitivity and specificity of 92% and 51%, respectively (Martinot-Peignoux et al. 2013).

The community-based REVEAL-HBV study has externally validated the use of one-time baseline measurement of HBsAg <1000 IU/mL and HBV DNA <2000 IU/mL for distinguishing inactive carriers from active CHB with a sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of 71%, 85%, 83%, 74%, and 78%, respectively (Liu et al. 2016). This study also showed that patients identified as inactive carriers had a lower incidence of HCC and cirrhosis and higher rate of spontaneous HBsAg seroclearance (Liu et al. 2016).

A multi-center European cohort of 1582 consecutive HBeAg-seronegative patients assessed the diagnostic value of HBcrAg on the differential diagnosis between HBeAg-negative infection (HBV DNA ≤20,000 IU/mL and ALT < 40 U/L) and chronic hepatitis B (HBV DNA >20,000 IU/mL and elevated ALT) defined according to EASL guidelines (Brunetto et al. 2021). This study found the HBcrAg assay provides an accurate, single-point differential diagnosis for HBeAg-seronegative patients.

8 The Development of Cirrhosis

Chronic HBV infection can progress to advanced liver diseases, including cirrhosis. Worldwide, at least one-third of liver cirrhosis cases can be attributed to HBV infection (Perz et al. 2006). A Taiwanese prospective study estimated the annual incidence of cirrhosis among asymptomatic HBV carriers to be 0.7% (Yu et al. 1997). In a study of 1400 Alaskan HBsAg-seropositive Natives cohort study, 8 cases of cirrhosis were confirmed after liver biopsy, with an incidence rate of 107 and 95 per 100,000 person-years in men and women, respectively (McMahon et al. 1990). Advanced age, male gender, HBeAg serostatus, elevated ALT levels, serum HBV DNA levels, serum quantitative HBsAg levels, HBV genotype C, precore G1896A and BCP A1762T/G1764A variants, and serum HBcrAg levels have been reported as risk factors of cirrhosis for chronic HBV carriers (Table 7.5) (Chen and Yang

Table 7.5 Determinants of cirrhosis development in the natural history of chronic hepatitis B

Advanced age
Male
HBeAg-seropositivity
Elevated ALT levels
Increasing serum HBV DNA levels (dose-response relationship)
Increasing serum HBsAg levels (in a dose-dependent manner particularly in HBV DNA $<10^6$ copies/mL)
HBV genotype C
Precore G1896A variant (protector)
BCP A1762T/G1764A variant
Serum HBcrAg levels (≥10 KU/mL for patients with intermediate viral load [2000–19,999 IU/mL])

2011; Iloeje et al. 2006; Yu et al. 1997; Lee et al. 2013; Tseng et al. 2013a, 2015, 2021).

In the REVEAL-HBV study, 365 individuals were newly diagnosed with liver cirrhosis over an average of 11 years of follow-up, giving an incidence rate of 912 per 100,000 person-years. The incidence of cirrhosis increased with elevated serum HBV DNA levels at study entry, from 339 to 2498 per 100,000 person-years for serum HBV DNA levels <300 to ≥1000,000 copies/mL (Iloeje et al. 2006). The dose-response relationship between serum HBV DNA levels and liver cirrhosis risk was observed after adjustment for age, gender, cigarette smoking and alcohol drinking, HBeAg serostatus, and serum ALT levels at study entry, and still seen even after stratification by sex, age, cigarette smoking, and alcohol consumption. In addition, the relative risk for mortality from chronic liver diseases and liver cirrhosis increased with increasing serum HBV DNA levels in a dose-dependent manner (Iloeje et al. 2007). In the subsequent study, quantitative serum HBsAg levels and cirrhosis risk were also evaluated in the REVEAL-HBV study (Lee et al. 2013). The cumulative lifetime risk for 30 to 75 years of age for cirrhosis was 11.4%, 23.3%, and 36.8% for individuals with serum HBsAg levels of <100, 100–999, and ≥1000 IU/mL, respectively (Chen et al. 2016). There was a dose-response relationship between serum HBsAg levels and cirrhosis when examining the multivariable-adjusted relative risk. Risk of cirrhosis has been evaluated with various combinations of HBV DNA and HBsAg levels. The results showed that serum levels of HBsAg could further predict long-term incidence of cirrhosis, particularly for individuals with serum HBV DNA levels lower than 10^6 copies/mL. Apart from serum HBsAg and HBV DNA levels, HBV genotype and mutant types were also significantly associated with cirrhosis risk (Chen and Yang 2011).

The hospital-based ERADICATE-B study also showed that in HBeAg-negative patients with low viral loads (<2000 IU/mL), a higher HBsAg level can predict disease progression, including HBeAg-negative hepatitis, hepatitis flare, and cirrhosis. Serum HBsAg levels <1000 IU/mL in combination with low levels of HBV DNA and ALT can help define minimal-risk HBV carriers (Tseng et al. 2013a). In addition, a subset of the SEARCH-B cohort has been used for investigating BCP

A1762T/G1764A variants associated with cirrhosis. The result showed that the BCP A1762T/G1764A variant was an independent risk factor for cirrhosis development (hazard ratio: 4.26; 95% CI: 1.32–13.77). Further pyrosequencing quantitative analysis demonstrated that the cirrhosis risk was higher in patients with BCP A1762T/G1764A variants ≥45% compared to <45% (Tseng et al. 2015).

A non-invasive score was developed by incorporating host and virus profiles for the prediction of 3-year, 5-year, and 10-year cirrhosis risk (Lee et al. 2013). Age, gender, serum ALT levels, HBeAg serostatus, serum HBV DNA and HBsAg levels, and HBV genotype were risk predictors for this score. The risk prediction model is suggested to be used in a stepwise manner. Patients should first be tested for HBeAg serostatus. Serum HBV DNA levels should be further examined for HBeAg-seronegatives, then quantitative HBsAg levels should be tested if patients have serum HBV DNA levels $<10^6$ copies/mL. In addition, HBV genotype may be measured for patients seropositive for HBeAg or with serum HBV DNA levels $\geq 10^6$ copies/mL. This scoring system with total risk scores ranging from 0 to 26 can predict 3-year, 5-year, and 10-year cirrhosis risk with AUROC of 0.86, 0.86, and 0.83, respectively. The predictive accuracy evaluated by AUROC was 0.79, 0.80, and 0.82 for the prediction of 3-year, 5-year, and 10-year cirrhosis risk, respectively, when the internal validation was performed.

Recently, the Taiwanese ERADICATE-B cohort with a total of 1673 treatment-naïve non-cirrhotic HBeAg-seronegative patients with ALT level <40 U/L showed that higher HBcrAg levels were associated with increased incidence of cirrhosis, cirrhosis-related complications, and liver-related death (Tseng et al. 2021). HBcrAg <10 KU/mL can define a low-risk group for disease progression among those with intermediate viral load (HBV DNA 2000–19,999 IU/mL).

9 The Development of Hepatocellular Carcinoma

A landmark cohort study of 22,707 Taiwanese government employees who were followed up for 3.3 years (75,000 person-years) found that HBsAg-seropositive individuals had a 223 fold increased risk of developing HCC compared to HBsAg-seronegative ones (Beasley et al. 1981). Multiple risk factors including older age, male gender, alcohol consumption, the presence of cirrhosis, elevated serum ALT levels, family history, metabolic factors, obesity, and co-infection with hepatitis C virus contribute to the risk of HCC in chronic HBV carriers (Table 7.6) (Chen et al. 1993, 1997, 2006a, 2008, 2014, Loomba et al. 2013a, b; Yang et al. 2008; Yu et al. 2000, 2008; Chao et al. 2011; Huang et al. 2011).

Hepatitis B viral factors play important roles in hepatocarcinogenesis. A previous study originated from the REVEAL cohort found that HBeAg-seropositive HBV carriers had an increased risk of developing HCC compared with HBeAg-seronegatives, which remained significant even after stratification analyses by age, gender, cigarette smoking, alcohol consumption, serum ALT levels and cirrhosis status, implying that active viral replication is crucial for HCC development (Yang et al. 2002). A further nested case control study showed a significant dose-response

Table 7.6 Determinants of HCC development in the natural history of chronic hepatitis B

Older age
Male
Alcohol consumption
Cirrhosis
Family history of HCC
Metabolic factors and obesity
Co-infection with HCV
Elevated ALT levels
HBeAg-seropositivity
Increasing serum HBV DNA levels (dose-response relationship)
Increasing serum HBsAg levels (in a dose-dependent manner particularly in HBV DNA <2000 IU/mL)
HBV genotypes C (vs. B) and F (vs. D)
Precore G1896A variant (protector)
BCP A1762T/G1764A variant
Pre-S deletion
Serum HBcrAg levels (≥10 KU/mL for patients with intermediate viral load [2000–19,999 IU/mL])
Single nucleotide polymorphisms including rs3077 (3′UTR of *HLA-DPA1*), rs9277535 (3′UTR of *HLA-DPB1*), rs9272105 (intergenic region between *HLA-DQA1* and *HLA-DRB1*), rs455804 (first intron of *GRIK1*), rs9275319 (intergenic region between *HLA-DQB1* and *HLA-DQA2*), rs7574865 (third intron of *STAT4*), and rs2296651 (S267F variant of *SLC10A1*)
Aflatoxin B1 exposure

relationship between serum HBV DNA levels measured by the branched-chain assay and the risk of HCC (Yang et al. 2002). The REVEAL-HBV study further comprehensively examined the association between serum HBV DNA levels and HCC in 3653 individuals who were seropositive for HBsAg and seronegative for antibodies against hepatitis C virus at study entry (Chen et al. 2006a). A strong biological gradient of HCC risk was observed across serum HBV DNA levels. The corresponding relative risks with 95% confidence intervals could be as high as 6.1 (2.9–12.7) for serum HBV DNA levels ≥1000,000 copies/mL compared to serum HBV DNA levels <300 copies/mL. The HCC risk can also be determined by repeated serum HBV DNA tracking in addition to a one-shot measurement (Chen et al. 2011; Chen 2005).

Recently, qHBsAg levels were found to be an independent predictor of HCC, especially in patients with low viral load (HBV DNA level <2000 IU/mL) (Lee et al. 2013; Tseng et al. 2012c). In the hospital-based ERADICATE-B study of 2688 non-cirrhotic Taiwanese CHB patients, elevated HBV DNA and HBsAg levels were both positively associated with HCC development in a dose-dependent manner. However, advanced age, male sex, and elevated ALT and qHBsAg level, but not HBV DNA level, were found to be independent risk factors for HCC development for 1068 HBeAg-negative patients with HBV DNA level <2000 IU/mL. The multivariable-adjusted hazard ratio of HCC was 13.7 (95% CI: 4.8–39.3) for patients with HBsAg level ≥1000 IU/mL compared to patients with HBsAg level <1000 IU/

mL (Tseng et al. 2012c). The ERADICATE-B study also demonstrated that HBsAg could stratify HCC risk for patients with intermediate viral load (HBV DNA levels 2000–20,000 IU/mL) but not for those with high viral load (HBV DNA levels >20,000 IU/mL) (Tseng et al. 2013b). In the REVEAL-HBV cohort, the cumulative lifetime HCC risk from 30 to 75 years of age was 3.3%, 12.0%, and 28.3% for those with baseline serum HBsAg levels of <100, 100–999, and ≥1000 IU/mL (Chen et al. 2016). The multivariate-adjusted hazard ratios (95% CI) for HCC were 2.83 (1.55–5.18) and 4.06 (2.24–7.36), respectively, for serum HBsAg levels of 100–999 and ≥1000 IU/mL, when compared to HBsAg levels <100 IU/mL (p for trend<0.001) (Lee et al. 2013). The REVEAL-HBV study showed that the dose-response relationship between serum HBsAg levels and HCC was only observed in participants with serum HBV DNA levels $<10^6$ copies/mL.

Algorithms for the management of CHB patients according to their HBV DNA and HBsAg levels has been proposed (Lin and Kao 2016; Tseng and Kao 2013). HBV DNA levels of 2000 IU/mL and 20,000 IU/mL have been used to categorize patients into low-, intermediate-, and high-risk groups. Minimal-risk patients with low levels of HBsAg (<1000 IU/mL) and HBV DNA (<2000 IU/mL) were considered as having minimal risk of HCC and recommended follow-up only, while patients with HBV DNA ≥20,000 IU/mL were recommended to start early antiviral treatment. For the HBeAg-seronegative patients with HBV DNA levels between 2000 and 19,999 IU/mL (intermediate viral load), additional biomarkers were needed for risk stratification. Recently, hepatitis B core-related antigen (HBcrAg) quantification has emerged as a surrogate marker for evaluating covalently closed circular DNA (cccDNA) level, which is the intranuclear template of HBV replication. Its clinical value was investigated in 1031 CHB patients who were not treated with nucleos(t)ide analog therapy (Tada et al. 2016). Cox proportional hazards models showed that HBcrAg >2.9 log U/ml was significantly associated with the incidence of HCC (hazard ratio = 5.05; 95% CI 2.40–10.63) after adjustment for HBV genotype, HBV DNA levels, HBeAg status, and BCP mutant status. In addition, in the ERADICATE-B study with the 2666 non-cirrhotic CHB patients who were free of antiviral treatment during the follow-up period from 1985 to 2000, HBcrAg level was found to be an independent risk factor in the multivariable analysis. In HBeAg-seronegative patients with intermediate viral load and normal levels of ALT, HBcrAg levels ≥10 KU/mL can identify patients at increased risk of HCC (hazard ratio, 6.29; 95% CI 2.27–17.48) (Tseng et al. 2021).

At least 10 HBV genotypes (A to J) have been identified to date according to more than 8% differences in the genome sequence, and there are large geographical variations in their distributions (Sunbul 2014). Some retrospective studies have demonstrated that CHB patients with genotype C infection have more severe liver diseases (including cirrhosis and HCC) than those infected with genotype B (Kao et al. 2000; Chan et al. 2004). One study in Alaska with predominant genotype D and F found that native Alaskans with HBV genotype F had a higher risk of developing HCC than other genotypes (Livingston et al. 2007b). A Taiwanese nested case control study, where HBV genotype B and C are predominant, found that genotype C was associated with increased risk for HCC (Yu et al. 2005). Data from Hong

Kong also found that HBV genotype C, particularly subgenotype Ce, increased the risk of HCC in CHB patients (Chan et al. 2008). The REVEAL-HBV study has estimated that incidence rates for HCC in participants with genotype B and genotype C were 306 and 786 per 100,000 person-years (Yang et al. 2008). Individuals with HBV genotype C had 2.4 times higher risk of developing HCC than individuals with genotype B. In addition to HBV genotype, individuals with the precore G1896A mutation had a reduced risk for HCC, compared to individuals with the wild-type virus. Moreover, consistent with the findings from a hospital-based study (Kao et al. 2003), individuals with the BCP region A1762T/G1764A double mutation had increased risk for HCC (Yang et al. 2008). Some other studies also found that patients with pre-S deletion and BCP mutation have been significantly associated with the development of progressive liver diseases (Chen et al. 2006b, 2007; Lin et al. 2007; Liu et al. 2009; Pollicino et al. 2014).

Apart from viral factors, several host factors have been documented as risk predictors of HCC. Some single nucleotide variants were found to be associated with HCC progression in CHB patients using genome-wide association studies (GWAS) (O'Brien et al. 2019). Among them, rs3077 in the 3′ untranslated region (UTR) of *HLA-DPA1* and rs9277535 in the 3'UTR of *HLA-DPB1* were frequently reported. (O'Brien et al. 2019). In addition, rs9272105 in the intergenic region between *HLA-DQA1* and *HLA-DRB1* region and rs455804 within the first intron of the glutamate receptor ionotropic kainite 1 gene (*GRIK1*) were found to be associated with HCC in a Han Chinese population. rs9275319 and rs7574865, which lies between *HLA-DQB1* and *HLA-DQA2* and locates in the third intron of *STAT4*, respectively, were found to be associated with HCC risk in a Chinese GWAS. A Taiwanese study showed that the S267F variant of *SLC10A1* (rs2296651), which encodes the sodium taurocholate co-transporting polypeptide (NTCP; the receptor of HBV on the human hepatocyte), was not only associated with resistance to CHB but also with reduced risk of HCC in CHB patients (Hu et al. 2016).

A case control study nested within the REVEAL-HBV cohort assessed the effect of aflatoxin B1 (AFB1) exposure on cirrhosis and HCC in CHB patients (Chu et al. 2017). Serum AFB1-albumin adduct levels at study entry were measured in 232 cirrhosis cases, 262 HCC cases and 577 controls and the analysis demonstrated that AFB1 exposure might increase the risk of cirrhosis and HCC in a dose-response manner among chronic HBV carriers.

In the past few years, risk calculators for predicting HCC risk have been developed (Lee et al. 2013; Yang et al. 2010, 2014). These easy-to-use risk scores are based on non-invasive clinical characteristics and have helped stratify patients' HCC risk according to their personal profiles, including age, sex, family history, alcohol consumption, serum ALT levels, HBeAg serostatus, serum HBV DNA and HBsAg levels, and HBV genotypes. The REACH-B HCC risk calculator was developed in the community-based REVEAL-HBV cohort using reliable and easily accessible clinical parameters and has been externally validated in clinical settings in Hon Kong and Korea, allowing the identification of treatment-naïve individuals at high risk who need intensive care (Yang et al. 2011). Based on the subsequent studies, HBsAg level has been shown to be a complementary marker for the risk of HCC in the low viral load.

HBsAg level was therefore incorporated into the HCC risk prediction model using the REVEAL-HBV cohort (Lee et al. 2013). With the establishment of quantitative HBsAg levels as an important seromarker in the natural history of CHB, the REACH-B score was updated by adding a parameter of HBsAg level (REACH-B IIa) (Yang et al. 2016). In addition, as testing serum HBV DNA levels for applying risk calculators is relatively costly, serum HBsAg levels were used to replace serum HBV DNA levels in the REACH-B IIb model (Yang et al. 2016). These modified systems identified patients who developed HCC with similar levels of accuracy as the original REACH-B score in the ERADICATE-B cohort and the cohort of the Chinese University of Hong Kong. The REACH-B IIa model was therefore suggested to be used in clinical practice by hepatologists for the management of CHB patients, while the REACH-B IIb model could be a first-line risk calculator for general practitioners, community surveys and countries with limited medical resources (Yang et al. 2016).

10 Occult Hepatitis B Infection

In spite of active maintenance of robust antiviral T-cell immunity, eradicating HBV from the body is very challenging, as cccDNA can be persistently detected in the liver of patients with resolved HBV infection (Rehermann et al. 1996; Michalak et al. 1999; Loriot et al. 1997). The lasting cccDNA in the hepatocytes seems to be replication competent, as HBV may reactivate in patients who receive immunosuppressive agents (Xunrong et al. 2001; Ishiga et al. 2001; Yeo et al. 2009; Hsu et al. 2014). Some HBsAg-seronegative individuals can be identified as having OBI by the presence of HBV in the blood or liver using highly sensitive HBV DNA PCR assays.

OBI has been reported in HCC patients with chronic HCV infection, liver transplant recipients from anti-HBc-seropositive donors, anti-HBc-seropositive patients co-infected with hepatitis C virus, patients with cryptogenic cirrhosis or advanced fibrosis, intravenous drug users, and routine blood donors (Torbenson and Thomas 2002). A population-based long-term follow-up study in a randomly selected cohort that includes repeated measurements of HBV infection markers was suggested to be the best approach to studying OBI (Chen 2005). Data on the natural history of OBI in the community is still lacking, nevertheless, clinic-based OBI studies which performed measurements at the point of spontaneous HBsAg seroclearance provided important clues.

A Taiwanese study with 218 patients (mean age, 44.8 years) who had undergone spontaneous HBsAg seroclearance followed up for 12–179 months found serum HBV DNA measured by PCR was detectable in 6 of 106 (5.7%), 4 of 128 (3.1%), and 2 of 158 (1.3%) available serum samples collected at the time of HBsAg seroclearance, 6 months, and 1 year after HBsAg seroclearance, respectively (Chen et al. 2002). Notably, among the samples with undetectable HBV DNA 1 year after HBsAg seroclearance, around half (56%) were anti-HBs seronegative. In OBI patients, the anti-HBs seropositivity at different time points was all less than half (3 at the time of HBsAg seroclearance, 2 at 6 months after seroclearance, and 0 at 1 year after seroclearance). Unfortunately, intrahepatic HBV DNA and associated HCC risk were not evaluated in this study.

Another Hong Kong study investigated 298 patients (median age, 49.6 years) with HBsAg seroclearance, most of whom (96%) did not receive any treatment (Yuen et al. 2008). Anti-HBs was detectable till the end of follow-up in 52% of the patients after HBsAg seroclearance, while cumulative rates of developing HCC were not different between patients with and without anti-HBs. After HBsAg seroclearance, 13.4% had detectable HBV DNA (median level: 7 IU/mL) within 1 year. Subsequent follow-up showed 6.1% of samples had detectable HBV DNA (median level: 13.9 IU/mL) 5 to 10 years after HBsAg seroclearance, and 3.7% of samples had detectable HBV DNA more than 10 years after HBsAg seroclearance. This study also assessed intrahepatic HBV DNA and messenger RNA in a small portion of patients and showed that all patients had detectable intrahepatic HBV DNA, and 79.3% had detectable cccDNA (median levels: 1.7 copies/cell and 0.031 copies/cell, respectively). However, except for only one patient with detectable X gene mRNA expression, all samples were undetectable for mRNA expression on surface and precore/pregenomic genomes. This study also reported that at diagnosis, two out of five patients had low serum levels of HBV DNA (23.3 and 169.5 copies/mL, respectively).

By measuring serum HBV DNA at or after HBsAg seroclearance, a substantial number of OBI subjects were discovered with a gradually declined proportion over time. However, by measuring HBV DNA in liver tissues, HBV existed inside the liver after HBsAg seroclearance, although low replicative and transcriptionally inactive (Yuen et al. 2008). Large-scale longitudinal population-based studies are needed to further investigate the natural history of OBI in terms of molecular mechanisms, dynamic fluctuations, and health risk (Chen 2005). Furthermore, there is an urgent need for emerging biomarkers that can be used to detect OBI easily and accurately. Such biomarkers may not only be helpful for investigating the association between OBI and long-term disease risk but also for managing patients after HBsAg seroclearance in HBV endemic areas.

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Hepatitis B Vaccines

8

John W. Ward

Abstract

Since 1982, safe and effective hepatitis B virus (HBV) vaccines have been commercially available first derived from plasma of HBV-infected persons and later from yeast cells using recombinant DNA technology. Hepatitis B vaccines have an overall efficacy of 80–100% and provide virtually complete protection against acute and chronic hepatitis B infection among persons who complete the three-dose vaccination series; a two-dose vaccine is now available. Despite decrease or loss of serologic evidence of a protective immune response over time, immune protection from hepatitis B vaccination lasts for more than 30 years. New hepatitis B vaccines provide new options for achieving seroprotective levels of immunity among populations (e.g., HIV-seropositive patients, the elderly) typically hyporesponsive to vaccination and to decrease the number of doses required to achieve an effective immune response. The greatest health impact is achieved through childhood vaccination beginning at birth, as children infected with HBV at birth or early childhood are at greatest risk for developing chronic HBV infection and HBV-related liver disease in later life. Globally, universal hepatitis B vaccination is recommended for all infants beginning preferably within 24 h of birth, full immunization of infants by routine immunization programs in the first 3 years of life, and catch-up vaccination of unimmunized older children and adults. Evidence is growing that antiviral prophylaxis given in the last trimester of pregnancy to women with a high viral load of HBV can lower the risk for perinatal transmission of HBV among newborns who receive a timely dose of hepatitis B vaccine. As of 2019, global coverage of infant HepB three-dose immunization was 85% with 189 countries (>95%) routinely vaccinating infants

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against HBV infection approaching the HBV "elimination" goal of 90% coverage. As of 2016, the 43% global coverage of hepatitis B birth dose vaccination falls short of this 90% coverage goal. Rates of HBV incidence, prevalence, and liver cancer fall dramatically among vaccinated cohorts. As a result, hepatitis B vaccination is one of the most cost-effective public health interventions available, yielding a cost per life saved of $4–$36 in low-income countries. The WHO has developed a framework for global action, with the goal of eliminating HBV and hepatitis C virus (HCV) as public health threats by 2030. Hepatitis B elimination can be achieved when countries implement comprehensive hepatitis B virus immunization programs including hepatitis B vaccine in national childhood immunization schedules, vaccinating newborns for hepatitis B, and providing catch-up vaccination for children or adolescents, and adults.

Keywords

Hepatitis B · Hepatitis B virus (HBV) · Vaccination · Birth dose · Immunization Hepatocellular carcinoma · Pre-exposure prophylaxis · Post-exposure prophylaxis · Vaccine efficacy · Adverse events · Hepatitis B elimination

1 Development of Hepatitis B Vaccines for Active Immunization

Safe and effective hepatitis B virus (HBV) vaccines have been commercially available since 1982. The American microbiologist Maurice Hilleman at Merck used three treatments (pepsin, urea, and formaldehyde) of blood serum together with rigorous filtration to yield a product that could be used as a safe vaccine. Hilleman hypothesized that he could make a hepatitis B vaccine by injecting hepatitis B surface protein. The first available vaccines were indeed produced by harvesting HBsAg (the 22-nm particle) from the plasma of persons with chronic HBV infection. Hepatitis B vaccine elicits development of antibody response to a determinant, located in the surface antigen of HBV (Coleman 2006). Anti-HBs is the serologic marker of vaccine-induced protection. The efficacy of pre-exposure hepatitis B vaccination has been demonstrated in randomized double-blind placebo-controlled clinical trials involving high-risk groups such as men who have sex with men (MSM), healthcare workers, hemodialysis staff, and hemodialysis patients (Crosnier et al. 1981; Szmuness et al. 1980, 1982; Desmyter et al. 1983; Stevens et al. 1984; Jack et al. 1999; Hadler et al. 1986; Francis et al. 1982). These studies demonstrated an overall efficacy of 80%–100% and virtually complete protection against acute and chronic hepatitis B infection among persons who developed anti-HBs concentrations of 10 mIU/mL or greater, even if subsequently, over time, anti-HBs concentrations declined to less than 10 mIU/mL.

Subsequently, the development of recombinant DNA technology to express HBsAg in other organisms offered the potential to produce unlimited supplies of vaccine (Sitrin et al. 1993; Emini et al. 1986), and nowadays recombinant DNA

vaccines have completely replaced the plasma-derived vaccines. Hepatitis B vaccines are formulated to contain 2.5–40 μg of HBsAg protein and an aluminum phosphate or aluminum hydroxide adjuvant (WHO 2017a): 0.25 mg in pediatric dosed vaccines and 0.5 mg in adult dosed vaccines. Other HepB vaccines incorporate other adjuvants to boost the immune response. Heplisav-B approved by the US FDA in 2017, with each dose combining 20 mcg of HBsAg and 3000 mcg of the toll-like receptor 9 (TLR9) agonist, CpG 1018, as the adjuvant formulated without preservatives (Food and Drug Administration 2018). A recombinant hepatitis B vaccine that is intended for adult patients with renal insufficiency uses alum and lipid A as adjuvants (Beran 2008). Since 1999, hepatitis B vaccines available in industrialized countries have not contained thimerosal as a preservative because of ill-founded concerns about possible neurodevelopmental effects of mercury (Centers for Disease Control and Prevention 1999). However, there is no evidence of any harmful effects from the small amounts of thimerosal in hepatitis B vaccine (Stratton et al. 2001). Thimerosal continues to be used as a preservative in multi-dose hepatitis B vaccine vials available in many countries and is particularly important in preventing bacterial contamination.

1.1 Recombinant DNA Vaccines

Most licensed recombinant DNA hepatitis B vaccines consist of a 226-amino-acid S gene product (HBsAg protein) (Sitrin et al. 1993). The yeast-produced vaccines, which are the most widely used, are manufactured by expression of HBsAg protein in genetically engineered yeast cells (*Saccharomyces cerevisiae*) that contain the S gene (McAleer et al. 1984). The expression plasmid generally contains only the 3′ portion of the S gene, and only the major HBsAg protein, without pre-S epitopes, is produced. As a result of biochemical and biophysical purification, there is no detectable yeast DNA and only trace amounts of yeast proteins (1%–5%) in the final vaccine products (DiMiceli et al. 2006). In the production of recombinant DNA hepatitis B vaccines, working seeds are derived from a master seed of the transformed yeast. Whenever a lot of vaccine is needed, yeast from the working seed is used to start the fermentation in large vessels. The HBsAg is then purified to eliminate yeast components by various physical separation techniques, including chromatography and filtration. The expressed HBsAg polypeptide self-assembles into immunogenic spherical particles closely resembling the natural 22-nm particles found in the serum of persons with chronic HBV infection. The *a* determinant that is responsible for the most important immune response is exposed on the surface of the artificial HBsAg particle, comparable to the natural particle. The artificial particles differ from natural particles only in the glycosylation of HBsAg. Similarly, HBsAg for Heplisav-B is expressed in a recombinant strain of *Hansenula polymorpha* yeast (Food and Drug Administration 2018).

In contrast to the production of yeast-derived non-glycosylated small envelope antigen, mammalian cells transfected with the genes coding for 2–3 of the HBV envelope proteins secrete the glycosylated viral surface antigen particles into the

growth medium from which they are purified by physical means. Mammalian-cell-derived vaccines (e.g., continuous mammalian cell line, like the mouse c127 clonal cell line) containing glycosylated pre-S1 and pre-S2 proteins, in addition to the major HBsAg protein, have also been produced in France, Germany, and Israel and are licensed in Israel, Hong Kong, India, the Philippines, and Vietnam (Shouval 2003; Zuckerman 2006). Evidence exists that vaccines containing the pre-S1 and pre-S2 proteins induce a faster and higher anti-HBs response as compared to yeast-derived S-containing vaccines and might be effective in persons who have genetic nonresponse to the major HBsAg protein. However, the high cost of production for vaccines with pre-S1 and pre-S2 antigens limits their use and availability (Zuckerman et al. 2001; Rendi-Wagner et al. 2006; Shouval et al. 2015).

1.2 Combination Vaccines

Several vaccine manufacturers have produced combination vaccines containing a hepatitis B vaccine component, in particular pediatric vaccines including diphtheria and tetanus toxoids and whole-cell pertussis (DTwP) or acellular pertussis (DTPa), *Haemophilus influenzae* type b conjugate (Hib), or inactivated poliovirus vaccine (IPV). For each of these combination vaccines, the manufacturer has shown that the components remain sufficiently immunogenic to elicit protective levels of anti-HBs (Diez-Delgado et al. 1997; Bruguera et al. 1996; West et al. 1997; Halperin et al. 2014).

2 Development of HBIG for Passive Immunization

The discovery that passively acquired anti-HBs can provide protection against acute hepatitis B and chronic HBV infection when administered soon after exposure led to the development and therapeutic use of HBIG, a specific immune globulin containing high concentrations of anti-HBs and later on to the development of hepatitis B vaccines. HBIG is prepared by fractionation from serum containing high concentrations of anti-HBs and that has been screened for HBsAg and antibodies to HIV and HCV. The process inactivates the potential presence of HIV and other viruses in the final product (Centers for Disease Control and Prevention 1986, 2011; Wells et al. 1986).

A major use of HBIG is as an adjunct to hepatitis B vaccine in preventing perinatal HBV transmission. Untreated, 70%–90% of infants born to HBeAg-positive mothers become infected at birth and develop chronic HBV infection (Xu et al. 1985; Stevens et al. 1979). Immunoprophylaxis with both HBIG and hepatitis B vaccine increases the efficacy of preventing perinatal HBV transmission from 85% to 95% and provides long-term protection (Xu et al. 1985; Stevens et al. 1985; Schillie and Murphy 2013). The lack of HBIG availability and costs can limit access to this intervention.

3 Vaccine Dosage, Schedule, and Duration of Protection

3.1 Vaccine Dosage

The quantity of HBsAg protein per dose that induces a protective immune response in infants and children varies by manufacturer (range, 2.5–10 μg) and by composition of the envelope protein(s) and is partially related to vaccine-production processes. In general, the vaccine dosage for infants and adolescents is 50% lower than that required for adults. There is no international standard of vaccine potency expressed in micrograms of HBsAg protein, and the relative efficacy of different vaccines cannot be assessed only on the basis of differences in HBsAg content (Costa et al. 2011).

Given the differences in the manufacturing processes, the quantity of HBsAg protein per dose that will induce a protective immune response differs among the various vaccine products depending on the recipient's age. Vaccines produced by each manufacturer have been evaluated in clinical trials to determine the age-specific dosage that achieves the maximal seroprotection rate. Persons who respond to hepatitis B vaccine (complete and correct schedule) with concentrations of anti-HBs of 10 milli-International Units (mIU)/mL or greater are protected against acute hepatitis B and chronic infection. Internationally marketed hepatitis B vaccines are considered immunologically comparable and can be used interchangeably (Seto et al. 1999).

Hepatitis B vaccine should be administered by intramuscular injection in the anterolateral aspect of the thigh of infants and children less than 24-months old, and in the deltoid muscle of older children, adolescents, and adults. Administration in the buttock is not recommended because of an association with decreased protective antibody levels in some studies, probably because of inadvertent subcutaneous injection or injection into deep fat tissue (Shaw et al. 1989).

3.2 Vaccine Schedule

Historically, the standard three-dose hepatitis B vaccine series has consisted of two priming doses administered 1 month apart and a third dose administered 6 months after the first dose (Schillie et al. 2018a). Multiple schedules have been used successfully: at birth and at 1 and 6 months of age; at 2, 4, and 6 months of age; at 3, 5, and 11 month of age; at 8, 12, 16 weeks and 12 or 15 months; and at 6, 10, and 14 weeks of age (Greenberg et al. 1996; Hadler and Margolis 1992; Schillie et al. 2018a; Da Villa et al. 1997; Aspinall and Kocks 1998; Goldfarb et al. 1994; Yusuf et al. 2000) (Table 8.1). Increasing the interval between the first and second dose of hepatitis B vaccine has little effect on immunogenicity or final antibody concentration (Middleman et al. 2001; Jilg et al. 1989). Longer intervals between the last two doses result in higher final antibody concentrations but not seroconversion rates (Thisyakorn et al. 2011). An anti-HBs concentration of 10 mIU/mL or more measured 1–3 months after administration of the last dose of the primary vaccination

Table 8.1 Hepatitis B vaccine schedules for infants, by infant birthweight and maternal HBsAg status

Birthweight	Maternal HBsAg status	Single-antigen vaccine		Single-antigen + combination vaccine	
		Dose	Age	Dose	Age
≥2000 g	Positive	1	Birth (≤12 h)	1	Birth (≤12 h)
		HBIG[a]	Birth (≤12 h)	HBIG	Birth (≤12 h)
		2	1–2 mos	2	2 mos
			6 mos[b,c]	3	4 mos
				4	6 mos[b,c]
	Unknown[d]	1	Birth (≤12 h)	1	Birth (≤12 h)
		2	1–2 mos	2	2 mos
		3	6 mos[b,c]	3	4 mos
				4	6 mos[b,c]
	Negative	1	Birth (≤24 h)	1	Birth (≤24 h)
		2	1–2 mos	2	2 mos
		3	6–18 mos[b,c]	3	4 mos
				4	6 mos[b,c]
<2000 g	Positive	1	Birth (≤12 h)	1	Birth (≤12 h)
		HBIG	Birth (≤12 h)	HBIG	Birth (≤12 h)
		2	1 mos	2	2 mos
		3	2–3 mos	3	4 mos
		4	6 mos[b,c]	4	6 mos[b,c]
	Unknown	1	Birth (≤12 h)	1	Birth (≤12 h)
		HBIG	Birth (≤12 h)	HBIG	Birth (≤12 h)
		2	1 mos	2	2 mos
		3	2–3 mos	3	4 mos
		4	6 mos[b,c]	4	6 mos[b,c]
	Negative	1	Hospital discharge or age 1 mo	1	Hospital discharge or age 1 mo
		2	2 mos	2	2 mos
		3	6–18 mos[b,c]	3	4 mos
				4	6 mos[b,c]

Abbreviations: HBIG = hepatitis B immune globulin; HBsAg = hepatitis B surface antigen

[a]HBIG should be administered at a separate anatomical site from vaccine

[b]The final dose in the vaccine series should not be administered before age 24 weeks (164 days)

[c]Mothers should have blood drawn and tested for HBsAg as soon as possible after admission for delivery; if the mother is found to be HBsAg positive, the infant should receive HBIG as soon as possible but no later than age 7 days

[d]Pediarix should not be administered before age 6 weeks

Schillie S, Vellozzi C, Reingold A, Harris A,Haber P, Ward JW, Nelson NP. Prevention of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on immunization practices. MMWR Recomm Rep 2018;67 (No. RR-1): 1–31

series is considered a reliable marker of protection against infection. Protection persists even if anti-HBs concentration declines to less than 10 mIU/mL over time.

A variety of hepatitis B vaccine schedules have been shown to induce levels of seroprotection of >95% in infants. Hepatitis B vaccine schedules that have been

demonstrated to induce seroprotection rates of >95% in adolescents include doses administered at 0, 1, and 6 months; 0, 2, and 4 months; and 0, 12, and 24 months (Jilg et al. 1989; Cassidy et al. 2001; Schiff et al. 1995; Milne et al. 1988; Centers for Disease Control and Prevention 2000). In addition, for adolescents aged 11–15 years, the adult dose of hepatitis B vaccine can be used for administration at 0 and at 4–6 months (Schillie et al. 2018a; Milne et al. 1988; Centers for Disease Control and Prevention 2000) (Table 8.2). This two-dose schedule produces

Table 8.2 Hepatitis B vaccine schedules for children, adolescents, and adults

Age group	Schedule[a] (interval represents time in months from first dose)
Children (1–10 years)	0, 1, and 6 mos
	0, 1, 2, and 12 mos
Adolescents (11–19 years)	0, 1, and 6 mos
	0, 12, and 24 mos
	0 and 4–6 mos[b]
	0, 1, 2, and 12 mos
	0, 7 days, 21–30 days, 12 mos[c]
Adults (≥20 years)	0, 1, and 6 mos
	0, 1, 2, and 12 mos
	0, 1, 2, and 6 mos[d]
	0, 7 days, 21–30 days, 12 mos[c]
	0,1 month[e]

[a]Refer to package inserts for further information. For all ages, when the HepB vaccine schedule is interrupted, the vaccine series does not need to be restarted. If the series is interrupted after the first dose, the second dose should be administered as soon as possible, and the second and third doses should be separated by an interval of at least 8 weeks. If only the third dose has been delayed, it should be administered as soon as possible. The final dose of vaccine must be administered at least 8 weeks after the second dose and should follow the first dose by at least 16 weeks; the minimum interval between the first and second doses is 4 weeks. Inadequate doses of hepatitis B vaccine or doses received after a shorter-than-recommended dosing interval should be readministered, using the correct dosage or schedule. Vaccine doses administered ≤4 days before the minimum interval or age are considered valid. Because of the unique accelerated schedule for Twinrix, the 4-day guideline does not apply to the first three doses of this vaccine when administered on a 0-day, 7-day, 21–30-day, and 12-month schedule (new recommendation)

[b]A2-dose schedule of Recombivax adult formulation (10 μg) is licensed for adolescents aged 11–15 years. When scheduled to receive the second dose, adolescents aged >15 years should be switched to a 3-dose series, with doses 2 and 3 consisting of the pediatric formulation administered on an appropriate schedule

[c]Twinrix is approved for use in persons aged ≥18 years and is available on an accelerated schedule with doses administered at 0, 7, 21–30 days, and 12 months

[d]A 4-dose schedule of Engerix administered in two 1 mL doses (40 μg) on a 0-, 1-, 2-, and 6-month schedule is recommended for adult hemodialysis patients

[e]HepB-CpG may be used as a HepB vaccine in persons aged ≥18 years recommended for vaccination against HBV

Schillie S, Vellozzi C, Reingold A, Harris A, Haber P, Ward JW, Nelson NP. Prevention of hepatitis B virus infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep 2018;67(No. RR-1): 1–31

Schillie, S, Harris A, Link-Gelles R, Romero Ward J, Nelson N. Recommendations of the Advisory Committee on Immunization Practices for use of a hepatitis B vaccine with a novel adjuvant. MMWR 2018 Apr 20; 67(15): 455–458

anti-HBs concentrations equivalent to those obtained with the pediatric dose administered on a three-dose schedule (Marsano et al. 1998).

Heplisav-B is a 2-dose vaccine series with each dose separated by 1 month. This two-dose series stimulates seroprotection of anti-HBs concentration of 10 mIU/mL or more among 90%–100% of persons 18 through 70 years of age versus 70.5%–90.2% with a licensed vaccine requiring three doses (CDC 2021).

3.3 Duration of Protection

Hepatitis B vaccine induces a protective antibody response in approximately 30%–55% of healthy adults aged <40 years after the first dose, in 75% after the second dose, and in more than 90% after the third dose (Zajac et al. 1986; Andre 1989). In adults older than 40 years, response rates decline with age; by age 60 years, protective levels of antibody develop in only 75% of vaccinated persons (Averhoff et al. 1998).

After primary immunization with hepatitis B vaccine, anti-HBs concentrations decline rapidly within the first year and more slowly thereafter. Among children who respond to a primary three-dose vaccination series with anti-HBs concentrations of 10 mIU/mL or greater, 15%–50% have low or undetectable concentrations of anti-HBs 5 to 15 years after vaccination (Huang et al. 1999; Resti et al. 1997; Ding et al. 1993; Liao et al. 1999; Coursaget et al. 1994; Viviani et al. 1999; Mintai et al. 1993; Schönberger et al. 2013).

Among adult vaccinees, anti-HBs concentrations decline to less than 10 mIU/mL in 7%–50% within 5 years after vaccination and in 30%–60% within 9–11 years after vaccination (Hadler et al. 1991; Wainwright et al. 1997; Goh et al. 1995; Couroucé et al. 1988; Gibas et al. 1988). Despite decrease or loss of vaccine-induced anti-HBs over time, observational studies have shown that a primary series of hepatitis B vaccine can prevent infection for more than 25 years (Wu et al. 2011; But et al. 2008; Poovorawan et al. 2011; Roznovsky et al. 2010; Bialek et al. 2008; Fitzsimons et al. 2005; Kao et al. 2009; Su et al. 2007; Hammitt et al. 2007; van der Sande et al. 2006, 2007; Jafarzadeh and Montazerifar 2006; Alfaleh et al. 2008; FitzSimons et al. 2013; Gilca et al. 2013; Poovorawan et al. 2013; Spada et al. 2014; Zhu et al. 2011; Ni et al. 2012; Mendy et al. 2013; Shen et al. 2015; Bruce et al. 2016).

A recent follow-up and response to a hepatitis B vaccine challenge study in Alaska confirms earlier observation of long-term protection, showing evidence of protection (anti-HBs >10 IU/L or response to challenge dose) in 94% of persons and no chronic infections 30 years after vaccine administration (Bruce et al. 2016). This is similar to other long-term cohort studies conducted in other parts of the world carried out through 20–25 years of follow-up (Ni et al. 2012; Mendy et al. 2013). Indeed, in immunocompetent individuals, the specific immunity to HBsAg outlasts the presence of vaccine-induced antibodies, conferring effective long-term protection against acute disease and development of HBsAg carriage, even in those showing waning or disappearance of anti-HBs (Banatvala et al. 2000; Banatvala and Van

Damme 2003; Jilg et al. 1990; West and Calandra 1996; Hall 2010). The mechanism for continued vaccine-induced protection is thought to be preservation of immune memory through selective expansion and differentiation of clones of antigen-specific B and T lymphocytes (Banatvala et al. 2000; Banatvala and Van Damme 2003).

To illustrate the prolonged duration of protection against hepatitis B even after disappearance of antibodies, an anamnestic response was measured shortly after offering a challenge dose (i.e., the "boost ability") in cohorts of vaccinees up to 30 years after primary vaccination (Hammitt et al. 2007; van der Sande et al. 2007; Jafarzadeh and Montazerifar 2006; Jan et al. 2010; Zanetti et al. 2005; Lu et al. 2004; Williams et al. 2003; Boxall et al. 2004; Gabbuti et al. 2007; Duval et al. 2005; Samandari et al. 2007; Lu et al. 2008; Zinke et al. 2009). Anamnestic responses have been demonstrated in 62% to more than 80% of subjects vaccinated 15 to 30 years earlier, indicating that a high proportion of vaccine recipients retain immune memory and would develop an anti-HBs response on exposure to HBV (Bruce et al. 2016).

Recently, increasing evidence suggests that the immune memory may begin to wane after the second decade of vaccination; however, this does not imply increased susceptibility to clinically significant HBV disease, as no acute or chronic HBV cases were reported in any of the ongoing follow-up studies (Bialek et al. 2008; Su et al. 2007; Jan et al. 2010; Samandari et al. 2007; Lu et al. 2008). Thus, absence of an anamnestic response after such booster vaccination may not necessarily mean that individuals are susceptible to HBV infection (Hammitt et al. 2007; Leuridan and Van Damme 2011). Further research in this area is needed.

Some long-term studies have documented breakthrough infections illustrated by the seroconversion to anti-HBc, but virtually no clinically significant infections (acute diseases or carriage) were reported (Wainwright et al. 1997; van der Sande et al. 2006; Jafarzadeh and Montazerifar 2006; Lu et al. 2004, 2008; Boxall et al. 2004; Dentinger et al. 2005; McMahon et al. 2005; Mele et al. 2001; Young et al. 2003; Poovorawan et al. 2009). From a public health perspective, prevention of carriage remains of utmost importance: in a 20-year evaluation of the hepatitis B universal immunization program in the Gambia, Viviani and coworkers showed a vaccine efficacy of 67% against development of anti-HBc, and 97% against HBsAg carriage (Viviani et al. 2008). In a more recent evaluation of the infant vaccination program in the Gambia, a 94% vaccine effectiveness was observed in fully vaccinated infants. Many of these fully vaccinated individuals had at some time been infected and experienced a nonsignificant breakthrough infection (anti-HBc positivity of 27%); chronic active hepatitis was not common and probably a consequence of perinatal infection. The study concluded that full infant HBV vaccination does provide strong protection against chronic HBV infection, but less protection against ever having HBV infection (Peto et al. 2014). Persons who have received only one or two doses of vaccine can also have detectable anti-HBs. However, long-term protection has been demonstrated only for immunocompetent persons who have completed a licensed vaccination series and have ever had an anti-HBs concentration of 10 mIU/mL or greater (Leuridan and Van Damme 2011).

On the basis of currently available scientific evidence, the WHO and advisory groups in the USA and Europe do not recommend routine booster doses of hepatitis B vaccine or periodic serologic testing to monitor anti-HBs concentrations for immunocompetent persons who have responded to vaccination or in universal immunization programs (Schillie et al. 2018a; Fitzsimons et al. 2005; European Consensus Group on Hepatitis B Immunity 2000). For the next decade, additional data are needed regarding the potential need for boosters and the duration of protection against infection and disease after hepatitis B vaccination, including information on the potential role of natural subclinical boosting.

4 Vaccine-Associated Adverse Events

Adverse events after immunization against hepatitis B are infrequent and generally mild. With the exception of localized pain, placebo-controlled studies have revealed that reported events (e.g., myalgia and transient fever) occur no more frequently among vaccinees than among persons receiving placebo (<10% among children, 30% among adults) (World Health Organization 2011). Data from numerous long-term studies fail to causally link serious adverse events to hepatitis B vaccination (Institute of Medicine 2002). Reports of severe anaphylactic reactions are very rare, and data do not indicate a causal association between hepatitis B vaccine and Guillain-Barré syndrome or demyelinating disorders, including multiple sclerosis. However, hepatitis B vaccine has been the subject of greatest concern regarding multiple sclerosis in France. In 1998, French media revealed possible occurrence of post-hepatitis B immunization multiple sclerosis. That same year the French health authorities abruptly ended routine school-based vaccination of adolescents, and adult vaccination began to be less widespread. Several epidemiologic studies conducted to evaluate the association between hepatitis B vaccination and multiple sclerosis have found no link. A recent nested case-control study using data from an US health maintenance organization (2008–2011) showed no longer-term association between hepatitis B vaccination and the risk of multiple sclerosis or other acquired central nervous system demyelinating syndromes, which argues against a causal association (Langer-Gould et al. 2014). Furthermore, no epidemiologic data support a causal association between hepatitis B vaccination and chronic fatigue syndrome, arthritis, autoimmune disorders, asthma, sudden infant death syndrome, alopecia, or diabetes (Mikaeloff et al. 2007; Yu et al. 2007; Duclos 2003; Schwalbe et al. 1998). The WHO's Global Advisory Committee on Vaccine Safety as well as the recent report of the Institute of Medicine confirms the excellent safety profile of hepatitis B vaccine and continues to monitor the safety of this vaccine (Stratton et al. 2011).

5 Development of New Hepatitis B Vaccines

Existing hepatitis B vaccines are highly effective, and there is no evidence that new vaccines will be needed to eliminate HBV transmission in immunocompetent populations with recommended immunization strategies. Potential uses for new hepatitis

B vaccines include enhancing seroprotection in nonresponders to existing vaccines (using pre-S1 and/or pre-S2, or adding new vaccine adjuvants instead of aluminum hydroxide) (Shouval et al. 2015; Rottinghaus et al. 2003; Jacques et al. 2002; Cooper and Mackie 2011; Leroux-Roels 2015; Janssen et al. 2015a; Said and Abdelwahab 2015), decreasing the number of doses required for seroprotection, and providing protection against HBV infection with S-mutant viruses (Zuckerman 2006; Janssen et al. 2015a, b; Shapira et al. 2001).

On November 9, 2017, Heplisav-B (HepB-CpG), a single-antigen HepB vaccine with a novel immunostimulatory sequence adjuvant, was approved by the Food and Drug Administration for the prevention of HBV in persons aged ≥18 years. HepB-CpG contains yeast-derived recombinant HepB surface antigen (HBsAg) and is prepared by combining purified HBsAg with small synthetic immunostimulatory cytidine-phosphate-guanosine oligodeoxynucleotide (CpG-ODN) motifs (1018 adjuvant). The 1018 adjuvant binds to toll-like receptor 9 to stimulate a directed immune response to HBsAg (WHO 2017a; Shapira et al. 2001; Schillie et al. 2018b). In studies involving 7056 persons receiving two doses of HepB-CpG compared with protection among 3214 persons receiving three doses of Engerix-B, seroprotection (antibodies to HBsAg, anti-HBs ≥10 mIU/mL), was achieved in 90.0%–100.0% of Heplisav-B compared with 70.5%–90.2% of subjects receiving a three-dose series of Engerix-B (Shapira et al. 2001; Schillie et al. 2018b; Janssen et al. 2013; Halperin et al. 2006, 2012; Heyward et al. 2013; Jackson et al. 2018). The addition of the new adjuvant increases the immune response eliciting a more robust immune response and higher levels of seroprotection with fewer doses. When comparing mild adverse events, serious adverse events, and cardiovascular events, subjects receiving Heplisav-B and Engerix had 45.6%, 5.4%, and 0.27% and 45.7%, 6.3%, and 0.14%, respectively. Monitoring to detect cardiovascular events after vaccination will be conducted as part of Heplisav-B post-marketing surveillance (Schillie et al. 2018b). The two-dose Heplisav-B vaccine is considered non-inferior to three-dose vaccines licensed for use in the USA. However, the higher levels of protection observed for persons with chronic liver disease, diabetes mellitus, and on renal dialysis suggest this hepatitis B vaccine, when used in routine clinical practice, can help achieve long-term protection against hepatitis B in populations often hyporesponsive to hepatitis B vaccination. Indeed, an economic model suggests the adult two-dose vaccine series of Heplisav-B is a cost-saving option for improving series completion and reducing morbidity and mortality among at-risk adult populations including adults with diabetes, chronic kidney disease, obesity, and HIV and for older adults and persons who inject drugs (Rosenthal et al. 2020).

It has been hypothesized that a lack in vaccine efficacy might be explained by a mismatch between vaccine genotype and endemic HBV genotype (adw versus ayw); if this hypothesis is proven, the use of a modified HBV vaccine in specific regions could be considered (Davies et al. 2013; Littlejohn et al. 2014). Vaccines generating strong T-cell immunity with purified or recombinant vaccine antigens, along with intradermal, oral, and nasal hepatitis B vaccines that have potential to simplify administration and cost less than injections, are also under investigation (Langer-Gould et al. 2014; Filippelli et al. 2014; Rajkannan et al. 2006; Coffman et al. 2010; Almeida and Borges 2015).

To date, chronic hepatitis B remains an incurable disease. Despite the tremendous progress made in developing nucleoside/nucleotide analogs capable of efficiently suppressing HBV replication, these agents do not eliminate the cccHBV-DNA residing in the nuclei of infected hepatocytes. Consequently, developing therapeutic immunization for persons with chronic HBV infection and restoring the defective immune tolerance to HBV remain important goals (Michel et al. 2015). Based on a small number of clinical trials, the available therapeutic vaccine candidates do not appear to be efficacious for the treatment of chronic HBV (Lim et al. 2019; Van Damme et al. 2018).

6 Recommendations for Hepatitis B Vaccination

HBV infection can occur at any age through multiple routes of transmission, and all persons at risk for HBV infection can benefit from immunization. However, the greatest health impact is achieved through childhood vaccination, as children infected with HBV at birth or early childhood are at greatest risk for developing chronic HBV infection and HBV-related liver disease in later life. Approximately 90% of HBV-infected infants <1 year of age develop chronic HBV infection compared with 30% of children infected between ages 1 and 4 years and <5% of persons infected as adults (Michel et al. 2015). Approximately 25% of persons who became chronically infected during childhood are at risk for death from HBV-associated liver cancer and cirrhosis (Beasley and Hwang 1991), a risk that is highest for children infected at birth. As a result, an estimated 21% and 48% of deaths from hepatitis are attributed to HBV infections occurring at birth or in early childhood, respectively (Goldstein et al. 2005).

Global recommendations for HBV prevention and control are set by the WHO in consultation with the Strategic Advisory Group of Experts on Immunization (SAGE). In 2017, the WHO updated the recommendations for hepatitis B vaccination (WHO 2017a). The WHO recommends all children worldwide receive the three-dose hepatitis B vaccination series. Reaching all children with at least 3 doses of hepatitis B vaccine should be the standard for all national immunization programs. The WHO also recommends all infants receive a birth dose of hepatitis B vaccine within 24 h of delivery. National strategies to prevent perinatal transmission should ensure high and timely coverage of the birth dose through a combination of strengthened maternal and infant care at birth with skilled health workers present to administer the vaccine, and innovative outreach strategies to provide vaccine for infants born at home. Lastly, the WHO recommends, when resources are available, hepatitis B vaccination of persons at high risk of HBV infection in older age groups and catch-up vaccination of unvaccinated cohorts.

In 2016, the WHO sets goals for the elimination of hepatitis B and hepatitis as a public health threat defined as a 90% reduction in incidence and 65% reduction in mortality by 2030 (WHO 2016a). The hepatitis B vaccination is the cornerstone of this global initiative to eliminate hepatitis B. The United Nations, with the launch of Sustainable Development Goals, called on the world to combat viral hepatitis

(United Nations General Assembly 2020). For the elimination of HBV mother to child transmission (EMTCT), the UN SDG and the WHO set a target for reducing global HBsAg prevalence to <1% among children <5 years of age by 2020 and as targeted by the WHO to <0.1% by 2030. Achievement of global goals for hepatitis B elimination will require continued improvements in hepatitis B vaccination coverage and integrating HepB vaccination with other strategies that improve prevention of HBV transmission and disease.

Recommendations for Pre- and Post-Exposure Prophylaxis

6.1 Infants Born to HBsAg-Positive Mothers

For infants born to HBsAg-positive mothers, protection increases to 95% when hepatitis B vaccination is augmented with a dose of hepatitis B immunoglobulin (HBIG) (Lee 2006; Xu et al. 1985; Schillie et al. 2015, 2018a). The USA and other countries recommend HBsAg screening of pregnant women and delivery of both HBIG and hepatitis B vaccination immediately after birth (i.e., <12 h) to infants of HBsAg-positive mothers.

6.2 HBeAg-Positive Pregnant Women

Evidence is growing that antiviral prophylaxis given in the last trimester of pregnancy to HBeAg-positive women or women with a high viral load of HBV can lower the risk for perinatal transmission of HBV (Funk et al. 2013, 2021; Greenup et al. 2014; Pan et al. 2016; Shi et al. 2010; Bzowej 2010; Dionne-Odom et al. 2015; Bleich and Swenson 2014; WHO 2020a). Infants born to these women are at greatest risk for "breakthrough" HBV infection despite receiving HBIG and timely birth dose of hepatitis B vaccine. In a recent trial involving pregnant women with high viral loads (i.e., >200,000 IU/ml) and their infants (all of whom received HBIG/hepatitis B vaccine within 12 h of birth), HBV transmission was detected in only 5% of infants born to mothers receiving antiviral prophylaxis compared with 18% of infants who received HBIG/hepatitis B vaccination alone (Pan et al. 2016).

A meta-analysis of TDF prophylaxis (300 mg/day) for pregnant women plus a timely HepB birth dose and HBIG for their infants had a protective benefit with an odds ratio for transmission of 0.10 (95% CI: 0.03–0.35) with no significant findings of major adverse events for mothers or infants (Shi et al. 2010). In 2018, ACIP and CDC recommended HBV-DNA testing for all HBsAg+ pregnant women to guide clinical decisions for TDF prophylaxis particularly for women with HBV-DNA > 200,000 IU/ml (Schillie et al. 2018a). In 2020, the WHO also recommended HBV-DNA testing for HBsAg+ women and. In 2020, the WHO recommended HBsAg testing for all pregnant women, HBV PCR or HBeAg testing for HBsAg+ expectant mothers, and TDF prophylaxis for women with HBV-DNA level > 200,000 IU/mL (WHO 2020a).

6.3 Persons with Percutaneous or Sexual Exposures

HBIG is indicated for post-exposure prophylaxis (PEP) after needle stick or other percutaneous injuries (Schillie et al. 2018b; Seeff et al. 1978; Redeker et al. 1975) and for preventing clinical hepatitis B or chronic HBV infection after sexual exposure to an acutely infected partner when administered within 7 days of exposure (Gallagher and Lipsitch 2019). Studies estimate about 85% effectiveness of HepB vaccine and HBIG prophylaxis in prevention of HBV transmission after percutaneous exposures in occupational and non-occupational settings (Gallagher and Lipsitch 2019). Both passive-active PEP with HBIG and hepatitis B vaccine and active PEP with hepatitis B vaccine alone have been demonstrated to be highly effective in preventing transmission after exposure to HBV (Mitsui et al. 1989a, b; Roumeliotou-Karayannis et al. 1985; Papaevangelou et al. 1988).

For management of occupational percutaneous exposures to blood and other body fluids of HBsAg + patients or those of unknown HBsAg status: The US ACIP recommends (1) no HepB vaccine for healthcare staff with verified receipt of a three-dose HepB vaccination series and documented anti-HBs ≥10 mIU/mL and (2) one dose of HBIG and one dose of HepB vaccine administered as soon as possible after the exposure for previously unvaccinated staff and for staff with verified receipt of the three doses of HepB vaccination series and documented anti-HBs <10 mIU/mL. All staff should complete the HepB vaccination series (Schillie et al. 2018b).

For identifiable exposures to blood or body fluids that contain blood of HBsAg+ among persons in non-occupational settings, ACIP recommends (1) a single dose of HepB vaccine for exposed persons with documented receipt of complete HepB vaccine series and (2) HBIG and HepB vaccine as soon as possible after the exposures preferably within 24 h for unvaccinated persons and persons in the process of receiving a HepB vaccination series. All persons should complete the HepB vaccination series.

7 Recommendations for Pre- and Post-vaccination Serologic Testing

Globally, prevaccination serologic testing is not recommended as routine practice. HBV testing is not a requirement for vaccination, and in settings where testing is not feasible, vaccination of recommended persons should continue (Schillie et al. 2018b). However, serologic screening with a single test (anti-HBc) or with a panel of tests (e.g., anti-HBs, anti-HBc, and HBsAg) can identify HBsAg-positive persons in need of evaluation for chronic HBV and related liver disease while also defraying costs by avoiding unnecessary vaccination of persons already infected with HBV (Schillie et al. 2018b; Weinbaum et al. 2008). In 2018, the US CDC recommended prevaccination testing for ten categories of potential vaccine recipients:

- Household, sexual, or needle contacts of hepatitis B surface antigen (HBsAg)-positive persons
- HIV-positive persons

- Persons with elevated alanine aminotransferase/aspartate aminotransferase of unknown etiology
- Hemodialysis patients
- Men who have sex with men
- Past or current persons who inject drugs
- Persons born in countries of high and intermediate hepatitis B virus (HBV) endemicity (HBsAg prevalence ≥2%)
- US-born persons not vaccinated as infants whose parents were born in countries with high HBV endemicity (≥8%)
- Persons needing immunosuppressive therapy, including chemotherapy, immunosuppression related to organ transplantation, and immunosuppression for rheumatologic or gastroenterologic disorders
- Donors of blood, plasma, organs, tissues, or semen (Schillie et al. 2018b).

8 Strategies for Increasing Hepatitis B Vaccination of Newborns

Given the strong association between risk for chronic HBV infection, liver disease, and age at infection, hepatitis B vaccination is most important for children aged <5 years, particularly newborns. Vaccination of children in this age group requires an immunization strategy unique to hepatitis B prevention: delivery of a "birth dose "immediately after birth of the child. Among infants born to HBsAg-positive mothers, a birth dose of hepatitis B vaccine reduces the risk for perinatal HBV transmission by at least 72% (Schillie and Murphy 2013); additional receipt of HBIG increases efficacy to 95%–97%. The timing of the delivery of hepatitis B vaccination is important, because protection afforded by vaccination wanes over time. Accordingly, the WHO recommends a dose of hepatitis B vaccine be provided to all infants as soon as possible after birth, preferably within 24 h (WHO 2017a).

Ensuring that all infants receive a dose of hepatitis B vaccine within the first 24 h of life requires integration of hepatitis B vaccination with other maternal and child health services provided by birth attendants (WHO 2015). As such, delivery of a birth dose of hepatitis B vaccine is most readily achieved when deliveries are managed in birthing facilities. In Laos from 2011 to 2012, 70% of infants delivered in 31 birth facilities received a birth dose of hepatitis B vaccine, whereas only 41% of newborns delivered at home were vaccinated at birth despite provision of assistance by 17 healthcare facilities (CDC 2013).

In many areas of the world with high prevalence of HBV infection, most births occur at home rather than in healthcare settings. In sub-Saharan Africa, only 24.6% (173,885/708143) of women delivered their babies in a health facility (Breakwell et al. 2017). Interventions that improve birth dose coverage for home births include training for birth attendants, systems to notify attendants of pending deliveries, and education to help communities understand the benefits and safety of hepatitis B vaccine (Creati et al. 2007; Nguyen et al. 2014; Wu et al. 2015; Levin et al. 2005). Because hepatitis B vaccines are heat stable, they can be used outside the cold chain

for newborn vaccination in settings that lack refrigeration, including patient's homes (Averhoff et al. 1998).

9 Strategies for Increasing Hepatitis B Vaccination of Children, Adolescents, and Adults

All infants should receive hepatitis B vaccine as part of the routine infant immunization schedule (see Dosage, Schedule, and Duration of Protection) (WHO 2017a). Over time, as cohorts of vaccinated children age, an increasing proportion of the population is immune to HBV infection, further helping reduce new infections. "Catch-up" vaccination strategies can expedite this process by targeting older children, adolescents, and adults too old to receive vaccine when routine hepatitis B vaccination of infants began. The WHO recommends the following as possible targets for HepB catch-up vaccination: age-specific cohorts (e.g., young adolescents before initiation of sexual activity) and persons with risk factors for acquiring HBV infection (e.g., prisoners, transplant recipients, injecting drug users, sex workers, those living with HBV-infected persons) (WHO 2017a).

Mandatory hepatitis B vaccination for school and university entry can increase acceptance of hepatitis B vaccination and vaccination coverage among older children and adolescents. Vaccination programs also can target adolescents and adults with behavioral or occupational risks for HBV infection. Populations identified by the WHO as possible targets for hepatitis B vaccination programs include recipients of blood or blood products, dialysis patients, recipients of solid organ transplantations, incarcerated persons, persons who inject drugs, household and sexual contacts of people with chronic HBV infection, people with multiple sexual partners, and healthcare workers and others with occupational exposures to blood and blood products. The WHO also recommends that travelers to HBV-endemic areas complete the hepatitis B vaccination series before departure (WHO 2017a).

The integration of hepatitis B vaccination into settings where persons at risk for HBV infection receive care (e.g., HIV and correctional care settings) can improve vaccination coverage (Schillie et al. 2018b). Employee standards and mandates improve hepatitis B vaccination coverage among healthcare workers, first responders, and other groups at risk for occupational exposure (Lehman et al. 2012; Agerton et al. 1995; Roup 1993).

10 Rates of Vaccine Coverage

Hepatitis B vaccine has been available since the early 1980s. However, the cost of vaccine, concerns about plasma-derived vaccines transmitting the agent causing AIDS, and the lack of global vaccine policies impeded efforts to immunize infants and children against hepatitis B. By 1991, only 20 countries had implemented routine infant immunization against hepatitis B (Schillie et al. 2018a; Kane 1998).

In 1992, the WHO recommended all countries incorporate hepatitis B vaccination into their national childhood immunization services (Kane 1998; World Health Organization 1992), and by 1998, a total of 90 countries were routinely administering hepatitis B vaccine to infants. However, by the end of 2000, only 32% of the global birth cohort had received three doses of hepatitis B vaccine, with most unvaccinated infants living in developing countries (Beeching 2004).

In the following decade, hepatitis B vaccination coverage grew rapidly. By 2010, hepatitis B vaccination coverage among infants had reached an estimated 75% worldwide (CDC 2011). Contributing to improvements in vaccination coverage was availability of low-cost recombinant vaccines, which decreased the price of hepatitis B vaccine from $3 to $6 per dose in the 1990s to $0.25 per dose over the following decade (Zuber et al. 2011). Further, hepatitis B vaccine was originally available only in single-antigen formulations. By the early 2000s, HBV antigens were incorporated into combination vaccines (first as tetravalent [DTwP-HepB] and then pentavalent [DTwP-HepB-Hib] formulations), greatly simplifying vaccine delivery.

In 2000, the GAVI Alliance (previously the Global Alliance for Vaccines and Immunization) was formed to help low-income countries purchase and deliver childhood vaccinations. The first GAVI-supported vaccine initiative was hepatitis B vaccination. From 2000 through 2008, GAVI facilitated the immunization of 194 million (68%) of the 286 children fully immunized against hepatitis B during this period. By 2004, 50% of the low-income countries receiving GAVI Alliance support had included hepatitis B vaccines in their routine immunization program, and by the end of 2014, all GAVI-eligible countries had done so. However, of the 48 countries eligible for financial support from GAVI (as of January 2018), 37 countries (77%) had not introduced universal HepB birth dose vaccination (Li et al. 2018). In 2018, the GAVI Board, depending on funding availability for 2021–2025, extended support for implementation of hepatitis B birth dose for Gavi-eligible countries (Annex C 2018).

In China, GAVI Alliance collaborations resulted in administration of a birth dose of hepatitis B vaccine, free of charge, to more than 25 million newborns living in the poorest and most remote provinces of western and central China; this initiative was expanded in 2002 to provide free hepatitis B vaccine to all newborns born in China (Cui et al. 2013). Similar GAVI collaborations have occurred worldwide. It is anticipated that by 2020, hepatitis B vaccination in GAVI-eligible countries will have averted more than 4.8 million HBV-related deaths (Cui et al. 2013).

As of 2019, global coverage of infant HepB three-dose immunization was 85% with 189 countries (>95%) routinely vaccinating infants against HBV infection (WHO 2019a). HepB three-dose coverage varies regionally ranging from 94% coverage in the Western Pacific Region to 73% coverage in the African region (Li et al. 2018; WHO 2021). As of 2016, global coverage of hepatitis B birth dose vaccination was 43% with 101 (52%) of 194 WHO Member States having policies for universal HepB birth dose vaccination with another 10 countries vaccinating newborn of HBsAg+ mothers. In 2016, HepB birth dose coverage is highest for the Western Pacific, 83%, and lowest, 10%, coverage in the African region (Li et al. 2018).

11 Impact of Vaccination

Hepatitis B immunization is significantly reducing HBV incidence and prevalence among vaccinated cohorts. From 1990 to 2015, pediatric HepB vaccination prevented an estimated 310 million new chronic HBV infections. Globally, from 2000 to 2019, hepatitis B vaccination of newborns and infants prevented 22 million deaths averting one death for every 13 persons vaccinated (Fig. 8.1). Only the measles vaccine has a greater impact in lives saved. As deaths from HBV infection tend to occur in mid to late adulthood, improvements in HepB vaccination over the last several decades are projected to prevent an additional 16 million deaths from 2020 to 2030.

Hepatitis B vaccines are affordable, and effective prevention strategies are being deployed worldwide. Hepatitis B vaccination is now one of the most cost-effective public health interventions available, yielding a cost per life saved of $4 to $36 in low-income countries (Nayagam et al. 2016). Indeed, in a report to the UN secretary-general, delivery of hepatitis B immunization was considered a "best buy" intervention because it is inexpensive, highly cost-effective, and culturally acceptable. Cost-benefit analysis has shown that perinatal hepatitis B prevention and routine infant hepatitis B immunization can save a net $1 in medical and work-loss costs for each $1 spent on immunization (Margolis et al. 1995). Other vaccine-based strategies, including universal adolescent vaccination and vaccination of at-risk adult populations, also have been found to be cost-effective or cost-saving (Margolis et al. 1995; Hu et al. 2008; Beutels et al. 2002; Tu et al. 2009).

Many studies conducted in high-HBV-endemicity areas have demonstrated declines in the prevalence of chronic HBV and related disease after implementation of routine infant immunization. For example, Taiwan, a country with previously high endemicity for hepatitis B, substantially decreased the burden of hepatitis B after the 1984 launch of routine hepatitis B vaccination of newborns. From 1984 to 2004, the HBsAg prevalence in persons <20 years of age decreased from 9.8% to 0.6% (Ni et al. 2007; Chen et al. 1996). A study conducted 22 years after

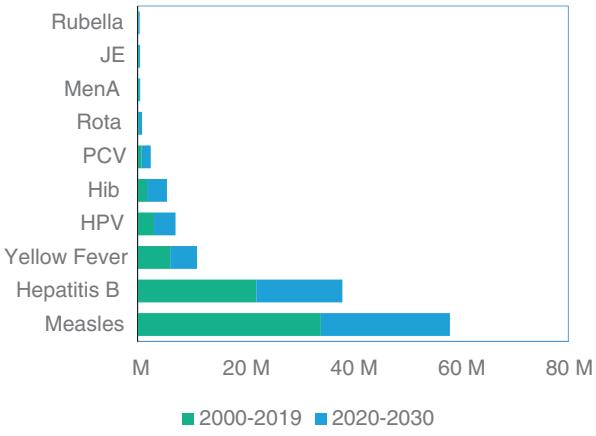

Fig. 8.1 Deaths averted by hepatitis B vaccination in comparison to other vaccine preventable diseases, 2000–2019 and 2020–2030. Source Li X, et al. Lancet 2021;39:398–408

introduction of HepB vaccination in Thailand found a reduction in HBsAg+ prevalence from 4.5% to 0.6% when comparing persons born before and after the start of routine HepB (Posuwan et al. 2016). In 1984, the Gambia implemented a universal infant immunization program. By 2003, childhood HBsAg prevalence had decreased in that country from 10% to 0.5% (Whittle et al. 2002). In Malaysia, after hepatitis B vaccination was implemented, HBsAg seroprevalence among children aged 7–12 years decreased from 1.6% (1997) to 0.3% (2003) (Ng et al. 2005).

In 2002, the national government of China, another highly endemic country, committed to covering hepatitis B vaccine-related costs and integrated hepatitis B vaccination into the routine infant immunization schedule, with a priority for administering a birth dose of hepatitis B vaccine to newborns within 24 h of delivery, dramatically reduced HBV prevalence through vaccination (Liu et al. 2019; Cui et al. 2009, 2017). From 2002 to 2015 infant and birth dose HepB vaccination coverage increased from 70% to >99.6% and 71% to 94%, respectively. To assess progress in HBV prevention, national surveys were conducted in 1992, 2006, and 2014. HBsAg prevalence among children <5 years of age progressively declines from 9.9% in 1992 to 1.0% in 2006 to 0.3% in 2014 (Cui et al. 2009). Over this period, HBsAg prevalence among 1–29-year-olds declined from 10.1% to 2.6%. In total, the HepB vaccination program in China prevented 28 million chronic HBV infections and 5 million deaths from HBV-related deaths (Liu et al. 2019).

Routine hepatitis B vaccination has also impacted rates of HBV infection in non-endemic countries. In 1991, Italy implemented universal infant and adolescent hepatitis B vaccination. Since introduction of routine hepatitis B vaccination, the overall incidence of acute hepatitis B declined from 5 cases per 100,000 in 1990 to 0.9 cases in 2010 (Romanò et al. 2012).

Routine hepatitis B vaccination for infants was implemented in the USA during 1991 (Schillie et al. 2018b). In future years, this strategy was expanded to include recommendations for catch-up vaccination of older children and adolescents (1996), all newborns (2006), and persons with diabetes (2011). In 2018, the national guidance from the US CDC recommended a birth dose of hepatitis B vaccine within 24 h of birth. To guide management, pregnant women found to have HBsAg on maternal testing are recommended to receive an HBV-DNA test to guide the administration of antiviral prophylaxis for women with high viral loads of HBV. The guidelines propose maternal antiviral therapy for women with HBV-DNA > 200,000 IU/ (Schillie et al. 2018a). In 2016–2017, a total of 91.4% of infants received at least three doses of HepB vaccine (CDC 2016). A total of 76% of infants received a birth dose within the first 3 days of life. However, HepB birth dose coverage ranges widely, from 31% to 82%, across states in the country. However, hepatitis B vaccination coverage remains low among adults (CDC 2017). In 2017, reported hepatitis B vaccination coverage (>3 doses) was 25.8% for adults >19 years, 34.3% for adults 19–49 years, and 16.6% for adults >50 years.

In the USA, improvements in vaccination have contributed to large declines in the incidence of hepatitis B (CDC 2018). From 1985 to 2018, the rates of reported acute HBV infection declined from 11.5 per 100,000 to 1.0 per 100,000 cases. From this rate of reporting in 2018, the US CDC estimates approximately 21,600 persons

were newly infected with HBV. From 2011 to 2018, HBV incidence has remained relatively stable with an estimated 18,000–22,000 acute HBV infections annually. In 2015, persons aged ≤19 years had the lowest incidence (0.02 cases per 100,000 population) likely a result of routine infant vaccination. National health surveys conducted in 2015–2016 of the non-institutionalized US population demonstrate approximately 23% of the population has serologic evidence of vaccine-induced protection (i.e., anti-HBs alone) which is highest among younger adults (18–29 age group) at 45.5%, followed by the 30–49 age group at 24.4%, and lowest among the older age group (50 and above) at 12.8% (Roberts et al. 2015, 2021; Le et al. 2020). Data from the same national surveys estimate 11.10 million (95% CI, 9.91–12.44 million) persons have been exposed to HBV (HBcAb+) in the USA. From 1999–2016, the prevalence of HBV exposures in the USA declined from 5.80% to 4.79%. Of persons exposed to HBV infection, an estimated 817,000 [95% CI (613,000, 1,100,000)] or 0.3% [95% CI (0.2, 0.4)] non-institutionalized persons >15 years of age have chronic hepatitis B infection. Non-Hispanic Asian persons had the highest HBV prevalence (3.41%) followed by non-Hispanic blacks (0.69) and whites (0.11). Of HBsAg+ persons in the USA, an estimated 563,000 [95% CI (445,000, 657,000)] (or 68.9%) were foreign born. Of foreign-born HBsAg+ persons, 69% migrated from Asia and 14% from Africa. This trend in hepatitis B prevalence is a consequence of the migration of HBV-infected persons to the USA (Le et al. 2020; Wong et al. 2021; Mitchell et al. 2011). Indeed, an estimated 1.47 million foreign-born persons with CHB are residing in the USA (Wong et al. 2021). Improvements in hepatitis B vaccination not only impact countries with moderate-to-high prevalence of hepatitis B, but benefit the USA and other low-endemic countries receiving migrants from areas of the world with higher HBV prevalence.

12 Targets and Goals for Eliminating New Cases of HBV

Elimination of HBV transmission is now formally established as a global health goal (WHO 2016a; United Nations General Assembly 2020). In 2015, the United Nations, with the launch of Sustainable Development Goals, called on the world to "combat viral hepatitis" (United Nations General Assembly 2020). In response to a request from the World Health Assembly, the WHO developed a framework for global action, with the goal of eliminating HBV and hepatitis C virus (HCV) as public health threats by 2030 (WHO 2016a). The WHO set goals for a 90% reduction in HBV transmission and 65% reduction in HBV mortality by 2030, with interim goals of 30% and 10% reductions by 2020, respectively. To achieve these goals, the WHO is calling on countries to implement a comprehensive hepatitis B virus immunization program with four attributes: (a) including hepatitis B vaccine in national childhood immunization schedules; (b) strengthening hepatitis B birth dose; (c) providing catch-up vaccination for children or adolescents with low coverage; and (d) offering hepatitis B virus vaccination to people who are at increased risk of HBV transmission (WHO 2016a). To reach these elimination goals, the WHO has targeted improvements in global coverage of HepB infant and birth dose

vaccination to 90% and 50%, respectively, by 2020 and 90% and 90%, by 2030 (WHO 2019a, 2020a). The 85% coverage of HepB vaccination indicates the excellent global progress in reaching the vaccine coverage target necessary to achieve elimination goals. Hepatitis B immunization is significantly reducing HBV incidence and prevalence among vaccinated cohorts. In 2020, HBsAg prevalence fell to <1% among children <5 years of age achieving the UN SDG goal and the interim target for the WHO global strategy for HBV elimination (WHO 2020b). The greatest remaining challenge is to more than double the current 43% global coverage for HepB birth dose vaccination joined with other related strategies including maternal HBsAg testing to deliver other interventions (i.e., infant HBIG administration, maternal antiviral prophylaxis) necessary to eliminate maternal to child transmission of HBV.

Elimination of hepatitis B virus transmission is achievable given the long experience with hepatitis B vaccination and demonstrated effectiveness and safety of hepatitis B vaccine (WHO 2017a, 2020b; Schillie et al. 2018b; Ghendon 1990; CDC 1992, 1999). Over 95% of persons who complete the three-dose vaccination series achieve immunoprotection (WHO 2017a; Schillie et al. 2018b; Michel et al. 2015), with levels of protection persisting for at least 23–30 years after vaccination; a booster dose is rarely recommended (Spradling et al. 2015; Middleman et al. 2014). Hundreds of millions of persons of all ages have been vaccinated over several decades with hepatitis B vaccines.

The WHO regional offices and the countries of the respective regions set goals for HepB vaccination coverage and reductions in HBV prevalence among young children; the following regional summaries reveal the remarkable progress in prevention of chronic HBV infection and the remaining challenges that must be met and overcome to eliminate hepatitis B.

12.1 WHO Western Pacific Region

In September 2005, the WHO Regional Office for the Western Pacific Region (WPR) became the first WHO region to adopt a goal of hepatitis B control. WPRO aimed to reduce the prevalence of chronic HBV infection to <2% in children at least 5 years of age by 2012, with an ultimate goal of an HBsAg seroprevalence of <1% in children ≤5 years of age (Woodring et al. 2019; Wiesen et al. 2016). By 2019, a total of 21 of 36 countries and areas were verified as having achieved the regional target of <1% HBsAg seroprevalence among children under 5 years of age. As a result, HBsAg prevalence in the region declined to 0.93% meeting the regional target.

In 2017, WPR established a goal for a reduction to <0.1% HBsAg prevalence for children <5 years by 2030 as the goal for EMTCT. WPR countries are adopting the triple elimination framework for HIV, HBV, and syphilis. Although 93% of countries in the region have a policy for HepB birth dose vaccination, improvements in antenatal HBV testing are needed. A total of 20 (56%) of countries or areas have a national policy for routine antenatal HBsAg testing including eight countries providing antivirals to HBV-infected mothers.

12.2 WHO Southeast Asia Region

In 2016, member countries of SEAR set a regional target of <1% HBsAg prevalence among children aged ≥5 years by 2020. In 2019, the region had 54% and 91% coverage of HepB birth dose and third dose (HepB3) immunization, respectively; 9 of 11 countries achieved ≥90% HepB3 coverage nationally (Sandhu et al. 2020). As a result, HBsAg among 5-year-olds declined to 1.2% prevalence approaching the regional target. The improvements in health as a result of HepB vaccination are substantial. From 1992 to 2015, HepB immunization in the region prevented an estimated 16 million chronic HBV infections and averted 2.5 million deaths (Childs et al. 2018).

12.3 Eastern Mediterranean Region

In 2009, the member states adopted a target for hepatitis B control through childhood vaccination to reduce HBsAg prevalence to <1% by 2015. In 2014, all countries had introduced infant three-dose vaccination with 83% regional coverage (Allison et al. 2016). A total of 14 (64%) of 22 countries had introduced universal birth dose vaccination increasing birth dose coverage to 71% in these countries and 24% in the region. Preliminary data suggest 15 (68%) of 22 countries in the region achieved <1% chronic HBV infection prevalence by the end of 2014. The regional HBsAg prevalence is 1.21% approaching the regional target. Among children born between 2005 and 2014, hepatitis B vaccination prevented 5 million chronic HBV infections and 700,000 future deaths from hepatitis B-related disease.

12.4 WHO European Region (EUR)

A regional action plan for 2016–2021 set coverage targets for three-dose (95%) and birth dose (90%) HepB vaccination, maternal HBV screening (85%), and post-exposure prophylaxis for PMCT to achieve ≤0.5% HBsAg prevalence in vaccinated cohorts (WHO 2016b). In 2017, a total of 47 of 53 countries routinely conducted infant HepB vaccination with coverage exceeding the >90% regional target (Duffel et al. 2021). All countries have implemented strategies to prevent perinatal HBV transmission either via universal newborn vaccination, universal screening of pregnant women followed by vaccination (Shouval et al. 2015), and other strategies. Regional prevalence was 0.4% exceeding the target of ≤0.5% HBsAg prevalence among vaccinated cohorts. Many of the European Union-affiliated countries have integrated maternal screening for infectious diseases including syphilis (26/26), HIV (24/26), and hepatitis B (WHO 2017b).

12.5 WHO Americas Region (AMR)

In 2015, the region was committed to reducing HBsAg prevalence to <0.1% among 4- to 6-year-old children (PAHO: EMTCT Plus 2020). The regional coverage of

three-dose infant immunization is 89% with all 52 countries and territories in the region including HepB vaccine in pediatric immunization schedules. Additionally, 36 (69%) countries/territories include an HBV birth dose including 22 with universal HepB vaccination policies. In countries with universal vaccination policies, 83% of newborns receive HepB vaccination. The high proportion of births managed by skilled birth attendants provides the settings for scale-up of birth dose vaccination and maternal HBV testing as part of the strategies that also includes HIV, syphilis, and, where appropriate, Chagas disease.

12.6 African Region (AFR)

HBV is highly endemic in sub-Saharan Africa with an estimated 8.8% HBV prevalence and most member states having a HBsAg prevalence of >2% and exceeding 8% for some countries particularly in western Africa (Breakwell et al. 2017). The African region is the only WHO region that has not approached or exceeded the target of reducing HBsAg prevalence rates to less than 1% in children younger than 5 years (Cohn et al. 2021). In November 2014, the WHO African Regional Committee endorsed a resolution to reduce HBsAg prevalence to <2% in children less than 5 years of age in all members states by 2020. All 47 countries in the WHO Africa Region have introduced HepB into the routine infant immunization schedule. By 2015, regional coverage for infant HepB immunization was 76%; a total of 6 (34%) countries reported >90% HepB3 coverage. However, in 2018, only 23% (11/47) of countries had adopted policies to begin HepB birth dose vaccination resulting in <10% regional HepB BD coverage (WHO 2019b). As a result, an estimated 1% of newborns annually are infected with HBV.

The most cost-effective intervention to reduce HBV infection rates in sub-Saharan Africa is timely birth dose vaccination followed by completion of the three-dose infant vaccination series. While assistance for AFR countries to implement HepB birth dose vaccination continues, the integration of maternal HBV and HIV testing affords an additional option for improved prevention of perinatal HBV infection.

In May 2016, the WHA approved the framework and the proposed hepatitis B elimination targets. In the USA, the National Academies of Sciences, Engineering, and Medicine (NAS) convened a consensus committee to examine the feasibility of setting goals for the elimination of hepatitis B and C as public health threats and to propose a strategy for and actions needed to reach national elimination goals. In May 2016, the committee released a report concluding that given political will and sufficient resources, hepatitis B could be eliminated as a public health problem in the USA (National Academies of Sciences, Engineering, and Medicine 2016). In 2021, the US government released The *Viral Hepatitis National Strategic Plan for the United States: A Roadmap to Elimination (2021–2025)* with targets for improvements in HBV prevention including increased hepatitis B vaccination coverage and the elimination of perinatal HBV transmission (U.S. Department of Health and Human Services 2020).

Universal infant vaccination beginning at birth on a global scale, along with targeted efforts to vaccinate all children and adults at risk for HBV infection, can reduce the risk for HBV infection to zero. Sustained high coverage of infant HepB immunization and effective implementation of strategies for EMTCT of HBV will be determinative in achieving global goals for HBV elimination. Integrating hepatitis B testing into routine maternal child health program increases adoption of additional strategies to prevent HBV transmission among the population at highest risk for chronic HBV infection. Through HBV screening of all populations at risk for HBV infection, public health programs and clinical care providers have an opportunity to ensure that HBV-infected persons and their contacts receive needed care, such as counseling, medical evaluation, and treatment. Likewise, providers of hepatitis B care and treatment can refer susceptible close contacts of HBsAg-positive patients for hepatitis B vaccination, and therapies can improve the effectiveness of vaccination programs. Commitment of public health resources to eliminate HBV transmission will require recognition of the large global burden of disease, demonstration of the benefits of vaccination, and patience to realize the goals of vaccine-associated disease reduction.

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9 Viral and Host Factors Affecting Disease Progression of Hepatitis B Virus Infection

Hung-Chih Yang and Tung-Hung Su

Abstract

The clinical outcomes of chronic hepatitis B virus (HBV) infection are heterogeneous, ranging from spontaneous seroconversion of hepatitis B surface antigen (HBsAg) to severe detrimental consequences, including hepatic failure, cirrhosis, and hepatocellular carcinoma (HCC). Four distinctive clinical phases are recognized in the natural course of chronic hepatitis B (CHB), namely, immune tolerance, immune clearance, inactive carrier and HBV reactivation phases. Patients with prolonged immune active phases are prone to develop cirrhosis and HCC. However, a small portion of HBsAg carriers will eventually lose HBsAg and even undergo HBsAg seroconversion. Conventionally, serum HBV DNA level and HBeAg serostatus combined with serum ALT level are utilized to distinguish the disease states in the natural history of CHB. A number of other viral factors, including HBV genotype, naturally occurring viral mutations as well as serum levels of HBsAg and HBV core-related antigen (HBcrAg) have been demonstrated the utility in predicting the long-term prognosis of CHB patients. Additionally, host factors including human leukocyte antigen, serum anti-HBc

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level, and factors involved in the risk of fibrosis and HCC are also associated with the outcomes of CHB. All these qualitative and quantitative viral factors contribute to the disease progression. With the advance of technology, more new viral biomarkers are emerging. Combination of all current and new viral factors may allow for more sophisticated delineation of disease states, the deeper mechanistic insight into the pathogenesis, and the more optimal management for patients.

Keywords

Hepatitis B virus · Chronic hepatitis B · Viral factors · Host factors · Natural history

1 The Natural History and Disease Progression of Chronic Hepatitis B

Chronic hepatitis B (CHB) undergoes prolonged and complex virus–host interactions and exhibits heterogeneous clinical outcomes, ranging from spontaneous seroconversion of hepatitis B surface antigen (HBsAg) to severe detrimental consequences, including hepatic failure, liver cirrhosis and hepatocellular carcinoma (HCC). Conventionally, based on the degree of hepatic necroinflammation, the serostatus of hepatitis B e antigen (HBeAg), and the HBV DNA level, the natural history of CHB is characterized as four distinctive phases, including the immune tolerance, immune clearance, inactive carrier, and reactivation of HBV (Yang et al. 2002; Liaw and Chu 2009; Yang and Kao 2016; McMahon 2009; Fattovich et al. 2008). In the immune tolerance phase, patients have very high levels of HBV DNA, normal ALT and positive HBeAg. Upon entry to the immune clearance phase, HBsAg carriers experience an elevation of ALT and decline of serum HBV DNA. HBeAg seroconversion, defined as HBeAg loss and appearance of anti-HBe antibody, is a milestone in the natural history of CHB, and often signifies the transition from the immune clearance to an inactive carrier state. Early entry to an inactive carrier state usually confers a favorable clinical outcome (Hsu et al. 2002; Bortolotti et al. 2006; Ni et al. 2007). Some HBeAg seroconverters can finally achieve HBsAg loss or seroconversion, an indication of resolved hepatitis B or a state of a functional cure. However, some patients with HBeAg seroconversion suffer from reactivation of HBV and recurrent hepatitis flares, named HBeAg-negative hepatitis. Although the majority of CHB patients have spontaneous HBeAg seroconversion in an early period of life and enjoy an uneventful clinical course, some of them have a prolonged immune clearance phase or HBeAg-negative hepatitis and suffer from persistent or recurrent hepatitis flares, eventually leading to cirrhosis and HCC.

The lifetime risk of end-stage liver disease, namely, cirrhosis and HCC, in HBsAg carriers is up to 15–40% (Liaw and Chu 2009; Kao 2007; Kao et al. 2010; El-Serag 2012). The annual incidence of cirrhosis is 3.5% in CHB patients with

persistent HBeAg seropositivity and 2.9% in those with HBeAg-negative hepatitis (Liaw and Chu 2009). Cirrhotic patients have been shown to exhibit a higher risk of HCC than those without cirrhosis, and the annual incidence of HCC is around 5–6% (Yang et al. 2002). A systemic review on the longitudinal studies estimated the incidence rates of HCC in HBsAg carriers in East Asian countries to be 0.2 per 100 person-years in inactive carriers, 0.6 in those with chronic HBV infection but without cirrhosis, and 3.2 in patients with compensated cirrhosis; the 5-year HCC accumulative incidence among cirrhotic patients is 15% (Fattovich et al. 2004). In contrast, the summary HCC incidence rate in individuals with chronic HBV infection in Europe was 0.02 per 100 person-years in inactive carriers, 0.1 in patients with CHB but without cirrhosis, and 2.2 in subjects with compensated cirrhosis; the 5-year HCC accumulative incidence was 10% among those with cirrhosis.

Early identification of CHB patients at risk of cirrhosis and HCC for timely antiviral treatment is critical to prevent the disease progression and reverse the detrimental outcomes. Therefore, the discovery of important risk factors that contribute to the disease progression and delineate the natural history of CHB should help early diagnosis of patients at risk and provide the optimal care (Yang and Kao 2016). In this Chapter, we will discuss the viral and host factors affecting the disease progression of patients with chronic HBV infection.

2 Viral Factors That Affect the Disease Progression of CHB

It has been known that HBV is non-cytopathogenic and causes liver injury mainly through the immune-mediated mechanisms (Guidotti and Chisari 2006; Rehermann and Nascimbeni 2005). Nevertheless, a body of evidence has demonstrated that viral replication is the primary driving force for hepatitis flares and disease progression in CHB patients (Chen et al. 2006a). Because HBV replicates through the reverse transcription, which lacks the proofreading ability, emergence of viral mutants and rapid evolution within an infected individual often occur under the host immune pressure along the long course of chronic HBV infection (Seeger and Mason 2015). In addition, diversification of viral genomes among different infected populations and across different geographic regions also results in different genotypes of HBV. Studies in the past decades have identified a number of qualitative and quantitative viral factors that affect the disease progression, including HBV DNA levels, HBeAg serostatus, HBsAg levels, HBV genotypes, naturally occurring mutants, and HBV core-related antigen (HBcrAg) levels. Recently, serum HBV RNA has also emerged as a surrogate biomarker for intrahepatic cccDNA. All these viral factors, to some extent, cause and/or reflect the complex virus–host interactions and liver injury and can serve as the predictive biomarkers for the clinical outcomes of CHB. The dynamic change of each viral factor along with the natural history of CHB is summarized in Fig. 9.1. We will describe and discuss these viral factors by emphasizing their impact on the disease progression and predictive roles in clinical outcomes.

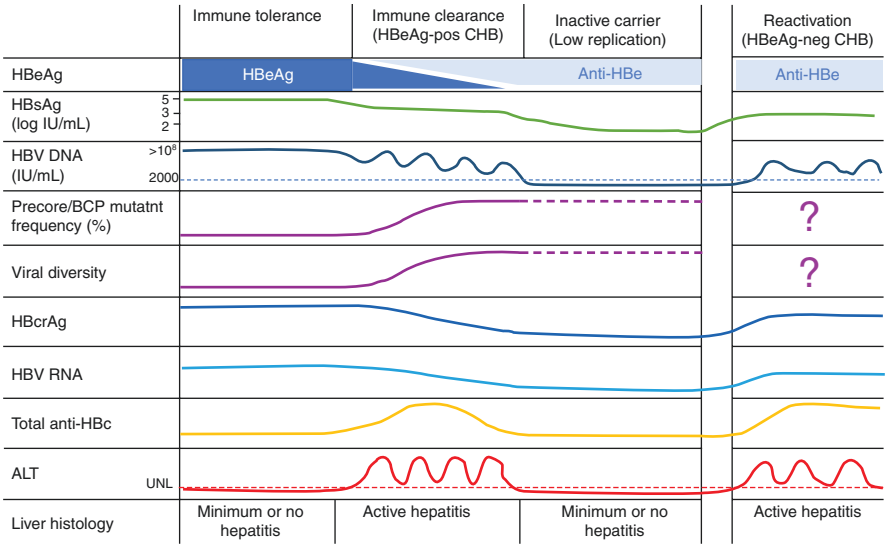

Fig. 9.1 The natural history of CHB by integrating new quantitative viral and host markers. *UNL* upper normal limit

3 HBV DNA Level

HBV viral load reflects the viral replicative capacity. Several prospective studies have demonstrated the risk of high HBV DNA levels in the development of HCC (Chen et al. 2006a, b, 2011; Yu et al. 2005). A large Taiwanese community-based REVEAL-HBV (Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus) cohort study that recruited mostly non-cirrhotic HBsAg carriers who were HBeAg-negative and >30 years old reported that the risk of HCC is positively correlated with the increase of viral load at the entry of study across a wide spectrum of HBV DNA levels. The cumulative incidence rates of HCC for patients with HBV levels ranging from 300 copies/mL to 1 million copies/mL were 1.3% and 14.9%, respectively (Chen et al. 2006a). Particularly, HBV DNA ≥ 10,000 copies/mL is a strong predictor for the risk of HCC. The following study also demonstrated that, among patients with HBV DNA ≥ 10,000 copies/mL at the entry of the study, the long-term follow-up HBV DNA level was also a predictor for risk of HCC (Chen et al. 2011). Compared with participants with HBV DNA < 10,000 copies/mL at baseline, those with HBV DNA ≥ 10,000 copies/mL at baseline, but follow-up HBV DNA levels <10,000 copies/mL had only a slightly increased HCC risk (hazard ratio 2.25), but the hazard ratio for those with long-term HBV DNA levels that remained as high as 1,000,000 to 10,000,000 copies/mL was 16.78. HBV DNA levels were also correlated with the risk of cirrhosis and HBV-related mortality in a dose-dependent manner (Iloeje et al. 2006, 2007). In addition, the HBV DNA level also strongly predicted the progression to cirrhosis with the

relative risk 2.5, 5.6 and 6.5 for HBV DNA levels $\geq 10^4$–$<10^5$, $\geq 10^5$–$<10^6$, and $\geq 10^6$ copies/mL, respectively, as compared to the HBV DNA level < 300 copies/mL (Iloeje et al. 2006).

Although the above studies suggest the strong association of serum HBV DNA level with the development of cirrhosis and HCC independent of ALT levels, HBV genotype and HBeAg serostatus, the long-term risk of HCC and cirrhosis in CHB patients in the immune-tolerant phase seems to be low. A prospective study evaluated the changes of liver histology within a period of 5 years in 57 immune-tolerant CHB patients with the median age of 31 and found that in 48 patients who had no ALT elevation and remained in the immune-tolerant phase, there was only minimal fibrosis progression. In contrast, five of nine who had an elevation of ALT and progressed to the immune clearance phase faced significant disease progression (Hui et al. 2007). In addition, unlike the REVEAL-HBV study, which used ALT <45 U/L as the upper limits of normal (ULNs), two studies used more stringent ALT cutoffs (normal ALT levels of healthy adults are ≤30 U/L for males and ≤ 19 U/L for females (Lok and McMahon 2007)) showed that only a minority (~20%) of HBeAg-positive patients with high HBV DNA (>10^6 IU/mL) exhibited significant histologic fibrosis and necroinflammation (Andreani et al. 2007; Lai et al. 2007). Therefore, despite their high HBV DNA levels, most immune-tolerant CHB patients have minimal liver necroinflammation and fibrosis. Based on currently available evidence, patients who are in a truly immune-tolerant phase are advised against antiviral therapy (Tseng and Kao 2015; Terrault et al. 2016). However, the long-term prognosis of CHB patients in a true immune-tolerant phase, particularly in those older than 40 years, requires to be determined in a large longitudinal cohort study in the future.

4 HBeAg Serostatus and Level

The biological role of HBeAg remains elusive because it is not required for viral assembly and replication (Milich and Liang 2003). Nevertheless, the positivity of HBeAg indicates the active replication of HBV, whereas HBeAg seroconversion is often accompanied by the reduction of viral replication and remission of hepatic necroinflammation. The convincing evidence for the adverse effect of HBeAg positivity on the long-term outcomes of CHB patients came from the community-based REVEAL-HBV cohort study, which demonstrated the association between the positivity for HBeAg and the risk of HCC (Yang et al. 2002). After adjustment of other confounding factors, the relative risk of HCC was 9.6 among men with HBsAg positivity alone and 60.2 among those who were positive for both HBsAg and HBeAg as compared to those who were negative for both. It has also been shown that delayed HBeAg seroconversion or relapse of hepatitis after HBeAg seroconversion is an independent risk of cirrhosis (Chu et al. 2004).

Recently, quantitative HBeAg level has been proposed to guide antiviral therapy (Fried et al. 2008; Lee et al. 2011). In treatment-naïve CHB patients, HBeAg level is found to correlate with HBV DNA level, but the emergence of precore/basal core

mutations can affect this relationship (Thompson et al. 2010). Nevertheless, the role of HBeAg levels in long-term clinical outcomes remains unclear.

5 HBsAg State and Level

HBsAg loss or seroconversion is a landmark in the natural history of CHB as well as the ultimate goal for antiviral treatment. Mechanistically, the HBsAg level can be considered a surrogate marker of HBV covalently closed circular DNA (cccDNA), the transcriptional and replicative template of HBV, although HBsAg can also be produced from the integrated HBV genomes (Thompson et al. 2010; Fried et al. 2008). HBsAg loss or seroconversion represents the resolution of hepatitis B or a state of a functional cure. In the latter scenario, replication-competent latent viruses can still be recovered from patients with resolved hepatitis B (Loriot et al. 1997; Marusawa et al. 2000; Rehermann et al. 1996). HBsAg loss or seroconversion used to be considered a rare event in HBsAg carriers. However, previous cohort studies revealed that HBsAg loss occurs at an annual rate from 0.5% to 2.3%, depending on the age at enrollment and disease status (McMahon et al. 2001; Chu and Liaw 2007; Kim et al. 2008; Liu et al. 2010; Simonetti et al. 2010; Tseng et al. 2011). HBsAg loss or seroconversion usually indicates a favorable clinical prognosis unless cirrhosis or HCC has already occurred (Chu and Liaw 2007; Yuen et al. 2004, 2008; Ahn et al. 2005; Arase et al. 2006; Chen et al. 2002).

Recently, HBsAg levels can be measured quantitatively. It has been found that HBsAg levels evolve along with distinct phases of CHB (Chan et al. 2010; Jaroszewicz et al. 2010; Nguyen et al. 2010; Matsumoto et al. 2012). The immune tolerance phase exhibits the highest HBsAg level, approximately 5 log IU/mL, which subsequently decreases to 4 log IU/mL in the immune clearance phase and continues to decline in inactive carriers (Brunetto et al. 2010; Brouwer et al. 2016; Liu et al. 2016). However, HBsAg level rebounds upon HBV reactivation in HBeAg-negative hepatitis B patients. It has been shown that a combination of HBsAg (<1000 IU/mL) and HBV DNA (≤2000 IU/mL) is able to accurately identify inactive carriers of genotype D with 94.3% diagnostic accuracy and 87.9% positive predictive value (PPV) (Brunetto et al. 2010). The same criteria could be utilized to determine inactive carriers with 78% diagnostic accuracy and 83% PPV in genotype B and C-infected carriers (Liu et al. 2016).

HBsAg level has been demonstrated as a useful biomarker to predict the HBsAg loss and the risk of HCC. In a hospital-based cohort study from Taiwan that enrolled 390 HBeAg seroconverters of genotype B and C, it was found that low serum HBsAg levels alone or combined with low HBV DNA levels, at 1 year after HBeAg seroconversion is a predictor of HBsAg loss (Tseng et al. 2011). Compared to patients with HBsAg ≧ 1000 IU/mL, patients with HBsAg of 100 to 999 and <100 IU/mL had a higher chance of HBsAg loss within 6 years at the hazard ratios of 4.4 and 24.3, respectively. In addition, low HBV DNA level (<2000 IU/mL) and low HBsAg level < 10 IU/mL, combined together, are able to predict HBsAg loss in HBeAg-negative HBV carriers (Tseng et al. 2012a). Furthermore, compared to

individuals with HBsAg levels >1000 IU/mL, the rates of HBsAg loss in HBeAg-negative HBV carriers with HBsAg levels of 100–999, 10–99, and <10 IU/mL, were significantly higher with hazard ratios of 2.5, 2.8, and 13.2, respectively.

The HBsAg level of HBeAg-negative patients with low viremia (<2000 IU/mL), is also associated with the risk of disease progression (Tseng et al. 2013, 2014). The study on a hospital-based Taiwanese cohort (ERADICATE-B) of HBsAg carriers reported that in genotype B or C HBeAg-negative patients, the adjusted hazard ratios for HBeAg-negative hepatitis and HCC were 1.5 and 13.7, respectively, in those with HBsAg level ≥ 1000 IU/mL versus <1000 IU/mL. As a result, a combination of low HBV DNA (<2000 IU/mL) and low HBsAg (<1000 IU/mL) is a useful biomarker for identifying HBsAg carriers with minimal risk of HCC (Tseng et al. 2012b). Consistently, the community-based study from the REVEAL-HBV cohort also showed that the combination of HBV DNA < 2000 IU/mL and HBsAg <1000 IU/mL was a predictor for a low risk of cirrhosis and HCC and a high chance of HBsAg seroclearance with the adjusted hazard ratios of 0.36, 0.36 and 6.97, respectively (Liu et al. 2016).

6 HBV Genotype

Because HBV polymerase lacks the ability of proofreading, viral mutations often occur during the long course of chronic HBV infection. Accumulation of mutations drives the HBV evolution and phylogenetic diversification across different geographic regions. The genotypes of HBV are defined by an intergroup divergence of more than 8% in the complete genome sequence. Currently, at least ten genotypes of HBV, from A to J, have been discovered. Different HBV genotypes are associated with distinct virological and epidemiological properties (Croagh et al. 2015; Liu and Kao 2013; Kramvis 2014; Schaefer 2007). For example, Genotype A is widespread in sub-Saharan Africa, Northern Europe, and Western Africa. Genotypes B and C are more common in Asia. Genotype D is primarily prevalent in Africa, Europe, the Mediterranean region and India. Genotype E is found in West and Central Africa. Genotype F is distributed over North and South America.

Since different genotypes of HBV are distributed in different geographic regions and ethnic groups, it is sometimes difficult to evaluate the risk potential of viral genotype on the long-term outcomes of CHB because of these confounding factors. Nevertheless, several lines of evidence have started to show that HBV genotype is associated with clinical outcomes. Previous studies found that the genotype influences the rate of HBeAg seroconversion. Compared to patients with genotype B infection, those with genotype C infection exhibited delayed or lower rate of spontaneous HBeAg seroconversion (Ni et al. 2004a). Furthermore, in a prospective cohort study that enrolled 1158 HBV carriers with the infection of genotypes A, B, C, D, and F throughout Alaska, it also found that genotype C infection had a delayed HBeAg seroclearance. Besides, individuals with genotypes C and F infection had a higher risk of HBeAg reversion after HBeAg seroclearance (Livingston et al. 2007). A longitudinal study that enrolled 258 Spanish patients with genotypes A, D, and F

reported that, although the HBeAg seroconversion rate was unrelated to HBV genotype, genotype A patients exhibited a higher rate of sustained remission after HBeAg seroconversion than those with genotype D infection (Sanchez-Tapias et al. 2002). Furthermore, compared to patients with the infection of genotypes C and D, those with genotypes A and B infection had a higher rate of the HBsAg seroclearance (Yuen et al. 2004; Sanchez-Tapias et al. 2002).

Previously, it also has been shown that HBV genotypes are associated with the risk of cirrhosis and HCC (Liu et al. 2006). Patients with the infection of genotype D have more active liver disease and advanced liver fibrosis compared to those with genotype A infection. In addition, compared to genotype B infection, genotype C infection is associated with a higher risk of cirrhosis and HCC (Liu and Kao 2013, 2015; Kao et al. 2000; Yang et al. 2008). Interestingly, whereas genotype C infection is associated with the development of HCC at older age, genotype B infection is associated with HCC that occurs at younger age (Ni et al. 2004a; Kao et al. 2000).

The natural history of HBV carriers with genotype E infection has been unclear until recently. A longitudinal cohort study that recruited 405 HBV carriers primarily with genotype E infection (>95%) in Gambia, West Africa, reported that the genotype E infection has similar annual rates of HBeAg (7.4%) and HBsAg (1.0%) seroclearance with other genotypes. However, treatment-naive male HBsAg carriers in the Gambia had the HCC incidence of 55.5/100,000 carrier-years. The risk of HCC was higher than HBsAg carriers in Europe but lower than those in East Asia (Shimakawa et al. 2016).

7 HBV Mutants

Due to the error-prone nature of HBV polymerase and the host immune selection pressure after entry of the immune clearance phase, viral mutants often emerge during chronic HBV infection, leading to viral quasispecies (Revill et al. 2020). Three naturally occurring HBV mutations, namely, G1896A precore premature stop codon mutation, A1762T and G1764A dual basal core promoter (BCP) mutations and deletions in the pre-S/S genes are commonly encountered in the natural course of CHB (Lin and Kao 2015; Chotiyaputta and Lok 2009; Chu et al. 2003). These HBV mutants have been suggested to affect the natural history of CHB, including HBeAg seroconversion and the risk of cirrhosis and HCC. In addition to HBV mutants, the viral quasispecies diversity per se is also associated with the clinical outcomes. Increased viral quasispecies diversity has been associated with HBeAg seroconversion (Lim et al. 2007). However, compared to patients without HBsAg loss, patients with HBsAg loss had lower viral diversity in structural genes but had higher viral diversity in the regulatory regions (Bayliss et al. 2016).

It has been shown that the precore and BCP mutations are associated with HBeAg seroconversion (Chu et al. 2003; Lok et al. 1995; Chang et al. 1998; Chan et al. 1999; Yuen et al. 2002; Ni et al. 2004b). The precore premature stop codon mutation results in failure of HBeAg production, whereas the dual BCP mutations are suggested to cause the reduction of the HBeAg expression (Chotiyaputta and Lok

2009). Previous studies found that precore mutation emerges before the occurrence of HBeAg seroconversion. In addition, following HBeAg seroconversion, the majority (>90%) of patients had core promoter or precore mutations (Chan et al. 1999). Interestingly, using the quantitative amplification-created restriction site assays for measurement of the precore mutant ratio, Chu et al. found that the higher ratio of the precore mutant is associated with earlier HBeAg seroconversion, but the patients who had significantly higher ratio of precore mutant tended to have high viremia and high ALT after HBeAg seroconversion (Chu et al. 2002). In addition, using a more quantitative SimpleProbe real-time PCR analysis of these mutations, Nie et al. discovered that the steady increase of the precore and/or BCP mutant frequencies usually occurs within 3 years before HBeAg seroconversion (Nie et al. 2012) (Bayliss et al. 2016). Taken together, these observations on the temporal relationship between viral mutations and HBeAg seroconversion suggest that the emergence of precore and BCP mutations are likely to be the consequence of the immune selection pressure rather than the causative driving force for HBeAg seroconversion.

The biological roles of precore and BCP mutations in chronic HBV infection remain elusive, and several studies have suggested that they may exert different effects on the outcomes of CHB. It has been shown that the dual A1762T/G1764A (TA) mutations in combination with T1753A, T1768A cause cell cycle dysregulation by upregulation of SKP2 and downregulation of p21, leading to the development of HCC (Huang et al. 2011). BCP mutations have been associated with the risk of cirrhosis and HCC, but the role of precore mutation is somehow controversial (Liu et al. 2006; Baptista et al. 1999; Lindh et al. 1999; Kao et al. 2003; Tong et al. 2006; Liu et al. 2009). Moreover, in the community-based REVEAL-HBV cohort study in Taiwan, BCP mutations are associated with the increased risk of HCC (adjusted hazard ratio: 1.73), whereas the precore mutation is associated with the decreased risk of HCC (adjusted hazard ratio: 0.34) (Yang et al. 2008). A meta-analysis of 43 studies also demonstrated that BCP mutations, but not precore mutation, increase the risk of HCC with the odds ratio of 3.79 (Liu et al. 2009). A recent hospital-based cohort (ERADICATE-B) quantitatively analyzed the precore and BCP mutations and showed that higher percentage of the BCP mutation at 1 year after HBeAg seroconversion is associated with higher risk of cirrhosis (Tseng et al. 2015). Recently, we also found that the percentage of the precore mutation increases during interferon-induced HBeAg seroconversion, whereas the percentage of BCP mutations do not change significantly (Yang et al. 2013). Taken together, the precore and BCP mutations are likely to play distinctive roles in the natural course of CHB.

The HBV pre-S/S deletion mutation is also commonly encountered in chronic HBV infection and is likely the consequence of the antiviral immune selection pressure (Fan et al. 2001; Chen et al. 2006c). The pre-S deletion has been suggested to cause endoplasmic reticulum (ER) ER stress and carcinogenesis of hepatocytes due to the accumulation of large surface protein ER. In addition, pre-S2 protein has been shown to activate hTERT transcription that leads to the development of HCC (Luan et al. 2009). Previous studies also reported that Pre-S deletion is associated with the

risk of advanced liver disease and HCC in HBeAg-negative patients (Chen et al. 2007; Pollicino et al. 2014).

It has also been shown that HBV mutations are associated with the HBV genotypes (Rodriguez-Frias et al. 1995). The precore stop codon mutation G1896A is rare in genotype A and more common in genotype D (Grandjacques et al. 2000). It has been shown that the precore mutation is restricted to the virus having T at nucleotide 1858 (Lok et al. 1995; Lin and Kao 2015). Likewise, precore premature stop codon mutation is also seldom observed in genotypes A and H and subgenotypes C1, F2 and F3 because of the same structural restriction (Tong and Revill 2016). Furthermore, patients with genotype D were more likely to have persistent HBV infection by the selection of precore mutants. Core promoter changes were significantly more common in patients infected with HBV who have C at nucleotide 1858 (Chan et al. 1999; Lindh et al. 1999). Therefore, studies on the role of HBV mutants in disease progression should take into consideration the effect of viral genotype.

8 Quantitative HBcrAg

HBcrAg combines the antigenic reactivity against hepatitis B core antigen (HBcAg), HBeAg and a 22-kDa precore protein without C-terminal arginine-rich domain (p22cr), so it can detect all these three antigens simultaneously (Kimura et al. 2002; Kimura et al. 2005). HBcrAg levels have been shown to correlate well with serum HBV DNA levels as well as intrahepatic HBV DNA levels, particularly in HBeAg-positive patients (Rokuhara et al. 2005; Wong et al. 2007). More importantly, the HBcrAg level is positively correlated with the intrahepatic cccDNA level and its transcriptional activity (Suzuki et al. 2009; Matsuzaki et al. 2013; Testoni et al. 2019). Mechanistically, the HBcrAg level is an indicator of the gene expression of cccDNA, so it can serve as an ideal marker for HBV replication or residual replicative-competent cccDNA. Recently, accumulative evidence has demonstrated the role of HBcrAg as a biomarker to predict the clinical outcomes of CHB (Mak et al. 2019; Baudi et al. 2020). The cohort studies in Asian and European have shown that HBcrAg levels vary along the natural history of CHB. The immune tolerance and immune clearance phases have higher HBcrAg levels, which are followed by that in HBeAg-negative hepatitis. The HBeAg-negative quiescent/inactive carrier phase has lower HBcrAg levels (Seto et al. 2014; Maasoumy et al. 2015). The HBcrAg level is lowest in patients with HBsAg seroclearance. Lower HBcrAg has also been associated with early spontaneous HBeAg seroconversion in studies from China and Japan (Bae et al. 2012; Song et al. 2017). Besides, a combination of HBcrAg $\leq$3 log U/mL plus HBV DNA $\leq$ 2000 IU/mL was shown to have good predictive ability for an inactive carrier state (Riveiro-Barciela et al. 2017). Of note, HBcrAg remains detectable in a portion of HBsAg seroconverters (Seto et al. 2014), and thus can be used to predict the risk of HBV reactivation in occult HBV infection under immunosuppressive therapies (Seto et al. 2016). In addition, HBcrAg has been associated with the risk

of liver inflammation, advanced fibrosis and HCC. A longitudinal cohort study that enrolled 1031 treatment-naïve HBsAg carriers in Japan reported that HBcrAg >2.9 log U/ml is an independent risk factor of HCC (adjusted hazard ratio: 5.05) (Tada et al. 2016). Recently, in a hospital-based study of 2666 Taiwanese HBsAg carriers (genotype B or C), HBcrAg was shown to be an independent risk factor of HCC. A subgroup analysis on HBeAg-negative patients with normal ALT and intermediate viral load (serum HBV DNA: 2000–19,999 IU/mL) further demonstrated patients with high levels of HBcrAg (≥10 KU/mL) were at high risk for HCC (Hazard ratio: 6.29) (Tseng et al. 2019).

9 Serum HBV RNA

Serum HBV RNA has recently emerged as a novel surrogate biomarker for intrahepatic cccDNA. Although it used to be considered that only mature virion containing rcDNA can be secreted from infected hepatocytes (Gerelsaikhan et al. 1996), accumulative evidence has demonstrated the existence of HBV RNA in serum (Su et al. 2001; Wang et al. 2016; Giersch et al. 2017). The major form of serum HBV RNA is likely the pregenomic RNA contained in secreted virions (Mak et al. 2019; Wang et al. 2016; Liu et al. 2019a). Serum HBV RNA represents the transcription activity of cccDNA and thus can serve as its surrogate biomarker. It has been shown that serum HBV RNA is well correlated with serum HBV DNA, HBcrAg, and intrahepatic cccDNA in naïve HBV carriers (Mak et al. 2019). Generally, serum HBV RNA level is lower than serum HBV DNA level by 1–2 log (Butler et al. 2018). The level of HBV RNA in the natural history of HBV infection has been shown to vary across different phases of CHB in a pattern similar to HBcrAg (van Campenhout et al. 2018; Wang et al. 2018). Serum HBV RNA level is highest in the immune-tolerant phase, followed by the immune clearance phase and further declines in inactive carriers. Because serum HBV RNA is correlated with the transcriptional activity of cccDNA, it has been suggested to serve as a potential biomarker in predicting the response to antiviral therapy, particularly for the purpose of HBV cure (Mak et al. 2019; Coffin et al. 2019). Nevertheless, the role of serum HBV RNA in the long-term outcomes of CHB remains unexplored.

10 Host Factors

In addition to viral factors, host factors also affect the disease progression of CHB. Here, we focus on the role of human leukocyte antigen, anti-HBc level, liver fibrosis, and the factors that are associated with the risk of HCC. We will introduce two new biomarkers, Mac-2 binding protein glycosylation isomer (M2BPGi) and prothrombin induced by vitamin K absence II (PIVKA-II), which have been recently associated with liver fibrosis and HCC, respectively. Their biological roles and clinical usage are also summarized in Table 9.1.

Table 9.1 Two new host factors contribute to HBV disease progression

	M2BPGi	PIVKA-II
Purpose	Liver fibrosis marker	HCC diagnostic marker
Origin	Altered N-glycosylation of Mac-2 binding protein	Altered prothrombin because of vitamin K deficiency (i.e., des-γ-carboxyprothrombin, DCP)
Produced by	Hepatic stellate cells (HSC)	Hepatocytes
Function	Reflects the activation of HSCs during fibrogenesis	May induce HCC cell proliferation, enhance angiogenesis
Clinical usage	1. Fibrosis evaluation 2. Prediction risk of HCC 3. Prediction of mortality	1. Diagnostic marker for HCC (complementary to AFP) 2. Prognostic marker for HCC (correlates tumor characteristics) 3. Predictive marker for HCC

M2BPGi Mac-2-binding protein glycosylation isomer; *PIVKA-II* protein induced by Vitamin K absence or Antagonist-II; *HCC* hepatocellular carcinoma; *AFP* alpha-fetoprotein

11 HLA

T cells have been demonstrated to play an essential role in control HBV infection (Bertoletti and Ferrari 2016). $CD8^+$ and $CD4^+$ T cells exert the antiviral activity through recognition of epitope peptides presented by class I and class II major histocompatibility complex, also named human leukocyte antigen (HLA), respectively. Thus, it is not surprising that HLA has been associated with the outcomes of CHB. Previous studies more commonly associated class II HLAs with the outcomes of CHB. For example, HLA-DRB1*1302 was associated with the protection against chronic HBV infection among both children and adults in Gambia (Thursz et al. 1995). Two subsequent studies confirmed the findings in Caucasian and Korean populations (Ahn et al. 2000; Hohler et al. 1997). A genome-wide association study (GWAS) on 786 Japanese CHB cases and 2201 controls discovered that 11 single-nucleotide polymorphisms (SNPs) in the region, including HLA-DPA1 and HLA-DPB1 were associated with CHB. Further study on three additional Japanese and Thai cohorts with 1300 cases and 2100 controls validated the results and identified risk haplotypes (HLA-DPA1*0202-DPB1*0501 and HLA-DPA1*0202-DPB1*0301) and protective haplotypes (DPB1*0402 and HLA-DPA1*0103-DPB1*0401) (Kamatani et al. 2009). The role of HLA-DPA1 and HLA-DPB1 in HBsAg seroclearance was further supported by analysis of different ethnics, including Asians and Caucasians, in the following studies (Mbarek et al. 2011; Nishida et al. 2012; Koukoulioti et al. 2019). The association of Class I HLAs with the outcomes of HBV infection was also noted, although it was less common than class II HLAs. Studies from Taiwan showed that HLA-B*4001 was associated with a higher rate of HBsAg seroconversion (Wu et al. 2004), and HLA-B61 was associated with earlier HBeAg seroconversion (Wu et al. 2006). The association of HLA-A*33:03 with persistent HBV infection and the association of HLA-B*13:01 with HBsAg clearance were also found in the Chinese Han population (Miao et al. 2013). In addition, the meta-analysis on 1652 healthy controls and 659 CHB patients from eight studies showed the protective role of HLA-B*07 and B*58 against CHB (Seshasubramanian

et al. 2018). Nevertheless, all the above studies only showed the association between HLA alleles and outcomes of CHB. Future studies are required to explore the underlying mechanisms that exert viral control through HLA-dependent pathways.

12 Total Anti-HBc Level

Anti-HBc antibody reacts against HBcAg and is a conventional serological marker for HBV infection. IgM anti-HBc represents an indicator of acute hepatitis B or flares during chronic HBV infection, whereas the IgG anti-HBc antibody lasts almost a lifetime in individuals with exposure to HBV. However, the evolution and dynamics of total anti-HBc levels in the natural history of CHB remain unclear. Recently, a quantitative anti-HBc (qAnti-HBc) has been developed and tested for its clinical utility (Li et al. 2010).

Quantitative qAnti-HBc levels were found to correlate well with ALT and hepatic inflammation levels in both HBeAg-positive and HBeAg-negative patients (Yuan et al. 2013, 2015; Jia et al. 2014; Song et al. 2015). In addition, a previous study also observed the variation of qAnti-HBc levels across different disease phases of HBV infection (Song et al. 2015). The levels of qAnti-HBc are higher in the immune clearance and reactivation phases than those in the immune tolerance and inactive carrier phases (Fig. 9.1). Of note, qAnti-HBc levels of the immune-tolerant patients are similar to those of inactive carriers, despite that the immune-tolerant patients exhibit very high levels of HBV DNA. This further supports the association between ALT and qAnti-HBc levels. In addition, serum qAnti-HBc level of HBsAg-positive patients was higher than those of HBsAg-negative individuals. Interestingly, among HBsAg-negative persons, subjects with occult HBV infection had higher serum qAnti-HBc levels than those with past HBV infection. A study on 397 patients from the REVEAL-HBV cohort, followed for 6.8 years, reported that baseline qAnti-HBc level was associated with HBeAg seroclearance (Liu et al. 2019b).

Recently, serum anti-HBc levels were found to positively correlate with intrahepatic cccDNA in HBsAg-negative patients with occult HBV infection. Moreover, higher baseline qAnti-HBc levels predict a higher risk of HBV reactivation in lymphoma patients with resolved hepatitis B receiving rituximab-containing chemotherapy (Hazard ratio: 4.52 for qAnti-HBc <6.41 IU/mL versus ≥ 6.41 IU/mL) (Yang et al. 2018). Taken together, given the correlation between qAnti-HBc and ALT, total qAnti-HBc level may serve as a serological marker for HBV-induced hepatic necroinflammation and is complementary to other quantitative viral markers, like HBsAg HBV DNA, and HBcrAg levels.

13 Fibrosis, A Key Step of Disease Progression

Following chronic hepatitis, liver fibrosis, cirrhosis, and HCC are subsequent steps of disease progression. In addition to the above-mentioned viral factors, liver inflammation and fibrosis are two key host factors leading to disease progression in CHB.

Two factors contribute to cirrhosis progression: HBV viral factors (genotype C, high viral load, basal core promoter mutation, and HBx protein) and HBV-induced hepatic inflammation (Su et al. 2014). Early studies indicated no fibrosis progression in patients in the immune-tolerant phase with normal liver function but elevated (0.21 fibrosis unit/year) in those with high ALT levels (Hui et al. 2007). Long-term hepatic necroinflammation is the main contributor to both fibrogenesis and carcinogenesis of the liver (Su et al. 2014). Successful antiviral therapy to suppress the viral replication, reduce liver inflammation, may halt or reverse liver fibrosis, and reduce the development of cirrhosis-related complications, HCC, and mortality even in patients with established cirrhosis (Su et al. 2016). In recent years, measurement of liver fibrosis is a new prerequisite in the management of CHB. Non-invasive tests of liver fibrosis are emerging and acceptable by the patients, including fibroscan, shear-wave elastography (Su et al. 2018), and blood-based biomarkers, such as Fib-4 index (Zoutendijk et al. 2013), and M2BPGi (Su et al. 2020).

The Mac-2 binding protein (M2BP) is a secreted glycoprotein (~90 kDa) with 7 N-glycans per monomer (Narimatsu 2015), which is polymerized in serum to form a sweet doughnut-like structure (Kuno et al. 2013). The N-glycosylation of M2BP alters during the progression of liver fibrosis. These altered form of M2BP becomes the M2BPGi, which is secreted by hepatic stellate cells (HSCs) in the liver (Shirabe et al. 2018). M2BPGi may be detected specifically by the *Wisteria floribunda* agglutinin (WFA) lectin probe (Kuno et al. 2013), and become a simple blood test for clinical usage. Several studies demonstrated the clinical usage of M2BPGi in the management of CHB. M2BPGi could be used for assessing liver fibrosis (Ishii et al. 2017; Yeh et al. 2019). M2BPGi may predict HBeAg seroconversion (Nishikawa et al. 2016).

Previous studies indicated that Mac-2 (Galectin 3) protein may stimulate cancer progression (Wang and Guo 2016), and thus the M2BPGi mediated by Mac-2 protein may be associated with HCC development in patients with liver fibrosis (Shirabe et al. 2018). Several studies investigate the role of M2BPGi in prediction the HCC risk in patients with CHB (Heo et al. 2016; Ichikawa et al. 2017). From 1070 patients of the REVEAL-HBV cohort, the M2BPGi is demonstrated to be a short-term predictor of HCC in untreated CHB (Liu et al. 2017). The role of M2BPGi in patients undergoing antiviral therapy had been investigated. A higher pre-treatment M2BPGi level was associated with an increased risk of HCC development in patients with undetectable HBV DNA under nucleos(t)ide analog (NA) therapy (Cheung et al. 2017; Hsu et al. 2018). Serum M2BPGi level significantly decreases after NA treatment in CHB patients (Hsu et al. 2018). In CHB patients receiving long-term NA treatment, serum M2BPGi level not only serves as an independent HCC predictor but also complements PAGE-B in stratifying HCC risks (Tseng et al. 2020). Not only baseline M2BPGi level, the M2BPGi level after viral suppressed by long-term NA therapy also predict the subsequent HCC risks, and even the risk of mortality in CHB patients with cirrhosis (Su et al. 2020), which may benefit the on-treatment individuals. M2BPGi level had been reimbursed by Japan and Korea and may become a clinical biomarker for confirmation, prediction, and monitoring of liver fibrosis, cirrhosis, HCC, and mortality in patients of CHB.

14 HCC Surveillance

HCC is the disastrous outcome of CHB. Contributing factors and predictors for HCC are important to be monitored during the management of CHB. AFP is commonly used for the surveillance of HCC, but AFP level was usually not elevated in small HCCs (Chen et al. 1984), and has some limitations. PIVKA-II, also called des-γ-carboxyprothrombin (DCP), is abnormal prothrombin without proper process of it γ-carboxyglutamic acid residues, produced by hepatocytes because of vitamin K deficiency. The abnormal prothrombin could be the product of a number of different defects in the vitamin K-dependent carboxylation system of the malignant hepatocytes, which include decreased cellular uptake of vitamin K or acquired enzyme deficiencies in the vitamin K cycle (Liebman et al. 1984), or a decline of γ-glutamyl carboxylase in HCC tissues (Inagaki et al. 2011), or the overexpression of prothrombin precursors (Ono et al. 1990). Preclinical studies showed PIVKA-II could induce proliferation of HCC cells through the STAT3 pathway by functioning like HGF and enhance the production of various angiogenic factors to promote angiogenesis around HCC tissues (Inagaki et al. 2011). Because the serum PIVKA-II level was not elevated in patients with chronic liver disease or cirrhosis, it becomes a specific diagnostic marker for HCC (Liebman et al. 1984; Inagaki et al. 2011). After the improvement of the sensitivity in the measurement of PIVKA-II by enzyme immunoassay, it becomes a commercial diagnostic test.

PIVKA-II has been approved for diagnosis of HCC by the Japan Society of Hepatology since 2008 (Kokudo et al. 2015) and has been used singly or in combination with other biomarkers. The GALAD score (gender, age, alpha-fetoprotein (AFP)-L3, AFP, and DCP) was created to detect HCC, which performed better than the detection by abdominal ultrasound (Yang et al. 2019). Several studies showed PIVKA-II level outperformed the AFP level to be an early diagnostic marker (Wu et al. 2018) and a poor prognostic marker for HCC (Kim et al. 2009). According to a recent metanalysis of 6 studies, PIVKA-II is an ideal marker for diagnosis of HBV-related HCC with an AUROC of 0.91 (95% CI: 0.88–0.93) (Chen et al. 2018). Meanwhile, PIVKA-II level had been associated with portal vein thrombosis in HBV-related HCC (Li et al. 2019), and a positive correlation between higher PIVKA-II and tumor size, differentiation, and microvascular invasion had been observed (Loglio et al. 2020). The combination of AFP and PIVKA-II further improves the diagnostic accuracy (Ji et al. 2016). Not only Asians, a recent study demonstrated the combination of PIVKA-II and AFP increases the detection rate for HCC in long-term NA-treated HBV Caucasian cirrhotic patients, suggesting a potential new approach for HCC surveillance (Loglio et al. 2020). However, a recent Korean study failed to show any benefit adding PIVKA-II to the combination of AFP and AFP-L3 in HBV patients treated with NA therapy during HCC surveillance (Choi et al. 2019). More studies are needed to determine the timing for the measurement of PIVKA-II for the surveillance of HCC.

Finally, the prognostic role of PIVKA-II for HCC had been investigated. Elevated preoperative AFP and PIVKA-II levels were associated with a higher recurrence

rate and shorter disease-free survival in patients with HBV-related HCC after curative resection (Chon et al. 2012).

15 Development of Risk Scores for Cirrhosis and HCC by Integration of Viral and Host Factors in CHB Patients

Accurate prediction for the risk of cirrhosis and HCC is required for timely and appropriate treatment of CHB. Since several aforementioned viral factors that affect the disease progression have been identified, it allows to better calculate the risk of HCC by incorporation of several viral and host factors in a risk scoring system in order to perform optimal and personalized surveillance for CHB patients (Yang et al. 2014; Wong and Janssen 2015). Previously, several risk scoring systems had been developed to predict the risk of HCC (Han and Ahn 2005; Yuen et al. 2009, 2010; Wong et al. 2010). Most of them were hospital-based cohort studies except the community-based Taiwanese cohort (REVEAL-HBV). These risk scoring systems faced the challenges of lacking external validation. To solve this issue, these study groups cooperated to develop a 17-point risk scoring system for HCC (REACH-B), which was derived from non-cirrhotic naïve CHB patients in the REVEAL-HBV cohort and validated by a composite hospital-based cohort from Hong Kong and Korea. The REACH-B risk score integrates several host and viral factors including age, sex, ALT level, HBeAg serostatus, and serum HBV DNA level, and accurately predict the risk of HCC with a wide range, from 0.0% to 23.6% at 3 years, 0.0% to 47.4% at 5 years, and 0.0% to 81.6% at 10 years for patients with the lowest through the highest scores (Yang et al. 2011). Recently, the HBsAg level has been demonstrated to predict the risk of long-term adverse outcomes and HCC (Tseng et al. 2012b, 2013), so the original REVEAL risk scoring system was upgraded by incorporation of HBsAg level and internally validated (Lee et al. 2013). In addition to its use in the prediction of HCC, this new risk calculator also accurately predicts the risk of cirrhosis. The sum risk scores ranged from 0 to 26 in the prediction model for cirrhosis and from 0 to 19 in the prediction model for HCC, respectively. The cirrhosis risk ranged from 0.08%–43.15% at 3 years, 0.13%–60.11% as 5 years, and 0.36%–91.98% at 10 years. For the HCC risk, it ranged from 0.01%–36.19% at 5 years, 0.03%–79.72% at 10 years, and 0.07%–98.16% at 15 years. Of note, external validation is still required for this new scoring system to prove its applicability in other CHB cohort patients.

16 Conclusions

Since the risk of long-term adverse outcomes in CHB patients is high, early diagnosis of patients at risk is critical to provide optimal and timely management for them. Previous studies, particularly the longitudinal cohort studies, have identified viral and host factors that affect the disease progression. The discovery of these viral and

host factors not only allows to gain mechanistic insight into the pathogenesis of CHB, but also helps the risk stratification of patients in order to improve the quality of care. Integration of several viral and host factors into a simple risk scoring system is even more useful for clinicians to evaluate the disease status of a patient. With the advance of quantitative and qualitative assays, new viral and host biomarkers will emerge and help fine-tune the delineation of disease status and the risk stratification of CHB patients. Understanding the impact of viral and host factors on disease progression and long-term outcomes of CHB will shed light on the complex virus–host interactions and eventually lead to optimal care of patients with the ultimate hope of HBV cure.

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Novel Biomarkers for the Management of Chronic Hepatitis B Virus Infection

10

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Abstract

On the basis of innovations in molecular medicine and genomics, several novel hepatitis B viral and host biomarkers associated with diagnosis and disease progression of chronic hepatitis B virus (HBV) infection have been elucidated. For large-scale screening of chronic hepatitis B (CHB), point-of-care tests provide simple and rapid methods for HBV detection. Regarding the monitoring of CHB progression, hepatitis B core-related antigen (HBcrAg) and HBV RNA are positively correlated with intrahepatic cccDNA. The serum HBcrAg level is a predictor of cirrhosis and HCC development, whereas HBV RNA is associated with the response of antiviral therapy and can serve as a biomarker to predict viral relapse after discontinuation of antiviral therapy. Total anti-HBc level may reflect the host immune response to HBV infection and is significantly correlated with the severity of hepatic inflammation and fibrosis as well as HBsAg seroclearance. For hepatocellular carcinoma (HCC) surveillance, Mac-2 binding protein glyco-

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sylation isomer (M2BPGi) is associated with HCC development in treatment-naïve patients and at virological remission in those on antiviral therapy. Finally, the circulating cell-free DNA or virus–host chimeric DNA and microRNAs play a functional role in hepatocarcinogenesis and have a high predictive value in the diagnosis of HBV-related HCC. In conclusion, these novel biomarkers help clinicians monitor the natural course and treatment response of CHB. The combination of novel and already in use biomarkers will improve the ability of early diagnosis and prognostic prediction of HBV-related HCC.

Keywords

Chronic hepatitis B · Circulating cell-free virus–host chimeric DNA · HBcrAg · HBV RNA · M2BPGi · microRNA · Total anti-HBc

1 Introduction

Hepatitis B virus (HBV) is one of the most important infectious diseases worldwide. Although the effective HBV vaccine successfully reduced the prevalence of HBsAg in young people (Lin and Kao 2020), it is estimated that there are 257 million patients with chronic HBV infection globally (Schweitzer et al. 2015). The host immune reaction to HBV leads to persistent and chronic hepatic inflammation and is responsible for the long-term adverse consequences, including chronic hepatitis, cirrhosis, hepatic failure and hepatocellular carcinoma (Kao and Chen 2002; Yuen et al. 2018). If left untreated, CHB results in cirrhosis and HCC. The annual incidence of cirrhosis varies from 2% to 10%. The cumulative incidence of cirrhosis at 5 years reaches approximately 20%. Furthermore, the annual incidences of HCC in non-cirrhotic patients and cirrhotic patients are less than 1% and up to 3%, respectively (Lin and Kao 2008). Therefore, patients with CHB must be diagnosed as early as possible, followed by disease monitoring and receiving anti-HBV therapy if the treatment criteria are met.

After decades of the development of anti-HBV therapy, the prevention of disease progression and reduction of HCC incidence in HBV patients have been achieved (Lin and Kao 2018). Several cohort studies demonstrated that the risk of HCC development was reduced by 45–60% in CHB patients receiving high potency and low resistance of anti-HBV therapy (Lin and Kao 2018). However, the optimal treatment duration and the predictors of treatment response need further clarification.

With recent advances in molecular medicine and genomics, several novel biomarkers concerning the management of chronic HBV infection have been elucidated (Coffin et al. 2019). Taking these biomarkers with clinical manifestations together will provide valuable medical care to improve survival and quality of life for CHB patients. In this article, the novel biomarkers for monitoring disease progression and surveillance of HCC in CHB are reviewed and discussed (Fig. 10.1).

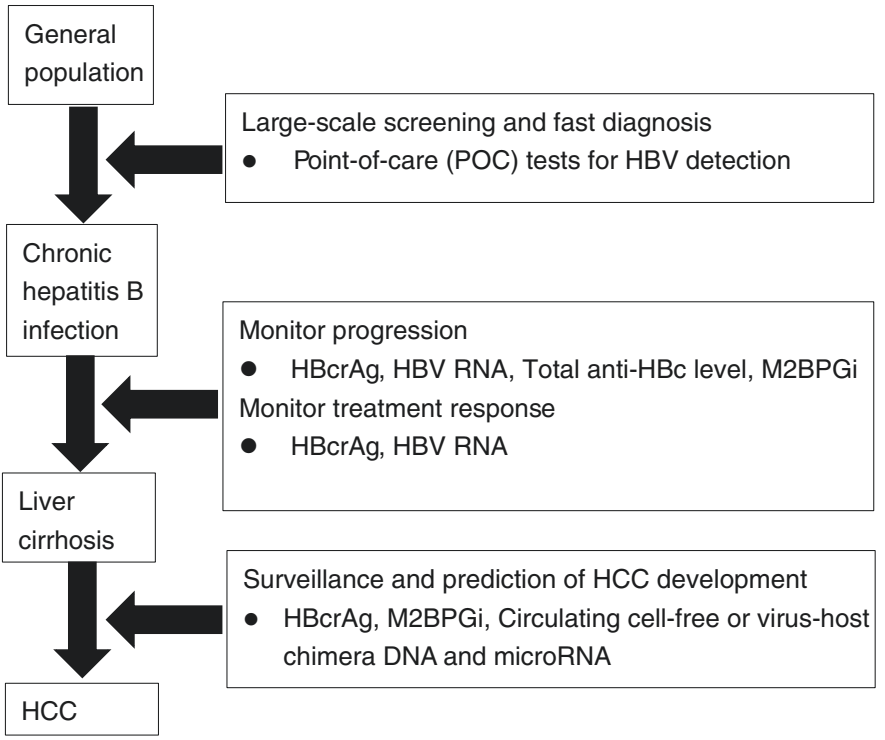

Fig. 10.1 Novel biomarkers for the management of chronic hepatitis B. *HBV* hepatitis B virus; *HBcrAg* hepatitis B core-related antigen; *HCC* hepatocellular carcinoma; *M2BPGi* Mac-2 binding protein glycosylation isomer

2 Viral Biomarkers for the Diagnosis and Screening of CHB

The conventional diagnosis for HBV infection is based on the detection of hepatitis B surface antigen (HBsAg). However, serum or liver HBV DNA is still detectable in subjects with occult HBV infection (OBI), who are HBsAg-negative/anti-HBc-positive (Mortensen et al. 2016). Detectable HBV DNA in plasma or serum is the molecular diagnosis of hepatitis B in all guidelines (Terrault et al. 2018; European Association for the Study of the Liver, European Association for the Study of the Liver. 2017; Sarin et al. 2016). However, these laboratory-based diagnostic methods may not be easily approachable or affordable, particularly in resource-limited areas. Therefore, affordable alternative methods for the rapid diagnosis or screening of HBV infection are required. The recent point-of-care (POC) tests for HBV detection can be used immediately at the time and place of patient care. POC tests include an immunologic test for viral antibodies and/or antigens and virologic test for nucleic acid of HBV. POC tests are easy to use and obtain results faster and can be applied to large-scale HBV screening (Chevaliez and Pawlotsky 2018). The recent

meta-analysis of 23 studies revealed that the sensitivity and specificity of POC testing with dried blood spots were 98% (95% CI: 95%–99%) and 100% (95% CI: 99–100%) for HBsAg detection (Lange et al. 2017). At present, several POC platforms have been developed. The technologic methods include microfluidic chip, lateral flow technology, electrochemical biosensors, and ultrasonic diagnostics (Yildiz et al. 2015). For example, a paper-based electrochemical sensor was developed for HBV DNA detection. This quantitative assay for HBV DNA combined slip paper-based analytical devices method and electrochemical detection technique. The assay provided a simple procedure to capture a detection limit of 85 picomole (Li et al. 2015). Recently, the POC platform based on a microfluidic chip can extract HBV nucleic acid in less than 1 min. The limit of detection was 200 IU/ml (Zhang et al. 2019a). Based on the recent development of POC platform for HBV DNA detection, the POC platform for diagnosis of HBV infection will be user-friendly, low cost with a simple procedure. However, it is essential to improve the detection limit of HBV DNA concentration before its application in large-scale HBV screening.

3 Viral Biomarkers Predictive of Disease Progression and Response to Therapy

3.1 HBV DNA and Quantitative HBsAg

With recent advances in molecular investigations of HBV, several viral biomarkers for the monitoring of natural course and treatment response of chronic HBV infection have been introduced (Lin and Kao 2016). Among the existing markers, hepatitis B viral load and quantitative HBsAg level are widely available and considered as standard virologic markers in clinical practice (Table 10.1). In brief, hepatitis B viral load is a strong predictor for liver disease progression. The monitoring of hepatitis B viral kinetics is the key marker for the response of antiviral therapy (Lin and Kao 2013). Serum HBsAg level serves as a complementary marker to hepatitis

Table 10.1 Existing viral biomarkers for the management of chronic hepatitis B

Hepatitis B viral biomarker	Clinical application
HBV DNA	• Associated with HCC risk in treatment-naïve patient. • Hepatitis B viral kinetics is the key marker for the response of antiviral therapy.
Quantitative HBsAg	• Positive correlated with intrahepatic cccDNA. • Predictor of spontaneous HBsAg loss in HBeAg-negative carriers with a low HBV DNA level • Complementary to HBV DNA level in predicting HCC risks, especially in patients with low HBV DNA level. • Associated with virologic relapse after cessation of anti-HBV therapy

B viral load for the prediction of HBV-related cirrhosis and HCC in patients with low viral load (Tseng et al. 2012, 2013). In addition, the HBsAg level at the time of discontinuation of anti-HBV therapy is associated with the rate of virologic relapse after cessation of anti-HBV therapy (Chen et al. 2015; Wang et al. 2016a; Jeng et al. 2018). However, the clinical course of CHB varies greatly among infected individuals. HCC still occurs in patients with sustained viral suppression and even in those with HBsAg seroclearance (Liu et al. 2014; Kim et al. 2015). Furthermore, there are no surrogate markers to decide the optimal duration of anti-HBV therapy in real-world practice (Chong and Lim 2017). Therefore, new biomarkers for the accurate determination of treatment duration for CHB are urgently awaited (Table 10.2).

Table 10.2 Emerging viral biomarkers for the management of chronic hepatitis B

Hepatitis B viral biomarker	Clinical application
Point-of-care (POC) test: immunologic test for viral antibodies and/or antigens and virologic test for the nucleic acid of HBV	• Large-scale HBV screening and diagnosis
Hepatitis B core-related antigen	• Positive correlated with serum HBV DNA and intrahepatic cccDNA • Predictor of spontaneous HBsAg loss in HBeAg-negative carriers with a low HBV DNA level • Predict HBV reactivation during antiviral therapy • Identify patients can discontinue antiviral therapy • Complementary to HBV DNA level in predicting HCC or cirrhosis risks, especially in patients with intermediate (2000-19,999 IU/ml) or low viremia (< 2,000 IU/ml) • Predict the risk of recurrence after primary HCC receiving curative resection
HBV RNA	• Positive correlated with intrahepatic cccDNA • Distinguish inactive and active hepatitis in HBeAg-negative CHB patients • Predict treatment response in patients receiving antiviral therapy • Predict clinical relapse after stopping NA treatment
Quantification of HBV core antibodies	• Distinguish the four phases of chronic HBV infection • Predict the transition from inactive hepatitis to active hepatitis • Predict HBsAg seroclearance in HBeAg-negative patients • Positively correlated with the severity of hepatic inflammation and fibrosis • Predict treatment responses in patients receiving antiviral therapy • Predict HBV reactivation in patients with immunosuppressive therapy

3.2 Hepatitis B Core-Related Antigen

Of the emerging hepatitis B viral markers, hepatitis B core-related antigen (HBcrAg) has caught the eyes of clinicians. HBcrAg consists of three proteins coded with the precore/core region, including hepatitis B core antigen (HBcAg), HBeAg and a precore protein (p22cr) (Kimura et al. 2002). HBcAg is an inner nucleocapsid surrounding the viral DNA. HBeAg is a circulating peptide derived from the core gene, then modified and secreted from liver cells. Both HBcAg and HBeAg can serve as inducer antigen of T-helper cell and are the target of cytotoxic T-cell. Therefore, HBcrAg may induce T-cell functions which is associated with viral elimination, remission, and acute exacerbation of chronic HBV infection (Lin and Kao 2016).

A previous report from Japan indicated that serum HBcrAg concentration was positively correlated with serum HBV DNA as well as intrahepatic cccDNA level. Even in serum HBV DNA-negative patients, the HBcrAg was still significantly correlated with intrahepatic cccDNA (Suzuki et al. 2009). Recent study further revealed that serum HBcrAg levels not only correlated with intrahepatic cccDNA, also associated with intrahepatic total HBV DNA, pregenomic RNA, transcriptional activity and severity of hepatic necroinflammation and fibrosis (Testoni et al. 2019). In several cohort studies of treatment-naive patients, the serum HBcrAg levels from high to low is the immune tolerance phase, immune clearance phase, HBeAg-negative chronic hepatitis and HBeAg-negative inactive carrier state in sequence (Maasoumy et al. 2015; Gou et al. 2017). In a follow-up study of HBeAg-positive patients at the immune clearance phase, the lower baseline HBcrAg levels and the larger reduction of HBcrAg levels during follow-up were associated with spontaneous HBeAg seroconversion (Song et al. 2017). Recently, the diagnostic accuracy of HBcrAg in predicting cirrhosis was investigated. Based on the *area under the receiver operating characteristic* (AUROC) curve analysis, HBcrAg levels had superior accuracy than HBV DNA levels for the prediction of cirrhosis in HBeAg-positive and HBeAg-negative patients. The AUROC were 0.700 and 0.837, respectively (Zhang et al. 2020a).

In the era of anti-HBV therapy, whether and when to stop nucleos(t)ide analogs (NAs) in HBeAg-negative CHB patients with sustained viral suppression arouse widespread discussion (Liaw 2019). In a prospective multicenter study on 135 CHB patients who stopped NA after sustained viral suppression, the serum HBcrAg level at stopping NA treatment was significantly associated with the incidence of clinical relapse (HR for clinical relapse was 1.48 (95% CI: 1.20–1.83) per log U/mL) (Hsu et al. 2019). In a prospective European study with HBeAg-negative patients receiving NA treatment, the undetectable HBcrAg at discontinuation of treatment was strongly correlated with HBsAg loss. On the contrary, patients with detectable HBcrAg had a significantly higher probability of retreatment (Papatheodoridi et al. 2020). Therefore, HBcrAg may help aid decision-making of cessation NA therapy in HBeAg-negative CHB patients.

Taken together, HBcrAg can be regarded as a surrogate marker of transcription activity of intrahepatic cccDNA and can be used to differentiate clinical phases of CHB, monitor liver disease deterioration and predict response and reactivation during antiviral therapy (Fig. 10.2).

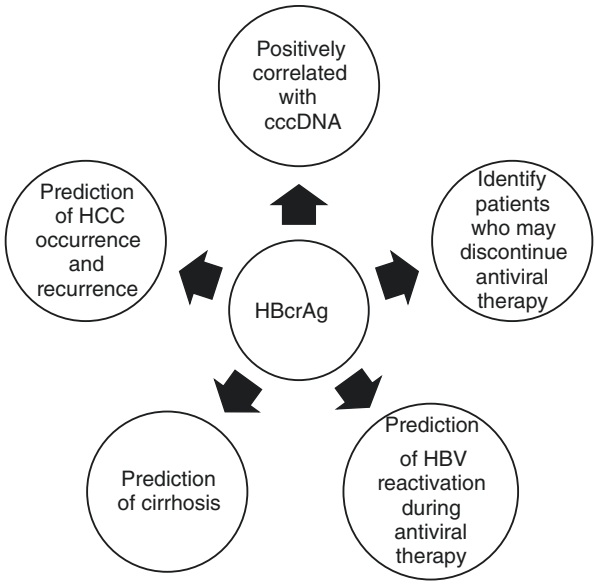

Fig. 10.2 Clinical application of hepatitis B core-related antigen. *HCC* hepatocellular carcinoma

3.3 HBV RNA

In HBV replication cycle, HBV pregenomic RNA is a temple for reverse transcription to HBV DNA in the nucleocapsid (Wu et al. 2019). It was reported that HBV RNA could be detected in peripheral blood in the early 1990s (Baginski et al. 1991; Köck et al. 1996). Further mouse model revealed that serum HBV RNA was pregenomic RNA and may represent the activity of intrahepatic cccDNA (Wang et al. 2016b). HBV RNA may serve as an alternative biomarker to monitor the natural course of CHB. In a cross-sectional study, Liu et al. reported that serum HBV RNA levels were highest in patients with HBeAg-positive, followed by HBeAg-negative patients, and the lowest in inactive carriers. Serum HBV RNA level will increase when the patients with HBeAg-negative CHB progressed from inactive phase to active phase. HBV RNA was superior to HBV DNA and HBsAg levels in distinguishing inactive and active hepatitis in HBeAg-negative CHB patients (Liu et al. 2019).

Serum HBV RNA is also useful for the prediction of treatment response in patients receiving antiviral therapy. In Asian HBeAg-negative CHB patients receiving pegylated interferon-based therapy, the serum HBV RNA levels at baseline and at week 12 of treatment were correlated with maintained virologic response and HBsAg clearance after treatment. At week 12 of treatment, patients without HBV RNA reduction had very low probability to achieve sustained virologic response (Limothai et al. 2019). For HBeAg-positive CHB patients receiving NA therapy, Ji et al. found that patients with serum HBV RNA level decline at week 12 of treatment had a higher rate of HBeAg seroconversion than those without HBV RNA decline (OR 3.560, 95% CI: 1.39–9.110, $P = 0.008$) (Ji et al. 2020).

Regarding the clinical end-point of NA treatment, HBV RNA may play a role in predicting relapse after NA discontinuation. In a recent study, Fan et al. followed HBeAg-positive CHB patients with NA treatment. The NA was stopped when HBeAg seroconversion with 48 weeks of consolidation treatment. After 4-year follow-up, patients with both undetectable HBV DNA and HBV RNA at the time of NA discontinuation had a significantly lower incidence of clinical relapse than patients with either HBV DNA or HBV RNA was detectable (Fan et al. 2020a). Furthermore, they investigated the combination of HBV RNA and HBcrAg in predicting clinical relapse after stopping NA treatment in HBeAg-positive patients who reached the end-point of treatment. During 4-year follow-up, patients with undetectable HBV RNA and low levels of HBcrAg (less than 4 $\log_{10}$ U/mL) at the end of treatment had significantly lower incidence of clinical relapse than patients with positive HBV RNA and high levels of HBcrAg (greater than 4 $\log_{10}$ U/mL) at the end of treatment (0% vs. 69.4%, $P < 0.001$) (Fan et al. 2020b).

3.4 Quantification of HBV Core Antibodies (Total Anti-HBc)

After exposure to HBV, the human immune system produces immune responses to HBV-encoded antigens. Immunoglobulin antibody to HBcAg (anti-HBc) is the earliest antibody to develop after infection. Anti-HBc has no protective effect against the virus. The clinical meaning of anti-HBc positivity includes resolved HBV infection, healthy HBV carriers or chronic hepatitis B (Liaw and Chu 2009). Therefore, the anti-HBc titer may reflect the immune responses in different phases of HBV infection. Through a novel assay for the quantification of total anti-HBc level (Li et al. 2010), the association between the total anti-HBc titer and the natural course of chronic HBV infection has been elucidated. In a clinical observational study, the serum levels of total anti-HBc were highest in patients with chronic HBV infection, followed by patients with occult infection, and lowest in patients with past HBV infection (Song et al. 2015). In a cross-sectional study, Jia et al. investigated the total anti-HBC levels in 211 treatment-naïve CHB patients. In the four phases of chronic HBV infection, total anti-HBc levels are significantly higher in the immune clearance and HBeAg-negative hepatitis than in the immune tolerance and inactive carrier phases. The low total anti-HBc levels in patients under the immunetolerant phase, who were expected to have high levels of HBV DNA, suggesting the immune response in the immune tolerance phase was similar to that of the inactive carrier state (Jia et al. 2014). In a cohort study enrolling 153 low viremic HBeAg-negative patients with normal serum ALT, the high baseline total anti-HBc levels was a powerful predictor to identify the patients who progressed to active HBeAg-negative chronic hepatitis B after 1 year of follow-up (4.84 vs. 3.90 $\log_{10}$IU/mL, OR: 10.221, 95% CI: 1.677–62.278, P = 0.012). The AUROC curve to predict the transition from normal to abnormal serum ALT was 0.884 (95% CI: 0.775–0.992, $P < 0.001$) (Oliveri et al. 2017). In a retrospective study that included 624 CHB patients without antiviral therapy, Li et al. found that total anti-HBc levels were positively

correlated with the severity of hepatic inflammation and fibrosis (Li et al. 2016, 2018). The optimal cutoff values of total anti-HBc level for the prediction of hepatic inflammation in HBeAg-positive and HBeAg-negative CHB patients without antiviral therapy were 4.36 log10 IU/mL and 4.62 log10 IU/mL, respectively (Li et al. 2016). In addition, the AUROC curve analysis revealed that the total anti-HBc level could be used for the diagnosis of significant fibrosis with modest accuracy (the AUROC was around 0.7) (Li et al. 2018). However, total anti-HBc level alone is insufficient for the prediction of hepatic inflammation and fibrosis. In combination with serum HBsAg and HBV DNA levels, total anti-HBc levels significantly improved the ability of these biomarkers in predicting hepatic inflammation and fibrosis (Zhang et al. 2019b). In a retrospective study in Taiwan, Hu et al. found that HBeAg-negative CHB patients with low baseline levels of anti-HBc (<3 log IU/ml) had a higher proportion of HBsAg seroclearance than patients with high levels of anti-HBc during long-term follow-up (adjusted rate ratio was 17.95). Compared to the models including HBV DNA and HBsAg, the prediction model including anti-HBc, HBV DNA and HBsAg had significantly higher predicting value to identify the possibility of HBsAg seroclearance in HBeAg-negative patients within 10 years. The AUROC was 0.82 (Hu et al. 2019).

The baseline total anti-HBc level was also used to predict treatment responses in CHB patients receiving antiviral therapy. In a retrospective study including HBeAg-positive patients with pegylated-interferon or NA therapy, the baseline anti-HBc level was significantly associated with the possibility of HBeAg seroconversion. Regardless of pegylated-interferon or NA therapy, patients with baseline anti-HBc levels ≥4.4 log10 IU/mL had a higher rate of HBeAg seroconversion (Fan et al. 2016). In another retrospective study, Xu et al. investigated the role of anti-HBc level in predicting response in HBeAg-positive patients receiving long-term entecavir treatment. The baseline anti-HBc level was the most significant factor associated with HBeAg seroconversion (OR: 5.78, 95% CI: 2.05–16.34, $P = 0.001$) (Xu et al. 2017). Recently, a similar study further revealed that the high ability of total anti-HBc levels in predicting HBeAg seroconversion after NA therapy with AUROC of 0.714 (Fu et al. 2020). Accordingly, total anti-HBc level may reflect the host adaptive immunity against HBV and serve as a biomarker in monitoring the natural course and treatment responses of CHB.

Patients with HBV infection, even resolved HBV infection, are vulnerable to HBV reactivation during immunosuppressive therapy or chemotherapy (Lin and Kao 2017). In a prospective study, Yang et al. combined serum levels of anti-HBs and anti-HBc to predict HBV reactivation in lymphoma patients with resolved HBV infection receiving rituximab-containing chemotherapy. They found that both high anti-HBc and low anti-HBs at baseline were associated with HBV reactivation with an HR of 17.29 (95% CI 3.92–76.30; $P < 0.001$) (Yang et al. 2018). Similarly, the study from Japan also demonstrated that the risk of HBV reactivation was significantly higher in lymphoma patients with high anti-HBc levels at baseline than those with low anti-HBc levels (Matsubara et al. 2017). Therefore, the combination of anti-HBc and anti-HBs levels may be a clinically effective predictor for the management of HBV reactivation (Nishida et al. 2019).

4 Novel Biomarkers for Predicting HBV-Related HCC

HBV-related HCC accounts for a large proportion of HCC worldwide, especially in the Asia-Pacific region (Global Burden of Disease Liver Cancer Collaboration 2017). The development of new viral and host biomarkers for monitoring and early HCC detection will provide the opportunity for curative treatment to further reduce HCC mortality (Tables 10.2 and 10.3).

4.1 Hepatitis B Core-Related Antigen

Although a previous study revealed that serum HBV DNA levels were strongly correlated with an increased risk of HCC development (Chen et al. 2006), the risk of HCC still present in patients with a lower level of or undetectable HBV DNA. Therefore, new biomarkers for HCC prediction, especially for patients with intermediate (2000–19,999 IU/mL) or low viremia (< 2000 IU/mL), are urgently needed. In a study of 1031 CHB patients without NA treatment, HBcrAg was superior to HBV DNA in predicting HCC development. The hazard ratio (HR) was 5.05 (95% confidence interval (CI), 2.40–10.63) at the cutoff value of 2.9 $\log_{10}$ U/mL (Tada et al. 2016). For CHB patients with spontaneous HBeAg seroconversion, high HBcrAg (≥5.21 $\log_{10}$ U/mL) within 3 years after HBeAg seroconversion was correlated with HCC development with HR of 1.75 (P = 0.032) (To et al. 2019).

In a long-term follow-up (mean: 15.95 years) study of 2666 treatment-naïve, non-cirrhotic CHB patients from Taiwan, HBcrAg was associated with HCC development in HBeAg-negative patients with intermediate HBV DNA levels. Patients with HBcrAg levels greater than 10 KU/mL had an increased risk of HCC by 6 folds (HR: 6.29; 95% CI: 2.27–17.48) (Tseng et al. 2019). A subsequent study showed that in HBeAg-negative patients with normal ALT levels, higher HBcrAg levels were associated with an increased risk of cirrhosis. Among those with intermediate

Table 10.3 Emerging host biomarkers for the management of chronic hepatitis B

Host biomarkers	Clinical application
Mac-2 binding protein glycosylation isomer (M2BPGi)	• Associated with the severity of liver fibrosis • Associated with HCC risk in patients with antiviral therapy
Circulating cell-free or virus–host chimera DNA	• Total plasma circulating cfDNA concentration was significantly correlated with advanced HCC stage and early recurrence • Circulating cell-free virus–host chimera DNA was associated with HBV-related HCC • Circulating hypermethylation levels of tumor suppressor genes (APC, COX2, RASSF1A) were associated with HBV-related HCC
Circulating microRNA	• The majority of circulating microRNAs were associated with HCC, regardless of upregulated or downregulated expression.

viral load, HBcrAg < 10 KU/mL defines a low-risk group of disease progression (Tseng et al. 2021).

Regarding patients receiving antiviral therapy with the sustained virologic response, HBcrAg was also associated with HCC occurrence (Cheung et al. 2017; Hosaka et al. 2019). In a case-controlled study on patients receiving NA therapy with undetectable HBV DNA, both the baseline and post-treatment HBcrAg levels in HCC patients were significantly higher than those without HCC occurrence (OR: 3.27) (Cheung et al. 2017). In a similar study enrolling 1268 patients with NA treatment, persistently high levels of HBcrAg after NA treatment was strongly correlated with HCC developed during follow-up (Hosaka et al. 2019). For patients with primary HCC, the HBcrAg levels before HCC treatment were also associated with the risk of recurrence after curative treatment. In a study of 89 HCC patients receiving curative resection, the recurrence rates were significantly higher in patients with high HBcrAg levels than those with low levels ($P = 0.003$) (Chen et al. 2018).

4.2 Mac-2 Binding Protein Glycosylation Isomer (M2BPGi)

More than 90% of the protein in the human body is glycoprotein. The changes of glycan structure of glycoprotein were related to cell inflammation and neoplastic transformation (Stowell et al. 2015). The development of glycoprotein-based biomarkers (glyco-biomarkers) related to cancer is an important research area. In 2013, serum Mac-2 binding protein glycosylation isomer (M2BPGi), formerly named *Wisteria floribunda* agglutinin-positive Mac-2-binding protein, was first introduced as a novel glyco-biomarker associated with liver fibrosis progression in Japan (Kuno et al. 2013). Subsequently, the association between the serum M2BPGi level and severity of hepatic fibrosis was determined in CHB patients. In a study with CHB patients receiving liver biopsy, serum M2BPGi concentration increases with the severity of liver fibrosis. The AUROC curve analysis revealed that M2BPGi had better performance in diagnosing significant fibrosis than other noninvasive biomarkers (Zou et al. 2017). Another similar study from Taiwan also indicated that serum M2BPGi levels were positively correlated with histologic fibrosis stage in patients with chronic HBV infection. The AUROC curve of M2BPGi for diagnosis of advanced fibrosis and cirrhosis were 0.785 and 0.769, respectively. Serum M2BPGi level was a risk factor of advanced fibrosis with OR of 1.97 (95%CI: 1.299–2.984, $P = 0.001$) (Yeh et al. 2019).

The clinical application of serum M2BPGi level in the prediction of HBV-related HCC was recently investigated (Baudi et al. 2020). In a large-scale retrospective study, Kim et al. showed that M2BPGi level was significantly associated with HCC development (adjusted HR: 1.143, 95% CI: 1.139–1.829) (Kim et al. 2017). In a case-control study including 1070 treatment-naïve CHB patients, Liu et al. found that M2BPGi level was a risk factor of HCC development within 5 years. For cirrhotic patients, M2BPGi level ≥ 2 cutoff index (COI) had a significantly higher risk of HCC development within 5 years (OR within 2 years and within 2–5 years were 10.07 ($P < 0.001$) and 7.07 ($P < 0.001$)) (Liu et al. 2017). In patients receiving antiviral

therapy, Hsu et al. found that serum M2BPGi decreased with the treatment period in patients receiving antiviral therapy, from 1.68 COI at initial treatment decreased to 0.88 after 2 years of treatment (Hsu et al. 2018). The serum M2BPGi levels at initial treatment, but not the levels at 1 and 2 years after treatment, were significantly associated with HCC development in cirrhotic patients (Hsu et al. 2018). In another study on patients receiving entecavir therapy, Tseng et al. showed that baseline M2BPGi level was positively correlated with HCC development. Compared to patients with low level of M2BPGi, patients with M2BPGi level ≥ 1.73 COI had a significantly higher risk of HCC development with HR of 4.40 (95% CI 1.90–10.20) in non-cirrhotic patients and 2.28 (95% CI 1.19–4.40) in cirrhotic patients. In addition, the M2BPGi levels in patients with HCC development were persistently higher than those without HCC development during follow-up (Tseng et al. 2020). The predictive ability still existed in treated patients with virologic remission. In a retrospective cohort study, Su et al. included compensated cirrhotic patients receiving long-term antiviral therapy with undetectable HBV DNA. M2BPGi level at virologic remission was a significant predictor for HCC development (HR: 1.58, 95% CI: 1.19–2.10, $P = 0.002$) and death (HR: 2.17, 95% CI: 1.02–4.62, $P = 0.044$) (Su et al. 2020). Taking these lines of evidence together, M2BPGi may serve as a valuable biomarker to predict both liver fibrosis severity and HCC risk in CHB patients.

4.3 Circulating Cell-Free or Virus–Host Chimera DNA and microRNA

It is well known that HCC is partially attributed to human genetic and epigenetic heterogeneity (An et al. 2018). Circulating cell-free DNA (cfDNA), also known as liquid biopsy, has rapidly developed as potential noninvasive biomarkers, not only for early diagnosis of HCC but also for predicting HCC prognosis and response to treatment (Pezzuto et al. 2018). The application of circulating cfDNA on the management of HCC includes the amount of circulating cfDNA, somatic mutations and epigenetic methylation status of cfDNA. The earlier studies have already shown that HCC patients had significantly higher circulating cfDNA concentration than non-HCC patients (Ren et al. 2006; Tokuhisa et al. 2007; Huang et al. 2011). In a study that enrolled HBV-related HCC patients receiving hepatectomy, Wang et al. found that total plasma circulating cfDNA concentration was significantly correlated with advanced HCC stage and early recurrence after tumor resection (Wang et al. 2019). However, the increased concentration of cfDNA may also be caused by hepatic inflammation or cell necrosis. Therefore, the HCC-specific genetic mutations from cfDNA were further investigated.

Based on a whole-genome approach, several genetic variation associated with HBV-related HCC were identified. Recently, Li et al. investigated the sequence of HBV integration in HCC chromosome, namely virus–host chimera DNA, in HCC patients receiving surgical resection. The most common sites of HBV integration located at the TERT, MLL4, and CCNA2 gene. They further found that this virus–host chimera DNA, a circulating cell-free tumor-specific DNA, can be detected in

plasma by droplet digital PCR (dd-PCR). The circulating cell-free virus–host chimera DNA was detected in 97.7% of HBV-related HCC patients before surgery. In addition, the presence of postoperative circulating virus–host chimera DNA could be a predictor of HCC recurrence (Li et al. 2020).

DNA methylation is a process of DNA chemical modification, which can change the genetic expression without changing the DNA sequence. Changes of methylation pattern in specific DNA are considered to be associated with hepatocarcinogenesis (Sceusi et al. 2011). Lu et al. identified significantly higher circulating hypermethylation levels of three tumor suppressor genes (APC, COX2, RASSF1A) and one microRNA (miR-203) in patients with HCC, especially in HBV-related HCC, than non-HCC group. A cfDNA methylation predictive model was further established by combination with these candidate genes and microRNA. The AUROC was 0.87 for diagnosis of HBV-related HCC (Lu et al. 2017). Recently, Zhang et al. conducted a meta-analysis, including 33 studies with 4113 patients, to determine the predictive ability of circulating tumor DNA in screening and diagnosis of HCC. The summary of AUROC of quantitative circulating tumor DNA in diagnosis of HCC was 0.88. Furthermore, the circulating RASSF1A methylation had a high predictive value in distinguishing HCC patients from non-HCC patients with AUROC of 0.841 (Zhang et al. 2020b).

MicroRNA is a non-coding RNA with a 21 to 23 nucleotide long molecule to regulate gene expression. Several studies have reported that deregulation of microRNA was associated with cancer development (Romano et al. 2017). Numerous microRNAs were found to be associated with HBV-related HCC, such as microRNA-18a (Li et al. 2012), microRNA-125b (Giray et al. 2014; Chen et al. 2017), microRNA-223 (Giray et al. 2014), microRNA-150 (Yu et al. 2015). Some microRNAs are upregulated in HCC, which may act like oncogenes. Song et al. found the overexpression of microRNA-155 and reduction of Zinc fingers and homeoboxes 2 (ZHX2) gene expression in HBV-related HCC (Song et al. 2018). ZHX2 was known as a tumor suppressor gene of HCC (Yue et al. 2012). Therefore, HBV may promote hepatocarcinogenesis by inhibiting tumor suppressor genes via microRNAs-dependent pathway (Oura et al. 2020). On the contrary, some microRNAs play a role of tumor suppressor gene. In a retrospective study, plasma microRNA-125b was determined in patients with HBV-related liver disease. Compared to patients without HCC, the concentration of microRNA-125b was significantly lower in patients with HCC. The AUROC value in the diagnosis of HCC among patients with chronic HBV infection was 0.958 (95% CI: 0.928–0.988) (Chen et al. 2017). In a meta-analysis, Jin et al. included 25 studies with 2290 HBV-related HCC patients and 1151 non-HCC HBV carriers. The majority of circulating microRNAs had high predictive values in the diagnosis of HCC with AUROC of 0.87 (95% CI: 0.83–0.89), regardless of upregulated or downregulated expression. In particular, the AUROC of microRNA-125b was 0.95 (95% CI: 0.92–0.96) for the diagnosis of HCC (Jin et al. 2019). The microRNA-125b could thus be a promising genetic biomarker for early detection of HBV-related HCC if these findings can be confirmed. Another similar meta-analysis enrolled 869 HBV-HCC patients and 1338 non-HCC controls from 8 studies. The pooled AUROC value of circulating

microRNAs for the diagnosis HBV-related HCC with low levels of AFP (<20 ng/ml) was 0.88 (95% CI: 0.85–0.90) (Peng et al. 2020).

Taking these promising findings together, the circulating cfDNA or virus–host chimera DNA and microRNAs may play a functional role in the development and progression of HBV-related HCC. These molecules have the potential to become emerging biomarkers for diagnosing and tracking the progression of HCC in patients with chronic HBV infection.

5 Conclusion

In 2016, World Health Organization (WHO) declared to eliminate the threat of HBV and HCV to public health and set a goal of reducing 90% new chronic infection and 65% mortality by 2030 (Yang and Kao 2020). To meet the WHO's goals, more effective strategies for the management of CHB are urgently needed. Global hepatitis B vaccine implementation has successfully reduced the incidence of hepatitis B infection in infants, children, teenagers, and young adults. New low price, fast, simple POC tests will scale up the detection of individuals infected with HBV. Through meticulous research on the molecular aspects of HBV infection, several emerging hepatitis B viral biomarkers associated with CHB progression have been identified, including HBcrAg, HBV RNA, and total anti-HBc levels. The integration of these new biomarkers with existing markers, such as HBV viral load, HBV genotype/mutants and quantitative HBsAg, will help clinicians evaluate the eligibility of patients to timely receive effective antiviral treatment and monitor treatment response. Finally, HCC surveillance in CHB patients by the combination of novel HBV viral and host biomarkers, including HBcrAg, M2BPGi, circulating cell-free or virus–host chimera DNA and microRNA, will improve the ability of early diagnosis and prognostic prediction of HBV-related HCC.

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Chronic Hepatitis B Virus Infection: Noninvasive Assessment of Liver Disease

11

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Abstract

Liver fibrosis and cirrhosis are one of the strongest risk factors of hepatocellular carcinoma and liver-related complications in patients with chronic hepatitis B. Because current therapies for chronic hepatitis B are safe and highly effective, routine liver biopsy for prognostication and selection of patients for treatment cannot be justified. Instead, noninvasive tests of liver fibrosis such as physical measurement of liver stiffness (e.g., transient elastography, point-shear wave elastography, two-dimensional shear wave elastography and magnetic resonance elastography) and serum tests of fibrosis (including both proprietary and generic markers) have largely replaced liver biopsy as the initial assessment of disease severity. This chapter focuses on the nature and diagnostic performance of these noninvasive tests. We also discuss their role in detecting portal hypertension and predicting adverse outcomes.

Keywords

Liver fibrosis · Cirrhosis · Biomarkers · Transient elastography · FibroScan Liver stiffness measurement

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1 Introduction

Chronic hepatitis B virus (HBV) infection is characterized by a complex natural history with variable bouts of hepatitic activities ranging from subclinical fluctuation in aminotransferase levels to acute-on-chronic liver failure. Disease progression depends on the frequency and severity of these hepatitis flares (see Chap. 7 for details). Patients who enter the low replicative phase or achieve hepatitis B surface antigen seroclearance early in their lives have a benign clinical course and low risk of liver-related complications (Yip et al. 2017). In contrast, patients who continue to have the active disease are at a high risk of developing cirrhosis and hepatocellular carcinoma (HCC).

In any case, liver fibrosis is central in the natural history of chronic hepatitis B. Like other chronic liver diseases, hepatic necroinflammation triggers a wound-healing process with deposition of fibrous tissue. While fibrosis limits the degree of injury during acute liver insult, it becomes maladaptive when liver injury is chronic. With accumulating fibrous tissue and distortion of liver architecture, cirrhosis develops and sets the stage for different cirrhotic complications and HCC. Moreover, as chronic hepatitis B is a dynamic disease, it is difficult to determine the phase of disease accurately based on one single set of laboratory results. For example, a patient with positive hepatitis B e antigen (HBeAg) and high serum HBV DNA level can be in the immune-tolerant phase or HBeAg-positive immune-active phase. A patient with negative HBeAg can be in the inactive chronic hepatitis B phase or HBeAg-negative immune reactivation phase. A normal aminotransferase level cannot be used to distinguish among the phases because hepatic necroinflammation may fluctuate with time. In this situation, the detection of significant fibrosis can inform doctors whether the patient has already experienced liver injury. Therefore, assessing the degree of liver fibrosis allows doctors to understand the phase of the disease and predict the prognosis of patients with chronic hepatitis B. On the management side, the presence of significant fibrosis is an important indication for antiviral therapy. The severity of fibrosis and cirrhosis determines if screening for HCC and varices is needed. Serial evaluation of fibrosis also reflects disease progression and response to treatment.

The diagnosis of cirrhosis is obvious when the liver is already shrunken with regenerative nodules or in the presence of clinical or radiological features of portal hypertension. However, routine imaging techniques like abdominal ultrasonography and computed tomography cannot reliably diagnose early cirrhosis, not to mention fibrosis. Traditionally, liver biopsy was considered the gold standard for fibrosis staging. Nevertheless, liver biopsy is invasive and poorly accepted by patients. It is also undesirable to perform a repeated liver biopsy to assess disease progression. Finally, liver biopsy is an imperfect "gold standard." It is important to remember that only a small portion of the liver is sampled during biopsy. Sampling variability involving the less severe part of the liver would lead to underestimation of the fibrosis stage. For all these reasons, reliable and accurate noninvasive tests of fibrosis are required.

In this chapter, we will first introduce different noninvasive tests of fibrosis. This is followed by a discussion on the use of these tests in fibrosis staging and estimation of portal hypertension and HCC risk. Although most data on noninvasive tests of fibrosis came from studies involving other liver diseases, we will refer to studies on chronic hepatitis B whenever possible and highlight cases when the data were from other liver diseases.

2 Noninvasive Tests of Liver Fibrosis

Noninvasive tests of fibrosis can be divided into serum tests and physical measurements of liver stiffness or elasticity. Serum tests can be done in virtually all patients in the out-patient setting. The cost depends on the biomarkers involved. In comparison, physical measurements are probably more accurate overall. On the other hand, they can only be done in centers equipped with machines. Measurements may also fail in some patients and be influenced by factors unrelated to fibrosis.

2.1 Serum Tests

Serum biomarkers of fibrosis can be divided into class I biomarkers, which directly measure fibrogenesis and fibrinolysis, and class II biomarkers, which are parameters that correlate with fibrosis (Table 11.1). Class II biomarkers are routinely performed in clinical practice and are therefore inexpensive, but they are also expected to be less accurate. Because no single biomarker has been shown to be sufficiently accurate, most serum tests are formulae from a mixture of biochemical and/or clinical parameters. It should be noted that these formulae were modeled against liver histology. As a result, the reliability of liver histology would be the ceiling for the accuracy of serum tests (Mehta et al. 2009). This may explain why serum tests often perform less well in independent validation cohorts and also appear less accurate than physical measurements of liver stiffness. For the same reason, the results of serum tests must be validated independently before they can be considered reliable.

2.1.1 Class I Biomarkers

FibroTest (BioPredictive, Paris, France), also known as FibroSure in the USA, is a patented biomarker panel comprising of α2-macroglobulin, gamma-glutamyl transpeptidase, apolipoprotein A1, haptoglobin, total bilirubin, age, and gender. The test was first developed using liver histology as the reference standard in patients with chronic hepatitis C (Imbert-Bismut et al. 2001; Poynard et al. 2003). In a meta-analysis of 16 studies, FibroTest had a hierarchical summary receiver operating curve of 0.84 for significant liver fibrosis and 0.87 for cirrhosis in patients with chronic hepatitis B (Salkic et al. 2014). At a cutoff of 0.48, the sensitivity and specificity for the detection of significant fibrosis are 62.3% and 79.4%, respectively. At a cutoff of 0.74, the sensitivity and specificity for the detection of cirrhosis are 61.5% and 90.8%, respectively. When alanine aminotransferase (ALT) is added to

Table 11.1 Serum tests of fibrosis for chronic hepatitis B

Tests	Formulae	AUROC (histologic system)	Limitations
Class I biomarkers			
FibroTest (Poynard et al. 2009; Kim et al. 2012; Myers et al. 2003)	Patented formula including α2-macroglobulin, gamma-glutamyl transpeptidase, apolipoprotein A1, haptoglobin, total bilirubin, age, and gender	0.78–0.90 for F2–4, 0.87 for F4 (METAVIR)	– Some of the tests are expensive and not routinely performed. – Haptoglobin affected by hemolysis. – Total bilirubin affected by hemolysis and Gilbert's syndrome.
FibroMeter VIRUS (Cales et al. 2005, 2008; Leroy et al. 2014)	Patented formula including platelet count, α2-macroglobulin, ALT, urea, prothrombin index, gamma-glutamyl transpeptidase, AST, age, and gender	0.91 for F2–4, 0.85 for F3–4, 0.87 for F4 (METAVIR)	– Some of the tests are expensive and not routinely performed. – Platelet count affected by immune thrombocytopenia purpura. – Both AST and ALT affected by hepatitis flare.
Enhanced liver fibrosis panel (Lichtinghagen et al. 2013)	Patented formula including type III procollagen peptide, hyaluronic acid and tissue inhibitor of metalloproteinase-1	0.90 for F3–6, 0.95 for F5–6 (Ishak)	– The tests are expensive and not routinely performed.
Wisteria floribunda agglutinin-positive Mac-2 binding protein (Ito et al. 2017)	A glycoprotein	0.78 for F2–4, 0.79 for F3–4, 0.77 for F4 (METAVIR)	– The test is expensive and not widely available.
Class II biomarkers			
AST-to-ALT ratio (Wai et al. 2006)	AST (IU/l)/ALT (IU/l)	0.58 for F3–6 (Ishak)	– Both AST and ALT affected by hepatitis flare.
AST-to-platelet ratio index (Wong et al. 2010b)	(AST [IU/l]/upper limit of normal for AST [IU/l])/ platelet count ($\times 10^9$/l) x 100	0.55 for F3–4 (METAVIR)	– AST affected by hepatitis flare. – Platelet count affected by immune thrombocytopenia purpura.

(continued)

Table 11.1 (continued)

Tests	Formulae	AUROC (histologic system)	Limitations
FIB-4 index (Wong et al. 2010b)	Age (years) × AST (IU/l) / platelet count (× 10^9/l) / $\sqrt{}$ALT (IU/l)	0.64 for F3–4 (METAVIR)	– Both AST and ALT affected by hepatitis flare. – Platelet count affected by immune thrombocytopenia purpura.
Forns index (Forns et al. 2002)	7.811–3.131 ln (platelet count [× 10^9/l]) + 0.781 ln (gamma-glutamyl transpeptidase [IU/l]) + 3.467 ln (age [years]) – 0.014 × cholesterol (mg/dl)	0.72 for F3–4 (METAVIR)	– Platelet count affected by immune thrombocytopenia purpura. – Cholesterol affected by lipid-lowering drugs.
Hui score (Hui et al. 2005)	3.148 + 0.167 × body mass index (kg/m^2) + 0.088 × total bilirubin (μmol/l) – 0.151 × albumin (g/l) – 0.019 × platelet count (× 10^9/l)	0.77 for F3–6 (Ishak)	– Total bilirubin affected by hemolysis and Gilbert's syndrome.

ALT alanine aminotransferase; *AST* aspartate aminotransferase; *AUROC* area under the receiver operating characteristics curve

the parameters of FibroTest, the combined formula of ActiTest can also be used to diagnose significant necroinflammation. FibroTest is reported on a scale of 0 to 1.0, with increasing values suggestive of higher fibrosis stages. In the report, the FibroTest result is also translated into corresponding fibrosis stages by the METAVIR system (F0–4), though in reality, there is some overlap in FibroTest values in patients with different fibrosis stages.

In patients receiving antiviral therapy for chronic hepatitis B, FibroTest values decrease with time (Poynard et al. 2009; Poynard et al. 2005). While some of the changes may reflect fibrosis regression, this may also be contributed by reduced hepatic necroinflammation. At present, the accuracy of FibroTest in diagnosing changes in histological fibrosis has not been formally evaluated.

FibroMeter (Echosens, Paris, France) is another patented biomarker panel comprising of platelet count, α2-macroglobulin, ALT, urea, prothrombin index, gamma-glutamyl transpeptidase, aspartate aminotransferase (AST), age, and gender. Separate formulae were developed for viral hepatitis, nonalcoholic fatty liver disease and alcoholic liver disease, with FibroMeter VIRUS designed for chronic hepatitis B and C. All reported results are on a scale of 0 to 1, which indicate the probability of significant fibrosis (fibrosis score/FibroMeter), cirrhosis (cirrhosis score/CirrhoMeter) and significant necroinflammation (activity grade/InflaMeter). In early works involving patients with chronic viral hepatitis (mainly hepatitis C but some hepatitis B), FibroMeter VIRUS had an area under the receiver operating

characteristics curve (AUROC) of 0.84–0.91 for significant fibrosis (F2), 0.85 for advanced fibrosis (F3) and 0.87 for cirrhosis (Cales et al. 2005, 2008; Leroy et al. 2014). The performance of both FibroTest and FibroMeter appears to be similar in patients with chronic hepatitis B and chronic hepatitis C (Leroy et al. 2014).

The enhanced liver fibrosis panel (ELF) is a patented panel of three class I biomarkers: type III procollagen peptide (PIIINP), hyaluronic acid and tissue inhibitor of metalloproteinase-1. The normal range and optimal cutoffs of ELF were derived from 400 healthy controls and 79 patients with chronic hepatitis C in Germany (Lichtinghagen et al. 2013). The AUROC was 0.90 for significant fibrosis (F3–6 by the Ishak system) and 0.95 for cirrhosis (F5–6). Importantly, the diagnostic performance of ELF was higher than that of its individual components. Based on this study, the recommended interpretation of the ELF score was: <7.7 = none to mild fibrosis, 7.7–9.7 = moderate fibrosis, and ≥ 9.8 = severe fibrosis. The test has been validated in Asian patients with chronic hepatitis B (Kim et al. 2012; Wong et al. 2014a).

Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA^+-M2BP) was identified as a liver fibrosis marker through glycoproteomic biomarker screening. In a meta-analysis of 21 studies, the sensitivities and specificities of WFA^+-M2BP in detecting significant fibrosis, advanced fibrosis and cirrhosis were 0.69 and 0.78, 0.76, and 0.76, and 0.82 and 0.84, respectively (Ito et al. 2017). However, the diagnostic accuracy of WFA^+-M2BP appears to be lower in chronic hepatitis B than chronic hepatitis C. In a study of 72 patients with chronic hepatitis B treated with entecavir for 26 weeks followed by entecavir plus peginterferon alfa for 52 weeks, the change in WFA^+-M2BP level from week 26 to week 52 was an independent predictor of fibrosis regression at week 78 (Liu et al. 2019). Because the combination of oral nucleos(t)ide analogs and peginterferon is not a standard treatment for chronic hepatitis B, the interesting findings should be validated in patients receiving standard treatments.

Pro-C3 is a neo-epitope-specific competitive ELISA test for PIIINP. It measures the propeptide cleaved off from the intact collagen molecule and is specific for liver collagen formation (Wong et al. 2018a). However, this biomarker was mainly evaluated in patients with nonalcoholic fatty liver disease. Its performance in patients with chronic hepatitis B is currently unclear.

2.1.2 Class II Biomarkers

Models involving Class II biomarkers utilize parameters associated with fibrosis and cirrhosis (Table 11.1). Although the formulae differ, similar clinical and laboratory parameters have been chosen. For example, since fibrosis develops over a long period in patients with chronic viral hepatitis, age is an important factor associated with fibrosis. Similarly, hepatic necroinflammation is the driver towards fibrosis progression and cirrhosis, and therefore patients with elevated aminotransferases are more likely to have fibrosis. Patients with cirrhosis may also have decreased protein synthesis (reflected by serum albumin), increased bilirubin level and hypersplenism (with thrombocytopenia). The latter markers may reflect cirrhosis better than earlier stages of fibrosis.

The AST/ALT ratio, AST-to-platelet ratio index (APRI) and FIB-4 index were first evaluated in patients with chronic hepatitis C and/or HIV co-infection (Williams and Hoofnagle 1988; Wai et al. 2003, 2006). While easy to calculate, the accuracy of these generic formulae is modest when applied to patients with chronic hepatitis B (Wai et al. 2006; Wong et al. 2010b). Importantly, the accuracy of these scores is low in patients with normal ALT levels or those treated with oral nucleos(t)ide analogs. Because AST and ALT improve promptly with antiviral therapy before fibrosis improves, an improvement in these fibrosis scores in this setting can be deceiving (Kim et al. 2016).

Apart from liver biochemistry and markers of liver dysfunction, the Forns index and the Hui score also include metabolic parameters (cholesterol for the Forns index and body mass index for the Hui score). By head-to-head comparison, both scores performed better than the AST/ALT ratio and APRI (Wong et al. 2010b). This underscores the fact that there are numerous metabolic changes in patients with cirrhosis. Besides, metabolic syndrome is a risk factor for the development of cirrhosis and HCC in patients with chronic hepatitis B (Wong et al. 2009a; Chen et al. 2008; Yip et al. 2018).

Some of the biomarkers may be affected by factors other than fibrosis. The limitations and potential source of misinterpretation are listed in Table 11.1.

2.2 Physical Measurements

Table 11.2 summarizes the characteristics of the machines for physical measurement of liver stiffness or elasticity. Other than the listed machines, some other ultrasound machines also have built-in technologies to measure tissue elasticity. However, they will not be discussed further because they are not specifically designed for liver assessment and performance data are limited.

For transient elastography, (FibroScan, Echosens, Paris, France), shear wave elastography (SuperSonic Imagine, Aix-en-Provence, France) and magnetic resonance elastography, shear waves or vibrations are generated externally by a probe or transducer and propagated across the liver parenchyma. Based on physical principles, waves travel faster in a stiffer medium. It is therefore possible to estimate liver

Table 11.2 Physical measurement of liver stiffness/elasticity

Tests	Mode of examination	Stress or vibration	Visualization of liver parenchyma
Transient elastography (FibroScan)	Ultrasound	External	No
Acoustic radiation force impulse	Ultrasound	Internal “push”	Yes
Shear wave elastography	Ultrasound	External	Yes
Magnetic resonance elastography	Magnetic resonance imaging	External, continuous	Yes

stiffness and thereby fibrosis by measuring the velocity of the shear waves. Because of potential interferences, ultrasound techniques use a single transient pulse to generate mechanical vibration. In contrast, continuous mechanical excitation is used in magnetic resonance elastography. Whether this accounts for superior diagnostic accuracy is currently unknown. On the other hand, acoustic radiation force impulse uses acoustic pulses to "push" tissue internally and capture tissue motion.

When an ultrasound machine or magnetic resonance imaging is used, it is possible to visualize the liver parenchyma. One may therefore combine fibrosis assessment and HCC screening during the same examination. In comparison, only M mode ultrasound can be done with transient elastography (FibroScan); a separate examination is needed for anatomical evaluation.

Transient elastography has moderate accuracy to diagnose significant fibrosis and high accuracy in diagnosing cirrhosis (Xu et al. 2019). One limitation of transient elastography is the low success rate of measurement in obese patients (Wong et al. 2011a). The development of the XL probe has largely but not completely solved this problem (de Ledinghen et al. 2012; Wong et al. 2012). The XL probe generates a more forceful mechanical impulse to produce shear waves, and it uses lower frequency ultrasound (2.5 MHz instead of 3.5 MHz) to gain access to deeper tissues. Although initial studies consistently show that the XL probe yields lower liver stiffness values than the M probe when applied on the same patient (Wong et al. 2012), subsequent studies in patients with nonalcoholic fatty liver disease suggest that the same liver stiffness cutoffs can be used if the probes are used according to the patients' body build (Wong et al. 2019a).

Based on studies on chronic hepatitis B and other liver diseases, it appears that point-shear wave elastography and two-dimensional shear wave elastography have at least similar diagnostic performance as transient elastography, and the success rate of examination may be higher because of real-time visualization of the region of interest (Xu et al. 2019; Friedrich-Rust et al. 2009; Rizzo et al. 2011; Leung et al. 2013).

Magnetic resonance elastography can examine the whole liver and is not affected by obesity and ascites. Its diagnostic accuracy is higher than that of ultrasound-based techniques, with AUROC approaching 95–100% for the diagnosis of significant fibrosis and cirrhosis (Huwart et al. 2008; Wang et al. 2012; Venkatesh et al. 2014). In head-to-head comparison in patients with nonalcoholic fatty liver disease, magnetic resonance elastography had a higher success rate and overall accuracy than transient elastography (Imajo et al. 2016). The main hurdle to the widespread use of magnetic resonance elastography is the availability of the technology and cost of the examination. A few patients may also have contraindications to magnetic resonance imaging such as metallic implants and claustrophobia. In addition, while it is possible to perform ultrasound-based elastography as a point-of-care test, patients need a separate appointment for magnetic resonance elastography.

It is important to understand that liver stiffness can be affected by factors other than fibrosis. Although most data in this area came from studies of transient elastography, other physical measurements should be similarly affected. The single most important confounding factor is active hepatic necroinflammation (Table 11.3).

Table 11.3 Conditions leading to false-positive liver stiffness measurement

Conditions	Clinical implications
Active hepatitis	– Use higher liver stiffness cutoffs in patients with elevated alanine aminotransferase. – Avoid liver stiffness measurement in patients with very high alanine aminotransferase.
Food intake	– Fast for at least 2 hours (preferably 6 hours) before liver stiffness measurement.
Biliary obstruction	– Avoid liver stiffness measurement in patients with mechanical biliary obstruction.
Congestive heart failure	– Avoid liver stiffness measurement in patients with active heart failure.
Amyloidosis	– Avoid liver stiffness measurement in patients with amyloidosis involving the liver.
Severe steatosis	– The influence of steatosis on liver stiffness remains controversial. – Caution in the interpretation of liver stiffness in patients with severe steatosis and morbid obesity.

Patients with ALT above five to ten times the upper limit of normal often have grossly elevated liver stiffness in the range of cirrhosis; the stiffness typically falls with the resolution of hepatitis. This may occur in patients with acute exacerbation of chronic hepatitis B (Wong et al. 2009b). Furthermore, a milder degree of inflammation (ALT 1–5 times the upper limit of normal) has also been shown to increase liver stiffness (Chan et al. 2009). An ALT-based algorithm for the interpretation of liver stiffness has thus been proposed. Besides, food intake increases portal blood flow and can raise liver stiffness by 1–2 kPa (Mederacke et al. 2009). While congestive heart failure, mechanical biliary obstruction and amyloidosis can also substantially increase liver stiffness, these conditions should be clinically apparent. The more controversial issue is the impact of severe steatosis. Theoretically, hepatic steatosis should make the liver softer, but a few but not all studies showed that patients with severe steatosis had higher liver stiffness (Petta et al. 2015). This may alternatively be due to increased liver stiffness in patients with extreme body mass index (Das et al. 2012; Wong et al. 2013).

3 Combination of Serum Tests and Physical Measurements of Liver Stiffness

Because serum tests and physical measurements work by different mechanisms, it is reasonable to use them together to improve the diagnostic accuracy. One approach is to do both tests together as an initial assessment. If both tests agree with each other, the estimation of the fibrosis stage is reliable, and treatment decisions can be made. In case the tests show conflicting results, one should explore factors that may affect the performance of the tests and consider repeating the tests later. Liver biopsy can be reserved for cases with indeterminate results after the above workup. This approach is endorsed by the European Association for the Study of the Liver and

Asociación Latinoamericana para el Estudio del Hígado for patients with chronic hepatitis C, but the algorithm has not been adequately tested in chronic hepatitis B (European Association for Study of the Liver, Asociacion Latinoamericana para el Estudio del Higado 2015). A few studies have tested the possibility of adding a serum test like the Forns index and enhanced liver fibrosis panel to transient elastography to improve the confidence of ruling in advanced fibrosis in patients with chronic hepatitis B (Wong et al. 2014a, 2010b).

Since routine use of two noninvasive tests of fibrosis would invariably increase healthcare costs, an alternative approach is to perform a second test only when the diagnosis is uncertain. After all, although recommended cutoffs are used to facilitate interpretation, the results of the noninvasive tests are continuous variables. The confidence of excluding and ruling in different fibrosis stages increases when the noninvasive test results are more extreme (Wong et al. 2015a).

4 Portal Hypertension

Portal hypertension accounts for most of the complications of cirrhosis, such as variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy and hepatorenal syndrome. The gold standard for the measurement of portal pressure is hepatic venous pressure gradient (HVPG). The normal HVPG is below 5 mmHg; a level of 10 mmHg or more is described as clinically significant portal hypertension when varices start to develop. There is a strong mechanistic basis for noninvasive tests of fibrosis to predict portal hypertension. First, both serum test results and liver stiffness increase from early to more advanced cirrhosis. They can therefore be used to diagnose not only cirrhosis but also advanced cirrhosis. Second, elastography-based assessments can also reflect portal blood flow, which is a major mechanism leading to portal hypertension. While studies on portal hypertension were seldom limited to patients with chronic hepatitis B, it is reasonable to extrapolate the findings to this population.

Serum tests such as the FIB-4 index have a moderate correlation with HVPG (Park et al. 2009). Liver stiffness measurement also has a moderate to good correlation with HVPG and the presence of large varices (Wong et al. 2015b). In the Baveno VI guidelines, a liver stiffness of ≥20–25 mmHg is considered sufficient to rule in clinically significant portal hypertension (de Franchis and Baveno 2015). In contrast, patients with liver stiffness <20 mmHg and normal platelet count >150 x 10^9/l have a minimal risk of having varices that require treatment and may be spared from screening endoscopy. They should, however, undergo yearly assessment for disease progression.

Splenomegaly is a feature of portal hypertension, and therefore platelet count (reflecting hypersplenism) and spleen size by abdominal ultrasonography have been used to identify patients for varices screening. For the same reason, spleen stiffness may reflect portal hypertension more directly than liver stiffness. A few studies have confirmed the association between spleen stiffness and HVPG or large varices (Colecchia et al. 2012; Stefanescu et al. 2011; Takuma et al. 2013).

In a randomized controlled trial of 548 patients with radiological cirrhosis, the use of liver and spleen stiffness as initial assessment was non-inferior to routine endoscopy in detecting varices needing treatment (Wong et al. 2018b). At a mean follow-up of 41 months, the two groups had an equally low incidence of variceal hemorrhage (4%) (Wong et al. 2019b). The findings support the feasibility and safety of a less invasive approach for varices surveillance.

5 Hepatocellular Carcinoma and Mortality

Cirrhosis is the single most important risk factor of HCC development in patients with chronic hepatitis B (Wong et al. 2010a). It is therefore not surprising that patients with abnormal noninvasive tests of fibrosis have an increased risk of HCC. While HCC risk increases in a stepwise manner as cirrhosis becomes more advanced (Jung et al. 2011), such cases are clinically apparent and do not need additional workup by noninvasive tests. Instead, the main role of noninvasive tests is to detect subclinical advanced fibrosis and cirrhosis that would otherwise be missed by routine imaging and blood tests (Kim et al. 2015).

In a multicenter French study of 1312 patients with chronic hepatitis B, FibroTest was associated with HCC risk in a dose-response manner (Poynard et al. 2014). Similarly, ELF and WPA^{+}-M2BP have been shown to predict HCC development and recurrence in patients with chronic hepatitis B (Kim et al. 2014, 2020; Kawaguchi et al. 2018).

The incidence of HCC increases with increasing liver stiffness measurement by transient elastography (Jung et al. 2011). Since HCC can develop in a non-cirrhotic liver and there are other important risk factors of HCC in patients with chronic hepatitis B, liver stiffness has been combined with other risk factors in some HCC risk scores (Table 11.4). On the whole, the LSM-HCC and mREACH-B scores, both incorporating liver stiffness, perform better than similar scores based on clinical characteristics alone in predicting HCC development in the next 3 to 5 years (Wong et al. 2014b; Jung et al. 2015). Likewise, liver stiffness and serum tests of fibrosis are at least as good as histological fibrosis staging in predicting mortality in chronic hepatitis B patients (de Ledinghen et al. 2013).

6 Unresolved Questions

Most studies to date validated noninvasive tests of fibrosis against liver histology or portal hypertension, or used a single baseline result to predict clinical outcomes. In clinical practice, however, doctors do not see a patient once but have to evaluate him/her repeatedly over time. It is unclear how well changes in noninvasive tests correlate with histological changes. For example, APRI and the FIB-4 index have been shown to decline during antiviral therapy even in patients without fibrosis regression (Kim et al. 2016). This is because AST and/or ALT are integral components of these scores. Antiviral therapy can reduce aminotransferases rapidly well

Table 11.4 Liver stiffness-based hepatocellular cellular prediction scores for chronic hepatitis B

Score	Calculation			HCC risk		
					3 years	5 years
LSM-HCC score (Wong et al. 2014b)	Age (years)	>50	10 points	0–10 points	0%	0.3%
	Albumin (g/l)	≤35	1 point	11–20 points	2%	5%
	HBV DNA (IU/ml)	>200,000	5 points	21–30 points	11%	12%
	Liver stiffness (kPa)	8.1–12.0	8 points			
		>12.0	14 points			
	Total		0–30 points			
mREACH-B score (Jung et al. 2015)	Age (years)	30–34	0 point	0–6 points	0.6%	0.9%
		35–39	1 point	7–9 points	1.9%	3.7%
		40–44	2 points	10–11 points	5.6%	11.6%
		45–49	3 points	12–13 points	16.0%	21.6%
		50–54	4 points	14–16 points	23.3%	30.0%
		55–59	5 points			
		60–65	6 points			
	Male sex		2 points			
	ALT (U/l)	<15	0 point			
		15–44	1 point			
		≥45	2 points			
	Hepatitis B e antigen	Positive	2 points			
	Liver stiffness (kPa)	8.0–13.0	2 points			
		>13.0	4 points			
	Total		0–16 points			

HBV hepatitis B virus; *HCC* hepatocellular carcinoma

before fibrosis regresses. The same problem occurs with transient elastography because an improvement in hepatic inflammation also reduces liver stiffness (Wong et al. 2011b).

Along the same line, it is now established that patients can achieve reversal of cirrhosis with prolonged antiviral therapy (Marcellin et al. 2013). Incident varices are rare in patients receiving antiviral therapy (Lampertico et al. 2015). It is important to evaluate if noninvasive tests can be used to diagnose reversal of cirrhosis and change the practice of HCC and varices screening in some patients.

7 Conclusion

Fibrosis assessment is an important part of the management of chronic hepatitis B. Serum- and image-based noninvasive tests of fibrosis can diagnose advanced fibrosis and cirrhosis with reasonable accuracy and have already changed clinical practice and reduced the need for liver biopsy in some patients. These tests also help doctors predict prognosis and select patients for HCC and varices screening. Developing tools to monitor disease progression in treated and untreated patients should be the next research priority.

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Chronic Hepatitis B Virus Infection: Interferon Therapy and Long-Term Outcomes

12

Margarita Papatheodoridi and George Papatheodoridis

Abstract

Pegylated interferon-alfa (PegIFNa) is currently one of the first-line treatment options for patients with chronic hepatitis B. Advantages of PegIFNa therapy include the finite duration and the possibility of sustained immunological control, including even HBsAg loss, while its major limitations are the risk of adverse effects and the relatively limited response rates. Therefore, careful patient selection for PegIFNa therapy is warranted. In the last decades, several studies have focused on assessing the rates and predictors of response to PegIFNa treatment in HBeAg-positive and HBeAg-negative chronic hepatitis B. Reliable stopping rules based on on-treatment HBsAg levels have now been developed and should be applied in order to promptly discontinue PegIFNa in patients with no or poor chances of response. PegIFNa has also been investigated as part of combined regimen with a nucleos(t)ide analog, but no clear advantage of any such combination has been shown to date. Sustained responses after PegIFNa treatment in chronic hepatitis B patients are usually maintained over time, and therefore, the long-term outcome of such sustained responders is usually excellent, as they have amelioration of liver histological lesions, reduced risk of hepatocellular carcinoma and improved overall survival.

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1 Introduction

To date, interferon-alfa (IFNa), particularly in its pegylated form (PegIFNa), represents one of the two main treatment options for chronic hepatitis B (CHB), alongside monotherapy with a nucleos(t)ide analog (NA).(Lampertico et al. 2017; Sarin et al. 2016; Terrault et al. 2018; Trépo et al. 2014) The rationale behind PegIFNa treatment, which still reserves its place in the armamentarium against hepatitis B virus (HBV) despite the triumphant and robust virological responses of NAs, is the induction of long-term immunological control after a course of finite duration. Nonetheless, many patients are not willing or eligible to receive PegIFNa due to its major pitfalls, including limited response rates and high risk of adverse events (Lampertico et al. 2015, 2017; Terrault et al. 2018; Papatheodoridis et al. 2008). Therefore, to harness the most clinical utility from IFNa/PegIFNa therapy, clinicians have tried to identify the patient population that would benefit from it.

In this chapter, we will review the principles of IFNa/PegIFNa therapy and data on the efficacy and long-term outcomes of IFNa/PegIFNa regimens in CHB.

2 Mechanisms of Action of IFNa

Interferons-alfa, −beta, −gamma are potent cytokines released by host cells in response to viral infections with antiviral, antiproliferative and immunomodulatory effects. IFNa and IFN-beta have predominantly antiviral effects, while IFN-gamma has more immunomodulatory action (Yeh et al. 2019). IFNa is the most studied and widely approved for CHB treatment worldwide. Standard IFNa was initially used, but it has now been replaced by PegIFNa, which was licensed as therapy for CHB in early 2000. PegIFNa has the advantage of an attached polyethylene glycol molecule to standard IFNa, which lowers the absorption rate after the subcutaneous injection as well as renal and cellular clearance rate, thereby enhancing drug half-life and allowing weekly administration. PegIFNa-2a has been approved in most countries, while PegIFNa-2b is only approved in few, mostly Asian countries.

The antiviral effect of IFNa consists in decreasing the viral load, HBV antigens and the number and viability of HBV infected cells. This is achieved by blocking RNA-core particle formation, damaging replication-competent core particles and lowering transcription rates of pregenomic and subgenomic RNA, as well as inducing epigenetic changes of HBV covalently closed circular DNA (cccDNA) (Wieland et al. 2000; Li et al. 2010; Xu et al. 2010; Belloni et al. 2012; Lucifora et al. 2014). Additionally, IFNa acts as an immune modulator on both innate and adaptive immune response. Thus, IFNa leads to increased TNF-related

apoptosis-inducing ligand expression from natural killer cells, activation of CD56-NK cells and increased IFN-gamma yield which affects CD4 T-cell response, thus resulting in better control of HBV infection and durable off-treatment responses (Wieland et al. 2000; Li et al. 2010; Xu et al. 2010; Belloni et al. 2012; Lucifora et al. 2014).

3 Indications and Contraindications for PegIFNa

PegIFNa can be considered for treatment-naïve, immunocompetent CHB patients who require treatment and do not have a contraindication for other reasons (Table 12.1). Standard IFNa may be considered only in the absence of other treatment options such as PegIFNa and NAs (Lampertico et al. 2017; Sarin et al. 2016; Terrault et al. 2018).

More specifically, PegIFNa treatment may be preferred in patients who would benefit from a finite duration of treatment (i.e., young adults and women of childbearing age). Although experts' opinions may differ, patient groups that might benefit from PegIFNa include HBeAg-positive CHB patients particularly if they have infection with HBV genotype A or HBeAg-negative CHB patients who have low probability of effectively discontinuing NA therapy (Vlachogiannakos and Papatheodoridis 2014, 2015). However, it should always be considered that PegIFNa has a far worse safety and tolerability profile compared to NAs, which implies careful discussion between the physician and the patient and a full explanation of the potential risks and benefits before the onset of such treatment.

IFNa/PegIFNa use is contraindicated in patients with decompensated cirrhosis due to high risk for serious infections and hepatic failure (Hoofnagle et al. 1993;

Table 12.1 Possible indications and main contraindications for PegIFNa therapy in chronic hepatitis B (CHB)

Possible indications
Treatment-naive immunocompetent patients, usually without cirrhosis
Young adults who prefer finite duration of treatment
Patients with HBeAg-positive CHB infected with genotype A
Patients with HBeAg-negative CHB and low probability of effectively discontinuing nucleos(t)ide analogs
Main contraindications
Decompensated cirrhosis
Compensated cirrhosis and signs of portal hypertension
Psychiatric history or history of suicidal tendencies
Pregnancy
Any autoimmune disease
Thyroid disorders
Leukopenia or thrombocytopenia
Organ transplantation
Severe cardiopulmonary or any other systemic comorbidity

Perrillo et al. 1995). IFNa/PegIFNa treatment may be safe and effective in patients with compensated cirrhosis but no sign of portal hypertension, but due to the higher risk of side effects, its use is not encouraged except for selected cases with compensated cirrhosis. (Lampertico et al. 2017) Moreover, IFNa/PegIFNa has several other contraindications including pregnancy due to a certain risk of pregnancy loss (Table 12.1) (Trotter and Zygmunt 2001).

4 Safety OF PegIFNa

Adverse events are an important issue for IFNa/PegIFNa treatment, as they may lead to dose reduction or discontinuation. The most common adverse event is a flu-like syndrome occurring in most patients (Konerman and Lok 2016). In addition, fatigue, anorexia, nausea, diarrhea, weight loss, hair loss, depression, bone marrow suppression, thyroid abnormalities and/or onset or deterioration of autoimmune diseases are also commonly reported (Fattovich et al. 1996; Marcellin et al. 2008).

IFNa/PegIFNa therapy has been associated with hepatic flares defined as ALT increase at least twofold over the upper limit of normal (>2xULN), which may occur in up to 30%–50% of patients (Nair and Perrillo 2001). Some, but not all, hepatic flares have been considered to be caused by immune-mediated lysis of infected hepatocytes representing a sign of favorable response (Flink et al. 2005).

5 Regimens and Monitoring of PegIFNa Treatment

PegIFNa therapy is usually given for 48 weeks (approximately 12 months) in CHB, but extending the duration of PegIFNa up to 96 weeks may be considered in quite selected HBeAg-negative CHB patients (Lampertico et al. 2017; Sarin et al. 2016; Terrault et al. 2018). PegIFNa is administered as weekly subcutaneous injections of 180 or 100 μg in case of PegIFNa-2a or PegIFNa-2b, respectively (Lampertico et al. 2017; Sarin et al. 2016; Terrault et al. 2018).

All CHB patients starting PegIFNa should undergo careful baseline assessment including among others HBV DNA and HBsAg levels as well as determination of HBV genotype, if possible. Careful monitoring both for treatment response and safety is warranted during and after the end of PegIFNa treatment (Lampertico et al. 2017; Sarin et al. 2016; Terrault et al. 2018). Thus, patients are usually followed every 4 weeks during treatment as well as at weeks 12, 24, and 48 after the end of treatment. Full blood count and ALT should be tested at every visit and thyroid-stimulating hormone every 12 weeks during treatment and at 12 weeks after treatment. HBV DNA and HBsAg levels in all CHB patients and HBeAg/anti-HBe in patients with initially HBeAg-positive CHB should be checked at 12, 24, and 48 weeks of therapy and at 24 and 48 weeks after the end of therapy (Fig. 12.1) (Lampertico et al. 2017).

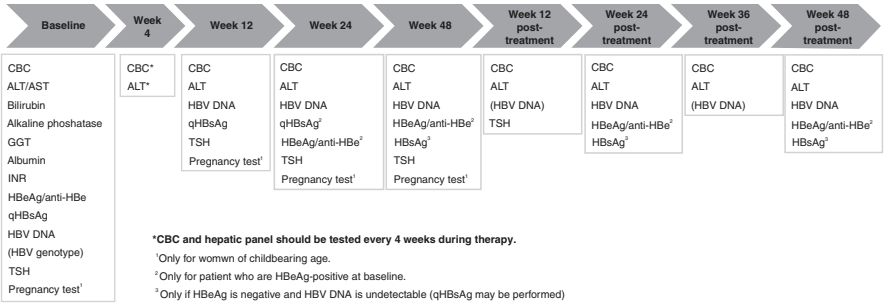

Fig. 12.1 Monitoring of chronic hepatitis B patients treated with pegylated interferon-alfa for 48 weeks. *CBC* complete blood count with differential; *ALT* alanine aminotransferase; AST: aspartate aminotransferase; *GGT* gamma-glutamyl-transpeptidase, *INR* international normalized ratio; *HBeAg* hepatitis B e antigen; *anti-HBe* hepatitis B e antibody; *qHBsAg* quantitative hepatitis B surface antigen; *HBsAg* (qualitative) hepatitis B surface antigen; *HBV* hepatitis B virus; *TSH* thyroid-stimulating hormone

Table 12.2 Definitions of response for PegIFNa therapy in chronic hepatitis B

Type of response	Definition of response
Virological	Serum HBV DNA <2000 IU/mL
HBeAg serological response (only for HBeAg-positive CHB at baseline)	HBeAg loss and seroconversion (development of anti-HBe)
HBsAg serological response (functional cure)	HBsAg loss with or without seroconversion (development of anti-HBs)
Biochemical	Normalization of ALT
Histological	Improvement in necroinflammatory activity (decrease by 2 points using Ishak's classification system or equivalent) without worsening fibrosis, compared to baseline

6 Goals of PegIFNa Treatment and Definitions of Response

The main goal of any therapeutic intervention in CHB patients should be to prevent the progression of liver disease, improve the existing liver histological lesions, decrease or ideally eliminate the risk of hepatocellular carcinoma (HCC) and eventually prolong survival (Lampertico et al. 2017; Sarin et al. 2016; Terrault et al. 2018). The specific goal of any CHB therapy of finite duration, including PegIFNa should be to ideally achieve HBsAg loss or at least to induce sustained biochemical and virological remission after treatment discontinuation ((Lampertico et al. 2017; Sarin et al. 2016; Terrault et al. 2018),(Papatheodoridis et al. 2008)).

There are different types of response classified as virological, serological, biochemical, and histological (Table 12.2) (Lampertico et al. 2017; Sarin et al. 2016; Terrault et al. 2018). Responses to PegIFNa treatment can be evaluated at different times, but response assessments at 12, 24, and 38 weeks of therapy as well as at 24 and particularly 48 weeks after the end of therapy are of most clinical relevance (Lampertico et al. 2017).

7 Efficacy OF PegIFNa

7.1 *HBeAg-Positive CHB*

In the late 1980s and early 1990s, standard IFNa regimens were shown to achieve HBeAg loss and virological remission by insensitive HBV DNA assays in approximately one-third of HBeAg-positive CHB patients. In a large meta-analysis (Wong et al. 1993) of 15 randomized placebo-controlled trials including 837 HBeAg-positive CHB patients treated with standard IFNa for 3–6 months, IFNa compared to placebo was reported to offer higher rates of 6-month off-treatment response defined by HBeAg loss (33% vs. 12%) or seroconversion, HBV DNA undetectability (37% vs. 17%), HBsAg loss (8% vs. 2%) or ALT normalization.

Follow-up studies of HBeAg-positive CHB patients treated with standard IFNa showed that responses were usually long-lasting after the end of treatment. One of the largest such studies by van Zonneveld et al. included 165 HBeAg-positive CHB patients treated with IFNa (median dose 30 MU/week) for a median of 16 weeks and followed for a median of 8.8 years (van Zonneveld et al. 2004). Response defined as HBeAg loss within 12 months after the end of therapy occurred in 54 (33%) patients and was maintained throughout follow-up in 87% of the 54 responders. HBsAg clearance was observed in 52% of responders and only 9% of non-responders.

In the registrational trial of PegIFNa-2a, 814 HBeAg-positive CHB patients (87% Asians) were randomized to receive 48 weeks of PegIFNa-2a (180 μg/week), lamivudine (LAM, 100 mg/day) or both. (Lau et al. 2005) At 24 weeks after end of treatment, PegIFNa-2a monotherapy or combined with LAM compared to LAM alone achieved more frequently HBeAg loss (34% or 28% vs. 21%, $P \leq 0.040$) or seroconversion (32% or 27% vs. 19%, $P \leq 0.020$), HBV DNA <20,000 IU/mL (32% or 34% vs. 22%, $P \leq 0.010$), ALT normalization (41% or 39% vs. 28%, $P \leq 0.006$) or combined serological, virological and biochemical response (23% or 21% vs. 10%, $P < 0.001$). HBsAg loss occurred in 3% of patients receiving PegIFNa-2a (alone or in combination with LAM), but in no patient under LAM monotherapy ($P = 0.001$). Similar to standard IFNa therapy, responses after PegIFNa are usually maintained in the long term. In a follow-up study of the PegIFNa-2a registrational trial in HBeAg-positive CHB, 83% of patients who achieved HBeAg seroconversion with PegIFNa-2a maintained this serological response at 12 months post-therapy. Furthermore, 69% of patients with HBeAg seroconversion at 12 months post-therapy had serum HBV DNA <2000 IU/mL and 38% of patients had HBV DNA <80 IU/mL (Piratvisuth et al. 2008).

The optimal dose and duration of PegIFNa-2a (90 vs. 180 μg/week for 24 or 48 weeks) was assessed in another large trial of 544 HBeAg-positive CHB Asian patients with non-A genotype (Liaw et al. 2011). The 180 μg weekly dose given for 48 weeks was proven to be significantly superior, offering HBeAg seroconversion rate of 36% compared to 26% or 23% for 48-week/90 μg group or 24-week/180 μg group and only 14% for the 24-week/90 μg group.

PegIFNa-2b therapy was evaluated in a study including 266 HBeAg-positive CHB patients randomized to receive a 52-week course of PegIFNa-2b (100 μg/week for weeks 0-31 and 50 μg/week for weeks 32-52) alone or combined with LAM (LA Janssen et al. 2005). At 24 weeks after the end of treatment, rates of HBeAg loss (36% vs. 35%), HBV DNA suppression and ALT normalization were similar between the PegIFNa-2b monotherapy and combination group. Long-term follow-up data (average 3.5 years after the end of treatment) from 65% of those study patients (n = 172) showed that HBeAg and HBsAg loss rates were 37% and 11%, respectively, while 81% of the initial responders again had sustained HBeAg loss and 30% achieved HBsAg loss (Buster et al. 2008).

PegIFNa-2b was also evaluated in another randomized trial including 100 Chinese HBeAg-positive CHB patients who were randomly assigned to receive either PegIFNa-2b (1.5 μg/kg/week, maximum 100 μg/week) for 32 weeks plus LAM for 52 weeks or LAM monotherapy for 52 weeks. After at least 24 weeks following the end of treatment, the rate of combined serological and virological response (defined as HBeAg seroconversion and HBV DNA <100,000 IU/mL) was 36% with the PegIFNa-2b and LAM combination, being higher than that with LAM monotherapy (14%) (Chan et al. 2005).

Very recently, long-term rates of HBsAg loss were published from the large S-Collate real-world prospective multicentre study that included 1842 patients from 26 countries. In HBeAg-positive CHB patients, HBsAg loss rate at 3 years of post-therapy follow-up was 2% in the intention-to-treat analysis and 5% in patients with available data (Marcellin et al. 2020).

According to the above and additional data (van Zonneveld et al. 2004; Piratvisuth et al. 2008; Buster et al. 2009; Korenman et al. 1991; Lau et al. 1997; Wong et al. 2010), it is evident that serological responses and particularly HBeAg loss and seroconversion are maintained in the long-term for ≥5-10 years after the end of IFNa/PegIFNa in most responders. Given that HBeAg seroconversion and mostly HBsAg clearance may be delayed in several cases (Piratvisuth et al. 2008; Marcellin et al. 2020), long-term post-treatment monitoring of PegIFNa treated patients is crucial.

Another characteristic of IFNa/PegIFNa therapy in HBeAg-positive CHB is that HBeAg seroconversion may not be combined with serum HBV DNA undetectability determined by sensitive polymerase chain reaction (PCR) assays, which ranges widely from 50-100% in patients who clear both HBeAg and HBsAg and is more frequently observed in case of HBsAg loss and rarely in patients with HBeAg seroconversion who remain HBsAg positive (Korenman et al. 1991). Furthermore, despite the higher probability of HBsAg loss with PegIFNa compared to NAs, the actual HBsAg loss rates remain rather low (usually <10%) (Konerman and Lok 2016; Marcellin et al. 2020).

7.2 HBeAg-Negative CHB

Early cohort studies in HBeAg-negative CHB reported that courses of standard IFNa (usually 3–5 MU thrice weekly for 6–24 months) achieved sustained

long-term off-therapy biochemical and virological responses by insensitive HBV DNA assays in 20–30% of patients (Fattovich et al. 1992; Brunetto et al. 1993; Lampertico et al. 2003; Manesis and Hadziyannis 2001). Despite the lack of data from randomized trials, a longer treatment duration of standard IFNa (12 or even 24 months) was considered to offer a higher probability of sustained off-IFNa response, mainly due to a higher risk of post-therapy relapse with a shorter duration of treatment. (Lampertico et al. 2003; Manesis and Hadziyannis 2001) A substantial proportion (>40%) of sustained responders to IFNa therapy was reported to achieve clear HBsAg loss during long-term follow-up (Manesis and Hadziyannis 2001).

The registrational trial of PegIFNa-2a in HBeAg-negative CHB included 537 patients who were randomly assigned to receive 48-week treatment with PegIFNa-2a (180 μg/week) alone or combined with LAM or LAM monotherapy.(Marcellin et al. 2004) At 24 weeks post-treatment, PegIFNa monotherapy or combined with LAM compared to LAM alone achieved higher rates of ALT normalization (59% or 60% vs. 44%, $P \leq 0.004$), serum HBV DNA suppression (<4000 IU/mL) (43% or 44% vs. 29%, $P \leq 0.007$) or both (36% or 38% vs. 23%, $P < 0.012$), or serum HBV DNA undetectability (<80 IU/mL) (19% or 20% vs. 7%, $P < 0.001$). HBsAg loss was observed in 4% or 3% of patients treated with PegIFNa-2a monotherapy or combination therapy and in no patient treated with LAM monotherapy ($P < 0.030$) (Marcellin et al. 2004). Long-term follow-up of 59% of patients of the original trial showed that the superior efficacy of PegIFNa was maintained over time (Marcellin et al. 2009). In particular, at 3 years of follow-up, rates of normal ALT were still higher in patients treated with PegIFNa monotherapy or combination therapy compared to LAM alone (31% vs. 18%, $P = 0.032$), as were rates of HBV DNA ≤2000 IU/mL (28% vs. 15%, $P = 0.039$) and HBsAg loss (9% vs. 0%) (Marcellin et al. 2009). Thus, it seemed that approximately 25–30% of HBeAg-negative CHB patients treated with a 48-week course of PegIFNa were in sustained biochemical and virological remission after 3 years. However, the actual response rates might have been overestimated due to patient selection. Intention-to-treat analysis including all patients of the original trial revealed that the rates of ALT normalization, HBV DNA decline and HBsAg loss were lower than the abovementioned (ALT normalization: 20% vs. 8%; HBV DNA ≤2000 IU/mL: 17% vs. 7% for PegIFNa containing arms vs. LAM alone, respectively) (Marcellin et al. 2009). Interestingly, the cumulative HBsAg loss rate seemed to increase further with prolongation of follow-up reaching 12% of the total patient population at 5 years post-therapy (Marcellin et al. 2013).

Recently, long-term data on HBsAg loss after PegIFNa-2a therapy in HBeAg-negative CHB became also available from the large S-Collate cohort study, which reported that 5% or 10% of such patients achieve HBsAg clearance at 3 years after treatment in modified intention-to-treat analysis or analysis of patients with available data (Marcellin et al. 2020).

The effectiveness of longer (96 weeks) duration of PegIFNa-2a therapy in HBeAg-negative CHB was also evaluated in one study from Italy (Lampertico et al. 2013a). In particular, 128 patients with HBeAg-negative CHB genotype D were randomly assigned to PegIFNa-2a 180 μg/week for 48 weeks, or PegIFNa 180 μg/

week for 48 weeks followed by 135 μg/week for another 48 weeks, or combination of PegIFNa 180 μg/week and LAM for 48 weeks followed by PegIFNa 135 μg/week for another 48 weeks. After 48 weeks from the end of treatment, rates of virological response defined as HBV DNA <2000 IU/mL were higher in patients treated with PegIFNa monotherapy for 96 weeks than for 48 weeks (29% vs. 12%, $P = 0.030$). Combination with LAM did not offer additional benefit in terms of virological response. Adverse events or discontinuation rates did not differ between the two groups of different durations of PegIFNa monotherapy. Nevertheless, the clinical usefulness of such an extended PegIFNa treatment needs to be evaluated further given the increasing cost, the potential of adverse events and its negative impact on the patients' quality of life. Therefore, a longer treatment duration of PegIFNa may be used only in very selected HBeAg-negative CHB patients who will be treated in experienced centers after discussion of the advantages and disadvantages of such an approach.

8 Predictors of Response and Stopping Rules for PegIFNa

8.1 HBeAg-Positive CHB

There are some pretreatment factors that can be helpful in predicting the probability of response to PegIFNa in **HBeAg-positive CHB** (Vlachogiannakos and Papatheodoridis 2015). In particular, higher pretreatment ALT (at least >2xULN), lower HBV DNA levels ($<2 \times 10^8$ IU/mL), high histological activity scores and genotype A (compared to all other genotypes) or genotype B compared to C have been associated with increased probability of HBeAg seroconversion and even HBsAg loss (Lau et al. 2005; LA Janssen et al. 2005; Buster et al. 2009; Flink et al. 2006; Fried et al. 2008). In contrast, PegIFNa is usually ineffective in HBeAg-positive CHB patients with normal ALT, irrespective of HBV DNA levels, who have the probability of response <10% (Lok et al. 1988; Lok 1993; Lai et al. 1987). Such patients are more frequently of Asian ethnicity and, particularly, children who were infected perinatally.

Over the last decade, the wide availability of quantitative determination of serum HBsAg levels has revived the interest in the use of such a marker in the prediction of treatment response in CHB (Vlachogiannakos and Papatheodoridis 2015). In HBeAg-positive CHB, baseline HBsAg levels did not seem to be associated with responses to PegIFNa.(Sonneveld et al. 2010; Marcellin et al. 2010) In contrast, in two studies including HBeAg-positive CHB patients, HBsAg levels <1500 IU/mL at 12 or 24 weeks of therapy were associated with high cumulative rates of HBeAg seroconversion at 6 months post-PegIFNa (>55%), while HBsAg levels >20,000 IU/mL at week 12 or 24 were associated with poor HBeAg seroconversion rates (0–15%) (Sonneveld et al. 2010; Marcellin et al. 2010). Finally, in 803 HBeAg-positive CHB patients included in three global studies, the probability of serological and virological response (HBeAg loss and HBV DNA <2000 IU/mL) at 6 months post-treatment was high (45%) in patients with HBsAg levels <1500 IU/mL at

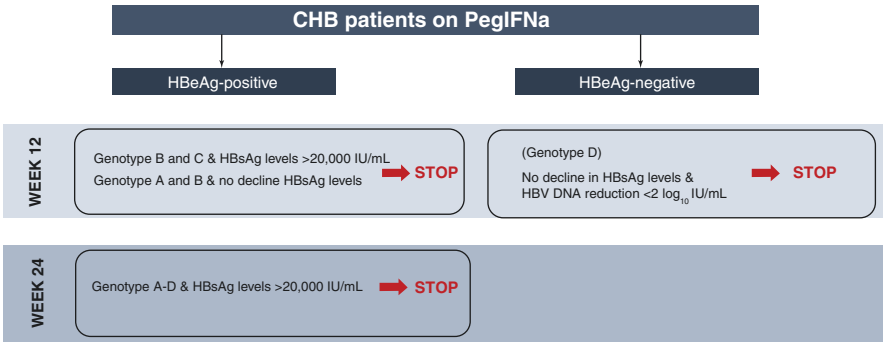

Fig. 12.2 Stopping rules algorithms for patients with chronic hepatitis B (CHB) who are treated with pegylated interferon-alfa (PegIFNa)

12 weeks of PegIFNa and poor in patients with HBV genotype B or C with HBsAg >20,000 IU/mL (2–8%) or in patients with HBV genotype A or D without any decline of HBsAg levels at 12 weeks (0–3%). In addition, almost all (99%) patients with HBsAg >20,000 IU/mL at week 24 did not respond to PegIFNa regardless of HBV genotype (Sonneveld et al. 2013). The latter data has driven the development of the 12−/24-week stopping rule for PegIFNa in HBeAg-positive CHB (Fig. 12.2) (Lampertico et al. 2017).

On-treatment HBsAg levels have been reasonably shown to affect HBsAg loss rates as well. In a large recent study of 2451 HBeAg-positive CHB patients treated with PegIFNa for 48 weeks, the lower end of treatment HBsAg levels were associated with higher rates of HBsAg loss after 5 years (Wu et al. 2020).

8.2 HBeAg-Negative CHB

Pretreatment predictors of response to PegIFNa in **HBeAg-negative** CHB have been evaluated in several studies but without encouraging and consistent results (Vlachogiannakos and Papatheodoridis 2014). In the PegIFNa-2a registrational trial in **HBeAg-negative** CHB, high baseline ALT, low baseline HBV DNA, younger age and female gender were independent predictors of combined biochemical and virological response at 24 weeks post-treatment, but they could not reliably predict other treatment end-points (Bonino et al. 2007). In the same trial, severe on-therapy ALT flares (>10× ULN) were associated with ALT normalization and histological improvement at 6 months post-treatment (Bonino et al. 2007). Furthermore, interleukin 28B polymorphisms and specifically the variant rs12979860 (C vs. T) were associated with a higher probability of sustained response to PegIFNa in HBeAg-negative CHB in one study (Lampertico et al. 2013b), but such findings were not confirmed in subsequent reports (Brouwer et al. 2013; Papatheodoridis et al. 2013).

The predictive role of HBsAg levels has also been evaluated for PegIFNa treatment in HBeAg-negative CHB. (Vlachogiannakos and Papatheodoridis 2014)

Baseline HBsAg levels were reported to be the only independent predictor of HBsAg loss at week 144 in a small study including 48 HBeAg-negative CHB patients who received PegIFNa and adefovir for 48 weeks (Takkenberg et al. 2013). In another small study including again 48 patients with HBeAg-negative CHB treated with PegIFNa for 48 weeks, rates of sustained virological response (undetectable HBV DNA) at 24 weeks post-treatment were high (89–92%) in patients who achieved HBsAg decline >0.5 log at week 12 or > 1 log at week 24 and poor (10%) in patients without such HBsAg decline. (Moucari et al. 2009) However, the latter findings were not confirmed in another study (Rijckborst et al. 2010).

In contrast to HBsAg levels alone, the on-treatment evaluation of both HBV DNA and HBsAg levels has been found to be a reliable predictor of post-PegIFNa response HBeAg-negative CHB. In 102 HBeAg-negative CHB patients infected mostly with genotype D (81/102) who received PegIFNa for 12 months, sustained biochemical and virological response (normal ALT and HBV DNA <2000 IU/mL) at 6 months after the end of treatment was observed in none of 20 patients with no HBsAg levels decline and a < 2 $\log_{10}$ HBV DNA decrease (Rijckborst et al. 2010). These results were confirmed in at least two additional cohorts of 91 and 95 HBeAg-negative CHB patients again infected with genotype D who were treated with PegIFNa for 48 or 96 weeks in one and for 48 weeks in the other study.(Rijckborst et al. 2012; Goulis et al. 2015) Based on these findings, this 12-week stopping rule (no decline of HBsAg and no reduction of HBV DNA ≥2 $\log_{10}$) has been included in the guidelines for the use of PegIFNa in the management of HBeAg-negative CHB (Fig. 12.2) (Lampertico et al. 2017).

On-treatment HBsAg levels have again been reported to affect subsequent HBsAg loss rates in HBeAg-negative CHB. In the PegIFNa registrational trial, on-treatment decline of HBsAg levels >1 $\log_{10}$ IU/mL and HBsAg <10 IU/mL at week 48 were strongly associated with HBsAg loss at 3 years after treatment (Brunetto et al. 2009). In addition, in the S-Collate cohort study, subsequent HBsAg loss rates were higher in HBeAg-negative CHB patients who achieved HBsAg <1500 IU/mL or decline ≥10% at week 12 of PegIFNa therapy (Marcellin et al. 2020).

9 Combinations OF PegIFNa with Current NAs

There are several hypotheses for potential benefit from the combination of PegIFNa with a NA, including, among others, the increased efficacy of PegIFNa in patients with low viremia that can be induced by NA as well as the possible increase of HBsAg loss rates that can be induced by PegIFNa (Vlachogiannakos and Papatheodoridis 2014, 2015). Therefore, various concomitant or add-on combinations of PegIFNa and a NA have been tried during the last two decades.

The efficacy of the combination of PegIFNa with LAM was evaluated even within the initial phase III trials of PegIFNa, always showing no benefit from the addition of LAM compared to PegIFNa monotherapy (Lau et al. 2005; LA Janssen

et al. 2005; Marcellin et al. 2004). The combination of PegIFNa with adefovir dipivoxil was assessed in a very limited number of CHB patients, and therefore safe conclusions cannot be drawn (Wursthorn et al. 2006; Lutgehetmann et al. 2008). The combination of PegIFNa with telbivudine is contraindicated, as it was assessed in a large randomized trial of 300 HBeAg-positive CHB patients, which was terminated early due to serious adverse events mainly peripheral neuropathy (Marcellin et al. 2015). Subsequently, research interest focused on the combination of PegIFNa with a NA of high genetic barrier to HBV resistance, namely entecavir (ETV, 0.5 mg/day) and tenofovir disoproxil fumarate (TDF, 300 mg/day).

Several clinical trials have assessed the combination of PegIFNa with ETV or TDF in CHB who start treatment, but the results remain inconclusive. A randomized open-label study included 218 treatment-naïve HBeAg-positive CHB patients from China who were randomly assigned to receive 48 weeks of PegIFNa-2a 180 μg/week alone or with 24 weeks ETV added prior to or after PegIFNa (Xie et al. 2014). There was no benefit from the addition of ETV, as HBeAg seroconversion rates at 24 weeks post-treatment were lower in the two combination arms compared to PegIFNa monotherapy (25% for add-on ETV, 26% for ETV pretreatment vs. 31% for PegIFNa monotherapy). Another randomized open-label study included 175 HBeAg-positive CHB patients treated with ETV for 24 weeks who were randomized to add-on PegIFNa-2a 180 μg/week from week 25 to 48 or to continue with ETV monotherapy until week 48 (Brouwer et al. 2015). Patients with HBeAg loss and virological response defined as serum HBV DNA <200 IU/mL discontinued ETV at week 72, and all patients were followed up to week 96. Although response rates were numerically higher in the PegIFNa add-on group compared with ETV monotherapy, the differences did not reach statistical significance at week 48 and 96 after the end of treatment. In addition, there was no arm to assess the efficacy of PegIFNa monotherapy. A recent randomized study from China including 144 CHB patients treated with ETV monotherapy or PegIFNa add-on therapy from week 26 to 52 also showed that PegIFNa and ETV combination was not associated with increased rates of HBsAg loss, HBeAg seroconversion or sustained virological response (Yang et al. 2020).

The combination of PegIFNa-2a and TDF was evaluated in a large randomized clinical trial including 751 CHB patients (58% HBeAg-positive), who received (A) PegIFNa 180 μg/week plus TDF for 48 weeks, (B) PegIFNa plus TDF for 16 weeks followed by TDF alone for 32 weeks, (C) TDF monotherapy for 120 weeks, or (D) PegIFNa monotherapy for 48 weeks (Marcellin et al. 2016). Patients receiving PegIFNa and TDF combination for 48 weeks had significantly higher rates of HBsAg loss (9%) at 72 weeks compared to those receiving monotherapy with TDF (0%, $P < 0.001$) or PegIFNa (3%, $P = 0.003$). HBsAg loss occurred more frequently in HBeAg-positive than HBeAg-negative patients and in those with genotype A infection compared to all other genotypes. At week 72, HBeAg loss and seroconversion rates were also higher in PegIFNa and TDF 48-week combination than TDF monotherapy (30% vs. 15%, $P = 0.009$ and 25% vs. 13%, $P = 0.025$, respectively), while biochemical and virological response rates were superior in patients remaining on TDF monotherapy, but they did not

differ among the other three groups (Marcellin et al. 2016). These data suggested that the combination of PegIFNa and TDF may increase the probability of functional cure, especially in CHB patients infected with HBV genotype A. However, the limited rates of HBsAg loss, even with such a combination do not justify its wide use in clinical practice today. Rates of HBsAg loss did not show further increase at 120 weeks (groups A, B, C and D: 10%, 3.5%, 0%, and 3.5%, respectively) (Ahn et al. 2018).

The use of PegIFNa has also been tried in patients who are under long-term therapy with ETV or TDF in an effort to increase the probability of safe and effective treatment discontinuation. In one study, (Ning et al. 2014) 200 initially HBeAg-positive CHB patients under ETV for 9–36 months were randomly assigned to continue ETV monotherapy for another 48 weeks or to switch to PegIFNa-2a 180 μg/weekly for 48 weeks after an 8-week overlap with ETV. At 48 weeks, HBeAg seroconversion rates in the ETV-PegIFNa group were more than two times higher than the rates in the ETV monotherapy group (15% vs. 6%), while eight patients of the first group and nobody from the second group achieved HBsAg loss. However, these patients had low HBeAg (<100 IU/mL) and HBV DNA levels (<200 IU/mL) at baseline, while approximately half of them had already achieved HBeAg seroconversion at randomization. Another study included 77 initially HBeAg-positive CHB patients treated with ETV or TDF for >48 weeks having serum HBV DNA <2000 IU/mL who were randomized to continue with ETV/TDF monotherapy or with add-on PegIFNa therapy for 48 weeks (Chi et al. 2017). At 96 weeks, the rates of HBeAg seroconversion and HBV DNA suppression <200 IU/mL were only numerically but not statistically higher in the add-on PegIFNa group than in the ETV/TDF monotherapy group (Chi et al. 2017). In a recent prospective non-randomized open-label trial, the combination of PegIFNa-2a with ETV or TDF was reported to achieve greater HBsAg decline and HBsAg loss rate (8% vs. 0%) compared to ETV/TDF monotherapy. In the latter study, however, a significant percentage of patients (22%) had to discontinue PegIFNa because of adverse events highlighting that safety may be limiting the usefulness of such approach (Broquetas et al. 2020). Finally, another recent study included 185 HBeAg-negative CHB patients with undetectable HBV DNA under NA for ≥1 year who were randomized to continue with NA alone or to receive add-on PegIFNa for 48 weeks. PegIFNa was reported to be poorly tolerated with early discontinuation due to adverse events in 20% of patients, while it did not significantly increase the 96-week HBsAg loss rates (7.8% for PegIFNa add-on vs. 3.2% for NA monotherapy, $P = 0.15$) (Bourlière et al. 2017).

10 Long-Term Outcomes after IFNa

The data on the long-term outcomes come mainly from cohorts of CHB patients treated with standard IFNa. Reasonably, PegIFNa therapy is considered to have similar effects on the long-term outcomes of CHB, although relevant data is practically lacking. Two long-term cohort studies in the late 1990s showed that IFNa

therapy is associated with improved overall survival, particularly in HBeAg-positive or HBeAg-negative CHB patients with sustained off-treatment responses. (Niederau et al. 1996; Papatheodoridis et al. 2001) Moreover, IFNa therapy was shown to improve liver histological lesions in sustained responders as well (Papatheodoridis et al. 2005). Finally, the impact of IFNa on HCC development in CHB patients remains controversial in individual studies (Vlachogiannakos and Papatheodoridis 2013) and thus several metanalyses have been performed showing that IFNa therapy is associated with reduced HCC risk, which may be more evident in Asian than Caucasian CHB patients and generally in patients with higher baseline HCC risk (Sung et al. 2008; Yang et al. 2009; Cammà et al. 2001; Miyake et al. 2009). In any case, since HCC may develop after IFNa/PegIFNa therapy even in sustained responders, the individual HCC risk should be determined, and HCC surveillance may be recommended (Vlachogiannakos and Papatheodoridis 2013).

11 Conclusions

PegIFNa, an agent with both antiviral and immunomodulatory properties, represents a first-line treatment option for patients with CHB, ideally those without cirrhosis who are young and keen for a treatment of finite duration. However, PegIFNa cannot be given to several patient categories due to contraindications, while it is not preferred by the majority of CHB patients because of its unfavorable tolerability and safety profile compared to oral antivirals. For CHB patients without contraindications who are willing to be treated with PegIFNa, it offers the possibility of sustained off-treatment response and even HBsAg loss, although the probability of response is limited (20–30%). Therefore, careful patient selection for PegIFNa therapy is warranted. Although satisfactory predictors of response have not been identified, strong on-treatment predictors of no response to PegIFNa have been determined in both HBeAg-positive and HBeAg-negative CHB. Thus, reliable stopping rules based on on-treatment HBsAg levels have been developed and should be applied in order to promptly discontinue PegIFNa in CHB patients with no or poor chances of response. Despite several research efforts, no clear benefit of any combination of PegIFNa with NA has been shown to date. A clear advantage of sustained responses after PegIFNa treatment in CHB is that such responses are usually maintained over time. Thus, the long-term outcome of CHB patients with sustained response to PegIFNa is usually excellent, as they have amelioration of liver histological lesions, reduced risk of hepatocellular carcinoma and improved overall survival. Besides, 40-50% of sustained responders to PegIFNa may even achieve HBsAg loss or functional cure, which represents the most desirable treatment endpoint in CHB. However, because of the current intense efforts for the development of new anti-HBV agents aiming for a functional cure, it remains elusive whether PegIFNa will still be used in CHB in the future, either alone or perhaps in combination with new agents.

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Nucleos(t)ide Therapy and Long-Term Outcomes

13

Jonggi Choi and Young-Suk Lim

Abstract

The goal of therapy for chronic hepatitis B is to decrease the risk of liver-related complications, including progression to cirrhosis, decompensated cirrhosis, hepatocellular carcinoma and death. Given that complete elimination of hepatitis B virus (HBV) from the host is not possible with currently available treatment owing to the persistence of covalently closed circular DNA and viral genome integration into host chromosomes inside hepatocyte, the primary target for treatment should be to suppress HBV replication and reduce serum HBV DNA at the lowest possible levels to achieve the goals. Currently, several approved nucleos(t)ide analogs (NUC) are available for treating chronic hepatitis B (CHB) in most countries: L-nucleosides (lamivudine and telbivudine); deoxyguanosine analog (entecavir); and acyclic nucleotide phosphonates (adefovir dipivoxil and tenofovir). These NUCs act primarily by inhibiting the reverse transcription of the pregenomic HBV RNA to the first strand of HBV DNA. Most of the clinical practice guidelines recommend entecavir, tenofovir disoproxil fumarate, or tenofovir alafenamide as preferred first-line monotherapies due to their superior efficacy and high barrier to resistance over comparable drugs. NUCs are administered orally and have favorable safety profiles over the course of several years. Many data have consistently shown that long-term suppression of HBV DNA replication by NUCs leads to the improvement in hepatic inflammation and fibrosis, hepatic function, and survival of the patients, and reduction in the risk of hepatocellular carcinoma (HCC). However, it is still highly controversial regarding when is the optimal time to initiate the NUC treatment in the patients with CHB and which NUC is the best option to further reduce HCC risk.

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Chronic hepatitis B · Nucleos(t)ide analog · Resistance · Long-term outcome · Hepatocellular carcinoma

1 Nucleos(T)Ide Analogues

1.1 Lamivudine

Lamivudine is the first oral nucleos(t)ide analog (NUC) for treating chronic hepatitis B (CHB). It is an analog of cytidine and is phosphorylated to its active metabolite, which acts as a chain terminator after competing for incorporation into viral DNA (Fung et al. 2011). Lamivudine is not presently considered a first-line monotherapy for CHB due to its high rate of resistance and inferior efficacy compared with entecavir and tenofovir disoproxil fumarate (TDF) by all international guidelines (European Association For The Study Of 2012; Sarin et al. 2015; Terrault et al. 2016).

Short-Term Responses

A. HBeAg-Positive Patient
In a randomized placebo-controlled trial, the lamivudine-treated group, compared with placebo group, had a higher rate of histologic improvement (52% versus 23%, $P < 0.001$), loss of HBeAg (32% versus 11%, $P = 0.003$), sustained suppression of HBV DNA to undetectable levels (44% versus 16%, $P < 0.001$), and ALT normalization (41% versus 7%, $P < 0.001$) after 52 weeks of treatment (Dienstag et al. 1999).

B. HBeAg-Negative Patient
A 1-year course of lamivudine therapy has been reported to achieve ALT normalization in 96% and undetectable levels of HBV DNA in 68% of HBeAg-negative patients (Hadziyannis et al. 2000). The lower limit of detection for serum HBV DNA has changed over the years, and was $1x10^5$ copies/mL for most of the studies for lamivudine.

Long-Term Outcomes

A. Histological Improvement
A 3-year follow-up study investigating histologic outcome during lamivudine therapy reported that lamivudine therapy reduced necroinflammatory activity and reversed fibrosis (including cirrhosis) in most patients. However, the emergence of lamivudine resistance mutations could offset histologic improvement throughout the treatment period (Dienstag et al. 2003).

B. Clinical Trial
The effectiveness of lamivudine therapy in preventing liver-related complications has been demonstrated in a large randomized controlled study with a mean

duration of 32 months. Lamivudine-received patients had significantly lower rates of disease progression, defined as an increase in Child-Pugh score by ≥2 points, renal insufficiency, bleeding varices, spontaneous bacterial peritonitis, liver-related death, and hepatocellular carcinoma (HCC) compared with placebo-received patients (7.8% versus 17.7%, $P = 0.001$). HCC occurred in 3.9% of lamivudine group and 7.4% of placebo group (hazard ratio [HR], 0.49; $P = 0.047$) (Liaw et al. 2004).

C. Observational Study

In a 5-year long-term follow-up study with lamivudine (median follow-up 3.8 ± 1.4 years), virological and biochemical remission rate at 48 months was 34% and 36%, respectively. In this study, long-term lamivudine therapy significantly improved survival and reduced the risk of major complications, compared with untreated patients.(Papatheodoridis et al. 2005) In patients with decompensated cirrhosis defined as a Child-Pugh score of ≥10, lamivudine significantly improved hepatic function in 60.9% of treated patients versus none of the controls ($P < 0.001$). Time to death or orthotopic liver transplantation was longer in lamivudine-treated patients than in controls ($P < 0.001$). (Yao 2001) In a meta-analysis, lamivudine treatment versus no treatment reduced the risk of HCC (four observational studies, relative risk [RR] = 0.6, 95% confidence interval [CI] 0.4-0.96, $I^2 = 49.9\%$), all-cause mortality (one study, RR = 0.4, 95% CI 0.3-0.6), and decompensated liver disease (one study, RR = 0.3, 95% CI 0.3-0.5) (Lok et al. 2016).

Resistance

The major drawback of lamivudine is the high rate of emerging drug resistance throughout the treatment period. The overall incidence of lamivudine-resistant mutation increased from 23% in 1-year of lamivudine treatment to 46%, 55%, 71%, and 65% in 2-, 3-, 4-, and 5-year, respectively. Moreover, patients with lamivudine-resistant mutations experienced more hepatitis flares than those without lamivudine-resistant mutations ($P < 0.005$) (Lok et al. 2003).

The main mechanism for lamivudine resistance includes mutations in rtM204V/I (a methionine to valine or isoleucine substitution) alone or rtM204V/I along with rtL180M mutation (Allen et al. 1998). Antiviral drug resistance could manifest as a virological breakthrough which may be followed by biochemical breakthrough, hepatitis flares and hepatic decompensation. Once drug-resistant HBV mutants develop, they do not disappear even after the discontinuation of the treatment but are retained in the viral archive permanently causing resistance to subsequent multiple drugs, such as telbivudine, entecavir, and adefovir (Lok and McMahon 2009; Zoulim and Locarnini 2012).

Dosage, Safety, and Side Effects

Lamivudine is administered orally at a dosage of 100 mg daily in patients with normal renal function. In general, lamivudine is a very safe drug. Pancreatitis and lactic acidosis were reported in patients who received lamivudine treating for CHB. Lamivudine is classified as pregnancy category C.

1.2 Adefovir

Adefovir, a nucleotide analogue of adenosine monophosphate, was approved in 2002 for treating CHB (Fung et al. 2011). Considering its inferiority to TDF in terms of viral suppression and resistance profile, adefovir is no longer a first-line drug.

Short-Term Responses

A. HBeAg-Positive Patient
In patients with HBeAg-positive CHB, adefovir treatment group than the placebo group had more histologic improvement (53% versus 25%, respectively, $P < 0.001$) after 48 weeks of treatment in phase 3 randomized controlled trial (Marcellin et al. 2003).

B. HBeAg-Negative Patient
In a placebo-controlled randomized study with HBeAg-negative CHB patients, the adefovir group had histologic improvement compared with the placebo group (64% versus 33%, $P < 0.001$). Adefovir treatment resulted in a greater decrease in the median serum HBV DNA (-3.91 versus -1.35 $\log_{10}$copies/mL, $P < 0.001$). ALT normalization occurred in 72% of adefovir and 29% of placebo (Hadziyannis et al. 2003).

Long-Term Outcomes

A. Histological Improvement
An open-label phase study with adefovir for HBeAg-negative CHB for up to 5 years, 73% of patients had improvement in fibrosis, and 83% had improvement in necroinflammation. Compared with baseline, Ishak fibrosis score improved in 35%, 55%, and 71% of patients after 48, 192, and 240 weeks of adefovir treatment, respectively (Hadziyannis et al. 2006).

B. Clinical Trial.
A 5-year long-term follow-up study including a subset of the above trial demonstrated that -4.05 $\log_{10}$ copies/mL of the median changes from baseline in serum HBV DNA, 48% of HBeAg seroconversion, and 67% of histologic improvement with adefovir treatment (Marcellin et al. 2008a). Treatment with adefovir for up to 240 weeks led to ALT normalization in 69%, improvement in fibrosis in 73%, and HBV DNA suppression (HBV DNA levels <1000 copies/mL) in 67% of patients (Hadziyannis et al. 2006). In an open-label, international study involving 128 patients with decompensated cirrhosis who failed lamivudine therapy, 81% and 76% of the patients achieved undetectable HBV DNA (HBV DNA <400 copies/mL) and ALT normalization, respectively. Additionally, the Child-Pugh score improved in 92% of patients at 48 weeks of adefovir treatment (Schiff et al. 2003).

C. Observational Study.
A long-term follow-up study with adefovir for wait-listed liver transplantation patients with lamivudine-resistant CHB evaluated the efficacy after 96 weeks of

treatment with adefovir. Sixty-five percent of patients had undetectable HBV DNA levels (<1000 copies/mL), and a median decrease in Child-Pugh score was 2 (Schiff et al. 2007).

Resistance

The main mutations developing adefovir resistance are rtA181V/T and rtN236T (Angus Gastro, 2003). Adefovir resistance mutations rtA181V or rtN236T developed in 20% of patients with HBeAg positive at week 195 (Marcellin et al. 2008a). In a patients with HBeAg negative, cumulative adefovir resistance rate at 1,2,3,4,and 5 years are 0%, 3%, 11%, 18%, and 29%, respectively.(Hadziyannis et al. 2006) However, adefovir resistance appeared to present earlier and more frequently in lamivudine-resistant patients compared with those who were treatment-naïve. In a study comparing lamivudine-resistant and treatment-naïve patients, 18% of lamivudine-resistant patients were found to have developed adefovir-resistant mutations, whereas none of the treatment-naïve patients developed after 48 weeks (Lee et al. 2006).

Dosage, Safety, and Side Effects

For patients with normal renal function, the dosage of adefovir is 100 mg daily. However, increasing the interval for dose is required for patients with renal impairment. A long-term follow-up study with adefovir for up to 5 years reported 3% of renal toxicity from adefovir treatment, defined as an increase of serum creatinine ≥0.5 mg/dL above the pre-treatment value (Hadziyannis et al. 2006). Therefore, close monitoring of serum creatinine and phosphate at least 3-month intervals is required for patients who are likely to progress renal impairment or receives adefovir for more than 1 year.

1.3 Telbivudine

Telbivudine, a synthetic L-nucleoside analog of thymidine, has been used for the treatment of CHB since 2006. Telbivudine undergoes phosphorylation to its triphosphate form by cellular kinases, which can inhibit HBV DNA polymerase by competing with thymidine 5′-triphosphate. Incorporation of telbivudine leads to premature chain termination (Fung et al. 2011). Telbivudine has been demonstrated to be more potent than lamivudine against HBV. However, telbivudine has an intermediate rate of resistance and inferiority to TDF in its efficacy. Thus, telbivudine is not a first-line monotherapy for CHB by international guidelines.

Short-Term Responses

A. HBeAg-Positive Patient

In a double-blind phase 3 trial (GLOBE trial) comparing between telbivudine and lamivudine, telbivudine showed a higher rate of therapeutic response (75% versus 67%, $P = 0.005$) and histologic response (65% versus 56%, $P = 0.01$) at

1 year. With regard to the mean reduction in the HBV DNA levels from baseline and the proportion of patients with a reduction in HBV DNA to undetectable levels, telbivudine was superior to lamivudine (Lai et al. 2007). Another 2-year follow-up study including HBeAg-positive patients treated with telbivudine or lamivudine demonstrated that telbivudine treatment patients had a higher rate in therapeutic response (63% versus 48%, $P < 0.001$), and undetectable levels of HBV DNA (56% versus 39%, $P < 0.001$), compared with lamivudine treatment patients. However, in regard to HBeAg seroconversion, both groups were comparable (30% in telbivudine versus 25% in lamivudine, $P = 0.056$)(Liaw et al. 2009). A randomized study conducted with Chinese CHB treatment-naïve patients did not show any difference between telbivudine and entecavir in treatment effectiveness, including the mean reductions from baseline in serum HBV DNA, the rate of undetectable levels of HBV DNA, HBeAg seroconversion rate, and ALT normalization at 24 weeks (Zheng et al. 2010).

B. HBeAg-Negative Patient

In one of two above-mentioned trials comparing between telbivudine and lamivudine, telbivudine was shown a greater decrease in the mean HBV DNA levels (difference − 0.83 $\log_{10}$copies/mL, $P < 0.001$), a higher rate of HBV DNA undetectability than lamivudine (88% in telbivudine versus 71% in lamivudine. $P < 0.001$) at week 104. However, there were no significant differences in histologic response and ALT normalization (Lai et al. 2007). Another study demonstrated superior efficacy of telbivudine to lamivudine in therapeutic response, HBV DNA undetectability but ALT normalization (Liaw et al. 2009).

Long-Term Outcomes

A. Histological Improvement

Study data showing histological improvement after a long-term telbivudine therapy are sparse.

B. Clinical Trial

In a non-inferiority, double-blind randomized 2-year study evaluating the efficacy between telbivudine and lamivudine in treatment-naïve patients with decompensated cirrhosis, telbivudine was associated with a higher rate of patients with clinical response (HBV DNA <300 copies/mL and ALT normalization) compared with lamivudine (45.6% versus 32.9%, $P = 0.093$, respectively). In this study, improvement in Child-Pugh score (decrease ≥2) occurred in 38.6% of the telbivudine group and in 40.4% of the lamivudine group ($P = 0.83$) (Chan et al. 2012).

C. Observational Study

A 3-year extension of GLOBE study consisting of 484 telbivudine-treated patients reported 2 cases (0.5%) of HCC occurrence during the study period (Gane et al. 2011). Patients with cirrhosis in the GLOBE trial showed a sustained improvement of renal function with a long-term telbivudine therapy (4–6 years), particularly among patients with increased risk of renal impairment.(Gane et al. 2014).

Resistance

The resistance rate of telbivudine is relatively high at 4.4%/2.7% and 25%/11% at 1 year and 2 years for HBeAg positive/HBeAg-negative patients, respectively (Lai et al. 2007; Liaw et al. 2009). The main mutation responsible for telbivudine resistance is rtM204I with or without concomitant rtL80I/V and rtL180M mutations.

Dosage, Safety, and Side Effects

Telbivudine is administered orally at a dosage of 600 mg daily. Myopathy has been reported in patients treated with telbivudine. Data of 667 patients in the GLOBE trial reported that 13% of patients receiving telbivudine had an elevation of creatine kinase (CK) level compared with 4% of patients receiving lamivudine (Liaw et al. 2009). Telbivudine has also been reported to be associated with peripheral neuropathy, particularly when used along with pegylated interferon. In a randomized trial, of the 50 patients who had received pegylated interferon and telbivudine, 7 (14%) patients developed peripheral neuropathy (Marcellin et al. 2015).

1.4 Entecavir

Entecavir, a deoxyguanosine analog, is a potent inhibitor of HBV replication and converts into its active form of entecavir-triphosphate in vivo. Entecavir is a competitive inhibitor of HBV polymerase negative-strand synthesis from pre-genomic RNA and positive-strand replication. It also acts by inhibiting the HBV priming reaction (Levine et al. 2002). In vitro, entecavir demonstrated a 30- to 2200-fold efficacy in reducing viral DNA replication compared to lamivudine (Fung et al. 2011). Entecavir has been available since 2005 at a dose of 0.5 mg/day and 1 mg/day for treatment of treatment-naïve and lamivudine-resistant CHB, respectively. By all international guidelines, entecavir is preferred as a first-line monotherapy for CHB because of its high potency and low rate of drug resistance.

Short-Term Responses

A. HBeAg-Positive Patient.

A phase 3, double-blind randomized trial including 715 nucleos(t)ide-naïve, HBeAg-positive patients with the compensated liver disease compared the efficacy between entecavir 0.5 mg/day and lamivudine 100 mg/day for 48 weeks (Chang and Lai 2006). After 48 weeks, more patients in the entecavir group than in the lamivudine group had histologic improvement (72% versus 62%, $P = 0.009$), undetectable serum HBV DNA levels (67% versus 36%, $P < 0.001$) and ALT normalization (68% versus 60%, $P = 0.02$). HBeAg seroconversion rates were comparable with the entecavir group (21%) and lamivudine (18%) ($P = 0.33$) (Chang and Lai 2006). In a follow-up to the above study for up to 96 weeks, higher proportions of entecavir-treated than lamivudine-treated patients achieved cumulative confirmed HBV DNA <300 copies/mL (80% versus 39%, $P < 0.001$) and ALT normalization (87% versus 79%, $P = 0.006$) (Gish

et al. 2007). However, cumulative HBeAg seroconversion occurred in 31% of entecavir-treated versus 25% of lamivudine-treated patients through 96 weeks (P=NS) (Gish et al. 2007). In a study of open-label treatment with entecavir 0.5 mg/day or adefovir 10 mg/day for a minimum of 52 weeks treatment including 69 nucleos(t)ide-naïve HBeAg-positive patients, the mean reduction in serum HBV DNA level at week 48 was greater in entecavir-treated patients compared with adefovir-treated patients (−7.28 versus −5.08 $\log_{10}$copies/mL, $P < 0.001$). At 48 weeks, a higher proportion of entecavir-treated patients achieved HBV DNA <300 copies/mL compared with adefovir-treated patients (58% versus 19%). HBeAg seroconversion rates were similar in both groups (Leung et al. 2008).

B. HBeAg-Negative Patients.
In a double-blind, randomized, phase 3 trial, entecavir was shown to be superior to lamivudine in histologic improvement (70% versus 61%, $P = 0.01$), the rate of undetectable HBV DNA levels (90% versus 72%, $P < 0.001$), and ALT normalization (78% versus 71%, $P = 0.045$) at week 48 (Lai et al. 2006).

Long-Term Outcomes

A. Histological Improvement
The efficacy of entecavir and lamivudine were compared in 245 biopsy-proven cirrhotic patients, which demonstrated that the entecavir group achieved improvement in Ishak fibrosis at week 48 in 57%/59% of nucleos(t)ide-naïve HBeAg-positive/HBeAg-negative patients. Whereas, among HBeAg-positive patients, histologic improvement (defined as a ≥ 2-point improvement in the Knodell necroinflammatory score and no worsening of fibrosis) has occurred in 80% and 64% of entecavir and lamivudine group at 48 weeks, respectively. In HBeAg-negative patients, 75% of the entecavir group demonstrated histologic improvement compared with 60% of the lamivudine group (Schiff et al. 2008).

B. Clinical Trial
A randomized open-label comparative study of entecavir (1.0 mg/day) versus adefovir (10 mg/day) therapy in CHB patients with hepatic decompensation (Child-Pugh score ≥ 7) demonstrated the superiority of entecavir to adefovir in the mean reduction in HBV DNA at week 24 (treatment difference 1.74 $\log_{10}$copies/mL). About 2/3 of subjects in both groups showed improvement in Child-Pugh score and a decrease in Model for End-Stage Liver Disease score (MELD) was 2.6 for entecavir and 1.7 for adefovir at week 48. Cumulative HCC incidence rates were 12% for entecavir and 20% for adefovir. Cumulative death rate, moreover, was 23% for entecavir and 33% for adefovir (Liaw et al. 2011a).

C. Observational Study
Hosaka et al., reported that the incidence of HCC was lower in entecavir-treated patients than non-treated patients among 316 propensity score matching cohort, and the suppression of HCC development was greater in patients at higher risk of HCC (Hosaka et al. 2013). In a cohort study of 1980 patients with cirrhosis (a mean duration of follow-up: 52 months), treatment with entecavir compared

with controls reduced the risk of HCC (RR = 0.3, 95% CI 0.1-0.5) and death (RR = 0.6, 95% CI 0.3-0.98) (Wong et al. 2013). A recently published retrospective-prospective cohort study in Taiwan showed that entecavir was associated with a 60% HCC risk reduction (HR = 0.40, 95% CI: 0.28-0.57) compared with the untreated cohort (Su et al. 2016). A large retrospective study including 5374 patients with CHB treated with entecavir or lamivudine has been demonstrated that entecavir therapy was associated with a lower risk of death or transplantation (HR = 0.49, $P < 0.001$) but a similar risk of HCC (HR = 1.08, $P = 0.48$) compared with lamivudine. Entecavir also reduced the risk of death or transplantation in cirrhosis subset (HR = 0.42, $P < 0.001$), but risk for HCC did not show difference (HR = 1.00, $P = 0.999$) when comparing with lamivudine (Lim et al. 2014).

Resistance

Entecavir has a relatively low incidence of drug resistance as it requires a combination of three mutations before resistance develops. In addition to the rtM204V and rtL180M mutations being responsible for lamivudine resistance, an additional mutation at rtI169T, rtT184G, rtS202I, or rtM250V is required for entecavir resistance (two-hit mechanism).(Tenney et al. 2004) The rate of entecavir resistance is 1.2% at 5 years in treatment-naïve patients. On the contrary, the rate of entecavir resistance was as high as 51% at 5 years in lamivudine-refractory patients (Tenney et al. 2009).

Dosage, Safety, and Side Effects

For treatment-naïve patients with normal renal function, entecavir dosage is 0.5 mg daily whereas 1.0 mg daily for those who experienced lamivudine or telbivudine or has decompensated cirrhosis. Entecavir dosage should be adjusted according to the creatinine clearance of patients. Common adverse events (AEs) from entecavir were myalgia (5%), and neuropathy (4%) in long-term cumulative safety results and 1% of patients discontinued entecavir due to AEs.(Manns et al. 2012) A safety concern with entecavir relates to its possible carcinogenic potential based on rodent carcinogenicity studies. In two epidemiological studies, subjects with entecavir were followed for 8 years in the US study and for 11 years in the Taiwan study, the rates of malignancy development were comparable between entecavir and lamivudine group. However, the observational period might be short to exclude carcinogenicity risk, and long-term follow-up studies may be required.

1.5 Tenofovir Disoproxil Fumarate (TDF)

TDF is a prodrug of tenofovir that undergoes phosphorylation to competitively inhibit the natural substrate deoxyadenosine 5′-triphosphate. Once incorporated into HBV DNA polymerase, it functions as a chain terminator (Fung et al. 2011). Currently, TDF is a preferred first-line antiviral drug for CHB due to its high potency and high barrier to resistance.

Short-Term Responses.

A. HBeAg-Positive Patients

A phase 3 double-blind study compared the efficacy in patients with HBeAg-positive between TDF 300 mg and adefovir 10 mg for 48 weeks. Seventy-six percent of patients receiving TDF had an HBV DNA level of less than 400 copies/mL, and 13% of patients receiving adefovir showed HBV DNA suppression (<400 copies/mL). With regard to ALT normalization, a higher proportion of patients with TDF than adefovir achieved ALT normalization (68% versus 54%, $P = 0.03$). However, there was no significant difference in the rate of HBeAg seroconversion in both groups (24% in TDF versus 18% in adefovir, $P = 0.36$). (Marcellin et al. 2008b). In an extension of the above study, 60% of patients achieved HBV DNA suppression (HBV DNA <69 IU/mL), and 47% of patients showed ALT normalization after 5 years of treatment. HBeAg seroconversion was achieved in 40% of patients, and HBsAg seroconversion occurred in 9.7% of patients with TDF treatment for 7-year follow-up. In the on-treatment analysis, defined as excluding missing data and patients with emtricitabine added to their treatment regimen, HBV DNA suppression was maintained up to 99% of patients, and ALT normalization was detected in 74% at year 7 (Buti et al. 2015).

B. HBeAg-Negative Patient

In a phase 3 randomized trial, TDF was superior to adefovir with respect to the primary end point of antiviral efficacy (defined as HBV DNA <400 copies/mL and ≥ 2-point reduction in Knodell inflammatory score without worsening of fibrosis at 48 weeks). Among 250 patients with TDF and 125 patients with adefovir, 71% of patients receiving TDF achieved primary end point compared with 49% of patients receiving adefovir ($P < 0.001$). However, regarding the rate of ALT normalization, both treatment groups were comparable (Marcellin et al. 2008b). After the completion of the above trial, patients enrolled in a 7-year open-label study where all patients continued or switched to TDF for a total duration of up to 8 years. Seventy-seven percent of patients achieved HBV DNA suppression (HBV DNA <69 IU/mL), and 65% of patients achieved ALT normalization at year 7. On the other hand, in the on-treatment analysis, 99% and 84% of patients had HBV DNA suppression and ALT normalization in the same treatment period (Buti et al. 2015).

Long-Term Outcome

A. Histological Improvement

A study investigating the improvement of liver histology after TDF treatment for 5 years has demonstrated that long-term suppression of HBV with TDF can lead to regression of fibrosis and cirrhosis. In this study, 348 patients (54%) had biopsy results at both baseline and week 240. 304 (87%) of the 348 had histological improvement (defined as ≥2 point reduction in Knodell necroinflammatory score with no worsening of fibrosis), and 176 (51%) had regression of fibrosis (defined as ≥1 unit decrease by Ishak scoring system) at week 240 ($P < 0.001$). Of the 96

(28%) patients with cirrhosis (Ishak score 5 or 6) at baseline, 74% no longer had cirrhosis (≥1 unit decrease in score) at year 5 (Marcellin et al. 2013).

B. Clinical Trial

TDF has been shown to be effective in patients with decompensated cirrhosis (a CTP score of 7-12 or prior CTP score ≥ 7 and CTP ≤12 at screen) in phase 2 double-blind randomized study. At week 48, in this trial, HBV DNA was <400 copies/mL in 71%, the rate of ALT normalization was 57%, HBeAg seroconversion was 21% of the TDF treatment group. In addition to virological and biochemical improvement, all patients improved in Child-Pugh and MELD scores (Liaw et al. 2011b). Furthermore, TDF appears to reduce mortality and improve liver function in a small study of 27 patients who developed acute-on-chronic liver failure compared to placebo (probability of survival, 57% in TDF versus 15% in placebo, $P = 0.03$) (Garg et al. 2011).

C. Observational Study

TDF was associated with a reduced incidence of HCC among patients without cirrhosis compared with those who were not received antiviral therapy in a well-validated prediction model. With the Risk Estimation for Hepatocellular Carcinoma in Chronic Hepatitis B (REACH-B) model, which estimates HCC incidence for up to 10 years based on age, sex, ALT level, HBeAg status, and HBV DNA, the standardized incidence ratio of HCC was 0.40 (95% confidence interval, 0.20-0.80), representing a 60% reduction in the incidence of HCC in patients receiving TDF treatment at the end of the 384 weeks (Kim et al. 2015). In another large European retrospective cohort study of 1666 CHB patients with entecavir or TDF, the cumulative probability of HCC was 1.3%, 3.4%, and 8.7% at 1,3, and 5 years after treatment initiation. However, virological remission with entecavir or TDF was not associated with the HCC development (Papatheodoridis et al. 2015b). These findings are not different from those being at risk of HCC among published untreated or lamivudine-treated cohorts of patients. (Rapti and Hadziyannis 2015).

Resistance

In an in vitro study, the mutation in rtA194T, along with lamivudine resistance-associated mutations, was reported to confer resistance to tenofovir in HBV/HIV-coinfected patients (Sheldon et al. 2005). Another in vitro study has demonstrated that rtA194T polymerase mutation is associated with partial tenofovir resistance and negatively impacts viral replication, especially when it harbors lamivudine resistance mutations (rt180M + rtM204V), precore mutations, and basal core promotor mutations, suggesting patients with HBeAg negative may be at particular risk for developing tenofovir resistance.(Amini-Bavil-Olyaee et al. 2009) However, in the following study, rtA194T mutation (with or without rtL180M + rtM204V), regardless of precore or basal core promotor mutations, remained susceptible to tenofovir and rtA194T alone is susceptible to lamivudine (Zhu et al. 2011). In a long-term follow study of a clinical trial, no resistance to TDF was detected for 7 years (Buti et al. 2015). So far, no clinical case of TDF resistance has been reported in patients with CHB mono-infection (Lampertico et al. 2015).

Dosage, Safety, and Side Effects

The standard dose for TDF is 300 mg daily for patients with normal renal function. No dose adjustment is necessary based on renal function, but dose interval should be adjusted according to the patient's renal function. TDF is not eliminated by hepatic metabolism; thus it is not affected by hepatic impairment, so it can be safely administered to patients with poor liver function. With regard to the drug-related AEs of TDF, more evidences have been accumulated comparing with other NUCs because it has been used for one of the elements of antiviral therapy for HIV-infected patients prior to using for CHB treatment. Three major AEs that should be paid attention to are mitochondrial toxicity, proximal renal tubular damage, and osteomalacia. Mitochondrial toxicity can manifest as lactic acidosis, fat redistribution syndrome, hepatic steatosis, acute pancreatitis, or peripheral neuropathy (Duarte-Rojo and Heathcote 2010). TDF has been reported to cause proximal tubular dysfunction, e.g., Fanconi syndrome, and also other related nephrotoxicities including diabetes insipidus, calcium and phosphorus dysregulation with the bone disease have been reported (Gupta 2008). Fanconi syndrome may lead to severe complications such as loss of calcium and phosphorus due to proximal tubular dysfunction. However, this report was from the majority of the patients with concomitant use of other HIV-drugs, which also could cause renal dysfunction (Gupta 2008). Renal function, however, should be assessed before and during treatment periodically, in particular, in patients at high risk for renal dysfunction (old age, hypertension, diabetes, chronic kidney disease and renal calculi).(Terrault et al. 2016).

In a study of HIV-infected patients, patients with TDF, including regimen had a high urinary excretion of phosphate. Although TDF was not associated with hypophosphatemia, this report addressed the possible concern for chronic demineralization of bone due to TDF (Labarga et al. 2009). The long-term significance of TDF effect on kidney and bone remains uncertain. Annual bone mineral density assessment in a 7-year of long-term follow-up study with TDF showed no significant changes at years 4 and 7. Renal AEs developed infrequently (1.7%), were generally mild, and improved with dose modification (Buti et al. 2015).

In pregnancy, the FDA category assigned to TDF is B. Studies on carcinogenesis, mutagenesis, and fertility, whether in vitro or in animals, with doses over five times that seen in humans on standard doses of TDF have not shown any consistent adverse findings that raise concern for humans (Duarte-Rojo and Heathcote 2010). Therefore, TDF is a preferred choice for pregnant patients considering its high potency, low resistance rate, and safety data of use during pregnancy (Terrault et al. 2016; European Association For The Study Of 2012). In a cohort of HBeAg-positive mothers with an HBV DNA level of more than 200,000 IU per milliliter during the third trimester, the rate of mother-to-child transmission was lower among those who received TDF therapy than among those who received usual care without antiviral therapy (Pan et al. 2016). In this study, the maternal and infant safety profiles were similar in the TDF group and the control group.

1.6 Tenofovir Alafenamide

Tenofovir alafenamide (TAF), a novel prodrug of tenofovir, was developed to have greater stability in plasma than TDF. TAF showed higher antiviral potency, with higher intracellular tenofovir-diphosphate levels and lower plasma tenofovir concentrations, compared with TDF, at approximately 10% of the dose (Ruane et al. 2013). TAF is more stable in plasma than TDF, and TAF metabolism differs from that of TDF, with fewer adverse effects on renal and bone function compared to that observed with TDF. Based on two large-scale phase 3 trials comparing TAF and TDF, TAF has been approved as the first-line antiviral treatment for patients with CHB by current international guidelines along with entecavir and TDF.

Short-Term Responses

A. HBeAg-Positive Patients

A phase 3 randomized trial of 1473 HBeAg-positive CHB patients showed that TAF was non-inferior to TDF (Chan et al. 2016). In terms of virological response (HBV DNA <29 IU/mL), TAF treatment achieved virological response in 64% of the study population, whereas 67% of TDF-treated patients achieved virological response at week 48 after randomization. At week 96, the proportion of TAF-treated patients with HBV DNA suppression was 73% compared to 75% of those receiving TDF (Agarwal et al. 2018).

Regarding biochemical response, 72% of the TAF group and 67% of the TDF group achieved normal ALT levels at week 48, as determined by local laboratory criteria for ALT normalization. However, TAF treatment resulted in a significantly higher proportion of ALT normalization than TDF treatment both at weeks 48 and 96, as per the AASLD criteria for ALT normalization (Chan et al. 2016).

B. HBeAg-Negative Patients

A phase 3 randomized trial of 426 HBeAg-negative CHB patients showed non-inferiority of TAF compared with TDF in terms of virological response (Buti et al. 2016). The proportions achieving virological response at week 48 in the TAF and TDF treatment were 94% and 93%, respectively. After 96 weeks of randomization, the proportion of HBV DNA suppression did not differ between the two treatment groups (Agarwal et al. 2018). Achievement of ALT normalization at week 48 did not statistically differ between the TAF and TDF treatment groups, as per the local laboratory criteria for ALT normalization. When applying the AASLD criteria for ALT normalization, TAF treatment (50%) showed a significantly higher on-treatment ALT normalization than TDF treatment (32%) (Buti et al. 2016).

Long-Term Outcomes

Given the recent introduction of TAF, data regarding the long-term outcome of TAF treatment are not sufficient. In a 5-year observation study of clinical trials including more than 1600 patients, TAF treatment led to a significant reduction in HCC incidence when comparing the prediction rates based on the REACH-B score. Of note,

TAF treatment appeared to be associated with a lower risk for HCC (1.0%) than TDF treatment (1.9%) (Lim et al. 2019a). However, this trend in the incidence of HCC did not meet statistical significance.

Dosage, Safety, and Side Effects

TAF 25 mg daily is the recommended dose for patients with CHB. Dose adjustment is not required in patients with mildly decreased renal function (creatinine clearance ≥15 mL/min). However, those with severe renal dysfunction are not advised to receive TAF.

In the above-mentioned phase 3 trial of HBeAg-positive patients, TAF treatment showed a significantly smaller decrease in bone mineral density at the hip and spine at week 48 than TDF treatment. In addition, TAF treatment was associated with a smaller increase in serum creatinine at week 48 than TDF treatment (Chan et al. 2016). Similar to those with HBeAg-positivity, HBeAg-negative patients receiving TAF also had significantly smaller decreases in bone mineral density and renal function than those receiving TDF at week 48 (Buti et al. 2016). Another randomized phase 3 trial, in which treatment was switched from TDF to TAF in virologically suppressed patients, showed that switching to TAF had significantly increased bone mineral density at the hip and spine compared to that observed after continuing TDF at week 48 (Lampertico et al. 2020). In addition, patients receiving TAF, unlike those receiving TDF, showed a lesser decline in renal function at week 48. (Lampertico et al. 2020).

In the 96 weeks of extension study of phase 3 trials, adverse events greater than grade 3 occurred in 7% of patients receiving TAF. However, only 2% of TAF-treated patients discontinued treatment due to adverse events. The most common adverse events during the study period included headache, nasopharyngitis, and ALT elevation (Agarwal et al. 2018). In the phase 3 trial where TDF was switched to TAF, only 3% of the patients treated with TAF treatment experienced adverse events greater than grade 3. No TAF-related serious adverse events were observed during the 48-week study period (Lampertico et al. 2020).

2 Treatment Strategies

2.1 Definition of Treatment Response

Definitions of terms for treatment response are shown in Table 13.1.

2.2 On-Treatment Monitoring

Serum HBV DNA levels may be checked every 3 months by sensitive PCR method until HBV DNA is undetectable. HBeAg, anti-HBe (in patients with HBeAg positive) and ALT may be measured every 3-6 months during NUC therapy. Thereafter, every 3–6 months of serial monitoring is required for early detection of virological and biochemical breakthrough. Renal safety measurements including serum

Table 13.1 Definitions of terms related to HBV infection, oral antiviral therapy and resistance to nucleos(t)ide therapy

Terminology	Definition
HBeAg clearance	Loss of HBeAg in a person who was previously HBeAg-positive
HBeAg seroconversion	Loss of HBeAg and detection of anti-HBe in a person who was previously HBeAg positive and anti-HBe negative
HBeAg reversion	Reappearance of HBeAg in a person who was previously HBeAg negative and usually associated with increased HBV replication
HBsAg seroconversion	Loss of HBsAg and development of anti-HBs
Acute exacerbation or flare of hepatitis B	Intermittent elevation of aminotransferase to more than five times the upper limit of normal and more than twice the baseline value
Reactivation of hepatitis B	Reappearance of active necroinflammatory disease of the liver in a patient known to have the inactive chronic HBV infection state or resolved hepatitis B infection
Resolved hepatitis B Infection	Previous HBV infection, but now HBsAg negative and anti-HBs positive
Hepatic decompensation	Defined as significant liver dysfunction as indicated by raised serum bilirubin (more than 2.5 times the upper limit of normal) and prolonged prothrombin time (prolonged by more than 3 s), or INR[1.5 or occurrence of complications such as ascites and hepatic encephalopathy
Biochemical response	Normalization of serum ALT level
Virological response on NUC therapy	
Primary non-response	Reduction of serum HBV DNA <1 log IU/ml at 12 weeks of oral antiviral therapy in an adherent patient
Suboptimal or partial virological response	Reduction of serum HBV DNA >1 log IU/ml but still detectable at 24 weeks of oral antiviral therapy in an adherent patient
Virological response	Undetectable serum HBV DNA during therapy
Virological breakthrough	Increase of serum HBV DNA [1 log IU/ml from the nadir of initial response during therapy, as confirmed 1 month later
Sustained off-treatment virological response	No clinical relapse during follow-up after stopping therapy
Complete response	Sustained virological response with HBsAg seroclearance
Viral relapse	Serum HBV DNA [2000 IU/ml after stopping treatment in patients with virological response
Clinical relapse	Viral relapse along with ALT >2x ALT
Histological response	Decrease in histology activity index by at least two points and no worsening of fibrosis score compared to pre-treatment liver biopsy or fibrosis reduction by at least one point by Metavir staging
Drug resistance	
Genotypic resistance	Detection of mutations in the HBV genome that are known to confer resistance and develop during antiviral therapy
Phenotypic resistance	Decreased susceptibility (in vitro testing) to inhibition by antiviral drugs; associated with genotypic resistance
Cross-resistance	Mutation selected by one antiviral agent that also confers resistance to other antiviral agents

creatinine, phosphorus, urine glucose, and urine protein and bone profile should be assessed before treatment commencement and during treatment periodically if treated with adefovir or TDF. Muscle weakness and development of neuropathy should be monitored during telbivudine therapy. In patients receiving long-term therapy with NUCs, checking patient's compliance and antiviral resistance testing should be assessed when virological breakthrough occurs during therapy, especially in patients who are taking low genetic barrier drugs.

2.3 Treatment Failure

Primary non-response in treatment-naïve patients is relatively low for all available NUCs; adefovir seems to have a higher rate of primary non-response compared with other NUCs. The strategies for partial virological response depend on types of prior NUC. In patients on the drug with the low genetic barrier to resistance (lamivudine, telbivudine, or adefovir), change to a more potent drug (entecavir or TDF) is recommended considering their high rate of long-term antiviral resistance (European Association For The Study Of 2012). In patients with primary non-response or partial response on entecavir or TDF can continue monotherapy regardless of ALT even though it is still controversial. Testing for genotypic resistance may be performed in patients with virological breakthrough if the patient is compliant with treatment. Identification of drug resistance during entecavir therapy is time to change treatment strategies: switch to TDF monotherapy is the recommended rescue therapy (Terrault et al. 2016).

2.4 Endpoint of Treatment with NUCs

The ideal endpoint of treatment for CHB is HBsAg loss following anti-HBs seroconversion. However, given the rarity of HBsAg seroconversion rate with current NUCs therapies, long-term, life-long treatment is required in most of the patients regardless of the virological and/or serologic responses (Su and Kao 2015).

In HBeAg-positive patients, HBeAg seroconversion with undetectable HBV DNA might be considered criteria to discontinue antiviral treatment after at least 12 months of consolidation therapy. However, the long-term durability of HBeAg seroconversion induced by NUCs is still less satisfactory. Virological relapse with or without HBeAg reversion is common after cessation of treatment with a recurrence rate of 40–50% in 2-years. In HBeAg-negative patients, the virological relapse rate is >90% after cessation of antiviral treatment (Seto et al. 2015). Among CHB patients who discontinue NUCs treatment, the combination of serum HBV DNA and quantitative HBsAg levels may predict the risk of clinical relapse (Wang et al. 2016). Several aspects should be considered prior to decide cessation of antiviral treatment: risk for virological relapse, hepatic decompensation, development of HCC, liver-related death, and burden of sustained antiviral therapy (long-term monitoring, adherence, and potential for drug resistance).

Without evidence of cirrhosis, antiviral treatment can be considered to withdraw after HBsAg loss (Kim et al. 2014). In patients with compensated cirrhosis, HBsAg loss is regarded as an endpoint of antiviral treatment even though the HBsAg seroconversion rate is very low. After patients achieve HBsAg loss, cessation of treatment may be considered. However, periodic monitoring is required for early detection of virological relapse, hepatic flare to ensure retreatment as soon as possible. In patients with decompensated cirrhosis, antiviral treatment is recommended indefinitely irrespective of HBV DNA level. Even with effective antiviral treatment, serial HCC surveillance is mandatory for all patients with cirrhosis.

3 Treatment for Antiviral Resistant Cases

The rate of resistance to NUCs can vary with duration of treatment, pre-treatment of HBV DNA levels, the potency of antiviral agents, and prior exposure to NUCs. The long-term resistance rates are presented in Table 13.2. Once genotypic resistance is evident, rescue therapy with drugs that do not share cross-resistance should be initiated to minimize the emergence of multiple drug-resistant strains (Liver 2012).

3.1 Lamivudine Resistance

Current evidence advocates that switching to TDF monotherapy is preferred. In a randomized prospective trial, patients with lamivudine resistant were treated with either TDF or TDF/emtricitabine. In this study, 89.4% of patients in the TDF and 86.3% of TDF/emtricitabine had undetectable HBV DNA levels (HBV DNA <69 IU/mL, $P = 0.43$) at week 96 (Fung et al. 2014).

3.2 Adefovir Resistance

It has been reported that adefovir resistance is more likely developed in patients with previous exposure to lamivudine (Lee et al. 2006). Adefovir monotherapy in patients with lamivudine resistance had a 22% of cumulative adefovir resistance at 2 years (Fung et al. 2006). In a study with patients who were resistant to both lamivudine/adefovir or lamivudine alone, entecavir was less effective in reducing serum HBV DNA levels in the lamivudine/adefovir group at week 48 (10% versus 34%, $P = 0.006$, respectively).(Shim et al. 2009) In a prospective study of 60 patients who failed to treat with both lamivudine and adefovir, TDF monotherapy has been shown to be effective in reducing HBV DNA levels. (Patterson et al. 2011) In a case of treatment failure or resistance to adefovir, treatment with TDF achieved long-term viral suppression (HBV DNA <400 copies/mL) in 84% of patients at week 168 (Berg et al. 2014). In a prospective randomized trial (Lim et al. 2016b), patients who had adefovir-resistant HBV mutations with serum HBV DNA levels >60 IU/mL were treated with either TDF monotherapy or TDF and entecavir combination

Table 13.2 Approved oral nucleos(t)ide analog treatment in adults

	Lamivudine(%)	Adefovir(%)	Telbivudine(%)	Entecavir(%)	TDF(%)	TAF(%)
Dose	100 mg daily	10 mg daily	600 mg daily	0.5 or 1.0 mg daily	300 mg daily	25 mg daily
Pregnancy category	C	C	B	C	B	Not assigned
Potential side effects	Pancreatitis Lactic acidosis	Acute renal failure Fanconi syndrome Nephrogenic diabetes insipidus	Myopathy Peripheral neuropathy	Lactic acidosis	Nephropathy Fanconi syndrome Osteomalacia	Increase in blood lipids and body weight
HBeAg-positive patients						
Undetectable HBV DNA*	36-44	13-21	60	67	76	64**
ALT normalization‡	41-72	48-54	77	68	68	72
HBeAg seroconversion	16-18	12-18	22	21	21	10
HBsAg loss	0-1	0	0.5	2-3/4-5(1 yr./3 yrs)	3/8 (1 yr./3 yrs)	1
References	(Lau et al. 2005; Dienstag et al. 1999; Chang et al. 2006; Lai et al. 2007)	(Marcellin et al. 2003, 2008b)	(Lai et al. 2007)	(Chang and Lai 2006; Gish et al. 2007; Lok et al. 2012)	(Marcellin et al. 2008b; Heathcote et al. 2011)	(Chan et al. 2016)
HBeAg-negative patients						
Undetectable HBV DNA*	72-73	51-63	88	90	93	94**
ALT normalization‡	71-79	72-77	74	78	76	83

(contiuned)

	Lamivudine(%)	Adefovir(%)	Telbivudine(%)	Entecavir(%)	TDF(%)	TAF(%)
HBsAg loss	0	0	0	<1	0	0
References	(Lai et al. 2006, 2007; Marcellin et al. 2004)	(Marcellin et al. 2008b; Hadziyannis et al. 2003)	(Lai et al. 2007)	(Lai et al. 2006)	(Marcellin et al. 2008b)	(Buti et al. 2016)
Resistance						
At 1 year	24	0/4.4-18	2.7-4.4	0.2/6	0	
At 3 year	53	11/34.3		1.2/36	0	
At 5 year	>70	29/65.6		1.2/51	0	
Reference	(Lai et al. 2003; Lok et al. 2003)	(Hadziyannis et al. 2006; Lee et al. 2006, 2010; Yeon et al. 2006)	(Lai et al. 2007; Liaw et al. 2009)	(Tenney et al. 2009)	(Lampertico et al. 2015)	

Responses at 48 weeks of treatment, if not specified.
TAF: Tenofovir alafenamide, TDF: Tenofovir disoproxil fumarate
*HBV DNA <60-80 IU/mL
**HBV DNA <29 IU/mL
‡ALT normalization defined by laboratory normal

therapy for 48 weeks. TDF monotherapy achieved a virological response comparable to that of TDF/entecavir combination therapy at 48 weeks. In this trial, the extension of TDF monotherapy for 144 weeks showed durable viral suppression (Lim et al. 2017). Currently, switch to TDF is the recommended therapy for adefovir resistance by international guidelines (Sarin et al. 2016; Liver 2017; Terrault et al. 2016). Indeed, a 5-year clinical trial of patients with either adefovir- or entecavir-resistant HBV showed that long-term TDF monotherapy provided a virological response in most patients (Lim et al. 2019b).

3.3 Telbivudine Resistance

There is few evidence to support an optimal treatment regimen for telbivudine resistance. Given a similar profile of antiviral resistance with lamivudine, telbivudine resistance could be treated in a similar way to lamivudine resistance.

3.4 Entecavir Resistance

Entecavir resistance can develop in patients with prior resistance to lamivudine. The 5-year cumulative probability of emerging entecavir resistance is 51% in lamivudine-resistant patients (Tenney et al. 2009). In a randomized study of 90 patients with entecavir resistance treated with TDF alone or TDF/entecavir combination therapy (Lim et al. 2016a), the primary efficacy endpoints (HBV DNA <15 IU/mL) was not significantly different between TDF monotherapy and TDF/entecavir combination therapy (71% versus 73%, respectively, $P > 0.99$) at week 48. In addition, none developed additional resistance mutations. In the case of entecavir resistance, switch to TDF monotherapy is a preferred option (Sarin et al. 2016; Liver. 2017; Terrault et al. 2016). However, long-term TDF monotherapy was associated with a decreasing renal function and bone mineral density.

3.5 Tenofovir Resistance

To date, no tenofovir resistance has been reported in the clinical setting. A long-term follow-up study of tenofovir-treated patients showed an effective serum HBV DNA suppression with no evidence of tenofovir resistance up to 6 year of treatment (Kitrinos et al. 2014).

4 Risk of HCC Development with Oral Antiviral Treatment

With the advent of NUCs, mounting evidence has accumulated, supporting that HCC incidence has decreased with oral NUCs treatment. The first data with lamivudine by Liaw et al. demonstrated that HCC risk was reduced with

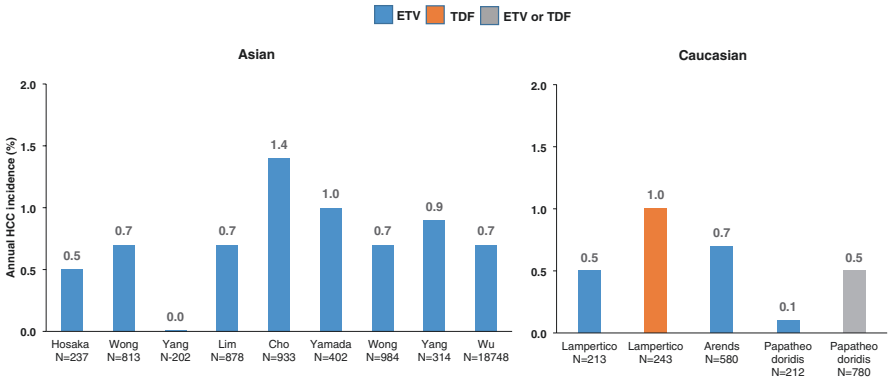

Fig. 13.1 Annual HCC incidence rates with entecavir or tenofovir in CHB without cirrhosis (Papatheodoridis et al. 2015a)

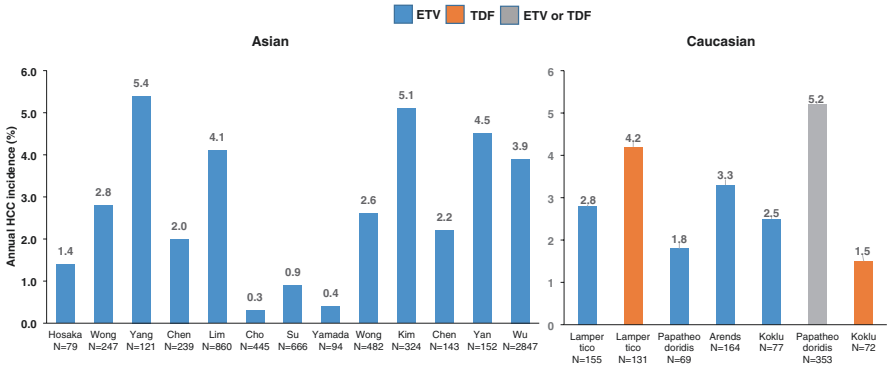

Fig. 13.2 Annual HCC incidence rates with entecavir or tenofovir in CHB with cirrhosis (Papatheodoridis et al. 2015a)

antiviral treatment compared to placebo in patients with cirrhosis or advanced fibrosis and active liver disease (HR = 0.49; P = 0.047) (Liaw et al. 2004). The annual incidence of HCC in patients treated with entecavir or TDF, which are the preferred agents for first-line therapy, is presented in Figs. 13.1 and 13.2. The observed HCC risk with entecavir or TDF ranges from 0.9 to 5.4% and 0.01 to 1.4% in patients with and without cirrhosis, respectively (Papatheodoridis et al. 2015a). Compared to untreated patients, treatment with entecavir or TDF showed a lower risk of HCC development ranging from an HR of 0.27 to 0.41. (Hosaka et al. 2013; Kumada et al. 2013; Wu et al. 2014; Su et al. 2014) In addition, a recent multinational, multicenter retrospective study of 1088 patients with CHB demonstrated that TDF treatment was independently associated with reduced risk of HCC (HR: 0.46) compared with untreated patients with CHB (Liu et al. 2019). However, it is still controversial whether the risk reduction for

HCC from entecavir or TDF is greater than other NUCs (i.e., lamivudine, adefovir, and telbivudine) (Lim et al. 2014; Papatheodoridis et al. 2015a).

5 Oral Antiviral Treatments May Not Have the Same Effect of HCC Prevention

Unlike the old generation of NUCs, such as lamivudine and adefovir, entecavir was expected to further decrease the development of HCC, considering its higher efficacy and a high genetic barrier to resistance. A Japanese study reported that entecavir treatment was associated with a significantly lower risk of HCC than treatment with lamivudine (Hosaka et al. 2013). However, another large-scale study of 5374 patients treated either with entecavir or with lamivudine showed that entecavir treatment did not differ from lamivudine treatment in terms of HCC risk (Lim et al. 2014). Interestingly, entecavir treatment was associated with a significantly lower risk of death or liver transplantation (HR: 0.49) compared with lamivudine (Lim et al. 2014). Therefore, it should be further investigated whether NUCs with higher efficacy in reducing viral load could lead to a proportional decrease in HCC development.

Current international guidelines for the treatment for CHB equally recommend entecavir, TDF or TAF as the first preferred antiviral treatment based on high efficacy in achieving surrogate markers, including ALT normalization and virological response (Sarin et al. 2016; Liver. 2017; Terrault et al. 2016). Nevertheless, this level of recommendation was not supported by the long-term clinical outcomes of both antiviral treatments. Notably, a nationwide cohort study from Korea addressed this issue by comparing entecavir and TDF in terms of HCC risk (Choi et al. 2019b). This Korean study showed that TDF treatment was associated with a significantly lower risk of HCC than entecavir treatment both in a population-based cohort and in a hospital-based cohort. A subsequent study from Hong Kong also showed that TDF treatment was associated with a lower risk for HCC than entecavir treatment, which was consistent with the results of a Korean study (Yip et al. 2020). Moreover, another study from Korea revealed that TDF was associated with a significantly higher recurrence-free and overall survival rate than entecavir in patients who underwent curative-intent liver resection for HBV-related HCC (Choi et al. 2020a). In contrast, a multicenter study showed no difference in HCC risk between these two treatments (Kim et al. 2019). Subsequent studies also failed to show any difference between entecavir and TDF treatment in terms of HCC development (Papatheodoridis et al. 2020; Lee et al. 2020; Hsu et al. 2020). Owing to these conflicting results, this issue of comparison is still under debate. A Meta-analysis comparing the preventive effect against HCC between these two treatments concluded that TDF was associated with a significantly lower risk of HCC than entecavir (Choi et al. 2020b). However, all studies comparing these two antiviral treatments were retrospective in nature and differed in terms of inclusion criteria, ethnicity, and inclusion of decompensated cirrhosis. Thus, the results regarding this issue need to be interpreted with caution.

6 Expanding Treatment Indication Based on HBV Viral Load May Reduce HCC Incidence and Mortality

Current international guidelines for CHB advise treatment initiation based on HBeAg-positivity, serum HBV DNA, ALT levels, and the presence of cirrhosis. In patients without cirrhosis, serum ALT and HBV DNA levels are the two main determinants for initiating antiviral treatment. However, the current criteria for antiviral treatment may have pitfalls. Patients who do not meet this indication may still develop HCC.

First, the ALT criteria for treatment indication (usually 2 × the upper limit of normal [ULN] by guidelines) may not be good for deciding to start antiviral treatment, given that there is a poor association between serum ALT levels and the degree of inflammation in the liver when ALT levels are slightly elevated (Nguyen et al. 2009; Park et al. 2008). In other words, current treatment indication may delay the initiation of antiviral treatment until serum ALT levels increase up to 2 x UNL. A Korean study of CHB patients with the high viral load but normal or slightly elevated ALT levels (<2 × ULN) showed a higher risk of HCC than that in patients treated with antivirals (Choi et al. 2019a). Thus, the current guidelines may put those who do not meet treatment indication at a risk for HCC by delaying antiviral treatment until the serum ALT levels increase beyond 2 x UNL. In addition, serum ALT levels are prone to be affected by other factors such as fatty liver and alcohol consumption, suggesting that ALT may not accurately reflect the inflammatory status of the liver parenchyma due to HBV itself.

Second, the concept of the immune tolerant (IT) phase, which is considered to be a clinically dormant stage, has been changing recently. Current guidelines indicate that patients with an IT phase can be observed without antiviral treatment. However, recent immunological studies have shown that patients with an IT and immune-active phase have similar profiles of HBV-specific T cell responses, indicating that patients with an IT phase are no longer considered to be immunologically benign (Kennedy et al. 2012). In addition, another in vitro study revealed that hepatocarcinogenesis through HBV DNA integration and clonal hepatocyte expansion initiated from the IT phase (Mason et al. 2016). A large-scale retrospective study conducted in Korea showed that untreated patients with an IT phase carried a higher risk of HCC than those with an immune-active phase subjected to antiviral treatment (Kim et al. 2018). Indeed, antiviral treatment in patients with an IT phase, despite normal levels of serum ALT, actually decreased the risk of HCC, compared to that in patients not receiving antiviral treatment. (Chang et al. 2017) Of note, starting antiviral treatment in patients with an IT phase is cost-effective compared with delayed antiviral treatment until active hepatitis phase development, especially with increasing HCC risk, decreasing drug costs, and productivity loss (Kim et al. 2020b). Therefore, those with a higher risk of HCC among "traditional" IT phase patients may benefit from early initiation of antiviral treatment to reduce the risk of HCC.

Lastly, the traditional notion, which is higher the serum HBV DNA level, the higher the risk of HCC, may need to be reconsidered. This notion stems mainly from a large-scale untreated CHB cohort (REVEAL study) (Chen et al. 2006).

However, this large cohort mostly included HBeAg-negative CHB patients and those with very high levels of HBV DNA classified into only one group without stratification. A large cohort study of 6949 treatment-naïve, non-cirrhotic, CHB patients with ALT <2 × ULN showed that those with baseline HBV DNA levels of 6–7 $\log_{10}$ IU/mL had the highest risk for HCC, and HBV DNA levels of >8 log10 IU/mL indicated the lowest risk for HCC. (Kim et al. 2020a) This parabolic association was independent of other predictive factors such as ALT and HBeAg status and remained statistically significant in all age subgroups (Kim et al. 2020a). These findings emphasize that baseline serum HBV DNA level is the main determinant of HCC risk.

Taken together, treatment indication based on the current guidelines may not be sufficient to further reduce the incidence of HCC. For better prevention of HCC than status quo, treatment indication could be expanded and guided by serum HBV DNA levels based on accumulating evidence from basic and clinical studies.

7 Summary and Conclusions

The goal of CHB treatment is to prevent liver-related morbidity and mortality through the persistent suppression of HBV viral replication with currently available oral NUCs. Entecavir, TDF, and TAF are the preferred choices for first-line antiviral treatment by all international practice guidelines due to their superior efficacy and high barrier to resistance over other drugs. However, currently available NUCs cannot completely eradicate HBV owing to the persistence of cccDNA and host genome integration in the hepatocytes. Mounting evidence suggests that NUCs can reduce the risk of development of HCC but not eliminate the risk. Accordingly, to optimize the HCC-preventive effect of NUCs in patients with CHB, further studies are urgently required regarding when is the optimal time to initiate the treatment and which NUC is the best option.

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Combination Therapy

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Abstract

Chronic hepatitis B virus (HBV) infection may progress to liver failure, cirrhosis, and hepatocellular carcinoma (HCC). Current approved antiviral treatments include nucleos(t)ide analogues (NUCs) and immunomodulators, such as pegylated interferon alpha (Peg-IFN). NUCs are safe and generally well-tolerated agents with direct antiviral activities. However, off-treatment durability of response to NUC therapy is low, requiring a long-term or lifelong course of treatment. Peg-IFN treatment has the advantage of a finite duration and a good chance of achieving sustained off-treatment response. But only a minority of patients have a success response to IFN alone, and high rates of side effects limit its clinical use. Elimination (complete cure) of HBV is hard to achieve with either therapy alone, given that the covalently closed circular DNA (cccDNA) persists stably in the nuclei of infected hepatocytes. Hepatitis B surface antigen (HBsAg) loss is therefore considered the optimal endpoint and a "functional/clinical cure" for HBV infection, despite the lack of HBV complete clearance. Theoretically, combination of Peg-IFN and NUC with differential mechanisms of actions on HBV is an alternative strategy to treat chronic hepatitis B. Recent studies demonstrated virological or serological benefits of de novo combination therapy with Peg-IFN and NUC, or addition of Peg-IFN (add-on or switch) to an ongoing NUC therapy, but few data exist about the long-term outcomes in patients receiving combination therapy. Currently, several new antiviral or immunomodulatory agents are being explored in experimental models or have reached clinical trials, which may have the potential to complement NUC or IFN-based therapy. This chapter summarizes the current status of combination therapy and novel therapeutic approaches developed to accomplish a cure of chronic HBV infection.

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Keywords

HBV · CHB · Peg-IFN · NUC · Combination therapy · Functional cure

Abbreviations

ADV	Adefovir dipivoxil
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
DAAs	Direct-acting antivirals
ETV	Entecavir
FTC	Emtricitabine
HAPs	heteroaryldihydropyrimidines
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B s antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
LAM	Lamivudine
LdT	Telbivudine
NTCP	Sodium taurocholate cotransporting polypeptide
NUC	Nucleos(t)ide analogue
Peg-IFN	Pegylated interferon
PPAs	Phenylpropenamides
TDF	Tenofovir disoproxil fumarate
TLR	Toll-like receptor

1 Goals of Therapy and Definition of "Functional Cure" for HBV Infection

Hepatitis B virus (HBV) infection remains a worldwide health burden. The World Health Organization (WHO) estimates that 257 million people are chronic carriers of hepatitis B surface antigen (HBsAg) globally; around 650,000 people die annually from the complications of chronic hepatitis B (CHB). Overall, HBV accounts for around 30% of cases of cirrhosis and 45% of hepatocellular carcinoma (HCC) (Ott et al. 2012; Guidelines for the Prevention 2015). Both viral and host factors are involved in the chronicity of HBV infection. The HBV genome forms covalently closed circular DNA (cccDNA), a stable minichromosome, within the nuclei of hepatocytes, which enables the infection to persist (Levrero et al. 2009). Data suggest that HBV is potentially capable of suppressing innate immunity (Op den Brouw et al. 2009; Lang et al. 2011), and persistent exposure to high concentrations of viral antigen may cause functional exhaustion of T cells in CHB patients (Das et al. 2008; Boni et al. 2007). The ultimate goal of antiviral therapy is to prevent or significantly delay the progression of HBV-related liver disease. This goal may be achieved

firstly through sustained immunological control of HBV infection and, eventually, by means of complete elimination of HBV (European Association for the Study of the Liver 2017; Lampertico and Liaw 2012; Scaglione and Lok 2012). But elimination of HBV (complete cure) is rarely experienced, given that HBV cccDNA persists stably, at very low level, even after the seroclearance of HBsAg. Serum HBsAg represents a surrogate marker for intrahepatic cccDNA, and loss of HBsAg is thought to be associated with a functional remission and improved long-term outcomes in CHB patients, even if HBV genome may not be eliminated and the few persisting infected hepatocytes are controlled by the host immune system (Martinot-Peignoux et al. 2014)]. HBsAg loss with or without HBsAb seroconversion is therefore considered the optimal endpoint and a "clinical or functional cure" for HBV infection, and represents the complete suppression of viral replication with sustained immunologic control of HBV infection (European Association for the Study of the Liver 2017; Ning et al. 2019). This ideal endpoint could be achieved, although relatively rarely, after therapy with available antiviral drugs. The suboptimal endpoint is defined by sustained off-therapy virological/serological response with biochemical and histological improvement and prevention of complications; under this circumstance, the antigen-specific immune response might be sufficient to suppress HBV replication.

2 Advantages and Disadvantages of Current Anti-HBV Therapies

Currently approved treatment options remain limited to two classes of antiviral agents: nucleos(t)ide analogues (NUCs) including lamivudine (LAM), telbivudine (LdT), adefovir dipivoxil (ADV), entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide fumarate (TAF), which suppress HBV replication efficiently by blocking the viral reverse transcriptase (European Association for the Study of the Liver 2017; Sarin et al. 2016; Terrault et al. 2016), and immunomodulatory agents including interferon-a (IFN) and pegylated interferon alpha (Peg-IFN), which bear both immune modulatory and antiviral effects (Sadler and Williams 2008). Both treatment strategies have advantages and limitations. NUCs have been widely used due to their convenience, well tolerability, and potent antiviral activity. Recent studies have shown significant reductions in cccDNA levels after long-term NUC treatment (Lai et al. 2017). However, even third-generation NUCs (ETV and TDF), recognized as first-line treatment options, have only small effects on the expression of viral antigens since NUCs do not directly inhibit the transcriptional abilities of cccDNA; thus, sustained immunological control is hard to achieve, and high rates of relapse after NUCs discontinuation require these oral antiviral treatments to be lifetime commitments, especially in HBeAg-negative patients. As a consequence, long-term duration can increase the likelihood of viral resistance, poor adherence, and potential adverse effects. Furthermore, several studies indicate that long-term NUC treatments reduce but cannot completely eliminate the risk of HCC (Arends et al. 2015). By contrast, IFN has synergic effect which can induce a

complex network of intracellular signaling and the production of antiviral proteins encoded by IFN-stimulated genes (ISGs), and enhances the activity of immune cells and expression of cytokines for control of viral replication (Sadler and Williams 2008). Moreover, recent studies report that IFN is capable of promoting degradation of pregenomic RNA and core particle, and inhibiting HBV transcription by inducing epigenetic regulation of the nuclear cccDNA minichromosome (Wieland et al. 2005; Xu et al. 2010; Belloni et al. 2012), thus reducing the production of viral antigen including HBsAg and inducing durable responses. Nevertheless, only a small proportion of patients achieve response to IFN monotherapy, and several factors are associated with favorable treatment outcomes, such as age, HBV-DNA levels, HBsAg titer, and alanine aminotransferase (ALT) levels. Moreover, due to the relatively high rates of adverse effects, IFN is often not well tolerated (Kim et al. 2011).

Despite being the optimal treatment endpoint for chronic hepatitis B, HBsAg loss is rarely achieved with NUC or Peg-IFN monotherapy (Marcellin et al. 2004; Lau et al. 2005; Marcellin et al. 2008). Theoretically, the combination of antivirals with different modes of action against HBV, such as NA and Peg-IFN, is a promising approach to generate synergistic and complementary effects and might achieve higher rates of HBsAg loss and, ideally, HBsAb seroconversion.

3 Combining Different NUCs as Rescue Therapy

Combining antiviral drugs with similar mechanisms of action, such as different NUCs, may not provide additional therapeutic benefits. Therefore, current guidelines (European Association for the Study of the Liver 2017; Sarin et al. 2016; Terrault et al. 2016) recommend combination therapy of NUCs with non-overlapping resistance profiles only as rescue regimen under highly selected circumstances such as for patients with suboptimal responses to NUC monotherapy and for patients infected with multidrug-resistant (MDR) strains of HBV. It is uncertain whether de novo combination therapy offers advantages over NUC monotherapy in treatment-naïve CHB patients, especially in patients with high baseline viral load; thus, more studies are required. A meta-analysis comparing ETV alone and LAM + ADV combination therapy demonstrated that combination therapy led to higher rates of serological and biochemical response at week 96, as compared to ETV monotherapy, and no viral resistance occurred in combination therapy and six patients receiving ETV alone experienced viral breakthrough (Liu et al. 2014). In a randomized clinical trial of 379 treatment-naïve patients, ETV alone and ETV + TDF combination demonstrated similar virological response rates by week 96. A post hoc subgroup analysis showed that combination therapy was superior to ETV alone in patients with positive HBeAg and baseline HBV DNA over 8 log IU/ml (Lok HT et al. 2012). ADV add-on rescue therapy in patients with resistance to LAM is more effective than ADV alone, especially in subjects with a significant viral load (Gaia et al. 2008; Kim et al. 2016). Recently, several studies investigated combination therapy with ETV and TDF in primary nonresponders, partial responders, or patients with virological breakthrough or MDR CHB patients. One of these studies has shown that combination therapy with ETV

plus TDF can provide high rates of viral suppression without treatment-emergent resistance to either agent in patients with previous NUC treatment failure (Zoulim et al. 2016). However, unfavorable respects of rescue combination therapy include higher treatment costs and potentially harmful side effects. Long-term data from a multicenter cohort study of patients with MDR chronic hepatitis B showed that the efficacy of TDF monotherapy was not different from that of the TDF-based combination therapy (Yim et al. 2020). A recent study reported that virological responses were durable after withdrawal of LAM in a majority of patients with LAM resistance who had achieved complete virological response with LAM and ADV combination therapy (Kim et al. 2017). In a follow-up study of MDR CHB patients, after achieving virological response, TDF was discontinued in ADV-resistant patients, and ETV was discontinued in LAM-resistant patients with ETV plus TDF combination therapy. The results demonstrated that switching to a monotherapy from combination therapy appeared to be efficient and safe (Petersen et al. 2014). A retrospective study showed that switching from an NUC combination to TAF was effective for HBV suppression and continued HBsAg reduction (Ogawa et al. 2020). This adapting step-down strategy requires larger comparative, prospective trials to investigate its safety and efficacy.

4 Interferon-Based Combination Therapy

NUC and Peg-IFN have different mechanisms of action; evidence has shown that combination of both treatments may enhance chances of serologic response and sustained off-treatment response, thereby facilitating the discontinuation of NUCs (Thimme and Dandri 2013; Wu et al. 2015). The updated Chinese Guidelines for the treatment of HBV recommend that sequential therapy with additional Peg-IFN or switching to Peg-IFN in patients who have achieved virological suppression on long-term NUC treatment may be advantageous to obtain a higher rate of HBeAg seroconversion and greater HBsAg decline than continuous NUC monotherapy (Hou et al. 2017). The 2015 updated Asian-Pacific guidelines recommend that the combination treatment with NUC and Peg-IFN could be considered the ideal treatment for chronic hepatitis B (Sarin et al. 2016). Sequential therapy with ETV followed by IFN is recognized as a first-line treatment for young HBeAg-negative patients with high viral load in the Japanese Guidelines (Hiromitsu Kumada et al. 2010). Currently, IFN and NUC combination treatment is not recommended either by AASLD Guidelines or by EASL Guidelines (European Association for the Study of the Liver 2017; Terrault et al. 2016). Clinical trials evaluated the de novo combination, as well as add-on or switch strategies with Peg-IFN and oral antivirals for CHB patients, but the results are inconclusive.

5 De Novo Combination Strategy

Early observation studies which evaluated Peg-IFN in combination with LAM or ADV started simultaneously demonstrated higher rates of on-treatment virological response, but not improved posttreatment response rates (Marcellin et al.

2004; Lau et al. 2005; Piccolo et al. 2009; Janssen et al. 2005). Previous studies reported that Peg-IFN plus ADV combination therapies contributed to remarkable reductions in levels of HBV DNA, intrahepatic cccDNA, and serum HBsAg titer (Wursthorn et al. 2006). A randomized multicenter study investigated the outcome of HBeAg-positive CHB patients treated with LdT plus Peg-IFN combination therapy and demonstrated that even though the combination therapy led to greater declines in HBV DNA and HBsAg levels, it carried an increased risk of unexpected severe peripheral neuropathy; thus, it should not be used (Marcellin et al. 2015). Recently, a large prospective, active-controlled randomized trial evaluated loss of HBsAg in patients receiving treatment with either TDF or Peg-IFN, or simultaneously combining TDF and Peg-IFN for 16 weeks or 48 weeks. This study showed that, at 24 weeks post-treatment, 48 weeks of combination therapy with TDF plus Peg-IFN led to a higher rate of HBsAg loss than either therapy given alone. But the overall rate of HBsAg clearance remained low (9.1%), with the highest rate occurring in genotype A patients (Marcellin et al. 2016). At week 120, 10.4% of patients treated with 48 weeks of TDF and Peg-IFN combination therapy developed HBsAg loss (Ahn et al. 2018). In a recent study, 26 patients with genotype C HBV infection were simultaneously administered ETV plus Peg-IFN for 48 weeks; the 5-year cumulative rate of HBsAg loss after the completion of combination therapy was found to be 15% (Hagiwara et al. 2018).

Simultaneous administrations of Peg-IFN and third-generation NUCs such as TDF and ETV might provide additional benefits. The therapeutic benefit and safety of combination of Peg-IFN and other NA such as TAF are worth exploring.

6 "Switch-to" Strategy

Late breaking clinical studies demonstrate that sequential combination therapy with IFN and NUC contributes to a better chance of HBsAg loss over NUC monotherapy. A prospective multicentered randomized and controlled trial (OSST study) reported that HBeAg-positive patients who did not seroconvert to HBeAb during 9–36 months of ETV treatment were randomly assigned to switch-to Peg-IFN treatment or to receive continuous ETV monotherapy for 48 weeks, as compared to continuous ETV monotherapy; sequential combination therapy using ETV and Peg-IFN led to significantly increased rates of HBeAg seroconversion and HBsAg seroclearance (8.5%) (Ning et al. 2014). During untreated follow-up in those patients who switched from ETV to Peg-IFN therapy, rates of HBeAg seroconversion improved from 17.7% at the end of therapy to 38.7% one year post-treatment; moreover, HBsAg seroclearance was sustained in six of seven patients (Han et al. 2016). Results from OSST cohort study were in line with previous studies in which patients received sequential combination therapy with NUC and IFN; however, these studies included a very limited number of patients (Moucari et al. 2011; Sarin et al. 2005). In the NEW SWITCH study, switching to 96-week course of Peg-IFN in HBeAg-positive patients who achieved HBeAg clearance by NA led to higher rates of HBsAg loss

(20.7%), as compared to that of 48-week course of treatment (14.4%), albeit not statistically significant (Hu et al. 2018). An exploratory study demonstrated that sequential treatment, specifically, 12 weeks of ETV alone, then ETV plus Peg-IFN for 12 weeks, followed by Peg-IFN alone for 36 weeks, led to significantly higher rates of HBeAg and HBsAg seroconversion in CHB patients with non-D genotypes and high HBV viremia, as compared with 48 weeks of Peg-IFN monotherapy (Boglione et al. 2013). The timing of switching may be an important factor associated with the treatment outcome. In a randomized trial, 21-week ETV lead-in pretreatment followed by 48-week Peg-IFN did not demonstrate superiority for off-treatment response over Peg-IFN alone in treatment-naïve HBeAg-positive patients (Xie et al. 2014). In a prospective study, of 41 HBeAg-positive patients who seroconverted to HBeAg during ETV treatment, 6 (15%) patients switching to 48-week course of Peg-IFN had HBsAg loss 24 weeks post-treatment (Chan et al. 2019).

7 "Add-on" Strategy

Another combination treatment approach, by adding Peg-IFN to ongoing NUC therapy, has recently been shown to be beneficial in improving the response rates. An observation study demonstrated that in CHB patients undergoing a stable oral therapy with undetected HBV-DNA, the addition of Peg-IFN led to HBsAg seroconversion in 2 out of 12 patients (Kittner et al. 2012). Another prospective study showed that in HBeAg-negative patients on a long-term NUC treatment with HBV DNA undetectable, add-on of Peg-IFN induced sustained HBsAg clearance and cessation of NUC therapy in 6 out of 10 patients (Ouzan et al. 2013). The PEGON study suggested that a 48-week add-on Peg-IFN therapy in HBeAg-positive patients treated with ETV or TDF led to a numerically higher rate of HBeAg seroconversion compared with NA monotherapy; however, this difference was not statistically significant (Chi et al. 2017). In a retrospective matched-pair study, Peg-IFN add-on in ETV-treated HBeAg-positive individuals without HBeAg seroconversion led to a higher rate of HBeAg seroconversion (44%) and is more likely to induce HBsAg loss (4%) than ETV monotherapy (6% and 0%). The further analysis demonstrated that a low baseline level of HBsAg <1000 IU/mL and HBsAg decline >0.5$\log_{10}$IU/mL at week 12 were associated with an optimal rate of HBsAg loss (Li et al. 2015). The HERMES study showed that adding Peg-IFN to ongoing NA therapy resulted in a significant decrease in HBsAg levels in HBeAg-negative patients with HBV genotype D infection (Lampertico et al. 2019). In the PEGAN study, at week 96, addition of a 48-week Peg-IFN in HBeAg-negative patients who achieved virological suppression by NA did not significantly improve HBsAg loss (7.8%) rate than continuous NA monotherapy (3.2%); however, it resulted in a more significant decline in HBsAg levels (Bourliere et al. 2017). A global multicentered randomized controlled trial (ARES study) investigated the effectiveness of Peg-IFN add-on therapy for 24 weeks in HBeAg-positive patients after 24 weeks of ETV monotherapy; when compared to continuous ETV monotherapy, this combination strategy of

adding Peg-IFN to ETV did not improve response rates which were defined as HBV DNA < 200 IU/mL with HBeAg clearance at week 48, but resulted in a higher rate of HBeAg seroconversion and more decline in HBV DNA, HBeAg, and HBsAg, and seemed to prevent post-treatment relapse, thereby allowing for NUC discontinuation (Brouwer et al. 2015).

8 Optimal Approach to Combination Therapy

A meta-analysis of 24 studies involving IFN and NA combination therapy revealed that the "NA-experienced" strategy was more effective in inducing HBsAg loss than the "de novo" strategy in CHB patients (8% versus 11%). Moreover, the "switch-to" strategy led to significantly higher rates of the pooled HBsAg loss than the "add-on" strategy (14% vs. 8%) (Qiu et al. 2018). A retrospective study demonstrated that both "switch-to" (9%) and "add-on" Peg-IFN treatment (15%) in ETV-treated HBeAg-negative patients significantly improved HBsAg loss rates than ETV monotherapy (0%). The response rate (HBsAg decline >1 log IU/mL) in the switch-to, add-on, and ETV monotherapy arms was 60%, 40%, and 2%, respectively, at week 48 (Yan et al. 2018). A non-randomized study in HBeAg-negative NA-treated patients with virological suppression showed that 8 of 10 patients switching to Peg-IFN and 2 of 11 patients adding Peg-IFN achieved HBsAg decline >1 log IU/mL, indicating that switch-to strategy might be more effective than add-on strategy in reducing HBsAg levels. NA discontinuation in switch-to strategy may activate host immune response which could favor a better response to Peg-IFN (Tatsukawa et al. 2018). A randomized controlled study comparing 48-week "add-on" or "switching-to" Peg-IFN in patients treated with long-term NA treatment showed that both add-on (9.0%) and switch (8.9%) treatment demonstrated higher overall HBsAg loss rates than controls (0%), whereas patients in the switch arm experienced significantly a higher virological relapse rate (30.2%) than controls (3.3%) and add-on arm (2.0%), respectively (WLY et al. 2017).

At present, it is difficult to draw firm conclusions from these studies regarding which combination strategy will be most beneficial. Considering that activation of innate immune response induced by Peg-IFN benefits from virological suppression, NA often needs long-term treatment to achieve complete suppression of viral replication and HBsAg levels decline, eventually allowing restoration of HBV-specific T cell responses. Choice of drugs (potent NA with Peg-IFN), the time schedule of combination (NA lead-in followed by Peg-IFN), and proper patient selection (virally suppressed patient with low HBsAg level) might be the key factors associated with the efficacy. Several studies have shown that host genetic background was associated with HBsAg loss during combination therapy (Jansen et al. 2014; Stelma et al. 2016; Tangkijvanich et al. 2016). One recent genome-wide association study in patients treated with ADV and Peg-IFN identified that one single-nucleotide polymorphism (SNP), rs12356193 located in the SLC16A9 gene, was strongly associated with HBsAg seroclearance (Jansen et al. 2014). A randomized clinical trial demonstrated that the combination therapy

with Peg-IFN plus ETV in treatment-naïve HBeAg-negative patients led to higher rates of undetectable HBV DNA at week 48, but did not increase HBsAg clearance and decline compared with Peg-IFN monotherapy. Interestingly, patients carrying the SNP rs3077 genotype GG with baseline HBsAg <1000 IU/mL had a good chance of attaining virological response and HBsAg loss. These studies indicate that the viral and host genetic characteristics may be beneficial to individualize decision-making before initiating Peg-IFN therapy (Tangkijvanich et al. 2016).

9 Roadmap for NA and Peg-IFN Combination Treatment in NA-Treated Patients

Baseline and early on-treatment HBsAg titers may predict which patients are more likely to achieve HBsAg loss with Peg-IFN treatment (Fig. 14.1). In NA-treated patients, at the time of switching, HBsAg <1500 IU/ml were associated with a higher chance of HBsAg loss at week 48 of Peg-IFN treatment than HBsAg level ≥ 1500 IU/ml. Moreover, patients with HBsAg levels of <200 IU/mL at week 12 or 24 had the greatest chance of HBsAg loss at week 48. By contrast, patients with HBsAg levels of ≥1500 IU/mL at week 12 or ≥ 200 IU/mL at week 24 had a minimal chance of achieving HBsAg loss; therefore, stopping Peg-IFN therapy may be considered (Ning et al. 2014; Hu et al. 2018). A prospective study evaluating "switch-to" Peg-IFN as a strategy to stop NA also showed that 20% of patients with baseline HBsAg <1500 IU/mL achieved HBsAg loss, and baseline HBsAg <500 IU/mL is the optimal predictor for HBsAg loss (50%) (Chan et al. 2019). Therefore, the NA-treated patients with low baseline level of HBsAg are more likely to achieve clinical cure by sequential Peg-IFN therapy, which is supported by several recent studies. The Endeavor study in ETV-treated patients with HBeAg loss demonstrated that triple combination of IFN, interleukin (IL)-2, and therapeutic vaccine led to higher rates of HBsAg loss (9.38%) than IFN or ETV alone, particularly in those with low baseline HBsAg levels <1500 IU/mL (27.3%) (Wu et al. 2019). Several studies in the selected patients with higher probability of clinical cure demonstrated the benefits of the baseline-guided strategy. A randomized controlled trial showed that in NA-treated patients with virological suppression and low HBsAg level (<2000 IU/ml), switching to 60-week Peg-IFN improved HBsAg loss (32.6%) and HBsAg seroconversion (27.9%) rates (Huang et al. 2017). Anchor study suggested that sequential combination therapy with Peg-IFN with or without granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with low HBsAg levels (<3000 IU/mL) led to significantly higher rates of HBsAg loss and HBsAg seroconversion than ETV alone (Meifang Han et al. 2017a). The I CURE study showed that add-on Peg-IFN in NA-treated patients with low HBsAg levels (<1000 IU/mL) and negative HBeAg led to a high rate of HBsAg loss (66.67%). After treatment discontinuation, 80% patients sustained complete response at week 24 of follow-up (Gao et al. 2018). In the PYRAMID study in NA-treated HBeAg-positive patients with virological

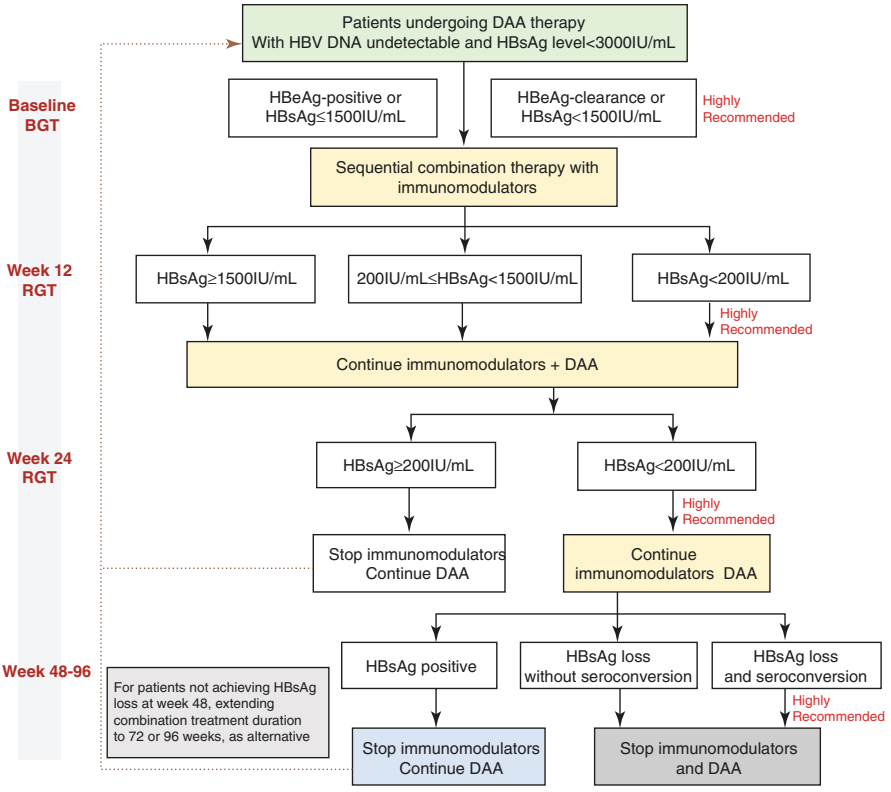

Fig. 14.1 Roadmap for combination therapy with direct-acting antivirals (DAAs) in DAA-treated patients [e.g., nucleos(t)ide analogues (NUC)] and immunomodulators (e.g., pegylated interferon). For patients undergoing long-term DAA (such as NUC) treatment with virological suppression and HBsAg level < 3000 IU/mL, sequential treatment with immunomodulators (such as interferon) can be considered. At week 24, patients with HBsAg level < 200 IU/mL should continue the combination treatment until 48 weeks; those with HBsAg level ≥ 200 IU/mL can stop immunomodulators and continue the NUC treatment. These patients may consider retreatment with immunomodulators if necessary. Abbreviation: *HBsAg* HBV surface antigen; *HBeAg* HBV e antigen; *BGT* Baseline-guided therapy; *RGT* Response-guided therapy; *DAA* direct-acting antiviral; *NUC* nucleos(t)ide analogues. Used with permission from (Ning et al. 2019)

suppression, HBsAg < 5000 IU/ml and HBeAg < 100PEIU/m, after receiving 24-week add-on Peg-IFN, patients with HBsAg < 200 IU/ml at week 24 continued combination treatment for a further 24 weeks; those with HBsAg ≥ 200 IU/mL were randomized to combination treatment or NUCs alone for 24 weeks. At week 72, 56.5% of patients with HBsAg < 200 IU/ml at week 24 achieved HBsAg loss, whereas only 4.5% of those with HBsAg ≥ 200 IU/ml at week 24 who continued combination treatment and none of those stopping Peg-IFN at week 24 achieved HBsAg loss (Qing Xie et al. 2018). These data suggest that the baseline- and response-guided treatment strategy could help identify patients most likely to respond to combination treatment.

10 The Mechanisms Involved in Immune Restoration Induced by Peg-IFN and NUC Combination Therapy

During persistent HBV infection, the impaired antiviral immune responses were reported through virus-mediated mechanisms, including the suppression of natural killer (NK) cell activity, the exhaustion of viral specific cytotoxic lymphocytes (CTLs), and the activation of regulatory T cells (Tregs). Current data emphasize the critical role of both innate and adaptive immune response in resolution of HBV infection (Maini and Schurich 2010; Thimme et al. 2003; Billerbeck et al. 2007). Further studies on the mechanism underlying enhanced immune response induced by IFN-based combination therapy may help guide future clinical trial design.

Accumulating data suggest that the host immune response can be impacted by NUC and Peg-IFN in different ways. It is widely accepted that Peg-IFN mediates significant augmentation of innate immunity**,** especially NK cells. Micco et al. investigated the immunomodulatory effects of Peg-IFN on NK cells and CD8 + T cells; the results showed that Peg-IFN was able to induce IL-15 and NKp46, accompanied by increases in expansion, activation, and antiviral ability of CD56bright NK cells with upregulated expressions of TRAIL and IFN-γ; in contrast, Peg-IFN resulted in a sustained depletion of effector CD8+ T cells and had a limited capacity to restore HBV-specific T cell functions (Micco et al. 2013). Studies conducted by Huang et al. revealed that significantly reduced expressions of Toll-like receptors (TLR) 3 and TLR9 on peripheral CD14+ monocytes in CHB patients could be restored by effective Peg-IFN therapy (Huang et al. 2013; Huang et al. 2014). However, different effects of Peg-IFN were observed on T cells. Consistent with the results obtained by Micco et al., Penna et al. reported that in HBeAg-negative CHB patients Peg-IFN did not improve early circulating HBV-specific T cell responses (Penna et al. 2012), which demonstrated a contrasting impact of Peg-IFN on innate and adaptive antiviral immune responses. Contrarily, potent NUC treatment was not able to restore the antiviral capacity of NK cells (Peppa et al. 2010; Zhang et al. 2016). But effect of NUC on T cell response seems different from IFN. Several studies demonstrated that the defective T cell function could be partially and transiently restored by NUC treatment. A recent study demonstrated that following in vitro culture, the dysfunctional HBV-specific T cells from patients achieving viral suppression with long-term NUC treatment had a significant functional recovery (Boni et al. 2012).

Accumulating lines of evidence emphasize the crucial role of immune restoration in achieving clinical cure of chronic hepatitis B. The different impacts of Peg-IFN and NUC on innate and adaptive immune response, as well as the findings that suppression of HBV replication by NUC prolongs innate immune response to Peg-IFN, give the mechanistic rationale for combination of these two agents in chronic HBV infection (de Niet et al. 2016; Tan et al. 2014). A recent study evaluated HBV-specific T cell function from patients with high HBV DNA level receiving Peg-IFN plus ADV combination therapy, and demonstrated a partial restoration of HBV-specific T cells in patients who achieved HBsAg clearance (de Niet et al. 2016). Control of HBV replication by prior long-term NUC treatment could contribute to partial functional restoration of HBV-specific T cell; subsequent administration of

immunomodulatory drugs such as Peg-IFN might further augment the immune response and increase the possibility of treatment success. According to this scenario, it is conceivable that sequential therapy with Peg-IFN on long-term NUC treatment might be an alternative strategy leading to a higher rate of HBsAg clearance. In line with this theory are intriguing immunology research findings based on OSST study. The OSST immunologic study showed that successful serological responses to sequential Peg-IFN-a therapy were associated with significant restoration of an impaired immune response before week 24. The restoration was manifested by increased proportions of NKG2C+ NK cells, higher proportions of TLR2+ CD14+ monocytes, and decreased proportions as well as diminished inhibitory function of Tregs (Yan et al. 2015b). Successful sequential therapy with ETV and Peg-IFN was also associated with altered expression of ISGs (Meifang Han et al. 2017b). During sequential treatment, restoration of CD56bright NK cells contributed to HBsAg and cccDNA clearance (Shi et al. 2018). This data suggests that, during the early phase of IFN-based combination treatment, the activation and recovery of immune system will be beneficial for CHB patients to obtain satisfactory endpoints. Obviously, comprehensive analyses of the innate and adaptive immune system in parallel with large prospective clinical trials are needed to further investigate the combination treatment strategies.

11 Novel Antiviral Strategies and Possible Combination Therapies toward a Cure of HBV Infection

Combination therapies with drugs acting on novel targets may help achieve a cure of HBV infection. It is suggested that complete HBV control does not only depend on the reduction in viral burden but also on the induction of effective antiviral immune response (Lin and Kao 2016). Currently, several strategies including small molecules targeting various stages of HBV life cycle (HBV entry, HBV cccDNA production and processing, viral replication, viral protein expression, etc.) as well as immunotherapeutic approaches are being explored in experimental models or have reached clinical testing, which may have the potential to complement NUC or IFN-based therapy (Fig. 14.2).

The sodium taurocholate cotransporting polypeptide (NTCP), a newly identified cellular receptor for HBV entry and a bile salt transporter, is a promising target enabling the development of entry inhibitors and new research possibilities (Huan Yan et al. 2012). Cyclosporine A or B is able to inhibit NTCP and prevents HBV entry in cell-culture models (Nkongolo et al. 2014; Watashi et al. 2014). Myrcludex-B can block HBV/hepatitis D virus (HDV) entry and has entered in clinical trial (Volz et al. 2013). These agents may prevent new infection (Kaneko et al. 2015; Yan et al. 2015a), but do not eliminate the preexisting HBV infection or directly target on cccDNA. Therefore, the combination regimens of NTCP inhibitors with other antivirals seem to be superior to their use as monotherapy, which needs to be further investigated.

Therapeutic strategies targeting the cccDNA for HBV cure aim to inhibit cccDNA formation, degrade cccDNA, or silence cccDNA transcription. Genome

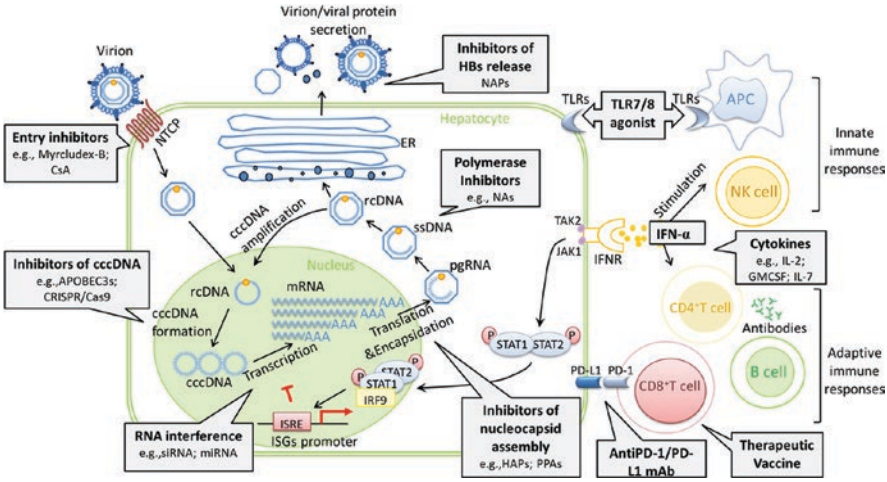

Fig. 14.2 Schematic representation of current treatment options and newly developed therapeutic strategies against HBV. Current treatment options involve NUCs, which efficiently inhibit the HBV DNA polymerase and thus block the viral replication pathway, and IFN which has both direct antiviral and immunomodulatory effects. Several strategies will appear in the near future, including direct-acting antivirals (DAAs) targeting different stages of the life cycle of the virus (HBV entry, HBV cccDNA production and processing, viral replication, viral protein expression, etc.) as well as immunotherapeutic approaches including Toll-like receptors (TLRs) agonist, pleiotropic cytokines (IL-2, GMCSF, IL-7), programmed cell death-1 (PD-1)/PD-L1 blockage, and therapeutic vaccines to revive host immunity and restore the function of the HBV-specific T cell. It is conceivable that the use of these additional strategies combined with NAs and IFN might be of synergic benefit in the restoration of innate and adaptive immune responses in CHB patients. Abbreviations: *NUC* nucleos(t)ide analogues; *IFN* interferon; *cccDNA* covalently closed circular DNA; *ER* endoplasmic reticulum; *hNTCP* human sodium taurocholatecotransporting polypeptide; *pgRNA* pregenomic RNA; *rcDNA* relaxed circular DNA; *CsA* cyclosporine A; *HAPs* heteroaryldihydropyrimidines; *PPAs* phenylpropenamides; *TLR* Toll-like receptors; *NAPs* nucleic acid polymers

editing approaches using vectors to deliver DNA cleavage enzymes into the hepatocytes, such as the clustered regularly interspaced short palindromic repeats (CRISPR)/CAS (Edward et al. 2015; Ramanan et al. 2015), might be promising approaches to degrade cccDNA. Epigenetic modification of histone inhibiting cccDNA transcription provides the proof of concept for silencing of cccDNA (Levrero et al. 2009; Teresa Pollicino et al. 2006). Activation of lymphotoxin-β receptor (LTβR) and IFN-a increased the APOBEC3 cytidine deaminases, and eventually contributed to degradation of cccDNA, but did not affect genomic DNA, thereby allowing the development of these novel strategies combined with other antiviral agents to cure CHB (Lucifora et al. 2014).

Several attempts are also made to develop nucleocapsid protein inhibitors. Phenylpropenamide (PPA) derivatives can interfere with the pregnomic RNA packaging, and heteroaryldihydropyrimidine (HAP) antiviral compound can decrease the stability of capsids. Both HAPs and PPAs synergize with nucleoside reverse transcriptase inhibitors (NRTIs) and are active against NRTI-resistant strains in vitro

(Delaney et al. 2002; Billioud et al. 2011), highlighting the potential for combination therapy with other antivirals such as NUC.

RNA interference (RNAi) is transcriptional process in which the introduction of microRNA (miRNA) or small interfering RNA (siRNA) contributes to gene silencing in a sequence-specific way (Bernstein et al. 2001; Hammond et al. 2000). Currently, several drug candidates have entered clinical development for CHB. Although the RNAi-based therapeutics are promising, there are lingering concerns about its long-term effects.

HBsAg is capable of suppressing host immunity permitting viral persistence. Nucleic acid polymer (NAP) can block HBsAg release from infected cells. In a phase 2 randomized trial, addition of REP 2139 or REP 2165 to TDF + Peg-IFN did not alter tolerability and significantly increased rates of HBsAg loss and HBsAg seroconversion during therapy and functional cure after therapy (Bazinet et al. 2020). Studies on the immunopathogenesis of HBV infection pave the way for the development of potential novel approaches, including Toll-like receptor (TLR) agonist(Amin et al. 2020), pleiotropic cytokines (Guptan et al. 2002), programmed cell death-1 (PD-1) and its ligand PD-L1 blockages (Edward Gane et al. 2019), and therapeutic vaccines (Boni et al. 2019). In virally suppressed HBeAg-negative patients, PD-1 inhibitor, with or without GS-4774, a HBV therapeutic vaccine, was well tolerated and led to HBsAg decline in most patients and sustained HBsAg loss in 1 patient (Edward Gane et al. 2019).

Given that the elimination of cccDNA may still be challenging even with the promising antivirals targeting the virus, combination strategies involving immunotherapies aiming at boosting the host immune response may be necessary. The difficulties in breaking the immune tolerance and eliminating cccDNA constitute the main obstacles for a cure of HBV infection. It is conceivable that combination of potent DAA with immunotherapeutic approach is encouraging and may ultimately help overcome these difficulties.

12 Conclusions

The combination treatment of NUC and Peg-IFN is an alternative strategy to optimize treatment efficacy and improve the chance of clinical cure. Although it is not possible to determine at this stage which antivirals or which combinations will be most beneficial, it can be reasonably assumed that sequential combination therapy with NUC and IFN might achieve better treatment outcomes than either treatment given alone because NUC reduces the viral load and thus subsequently enhances the immune response to IFN (Yang and Kao 2015). Novel therapeutic approaches will be approved for clinical use in the near future, including compounds targeting various stages of HBV life cycle and immunomodulators. We can speculate that combination of these new strategies with current available antivirals will synergistically help enhance the host immune responses and eliminate cccDNA, ultimately leading to a complete cure of HBV infection.

Conflict of Interest The authors declare no conflicts of interest.

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Treatment of HCV, HDV, or HIV Coinfections

15

Kali Zhou and Norah A. Terrault

Abstract

Due to shared routes of infection (parenteral and sexually transmitted), persons with hepatitis B may be infected with other viruses—specifically human immunodeficiency virus, hepatitis D, and hepatitis C. Coinfections lead to an altered natural history of hepatitis B, with higher risk of cirrhosis and liver cancer; thus, screening for coinfections is important in patients with hepatitis B to identify those at heightened risk. Managing patients with coinfections requires longitudinal monitoring of viral co-pathogen activity, awareness of the optimal timing of antiviral therapy for the different coinfections, and attention to the impact of viral interference and viral clearance on clinical outcomes.

Keywords

Superinfection · Viral interference · Cirrhosis · Liver cancer · Antivirals

Abbreviations

AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase
ART	antiretroviral therapy
AST	aspartate aminotransferase
CHB	chronic hepatitis B
CHD	chronic hepatitis D

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DAA	direct-acting antiviral
ETV	entecavir
FTC	emtricitabine
GFR	glomerular filtration rate
HBeAg	hepatitis B e-antigen
HBIG	hepatitis B immunoglobulin
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDV	hepatitis D virus
HIV	human immunodeficiency virus
LMV	lamivudine
LT	liver transplantation
MSM	men who have sex with men
NA	nucleos(t)ide analogues
Peg-IFN	peginterferon
PWIDs	persons who inject drugs
SVR	sustained virologic response
TAF	tenofovir alafenamide
TDF	tenofovir disoproxil fumarate

1 Introduction

Coinfection of hepatitis B virus (HBV) with other viral infections, namely human immunodeficiency virus (HIV), hepatitis D virus (HDV), and hepatitis C virus (HCV), has implications for the natural history and treatment of persons living with chronic HBV (CHB) infection. Owing to shared routes of infection, coinfection is not uncommon—of the 240 million persons infected with chronic hepatitis B worldwide, approximately 10–15 million are coinfected with HDV, 7–20 million are coinfected with HCV, and 3–6 million are coinfected with HIV (Stockdale et al. 2020a; Platt et al. 2020; Bini and Perumalswami 2010; Yu et al. 2020) (Fig. 15.1). Screening for coinfections is an essential consideration in the management of patients with chronic HBV given the potential for accelerated fibrosis progression and carcinogenesis, which, importantly, may be ameliorated in large part with advances in and access to treatment.

2 HBV and HIV Coinfection

2.1 Epidemiology of HBV and HIV Coinfection

There are approximately 38 million persons living with HIV worldwide. Globally, the prevalence of HIV/HBV coinfection among persons living with HIV is 7.6%, approximating a population of 2.7 million individuals (Platt et al. 2020). The vast majority of

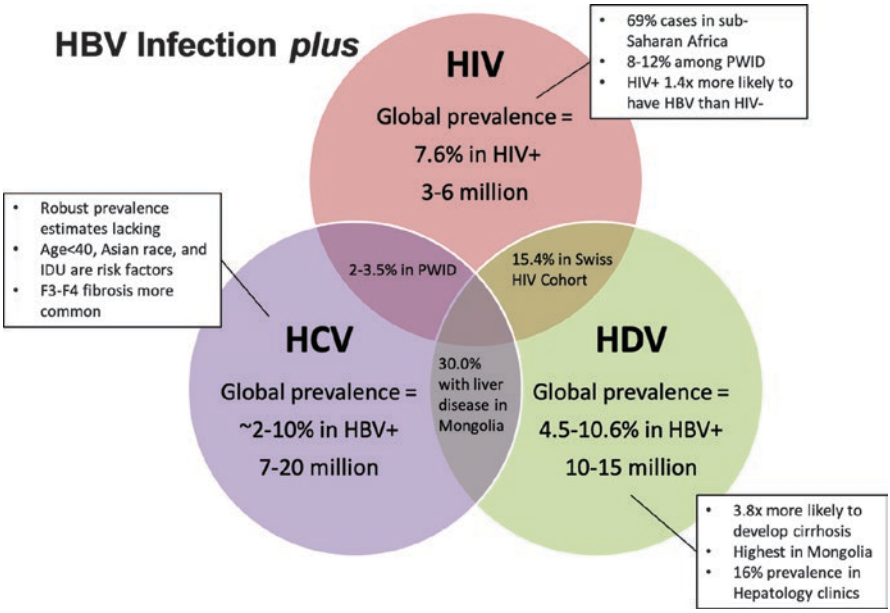

Fig. 15.1 Epidemiology of HIV, HCV, and HDV coinfections with HBV. The estimated prevalence of HBV-HIV coinfection is 3–6 million, HBV-HDV coinfection in 10–15 million, and HBV-HCV coinfection 7–20 million. The wide range of prevalence estimates reflects a lack of robust seroprevalence studies in many countries

the infection burden (69%) is in sub-Saharan Africa. The advent of highly active antiretroviral therapy (ART) has delayed progression of HIV-related disease to complications of the acquired immunodeficiency syndrome (AIDS), and dramatically increased the life expectancy of patients (Mocroft et al. 2003; Thio 2009; Thio et al. 2002; Palella Jr. et al. 2006; Mallet et al. 2011). In an aging and treated HIV population, cause of death among persons living with HIV has shifted from complications of AIDS to non-AIDS infections, heart disease, cancer, and liver-related deaths due to viral hepatitis (Antiretroviral Therapy Cohort C 2010; Croxford et al. 2017). Compared to HBV mono-infected, coinfected individuals are older, male, less often Asian, and more often report injection drug use and high-risk sexual activity (Cooper et al. 2020). Increasingly, coexisting fatty liver disease is being identified in the coinfected population, estimated at 30% in the North American Hepatitis B Research Network cohort, 10% with biopsy-proven steatohepatitis (Khalili et al. 2020).

3 Natural History of HBV and HIV Coinfection

Coexisting HIV has an impact on nearly all aspects of the natural history of HBV. First, HIV-positive individuals are 6 times more likely to progress to chronic HBV after acute exposure as an adult (Hadler et al. 1991), while 95% of HIV-uninfected adults are expected to clear the infection. In CHB, HBV DNA levels are

typically higher in coinfected compared to mono-infected persons, with lower spontaneous hepatitis B e-antigen (HBeAg) seroconversion rates, especially in individuals with lower CD4+ T helper cell counts (Bodsworth et al. 1991). Individuals with lower CD4 counts or higher HIV RNA levels have lower alanine aminotransferase (ALT) levels, postulated to be due to depressed HBV-specific CD4+ T-cell responses induced by HIV infection of CD4+ T cells (Chang et al. 2005). Functional cure or loss of hepatitis B surface antigen (HBsAg) has been reported to occur more frequently in coinfected compared to mono-infected persons. In one study of patients on ART, 15% had lost HBsAg by year 5 of treatment (compared to ~5–8% in mono-infected) (Audsley et al. 2020). These higher rates of HBsAg loss occur early on after institution of ART, suggesting that immune reconstitution and CD4+ T-cell recovery may play a role (Chihota et al. 2020; Audsley and Sasadeusz 2020).

Presently, liver disease is the second most common cause of death in HIV-infected patients (Falade-Nwulia and Thio 2011; Joshi et al. 2011). More than one-third of HIV-suppressed coinfected individuals have evidence of significant fibrosis on liver biopsy (Sterling et al. 2019). Estimates of liver-related mortality, from the Antiretroviral Therapy Cohort Collaboration in HIV-1-infected patients who initiated ART from 1996 through 2006, demonstrated that liver disease accounted for 113/792 or 7.0% of all deaths—3.9% were from viral hepatitis and ~3.1% were from other liver conditions. The rates of liver-related death were six-fold higher in persons who inject drugs (PWIDs) often due to drug dependency, alcohol abuse, and/or coinfection with HCV (Antiretroviral Therapy Cohort Collaboration 2010). Large observational studies have demonstrated heightened all-cause mortality as well among coinfected individuals, though no increase in AIDS-related mortality (Thornton et al. 2017). Furthermore, poorly controlled HIV infection, i.e., lower CD4+ count and higher HIV viral load, has been associated with progression to advanced hepatic fibrosis in a dose-dependent manner and independent of viral hepatitis, suggesting that early treatment of HIV has a beneficial effect on liver disease (Kim et al. 2016). Postulated mechanisms for increased fibrogenesis and HBV disease activity include depletion of CD4+ T cells, HIV-induced upregulation of tumor necrosis factor-related apoptosis inducing ligand receptor 2 leading to hepatocyte apoptosis, and entry of HIV into hepatic cell lines via CCR5 and CXCR4, the latter of which induces increases in intracellular HBsAg levels (Babu et al. 2009; Iser et al. 2010; Nikolopoulos et al. 2009). Incidence of HCC among HBV-HIV coinfected on treatment is 5.9 per 1000 person-years among those with cirrhosis and 1.2 per 1000 person-years among those without; there is no increase in HCC incidence over time among those stably on treatment (Wandeler et al. 2019).

Influence of HBV on HIV-related outcomes is less clear. Data from the Swiss HIV Cohort Study reported that HBsAg-positive patients had significantly impaired CD4+ T-cell recovery during the first 3 years of ART despite similar virologic effectiveness of ART compared to HBsAg-negative patients [504 cells/μL (95%CI: 496–511) vs 449 cells/μL (95%CI: 428–469)] (Wandeler et al. 2013). In this study, using the composite endpoint of AIDS-defining illnesses and deaths, the risk of an AIDS or death event was almost double among HIV-infected individuals with HBV

coinfection (adjusted hazard ratio (HR), 1.80; 95%CI: 1.20–2.69) (Wandeler et al. 2013), while others have reported no increase in AIDS-related mortality, albeit with HBV viral suppression (Chun et al. 2012; Tsai et al. 2019). Coinfection with HBV may also augment the risk of non-Hodgkin's lymphoma by nearly two-fold in HIV-infected patients (Wang et al. 2017).

Despite increased uptake of HBV therapy over time, gaps remain, and no clear reduction in risk of end-stage liver disease was observed between 1996 and 2010 across twelve North American coinfected cohorts (Klein et al. 2016). However, in a study from Taiwan, mortality in the coinfected population improved to that among mono-infected persons in the post-tenofovir ART era (Tsai et al. 2019)[32].

3.1 Diagnosis and Screening

Testing for HIV coinfection is recommended in all individuals with chronic HBV infection by all major society guidelines (Terrault et al. 2018a; European Association for the Study of the Liver 2017a; Sarin et al. 2016) and should be performed prior to initiation of HBV treatment. The appropriate screening is a serum test for HIV antibodies, which detects HIV infection 18 to 90 days after an exposure. If there is a concern for more recent exposure, testing for HIV RNA is available, though cost prohibits use as a general screening test. Rapid point-of-care HIV tests, including both finger-prick and oral fluid collection, are also available and have comparable sensitivity and specificity to conventional tests (Delaney et al. 2011). The Center for Disease Control also recommends repeat HIV screening at least once a year in high-risk individuals (Branson et al. 2006), including sexually active men who have sex with men (MSM), sex with an HIV-positive partner, more than one partner since last test, PWIDs, and those who have another sexually transmitted disease.

An assessment for liver fibrosis should be performed after confirmation of HIV-HBV coinfection, specifically to identify those with advanced fibrosis or cirrhosis. Laboratory testing for liver enzymes including ALT, aspartate aminotransferase (AST), and liver synthetic function tests (albumin, bilirubin, and internationalized normalized ratio) is helpful. The complete blood count, including platelet count, may be an indicator of portal hypertension due to cirrhosis. These tests can then be used to calculate noninvasive indices of fibrosis, including the Fibrosis-4 or FIB-4 index and the AST-to-platelet ratio (Sterling et al. 2006; Lin et al. 2011), although they are of moderate accuracy and thrombocytopenia may be caused by HIV infection as well as portal hypertension. In settings where transient elastography is available, this is the most ideal noninvasive test for fibrosis staging. In a recent study in HIV-HBV coinfected on the accuracy of transient elastography alone or in combination with FIB-4/AST-to-platelet ratio, with liver biopsy as the gold standard, an elastography cutoff of 8.8 kPa or greater had 92% sensitivity and 96% specificity for advanced fibrosis, while the addition of FIB-4 or AST-to-platelet ratio did not improve ability to discriminate (Sterling et al. 2020).

Patients with cirrhosis and portal hypertension also need to undergo upper endoscopy to screen for esophageal varices. Recently, various criteria for variceal

screening (e.g., Baveno VI, expanded Baveno VI, and HEPAVIR) were validated in persons living with HIV with cirrhosis, demonstrating effectiveness in sparing endoscopy, thus improving resource utilization, with few missed bleeding events (Merchante et al. 2019a). Finally, all HBV-HIV-coinfected individuals who meet HCC surveillance criteria for HBV (e.g., all with cirrhosis, Asian men over the age of 40, Asian women over the age of 50, black men over the age of 40, first-degree family history of HCC) should be enrolled into a surveillance program with liver US with or without serum alpha-fetoprotein every 6 months (Terrault et al. 2018a). Of note, performance of ultrasound in detection of small lesions may be inferior among HIV infected persons (Merchante et al. 2019b). There are insufficient data to support surveillance in all individuals with HIV-HBV coinfection. However, surveillance among patients with cirrhosis is well supported and uptake as low as 5–18% has been reported in the HIV-coinfected population with cirrhosis, an important area for improvement (Willemse et al. 2019).

4 Approach to Treatment for Hepatitis B and HIV Coinfection

HIV treatment with ART is recommended for all individuals with HIV, regardless of demographic or clinical factors, to reduce morbidity and mortality as well as transmission (Panel on Antiretroviral Guidelines for Adults and Adolescents 2020). HBV testing is required prior to initiation of ART for all HIV-infected adults and coinfected patients should be treated with ART that includes nucleos(t)ide analogues (NA) with dual anti-HBV-HIV activity (Table 15.1). Current first-line options for treatment of HBV include pegylated-interferon (Peg-IFN), entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF) (Terrault et al. 2018a). Peg-IFN is rarely used in clinical practice due to adverse side effect profile, and immunomodulatory mode of action is a further concern among persons with HIV. First-line options for a fully suppressive ART regimen for HIV/HBV-coinfected patients include a dual NA backbone with TAF or TDF plus lamivudine (LMV) or emtricitabine (FTC) in combination with an integrase inhibitor. These regimens are bictegravir/TAF/FTC, dolutegravir/FTC or LMV/TAF or TDF, and raltegravir/FTC or LMV/TAF or TDF. A co-formulated bictegravir, FTC, and TAF in a single tablet once a day is available, with favorable long-term safety and efficacy data (Stellbrink et al. 2019). If TDF or TAF cannot be used, ETV has weak anti-HIV activity and can be substituted, though it can promote HIV resistance to LMV/FTC and should only be used as part of a fully suppressive ART regimen.

There are additional considerations in resource-constrained settings, where cost may dictate that the only available treatment option for HBV is LMV (Soriano et al. 2008; Wiersma et al. 2011). However, this strategy is suboptimal especially with long-term LMV use (without a second anti-HBV drug), as the rate of developing drug resistance is upward of 90%, resulting in severe hepatitis and fatalities (Bruno et al. 2001; Benhamou et al. 1999; Ramos et al. 2007). Viral suppression (up to 5 years) among coinfected with LMV alone appears to be more durable among those with baseline HBV DNA <6 $\log_{10}$ IU/mL, and stratification on HBV DNA has

Table 15.1 Anti-HBV therapies and use in HBV-HIV-coinfected patients

Drug	Anti-HIV activity	Major side effects	Risk of HBV resistance	Recommendation for HIV/HBV-coinfected persons
Tenofovir disoproxil fumarate (TDF)	++	Nephrotoxicity Bone loss	–	First line with FTC as NRTI backbone of ART regimen
Tenofovir alafenamide (TAF)	++	Less nephrotoxicity and bone loss	–	First line with FTC as NRTI backbone of ART regimen
Emtricitabine (FTC)	++	None	++	First line with TDF/TAF regimens
Lamivudine (LMV)	++	None	++	In combination with TDF only
Entecavir (ETV)	+	None	+	Second line with full ART regimen if unable to receive TDF or TAF
Adefovir (ADV)	–	Nephrotoxicity	++	No
Telbivudine (LdT)	–	Myopathy Neuropathy	++	No
Pegylated interferon alfa-2a (peg-IFN)	–	Leukopenia Depression	–	Side effects limit clinical use; consider in patients with HDV coinfection

been suggested as one method to optimize use of LMV in settings with poor access to TDF (Dunn et al. 2021). There has been promising progress in the development of long-acting injectable formulations of ART for HIV, which could greatly simplify HIV treatment regimens and promote adherence. Unfortunately, injectable regimens do not include drugs with anti-HBV activity and represent a missed opportunity for implementation in restrained-constrained settings where HIV-HBV coinfection prevalence is highest (Bollinger et al. 2020).

4.1 Treatment Considerations in HIV-HBV Coinfection

Patients with HIV infection may experience elevated transaminase levels after initiation of ART, occurring as a consequence of immune reconstitution and immune-mediated HBV-induced liver injury, as a hepatotoxic side effect of certain antiretroviral agents, or as a marker of HBeAg seroconversion (Lascar et al. 2005; Drake et al. 2004; Carr and Cooper 1997). The Multicenter AIDS Cohort study reported HBV coinfection as an independent risk factor for ART-related hepatotoxicity (Thio et al. 2002). While the magnitude of transaminitis appears to be greater with HBV–HIV coinfection, typically elevations resolve without needing to stop therapy, although consideration should be given to stopping the likely offender if ALT levels exceed 5–10 times upper limit of normal (normal: <25 U/L in women; <35 U/L in men) or clinical signs/symptoms of liver disease appear (Neukam et al. 2016).

The potential for renal dysfunction should be considered. TDF is associated with infrequent but significant renal toxicity and metabolic bone disease, more

prominently in HBV-HIV-coinfected patients (Neukam et al. 2016). Although the exact mechanism of TDF renal toxicity is unclear, affected individuals present with decreased glomerular filtration rate (GFR) and proximal tubular dysfunction, with a higher risk if older age, low body weight, lower baseline estimated GFR, and other predisposing comorbidities (i.e., diabetes, hypertension, concomitant nephrotoxic medications) (Rodriguez-Novoa et al. 2009). Increasingly, TAF—a prodrug of TDF with more potent cellular activity at lower serum levels—is being utilized in clinical practice, and multiple fixed-dose TAF-containing combination therapies are available for HIV ART. Compared with those receiving TDF, HIV-positive patients on TAF experienced significantly smaller changes in estimated creatinine clearance, renal tubular proteinuria, and bone mineral density (Sax et al. 2014). Switching patients from TDF- to TAF-containing regimens has been shown to be safe and efficacious and ultimately leads to improvements in renal function (Gallant et al. 2016).

Clinicians need to assess on-treatment response to NA by monitoring serum HBV DNA (i.e., suppression of viral replication), serum liver transaminases, and serologic markers (HBeAg and HBsAg, as appropriate) at regular intervals (Terrault et al. 2018a; European Association for the Study of the Liver 2017a; Sarin et al. 2016). Many studies have demonstrated that achievement of these surrogate intermediate endpoints leads to reduced risk of end-stage liver disease, most dramatically with HBsAg loss (Marcellin et al. 2013). It should be noted that the duration of time needed to achieve these endpoints, including complete viral suppression to undetectable levels, can be longer among coinfected, on the order of years. If incomplete suppression is present, adherence, issues with absorption, and drug–drug interactions should be considered; key risk factors for incomplete HBV DNA suppression include high baseline HBV DNA as well as persistent HIV viremia (Hafkin et al. 2014). HBV therapy should always be continued in HBV–HIV-coinfected patients, and if any interruption in HIV therapy occurs, providers should ensure anti-HBV agents are continued or alternative therapy is instituted to prevent clinically significant HBV flares.

As in HBV-mono-infected patients, the primary endpoint of treatment in patients with coinfection is the achievement of functional cure, or HBsAg loss. Novel serum biomarkers predictive of HBsAg loss are of great interest, and a few studies have evaluated these tests in HBV–HIV-coinfected patients. Quantitative HBsAg (qHBsAg) titers have been proposed as a marker of viral replication and treatment efficacy when HBV DNA becomes undetectable (Sonneveld et al. 2011; Chan et al. 2011; Brunetto et al. 2010). Recent studies of qHBsAg levels demonstrated that declines in HBsAg levels on ART correlated with CD4+ cells, and similar to mono-infected, decline on therapy is generally slow and 6-month HBsAg titers were predictive of HBsAg loss in HBeAg-positive individuals (Thibault et al. 2011; Maylin et al. 2012; Jaroszewicz et al. 2012; Zoutendijk et al. 2012). Quantitative hepatitis B core-related antigen and anti-HBc titers have been associated with HBeAg seroclearance (Dezanet et al. 2020). Longitudinal decreases in both intrahepatic cccDNA and pregenomic RNA have also been demonstrated in coinfected patients on HBV therapy (Balagopal et al. 2020). Application of novel HBV therapies of finite

duration as well as on-treatment monitoring, safety, and efficacy in the coinfected population will be areas of great interest in future.

5 Hepatitis C and B Virus Coinfection

5.1 Epidemiology

The global seroprevalence of HCV coinfection among HBsAg-positive persons is poorly characterized, with estimates of 7–20 million affected (Fig. 15.1). Countries endemic for HBV are predicted to have highest numbers of coinfected individuals. In studies of persons with CHB from Southern Europe (Spain, Italy), India, and Southeast Asia (China, Taiwan, Japan, Thailand), anti-HCV positivity was found in 3–22% (Chu and Lee 2008). In contrast, in a Canadian study of 1.3 million persons in the province of British Columbia, 0.5% had HBV-HCV (McKee et al. 2018) and in a US study based on the Veteran's cohort with chronic HCV, 1.4% had HBV coinfection (Tyson et al. 2013). Among blood donors, the rates of HBV-HCV coinfection are <1%, even among countries with high prevalence of HBV and HCV among donors (Ehsan et al. 2020).

Within countries, HCV coinfection is associated with risk profiles, notably PWIDs, those on hemodialysis, hemophiliacs, and others with a history of multiple blood transfusions and HIV-infected persons. Unique risks may be related to regional practices. In a study from Rwanda, where only 0.02% had HBV-HCV coinfection, scarification or receiving an operation from traditional healer was associated with likelihood of coinfections (Makuza et al. 2020). In many regions, the ongoing syndemics of HBV, HCV, and HIV are fueled by injection drug use and high-risk sex practices. For example, in a study of HCV-infected patients from New York, 62% had prior exposure to HBV (anti-HCV positive) and 5.8% were coinfected (HBsAg positive) with age < 40 years, injection drug use, and a greater number of lifetime sexual partners as independent risk factors for dual infection (Bini and Perumalswami 2010). Targeting groups at higher risk for HBV-HCV coinfection, such as PWIDs, through harm reduction measures and HBV vaccination, can be expected to produce a decline in coinfection rates. For example, in a Canadian study of HBV-HCV coinfection rates from 1991 to 2007, a decline was evident from 2001 onward and was attributed to universal vaccination of sixth graders beginning in 1992 and of PWIDs beginning in 1992 with a target blitz of this population in 2000 (Fang et al. 2009).

6 Natural History HBV and HCV Coinfection

Acute coinfection of HBV and HCV is rare but described among PWIDs. In areas with a high prevalence of CHB such as Southeast Asian countries, where individuals acquire HBV infection at birth, HCV is typically acquired later as a superinfection, while in countries where HBV is of low or intermediate endemicity, such as

North America and Europe, HBV and HCV are more frequently acquired as a coinfection, typically during adolescent or adult years (Nguyen et al. 2011).

Spontaneous clearance of both, one, or neither virus is possible and biphasic hepatitis has been observed. Acute HCV superinfection in patients with chronic hepatitis B frequently presents with icteric hepatitis and can be severe with one study finding that 34% of patients develop decompensation, 11% acute-on-chronic hepatitis and liver failure, and 10% died (Liaw et al. 2004). Historically, dually infected patients also had a higher incidence of cirrhosis (48% at 10 years) and HCC (14% at 10 years, 21% at 15 years, and 32% at 20 years) than patients with CHB. Interestingly, these patients were also found to achieve earlier loss of HBsAg. Acute superinfection of HBV, though less frequent than HCV superinfection or HBV-HCV coinfection, has been associated with high rates of both HBsAg and HCV clearance. In a series of 8 patients with HBV superinfection who were untreated, spontaneous HCV clearance was seen in 5/8 (62.5%), while HBsAg clearance occurred in 6/8 (75%) (Papadopoulos et al. 2018).

Among those with chronic HBV-HCV coinfection, different profiles may be seen, reflecting the predominance of HBV, HCV, or both. Patients who have both HBV DNA and HCV RNA detectable in blood appear to be at highest risk of progression to cirrhosis and liver decompensation. Patients with active HCV infection (HCV RNA+) in the setting of inactive HBsAg (HBsAg+/HBV DNA-) seem to behave similarly to patients with HCV mono-infection, at least until HCV clearance is achieved. Finally, HBsAg-positive patients who have antibody to HCV but have undetectable HCV RNA, indicating prior HCV exposure and spontaneously HCV clearance, have a natural history similar to patients with chronic HBV alone.

An inverse relationship in HBV and HCV viral levels has been reported in cross-sectional studies, suggesting presence of viral interference (Alberti et al. 1986; Liaw 1995); however, longitudinal studies in coinfected patients show more variable patterns, with viral levels seeming to be independent of each other (Nguyen et al. 2011; Raimondo et al. 2006). That being said, studies of HCV treatment in HBV-HCV-coinfected patients have described reappearance of HBV DNA in patients achieving HCV clearance, lending support to the concept of viral interference (Potthoff et al. 2008a). Likewise, HBV reactivation has been reported in some HCV-HBV-coinfected patients after successful treatment of HCV (Bersoff-Matcha et al. 2017). Of note, HCV eradication has not been associated with higher rates of HBsAg loss among coinfected patients. In 111 patients with HBV-HCV coinfection and 111 propensity score-matched controls with HBV mono-infection from Taiwan, HCV coinfection was not associated with HBsAg loss in a cohort study of 10 years follow-up [1.7% per year (95% CI: 1.1–2.7) in coinfected and 1.4% (95% CI: 0.88–12.07) in mono-infected] (Yang et al. 2016).

Consistently, natural history studies have shown that patients with dual infection with HBV and HCV are at higher risk of liver-related outcomes than patients with HBV or HCV infection alone (Yang et al. 2016; Butt et al. 2020b; Zhang et al. 2016). Dual infection has also been associated with a higher incidence of HCC compared to mono-infected patients (Yang et al. 2016; Zampino et al. 2015; Benvegnu et al. 1994) and higher all-cause mortality (19.1 vs 5.1/1000 person-years

in HBV-HCV versus HBV mono-infected) (Butt et al. 2020a). A meta-analysis of 22 studies of patients with HBV-HCV coinfection demonstrated an additive risk of HCC, but with more recent studies showing a sub-additive effect of the copathogens compared to older (prior to 2000) studies (Cho et al. 2011).

As therapies for both HCV and HBV are more widely applied, the natural history and rates of progressive disease in patients with dual infection are anticipated to decline. For example, in a HBV-HCV-coinfected cohort from the USA, achievement of a sustained virologic response with HCV therapy was associated with a 50% reduction in risk of cirrhosis (Butt et al. 2020b).

7 Indications for Antiviral Therapy

As with any patient with chronic HBV infection, the primary goals of treatment are to reduce liver injury and progression to cirrhosis and HCC and, when possible, achieve viral clearance. In the case of HBV-HCV-coinfected patients, eradication of HCV should be possible in most patients with currently available direct acting agents (DAAs). Indeed, HBV-HCV-coinfected patients are considered a high priority group for HCV treatment (Sarin et al. 2016; AASLD/IDSA/IAS–USA 2016). Since HBsAg seroconversion is a very infrequent event, the interim endpoints of treatment of HBV-HCV-coinfected patients are sustained virologic response 12 weeks after treatment end (SVR12) for HCV (defined by undetectable HCV RNA for at least 6 months), low to undetectable levels of HBV DNA, and normalization of ALT levels.

Antiviral therapy in HBV-HCV coinfection should be tailored to the virus that is "active." Based on published data, the most common clinical scenario is active HCV with inactive HBV, although other combinations can occur. If both viruses are shown to be active, then treatment should be directed against both pathogens. Importantly, treatment of one virus may lead to changes in the activity of the other virus, a concept well demonstrated by HBV reactivation occurring during and after HCV treatment with DAAs; thus, monitoring is a necessary component of management.

8 Approach to Antiviral Therapy for HBV-HCV Coinfection

In the IFN era, the treatment of choice for patients with HCV RNA detectable (with or without active HBV) was Peg-IFN and ribavirin for 24–48 weeks, depending on HCV genotype, and moderate to high rates of HCV eradication and HBV suppression were reported with this combination (Kim et al. 2011; Liu et al. 2009; Uyanikoglu et al. 2013; Potthoff et al. 2008b). However, IFN-based therapy has been supplanted by DAAs in the treatment of HBV-HCV dually infected patients. In a recent clinical trial of ledipasvir/sofosbuvir in 111 HBV-HCV-coinfected patients (61% HCV GT 1; 16% compensated cirrhosis; 33% treatment-experienced), all patients (100%) achieved SVR12 (Liu et al. 2018). In a large real-world cohort, SVR after DAA treatment in coinfected was 95% and no difference in SVR was noted between coinfected and mono-infected groups (Moorman et al. 2018). Therefore, the current approach to

selection of a DAA regimen for treatment of HCV among coinfected individuals is the same as for mono-infected (AASLD/IDSA/IAS–USA 2016; European Association for the Study of the Liver 2020).

Drug–drug interactions need always to be considered. Based on current knowledge, the only potential concern with first-line HBV therapies is the increased concentration of TDF with certain regimens, including sofosbuvir/velpatasvir and sofosbuvir/ledipasvir. While these regimens are not contraindicated due to interaction, closer monitoring for tenofovir-related adverse effects such as renal toxicity is recommended. There are no known interactions with currently approved DAAs and ETV. For triply infected patients with HIV/HBV and HCV, more opportunities for drug interactions exist and a careful review of antiretroviral therapy pre-initiation of HCV or HBV therapy should be undertaken.

8.1 Risk of HBV Reactivation with DAAs for HCV

Reports of HBV reactivation, defined as an increase in HBV DNA in patients with inactive or resolved HBV infection, began to emerge after institution of DAAs, with 29 cases reported to the US Food and Drug Administration between late 2013 and 2016, with two cases resulting in death and one in liver transplantation (Bersoff-Matcha et al. 2017). Multiple observational studies since have sought to better define the incidence and risk of HBV reactivation (Moorman et al. 2018; Belperio et al. 2017; Butt et al. 2018). In a pooled meta-analysis, reactivation was shown to be fairly frequent, occurring in 24% of patients with chronic infection and 1.4% with resolved infection; reactivation-related hepatitis was further seen in 9% (Mucke et al. 2018). There was a differential risk by baseline HBV DNA level, with a 73% lower risk of reactivation if HBV DNA was undetectable. Reactivation risk is also higher among those with higher pretreatment quantitative HBsAg (Yeh et al. 2020). Compared to IFN-based therapy, HBV reactivation occurs earlier (i.e., within 4 to 12 weeks of treatment) and is more clinically significant with DAAs (Chen et al. 2017b). Preemptive anti-HBV therapy has been shown to substantially reduce the risk of reactivation (Jiang et al. 2018). Due to high potential for decompensation and death, those with cirrhosis must be on HBV therapy prior to initiation of DAAs. In individuals with viral load and ALT parameters that meet HBV treatment criteria, HBV therapy should also be started. For HBsAg-positive who do not meet treatment criteria, either one of two approaches can be considered: (1) initiate HBV antiviral prophylaxis during DAA treatment and continue for at least 12 weeks after completion of DAAs or (2) monitor HBV DNA levels monthly during and immediately after DAA therapy with initiation of anti-HBV agents if HBV DNA rises ten-fold or more above baseline or >1000 IU/mL in those with previously undetectable levels (Terrault et al. 2018b). The latter option should only be implemented in patients with adequate and reliable access to laboratory testing. European guidelines recommend prophylaxis in all HBsAg-positive patients and reserve monitoring with serum ALT and on-demand therapy for anti-HBc-positive patients only (European Association for the Study of the Liver. 2017b) (Table 15.2).

Table 15.2 Approach to risk management of HBV reactivation in HBV-HCV-coinfected patients undergoing DAA therapy

Society guidelines	CHB meeting treatment criteria	CHB not meeting treatment criteria	Resolved infection (HBsAg−/anti-HBc+)
American Association for the Study of Liver Diseases (AASLD)	Initiate or continue anti-HBV therapy[a]	Initiate prophylactic anti-HBV therapy concurrent with DAA and continue until 12 weeks after completion of DAA **OR** Monitor HBV DNA levels monthly during and for 3 months after DAA therapy; initiate anti-HBV therapy if: • HBV DNA increases >ten-fold above baseline • HBV DNA >1000 IU/mL if previously undetectable	ALT levels should be monitored at baseline, at end of treatment, and during follow-up Test HBV DNA and HBsAg if ALT levels increase or fail to normalize during or after DAA therapy
European Association for the Study of the Liver (EASL)	Initiate or continue anti-HBV therapy[a]	Anti-HBV prophylaxis should be considered concomitant with DAA therapy and until week 12 post-therapy, with monthly monitoring if anti-HBV therapy is stopped	ALT levels should be monitored monthly Test HBV DNA and HBsAg if ALT levels do not normalize or increase during or after DAA therapy
Asian Pacific Association for the Study of the liver (APASL)	Initiate or continue anti-HBV therapy[a]	Initiate prophylactic anti-HBV therapy concurrent with DAA and continue until 24 weeks after completion of therapy **OR** Close monitoring[a] during and through 24 weeks after DAA therapy	Recommend testing for HCV RNA, HBV DNA, and HBsAg if abnormal liver tests are observed[b] during DAA therapy and after end of therapy

[a]All patients with cirrhosis should be on anti-HBV therapy
[b]Frequency of monitoring not specified

9 Hepatitis D Virus Infection

9.1 Epidemiology

The prevalence of HDV coinfection varies geographically and within populations. An estimated 5–10% of HBsAg-positive persons are HDV infected (Stockdale et al. 2020b), translating into 10–15 million with HDV, though this figure is controversial due to a paucity of data from many countries, the variable definition of HDV infections used, and reliance on "at risk" cohorts to estimate general population rates. Additionally, since anti-HDV may disappear after resolved acute HDV infection, the true prevalence of HDV infection (resolved and current) across geographic areas and in subpopulations is unclear. Countries with the highest rates of HDV infection

include Taiwan, Pakistan, Mongolia, Italy, Turkey, the Amazon Basin, and Central Africa (Hughes et al. 2011; Stockdale et al. 2017). Within countries, the highest rates of HDV infection are reported among PWIDs, hemodialysis recipients, MSM, commercial sex workers, and those with HCV or HIV (Stockdale et al. 2020b).

HBV vaccination, especially targeting many of these higher risk groups, has reduced the prevalence of HDV in many countries over the past two decades; however, PWIDs and persons living with HIV continue to be a reservoir for HDV infection. For example, in Taiwan, the prevalence of HDV coinfection increased from 29.4% in 2001–2004 to 62.5% in 2009–2012 among injection drug users and from 38.5% to 89.8% among HIV-positive injection drug users, contrasting with rates of 1.9% and 4.1% in the general population of HBsAg-positive individuals (Lin et al. 2015). In a study from Peru conducted 23 years after the launch of their vaccination program, a decrease in the HBsAg carrier rate from 9.8% in 1991 to 1.2% in 2014 was seen and HDV seroprevalence decreased from 9% in 1990 to 5.2% in 2014, with no HDV under the age of 30 (Cabezas et al. 2020). In recent years, the impact of immigration, particularly in Europe, on HDV prevalence has resulted in HDV seroprevalence rates that have plateaued or increased, reflecting immigration from areas of high HBV endemicity. In Germany, the prevalence of anti-HDV antibody decreased from 18.6% in 1992 to 6.8% in 1997, but between 1998 and 2006 increased to between 8% and 14% (Wedemeyer et al. 2007) and the majority of cases were among immigrants from Turkey and Eastern Europe. Similarly, in a study from Italy of 794 HBsAg-positive patients from 9 tertiary centers from diverse regions, the overall seroprevalence decreased from 7.4% to 6.4% among Italians but increased from 12.2% to 26.4% among non-natives during 2001–2019 (Stroffolini et al. 2020). Such studies serve as a reminder of the changing global distribution of HDV infection.

10 Natural History of HBV and HDV Infection

HDV's requirement of the hepatitis B envelope protein to have complete and infectious virions is well established. Recently, in vitro cell culture experiments and animal models found that HDV can be enveloped by other enveloped viruses—specifically HCV and dengue virus (Perez-Vargas et al. 2019). The clinical relevance of this finding is unclear, with available studies suggesting a very low prevalence. A study from Germany of 323 HCV-infected, HBsAg-negative patients found none were HDV RNA positive but 8/316 (2.5%) were anti-HDV positive and all 8 patients were anti-HBc positive suggesting prior HBV-HDV infection with clearance (Pfluger et al. 2021). In a study of 160 HCV-infected, HBsAg-negative patients from clinical trials, 2 were anti-HDV positive (1.25%) with one also HDV RNA positive but neither had any markers of HBV infection (Chemin et al. 2020). More studies are needed to determine the clinical significance of HDV transmission with the help of these "nonconventional" enveloped viruses.

Acute hepatitis delta may occur in the setting of simultaneous HBV-HDV infection (coinfection) or as superinfection in person with chronic hepatitis B

(superinfection) (Fig. 15.2). Coinfection occurs much less frequently than superinfection, more frequently in the context of sexual transmission or injection drug use and is associated with a higher risk for acute liver failure (Smedile et al. 1982) and spontaneous resolution of HBV and HDV (Buti et al. 2011). In contrast, for the majority of patients with superinfection, chronic HDV will develop with a variable course of viremia. Cross-sectional studies report that HDV typically predominates—with more patients being HBeAg negative and with low or undetectable levels of HBV DNA compared to HBV-mono-infected patients (Mumtaz et al. 2011). Indeed, the typical clinical scenario in which HDV testing has been recommended is when ALT levels are elevated (suggesting active liver disease) and HBV DNA levels are undetectable. However, longitudinal studies reveal a more complex and dynamic picture. In a study of 25 HDV-infected patients followed with quantitative levels of HBV DNA, HDV RNA, and HBsAg over a period of 4 to 8 years (Schaper et al. 2010), replication profiles with fluctuating activity of one or both viruses and alternating periods of viral predominance were seen. Thus, there is a need for regular monitoring of HDV and HBV viral levels to guide treatment decisions.

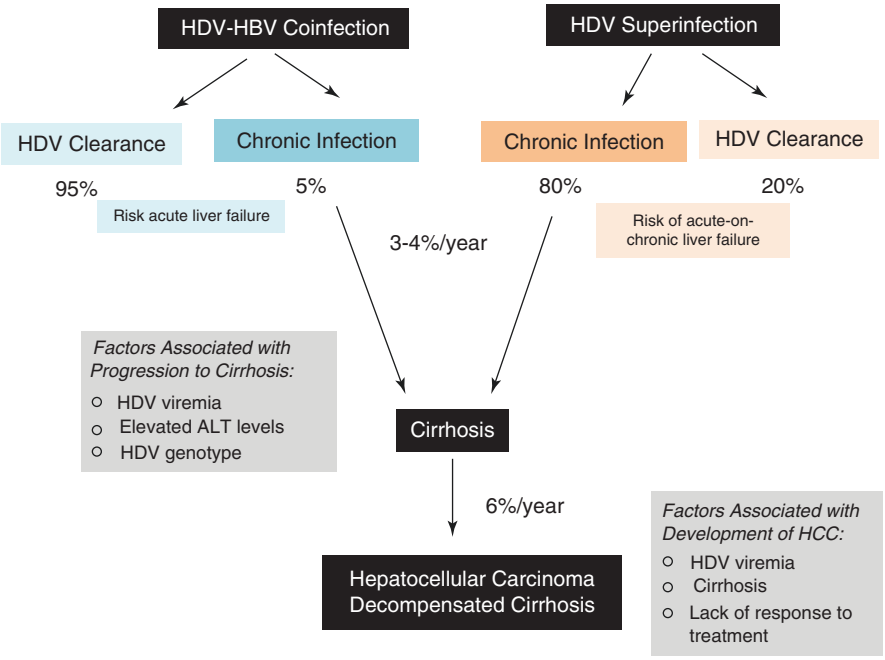

Fig. 15.2 The natural history of HDV superinfection and HDV coinfection. The likelihood of chronicity following acute infection differs if coinfection (HBV and HDV acquired simultaneously) or superinfection (HDV acquired in setting of CHB). Spontaneous resolution is higher with coinfection and superinfection. Both coinfection and superinfection can result in severe disease and manifest as acute liver failure in coinfection and acute-on-chronic liver failure in superinfection. For those with chronic HDV infection, the risk of cirrhosis and HCC is largely related to the presence of HDV viremia, cirrhosis (for HCC), and absence of response to therapy

Most studies report more rapid progression to cirrhosis and/or higher rates of cirrhosis and HCC among patients with HDV infection compared to those with HBV alone. In an Italian cohort with cirrhosis, the median age at presentation was 14 years younger in those with HDV versus HBV (Fattovich et al. 2000), and in another longitudinal study, the annual rate of progression from chronic hepatitis to cirrhosis was 4% per year (Romeo et al. 2009). HDV viremia and presence of cirrhosis are most frequently linked with adverse clinical outcomes (Wranke et al. 2020; Palom et al. 2020; Kamal et al. 2020). In a Swedish cohort, the incidence rate of liver-related events was higher in those with cirrhosis at entry and HDV RNA viremia was associated with a 3.8-fold higher risk for liver-related outcomes (Kamal et al. 2020). Among patients with HDV viremia without cirrhosis at cohort entry, the cumulative risk of cirrhosis or liver-related events was 18% and 36% after 5 and 10 years of follow-up (Kamal et al. 2020). In the French National Laboratory study, HDV viremia was associated with incident cirrhosis (HR 6.1, 95% CI 3.8–9.8) and liver decompensation (HR 2.6, 95% CI 1.4–4.6). In long-term follow-up of the Hep-Net-International-Delta-Hepatitis-Intervention-Study participants with cirrhosis, the rate of liver-related complications at 5 and 10 years was 30% and 65%, respectively (Wranke et al. 2020). Genotype-specific differences in natural history are sparse, but genotype 3 has been associated with acute liver failure (Casey et al. 1996), and African genotypes 5–8 associated with lower risk of cirrhosis (Roulot et al. 2020).

Most retrospective and cohort studies suggest that HDV coinfection is associated with an increased risk of HCC compared to HBV mono-infection. In a case-control study from Gambia, the odds of HCC were 30-fold and eight-fold higher for HDV- and HBV-infected patients, respectively, compared to uninfected after adjustment for covariates (including alcohol, anti-HCV, and aflatoxin B1 exposure) (Mahale et al. 2019).Viremia has been linked with HCC risk but a specific threshold of HDV RNA is unknown, and the oncogenic link is likely due to higher levels of inflammation and fibrosis, with earlier onset of cirrhosis (Puigvehi et al. 2019). In a systematic review and meta-analysis of 93 studies (68 case-control studies including 22,862 patients and 25 cohort studies including 75,427 patients), patients with HDV had a significantly increased risk of HCC compared to HBV-mono-infected patients (pooled odds ratio 1.28; 95% CI 1.05–1.57) but with substantial study heterogeneity (Alfaiate et al. 2020). A stronger association was seen in prospective cohorts (pooled odds ratio 2.77; 95% CI 1.79–4.28) and studies with HIV-infected patients (pooled odds ratio 7.13; 95% CI 2.83–17.92) and with less study heterogeneity.

11 Diagnosis and Screening

The European Association for the Study of the Liver and the Asian-Pacific Association for the Study of the Liver recommend routine screening for HDV infection in all HBsAg-positive persons (Sarin et al. 2016; European Association for the Study of the Liver 2012). The American Association for the Study of Liver Diseases recommends testing in select patient groups (Terrault et al. 2016), including PWIDs, HIV-infected and MSMs, immigrants from areas of high HDV endemicity, as well

as HBsAg-positive patients with low or undetectable HBV DNA levels but high ALT levels. The rate of HDV screening in clinical practice appears to be highly variable and generally underutilized (Kushner et al. 2015; El Bouzidi et al. 2015; Safaie et al. 2018). Given the importance of the diagnosis of HDV to the long-term management of HBV, universal screening of HBsAg-positive patients would seem appropriate in most clinical settings (Terrault and Ghany 2020).

The recommended screening test is the antibody to the Delta antigen (anti-HDV). In the appropriate setting (i.e., acute hepatitis), testing for anti-HBc IgM will assist in differentiating coinfection from superinfection, the latter confirmed with positive anti-HDV with negative anti-HBc IgM. Early studies of HDV infection among adults highlighted the typical serologic evolution among symptomatic HDV infection. In acute HDV infection, antigenemia was typically brief and followed by anti-HDV IgM (median 5 days after presentation) followed by or overlapping with anti-HDV IgG detection (median 10 days up to as long as 90 days) (Aragona et al. 1987). Importantly, a gap between HDV antigenemia, anti-HDV IgM, and IgG development was evident in a substantial proportion of patients, possibly reflecting the sensitivity of the assays used but highlighting the need to consider repeat testing in the appropriate clinical setting (Aragona et al. 1987).

For anti-HDV-positive patients, HDV RNA testing is needed to determine whether the antibody positivity reflects active HDV viremia. However, the performance characteristics of HDV RNA assays are variable as shown in a comparative study of HDV RNA sensitivity from 28 clinical labs in 17 countries worldwide; 16 laboratories (57.1%) were unable to confirm HDV RNA positivity in up to 10 of 20 clinical samples tested (Le Gal et al. 2016). Factors potentially influencing HDV RNA detection rates include method of RNA extraction (Bremer et al. 2019) and type of PCR assay used, and the distribution of HDV genotypes, with African HDV genotypes (i.e., HDV-1 and HDV-5 to −8) suboptimally quantified by some assays (Le Gal et al. 2016; Brichler et al. 2013). Newer assays are overcoming these limitations (Le Gal et al. 2017). As access to HDV RNA testing may be limited in some clinical settings, particularly in resource-limited regions, alternative means of defining patients with active HDV infection and/or those in need of treatment are needed. Anti-HDV IgM has a poor correlation with HDV RNA levels (Wranke et al. 2014), and HDVAg is transient during acute infection and not a useful marker of chronic infection (Shattock et al. 1989). A novel quantitative microarray antibody capture assay called Q-MAC anti-HDV has high positive predictive value for HDV viremia, with a fluorescence intensity value above 1.659 units identifying 100% of HDV RNA-positive samples allowing confirmation of infection and viremia with one test (Chen et al. 2017a).

12 Indications for Antiviral Therapy

As with any patient with CHB, persons infected with chronic hepatitis delta (CHD) require serial testing of ALT levels and viral levels over time to determine the need for treatment. For patients with elevated ALT levels, measurement of HBV DNA and HDV RNA will allow determination whether one or both viruses warrant

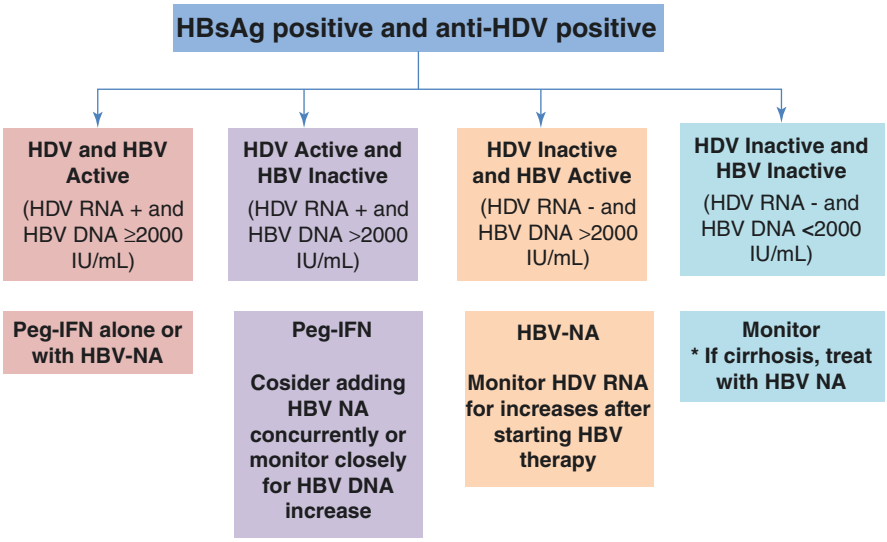

Fig. 15.3 Suggested treatment approach for chronic HBV (HBsAg positive) with HDV coinfection. Shown are the possible serologic/virologic profiles among coinfected patients and below each the suggested treatment approach. The general strategy is one of treating the "active" disease (ALT elevation)—using peg-IFN if HDV is active (HDV RNA detectable), NA if HBV is active (HBV DNA >2000 IU/mL), and both if HBV and HDV are active. The usual duration of Peg-IFN inclusive therapy is 12–18 months. *NA* nucleos(t)ide analogue; *Peg-IFN* peg-IFN

treatment. Treatment decisions should be directed by the virologic results (Fig. 15.3). For patients who do not meet criteria for treatment and have normal liver enzymes, monitoring ALT activity every 6 months and repeated HBV DNA and HDV RNA levels if ALT levels increase would be a reasonable and low-cost approach. The presence of underlying cirrhosis should modify treatment decisions, as is the case in HBV monotherapy. Treatment of HBV is indicated regardless of HBV DNA or ALT levels in persons with cirrhosis to insure sustained suppression of HBV and eliminate any contribution of HBV to HDV progression (Terrault et al. 2016). NAs have no efficacy against HDV infection and are not recommended in patients without cirrhosis who have suppressed or low (<2000 IU/mL) HBV DNA levels (Terrault et al. 2016). As HBV DNA levels may change over time, including during treatment of HDV infection, regular monitoring of HBV DNA is needed among HDV patients.

13 Interferon Therapy

Interferon (IFN) is the only antiviral therapy shown to have any efficacy in HDV infection, but efficacy is modest and there are significant side effects and a lack of applicability to patients with more advanced stages of cirrhosis. Standard interferon, with doses ranging from 3MU thrice weekly to 5MU daily, has been replaced by Peg-IFN as the drug of choice without differences in efficacy between Peg-IFN alpha-2a (180 ug

weekly) and 2b (1.5 ug/kg weekly) (Bahcecioglu et al. 2015; Gheorghe et al. 2011; Wedemeyer et al. 2011; Niro et al. 2006; Erhardt et al. 2006). Treatment success, defined by an undetectable HDV RNA level 24 weeks after completion of therapy, ranges from 17% to 43% with 1 year of treatment (Erhardt et al. 2006; Castelnau et al. 2006). Normalization of ALT levels parallels the virologic responses. The optimal duration of IFN therapy is unclear, but experts recommend treatment for 12–18 months initially (Sarin et al. 2016; Terrault et al. 2018b), with retreatment considered if virologic suppression is not maintained post-treatment. This strategy balances the patient's quality of life with the need to prevent liver disease progression. Adding NAs does not influence virologic response (Wedemeyer et al. 2011; Abbas et al. 2016; Yurdaydin et al. 2008). Factors predictive of a sustained virologic response (defined by undetectable HDV RNA 24 weeks after treatment end) are rate of HDV RNA decline and achievement of undetectable HDV RNA early in treatment (within first 24 weeks) (Castelnau et al. 2006; Abbas et al. 2014; Keskin et al. 2015). A less than 2-log copies/mL decline in HDV RNA after 24 weeks treatment is associated with only a 5% chance of achieving a sustained off-treatment response with continued peg-IFN (Keskin et al. 2015).

Late relapses occur leading to very low rates of HDV RNA undetectability with off-treatment follow-up period beyond 5 years. In the multicenter HIDIT-1 study of peg-IFNα2a for 48 weeks with or without adefovir, 40% of patients achieved an undetectable HDV RNA level 24 weeks after completing therapy, but at a mean follow-up 4.3 years later, only 12% were still undetectable (Heidrich et al. 2014). A more definitive endpoint for therapy, which rarely occurs with peg-IFN, is sustained suppression of HDV RNA plus loss of HBsAg. In one study of treatment of peg-IFN for up to 5 years, HBsAg loss occurred in 3 of 13 (23%) patients after 24, 37, and 202 weeks of treatment (Heller et al. 2014). In the HIDIT-1 long-term follow-up study (median 8.9 years) of 77 patients treated with peg-IFN, peg-IFN plus adefovir, or adefovir alone, loss of HBsAg occurred in 9% of patients and only among those who received peg-IFN (± adefovir) (Wranke et al. 2020).

IFN therapy has been associated with reduced rates of liver complications, including death. In a single-center study of 136 patients (39% with cirrhosis at entry), the rate of liver-related complications (decompensation, HCC, liver transplantation, or death) over a median follow-up of 5.2 years was four-fold higher in untreated and 2.2-fold higher in NA-treated HDV patients compared to IFN-treated patients (Wranke et al. 2017). The benefits of IFN on disease progression and clinical outcomes have been most closely associated with undetectability of HDV RNA during follow-up (Wranke et al. 2017). In a Spanish study with 8 years follow-up, 30% cirrhosis at baseline and 85% viremia, liver decompensation was reduced in IFN-treated patients (13% vs 38%, $p = 0.03$) (Palom et al. 2020).

14 New Therapies for HDV Infection

An enhanced knowledge of the HDV life cycle has paved the way for several new HDV therapeutics (Fig. 15.4). New HDV-specific drugs include inhibitors of viral entry, prenylation, and subviral particle production and release. Additionally,

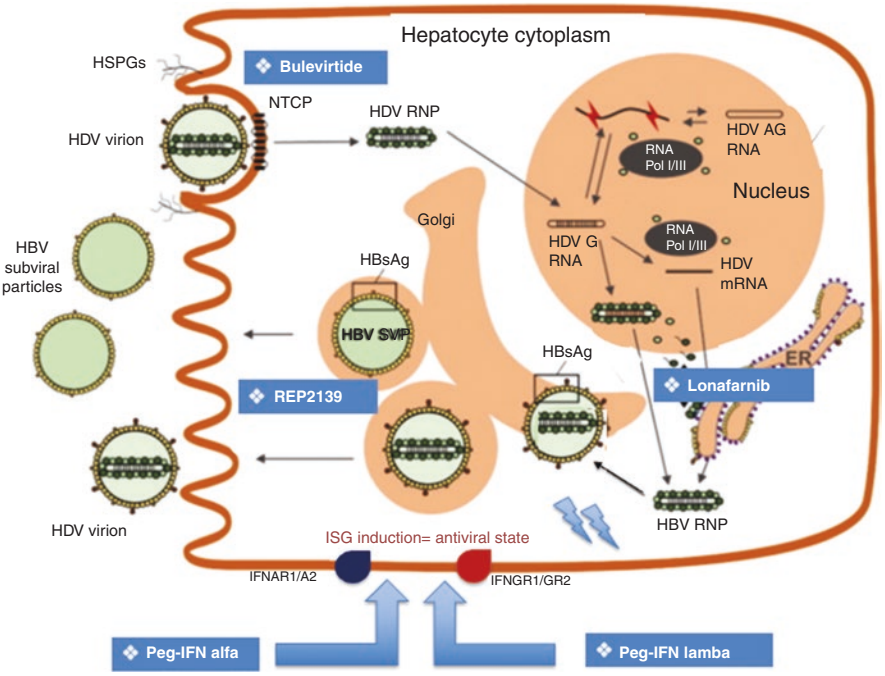

Fig. 15.4 HDV therapies target different aspects of the HDV and HBV life cyle. HDV entry is mediated by viral interaction with HSPGs initially, and then a specific interaction with the viral receptor, NTCP. This step is inhibited by bulevirtide. The viral RNP is then transported to the nucleus where it releases the viral genome that serves as template to transcription of HDV mRNA with replication mediated by cellular DNA-dependent RNA polymerases. Farnesylation of L-HDAg is inhibited by lonafarnib, a step necessary for viral assembly. HDV virions are thought to be secreted through the Golgi in parallel with HBV subviral particles and REP 2139 is believed to inhibit secretion of HBsAg and thus the intact HDV virions. Interferons (alpha and lambda acting via different cell receptors) lead to the induction of an antiviral state that reduced viral replication and likely cell-to-cell spread of virus. Not shown are drugs in development for HBV, such as the ribonucleic acid interfering drug, JNJ-73763989, which target HBV transcription including HBsAg. *ER, endoplasmic reticulum; G, genome; HBV, hepatitis B virus; HDV, hepatitis B virus; HSPGs, heparan sulfate proteoglycans; NTCP, sodium taurocholate co-receptor peptide; RNP, ribonucleoprotein; SVPs, subviral particles. Adapted with permission from Mentha* et al. *(*Mentha et al. 2019*)*

lambda interferon is being evaluated as an alternative antiviral-immunomodulatory to peg-IFNα. Additionally, new therapies for HBV focused on achievement of functional cure (Cornberg et al. 2020)—loss of HBsAg—will have the additional benefits of being effective therapies for HDV. For example, a phase 2 study of the ribonucleic acid interference (RNAi) drug, JNJ-73763989, which targets all the HBV transcripts including HBsAg, is being studied in patients with HDV (NCT04535544). Several of the HDV-specific therapies are in phase 3 study, and one (bulevirtide) has approval in the European Union, offering optimism for the millions affected by HDV infection, for whom effective and well-tolerated therapies have been lacking.

14.1 Prenylation Inhibitors

Prenylation of the HDV is an essential step in viral assembly. Specifically, the nascent HDV nucleoprotein complex is enveloped by hepatitis B surface antigen (HBsAg) within the hepatocyte cytoplasm, and this process involves the attachment of a 15-carbon prenyl group, farnesyl, to the large HDAg, a reaction catalyzed by farnesyl transferase. The small molecule prenylation inhibitor, lonafarnib, inhibits HDV viral assembly and release (Bordier et al. 2002). In an initial proof-of-concept study of oral lonafarnib for 28 days in 14 patients with CHD, the mean log HDV RNA decline from baseline was −0.73 log IU/mL and −1.54 log IU/mL in lonafarnib 100 and 200 mg twice daily groups, respectively, compared to −0.13 log IU/mL in placebo-treated patients (Koh et al. 2015). A subsequent dose optimization study called LOWR (lonafarnib with and without ritonavir) HDV-1 study included 20 patients with compensated liver disease treated with lonafarnib alone at higher doses (up to 300 mg twice daily), lower dose lonafarnib (100 mg twice daily) boosted with ritonavir, as a means of increasing hepatic levels of the drug while reducing systemic side effects, and lonafarnib (100–300 mg twice daily) with peg-IFNα2a 180 ug weekly for treatment periods of 8–12 weeks (Yurdaydin et al. 2018b). The combination of lonafarnib with low-dose ritonavir or peg-IFN achieved the best balance in terms of efficacy (decline in HDV RNA) and tolerability. In the subsequent LOWR HDV-2 study of 55 patients with CHD, lonafarnib at doses of 25 and 50 mg boosted with ritonavir 100 mg twice daily was best tolerated and triple therapy with Peg-IFNα achieved the highest efficacy, defined by >2-log decline of HDV RNA after 24 weeks, and the lower doses of ritonavir boosted with lonafarnib had substantially fewer gastrointestinal adverse events (Yurdaydin et al. 2018a). The phase 3 global study (NCT03719313) with a planned enrollment of 400 patients with CHD is underway comparing 48 weeks of ritonavir-boosted lonafarnib 50 mg twice daily (n = 175), ritonavir-boosted lonafarnib 50 mg twice daily plus peg-IFN alfa-2a (n = 125), peg-INF alfa-2a alone (n = 50), and placebo (n = 50), with the primary endpoint being a ≥ 2-log decline in HDV RNA with normalization of ALT at 24 weeks after end of treatment.

14.2 Entry Inhibitors

HDV initially binds with low affinity to hepatocyte heparin sulfate proteoglycans and then to the high-affinity human sodium/taurocholate cotransporting polypeptide (NTCP) and co-receptor, epidermal growth factor receptor (Herrscher et al. 2020). The first-in-class entry inhibitor, bulevirtide, is a synthetic lipopeptide of 47 amino acids of the HBV pre-surface (S1) protein that specifically binds to and competitively inhibits the NTCT receptor (Kang and Syed 2020) and in clinical trials was associated with reductions in HDV RNA (Bogomolov et al. 2016) and improved ALT levels. Bulevirtide at a recommended dose of 2 mg daily subcutaneously was approved in Europe for treatment of HDV in patients with compensated cirrhosis in July 2020 and can be used alone or in combination with NA or peg-IFN for “as long

as the patient benefits from it" (https://www.ema.europa.eu/en/medicines/human/EPAR/hepcludex#product-information-section n.d.). Approval was based on two phase 2 studies: MYR202 (NCT03546621) and MYR203 (NCT02888106). In MYR202, 90 patients were randomized to bulevirtide 2 mg ($n = 28$), 5 mg ($n = 32$), or 10 mg ($n = 30$) once daily in addition to TDF, or to TDF alone ($n = 28$) for 24 weeks. ALT normalization and HDV RNA declines ≥2 log IU/mL were more frequent in bulevirtide plus TDF (53.6% and 42.9%) than TDF only (3.6% and 7.1%) groups. Relapse of HDV RNA occurred in 60% after treatment end. In MYR203, 60 patients were randomized to bulevirtide 2 mg or 5 mg once daily plus PEG-IFNα-2a 180 μg weekly, bulevirtide 2 mg alone, or PEG-IFNα-2a alone for 48 weeks ($n = 15$ in each arm). At week 72 (24 weeks after stopping treatment), the proportion with undetectable HDV RNA was 53% in bulevirtide 2 mg + peg-IFN group, 27% bulevirtide 5 mg + peg-IFN, 7% bulevirtide 2 mg alone, and 0% in peg-IFN alone. ALT normalization at week 72 was also highest in the bulevirtide 2 mg + peg-IFN group (47%). The common side effects associated with bulevirtide are injection site rejections, dose-related increases in serum bile acids and mild neutropenia and thrombocytopenia that are reversible, and ALT flares occurring after discontinuation of bulevirtide. The increased serum bile acids are an anticipated side effect based on the drug's mechanism of action and without clinical consequences. Additional phase III (NCT03852719; MYR301) and phase IV studies (NCT04166266) are underway (Kang and Syed 2020).

Interestingly, other inhibitors of the NTCP receptor, such as ezetimibe, may be effective in reducing HDV RNA levels as suggested by a pilot study of 44 patients with CHD (mean HDV RNA level at baseline 5.4 log IU/ml) where a one-log reduction of HDV viral load was seen in 43% (18/42) of the patients who completed the 12 weeks of therapy (Abbas et al. 2020).

14.3 Subviral Particle Release Inhibitors

Another novel therapeutic approach is the use of highly negatively charged nucleic acid polymers that interfere with subviral particle assembly and secretion leading to reduced HDV virion production, the proposed primary mechanism leading to reduction in HDV RNA with REP-2139 therapy (Shekhtman et al. 2020). In a single-arm study of 12 patients with CHD (all genotype 1, median HDV RNA 6.6-log IU/mL, HBsAg greater than 1000 IU/mL), REP 2139 500 mg weekly at intravenous infusion for 15 weeks, followed by 250 mg REP 2139 weekly in combination with peg-IFNα-2a 180 ug once per week for 15 weeks, and then peg-IFN 180 ug weekly for 33 weeks were given. The mean reduction in serum HDV RNA during REP 2139 monotherapy was 4.2 log IU/mL with 9 of 12 (75%) of patients being HDV RNA negative at end of treatment, of whom 5 were also HBsAg negative. Adverse events occurred in 100% of treated patients, with pyrexia, chills, thrombocytopenia, and leukopenia the most frequent adverse events during the REP-2139 monotherapy phase. ALT or AST elevations were seen in the majority of patients during the peg-IFN phases, with one patient requiring early discontinuation due to ALT and

bilirubin elevation (Bazinet et al. 2017). Another phase 2 study in CHB of REP-2139 as subcutaneous formulation combined with TDF and peg-IFN is being planned (http://replicor.com/pipeline/ 2021).

14.4 Interferon Lambda

Interferon lambda is a novel type III interferon that binds to a receptor unique from type I interferons (e.g., peg-IFNα) and is highly expressed on hepatocytes but has limited expression on hematopoietic and other cell types, thereby yielding a drug with an improved side effect profile compared to typical alpha interferons. Previously studied with patients with HBV and HCV infection, this interferon has shown promising preliminary results in patients with CHD. In the LIMT HDV study, 33 patients (baseline HDV RNA 4.4 log-IU/mL) were randomized to lambda interferon 120 versus 180 ug weekly for 48 weeks with 24 weeks follow-up (Hamid et al. 2017). Initial HDV RNA levels after 24 weeks treatment showed 50% with a 2-log or greater decline in HDV RNA (40% undetectable). Adverse events and treatment discontinuations were seen in 15%; 12% experienced an ALT flare and 9% hyperbilirubinemia during treatment. Interference with bilirubin transporter molecules or immune-related genes with viral interactions within the hepatocytes are the proposed reason for the ALT and bilirubin elevations, but additional studies are needed. A phase 2 study of 33 CHD patients of ritonavir-boosted lonafarnib plus lambda interferon for 24 weeks is underway (NCT03600714).

15 Triple Infections with Chronic HBV

15.1 Epidemiology and Natural History

Epidemiologic data on the frequency of triple infections (i.e., HBV-HIV-HDV, HBV-HIV-HCV, or HBV-HCV-HDV) are limited. They are most frequently reported in high to intermediate endemic regions, especially in high-risk individuals, i.e., PWIDs and MSM (Zhou et al. 2012; Bagheri Amiri et al. 2016; Hung et al. 2014). Country-specific estimates of triple infections with HBV, HCV, and HDV are available from Mongolia, India, Taiwan, China, and Pakistan, with the highest rates reported from Mongolia. Among 179 Mongols with CHB tested in 2004, 35% were triply infected with HBV-HCV-HDV (Tsatsralt-Od et al. 2005). In contrast, a recent study from 2020 of Mongols living in the USA showed only 8.6% had evidence of triple infection (Fong et al. 2020). In two studies from Pakistan of outpatient liver clinics, among patients with CHB, triple infection (HBV-HCV-HCV) was identified in 4/76 (5.2%) (Baig et al. 2009) and 29/246 (11.8%) (Zuberi et al. 2008). In Taiwan, the prevalence of HBV-HCV-HDV coinfection was 5.2% (Lu et al. 2003). Although data are not abundant, higher rates of elevated ALT levels, cirrhosis, and HCC are reported among CHB-infected persons with triple infection (HBV-HCV-HDV) compared to those with mono or dual infections (Tsatsralt-Od et al. 2005; Jardi

et al. 2001). Viral interactions are likely. In a longitudinal study from Taiwan (Lu et al. 2003), HCV acted as the dominant factor in triple viral-infected individuals (Lu et al. 2003). This differs from an Italian cohort, in which HDV infection was the dominant virus in triple coinfection, with HDV having a greater influence on HCV than on HBV replication (Jardi et al. 2001).

Among persons living with HIV and CHB, triple infections are reported. A Serbian HIV cohort identified triple infections of HBV-HIV-HCV in 1.7% of patients (Ranin et al. 2018). In the Swiss HIV Cohort study, HIV-HBV-HDV infection was associated with 2.3-fold increased mortality: 7.7-fold for liver-related death and 9.3-fold for HCC (Beguelin et al. 2017). Similarly, triple infection with HIV-HBV-HCV conferred the highest risk of death in the British Columbia Hepatitis Testers Cohort (higher than both coinfected and mono-infected individuals), independent of high rates of substance use and social factors that are also key contributors to increased mortality in this group (Butt et al. 2020a). Therefore, in combination with harm reduction strategies, treatment of coinfections to reduce liver-related complications is a priority in individuals with multiple coinfections.

15.2 Approach to Treatment

Peg-IFN has activity against HBV, HDV, and HCV, and older literature focuses on the use of peg-IFN with ribavirin as a strategy to treat all viruses simultaneously (Hartl et al. 2012). However, with the availability of safe and highly effective DAAs for HCV, the current strategy would be to eradicate HCV and then treat HBV and HDV as defined by level of each viral activity (Fig. 15.3).

16 Liver Transplantation for Patient with Chronic HBV and Coinfections

The outcomes of liver transplantation (LT) among patients with chronic HBV are dependent upon the prevention and control of HBV infection post-LT. Over the past several decades, prophylactic strategies have evolved to yield high rates of success, such that CHB patients undergoing LT recipients typically are HBsAg negative and HBV free post-LT on lifelong prophylactic therapy (Fox and Terrault 2011). The presence of HCV, HDV, and/or HIV coinfection requires additional considerations for prophylactic therapy in the LT setting (Table 15.3).

Prior to the availability of DAAs, the posttransplant outcomes of patients coinfected with HBV and HCV were determined by the natural history of recurrence of HCV, as HBV recurrence was prevented by the use of prophylactic therapies. The most dreaded complication was severe cholestatic hepatitis, which led to rapid graft loss in the absence of effective HCV therapies. However, with the advent of DAA therapies, the management of HBV-HCV-coinfected transplant patients parallels those of recipients with HBV and HCV infection alone. HBV prophylaxis using NAs with or without hepatitis B immunoglobulin (HBIG) is used to manage HBV

Table 15.3 Management of coinfections in the setting of liver transplantation

Pre-transplant status	Prior to transplant antivirals	Perioperative period	Post-LT therapy
HBV + HCV	Entecavir, TDF, TAF HCV DAAs may be considered	Continue NA Individualize HBIG: Add if high HBV DNA	NA for HBV long term (± HBIG for limited duration) DAA for HCV, initiated within first 6 months post-LT
HBV + HDV	Entecavir, TDF, TAF	HBIG + NA	HBIG + NA long term, targeting anti-HBs titer of ≥100 IU/mL early post-LT, then >10 IU/mL
HBV + HIV	Entecavir, TDF, TAF	HBIG + NA	HBIG + NA long term, targeting anti-HBs titer of ≥10 IU/mL
HBV + HDV + HCV	Entecavir, TDF, TAF	HBIG + NA	HBIG + NA long term, targeting anti-HBs titer of ≥100 IU/mL early post-LT, then >10 IU/mL

and DAAs are used either prior to or post-LT to treat HCV. If DAA therapy is used post-LT (Terrault et al. 2017), it is recommended that treatment be started prior to the development of recurrent HCV disease and with close monitoring for immunologic consequences related to HCV eradication. As all post-LT recipients are on HBV prophylaxis, no additional monitoring of HBV status is needed during DAA therapy.

Historically, prior to the use of prophylactic therapies, HDV patients were shown to have a better prognosis after LT than patients with HBV infection alone (Samuel et al. 1993). This was presumably due to the lower HBV DNA levels present pre-LT in patients with HDV coinfection. However, recurrent HDV infection leading to graft loss occurs (Zignego et al. 1993); thus, prophylaxis for HBV infection is needed to prevent both HBV and HDV infections (Baskiran et al. 2020). Prevention of HBV recurrence effectively prevents HDV recurrence. Prophylactic therapies for LT recipients with HDV coinfection emphasize the use of long-term HBIG plus NA to prevent reappearance of HBsAg post-LT (Terrault et al. 2018b; Fox and Terrault 2011). Dosing of HBIG to achieve trough anti-HBs titers of ≥100 IU/mL during the early post-LT period (first 3–6 months) is a reasonable strategy (De Simone et al. 2016), with lower trough levels acceptable during the long-term maintenance period. As HBV recurrence is universal in LT recipients receiving HBsAg-positive grafts, such grafts are not recommended for use in recipients with HDV infection due to high risk of HDV recurrence (Franchello et al. 2005). New HDV-specific therapies may change the treatment paradigm for LT recipients in the future.

HIV was historically considered a contraindication for LT due to concerns of immunosuppression and accelerated HIV disease, especially in the era prior to the availability of highly active ART. However, several large cohort studies have demonstrated acceptable posttransplant outcomes with no increased risk of HIV

complications compared to HBV-mono-infected patients undergoing LT for HBV infection (Coffin et al. 2010; Tateo et al. 2009) when effective HBV prophylaxis is provided (Anadol et al. 2012). Thus, HBV-HIV-coinfected individuals with complications of cirrhosis should be offered LT as a potentially life-saving and curative therapy. Indeed, among persons living with HIV who have undergone LT, those with CHB have the highest survival rates compared to those transplanted for indications other than CHB (Cooper et al. 2011). Prophylactic therapy with HBIG plus NA has been shown to be highly effective in preventing graft loss for HBV-HIV-coinfected patients (Terrault et al. 2018b; Roche et al. 2015). While NA monotherapy using entecavir and tenofovir is effective prophylaxis in HBV-mono-infected LT recipients (Fung et al. 2017), there is no reported experience of using NA monotherapy as initial prophylaxis in HBV-HIV-coinfected patients. Even in patients on dual prophylactic therapy with HBIG and NA, intermittent, low-level HBV viremia occurs, though not associated with HBsAg detection or ALT elevation (Coffin et al. 2010). Given concerns that there are limited treatment options available to those who develop recurrent HBV disease after LT and that recurrence can rapidly progress to graft loss if uncontrolled, the prudent approach is to use HBIG plus NAs long term.

17 Summary

All HBsAg-positive persons should be tested for HCV, HDV, and HIV so that these important coinfections are not missed. Coinfected persons typically have a more accelerated natural history and higher risk of cirrhosis and HCC than HBV-mono-infected patients, and monitoring for these complications is critical to preventing liver-related deaths. Treatment paradigms are more complex in coinfected patients with viral activity varying over time and in response to antiviral therapy. Long-term monitoring of viral activity is useful in guiding the timing of interventions.

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Management of Chronic Hepatitis B Virus Infection in Children and Pregnant Women

16

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Abstract

Chronic hepatitis B virus (HBV) infection is a substantial global health burden. Despite immunoprophylaxis and advancement in antiviral therapies in the past decades, there is still an enormous load of afflicted people, mostly adults born before the HBV universal vaccination program and children without timely vaccination or immunization failure. Like adults, infected children may experience hepatitis activity and chronic consequences without awareness and thus require screening, followed by close monitoring and proper treatment. As of 2021, most antivirals licensed for adults are approved for children (including pegylated interferon and tenofovir). HBV-infected pregnant women are a particular group that needs screening to identify and monitoring hepatic inflammation and viral loads during pregnancy and postpartum to implement appropriate management for maternal/fetal health and to prevent mother-to-infant transmission of HBV. The best practice and long-term impacts in children and pregnant women/

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offspring are still evolving. To reach the goal of eliminating viral hepatitis of the World Health Assembly by 2030, infant vaccination should be aided with maternal late pregnancy prophylaxis. Screening, monitoring, antiviral therapy, and surveillance of cirrhosis and hepatocellular carcinoma are the secondary strategies toward the global elimination of chronic hepatitis B. They should include children and pregnant women as a whole.

Keywords

Hepatitis B vaccines · Vertical transmission · Mother-to-infant transmission Nucleos(t)ide analogues · Pegylated interferons

1 Introduction

Chronic hepatitis B virus (HBV) infection affects more than 257 million people worldwide. It may cause hepatic necroinflammation and fibrosis, marching into cirrhosis, hepatocellular carcinoma, and death in 15–40% of infected patients (Lok 2002; WHO 2020a). Most of these infections developed before the launch of hepatitis B vaccination. Since the implementation of the world's first universal infant vaccination program in Taiwan in 1984, the hepatitis B vaccine (HepB) for infants had been introduced nationwide in 189 countries by the end of 2019. Global coverage with three-dose HepB was estimated at 85%, and 109 countries (43%) introduced the birth dose to newborns and up to 84% in the Western Pacific Region (WHO 2020b; 2016). HepB has achieved 62–100% effectiveness in preventing hepatitis B surface antigen (HBsAg) carriage, especially in hyperendemic areas like Africa and Asia-Pacific regions (Harpaz et al. 2000; Mendy et al. 2013; Chen 2009; Ni et al. 2016). However, around 10% of infants from hepatitis B e antigen (HBeAg)-positive or high viral load carrier mothers still get infection despite passive-active immunoprophylaxis in infancy (Chen et al. 2012; Wen et al. 2013).

Through the advancement of antiviral therapy (AVT) in the past three decades and screening campaigns promoted by liver associations/health organizations, better management of chronic HBV-infected patients can be applied toward a goal to reduce detrimental outcomes. Leading international liver associations have established guidelines for managing chronic HBV infection and updated the recommendations according to new evidence. These guidelines are mostly based on prospective studies or randomized clinical trials aiming at adult populations, the major disease-ridden groups. Although HBV-infected children are usually experiencing an immune-tolerant course with mild inflammation, a small proportion indeed develops liver cancer even in the vaccination era (0.27 to $0.48/10^5$ person-year, 6–19 years of age, vaccinated birth cohort) (Chang et al. 2016). However, therapeutic guidelines in pediatric patients are limited due to less approved antivirals and long-term follow-up. Due to vulnerability and small numbers of pediatric patients, clinical trials of antivirals that have been licensed for adults usually take a longer time in

board reviewing and participant recruitment. Pediatric hepatologists often need to treat patients with hepatitis activity according to experiences extrapolated from adults' series or experts' opinions.

Another particular group is pregnant women with hepatitis B carriage, who may experience active hepatitis and need treatment or be asymptomatic but transmit HBV to their babies; the latter would contribute to the HBV reservoir across generations. The proper management of these two unique groups constitutes essential jigsaws in the global elimination of hepatitis B.

2 Management of Children with Chronic Hepatitis B

2.1 Identifying Infected Children

2.1.1 Postvaccination Serologic testing in High-Risk Infants

Identification of the infected persons is the first step to monitor or treat them. For children born after the launch of universal hepatitis B vaccination, the most critical risk factors of breakthrough infection are maternal HBsAg carriage and incomplete immunization. Currently, there are four main strategies to interrupt mother-to-infant transmission of HBV:

1. Universal maternal screening of HBsAg→universal neonatal HepB with additional hepatitis B immunoglobulin (HBIG) to infants of HBsAg-positive mothers (the United States, Italy, Korea, Japan 2015, etc.); HBV DNA testing in HBsAg-positive mother to implement antivirals in high viral load mothers (the United States).
2. Universal maternal screening of HBsAg and HBeAg→universal neonatal vaccine and additional HBIG to infants of HBsAg-positive mothers (in Taiwan since Jul 2019, Singapore since 2006, expanded from HBIG to infants of dual HBsAg- and HBeAg-positive mothers). Maternal HBeAg-positivity may serve for HBV DNA testing and maternal antiviral therapy (Taiwan 2018).
3. No maternal screening→universal neonatal vaccine (most middle income and developing countries).
4. Universal maternal screening of HBsAg→selective neonatal active and passive immunization only to infants of carrier mothers (the United Kingdom, Denmark, etc.) (Chen et al. 2012; Owens et al. 2019; Erika Duffell et al. 2020; Tanaka et al. 2019; Cho et al. 2017).

Different strategies would affect infant immunization policy and surveillance target on children at risk of infection. Serologic testing of HBsAg/anti-HBs in infants of carrier mothers at 9–12 months of age or one to two months after the last dose of vaccine is recommended and adopted by many countries (United States, United Kingdom, Taiwan, etc.) (Schillie et al. 2015a; O'Flanagan et al. 2009). This step is critical in understanding the global chronic HBV infection burden in the next generations and whether the elimination of HBV is achieved through universal immunization in the long run.

2.1.2 Screening Immune-Compromised Patients

Another risk group that may acquire hepatitis B infection even after vaccination is immune-compromised patients. Vaccine-induced immune memory may last for more than 20–30 years, and a booster dose is not suggested in immune-competent individuals with waning anti-HBs < 10 mIU/mL (West and Calandra 1996; Leuridan and Van Damme 2011; Bruce et al. 2016). In immune-compromised patients, HBV serology [anti-HBs, HBsAg, antibody to core antigen (anti-HBc)] checkups are recommended, and vaccine booster is advised if anti-HBs < 10 mIU/mL. In hematopoietic stem cell transplant or organ transplant, the donors' serology should also be tested, especially in high endemic areas (Hui et al. 2005). In liver transplantation from anti-HBc-positive donors, the recipients' anti-HBs should be boosted to >200 mIU/mL (or to >1000 mIU/mL if possible) to prevent de novo hepatitis B infection (Su et al. 2009; Lin et al. 2015). Close monitoring of liver biochemistry profiles and hepatitis B markers in potential occult infected immune-compromised (e.g., HBsAg-negative/anti-HBc-positive, or high titer anti-HBs without previous vaccine booster (Shahmoradi et al. 2012; Liu et al. 2006b; Hsu et al. 2014)) is mandatory to apply preemptive or therapeutic antivirals (Hui et al. 2006; Uhm et al. 2007).

2.1.3 Other Risk Groups

Household contacts or sexual partners of HBsAg carriers, injection drug users, or men who have sex with men are usually protected by complete primary vaccination if immunity is not impaired (Leuridan and Van Damme 2011).

2.2 Antiviral Therapy in Children with Chronic Hepatitis B Virus Infection

2.2.1 Natural History of CHB Infection and its Clinical Implication in Children

Most children with chronic hepatitis B (CHB) are infected at the neonatal period (mother-to-child transmission, the main transmission route in Asia) or before 6 years of age (horizontal infection, the primary transmission route in Africa). They have a 90% and ~ 30% probability of chronicity, respectively (Hyams 1995). Most carrier children are in the immune-tolerant phase, characterized by normal levels of aminotransferases, minimal liver histology, positive HBeAg, and high HBV DNA levels. Mechanisms of immune tolerance have been elucidated from clinical and experimental studies. Maternal transplacental HBeAg may induce in utero Th-cell tolerance and guarantee persistent perinatal infection (Milich et al. 1990; Hsu et al. 1992a), while secreted HBeAg in chronic stage may function as an age-dependent immunoregulator, tolerogenic or anti-inflammatory, in neonate and children (Milich and Liang 2003). Low expression of TAP1 (transporter associated with antigen processing gene 1) and LMP2 (low-molecular weight proteins 2) plays a pivotal role in defective viral antigen processing and presentation, resulting in immunotolerance (Sukriti et al. 2010).

After a variable period (generally lasting 10–30 years) of immune tolerance, the breakpoint commences, and immune clearance begins with an elevation of aminotransferases, HBV DNA fluctuation, and necroinflammatory activity in the liver histology. If the immune action is appropriate, alanine aminotransferase (ALT) level returns to normal, HBV DNA level decreases, and HBeAg loses with the development of anti-HBe (i.e., e-seroconversion), indicating a transition into the inactive carrier or low replicative phase.

Several host and viral factors affect the initiation of immune clearance: HLA class I and class II types, cytokine IL-10 and IL-12 polymorphism and phenotype, Toll-like receptors 4 and 5 polymorphism, early onset of puberty or menarche, and genotypes B and A (versus genotype C and D) are correlated with earlier or higher rate/sustenance of HBeAg seroconversion (Wu et al. 2006; 2010a, b, 2012a, 2014; Kao et al. 2004; Sánchez-Tapias et al. 2002).

Spontaneous clearance of HBeAg in children is uncommon in comparison with adults. The expected annual rates of e-seroconversion in genotype B and C infections were 15.5% and 7.9%, respectively, in HBeAg-positive adults followed up for 52 months (Kao et al. 2004). In an Alaska adult cohort, the age of the 50th percentile of persons at the time of HBeAg clearance by genotype was as follows: A, 19.4; B, 19.5; C, 47.8; D, 18.0; and F, 16.1 (Livingston et al. 2007). In contrast, annual HBeAg clearance rates were < 2% among children under three-years old, while around 5% among older ones (Chang et al. 1989). Spontaneous HBeAg seroconversion generally takes 2–7 years to happen and mostly asymptomatic in the seroconverters. Unless the children are identified by risk screening policy or symptomatic and regularly monitored, the elevation of ALT would be unnoticed. The appropriate cutoff ALT levels for chronic liver disease in children are reevaluated in the SAFETY (Screening ALT for Elevation in Today's Youth) study, considering only healthy weight, metabolically regular, liver disease-free pediatric participants in the United States. The 95th percentile levels for ALT for boys and girls are 25.8 and 22.1 U/L, respectively, much lower than the usual laboratory cutoff (40 U/L for males and 35 U/L for females) (Schwimmer et al. 2010). Serial monitoring of ALT levels in a group of HBeAg-positive children also disclosed the predictable span of 8.35, 5.14, 4.25, 3.95, and 2.80 years after the ALT levels over 20, 30, 40, 60, and 150 U/L, respectively, to achieve HBeAg clearance. Therefore, ALT >30 U/L may serve as a more suitable cutoff in children to detect the beginning of the immune clearance phase and guide AVT in real practice or clinical trials (Wu et al. 2012b).

HBeAg seroconversion is regarded as a turning point in chronic hepatitis B, indicating a breakdown of host immune tolerance and viral replication, which is usually considered as an advantage to the host and set as one of the endpoints in AVT (Liaw 2009). Most cases after HBeAg seroconversion will continue to stay in inactive status. However, those who experience multiple acute exacerbations, fail to sustain HBeAg clearance, encounter HBeAg seroreversion, or HBeAg-negative hepatitis may progress from perpetual hepatic inflammation/fibrosis to cirrhosis or even hepatocellular carcinoma (HCC). Two Italian pediatric series with longitudinal observation for 24 and 29 years, respectively, found 1.7 and 4.5% had cirrhosis, and two cirrhotic children in the latter series had HCC 9 and 16 years after HBeAg

seroconversion, one of them even with HBsAg clearance (Bortolotti et al. 2006; Iorio et al. 2007). Besides, early HBeAg seroconversion not necessarily designates a benign course with nearly half (14/30 liver biopsy at anti-HBe-positive stage) showing various degrees of fibrosis/cirrhosis and an early converter before three-years old developed HCC at eleven (Chang et al. 1995). After universal vaccination for more than 30 years in Taiwan, the HCC incidence in vaccinees 6–26 years of age has reduced more than 70 percent (0.11 ~ 0.41 per 10^5 person-year; relative risk 0.24 compared to pre-vaccination birth cohort). However, HCC preventive failure still exists, mainly in carriers born to HBeAg-positive carrier mothers (Chang et al. 2016). Infected children need continuous monitoring for hepatitis severity, viral activity, and CHB complication while they grow into adulthood.

Spontaneous HBsAg clearance in chronic hepatitis B is a rare event with an annual incidence of about 0.12%–2.38% in Asian countries and 0.54 to 1.98% in Western countries (Chu and Liaw 2010). The mean age of clearance in Taiwanese CHB patients enrolled at 16 to 76 is 47.8 ± 9.6 years of age (Chu and Liaw 2007). None of the cases less than 20 years of age had HBsAg clearance in a cohort of HBsAg carriers living in Okinawa (Furusyo et al. 1999). Hsu et al. found an average HBsAg loss rate of 0.6%/year in carrier children enrolled at one month to 17 years old and followed for 1–12 years in Taiwan (Hsu et al. 1992b). The higher HBsAg clearance rate was associated with HBeAg negativity (inactive carriers) (Furusyo et al. 1999; Hsu et al. 1992b) and older age at enrollment (Chu and Liaw 2007; Hsu et al. 1992b; Kato et al. 2000), HBsAg-negative mother (non-perinatal infection) (Hsu et al. 1992b), genotype C (Tseng et al. 2015), and more severe histology change or ALT elevation when they were HBeAg positive (Hsu et al. 1992b; Tseng et al. 2015). Although HBsAg loss or seroconversion usually indicates a better prognosis, some may still possess HBV DNA in serum (0–18%, (Hsu et al. 1992b; Kato et al. 2000)) or liver (91%, (Fong et al. 1993)). HCC risk is reduced but still present with an incidence of 36.8 (HBsAg loss) vs. 195.7 (HBsAg persistence) per 100,000 person-year (Hsu et al. 1992b; Kato et al. 2000; Liaw et al. 1991; Simonetti et al. 2010).

2.2.2 Current Treatment for Children with Chronic Hepatitis B (Table 16.1)

The ideal therapy for chronic HBV infection would eradicate the virus with HBsAg seroconversion or loss and undetectable viral DNA without substantial liver injury. Currently, there is no cure for chronic HBV infection. The treatment goal is to suppress disease activity and avoid the morbidity and mortality of cirrhosis or HCC. At present, the decision to start AVT in children is dependent on ALT elevation, HBeAg positivity, HBV DNA levels, the severity of liver disease by histology or noninvasive method, and family history of HCC. There are two main antiviral treatment regimens, interferon (IFN)-based (conventional or pegylated, pegIFN) and nucleos(t)ide analogs (NUCs). As of 2021, more antivirals were approved for children with CHB, including conventional IFN-α (≥1 year old), pegIFNα-2a (≥3 years old), lamivudine (LAM) (≥3 years old), adefovir dipivoxil (ADV) (≥12 years old), telbivudine (LdT) (≥16 years old), tenofovir disoproxil fumarate

Table 16.1 Clinical trials of antiviral therapies in children with chronic hepatitis B

Intervention	Age No	ALT normalization	HBeAg loss/ seroconversion	HBV DNA suppression	HBsAg loss	Predictors in responders
IFN-α (FDA approved in 1997 for children ≥ 1 year old)						
Ruiz-Moreno et al. (1991), (10 or 5 MU tiw 24 weeks, FU 15 months)	1.5–15.5y $N = 36$ $I_{10} = 12$ $I_5 = 12$ C = 12	42%	46 vs. - % / 46 vs. -%	50 vs.17%	—	—
Utili et al.1991, (3 MU tiw 12 months, FU 6 months)	6–14 y $N = 20$ I = 10 C = 10	30 vs.10%	20 vs.10%/ 20 vs.10%	30 vs.10%	—	Higher baseline ALT and histological activity
Lai et al. (1991), (5 MU/m^2 tiw for 16 weeks FU 24 months)	2-17 y $N = 90$ I = 29, P + I = 31 C = 30	only one with abnormal ALT at baseline	13 (P + I), 3 (I) vs. 0% / 13 (P + I), 3 (I) vs.0%	16 (P + I), 7(I) vs. 0%	3 vs. 0%	—
Barbera et al. (1994), (7.5 or 3 MU/m^2 tiw 6 months, FU 18 months)	Mean 8y $N = 77$ $I_{7.5} = 21$ $I_3 = 19$ C = 37	41 vs. 32% (EOT)	26 vs. 14%/ —	36 vs.22%	—	Higher baseline ALT
Vajro et al. (1996), (10 MU/ m^2, tiw 1 year, FU 24 months)	7.8 ± 2.7y $N = 31$ I = 13 P + I = 9 C = 9	67 vs. 44%	61 vs.33%/ 61 vs.33%	61 vs.44%	19 vs. 0%	ALT>120 U/L, higher histology activity, DNA <175 pg/mL
Gregorio et al. (1996), (5 MU/m^2 tiw 12 weeks, FU 12-18 months)	2-16 y $N = 95$ I = 30 P + I = 34 C = 31	—	39 vs. 16%†/ 38 vs. 13%†	39 vs.26%	—	Baseline DNA <1000 pg/mL

(continued)

Table 16.1 (continued)

Intervention	Age No	ALT normalization	HBeAg loss/ seroconversion	HBV DNA suppression	HBsAg loss	Predictors in responders
Sokal et al. (1998), (6 MU/m^2 tiw 24 weeks, FU 24 weeks)	1-17y *N* = 149 I = 72 C = 77	11 vs.7%	26 vs. 11† (EOT) 33 vs. 11%†/ —	33 vs. 11%†	10 vs. 1%†	Female
Peginterferon α-2a (FDA approved in 2017 for children ≥ 3 years old)						
Wirth et al. (2018) (180 μg/1.73 m2 once-weekly 48 weeks, FU 24 weeks)	3- < 18 y *N* = 161 pIFN = 101 C = 50 AF = 10	52 vs. 12%†	25.7 vs. 6%†/ 25.7 vs. 6%†	<2000 IU/mL 28.7 vs. 2%† Undetectable 16.8 vs. 2%†	8.9 vs. 0%†	Age < 12y, Genotype B, C, Lower baseline DNA and HBeAg levels
Lamivudine (FDA approved in 2000 for children 2–17 years old)						
Jonas et al. (2002), (3 mg/kg once daily for 52 weeks, EOT)	2-17 y *N* = 288 L = 191 C = 97	55 vs.12%†	26 vs. 15%†/ 22 vs.13%	61 vs.16%†	2 vs. 0%	Baseline ALT>2x, higher histology activity, DNA < 800 meq/mL
Sokal et al. (2006) (24 months extension of the above trial, HBeAg(+) Tx, HBeAg(−) Ob	2-18 y *N* = 276 L3y = 134, C-L2y = 79 L1y-Ob = 49 C-Ob = 14	3y 39% 2y 54%	26 /26% 35 /34% L1y-Ob 85/84% Pl-Ob 100/ 100%	21 vs. 30% L1y-Ob 86% Pl-Ob 92%	2 vs. 1%	VR at 24 m YMDD+ 5% YMDD- 54%
Adefovir Dipivoxil (Approved in May 2010 for children 12 to < 18 years old)						
Jonas et al. (2008) (2-7 y 0.3 mg/kg; 7- < 12 0.25 mg/kg 12- < 18 y 10 mg; once daily for 48 weeks; EOT)	2– 6 y N = 35, 7 -11 y *N* = 55, 12 – 17 y *N* = 83 ADV = 118 C = 58	2-6 y 30 vs. 25% 7-11y 58 vs.16%† 12-17y 64 vs. 22%† Total 56 vs. 21%†	Total 16 vs. 5%	2-6 y 17 vs. 8% 7-11y 19 vs.0% 12-17y 29 vs. 0%† Total 23 vs. 2%†	0.9 vs. 0%	Baseline ALT >90 DNA ≤ 8.8 $\log_{10}$ copies/mL

Intervention	Age No	ALT normalization	HBeAg loss/ seroconversion	HBV DNA suppression	HBsAg loss	Predictors in responders
Entecavir (Approved in Mar 2014 for children 2 to < 18 years old)						
Jonas et al. (2016), (0.015 mg/kg up to 0.5 mg/d for minimal 48 weeks)	2-6 y $N = 41$ 6-12 y $N = 46$ 12- < 18y $N = 93$ ETV = 120 C = 60	68 vs. 23%†	24 vs.10%†	49 vs.3%†	1.7 vs. 1.7% (week 48) 5.8 vs. 0% (week96)	HBV DNA < 8 $\log_{10}$ IU/mL, Non-D genotype
Tenofovir disoproxil fumarate (FDA approved in 2010 for children 12 to < 18 years old, in 2018 for ≥ 2 years)						
Murray et al. (2012) (300 mg daily for 72 weeks, open-label 120 weeks)	12-18 y $N = 106$ TDF = 52 C = 54	77 vs.39% † (72 week)	21 vs. 15%	89 vs. 0%†	2 vs. 0%	—
clinicaltrials.gov / NCT01651403 (2020) (<17 kg, 8 mg/kg powder; >17 kg, 150, 200, 250 or 300 mg tablets, once daily, for 48 weeks)	2 to <12y $N = 89$ TDF = 60 C = 29	52 vs. 17%†	25 vs. 24%	77 vs. 7%†	3.3 vs. 3.4%	—

C: control, †: $p < 0.05$

Interferon: I_{10}: Interferon 10 MU, I_5: Interferon 5 MU, C: control, P + I: prednisolone priming + Interferon, $I_{7.5}$: Interferon 7.5MU, I_3: Interferon 3MU; EOT: end of treatment

Peginterferon α-2a: AF, advanced fibrosis

Lamivudine: L3y: Lamivudine for 3 years, C-L2y: Placebo control, then LAM for 2 years, L1y-Ob: LAM for one year then observation, C-Ob: Placebo then observation, VR: virological response

(TDF) (≥ 2 years old), tenofovir alafenamide (TAF) (≥12 years old), and entecavir (ETV) (≥ 2 years old). In adult series, IFN-based therapy holds the benefit of immune control, finite course; sustained off-therapy response (HBeAg seroconversion, low HBV DNA, and normal ALT) occurs 20% more often in treated than in controls (Wong et al. 1993). In those who achieved HBeAg seroconversion, 80% cleared HBsAg within a decade after therapy (Korenman et al. 1991). However, IFN needs injection and has more adverse effects (flu-like syndrome, cytopenia that needs dose reduction or interruption, hair loss, depression, etc.). PegIFN has replaced conventional IFN in adult patients due to a more convenient, once per week injection and may be prescribed to CHB children ≥three (Wirth et al. 2018). NUCs are given in the oral route and have good tolerance. In adult series, HBeAg seroconversion, undetectable HBV DNA, and normal ALT occur in 10–20% treated cases compared to about 6% in controls after 1 year of therapy (Dienstag 2008; Bedre et al. 2016). However, a prolonged or indefinite course of treatment is necessary to maintain virological control. Prolonged usage of NUCs with low genetic barriers takes a high risk of the emergence of drug-resistant mutations in adults. Consequently, ETV and TDF with infrequent resistant mutants emerging after prolonged use have mostly replaced LAM and ADV as the first-line therapy (Chang et al. 2010; Gordon et al. 2013).

Interferon

Interferon inhibits HBV replication by blocking specific steps in the pregenomic RNA-primed assembly of core particles (Hayashi and Koike 1989), suppression of cccDNA transcription by reduction of acetylated histone H3 lysine 9 (H3K9) and 27 (H3K27) in cccDNA minichromosomes (Liu et al. 2013), and enhancement of expression of HBsAg/preS2 on hepatocytes to augment immune recognition (Lau et al. 1991). Conventional interferon has been applied in children since the 1990s. Several clinical trials were conducted in Chinese or Caucasian children with positive HBeAg, normal or > 1.5x ULN ALT levels, high viral DNA with or without prednisolone priming. The doses ranged from 5 MU to 10 MU subcutaneous injection three times per week for a duration of 16 weeks to 1 year. At the end of treatment and after 6 to 24 months follow-up, HBeAg loss/seroconversion occurred in 7–61%, and HBV DNA clearance ranged from 10 to 61% in the treated groups, which were higher than the untreated group (0–33% and 0–44%, respectively). HBsAg loss occurred in 2–19% in the treated but 0- < 1% in the untreated. ALT normalization was 11–67% in treated but 7–44% in the untreated (Table 16.1) (Lai et al. 1991; Ruiz-Moreno et al. 1991; Utili et al. 1991; Gregorio et al. 1996; Vajro et al. 1996; Sokal et al. 1998). Lower baseline HBV DNA, higher baseline ALT, moderate-to-severe histology, female, and age less than five predicted a better treatment response (Utili et al. 1991; Barbera et al. 1994; Gregorio et al. 1996; Vajro et al. 1996; Sokal et al. 1998; Kobak et al. 2004). The treatment benefit was challenged by no significant difference in cumulative HBeAg or HBsAg seroconversion rates in long-term (5 to 7 years after stopping therapy) comparison between IFN-treated and untreated children with elevated ALT despite an accelerated course of 1–3 years in treated children (Bortolotti et al. 2000; Vo Thi Diem et al. 2005; Hsu

et al. 2008). Beneficial short- and long-term virological outcomes were noted only in those children with pretreatment HBV DNA < 2×10^8 copies/ml (Hsu et al. 2008). In summary, conventional IFN 5–10 M.U. three injections per week for 6–12 months in ALT elevated, HBeAg-positive children may accelerate HBeAg seroconversion without the hazard of resistance.

Pegylated Interferon

Pegylated Interferon (PegIFN) alfa-2a has surpassed conventional IFNs in adults with CHB because of once-weekly (vs. three times weekly) subcutaneous injection and excellent efficacy and safety profile. PEG-B-ACTIVE (YV25718, NCT01519960) was a randomized, controlled, open-label, multicenter, phase III study conducted at 37 sites globally. It included 151 patients of 3 to <18 years, HBeAg-positive, HBV DNA >2000 IU/mL, ALT >1× but ≤10 × ULN, without advanced fibrosis randomized (2:1) to 48 weeks of PegIFN alfa-2a ($N = 101$) or an untreated control group ($N = 50$). Most patients (64%) were male, 56% were Asian, and the mean age was 10.7 years (range, 3–17). HBeAg seroconversion after 24 weeks end of treatment was significantly higher in treated (OR, 5.43; $P = 0.0043$). A higher proportion of treated groups achieved HBsAg clearance, HBV DNA <2000 IU/mL, and ALT normalization. Higher treatment-induced HBeAg seroconversion was noticed in younger (<12 years) patients, those with genotypes B and C, and patients with lower HBV DNA, HBeAg, and HBsAg at baseline (Wirth et al. 2018). Based on this study's results, both the US FDA and the European Medicines Agency (EMA) approved PegIFN alfa-2a to treat pediatric patients from 3 to <18 years with CHB on Oct 13, 2017, and Nov 10, 2017, respectively.

Nucleos(T)Ide Analogs (NUCs)

Although NUCs are potent HBV polymerase inhibitors, due to the persistence of cccDNA in the nucleus, prolonged treatment to maintain viral suppression is necessary. Hence, polymerase-resistant mutants' emergence is a significant concern, especially low-genetic barrier first-generation agents.

Lamivudine A multicenter 52-week RCT showed 23% of treated children cleared HBV DNA and had HBeAg seroconversion compared to 13% of the placebo group. ALT>2 times ULN, higher histology activity, and lower HBV DNA loads at baseline predict the virological response (Jonas et al. 2002). Subsequent 24-month open-label extension trial showed durability of virological response in around 90% of responsive cases. The prevalence of resistant mutants during LAM therapy was 19%, 49%, and 64% in children treated for 52, 96, and 144 weeks, similar to the cumulative incidence of resistant mutants in adult (23%, 46%, and 55%) (Jonas et al. 2002; Sokal et al. 2006; Zoulim and Locarnini 2009). Virological response decreased to 5% in those with YMDD (tyrosine, methionine, aspartate, aspartate) mutants (Sokal et al. 2006). In a Korean study, 2-year treatment-induced 65% HBeAg seroconversion with 89% in those younger than 7 years old compared to 43% in older ones. Notably, 42% of cases younger than 7 years old also cleared HBsAg compared to none of those beyond seven (Choe et al. 2007). In summary,

LAM 3 mg/kg once-daily for ≥one year may result in 20–65% HBeAg clearance and undetectable HBV DNA in children. However, a high incidence of resistant mutants had precluded it as first-line therapy when newer agents with higher genetic barriers were licensed.

Adefovir Dipivoxil ADV was approved in the year 2000 for children ≥12 years old after a 48-week RCT showed a significant (23%) virological response in the adolescents compared to 0% of the placebo (Jonas et al. 2008). An extended open-label phase was conducted in HBeAg-positive children up to 192 additional weeks (total 240 weeks), either monotherapy or combined with LAM (in LAM experienced cases). Nearly 40% of HBV DNA-positive subjects at 24 weeks discontinued ADV (virological failure). HBeAg seroconversion occurred in 55 of 101 continuous therapy groups (54% or 34% of intension-to-treat analysis), and five cases cleared HBsAg (5%). ADV-associated rtN236T mutation only developed in one monotherapy case at 240 weeks, and rtA181T mutant, which is dually resistant to ADV and LAM, in one patient with LAM added on. The incidence of ADV-resistant mutants was much lower than that in the adult series (cumulative incidence of 0%, 3%, 11%, 18%, and 29% at 1, 2, 3, 4, 5 years (EASL 2012).

Entecavir Due to high mutation profiles in prolonged usage of LAM, ADV, and LdT, ETV and TDF have become the first-line therapy in adults with CHB (Sarin et al. 2016; Terrault et al. 2016). ETV has been approved for children 2–18 years of age in Mar 2014. In the clinical trials with a minimum duration of 48 weeks, HBeAg seroconversion and undetectable HBV DNA (<50 IU/mL) were achieved in 24% at 48 weeks, significantly higher than the control group (3%). In the ETV-treated, HBV DNA suppression occurred in 49% compared to 3% in control; HBeAg seroconversion in 24% compared to 10% in control; ALT normalization in 68% compared to 23% in control. The entire efficacy endpoints continued to increase after 96-week therapy with a virological response to 36%. Resistant mutants (M204V, L180M, S202G) were found in one (0.8% of 120 ETV treated) at week 48 and three more at week 96 (cumulatively 2.6% at year 2) (Jonas et al. 2016). The incidence was higher than those in adult series (cumulative incidence 0.2, 0.5, 1.2, 1.2, 1.2 at year 1, 2, 3, 4, 5 (EASL 2012).

Tenofovir Disoproxil Fumarate A 72-week RCT of TDF in adolescents 12 to <18 years of age (85% antivirals experienced, 90% HBeAg-positive, 27% normal ALT, 52 TDF, and 54 placeboes) showed high viral suppression (HBV DNA <400 copies/mL; TDF: 89% vs. placebo, 0%) in either naïve or LAM-experienced cases with no resistant mutant at 72 weeks nor increased severe safety issues. In HBeAg-positive patients, 21% (10/48) of patients in the TDF group and 15% (7/48) in the placebo group lost HBeAg by week 72 ($p > 0.05$). A decrease in bone mineral density did not reach the safety endpoint of more than 6% in the

TDF group at week 72 (Murray et al. 2012). A clinical trial (NCT01651403) was conducted to evaluate the antiviral efficacy of TDF versus placebo in the pediatric CHB aged 2 to <12 years. TDF tablet (150, 200, 250, or 300 mg tablets based on body weight) or powder in a dose of 8 mg/kg was administered orally once daily for 48 weeks. Till 2020, 60 TDF-treated and 29 placeboes, aged 6 ± 2.8 years, were analyzed. The results showed 77% achieved the primary endpoint of HBV DNA <400 copies/mL at 48 weeks, significantly higher than the placebo group (7%, $p < 0.001$). More patients in the TDF treated had ALT normalization at 48 weeks (52% vs. 17%, $P < 0.001$). However, HBeAg seroconversion and HBsAg loss were not different between the TDF and placebo groups (25% vs. 24%, and 3.3% vs. 3.4%), respectively (NCT01651403 2020). US FDA and EMA had approved TDF for children ≥2 years to <18 years with CHB in 2018 and 2019, respectively.

Tenofovir Alafenamide (TAF) A randomized, double-blind trial in the evaluation of the pharmacokinetics, safety, and antiviral efficacy of TAF in children and adolescents with CHB was started in 2016 recruiting children 2 to <18 years (NCT02932150). EMA had approved TAF (25 mg) for children >12 years old and bodyweight of >35 kg (EMA 2017a, b).

Combination of IFN and NUC Immune-tolerant children with normal ALT and high viral loads did not respond to monotherapy with IFN-α2b in previous clinical studies (Sokal et al. 1998; Lai et al. 1987; Lai et al. 1991) or may develop drug resistance on prolonged NUCs. Accordingly, they are not good candidates to apply antiviral therapies. A pilot study in 23 immune-tolerant children employing 8-week LAM followed by 44-week combined LAM and IFN showed 78% HBV DNA negativity, 22% HBeAg seroconversion, and 17% HBsAg seroconversion (D'Antiga et al. 2006). However, a larger trial enrolled sixty immune-tolerant children, median age 10.9 (range, 3.4–17.9) years, and treated them with ETV once-daily for 48 weeks; pegIFN alfa-2a once weekly was added at the end of week 8 and continued until week 48. Fifty-five completed the treatment course. At 48 weeks after stopping treatment (week 96), two (3%) achieved the primary endpoint (HBeAg loss and HBV DNA <1000 IU/mL 48 weeks after stopping therapy) and were also HBsAg-seroconverted. ALT and HBV DNA levels at week 96 were similar to baseline in the remaining children. In summary, the combination of ETV and pegIFN for up to 48 weeks rarely led to HBeAg clearance or sustained suppression of HBV DNA levels in immune-tolerant children, and treatment was frequently associated with adverse effects (Rosenthal et al. 2019).

2.2.3 Who, When, and How to Treat Children with Chronic HBV Infection?

Based on the results of these trials and clinical data, the recommendation of administration of AVT in CHB children are as follows (Fig. 16.1):

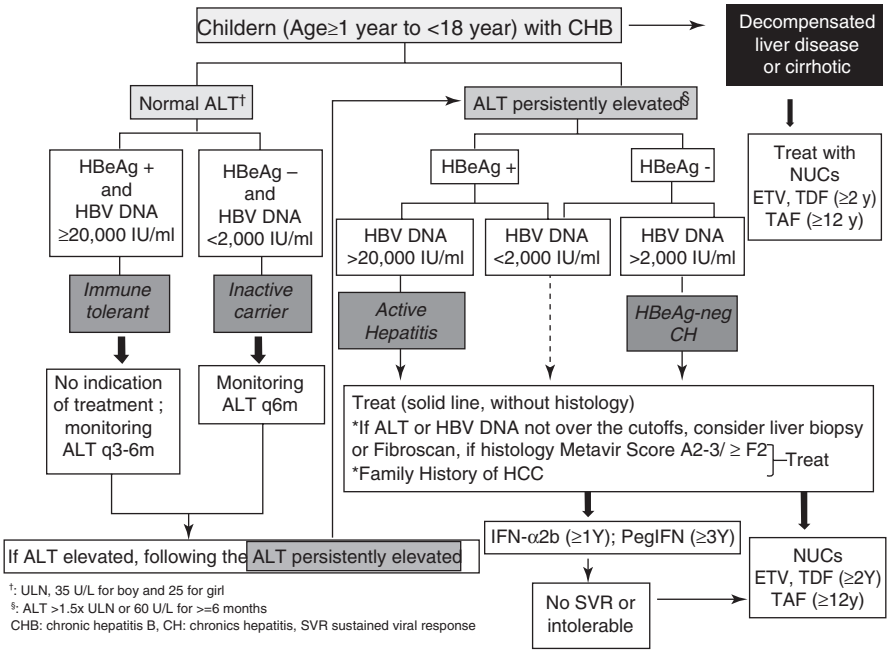

Fig. 16.1 Algorithm for management of children with chronic hepatitis B virus infection

Who and when

- In chronic HBV infected children with liver cirrhosis, either decompensated or compensated, or severe acute hepatitis flares, AVT is indicated. Liver transplantation may be essential if the condition deteriorates.
- In non-cirrhotic HBeAg-positive children entering the immune-active phase, the timing to start AVT varies in different society guidelines. Collective assessment of ALT levels, HBeAg status, HBV DNA levels, and fibrosis stage, together with consideration of the family history of HCC and other concurrent liver diseases, is the basis for decision-making. Commonly, the first clue is a persistently elevated ALT, usually set as >1.5-folds upper limit of normal (ULN, 35 U/L for boy and 25 for girl by AASLD (Terrault et al. 2018)) or > 60 U/L by ESPGHAN (Sokal et al. 2013). Other causes of hepatic inflammation should be excluded. As stated in the previous sect. 16.2.2.1, an ALT level over 60 IU/mL may predict spontaneous e seroconversion in 4 years, taking the toll of lingering hepatic necroinflammation and fibrosis. Whether to start antiviral treatment should be discussed with the child and the caregivers about the benefit (accelerated viral suppression and HBeAg clearance) and adverse effect of AVT (safety issues) versus natural course and consequences. As of 2020, most first-line AVTs (ETV, TDF, TAF (for >12 years), and pegIFN) have been approved for children with similar or even better efficacy and acceptable safety profiles, a comparable observation time as for adults may be adopted (no limit on duration in $AASLD_{2018}$

or $EASL_{2017}$ if HBV DNA >20,000 IU/mL; and ALT>2x ULN, two occasions three months apart in $NICE_{2017}$, ≥ one month between observations in $APASL_{2016}$). HBV DNA at this phase is mostly well above 20,000 IU/mL, a cutoff for treating adults with immune-active CHB. HBV DNA of 2000 to 20,000 IU/mL may represent anticipated HBeAg seroconversion. Therefore, monitoring ALT every 1–3 months, and if persistently elevated ≥6 months, treatment is recommended. If significant inflammation (Metavir Score A2 or A3) or fibrosis (Metavir Score ≥ F2) is documented by histology or noninvasive methods (such as Fibroscan or elastography) or the presence of a family history of HBV-related cirrhosis or HCC, AVT is recommended. Without significant fibrosis, therapy may be deferred to wait for spontaneous HBeAg seroconversion if viral loads are below 10,000 IU/mL ($AASLD_{2018}$) (Lampertico et al. 2017; Terrault et al. 2018; Sarin et al. 2016; NICE 2017; Indolfi et al. 2019).

- HBeAg-negative children with persistently elevated ALT indicate reactivation, and AVT is recommended. Usually, HBV DNA levels in these cases are over 2000 IU/mL. If viral loads are <2000 IU/mL, other causes of hepatic inflammation should be excluded.
- In those with normal ALT levels in the immune-tolerant phase, regular monitoring of ALT/HBeAg/or HBV DNA status is suggested. AVT is not recommended.
- In children with evidence of HBV infection (HBsAg-positive or anti-HBc-positive) who will receive immunosuppressant therapy, baseline HBV DNA level should be measured, and the risk of reactivation should be assessed (Table 16.2). The risk of reactivation is higher in HBsAg-positive than HBsAg-negative and depends on different immunosuppressants/clinical conditions. HBV reactivation is diagnosed when a patient with serologic evidence of HBV infection has a detectable HBV DNA level when they previously had an undetectable level, or a rise more than 2 $\log_{10}$ IU/mL above baseline. AVT is recommended for all patients who develop HBV reactivation. AVT prophylaxis, especially in moderate to very high-risk patients, can decrease the risk of HBV reactivation. AVT should be maintained for at least 6 months after immunosuppression withdrawal but should be kept for at least 18 months after discontinuing anti-CD20 and careful monitor-

Table 16.2 Risk of HBV reactivation in different HBsAg status in various immunocompromised scenarios

	HBsAg-positive	HBsAg-negative /Anti-HBc-positive
Anti-CD20 Hematopoietic stem cell transplantation	Very high risk	Moderate risk
High dose steroids (>20 mg for ≧ 4 weeks)	Moderate risk	Low risk
Cytotoxic chemotherapy without steroids Anti-TNF therapy Anti-rejection therapy for solid organ transplantation	Low risk	Rare
Methotrexate or azathioprine	Very low risk	Rare

ing for 12 months after AVT withdrawal (Bisceglie et al. 2015; Viganò et al. 2015). Among those at low to very low risk of reactivation, early detection of HBV reactivation by frequent monitoring and timely AVT are appropriate.

How

The choice of initial AVT depends on the age, hepatitis activity, drug availability/cost, and tolerability after a thorough discussion with the child and parents. Interferons may be considered due to a finite course. PegIFN (180 μg/1.73 m^2 to maximum 180 μg once weekly) may be given for children ≧ 3 years of age and conventional IFN-α (6 M.U./m^2 body surface area, maximum 10 M.U. three injections per week), for children ≧ one-year old. The standard IFN-α treatment is 6 months for HBeAg-positive cases (pediatric series) and 12 months for HBeAg-negative patients (from adults' practice), while a typical pegIFN protocol is 48 weeks. An on-treatment HBsAg kinetics is predictive of pegIFN response in adults. In HBeAg-positive patients, pegIFN may be discontinued due to low probability of HBeAg seroconversion if HBsAg levels >20,000 IU/mL (genotype B, C), or no decline (genotype A, D) at 12 weeks, or if HBsAg >20,000 IU/mL at 24 weeks for all genotypes (Sonneveld et al. 2013). IFN is contraindicated in patients with severe flares (ALT >fivefolds ULN) or hepatic decompensation (jaundice or coagulopathy) because in-therapy flares may precipitate liver failure. IFN is also contraindicated in children with cytopenia, autoimmune disorders, cardiac or renal failure, serious neuropsychiatric disease, and transplant patients (Jara and Bortolotti 1999; Shah et al. 2009).

- If NUCs are selected for therapy, drug of choice in children ≥ two years of age and weight ≥ 10 kg are ETV and TDF (ETV, a weight-based dose from 10 to 30 kg and 0.5 mg above 30 kg daily in treatment naïve cases and double dose in LAM-experienced; TDF, 8 mg/kg for <17 kg, weight-based tablet for >17 kg, maximum 300 mg daily). For those ≥12 years old, TAF is another choice. These NUCs are potent in viral suppression with acceptable safety profiles in children. NUCs with low barriers against HBV resistance (LAM, ADV, and LdT) are not advocated to treat CHB. The optimal duration of NUCs is not defined and dependent on HBeAg status, the effect of HBV DNA suppression, and the presence of cirrhosis/decompensation. Based on the kinetics of HBsAg change during NUCs therapy, a finite treatment course seems less achievable (Chevaliez et al. 2013). In non-cirrhotic HBeAg-positive children who seroconvert to anti-HBe, at least an additional 12 months of treatment with persistently normal ALT and undetectable HBV DNA (so-called consolidation) is indicated. In cirrhotic cases with HBeAg seroconversion on NUCs therapy, indefinite treatment is suggested. In those who stop NUCs following the guideline, careful monitoring monthly for initial 3 months, then 3-monthly for at least 1 year is advised to detect recurrent viremia, ALT flares, HBeAg seroreversion, or decompensation (Sarin et al. 2016; Terrault et al. 2016). In NUC-nonresponder or virological breakthrough cases, resistant mutations should be checked. Either ETV (for ADV-resistant and LAM-

naïve) or TDF (LAM- or ADV-resistant patients previously treated with LAM) may be substituted. IFN-α or PegIFN can be a possibility if no other NUCs available (Zoulim and Locarnini 2009; Sokal et al. 2013).

- HBeAg-negative chronic hepatitis is a progressive disease and difficult to cure in adult patients. In contrast, it is less prevalent in pediatric patients (4.8% in IFN-treated or naïve children (Iorio et al. 2007)). In adults, PegIFN alone for 48 weeks achieved a significantly higher rate of sustained response than LAM alone without additional benefit when combined with LAM (Marcellin et al. 2004). More elevated baseline ALT, lower HBV DNA levels, viral genotypes (B > C > D), IL28B (rs12979860 genotype CC vs. non-CC), and early on-treatment HBsAg kinetics (a decrease of 0.5 and 1 Log_{10}IU/mL HBsAg levels at weeks 12, and 24 of therapy, respectively) may predict better treatment response (Bonino et al. 2007; Lampertico et al. 2013b; Moucari et al. 2009; Lin and Kao 2013). The ideal endpoint of therapy in HBeAg-negative hepatitis is HBsAg clearance, which may be better achieved via PegIFN than NUC (4% vs. 0%, off-therapy 6 months; 8% in off-therapy year 3–5) (Chotiyaputta and Lok 2010; Lampertico et al. 2013a). If NUC is selected, an alternative stopping rule is at least three results of undetectable HBV DNA 6 months apart after a minimum of 24 months of treatment (Sarin et al. 2016).

2.2.4 HCC surveillance in Children with Chronic HBV Infection

Most children with chronic HBV infections present with mild or minimal liver inflammatory activity, yet 0.01–0.03% of these carriers may develop HCC before adulthood (Bortolotti et al. 2006; Wen et al. 2004). The childhood HCC incidence had decreased from 0.51–0.60 to 0.15–0.19 per 100,000 person-year in the birth cohort born after the HBV vaccination program (Chang et al. 2009). The risk of HCC in adults with CHB is predictable by age, gender, HBeAg serostatus, viral loads, ALT levels, quantitative HBsAg levels, and HBV genotypes (Chen et al. 2011; Lee et al. 2013). The risk is increased in those with precore/basal core promoter mutants (Yuen 2004; Liu et al. 2006a; Kao et al. 2003). Although a landmark study showed HBeAg seropositivity predicted high risk for HCC in the era that DNA quantification was not available (Yang et al. 2002), a later study found that HBeAg clearance was not sufficient to reduce HCC risk unless it was followed by undetectable DNA/HBsAg clearance (Liu et al. 2014). HCC has been described in children who had undergone early (<2 years of age) HBeAg seroconversion, indicating that there is still a risk for HCC after HBeAg seroconversion if DNA levels are not persistently low (<2000 IU/mL) (Wen et al. 2004; Chen et al. 2011). HBV genotype may influence HCC development. Compared to genotype B infection, genotype C with distinctive features of delayed HBeAg seroclearance, higher frequency of basal core promoter mutation, higher DNA levels are associated with cirrhosis and HCC in older patients in Taiwan (Kao 2003; Ni et al. 2004). The risk model to stratify HCC screening policy in adult carriers may not be practical in children. HCC surveillance in children using liver ultrasound should be employed every 6–12 months, depending on the stage of fibrosis and family history of HCC (Bruix and Sherman 2011; Yu et al. 2000). Alpha-fetoprotein (AFP) alone was

shown to offer insufficient sensitivity and specificity for cost-effective HCC surveillance in adults (Singal et al. 2009; Lok et al. 2010). Regardless of whether the virus is actively replicating, HBV infection is the absolute risk of HCC. Continuous surveillance for HCC in all ages and phases of HBV infection, even after HBeAg seroclearance or HBsAg clearance, is mandatory (Hsu et al. 2002; Wen et al. 2004; Bortolotti et al. 2006).

3 Management of Pregnant Women with HBV Infection

3.1 Acute Hepatitis B in Pregnancy

During pregnancy, acute hepatitis B is usually mild without increased mortality or teratogenicity (Sookoian 2006; Lobstein et al. 2011). Prematurity or low birth weight was reported in neonates from pregnant mothers with acute hepatitis B infection (Hieber et al. 1977; Jonas 2009). AVT will be considered if impending acute liver failure develops (Degertekin and Lok 2009). The mother-to-child transmission (MTCT) rate of HBV depends on the gestational ages at acute infection, about 10% in early pregnancy to as high as 60% approaching delivery (Sookoian 2006; Jonas 2009). Monitoring of maternal serology and HBV DNA is necessary to adopt preventive tasks for MTCT. If the mother is HBsAg-positive with detectable HBV DNA near delivery, HBIG needs to be given to the neonate at birth in addition to standard vaccination. If maternal DNA level is high, AVT is indicated to reduce MTCT. Otherwise, supportive management is generally acceptable with careful monitoring of liver biochemical and coagulation profiles. The choice of AVT in pregnant women will be based on safety, accessibility, and expected therapy duration and will be discussed below.

3.2 Chronic Hepatitis B Virus infection in Pregnancy

3.2.1 Effect of Pregnancy on Maternal Chronic HBV Infection

Most HBsAg carrier mothers can tolerate pregnancy well unless advanced liver disease already exists. However, pregnancy itself is a hormone-induced immune-tolerant status (to the fetus) with high adrenal corticosteroid levels that may modulate immune responses (Trowsdale and Betz 2006).

Acute Flares. Due to a pregnancy-related immune-tolerant status, ALT decreases significantly in a pairwise comparison between three trimesters (ALT in the first trimester > in the second trimester > in the third trimester, $P = 0.01$ and 0.02, respectively) while serum HBV DNA levels do not change significantly in three trimesters. ALT flares in the range of 38–1654 U/L were observed in 25% postpartum compared to 1.6% during pregnancy. HBeAg-positivity at baseline may predict postpartum flares. Most flares with ALT <5x ULN return to normal within 9–12 months after delivery. Those with ALT>10x ULN may take over 12 months,

and AVT is indicated (Giles et al. 2015). In those mothers who received AVT in the third trimester for prevention of MTCT, the occurrence or onset of flares does not differ no matter whether the AVT is stopped at 2 weeks (50%, at 8 weeks) or 12 weeks after delivery (49%, at ten weeks), or no antivirals (29%, at 9 weeks). Extending antiviral therapy does not protect against postpartum flares or affect HBeAg seroconversion rates (Nguyen et al. 2014).

3.2.2 Effect of Chronic HBV Infection on Pregnancy Outcomes

Some studies reported CHB imposed an increased risk of gestational diabetes mellitus, antepartum hemorrhage, preterm labor, premature birth, and perinatal morbidity (Tse et al. 2005; Lao et al. 2007; Hieber et al. 1977; Safir et al. 2010). However, others could not show any significant adverse effects on pregnancy (Livadas et al. 1979; Pastorek et al. 1988; Wong et al. 1999; Lobstein et al. 2011). A population-based study showed that cirrhotic mothers were more likely to deliver by cesarean section, to have maternal and fetal mortality, antepartum admission, and maternal and fetal complications, including gestational hypertension, placental abruption, uterovaginal hemorrhage; prematurity, and growth restriction in babies. Hepatic decompensation occurred in 15%, which resulted in 6 and 12% maternal and fetal mortality, respectively (Shaheen and Myers 2010).

3.3 Management of Pregnant Women with Chronic HBV Infection

Various factors need to be considered when encountering pregnant women's management with chronic HBV, including antivirals' indications, potential influences on the fetus, the expected duration of therapy, risk of developing drug resistance, and accessibility and cost of the antivirals.

3.3.1 Managing Women Who Become Pregnant when under Antiviral Therapy for CHB

Women should notify their clinician immediately if they get pregnant while taking AVT, and the risks and benefits of continuing treatment should be discussed. Interrupting treatment may pose a risk of hepatitis flare for the mother, while continuing treatment may affect the fetus. Discontinuing treatment can be a choice if the patient is non-cirrhotic. Most safety data of NUCs are from HIV-infected pregnant women. TDF and LdT are classified as pregnancy category B. The rest are rated pregnancy category C. Conventional IFN is contraindicated in pregnancy, and PegIFN is category C, but not recommended due to its antiproliferative effect. Although lamivudine is considered category C, there is a long history of safety data in HIV-infected women. Clinical trials evaluating the efficacy of LAM, LdT, and TDF to reduce the risk of mother-to-child transmission also support these agents' safety during pregnancy; however, long-term outcomes are still under surveillance. Therefore, women receiving ETV, ADV, or IFNs can be shifted to pregnant category B agents, TDF or LdT, if continuing treatment is favored. They should be carefully monitored during the switching to ensure no flare-ups.

3.3.2 Indications for Initiating Antiviral Therapy during Pregnancy

The decision to start AVT complies with the EASL, AASLD, and APASL guidelines in managing adult chronic HBV infection (Sarin et al. 2016; Lampertico et al. 2017; Terrault et al. 2018). However, some scenarios may need consideration:

- Although AVT is recommended for most patients with an ALT >2x ULN, women without evidence of cirrhosis may choose to defer therapy until after completion of childbearing if they have low viral loads and mild disease activity.
- Women with high viral loads should consider initiating therapy in the third trimester to prevent transmission to their child, even if ALT levels are normal (see sect. 16.3.4 AVT to reduce mother-to-child transmission).
- Management of cirrhosis in pregnant women does not differ from that of non-pregnant patients (Tran et al. 2016).
- In women with childbearing potential, indications for AVT are the same as other adults. However, several issues may be addressed. Those with mild disease, who are planning to conceive soon, may elect to defer treatment until they have completed childbearing. Those who are willing to receive treatment before pregnancy may choose Peg-IFN under contraception during therapy because of its finite duration (48 weeks). If the patient decides treatment with a NUC, TDF is preferred due to its potency, safety data in pregnancy, and low drug resistance risk.

3.4 Antiviral Therapy to Reduce Mother-to-Infant-Transmission of HBV

3.4.1 Risk of Transmission

The rate of mother-to-infant transmission (MTIT) of HBV from HBsAg-positive mothers was reported to be 40 ~ 50% in the pre-immunization era (Stevens et al. 1975). The risk is highest in HBsAg-positive/ HBeAg-positive/ anti-HBe-negative mothers (transmission rate: 70%–90%), lower for HBsAg-positive/ HBeAg-negative/ anti-HBe-negative mothers (transmission rate: 25%–40%) and lowest in HBsAg-positive/HBeAg-negative/anti-HBe-positive (0–12%) (Beasley et al. 1977; Stevens et al. 1979; Degli Esposti and Shah 2011; Borgia et al. 2012; Wong et al. 1984). Transmission can occur in utero (intrauterine), at birth (perinatal), or after birth (Lin et al. 1987; Tang et al. 1998). The MTIT has reduced significantly after the universal HBV vaccination program. It is around 1% in the United States (Schillie et al. 2015b) or 2.46% in Taiwan (Chen et al. 2012), affected by maternal HBV prevalence (6% in Asian ethnic, 0.14 ~ 1% in non-Asian ethnic in the United States, vs. 3.1 ~ 15.5% in Taiwan) (Euler et al. 2003; Lin et al. 2008) and immunoprophylaxis policy (HBIG and HepB to all infants born to HBsAg-positive mothers in the United States; HBIG to infants born to HBsAg-positive/HBeAg-positive mothers and HepB to all infants in Taiwan until 2019).

3.4.2 Risk Factors of Mother-to-Infant Transmission

The common risk factors of transmission are HBeAg-positive mothers, high maternal viral load, or younger maternal age; transmission is also associated with incomplete HepB or delayed immunoprophylaxis (Schillie et al. 2015b; Wen et al. 2013). Among those factors, the most important determinant for the propensity of MTIT is maternal HBV viral loads.

Maternal Viral Loads

An early study by dot-hybridization assay for HBV DNA showed that high maternal serum HBV DNA (>8000 pg/mL) at delivery predicted 100% MTIT despite immunoprophylaxis (Lee et al. 1986). Recent studies have documented a clearer correlation or cutoff of maternal viral titers to transmission rates by applying more sensitive quantitative PCR methods (Table 16.3). In HBV high endemic areas (Asia), higher maternal viral loads (from 6 to >8 $\log_{10}$ copies/ml) are correlated with higher infant transmission rates despite passive and active immunoprophylaxis (Zou et al. 2012; Wen et al. 2013). In low endemic countries (Australia and the United States), the transmission threshold may be higher (>8 $\log_{10}$ copies/ml) with lower infant infection rates in the same cutoffs (Wiseman et al. 2009; Kubo et al. 2014). There is still

Table 16.3 Maternal viral loads or seromarker status and infant transmission rates in different studies

Study	Mother–infant pair	HBIG/Vaccine	Definition of infant transmission	Maternal seromarkers or viral loads /transmission rate (%)	
Wiseman et al. (2009) (Australia)	138 infants of HBV DNA(+) mothers	<12 hr[a]/<12 hr., 2, 4, 6 months	HBsAg (+) at 9 months	DNA(+) HBeAg(+) DNA ($\log_{10}$ copies/ml) > 8 < 8	3 7 9 0
Zou et al. (2012) (China)	869 infants of HBsAg(+) mothers	<12 hr./<12 hr., another two doses <6 months	HBsAg (+) at 7–12 months	DNA($\log_{10}$ copies/mL) <6 6–6.99 7–7.99 >8	 0 3.2 6.7 7.6
Wen et al. (2013) (Taiwan)	303 infants of HBsAg(+) mothers	≤24 hr./0, 1, 6 months	HBsAg (+) at 4–8 monthsand/or 1–3 years	HBsAg (+) HBeAg (+) DNA($\log_{10}$ copies/mL) 5 6 7 8 9	3.3 12.3 0.9 2.6 6.6 14.6 27.7
Kubo et al. (2014) (us)	4446 infants of HBsAg (+) mothers	<12 hr./<12 hr., at 24–67, 64–214 days	HBsAg (+) at 9–15 months	HBsAg (+) HBeAg (+) DNA (IU/ml) $\geq 5 \times 10^7$ $< 5 \times 10^7$	0.75 3.37 3.61 0

[a]One of the infected infants inadvertently missed HBIG administration

no consensus on the threshold of maternal viral loads to target for preventive therapy for MTIT. Some advocates lower threshold (>200,000 IU/mL, ~ 5.3 $\log_{10}$ IU/mL or > 6 $\log_{10}$ copies/mL) to target, aiming to prevent all possible MTIT (Zou et al. 2012; Zhang et al. 2014). However, lower target levels may render a larger number of pregnant women under AVT exposure.

Maternal HBeAg Status

The significant difference in MTIT rates between HBeAg-positive and HBeAg-negative mothers has been well-documented in many studies before (85 vs. 31% (Beasley et al. 1977)) or after immunoprophylaxis programs (9.3 vs. 0.23% (Chen et al. 2012), 1.8 vs. 0% (Kubo et al. 2014), 3.2 vs. 0% (Schillie et al. 2015b), 9.8 vs. 0.7% (Xu et al. 2002)). HBeAg-positive CHB correlates with higher HBV DNA levels (25th to 75th centile, 6.8–8.3 $\log_{10}$ IU/mL) compared to HBeAg-negative CHB (25th to 75th centile, 3.2–5.8 $\log_{10}$ IU/mL, $p < 0.0001$) unless BCP/PC variants are present, which may result in dissociated HBeAg levels with viral replication (Thompson et al. 2010). In HBeAg-positive mothers, serum HBV DNA is significantly higher than HBeAg-negative mothers (7.4 ± 1.9 vs. 2.7 ± 1.4 $\log_{10}$ copies/mL, $p < 0.0001$) (Wen et al. 2013). Therefore, the link between maternal HBeAg seropositivity and MTIT is mainly a reflection of maternal viral loads (Kubo et al. 2014; Burk et al. 1994).

Maternal HBsAg Levels

Although maternal HBV DNA levels are the best predictors of MTIT, universal screening of HBsAg-positive mothers with quantitative PCR is hindered by its cost. Due to the correlation of HBsAg levels with HBV DNA, especially at high DNA levels or HBeAg-positive status (Thompson et al. 2010; Su et al. 2010), several studies evaluate the option of using maternal HBsAg levels as a surrogate predictor of MTIT (Sun et al. 2012; Wen et al. 2016). Using HBsAg titer above 4.1 log IU/mL as a cutoff could predict HBV DNA levels of ≥7.0 log IU/mL with good sensitivity (85%) and specificity (97%) (Sun et al. 2012). Wen et al. showed the estimated transmission rates at maternal HBsAg levels of 4, 4.5, and 5 $\log_{10}$ IU/mL were 2.4%, 8.6%, and 26.4% (Wen et al. 2016).

Amniocentesis and Other Factors Causing Maternal–Fetal Hemorrhage

Obstetric procedures or complications that may cause maternal–fetal hemorrhage, such as threatened abortion, chorionic villus sampling, amniocentesis, threatened preterm labor, emergent cesarean section after any period of labor, and forceps/vacuum delivery have been reported to increase the risk of MTIT (Lin et al. 1987; Xu et al. 2002). A study showed, in comparison to infants without amniocentesis, significantly higher transmission rate was noted in HBsAg-positive mothers with high viral loads >7 $\log_{10}$ copies/mL (4.5% vs. 50%); but no difference in those with viral loads <6.99 $\log_{10}$ IU/mL (1.5% vs. 1.8%) (Yi et al. 2014). Another statistic from Canada shows MTIT attributable to amniocentesis in HBsAg-positive mothers up to 1.4%. However, the rate may be as high as 16% in HBeAg-positive mothers

(Gagnon et al. 2014). Therefore, noninvasive methods of prenatal risk screening should be used to minimize the number of amniocenteses. Awareness of the viral loads or HBeAg status is important in counseling high-risk mothers about the transmission risk associated with amniocentesis.

Different modes of delivery also influence the transmission rate. A study compared infant-carrier mother pairs delivered vaginally (VD), elective cesarean section (ECS), or urgent cesarean section (UCS). A significantly lower rate of MTIT was noted by ECS (1.4%), compared to VD (3.4%, $p < 0.032$) or UCS (4.2%, $p < 0.02$). Based on this study, the authors suggested that ECS for HBeAg-positive mothers with pre-delivery HBV DNA >6 $\log_{10}$ copies/mL (or > 200,000 IU/mL) may reduce MTIT (Pan et al. 2013). However, a review from Australian, the United Kingdom, and New Zealand experts suggests there is no sufficient evidence to modify the delivery mode for MTIT reasons if neonatal immunization is used (Visvanathan et al. 2016).

Breastfeeding

Although HBsAg, HBeAg, and HBV DNA are present in breast milk, no differences in the rates of HBV infection have been reported between breast-fed infants versus formula-fed infants even before the era of immunoprophylaxis (Beasley et al. 1975; Hill et al. 2002; Montoya-Ferrer et al. 2015). Infants who received HBIG and the birth dose of HepB can be breastfed (Dionne-Odom et al. 2016). However, HBsAg-positive mothers should also avoid bleeding from cracked nipples. Carrier mothers should not donate breast milk (de Oliveira et al. 2009).

3.4.3 Algorithm to Prevent Mother-to-Infant Transmission (Fig. 16.2)

- Pregnant women should have HBsAg testing at the first prenatal visit. Those who are HBsAg-positive are recommended to test for HBeAg, HBV DNA, and aminotransferase levels.
- Women who have a high HBV DNA level (i.e., >200,000 IU/mL) and/or a positive HBeAg should be referred to a hepatologist to evaluate the indication of AVT.
- Women with low HBV DNA levels in the first trimester should have viral load testing again around weeks 20 to 28. If the levels are over the threshold of indication, AVT should be considered.
- Antiviral treatment for the prevention of MTIT should be started at the beginning of the third trimester. Hence, there is sufficient time for the HBV viral load to decrease, considering preterm labor.
- HBsAg-negative women at high risk for HBV infection (e.g., injection drug user, sexual partner, or household contact of HBsAg carriers) should be vaccinated if she is also anti-HBs-negative and anti-HBc-negative. HBsAg testing should be repeated in late pregnancy (approximately 28 weeks) if she is still susceptible.

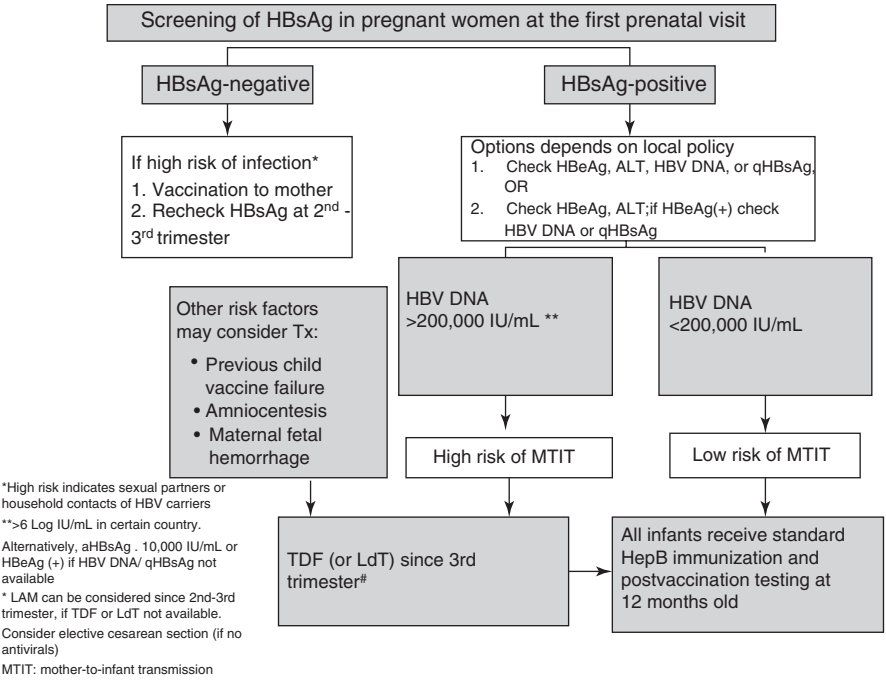

Fig. 16.2 Algorithm for management of pregnant women to prevent mother-to-child transmission of HBV

3.4.4 Choice of Antiviral Agents

For those who require treatment, TDF is the drug of choice, as recommended by WHO guidelines (WHO 2020c). This is important since many of these young mothers may require antiviral treatment for their liver disease in the future. In addition, this agent appears to be safe in pregnancy, and has been evaluated in several prospective clinical trials (Chen et al. 2015; Brown et al. 2016; Pan et al. 2016). Although other agents (e.g., LAM and LdT) also reduce MTIT and appear to be safe when administered during pregnancy (Han et al. 2011; Pan et al. 2012; Shi et al. 2010), they are associated with higher rates of antiviral resistance. LAM may be a reasonable alternative if cost is a barrier or category B medication is not available and treatment is going to be administered for a short duration (i.e., ≤3 months). However, it is essential to confirm that the patients have not received LAM in the past because the chance of LAM resistance is high; and also consider the fact that maternal viral load reduction is slower compared to newer AVT.

3.4.5 Efficacy and Safety of Antiviral therapy in Preventing MTIT of HBV

A growing number of studies using AVTs to prevent MTIT have been published in recent years showing promising results. A systemic review and meta-analysis included 26 controlled trials from 1988 to 2014 that enrolled 3622 pregnant women. Eleven compared LAM vs. control, nine compared LdT vs. control, three compared

TDF vs. control, two compared LAM vs. LdT, and another compared TDF vs. LAM. Treatments started in the second or third trimester with an average baseline HBV DNA level of 7.63 $\log_{10}$ IU/mL and ALT level of 37.7 U/L. All infants (except one study) received passive-active immunization. The infant outcome is defined by seropositivity of HBsAg or HBV DNA at 6–12 months of age. The meta-analysis concluded use of any AVT compared to control in pregnant women reduces the likelihood of MTIT (HBsAg seropositivity, risk ratio (RR) = 0.26; HBV DNA positivity, RR = 0.31). LdT, LAM, and TDF appear to be safe in pregnancy with no increased adverse maternal or fetal outcome (Brown et al. 2016). The study designs were highly variable in earlier trials, and lacked sufficient controls.

A prospective trial in Taiwan enrolled 118 pregnant women with HBV DNA ≥ 7.5 $\log_{10}$ IU/mL. Sixty-two mothers received TDF from 30–32 weeks of gestation until one month postpartum. Compared to untreated group ($N = 56$), infants from the TDF group had lower seropositivity rate of HBV DNA at birth (6.15% vs. 31.48%) and lower HBsAg seropositivity rate at 6 months of age (1.54% vs. 10.71%). Mothers in the TDF group experienced less incidence of ALT >2x ULN lasting more than 3 months, less extent of ALT elevation, and less severe flares (>5x) at postpartum week 8. An infant in the TDF group who was HBsAg-negative at 6 months old became seropositive at 12 months old, which was attributed to low anti-HBs response (11.1 mIU/mL at 6 months old) even after passive and active immunoprophylaxis. Thus, it illustrates the significance of adequate vaccine-induced immune response to protect infants at risk and the necessity of serological monitoring in children of carrier mothers (Chen et al. 2015). A follow-up study showed those in the TDF group had a significantly lower rate of detectable neonatal HBV DNA at birth (5.22% vs. 30.11%) and positive HBsAg at 6 months (1.74% vs. 11.83%) and 12 months (1.74% vs. 10.75%). Serum HBV DNA > 1.9 $\log_{10}$ IU/mL at birth was predictive of HBV infection (Chang et al. 2019). An open-label RCT included 200 Chinese HBsAg-positive pregnant mothers with HBV DNA level > 200,000 IU/mL, who were 1:1 randomly assigned to control or TDF from 30 to 32 weeks of gestation until postpartum week 4. At postpartum week 28, the rate of MTIT was significantly lower in the TDF group than in the control group, both in the intention-to-treat analysis (5% vs. 18%, $P = 0.007$) and the per-protocol analysis (0 vs. 7%, $P = 0.01$). After discontinuation of TDF, ALT elevations above the normal range occurred more frequently in mothers in the TDF group than those in the control group (46% vs. 30%, $P = 0.03$) (Pan et al. 2016). From the incubation period of HBV infection (average 75 days, range 30–180 days), surveillance of HBsAg or HBV DNA of children at risk when they are six months of age is appropriate to check MTIT status. Children of carrier mothers who received TDF in late pregnancy had comparable long-term growth, renal function, and bone development up to 6–7 years after birth with the control peers whose mothers did not receive TDF (Wen et al. 2020).

3.4.6 Postpartum Cessation of AVT

Suppose the ALT levels are normal during pregnancy and the goal of AVT is exclusively for preventing MTIT. In that case, mothers may stop antivirals soon after delivery. Most clinical studies continued treatment until postpartum week 4 to 12 to

reduce postpartum flares risk (Visvanathan et al. 2016). A prospective study showed extending AVT (10–12 weeks vs. two weeks or no AVT) beyond delivery did not appear to reduce the frequency of HBV flares (40% vs. 50%, no AVT, 29%, $p = 0.33$) over a median of 48 weeks of follow-up (Nguyen et al. 2014). However, if maternal ALT levels are elevated during pregnancy, a postpartum extension until achieving the therapeutic endpoints is recommended. A prospective observational study included 241 pregnant women who received LdT since 24 or 28 weeks of gestation and opted for a cessation of therapy ($N = 143$) at postpartum week (PPW) 12 or continuation ($N = 98$, shifting to ETV). Stratified by ALT elevation or not during pregnancy, in those who stopped AVT at PPW12, hepatic flares were significantly higher in those who had ALT elevation during pregnancy (25% vs. 6.8%, $p = 0.003$). Those who continued AVT did not develop virological or ALT flares from PPW 24 to 52. Besides, a higher HBeAg seroconversion rate was observed in those who had ALT elevation during pregnancy (36.6% vs. 8.8%, $p = 0.001$) (Liu et al. 2016). All the mothers receiving AVT treatment during pregnancy should be closely monitored during treatment and in the first six months after delivery, especially for those whose AVT is stopped postpartum.

3.4.7 Breastfeeding during Maternal Antiviral Therapy

Despite LAM and TDF's safety in pregnancy from the Antiretroviral Pregnancy Registry, the label of either drug recommends against their use during breastfeeding. However, most society guidelines (Lampertico et al. 2017; Terrault et al. 2018; NICE 2017; Coffin et al. 2018) and clinical studies support breastfeeding during LAM or TDF due to low oral bioavailability in breast-fed infants. In HIV-infected women treated with 300–600 mg/day, the median LAM serum level was 508 ng/mL, median breast milk LAM was 1214 ng/mL. Still, the infant's median serum levels continued to drop from 67 ng/mL to 24 ng/mL to undetectable at delivery, 6 and 24 weeks of age (Moodley et al. 1998; Shapiro et al. 2005; Ehrhardt et al. 2015). The median concentration of TDF measured in breast milk was approximately 3% of the median concentration measured in mother's serum, 0.5%–16% of the TDF dosage that fetuses experienced via placental transfer, and 0.01–0.04% of the recommended weight-adjusted therapeutic dose (Visvanathan et al. 2016; Benaboud et al. 2011; Hu et al. 2019). LdT has not been studied in nursing mothers treated for HBV infection, and no relevant published data of the maternal or infant drug levels are available to date (LactMed 2020).

4 Conclusions and Future Scope

Pregnant women and children with chronic HBV infection are two special populations in the global burden of HBV infection, both serving as patients themselves as well as infectious sources and, through mother-to-infant transmission, perpetuating the reservoir of HBV. To combat HBV infection before it damages the host or transmits to vulnerable people, we should treat it early if primary prevention by immunoprophylaxis fails. Currently, it is not recommended to introduce antivirals for

children in the immune-tolerant phase. Previously, an observation scheme was suggested even in children or pregnant women's immune-active phase due to these subjects' vulnerability. Thanks to the advent of more potent antivirals with acceptable safety profiles, therapies in children and pregnant women have entered a new era. The fundamental measure for global HBV elimination is to optimize hepatitis B immunization programs worldwide with a target of 90% 3-dose HepB coverage, and 90% of <12-hour birth dose, aided with antiviral therapy in high viral load pregnant mothers to prevent MTIT. Additionally, universal screening of high-risk populations, timely application of antivirals, close monitoring, and surveillance of HCC are essential for the successful management of CHB. Some unresolved issues still exist: when to implement AVT in the immune-active phase, the indication threshold of viral loads in children and pregnant women, the AVT-induced/vaccine escape mutants, and the proper time to stop NUCs. The development of new antiviral therapies to eradicate HBV infection from childhood to adulthood is highly anticipated.

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Occult Hepatitis B Infection

17

Tai-Chung Tseng and Chun-Jen Liu

Abstract

Occult hepatitis B virus infection (OBI) is defined as the presence of HBV replicative templates in the liver with/without circulating HBV DNA in patients with undetectable hepatitis B surface antigen (HBsAg). The prevalence of OBI is estimated to be ranging from <1 to 18% in general population. Usually, serum HBV DNA level is low and intermittently detected, which does not induce liver damage. However, there are some potential risk for patients. Firstly, OBI has been reported to be associated with the development HCC in patients with chronic hepatitis C in some studies, but other studies did not find such an association. It is still a debating issue whether OBI may accelerate the disease progression toward cirrhosis and the development of HCC in patients with other chronic liver diseases. Secondly, there is potential risk of HBV transmission through blood transfusion from OBI donors. The risk could be minimized by screening the blood products using nucleic acid testing. Thirdly, HBV reactivation from OBI is being increasingly recognized when patients receive potent immune-suppressive therapies including B-cell depleting agents. Although prophylactic antiviral therapy minimizes the risk of HBV reactivation, the best strategy to prevent HBV reactivation in these situations remains to be defined. More research is needed to develop a useful guideline to optimize clinical management of OBI.

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Occult hepatitis B · Epidemiology · HCC · Transfusion · Hepatitis C · Reactivation · Direct-acting antiviral · Immunosuppression · Prevention

1 Introduction

Occult HBV infection (OBI) is defined as the presence of HBV replicative templates, covalently closed circular DNA (cccDNA) in the liver, with/without circulating HBV DNA in patients with undetectable hepatitis B surface antigen (HBsAg) (Raimondo et al. 2019). Clearance of HBsAg months after acute HBV infection in adults or decades after being chronic HBsAg carriage is usually regarded as a functional cure of HBV infection (Lok et al. 2017). However, despite HBsAg seroclearance, the stable cccDNA in the long-lived hepatocytes is an obstacle to eliminating HBV infection and may lead to the development of OBI. It implies that HBV infection may last even under efficient host immune control.

In most cases of OBI with limited HBV replication and viral protein expression, HBV DNA is usually less than 200 IU/ml, which is usually intermittently detected in serum (Kazemi-Shirazi et al. 2000; Cacciola et al. 1999; Huang et al. 2012; Spreafico et al. 2015; El Chaar et al. 2010). HBV DNA between 200 and 2000 IU/mL is also evidenced in some OBI patients due to the limitation of detecting mutated HBsAg by some commercially available HBsAg assays (Huang et al. 2012; El Chaar et al. 2010; Hou et al. 1995; Chaudhuri et al. 2004; Mu et al. 2009; Torbenson and Thomas 2002). Antiviral therapy is practically not recommended for OBI patients under current international guidelines as viral load <2000 IU/mL is usually not associated with HBV-related liver necroinflammation (Raimondo et al. 2019).

2 Classifications of OBI Patients

OBI patients can be classified as seropositive and seronegative OBI by serological markers (Raimondo et al. 2019). Seropositive OBI is characterized as anti-hepatitis B core antibody (anti-HBc) positive with the presence or absence of anti-hepatitis B antibody (anti-HBs). Seronegative OBI is featured by double-negativity for anti-HBc and anti-HBs and accounts for 1 to 20% of OBI (Cacciola et al. 1999; Torbenson and Thomas 2002). Although it is possible that either no production or gradually reduction of anti-HBc and anti-HBs causes seronegative OBI, the clinical outcomes of seropositive versus seronegative OBI patients are still under investigation.

It has been reported that the rates of detectable HBV DNA among OBI categories are the highest in OBI with anti-HBc alone, followed by OBI positive for both anti-HBc and anti-HBs and seronegative OBI (Brechot et al. 2001; Pisaturo et al. 2020). However, the positive rate of HBV DNA in seronegative OBI patients might be underestimated because examination of HBV DNA is conducted systemically only in few studies.

3 Diagnosis

HBV DNA and HBsAg are common viral markers for OBI diagnosis. Although detection of HBV DNA in the liver is the gold standard for the diagnosis of OBI, determination of HBV DNA and HBsAg in the circulating compartment is practically applied instead because acquisition and examination of blood sample is easier than the liver biopsy specimens.

Measurement of HBsAg in OBI patients using insensitive HBsAg assays may be falsely negative thus resulting in diagnostic error of OBI in patients with overt HBV infection. Currently, the lower limit of the most commercial HBsAg detection assays is 0.05 IU/ml. It is demonstrated that 1 to 48% of HBsAg-negative samples were positive for HBsAg if determined by using high sensitivity assay, which detects both outer and inner epitopes of HBsAg, with the lower limit of 0.005 IU/ml (Seto et al. 2012; Ozeki et al. 2018; Yang et al. 2016). A recently advanced strategy for improving HBsAg detection is measuring the level of HBsAg releasing from the HBsAg-anti-HBs immune complex as well as the free-form HBsAg and the detection limit is 0.0005 IU/mL (Matsumoto et al. 2017). It is found that some OBI patients carry only HBsAg-anti-HBs immune complex and are at higher risk of HBV reactivation after receiving rituximab-containing chemotherapy by this novel assay (Kusumoto et al. 2020).

Similarly, detection of HBV DNA in OBI patients by insensitive HBV DNA assays may also lead to false negativity and thus underestimation of OBI cases. The lower limit of conventional HBV DNA assays is 10 to 20 IU/ml. Because HBV DNA level is usually low and intermittently detected in OBI, it is suggested that measurements of serum HBV DNA at multiple timepoints are needed to ensure the detection of OBI.

Anti-HBc had been used as a surrogate marker in OBI diagnosis (Raimondo et al. 2019). The presence of anti-HBc indicates the previous infection of HBV, whereas the presence of anti-HBs alone may denote the previous vaccination of HBV. It has been reported HBV reactivation occurs in HBsAg-negative and anti-HBc-positive individuals with undetectable HBV DNA in the blood (Yang et al. 2018; Huang et al. 2013; Seto et al. 2014). However, the presence of anti-HBc does not equal OBI because evidencing viral replication is mandatory for OBI diagnosis. Accordingly, patients with HBsAg-negative and anti-HBc-positive are better characterized as resolved hepatitis B.

In summary, the major limitation to diagnose OBI is the lack of standardized and validated assays. Therefore, data across different studies cannot be properly compared or integrated.

4 Epidemiology of OBI

The global prevalence of OBI has not been clarified yet because of several practical issues. Firstly, for general population or subjects without any evidence of liver disease, hepatitis B serology is not surveyed routinely. Secondly, for patients with known but resolved HBV infection, serum HBV DNA is almost not tested. Thirdly,

the HBV DNA level in serum of patients with OBI is usually too low to be detected by standard commercial assays. Previous studies have shown that the HBV DNA levels in patients with OBI were typically less than 10–20 IU/mL (Morales-Romero et al. 2014; Yuen et al. 2011). Finally, measuring HBV DNA in the liver tissues may help detection of OBI; however, the invasive nature of liver biopsy makes it an unpopular approach (Yuen et al. 2008, 2010, 2011; Song et al. 2009; Bhatti et al. 2007; Werle-Lapostolle et al. 2004; Reesink et al. 2008; Georgiadou et al. 2004; Fang et al. 2009; Minuk et al. 2005; Kim et al. 2007; Svicher et al. 2012).

5 Prevalence of OBI in the East

Chronic HBV infection is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) in the Asian-Pacific region (Chen 2000; Merican et al. 2000). Globally, more than 70% of HBV infections occur in the Asian-Pacific region.

The prevalence of persistent as well as past infection is high in South and Southeast Asia (Alter 2003). Mongolia is also highly endemic for HBV infection (Alter 2003). In these areas, more than 8% of the general population is positive for HBsAg, 40 to 90% of the adult population has serological evidence of previous HBV infection, and 4 to 25% of the HBsAg (−) and anti-HBc (+) subjects have detectable serum HBV DNA (Minuk et al. 2005; Lai et al. 1989; Wang et al. 1991; Iizuka et al. 1992; Nagaraju et al. 1994). In these highly endemic areas, the majority of infections occur perinatally or in early childhood, a high proportion of the infected adults have late chronic HBV with undetectable HBsAg; this phenomenon may account for the high rate of OBI in anti-HBc-positive populations in these areas.

In areas of Asia with an intermediate prevalence of HBV infection including Middle East countries, India, South Asia, and Korea, the prevalence of HBsAg ranges from 2 to 7%. About 16 to 55% of the population has serologic evidence of past HBV infection.

In well-developed countries of the Asia-Pacific area including Australia and Japan, the prevalence of chronic HBV infection is usually less than 1%. Only 4 to 15% of the adult population has evidence of HBV infection rate (Alter 2003). Among these low prevalence countries, HBV DNA could be found in less than 5% of the HBsAg (−), anti-HBc (+) blood units (Allain et al. 1999; Kleinman et al. 2003).

6 Prevalence of OBI in the West

The prevalence of OBI also varies in different geographical areas in the west. Recently, a systematic review and meta-analysis were conducted in Western Europe and in Northern America (Pisaturo et al. 2020). Interestingly, their data showed that the prevalence of OBI was high in HBsAg-negative patients. Besides, the presence of OBI was associated with anti-HBc positivity (Pisaturo et al. 2020). Totally, 34% of the general population had evidence of OBI; 28% (95% CI, 12–48%) in 329

subjects without chronic liver disease, and 35% in 2400 patients with chronic liver disease. Subgroup analysis further revealed that the prevalence of OBI was 51% and 19% among the 823 anti-HBc-positive subjects and the 1041 anti-HBc-negative subjects, respectively.

7 Prevalence of OBI in Different Clinical Situations

There is ample evidence in cross-sectional studies demonstrating the persistence of HBV DNA in patients with HBsAg-negative HBV infections. Overall, serum HBV DNA was documented in 5 to 55% of HBsAg-negative chronic hepatitis patients with chronic hepatitis (Torbenson and Thomas 2002; Brechot et al. 2001; Hu 2002). For patients with HCC, OBI was found in 14 to 100% of anti-HBc-only positive patients; and OBI was found in 8 to 87% of the seronegative patients without any markers of HBV infection (Paterlini et al. 1990; Thiers et al. 1993; Fukuda et al. 1996; Shintani et al. 2000).

For patients with fulminant hepatitis, OBI is documented in around 10% of HBsAg-negative patients. OBI can also be observed in apparently healthy individuals with normal liver function tests (Wang et al. 1991; Marusawa et al. 2000; Shih et al. 1990; Hennig et al. 2002). Again, the rate of HBV DNA is significantly higher in healthy individuals with anti-HBc alone. Allain et al. reported that for anti-HBc-positive subjects, the average HBV DNA detection rates were 7 and 13% among these subjects with versus without anti-HBs, respectively. In blood donors, the rates of OBI ranged from 0 to 17% (Allain 2004).

In the west, a recent review demonstrated that the prevalence of OBI ranges from 4 to 38% in patients with cryptogenic cirrhosis or advanced liver fibrosis (Squadrito et al. 2013; Hou et al. 2001; Chan et al. 2002), is about 45% in patients with a history of exposure to blood product, is 52% in chronic hepatitis C patients, ranges from 0% to 45% in patients infected with HIV, ranges from 0% to 22.7% in blood donors (Kishk et al. 2015; Sofian et al. 2010) and ranges from 0% to 54% patients receiving hemodialysis (Minuk et al. 2004).

8 Clinical Implications

8.1 Transmission of HBV through OBI

Any product containing full HBV viral particles is considered to be potentially infectious. According to a chimpanzee study, HBV DNA of only 10 copies can already achieve the minimum 50% infectious dose of HBV (Komiya et al. 2008). OBI donors, although usually having very low HBV viremia, may thus still transmit the HBV to susceptible individuals in the setting of blood donation. Several confounding factors in human situations further influence the transmission of HBV from OBI subjects, including the anti-HBs status of both the donors and the recipients, the kind and the volume of the blood products being transfused to the

recipients, and the immunological status of the recipients (Raimondo et al. 2013; Mosley et al. 1995; Satake et al. 2007).

Transmission by blood components negative for HBsAg can occur either in the acute phase of infection during the seronegative window period or during chronic stages of infection (i.e., OBI). Because of limitations in previous blood screening practices, OBI is a risky but overlooked source of HBV transmission.

The prevalence of OBI among blood donors varies globally. For example, in Egypt, the rate of OBI can be as high as 22.7% (Kishk et al. 2015). In Iran, none was found to have OBI (Sofian et al. 2010). Screening 14,937 young blood donors born between 1992 and 1997 in China (in the era of universal HBV vaccination) documented that 10 (0.067%) of these donors had detectable serum HBV DNA, indicating the presence of OBI (Tang et al. 2018).

Regarding the screening for HBV infection in blood donors, it would be useful to assess the relative contribution of two potential sources of transfusion-transmitted HBV infection from HBsAg-negative donations. Anti-HBc screening can eliminate the residual risk of occult HBV transmission by transfusion in low-endemic areas. On the contrary, nucleic acid amplification test (NAT) would be effective in the screening of blood donors for OBI in highly endemic countries. However, the cost-effectiveness of different blood screening strategies in different countries needs to be investigated further (Liu et al. 2006).

Although there are already many human studies examining the transmissibility rate of HBV from blood donors with OBI, most of these studies are retrospective in nature. We could not trace back the potential donors or the infectious origin. There are several studies performed to determine the HBV transmission rate from OBI donors. It was found that the transmission rate was low, at around 1–3% (Yuen et al. 2011; Mosley et al. 1995; Candotti and Allain 2009). The risk was furtherly reduced if the donor serum was anti-HBs positive (Mosley et al. 1995). There are studies showing that HBV transmission is possible from anti-HBc positive donors (Hoofnagle et al. 1978; Lander et al. 1978; Koziol et al. 1986). The transmissible rate is around 2.4–3.0% (Lai and Yuen 2009).

From another aspect, although OBI is transmissible through blood transfusion to HBV-naïve recipients, its impact on recipients with prevalent HBV infection in HBV hyperendemic areas is unclear. To address this issue, we consecutively collected HBV-naïve recipients indicated by anti-HBc-negative, with normal ALT, and followed their HBV DNA and serologic markers before and after transfusion in Taiwan (Liu et al. 2006). Among 4448 blood unit recipients, we collected 467 (10.5%) anti-HBc-negative recipients and completed the posttransfusion follow-up in 327 recipients. We identified 5 (1.5%) recipients who developed hepatitis B viremia 1 week after transfusion. Three were children with subclinical acute infection (anti-HBs positive from birth HBV vaccination in all 3 children), one had transient transfusion-transmitted HBV without seroconversion to anti-HBc and one had OBI. Our findings suggested that OBI was transmissible by transfusion in HBV endemic areas. The incidence of posttransfusion acute HBV infection was 0.9% (100 per million units) in naïve recipients in Taiwan, approximately 40-fold higher than in developed countries. Moreover, some vaccinated children with anti-HBs

were still susceptible to HBV infection. Our findings indicated that sensitive screening assays for OBI such as NAT should be considered in endemic areas.

We further conducted a look-back study to determine the clinical significance of OBI-positive blood transfusion in Taiwan (Su et al. 2011). In 2006, we identified 12 occult HBV blood donors from 10,824 repository samples by using NAT. The 74 corresponding recipients were further identified. Among the 74 recipients, 18 were alive and 12 were called back to our clinic. However, only 24 recipients had available posttransfusion serological profiles; none was seroconverted to be HBsAg positive. One recipient had an identical sub-genomic sequence of HBV surface gene (384 nucleotides) to his donor. Our findings suggested that in HBV hyperendemic areas, OBI was transmissible. However, the risk of transfusion-transmitted HBV infection is low.

9 HCC Development

It is widely debated whether OBI may accelerate the disease progression toward cirrhosis and the development of HCC in patients with other chronic liver diseases. While many studies have shown a significant association between OBI and HCC in patients with chronic hepatitis C (Squadrito et al. 2013; Shetty et al. 2008; Wang et al. 2018), other studies found no association (Lok et al. 2011; Chen et al. 2017). It is believed that the OBI-related HCC risk, if exists, is very limited although OBI potentially maintains the pro-oncogenic properties attributed to the HBV infection (Saitta et al. 2015). More large cohort studies are needed to address the issue.

10 HBV Reactivation after Immunosuppressant

HBV reactivation is defined as a sudden surge of viral load, which is attributed to inadequate host immune control over HBV replication and is followed by varying degrees of liver necroinflammation or even liver decompensation. Although spontaneous HBV reactivation is not rare in HBsAg-positive patients with a low viral load, (Tseng et al. 2013), HBV reactivation occurs more frequently in the HBsAg-positive CHB patients undergoing chemotherapeutic treatment and immunosuppressive therapy, such as stroid-containing regimen (Cheng et al. 2003). A substantial risk of HBV reactivation develops after host immune response is suppressed and prophylactic antiviral treatment is recommended in this clinical circumstance.

In patients with resolved HBV infection, HBV reactivation is characterized by either the reappearance of HBsAg, the reappearance of HBV DNA with a record of undetectable HBV DNA, or a tenfold increase of HBV DNA with previously detectable serum HBV DNA. HBV reactivation rarely occurs in patients with resolved HBV infection after traditional chemotherapy. However, it has become a more serious problem with the increasing use of monoclonal antibodies with potent immunosuppressive effects for autoimmune diseases and hematological malignancies (Raimondo et al. 2019; Yeo et al. 2009).

It is first shown that approximately 24% of anti-HBc-positive subjects receiving cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) plus anti-CD20 (rituximab) experienced HBV reactivation resulting in one death (Yeo et al. 2009). On the other hand, there is no HBV reactivation in anti-HBc-positive subjects if they received rituximab-free treatment regime. It strongly suggests that rituximab, a B cell-depleting agent, significantly increases the risk of HBV reactivation in patients with resolved HBV infection.

This issue is further studied in detail by other prospective studies. Of the 63 HBsAg-negative, anti-HBc-positive subjects (all with undetectable serum HBV DNA) receiving R-CHOP, 42% of subjects develop HBV reactivation (Seto et al. 2014). It is also found that the rate of HBV reactivation is lower in anti-HBs-positive subjects compared with anti-HBs-negative subjects (34% vs. 68% respectively, $p = 0.012$). Furthermore, the result from a randomized control study shows that entecavir prophylaxis minimizes the risk of HBV reactivation in patients with resolved HBV infection after R-CHOP (HBsAg seroreversion rates were 16.3% vs. 0%) (Huang et al. 2013).

Two important factors determine the risk of HBV reactivation in resolved HBV patients after R-CHOP. One is serum levels of anti-HBs, which reflects the host humoral immune response. A meta-analysis has demonstrated the protective value of detectable anti-HBs levels (Paul et al. 2017). The other is the presence of residual replicative cccDNA in the liver. Several biomarkers indicating residual replicative templates have been explored to predict the risk of HBV reactivation after R-CHOP treatment in patients with resolved HBV infection. The first biomarker is the detectable HBV DNA in serum, which is commonly used to define OBI. Although not all the data support its role in predicting HBV reactivation (Yang et al. 2018; Huang et al. 2013; Seto et al. 2014), the data from a large cohort study enrolling 266 Asian patients showed that patients with detectable HBV DNA level (2.2% of the overall cohort) are associated with HBV reactivation (Kusumoto et al. 2015). The second potential biomarker is hepatitis B core-related antigen (HBcrAg), which is a viral protein translated from cccDNA. Of the 124 patients receiving either rituximab-containing chemotherapy or allogeneic hematopoietic stem cell transplantation from a prospective study, they found that detectable HBcrAg level at baseline (17.7% of the overall cohort) is associated with higher risk of HBV reactivation (Seto et al. 2016). The third biomarker is quantitative anti-HBc, which has been shown to be positively associated with cccDNA levels in patients with resolved HBV infection (Caviglia et al. 2018). Of 197 patients receiving R-CHOP treatment from a prospective study, a higher anti-HBc level at baseline (≥6.41 IU/ml, 35.9% of the overall cohort) is associated with increased risk of HBV reactivation (Yang et al. 2018). The fourth biomarker is ultra-high sensitivity HBsAg assay, which detects the HBsAg contained in the immune complex and has a lower detection limit than conventional HBsAg assay (0.0005 IU/ml vs. 0.05 IU/mL) (Kusumoto et al. 2020). Of the 252 patients with HBsAg <0.05 IU/mL, 4 patients had detectable HBsAg by ultra-high sensitivity HBsAg assay and all of them had HBV reactivation. Although all these biomarkers do not directly detect the intrahepatic cccDNA, which indicates the presence of OBI, these surrogate biomarkers indicate the presence of residual cccDNA and help clarify the role of OBI in inducing HBV reactivation after R-CHOP.

HBV reactivation is also an important issue for patients with hematological malignancy because there is a high degree of bone marrow suppression by intense immunosuppressive therapy, especially for those receiving hematopoietic stem cell transplantation (HSCT). The rate of HBV reactivation in HSCT patients with resolved HBV infection is in a range of 2.4 and 43% as reported by different studies, which may be influenced by the definition of HBV reactivation (Vigano et al. 2011; Hammond et al. 2009; Seto et al. 2017; Chen et al. 2018). A prospective study including 62 HSCT patients shows that the HBV reactivation is as high as 40% within 2 years of follow-up but HBsAg seroreversion occurred only in one patient (7.7%) (Seto et al. 2016).

HBV reactivation has also been reported in patients with resolved HBV infection after biologic therapy. According to a large-scale study including 468 Asian HBsAg-negative and anti-HBc-positive patients, the use of antitumor necrosis factor was associated with the HBV reactivation rate of 1.7% (Lee et al. 2013), in contrast to the zero risk reported in Western countries (Pauly et al. 2018; Barone et al. 2015). To be noted, patients with rheumatology disease usually receive different kinds of biological therapy, and it is sometimes difficult to attribute the risk of HBV reactivation to a specific drug (Chen et al. 2020). The current data suggest the risk of HBV reactivation from patients with resolved HBV infection is limited after biological therapy except using B cell-depleting agent.

Prophylactic antiviral agents are recommended for chronic hepatitis B patients with risk of HBV reactivation >10% after chemotherapy or immunosuppressant treatment (Reddy et al. 2015). For OBI subjects, this practice is also widely adopted for those with detectable HBV DNA. A controversy, however, exists in HBsAg-negative, anti-HBc-positive patients with undetectable HBV DNA. Currently, prophylactic antiviral treatment is recommended for all the patients with resolved HBV infection receiving rituximab-containing chemotherapy. However, there is a high prevalence rate of resolved HBV infection in Asia. A cost-effective alternative is to identify the high-risk patients (>10% of reactivation rate) for prophylactic antiviral treatment while arranging a close observation for the rest of the patients. The risk stratification needs more data from different viral and host biomarker research. Several studies have shown that monitoring of HBV DNA or ultra-high sensitive HBsAg monthly for prompt antiviral treatment when HBV DNA or HBsAg detected is effective to avoid HBV-associated hepatitis (Kusumoto et al. 2020; Kusumoto et al. 2015). At present, there are no studies to show the best monitoring strategy and more studies are needed to optimize the management.

11 Reactivation of HBV in Chronic Hepatitis C Patients Receiving DAA Therapy

Reactivation of HBV activity has been an important clinical concern in HCV/HBV coinfected patients receiving anti-HCV therapy in the era of pegylated interferon plus ribavirin combination therapy (Liu et al. 2009).

After the introduction of direct-acting antiviral (DAA) for the treatment of chronic hepatitis C, the awareness of HBV reactivation was further increased.

During 108 weeks after DAA treatment, HBV virologic reactivation occurred in 73% of patients (81/111) (Liu et al. 2017). Clinical reactivation occurred in 9% of participants (10/111). Our data clearly indicated that among HCV/HBV coinfected patients treated with DAAs for HCV, HBV virologic reactivation occurred commonly and should be monitored.

In contrast to overt HBV/HCV coinfection, patients with chronic hepatitis C and coexisting OBI have a minimal risk of HBV reactivation after the start of DAA therapy in previous studies and meta-analysis (Liu et al. 2017; Pisaturo et al. 2019). Per regional guidelines, only serum ALT monitoring is recommended for HCV/OBI coinfected patients; and serum HBV DNA or HBsAg testing is reserved for those patients experiencing serum ALT elevation of unknown etiology.

12 Conclusion

In summary, OBI is now a disease entity with increasing attention in various aspects of liver diseases. The diagnosis of OBI could be affected by the different sensitivity of HBsAg and HBV DNA assays. The prevalence is yet to be studied comprehensively in different population across the world. OBI patients usually have low viral load thus may not need antiviral therapy in general conditions. There is a risk of HBV transmission through blood transfusion, which has been minimized by the application of NAT screening for blood products. HBV reactivation from OBI is being increasingly recognized after the introduction of potent B cell-depleting therapy. Although prophylactic antiviral therapy minimizes the risk of HBV reactivation, the best strategy to prevent HBV reactivation in these patients remains to be defined (Fig. 17.1).

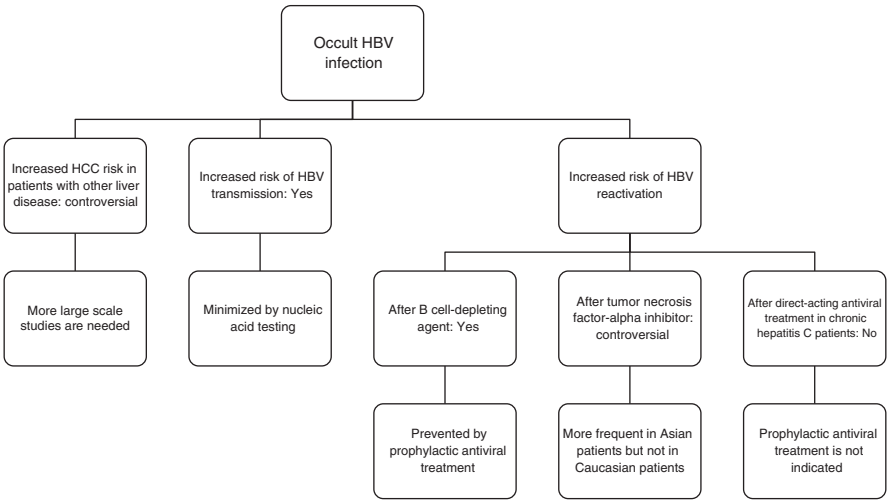

Fig. 17.1 Clinical significance and management of occult hepatitis B virus infection (OBI)

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Hepatitis B Virus Reactivation and Management of Patients Undergoing Immunosuppression

18

Prowpanga Udompap and W. Ray Kim

Abstract

As modern therapeutics continue to discover novel targets in immune pathways, an increasing number of patients with current or prior hepatitis B virus (HBV) infection are exposed to the risk of reactivation of the virus. Hepatitis B reactivation (HBR) is generally defined by a rise in HBV DNA in patients with chronic HBV infection or the reappearance of HBV DNA or HBsAg in the patients with resolved HBV infection. HBR is prevented and managed by identification of patients at risk for reactivation and initiation of antivirals for prophylaxis or therapy of HBR as indicated. Even as research endeavors are under way to achieve functional cure of HBV infection, HBR remains a relevant entity, as its occurrence would indicate persistence of ccc DNA.

Keywords

Hepatitis B · HBV · Reactivation · Immunosuppression

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1 Introduction

Previously dormant or inactive hepatitis B may reactivate, often in conjunction with medications that influence the host's immune function. Reactivation may occur in asymptomatic patients with chronic hepatitis B virus (HBV) infection or in individuals who have recovered from a past infection. HBV reactivation (HBR) has become a serious concern because an increasing number of patients receive medications that may impair the host immune system sufficiently to precipitate HBR. Prevention and treatment for HBR present a challenge, in part because not all patients with HBV infection are diagnosed or aware of their infection and there is no curative therapy for HBV infection. Further, individuals who recovered from a past infection are normally considered to be immune to HBV; however, they may remain at risk of HBR depending on the setting.

In the background of HBR is the balance between the replicative drive of the virus and the immune response from the host (Fig. 18.1) (Lok et al. 1991; Yeo et al. 2004b). In patients with inactive HBV infection, viral replication is inhibited as a result of the control by the host immune system. HBR occurs when this balance is perturbed by an environmental agent such as an immunosuppressive or cancer therapeutic compound that lifts the immune control allowing HBV replication to resume (Hoofnagle 2009; Keam et al. 2011). Another scenario that HBR could occur is when treating a coexistent hepatotropic virus such as hepatitis C virus (HCV). It has been shown that successful eradication of HCV, especially with recently available direct-acting antiviral agents, has been associated with HBR (Mucke et al. 2018).

Although HBR is often temporary and clinically silent, it may cause a symptomatic flare of hepatitis. While the flare in and of itself may evolve into a serious condition incurring morbidity and even mortality, another major clinical consequence of HBR is the need for interruption of the causative immunosuppressive or

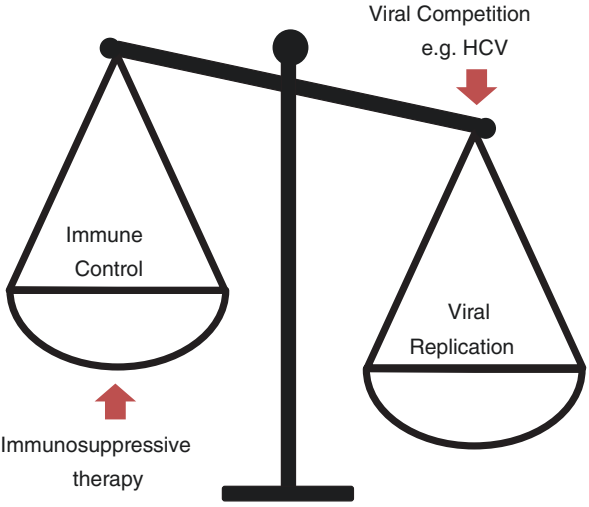


Fig. 18.1 The occurrence of HBR depends on the balance between the replicative drive of the virus and the immune response from the host

chemotherapy. Moreover, in patients whose HBV status is unknown or unsuspected, HBR may be a source of confusion and misdiagnosis, leading to a delay in appropriate clinical management. Hence, preventing HBR protects the patients from experiencing potentially dangerous flares and from failing to achieve the intended goals of the immunosuppressive therapy.

2 Definition of HBR

Uniform, standardized nomenclature, and definitions for HBR are unavailable. HBR may present as an abrupt reappearance of HBV DNA in those with previously resolved HBV viremia, or as a significant rise in serum HBV DNA from the baseline HBV DNA in chronic hepatitis B (CHB) patients, or as reverse seroconversion, i.e., an individual previously HBsAg negative becoming HBsAg positive (Loomba et al. 2017). Table 18.1 compares the definition of HBR from major professional societies (Terrault et al. 2018a; European Association for the Study of the Liver 2017; Sarin et al. 2016; Perrillo et al. 2015a). The current guidance on the Prevention, Diagnosis, and Treatment of Chronic Hepatitis B by the American Association for the Study of Liver Diseases (AASLD) (Terrault et al. 2018a) defines HBR in each patient category by the following criteria. For patients who are HBsAg-positive, HBR is diagnosed when there is either: (1) ≥2 log or 100-fold increase in HBV DNA compared to the baseline; (2) HBV DNA ≥ 3 log or 1000 IU/mL in those with previously undetectable HBV DNA; or (3) HBV DNA ≥ 4 log or 10,000 IU/mL, if the baseline level is not available. For patients who are anti-HBc-positive and HBsAg-negative, the criteria include: (1) emergence of detectable levels of HBV DNA or (2) reappearance of HBsAg, the latter likely connoting more serious consequences of HBR. Serum alanine aminotransferase (ALT) activities are not included in the criteria in part because of the lack of a broad consensus based on evidence about the diagnostic thresholds for ALT (Di Bisceglie et al. 2015; Hwang and Lok 2014).

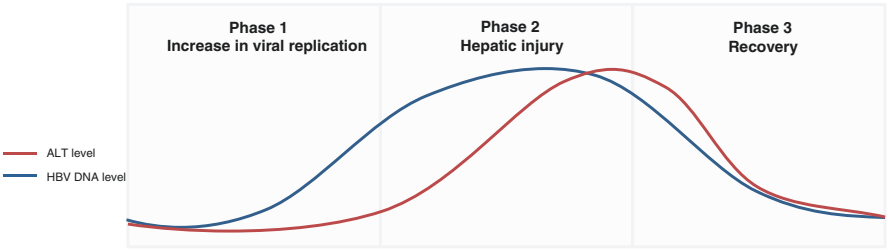
3 Clinical Manifestations of HBR

Clinical features of HBR vary from asymptomatic changes in the laboratories to fulminant hepatic failure leading to death. The course of HBR has been described in three phases (Fig. 18.2). The first phase is mainly a virological event, characterized by an abrupt increase in viral replication soon after immunosuppressive therapy is initiated. There are no apparent hepatitis symptoms, and serum aminotransferase levels are usually unchanged from baseline. HBV DNA levels continue to rise during the second phase and may be accompanied by an elevation in serum aminotransferases with or without symptoms such as fatigue. In severe cases, hepatitis activities may be severe enough to result in liver failure. As expected, these poor outcomes tend to occur more frequently in cirrhotic patients. In the third phase, HBV DNA levels and serum aminotransferases levels start to decrease and HBV markers may return to the baseline. Not all

Table 18.1 Comparison of the recommendation to prevent HBR from each professional society

			AASLD (2018a)	EASL (2017)	APASL (2016)	AGA (2015a)
Definition of HBR						
Chronic HBV infection	Baseline HBV DNA	Unavailable	Detection of HBV DNA ≥4 log or 10,000 IU/mL	Not defined in the guideline	Detection of HBV DNA ≥20,000 IU/ml	Not defined in the guideline
		Undetectable	A reappearance of HBV DNA to ≥3 log or 1000 IU/mL		A reappearance of HBV DNA to ≥2 log or ≥ 100 IU/ml	*De novo* detection of HBV DNA
		Detectable	HBV DNA ≥ 100-fold or 2 log increase from baseline		HBV DNA ≥ 100-fold or 2 log increase from baseline	HBV DNA ≥ ten-fold or 1 log increase from baseline
Resolved HBV infection			HBV DNA is detectable *or* the reappearance of HBsAg		HBV DNA is detectable *or* the reappearance of HBsAg	HBV DNA is detectable *or* the reappearance of HBsAg
Recommended screening labs			HBsAg, anti-HBc	HBsAg, anti-HBc, and anti-HBs	HBsAg, anti-HBc	HBsAg, anti-HBc
Recommended strategy						
HBsAg +			Start antiviral prophylaxis	Start antiviral prophylaxis	Start antiviral prophylaxis	– Start antiviral prophylaxis in patients with either high-risk or moderate risk
HBsAg-/antiHBc +			– Start antiviral prophylaxis in high-risk group, e.g., rituximab and hematopoietic stem cell transplant – Continue monitor and on-demand therapy in the rest	– Start antiviral prophylaxis in high-risk group, e.g., rituximab and hematopoietic stem cell transplant – Continue monitor and on-demand therapy in the rest	– Starting antiviral prophylaxis if detectable HBV DNA – For those receiving rituximab, start antivirals or close monitor with on-demand therapy – Continue monitor and on-demand therapy in the rest	

Choice of antivirals	High-genetic barrier antivirals, e.g., entecavir and tenofovir	High-genetic barrier antivirals, e.g., entecavir and tenofovir	Consider high-genetic barrier antivirals, e.g., entecavir and tenofovir	High-genetic barrier antivirals, e.g., entecavir and tenofovir
Duration of prophylaxis	6–12 months after immunosuppressive therapy (12 months if anti-CD20 therapy)	12–18 months after immunosuppressive therapy (18 months if anti-CD20 therapy)	12 months after immunosuppressive therapy	6–12 months after immunosuppressive therapy (12 months if anti-CD20 or B cell depleting therapy)
Post-prophylaxis monitoring	Up to 12 months after antiviral withdrawal	Up to 12 months after antiviral withdrawal	Not defined	Not defined



HBV DNA level	Increased	Further increase	Return to baseline
ALT level	Usually normal	Increase	Return to baseline
Hepatitis symptoms	None	None/Varied	None
Liver failure	-	In severe cases	-
Death	-	In severe cases	-

Fig. 18.2 Phases of HBV reactivation (HBR)

HBR patients go through these three phases. Many patients may develop only transient increased HBV DNA with or without ALT elevation with no clinical consequences.

4 Mechanisms of HBR

After entry into the hepatocytes, HBV releases nucleocapsids that contain partially double-strand viral genome, which is repaired into a full-length covalently closed circular DNA (cccDNA). The cccDNA is stable in the infected hepatocyte and may persist in a latent state as a potential reservoir for HBR, even after decades of recovery from HBV infection (Rehermann et al. 1996).

Immune control of HBV is mediated through HBV-specific cytotoxic T cells (Rehermann et al. 1996; Zhang et al. 2012) in conjunction with B cells which produce neutralizing antibodies (Chang and Lewin 2007). Despite this robust immune control, it is not sufficient to completely eradicate the cccDNA in the infected hepatocytes even in patients who apparently recover from HBV infection with HBsAg loss. Administration of immunosuppressive agents may lead to impairment of T and B cell functions to the degree to allow the virus to resume HBV replication, resulting in marked increase in the expression of HBV transcription intermediaries and products within hepatocytes (Keam et al. 2011). The following sections describe mechanisms by which different classes of immunosuppressive agents may influence the host immune response against HBV.

4.1 Corticosteroids

Of a number of pathways for which corticosteroids may promote HBV replication, the main mechanism is thought to be impairment of proliferation of T and B cells in part by inhibiting the production of interleukins (Loomba et al. 2017). The HBV genome also has a glucocorticoid responsive element that enhances replication of the virus (Tur-Kaspa et al. 1986, 1988; Calabrese et al. 2006). In a recent

prospective study, half of the study patients had increased HBV DNA levels within two weeks of starting a corticosteroid-containing chemotherapy regimen. This occurred well before the development of leukopenia, suggesting a direct stimulatory effect of corticosteroids on HBV DNA transcription (Cheng et al. 2003). Indirectly, corticosteroids have a number of immunosuppressive effects including inhibition of cytotoxic T cell function (Tur-Kaspa et al. 1988). The risk of HBR among those treated with corticosteroids varies by the dosage, duration of treatment, and HBV serologic status of the host.

4.2 B Cell Depleting Agents

Rituximab, obinutuzumab, and ofatumumab are the major monoclonal antibodies against CD20, a cell surface marker of B cells. They are used to treat hematologic malignancies and, less commonly, severe autoimmune diseases such as rheumatoid arthritis and vasculitis. This class of drugs is the most notorious as a cause of HBR. While the mechanism of rituximab/ofatumumab-associated HBR is not completely understood, the putative mechanisms are that depletion of B cells and the resulting disruption of antigen-presentation impairs CD8+ cytotoxic T cell's ability to kill HBV-infected hepatocytes. Anti-CD20 antibodies reduce the number of CD4 memory T cells, increase T cell subclasses Th1/Th2 and Tc1/Tc2 ratios and upregulate Fas ligands on Th1 and Th2 cells, further impairing the host immune control against the virus (Evens et al. 2011a; Misumi and Whitmire 2014; Tsutsumi et al. 2015). B cell depletion may lead to loss of anti-HBs (Pei et al. 2012).

4.3 Cytotoxic Chemotherapeutic and Immunosuppressant Agents

Cytotoxic cancer chemotherapeutic agents disrupt cell cycles, leading to DNA destruction, which sets in motion cellular DNA repair mechanisms, resulting in a cascade of responses including upregulation of promyelocytic leukemia protein (PML) and PML nuclear body (PML-NB), which have been linked with increased HBV pre-genomic transcription, HBV-core expression, and HBV DNA replication (Chung and Tsai 2009). Traditional immunosuppressants such as methotrexate, azathioprine, and 6-mercaptopurine also disrupt DNA synthesis. However, these are apparently not as detrimental from the standpoint of HBR as other chemotherapeutic agents (Calabrese et al. 2006; Droz et al. 2013; Flowers et al. 1990).

4.4 Biological Immunomodulants

The first widely used biological agents are the TNF-α inhibitors. TNF-α, similar to interferon α/γ can activate a unique noncytotoxic antiviral pathway, namely the APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) proteins, which degrades the cccDNA in infected hepatocytes (Kasahara et al. 2003;

Tzeng et al. 2014). Thus, the inhibition of TNF-α activity may lead to enhanced viral replication (Carroll and Forgione 2010). Commonly used TNF-α inhibitors currently, namely etanercept, infliximab, adalimumab, certolizumab, and golimumab, have all been implicated in HBR.

The second group of biologics that are increasingly utilized, especially in rheumatologic diseases, dermatologic diseases, and inflammatory bowel diseases is the cytokine and integrin inhibitors. The direct effects of these agents on T cell immunity raise concerns that the risk of HBR may be significant (Perrillo et al. 2015a). Generally, these agents block the localization and traffic of the activated lymphocytes, e.g., abatacept (blocks co-stimulation of T cells), ustekinumab (monoclonal antibody to interleukin-12 and interleukin-23), natalizumab, and vedolizumab (inhibit cell adhesion molecule, α4-integrin, found on lymphocytes). These agents potentially reduce the immune control of HBV replication in the liver, predisposing the host to HBR (Loomba et al. 2017).

4.5 Kinase Inhibitors and Proteasome Inhibitors

Imatinib and other tyrosine kinase inhibitors can inhibit T cell activation and proliferation. While bortezomib, the proteasome inhibitors for the multiple myeloma treatment, targets cellular pathways that affect the proliferation of malignant plasma cells, both could interfere with the function of healthy B- and plasmas cells that are important for the HBV immune control (Beysel et al. 2010).

4.6 Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICI) targeting programmed cell death protein 1 (PD-1)/PD ligand 1 (PD-L1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) have now been increasingly used for cancer therapy. CHB patients, in general, tend to have exhausted T cells that expressing high levels of co-inhibitory molecules including CTLA-4 and PD-1. Blocking these molecules could restore the function of exhausted T cells and could potentially lead to cccDNA eradication, suggesting the possible role of ICI in CHB therapy. The occurrence of HBR, however, could happen as a paradoxical effect as ICI treatment may disrupt the balance of chronic HBV infection and, in some conditions, leads to its reactivation rather than improvement (Godbert et al. 2020).

4.7 Chimeric Antigen Receptor T Cell Therapy

Chimeric antigen receptor (CAR) T cell therapy is a recent breakthrough treatment for B cell malignancies. Anti-CD19 CAR T cell therapy, used for relapsed/refractory diffused large B cell lymphoma, has been found to cause persistent B cell

depletion and hypogammaglobulinemia in patients with leukemia and lymphoma (Schuster et al. 2017) and potentially lead to HBR (Wei et al. 2019).

5 Incidence of HBR

The accurate incidence of HBR among patients with immunosuppressive therapy is difficult to define for a number of reasons. First, the settings in which HBR occurs are heterogeneous depending upon host characteristics, baseline HBV status, types of immunosuppressive therapy, and the underlying disease that requires the immunosuppressive therapy. Second, most studies are conducted in retrospective fashion and would be enriched with patients with severe HBR requiring medical attention. Finally, the criteria to diagnose HBR are not uniformed defined, creating further heterogeneity in study results. With these caveats, we summarize data regarding the Incidence of HBR according to the clinical scenario.

5.1 Patients Undergoing Cancer Chemotherapy

HBR was initially described in cancer patients undergoing chemotherapy. The most common scenario for HBR to occur in cancer therapy is patients with hematological malignancies receiving anti-CD20 antibodies for which the incidence of HBR ranged from 16 to 80% in those with HBsAg-positive (Evens et al. 2011b) and from 3 to 41% in those with HBsAg-negative/anti-HBc-positive (Perrillo et al. 2015a, b; Seto et al. 2014).

The incidence of HBR is much lower with cytotoxic chemotherapy for solid tumors, with breast cancer being tumors most frequently associated with HBR with a rate of 20–40% (Gonzalez and Perrillo 2016). The incidence of HBR in HBsAg-positive patients being treated for cancer has been reported to be 14–72%, whereas it is much lower (<3%) among patients who are HBsAg-negative/anti-HBc-positive (Lok et al. 1991; Kim et al. 2007; Kumagai et al. 1997; Yeo et al. 2000a, b, 2003; 2004a).

Regarding the incidence of HBR among patients undergoing anti-CD19 CAR T cell therapy for relapsed/refractory diffuse large B cell lymphoma, Yang recently reported 3 out of 15 patients experiencing HBR (Yang et al. 2020). The actual risk of HBR in this population is yet to be determined as almost all of the clinical trials of CAR T cell therapy excluded patients with HBV infection.

The incidence of HBR is much lower for ICI therapy. In fact, liver injury due to immune reconstitution is more common than HBR. However, there are several reports of HBR associated with ICI therapy. Zhang reported HBR in 5.3% of HBsAg-positive patients undergoing ICI therapy (Zhang et al. 2019). The majority of previously reported HBR in patients on ICI therapy are HBsAg-positive (Koksal et al. 2017; Pandey et al. 2018), with the exception of one case report which described HBR in a HBsAg-negative patient with concomitant HIV infection (Lake 2017).

In addition to systemic immunochemotherapy, HBR may also occur in patients undergoing regional therapy such as transarterial chemoembolization (TACE) for hepatocellular carcinoma (HCC). Although the incidence of HBR in those settings has not been accurately defined, it could be as high as 30–40% (Jang et al. 2004).

5.2 Patients Undergoing Treatment with Other Biologics

The incidence of HBR has been mostly assessed in patient undergoing anti-TNF therapy, partly because anti-TNF has been in existence longer than other novel biologics. A comprehensive review of HBR attributed to biologics, mainly anti-TNF, described an overall HBR frequency of 39% in HBsAg-positive and 5% in HBsAg-negative/anti-HBc-positive patients (Perez-Alvarez et al. 2011). In another report in rheumatological patients treated with anti-TNF, HBR was reported in 12% among patients with positive HBsAg and 2% among HBsAg-negative, anti-HBc-positive patients (Lee et al. 2013). In another observational study of 146 patients with resolved HBV infection who had been given long-term TNF inhibitor therapy, none developed HBR (Barone et al. 2015).

Data about HBR from agents targeting T cell activation including IL-23 and integrin inhibitors remain limited as these agents are relatively new to the market. One study on ustekinumab therapy reported HBR in 3 out of 54 patients with current or resolved HBV infection (Ting et al. 2018).

5.3 Patients Undergoing Organ/Cell Transplantation

5.3.1 Hematopoietic Stem Cell Transplantation

Patients undergoing allogeneic Hematopoietic Stem Cell Transplantation (HSCT) tend to be heavily immunosuppressed, including immunoablative therapy applied prior to the infusion of the donor marrow. The incidence of HBR after HSCT is almost universal among HBsAg-positive patients (Lalazar et al. 2007; Lau et al. 1997; Martin et al. 1995) and maybe up to 50% in HBsAg-negative/anti-HBc-positive patients (Hammond et al. 2009; Park et al. 2011; Seth et al. 2002; Vigano et al. 2011; Ramos et al. 2010; Knoll et al. 2004; Onozawa et al. 2005). In HSCT patients, the risk of reverse seroconversion persists for many years because of the delay in reconstitution of the recipient's immune response to HBV. Reverse seroconversion may occur in patients who are initially anti-HBs-positive: in studies measuring the anti-HBs titer serially, HSCT recipients gradually lost anti-HBs to become undetectable 1–3 years after transplantation. Meanwhile, HBV DNA increased and HBsAg reappeared in the serum. In one retrospective study of HBsAg-negative/anti-HBc-positive HSCT recipients, the cumulative probability of reverse seroconversion was 9% at the end of the first year, which more than quadrupled to 43% at the end of fourth year (Hoofnagle 2009; Hammond et al. 2009). In addition, patients with graft-versus-host disease (GVHD) are at a higher risk of HBR compared to HSCT

patients without GVHD, as they require treatment with high doses of steroids and/or anti-thymocyte globulins to further suppress the host immunity (Liang 2009). GVHD also delays reconstitution of the immune system for up to 12–18 months (Socie and Ritz 2014).

5.4 Solid Organ Transplantation

The calcineurin inhibitors, e.g., cyclosporine and tacrolimus, are commonly used in solid organ transplants (SOTs). These agents inhibit T cell activation and transcription of IL-2. The effect of HBV infection on the outcome of SOT has been studied most in kidney transplantation (KT). Patients with HBsAg-positive have an increased risk of graft loss and mortality (Reddy et al. 2011; Fabrizi et al. 2005). The risk of HBR is higher in HBsAg-positive recipients, especially in those with detectable HBV DNA or HBeAg-positive compared with HBsAg-negative recipients (Reddy et al. 2011). Degos demonstrated that HBR occurred in 11 of 12 (92%) HBsAg-positive recipients (Degos et al. 1988). A study by Fornairon also described that 85% of HBsAg-positive KT recipients developed histological progression, leading to cirrhosis and HCC in some patients (Fornairon et al. 1996). The reported HBR incidence among KT recipients with isolated anti-HBc-positive was lower, varying from 0% to 6.5% (Chen et al. 2013; Kanaan et al. 2012).

6 Risk Assessment for HBR

The risk of HBR depends on three important factors: (1) the baseline status of HBV infection, (2) host characteristics, and (3) the type of treatment exposure (Fig. 18.3).

For viral factors, the presence of HBsAg is a predominant determinant of HBR. In a study by Lau, individuals who have HBsAg-positive carried a greater risk for HBR compared with those who are HBsAg-negative (HR 33.3, 95%CI 7.4–142.9, $p < 0.01$). Among those with HBsAg-positive, the risk of HBR correlates with markers of viral replication status, namely, HBeAg and serum HBV DNA. In particular, HBV DNA levels exceeding 10^5 copies/mL were associated with the highest risk of HBR (Lau et al. 2002). Compared to undetectable HBV DNA, detectable viremia was associated with a HR of 9.35 (95%CI 1.65–52.6, $p = 0.01$). Several small studies suggested that non-A genotype HBV infection is more prone to HBR (Borentain et al. 2010). Lastly, although the presence of anti-HBs is protective in immune-competent hosts, it is not necessarily so in the context of HBR. Thus, in patients who are anti-HBc-positive and HBsAg-negative, HBR may still occur even if they are anti-HBs-positive.

In addition to the viral characteristics, host and treatment factors play an important role in determining the risk of HBR. With regard to the host factors, a study by Yeo et al. evaluated risk for HBR among cancer patients treated with chemotherapy. In addition to HBeAg positivity ($p < 0.01$), they found that male gender ($p = 0.045$) and diagnosis of lymphoma ($p = 0.03$) were associated with HBR (Yeo et al. 2000b).

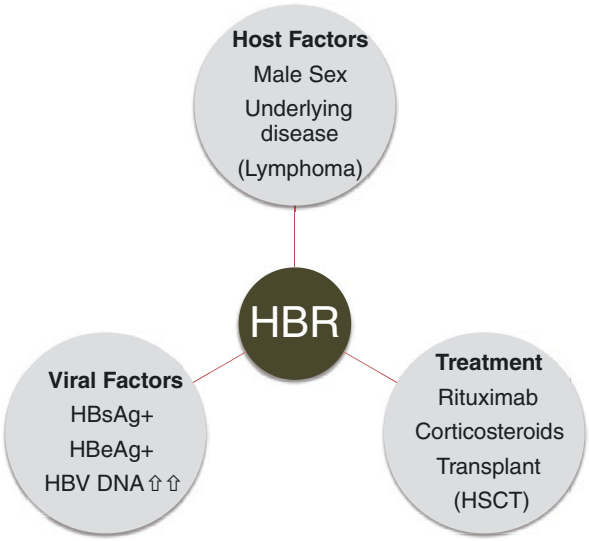


Fig. 18.3 Three important factors affecting the risk for HBR

Other studies showed that among patients with solid organ tumors, HBR occurs more commonly in breast cancer patients (41%) compared with other sites (7–29%) (Yeo et al. 2003, 2004b). Organ transplantation carries an immense risk of HBR, especially HSCT, which affects the host immune function most profoundly. The risk of HBR in patients receiving immunosuppression in settings other than transplantation correlates with the type and level of immunosuppression. For example, the risk is highest when the regimens contain rituximab or high-dose corticosteroid (Cheng et al. 2003; Abramson and Chung 2014; Mendez-Navarro et al. 2011; Mozessohn et al. 2015; Kim et al. 2010; Yeo et al. 2009).

Table 18.2 categorizes the risk of HBR based on the types of immunosuppressive agents and HBV serologic status of the patients. Three strata in HBR risk may be defined: high-, intermediate-, and low-risk groups, corresponding to anticipated incidence of >10%, 1–10%, and < 1% of cases, respectively. These categories inform patient management.

7 Management Strategies for HBR

Two main goals of the HBR management are (1) prevent liver-related morbidity and mortality and (2) allow the immunosuppressive or chemotherapy to continue unperturbed. To achieve these goals, the most effective strategy is to prevent HBR in the first place. This principle is best demonstrated in randomized controlled trials that compared prophylactic antiviral therapy in patients considered to be high risk versus withholding antiviral treatment until a diagnosis of HBR is established. Fig. 18.4 summarizes the results of trials in which lamivudine was used to prevent HBR (Lau et al. 2003; Hsu et al. 2008; Jang et al. 2006; Long et al. 2011). In patients who did not receive prophylactic antiviral therapy, HBR occurred in 30–50%—more

Table 18.2 Risk stratification for hepatitis B reactivation (HBR) based on the types of immunosuppressive agents and HBV serologic status

Risk of HBR	HBsAg + / anti-HBc +	HBsAg – / anti-HBc +
High	– B cell-depleting agents, e.g., rituximab and ofatumumab – Anthracycline derivatives, e.g., doxorubicin and epirubicin – Moderate- or high-dose corticosteroids* daily for ≥4 weeks	– B cell-depleting agents, e.g., rituximab and ofatumumab
Moderate	– TNF-α inhibitors, e.g., etanercept, adalimumab, certolizumab, and infliximab – Cytokine or integrin inhibitors, e.g., abatacept, ustekinumab, natalizumab, and vedolizumab – Tyrosine kinase inhibitors, e.g., imatinib, nilotinib – Low-dose corticosteroids daily for ≥4 weeks	– TNF-α inhibitors, e.g., etanercept, adalimumab, certolizumab, and infliximab – Cytokine or integrin inhibitors, e.g., abatacept, ustekinumab, natalizumab, and vedolizumab – Tyrosine kinase inhibitors, e.g., imatinib and nilotinib – Moderate- or high-dose corticosteroids daily for ≥4 weeks – Anthracycline derivatives, e.g., doxorubicin and epirubicin
Low	– Traditional immunosuppressive agents, e.g., azathrioprine, 6-mercaptopurine, and methotrexate – Intra-articular corticosteroids – Any dose of oral corticosteroids daily for ≤1 week	– Traditional immunosuppressive agents, e.g., azathrioprine, 6-mercaptopurine, and methotrexate – Low-dose corticosteroids daily for ≥4 weeks – Intra-articular corticosteroids – Any dose of oral corticosteroids daily for ≤1 week

High dose: >20 mg prednisone daily or equivalent
Moderate dose: 10–20 mg prednisone daily or equivalent
Low dose: <10 mg prednisone daily or equivalent
[a]Definitions of corticosteroid doses

frequently in lymphoma patients. Prophylactic lamivudine was able to virtually eliminate HBR. While lamivudine may not be the ideal agent today, these data are convincing that in high-risk patients, prevention is a preferred strategy than reactive treatment of HBR once it has occurred.

7.1 Screening and Risk Stratification

A crucial element in HBR management is to identify patients with HBV infection prior to initiation of immunosuppressive therapy. Various governmental and professional organizations have published guidelines about HBV screening in the general population (European Association for the Study of the Liver 2017; Sarin et al. 2016; Weinbaum et al. 2008; Baden et al. 2012; LeFevre and Force 2014; Hwang et al. 2015; Reddy et al. 2015; Terrault et al. 2018b). Although these guidelines vary in

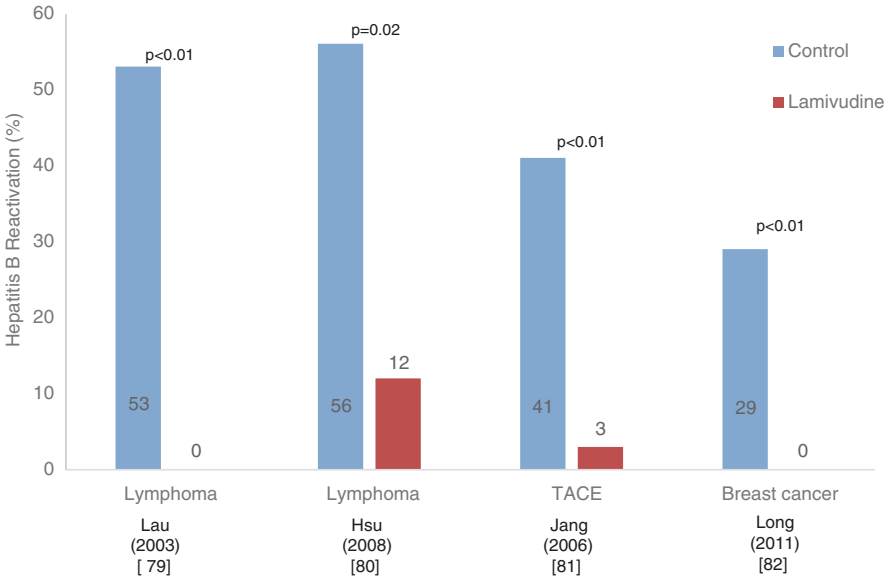


Fig. 18.4 Results from multiple trials showing the effect of lamivudine in the prevention of HBR

some of the details in their recommendations, all suggested that the initial screening should be performed with HBsAg and anti-HBc. Regarding anti-HBc testing, it can be either total anti-HBc or anti-HBc immunoglobulin G but, not immunoglobulin M.

An approach to diagnose all patients at risk of HBR would be universal screening—namely testing every patient for HBV infection before immunosuppressant therapy is instituted. For example, the US Food and Drug Administration (FDA) recommends healthcare providers to routinely screen all patients for HBV infection prior to the initiation of chemotherapy or immunosuppressive therapy. A study by Hwang found that case identification was substantially improved through universal screening compared to the usual practice (Hwang et al. 2012). Also, among patients with cancer, HBV screening rates based on risk factors have been reported to be low, ranging from 19% to 55% (Hwang et al. 2012, 2013; Visram et al. 2015) despite a high prevalence of HBV risk factors in this group of patients (Hwang et al. 2018). Universal screening is not widely practiced because oncologists often do not perceive its benefit to be large enough to justify the efforts and expenses needed, particularly in low HBV prevalence settings such as the United States general practice. To date, available cost-effectiveness analyses suggested that universal screening with HBsAg and anti-HBc is not cost-effective in palliative or adjuvant setting for solid tumors but it is cost-saving for lymphoma patients undergoing chemotherapy with a rituximab-containing regimen (Day et al. 2011; Zurawska et al. 2012).

An alternate strategy in screening for HBR prophylaxis candidates is to stratify individual patients according to their risk of HBR. Based on the immunosuppressive regimen and the serologic profile (see Table 18.2), the patient may be classified as high, moderate, and low risk. High- and moderate-risk patients should receive HBV

screening; whereas in low-risk patients, screening may be reserved for those who meet the standard HBV screening criteria. Patients are screened for HBsAg and anti-HBc, followed by HBV DNA, if either is positive. The advantage of this risk-stratification strategy is that it is more likely to be cost-effective than universal screening and reduce the potential harm of false-positive results. However, it is limited by the complexity of its application.

Regarding the utility of anti-HBs test and the management of HBR, it is often believed that the presence of anti-HBs makes it less likely that the patient will experience HBR. However, the role for screening for anti-HBs before immunosuppressive therapy has not yet been established. HBR may occur despite the presence of anti-HBs, particularly in patients undergoing the deepest level of immunosuppression (e.g., HSCT), in whom HBR may occur in conjunction with HBs seroreversion. Thus, it may be the safest not to use anti-HBs status in determining the need for antiviral prophylaxis regardless of the risk level.

7.2 Antiviral Prophylaxis Algorithm

The next set of questions in the management of patients at risk of HBR addresses (1) who are candidates for antiviral prophylaxis and (2) what antiviral regimen should be used. Although there are slight differences in the recommendations for the eligible patients for prophylactic antiviral therapy between professional society guidelines (Table 18.1), we propose an algorithm as shown in Fig. 18.5. Once a decision is made how screening is performed (universal versus risk stratified), the patient should be tested, at the minimum, for HbsAg, and anti-HBc. Depending on the patient's risk profile, additional testing for hepatitis C, human immunodeficiency virus, or hepatitis D (if HBsAg is positive) may also be considered.

In a patient who is negative for both HBsAg and anti-HBc, there is no need for antiviral prophylaxis. All patients with HBsAg-positive, however, should receive antiviral prophylaxis regardless of the risk. If the patient is only anti-HBc-positive, the risk of HBR needs to be assessed. If the patient meets the high-risk criteria (Table 18.2), prophylactic antiviral treatment is indicated, whereas in a patient who is low risk, prophylaxis is not recommended. In patients who are at moderate risk, antiviral prophylaxis is preferred. However, the evidence to support the recommendation is not very robust and an alternate approach may be to monitor HBV DNA levels for early detection and prompt treatment for HBR. There is no consensus about optimal ways to monitor for HBR both during and after cessation of immunosuppressive therapy, although some have suggested a monitoring interval of 3 months (Hwang and Lok 2014). In our opinion, the upfront institution of antiviral prophylaxis obviates the cost and inconvenience of repeated HBV DNA testing, especially if the antiviral therapy can be delivered inexpensively. In patients who place a higher value on avoiding any long-term use of antiviral therapy and costs associated with its use and consider avoiding the small risk of reactivation less important, it may be reasonable to choose no prophylaxis with close monitoring over antiviral prophylaxis, particularly if HBsAg is negative.

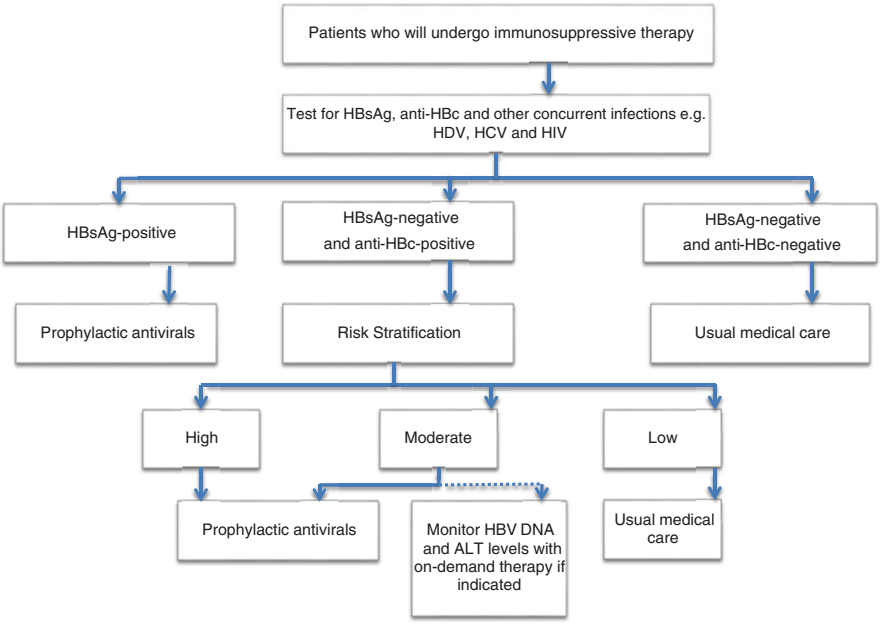


Fig. 18.5 Algorithm for HBV screening and antiviral prophylaxis to prevent HBR in nontransplant patients undergoing immunosuppressive therapy

With regard to the choice of prophylactic antiviral agents, lamivudine has been most widely studied (Yeo et al. 2004a, c; Lau et al. 2003; Hsu et al. 2008; Jang et al. 2006; Loomba et al. 2008; Ahmed and Keeffe 1999; Kohrt et al. 2006; Li et al. 2006; Rossi et al. 2001; Nagamatsu et al. 2004; Dai et al. 2004). Those studies showed that lamivudine improved the outcome of patients by reducing the occurrence of HBR, HBV-related acute liver failure, and HBV-related mortality, resulting in lower likelihood to delay or interrupt chemotherapy and, ultimately, positively impacted the outcome of the cancer therapy. While lamivudine was effective in proving the concept of antiviral prophylaxis of HBR, it has fallen out of favor in the treatment of chronic HBV infection in general, due in part to its susceptibility to viral mutations that negate its efficacy and to its lower potency compared to more modern agents. The majority of the guidance from professional societies prefers third-generation nucleoside/nucleotide analogues (NAs), e.g., tenofovir (TDF or TAF), entecavir over lamivudine for HBR prophylaxis. A randomized controlled trial showed the superiority of entecavir over lamivudine in decreasing the risk of HBR, hepatitis B flare, and interruption of immunosuppressive therapy (Huang et al. 2013, 2014). There are other studies with a similar conclusion, although the quality of those studies is not as robust.

A counterargument in favor of lamivudine is that most patients receiving antiviral prophylaxis have low or undetectable levels of HBV DNA at baseline and lamivudine failure is expected to be infrequent. In patients who put a higher value on the

cost of antiviral therapy and a lower value on avoiding the potentially small risk of resistance development, it may be reasonable to select the least expensive anti-HBV medication over more expensive antiviral drugs with a higher barrier to resistance. In patients with undetectable viral load with an expected duration of prophylaxis for 6 months or less, lamivudine may be acceptable.

Data are sparse as to the optimal timing of the initiation and discontinuation of antiviral prophylaxis. In our practice, we try to start HBR prophylaxis as soon as the need is determined. For patients with low or undetectable viremia, prophylaxis initiation concurrent to immunosuppressive therapy would be sufficient. In patients with higher levels of HBV DNA, we expect it to be advantageous if HBR prophylaxis can precede the onset of immunosuppression. However, immunosuppressive therapy, especially cancer chemotherapy, should not be delayed on account of achieving viral suppression. With regard to the duration of therapy, most experts recommend the antiviral prophylaxis to continue for 6–12 months after discontinuation of immunosuppressive therapy, although this duration of therapy has not been studied in a randomized controlled trial fashion. For individuals receiving B cell-depleting agents, e.g., rituximab, the prophylaxis should be continued for 12–18 months after the last dose of B cell-depleting agents. HBV DNA and liver test may be monitored every 3 months during prophylaxis and for 12 months after antiviral cessation as the immune recovery may be delayed, with HBR seen up to a year after the last dose (Table 18.1) (European Association for the Study of the Liver 2017; Terrault et al. 2018b; Ceccarelli et al. 2012).

7.3 Treatment of Established HBR

Any abnormalities in liver tests of patients undergoing immunosuppressive therapy or chemotherapy need to be carefully investigated. It is important to differentiate HBR from various potential causes including infections from another hepatitis virus (hepatitis A, C, D, or E), opportunistic pathogens (e.g., cytomegalovirus), and drug-induced liver injury or other causes (e.g., graft versus host disease or sinusoidal obstruction syndrome). In patients who have been screened for HBV infection and deemed to be at a moderate risk for HBR, and elect to be monitored without prophylactic antiviral therapy, HBR may be diagnosed early by rising HBV DNA levels before biochemical or clinical evidence of hepatitis activities emerges. Whether employing the so-called "on-demand" rescue therapy in that setting is inferior to upfront prophylaxis remains uncertain. In patients who were not screened initially and develop active hepatitis B, HBR may be misdiagnosed as acute HBV infection since anti-HBc IgM may be detected in severe hepatitis B flare (Law et al. 2016).

Once the diagnosis of HBR is established, the treatment goal is to prevent severe hepatitis and hepatic failure. This may be achieved by (1) effective and expeditious viral control and (2) monitoring and supportive treatment for hepatic insufficiency. To achieve viral control, potent NAs must be initiated as soon as possible, although high-quality evidence demonstrating the efficacy of antiviral therapy in reducing morbidity and mortality in patients with HBR is lacking (Liao et al. 2002). Delay in

the institution of antiviral therapy may lead to hepatic failure, liver transplantation, or death. Interferon-based therapy is inappropriate in this setting (Lok et al. 1991; Lau et al. 2003; Jindal et al. 2013; Hsu et al. 2014).

With regard to the choice of antiviral agents, there have been no randomized studies of the clinical effectiveness in HBR therapy, for example, comparing third-generation NAs with earlier generation agents. In part based on data in immunocompetent patients, most guidelines recommend entecavir or tenofovir in this setting (Perrillo et al. 2015a). In patients whose HBR progresses to symptomatic hepatitis and develops signs of hepatic insufficiency, urgent treatment with the most potent agent to stop the ongoing necro-inflammation and preserve functioning hepatocyte mass is particularly important. While the definitive treatment for liver failure would be liver transplantation, rarely patients with HBR are candidates for liver transplantation because of their underlying disease (Noterdaeme et al. 2011). However, we believe that these patients should be cared for by a team of healthcare providers with hepatology expertise to maximize support and afford a chance for recovery.

There are little data to define the optimal duration of therapy—it may take patients with established HBR longer to bring HBV replication under control compared to patients with low viral burden undergoing antiviral prophylaxis (Hwang and Lok 2014). In patients with satisfactory viral control, we believe the duration of antiviral therapy needs to be individualized based on (1) the severity of hepatitis activity, (2) baseline viral status, and (3) height of HBV DNA flare. In patients with mild asymptomatic HBR, antiviral therapy may be continued for 6–12 months after discontinuation of immunosuppressive therapy or 12–18 months in patients treated with a B cell depleting regimen. In contrast, if a HBsAg-positive patient presented with severe flare, applying the standard treatment guideline (indefinitely or until HBsAg loss) may be the safest course of action.

7.4 Management of Transplant Recipients

In addition to being subjected to immunosuppression, organ transplant recipients may develop HBR as a result of transmission of donor-derived HBV. The risk of HBV transmission is highest in liver transplantation, since hepatocytes are the primary site of HBV infection, moderate in kidney transplantation, and lowest in thoracic organ transplantation. Several management guidelines have been published in order to enhance the quality of care and improve the efficiency of HBR prevention in transplanted patients (Tomblyn et al. 2009; Kasiske et al. 2010). Care of liver transplantation patients is discussed elsewhere.

All potential organ donors and recipients should be tested for HBsAg, anti-HBs, and anti-HBc. Those with either HBsAg-positive or anti-HBc-positive should be tested for ALT and HBV DNA. Recipients who are HBsAg-positive or detectable HBV DNA should receive antiviral prophylaxis. Whenever possible, HBsAg-negative candidates should be immunized against HBV and the response to vaccination should be confirmed. All transplant candidates with evidence of active HBV

DNA replication (either HBsAg-positive or detectable HBV DNA) should be evaluated for the degree of liver fibrosis prior to the transplantation, since advanced fibrosis/cirrhosis can increase treatment-related morbidity and mortality. Transplant recipients who are HBsAg-negative/anti-HBc-positive may be managed in a similar fashion as immunosuppressed patients at moderate risk—they may be given antiviral prophylaxis or monitored for HBV DNA level for early detection of HBR followed by preemptive treatment.

Multiple studies have shown that non-liver solid organ transplant recipients with HBV infection have a higher liver-related complications and higher mortality rates with the largest experience in KT (Fabrizi et al. 2005; Lee et al. 2016). Prior to the availability of oral antiviral agents, recipients with HBsAg-positive had 2.5-fold increased risk of death and 1.4-fold increased risk of allograft loss compared to HBsAg-negative recipients (Fabrizi et al. 2005). More recent data in the era of oral antiviral agents indicate improved survival of KT recipients with HBV infection. Five-year survivals of KT recipients with and without HBV infection were 85% versus 86%, respectively. Graft survival was also similar approximately at 75% (Reddy et al. 2011). To effectively prevent HBR in HBsAg-positive recipients, antiviral therapy should begin before or at the time of surgery and continue indefinitely, regardless of ALT and HBV-DNA status, as the HBR after transplantation cannot be easily predicted with these parameters.

In the non-liver solid organ recipient with HBsAg-negative who receives an organ from HBsAg-negative but anti-HBc-positive donor, the risk of acquiring HBV infection is very low. In those with anti-HBs-positive, the risk is even lower. A shared decision may be made with the patient (1) monitoring for HBR without antiviral prophylaxis by following ALT and HBV DNA every 3 months for the first year posttransplant and after receipt of T cell depleting therapies, such as anti-thymocyte globulin or (2) proceeding to antiviral prophylaxis and continue for 6–12 months to cover the period of maximum immunosuppression. In those with anti-HBs < 10 mIU/mL, vaccination is highly recommended (Terrault et al. 2018b).

For patients undergoing HSCT, the majority of the society guidelines recommend starting antiviral prophylaxis for both HBsAg-positive and HBsAg-negative but anti-HBc-positive. In addition, the American Society for Blood and Marrow Transplant recommends that HBsAg-negative recipients receiving stem cells from an HBsAg-positive donor should be immunized prior to the chemotherapy. They should receive the initial two doses 3–4 weeks apart, followed by the third dose 6 months later, preferably all three doses given prior to HSCT. If the complete vaccination is not practical or the anti-HBs titer is <10 IU/L post-vaccination, the recipients should receive the hepatitis B immunoglobulin (HBIg) 0.06 mL/kg before the stem cell infusion. After the immune recovery, seronegative recipients who fail to raise the anti-HBs titer should be revaccinated.

HSCT donors with detectable HBV DNA should be treated with antivirals for at least 4 weeks or until HBV DNA becomes undetectable. The cell volume from HBsAg-positive and/or anti-HBc-positive should be minimized and all cell products are tested for HBV DNA at the time of harvest. If HBV DNA is detectable at harvest either in the donor or in the harvested cells, the recipients should receive antiviral

prophylaxis from day zero to at least 6 months after discontinuation of the immunosuppression, and optionally HBIg at four weeks after transplantation. If HBV is undetectable in the donor and harvested cells, recipients may be monitored with monthly ALT for the first 6 months. If ALT increases, HBV DNA and HBsAg should be tested. If there is detectable HBV DNA or HBsAg-positive, preemptive therapy is needed (Tomblyn et al. 2009).

8 HBR after Successful Treatment of Hepatitis C

HBV/HCV dual infection is not uncommon, especially in the endemic areas of HBV and among the high-risk population in which the two viruses share similar routes of transmission. The prevalence of dual infection with HBV has been reported from 5% to 20% of individuals with HCV infection (Chu and Lee 2008). In addition, occult HBV infection, defined by the presence of HBV DNA in the absence of HBsAg, may be found in 12% to 44% of HCV-infected patients (Fukuda et al. 1999). As commonly seen in patients with infection with multiple hepatotropic viruses, in HBV/HCV dual infection, one of the viruses predominates (as measured by the viral load), which tends to be HCV.

Direct-acting antivirals (DAAs) against HCV available today afford high rates of cure. Given that patients with HBV/HCV coinfection were excluded from most DAA clinical trials, the issue with HBR was brought to the attention only after the DAAs began to be used broadly. There have been increasing post-marketing reports suggesting that HBR could occur following DAA-induced control of HCV in both individuals with chronic and resolved HBV infection (Bersoff-Matcha et al. 2017). This concern prompted the US Food and Drug Administration (FDA) and the European Medicine Agency's Pharmacovigilance Risk Assessment Committee (PRAC) to issue warnings about the risk of HBR in patients with dual infection.

HBR in patients receiving DAA therapy reportedly occurs more frequently than those who were treated with interferon. An overall HBR rate of 14.5% was reported from patients following interferon-induced HCV eradication (Mucke et al. 2018). A meta-analysis by Mucke reported that the overall risk of HBR *during* DAA therapy was 24% in patients with untreated chronic HBV infection and 1.4% in those with resolved HBV infection. The risk of HBR-related hepatitis was 9% in the former, whereas no HBV-related hepatitis in the latter group. Although the majority of reported HBR events occurred between 4 and 12 weeks of DAA treatment (Chen et al. 2017), it may also occur *after* the end of DAA therapy especially in patients with concomitant HBV infection. The overall HBR rate after the end of DAA therapy was 41.4% in HBsAg-positive patients, compared to 0.9% in HCV patients with HBsAg-negative/anti-HBc-positive (Kanda et al. 2019).

Only limited data are available regarding the severity of liver disease of HBR in HBsAg-positive, HCV patients treated with DAAs (Bersoff-Matcha et al. 2017; Wang et al. 2017). Most reported cases were asymptomatic with the increase of HBV DNA (Wang et al. 2017). However, there have been at least two cases that

progressed to liver failure, resulting in death in one and liver transplantation in the other (Bersoff-Matcha et al. 2017). Risk factors associated with HBR during or after DAA therapy and the risk of progression to liver failure are yet to be determined. We believe it is also important to assess the liver fibrosis status, which is commonly performed in preparation of the HCV therapy.

Patients with HBV/HCV coinfection who meet the criteria for therapy for chronic HBV infection should be started on antivirals. For those with HBsAg-positive but do not meet the criteria for therapy, the European Association for the Study of the Liver (EASL) recommended antiviral prophylaxis in a similar fashion with the high-risk group of those undergoing immunosuppressive therapy given that the HBR occurrence rate is >10%, whereas AASLD and the Asian Pacific Association for the Study of the Liver (APASL) recommend close HBV DNA level monitoring. AASLD favor trending the level every 4–8 weeks during treatment and until week 12 post-DAA treatment while APASL recommends monitoring up to 24 weeks post-DAA treatment.

For those with resolved infection (HBsAg-negative, anti-HBc-positive), the risk of HBR is very low and all societies recommend ALT monitoring and testing for HBV DNA and HBsAg if ALT levels increase or fail to normalize during treatment or post-DAA treatment. We illustrate an algorithm for patients with potential HBR in the setting of DAA therapy in Fig. 18.6.

There is no data available to inform the optimal duration of HBV therapy in dual infection patients being treated for HCV. To the degree that there may be host immunological shift that underlies the development of HBR, we concur with the EASL guideline that the patient should continue the prophylaxis for another 12 weeks after discontinuation of DAAs. Also, in our practice, we monitor these patients for another 3 months to ensure the absence of HBR off anti-HBV prophylaxis. Obviously, in patients determined to be candidates for HBV therapy independent of HCV, therapy should be continued until the planned endpoint is met. Care must be taken in discontinuing anti-HBV prophylaxis in patients with cirrhosis, which may precipitate hepatic decompensation.

9 Current Challenges and Future Directions

HBR leading to a poor patient outcome such as liver failure or disruption of cancer chemotherapy represents an unnecessary clinical tragedy, which is eminently preventable by appropriate screening and prophylaxis. Despite a multitude of guidelines to inform clinicians caring for patients undergoing cancer treatment, transplantation, and immunomodulatory therapy, HBR continues to occur (Patel et al. 2016; Yuen 2016). Survey studies conducted in practicing physicians indicate that adherence to routine HBV screening prior to the immunosuppressive therapy remains low—approximately 20–40% of oncologists, 40% of dermatologists, and 70% of rheumatologists follow a guideline in some fashion (Hwang et al. 2012; Stine et al. 2010, 2011; Tran et al. 2010; Kawsar et al. 2012).

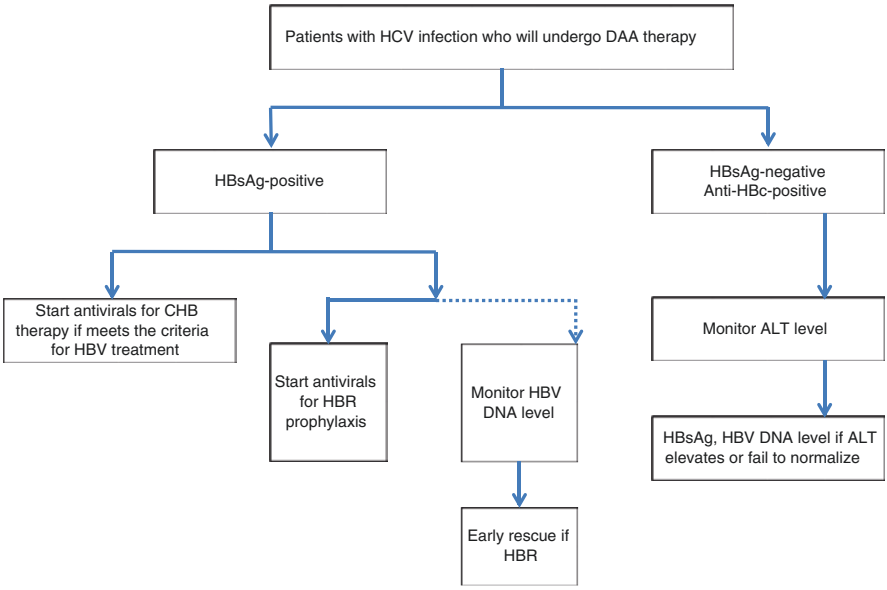


Fig. 18.6 Algorithm for HBV screening and antiviral prophylaxis to prevent HBR in HCV patients undergoing direct-acting antiviral therapy

This problem may be partly attributable to the inconsistency among the guidelines. Clearly, multi-society collaboration to develop a broadly applicable consensus is an essential step. Secondarily, efforts to disseminate the consensus guideline to all practitioners are needed. For healthcare providers that are not routinely involved in the care of patients at risk of HBR, notification and/or order sets in the electronic medical systems may be helpful to alert the provider to screen for HBV infection and to guide them to initiate appropriate prophylaxis. Such a proactive measure maybe even more important in the future, as increasingly more complex and potent immunosuppressive and chemotherapeutic regimens are being developed.

As investigators strive toward gaining more virological insight and immunopathogenetical knowledge of HBV infection, a deeper understanding of the basic mechanisms of HBR may help better inform clinical decisions. This may be particularly true of the HBV/HCV dual infection cases. In addition, the effect of new therapeutic agents that interact with the immune system in a nonconventional manner on the occurrence and course of HBR remains to be studied. Finally, as new diagnostic biomarkers and therapeutic agents are being actively developed for the goal of "cure" of HBV, additional tools may become available to provide more accurate risk stratification and then inactivate, if not cure, HBV in a sustainable fashion in patients undergoing increasingly sophisticated regimens that have a diverse effect on the immune system.

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Novel Therapy for Functional Cure of Chronic Hepatitis B Virus Infection

19

Lung-Yi Mak and Man-Fung Yuen

Abstract

Functional cure is the currently preferable and optimal treatment endpoint, which refers to sustained seroclearance of HBsAg with or without seroconversion of antibody to HBsAg. Functional cure is associated with improved clinical outcomes. The unsatisfactorily low rate of functional cure achieved by currently approved therapies [i.e., nucleos(t)ide reverse transcriptase inhibitor (NRTI) or pegylated interferon] triggers the ongoing search for new treatment approaches. Cessation of long-term NRTI aiming at subsequent functional cure has led to heterogeneous results in patients with different baseline characteristics. Meticulous patient selection is required with efforts to identify patients with favorable factors including Caucasian ethnicity and low HBsAg level. For novel agents, reduction of viral burden and enhancement/restoration of host immunity are equally important. Most agents currently in clinical phase of development demonstrated favorable results in suppression of viral proteins and genomic materials, and some agents will enter phase 3 clinical trials for further evaluation. Safety data is of paramount importance. The future treatment regime will likely entail a combination of NRTI, virus-directing agent, and immune boosting agent. The best cocktail therapy is still unknown, and will need to be revealed by well-designed randomized controlled trials.

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1 Introduction

Chronic hepatitis B infection (CHB) affects 292 million people globally (Polaris 2018), and is a major risk factor of liver-related diseases. Hepatitis B surface antigen (HBsAg) positivity confers twofolds increase in risk of mortality from various causes including cirrhosis and hepatocellular carcinoma (HCC) (Si et al. 2019). Vaccination programs have reduced the incidence of CHB, especially in the younger age group (Ni et al. 2012; Liu et al. 2019), but mortality from CHB has not been curbed due to the high load of existing chronically infected patients. Many of these patients will require antiviral therapy—currently available ones include nucleos(t)ide reverse transcriptase inhibitor (NRTI) and pegylated interferon (PEG-IFN). Antiviral therapy, especially NRTI, is highly effective in achieving short-term goals, namely liver biochemical normalization and hepatitis B virus (HBV) DNA suppression (European Association for the Study of the Liver 2017; Terrault et al. 2018). Long-term goals of antiviral therapy including risk reduction of HCC and mortality have been demonstrated (Ju et al. 2018; Lin et al. 1999; Seto et al. 2017; Thiele et al. 2013). Nevertheless, most NRTI-treated patients require lifelong therapy to suppress viral replication, which creates issues of cost, adherence, and risks of therapy-related adverse events (Mak et al. 2016). In view of these concerns, the goals of therapy have been redefined and new treatment approaches are being developed, which will be reviewed in the following sections.

2 Functional Cure: Definition and Implications

The goal of developing novel therapies is to enhance cure in CHB, which refers to elimination of HBV, following which antiviral therapy can be stopped with minimal risks of virological relapse and ongoing liver damage which eventually will reduce the risk of cirrhosis and HCC. However, due to the presence of covalently closed circular DNA (cccDNA) and integrated HBV DNA, a *complete cure* and *sterilizing cure* removing these two viral forms respectively, are clearly infeasible at present and are expected to be unachievable in the coming decade. Therefore, it is generally accepted that *functional cure* is the currently preferable and optimal treatment endpoint, which refers to sustained seroclearance of HBsAg with or without seroconversion of antibody to HBsAg (anti-HBs).

There are several clinical benefits of achieving functional cure. Firstly, antiviral treatment can be safely stopped (except in patients with cirrhosis with detectable DNA) with a low risk of HBsAg seroreversion or virological rebound (Yip et al. 2017b; Seto et al. 2016). Secondly, functional cure is associated with fibrosis

regression after ≥3 years of HBsAg seroclearance (Mak et al. 2019a). Thirdly, functional cure is associated with a significantly reduced risk of HCC, especially if the onset of HBsAg seroclearance is below the age of 50 years (Yuen et al. 2008; Yip et al. 2017a; Kim et al. 2015). Fourthly, the risks of liver decompensation, need of liver transplantation and death are significantly lowered in patients who achieved functional cure (Anderson et al. 2020; Kim et al. 2014). Although there are doubtless clinical benefits obtained by achieving functional cure, the rate of achieving HBsAg seroclearance has, however, been disappointing with the current antiviral therapy (~1–2% per year). Table 19.1 summarizes the reported rates of achieving

Table 19.1 Reported rates of HBsAg seroclearance

Author	Type	Country and sample size	Median FU/ timing of outcome assessment	Rate of HBsAg seroclearance (HBeAg status)
Liu J et al. (2010)	Spontaneous	Taiwan (n = 3087)	8.04 years	2.26% annually (mixed)
Tai DI et al. (2010)	Spontaneous	Taiwan (n = 662)	13.6 years	10.2% cumulative (mixed)
Simonetti J et al. (2010)	Spontaneous	Alaska (n = 1271)	19.6 years	0.7% annually, 12.4% cumulative (mixed)
Fung J et al. (2014)	Spontaneous	Hong Kong, China (n = 775)	25 years	23.6% cumulative (HBeAg−)
Lim TH et al. (2015)	Spontaneous	New Zealand (n = 572)	28 years	1.34% annually (mixed)
Hara T et al. (2014)	ETV treated	Japan (n = 553)	3 years	3.5% in 5 years (mixed)
Ko KL et al. (2020)	ETV-treated	Hong Kong, China (n = 1225)	6.6 years	5.2% (mixed) cumulative
Lam YF et al. (2017)	ETV treated	Hong Kong, China (n = 222)	7 years	2.5% (HBeAg−) cumulative
Buti M et al. (2015)	TDF treated	Spain (n = 585)	7 years	11.8% (HBeAg+) 1.3% (HBeAg−) Both are cumulative
Wong V et al. (2010)	PEG-IFN treated	Hong Kong, China (n = 85)	6.1 years	2.4% (HBeAg+) cumulative
Marcellin P et al. (2009)	PEG-IFN-treated +/− lamivudine	Multicenter (n = 230)	3 years	8% (HBeAg−) cumulative
Ahn SH et al. (2018)	PEG-IFN + TDF treated	Multicenter (n = 186)	2.3 years	10.4% (mixed) cumulative
Chan HLY et al. (2016)	TAF treated	Multicenter (n = 576)	1 year	1% (HBeAg+) cumulative
Buti M et al. (2016)	TAF treated	Multicenter (n = 285)	1 year	0% (HBeAg−) cumulative

ETV entecavir, *FU* follow-up, *HBeAg+* hepatitis B e antigen positive, *HBeAg−* hepatitis B e antigen negative, *PEG-IFN* pegylated interferon, *TAF* tenofovir alafenamide, *TDF* tenofovir disoproxil fumarate

functional cure in treatment-naive or antiviral-treated patients (Liu et al. 2010; Tai et al. 2010; Simonetti et al. 2010; Fung et al. 2014; Lim et al. 2015; Hara et al. 2014; Ko et al. 2020; Lam et al. 2017; Buti et al. 2015, 2016; Wong et al. 2010; Marcellin et al. 2009; Ahn et al. 2018; Chan et al. 2016).

3 Novel Therapeutic Approaches

In view of the low rates of achieving functional cure with the currently available therapy, novel treatment approaches have been explored, aiming to boost the rate of HBsAg seroclearance. These approaches can be divided into 3 types: induction of "good flare" by cessation of long-term NRTI, inhibition of alternative steps of viral replication (i.e., virus-directing agents) and enhancement of host immunity (immunomodulatory agents). Only agents that are already in the clinical phase of development will be discussed.

3.1 Induction of "Good Flare" by Cessation of Long-Term NRTI

The duration of long-term NRTI is not well defined. While some international guidelines suggest indefinite duration of NRTI (European Association for the Study of the Liver 2017; Terrault et al. 2018), others have recommended that NRTI can be discontinued in HBeAg-negative patients after a period of consolidation therapy following DNA undetectability (Sarin et al. 2016). This approach has been attempted, leading to variable rates of virological relapse (9–91% at the first year) after treatment cessation (Seto et al. 2015; Jeng et al. 2016, 2018; Wang et al. 2016; Berg et al. 2017; Liem et al. 2019). Such an approach also revealed that by inducing a virological flare through NRTI cessation, which is sometimes followed by a mild biochemical flare, some patients will develop HBsAg seroclearance afterward, i.e., a "good flare." In a recent study that assessed the peripheral lymphocyte populations as part of the investigation in patients who stopped long-term NRTI, 8/27 (30%) patients achieved HBsAg seroclearance at 34 months of follow-up. Biochemical flare (i.e., elevation of alanine aminotransferase, ALT) was observed in all patients who subsequently developed HBsAg loss and no patients developed decompensation. Although the HBV-specific cytotoxic T cell response following NRTI withdrawal did not differ significantly among patients who achieved HBsAg loss compared to those with virological relapse necessitating retreatment, the study findings support the notion of induction of "good flare" as a preceding event of functional cure (Garcia-Lopez et al. 2020).

Ethnicity is an important parameter that affects the rate of HBsAg seroclearance in HBeAg-negative patients who stopped long-term NRTI. For instance, the rate of HBsAg seroclearance was 19% in 3 years in patients of European descent (Berg et al. 2017) compared to 0–1.78% (in 1 year/ annually) in patients of Asian descent (Seto et al. 2015; Jeng et al. 2018) who stopped long-term NRTI. This observation is recently consistently demonstrated in the RETRACT-B study, which involved an

international cohort (88% Asian, 10% Caucasian) of HBeAg-negative patients who stopped long-term NRTI therapy. The rate of HBsAg seroclearance increased steadily over time: 3% at 1 year, 8% at 2 years, 12% at 3 years, and 14% at 4 years post-NRTI cessation. Caucasian (compared with Asian: 41% vs 11%, $P < 0.001$) was independently associated with a hazard ratio of 5.8 for HBsAg loss (Hirode et al. 2020). This highlights the importance of careful patient selection for adopting this approach to induce functional cure.

3.2 Inhibition of Alternative Steps of Viral Replication

NRTIs inhibit the DNA polymerase, i.e., only one of the many steps of HBV replication inside an infected hepatocyte. There are multiple classes of novel agents that target alternative steps of viral replication, including viral entry, interference of RNA transcriptional activity, capsid formation/encapsidation, and viral protein export (Fig. 19.1). Table 19.2 summarizes the virus-directing agents for CHB that are currently in clinical phase of development.

3.2.1 Inhibition of Viral Entry

Sodium taurocholate cotransporting polypeptide (NTCP) is the functional receptor for HBV entry, which uses its surface lipopeptide pre-S1 for docking to the

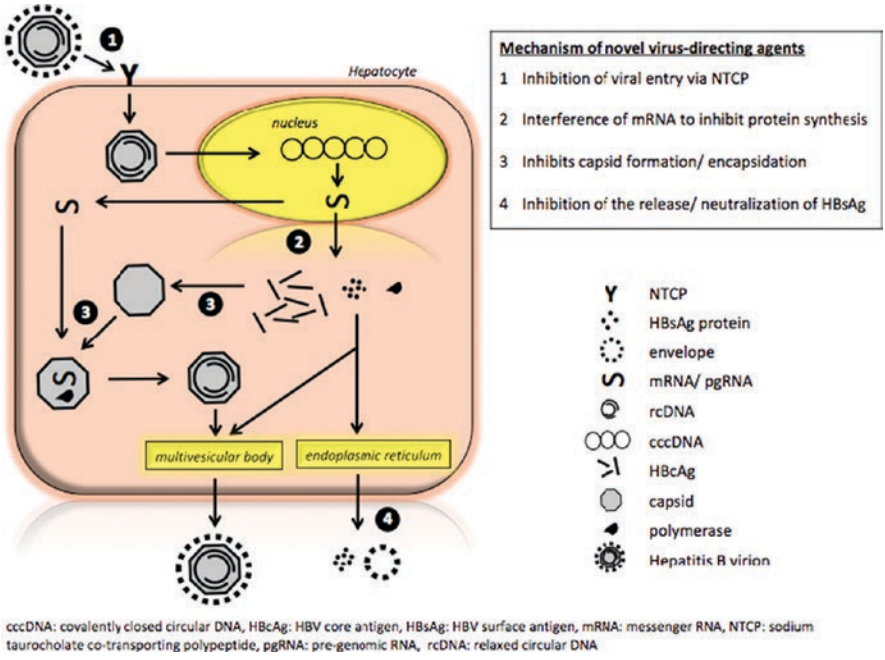

Fig. 19.1 Target sites and mechanisms of novel virus-directing agents. Adapted with permission from Mak LY et al. (2019b)

hepatocyte via NTCP (Yuen and Lai 2015). Myrcludex B, now named Bulevertide (brand name Hepcludex), works by blocking NTCP and thereby inhibits viral entry. This drug has been approved for medical use in the European Union in July 2020 for patients with chronic hepatitis D in the presence of CHB. Although initial 24-week combination of Myrcludex B with PEG-IFN in HBV + HDV did not show

Table 19.2 Novel antiviral agents in clinical phase of development

		Remarks	Drug names	Company	Phase
1	Inhibition of viral entry	NTCP	Myrcludex B/ Bulevertide	MYR GmbH, Germany	II
2	RNA interference	siRNA	Dicerna GAIXc-HBVS (RG 6346)	Roche, Switzerland (with Dicerna)	I and II
			JNJ 3989 (ARO-HBV 1 & ARO-HBV 2)	J&J with Arrowhead, USA	II
			ARB-729	Arbutus Biopharma, USA	I
			VIR-2218 (ALN-HBV)	Vir Biotech, USA	II
		ASO	GSK-836 (ISIS-358)/ GSK-404	Ionis with GSK, USA	II
3	Inhibition of capsid formation	CpAM/ CAM	GLS-4/ ritonavir	Sunshine Lake Pharma Co, Ltd., China	II
			ABI-HB0731 (Vebicorvir)	Assembly Biosciences, USA	II
			ABI-H2158	Assembly Biosciences, USA	II
			JNJ-6379 (JADE study)	Janssen, Ireland	II
			EDP-514	Enanta Pharma, USA	I
			Morphothiadin	HEC Pharma, China	II
			QL-007	Qilu, China	I
			ZM-H1505R	ZhiMeng Biopharma, China	I
			RG7907	Roche, Switzerland	I
			ABI-H3733	Assembly Biosciences	I
			ALG-000184	Aligos Therapeutics	I
4	Inhibition of HBsAg release	Nucleic acid polymer	REP 2139 or REP 2165	Replicor Inc., Canada	II
			ALG-010133	Aligos Therapeutics	I

Table 19.2 (continued)

		Remarks	Drug names	Company	Phase
5	Enhancement of innate/ adaptive immunity	TLR agonist	RG-7854 (TLR7)	Roche, Switzerland	I
			Vesatolimod (TLR7, GS-9620)	Gilead Sciences, USA	II
			Selgantolimod (TLR8 agonist, GS-9688)	Gilead Sciences, USA	II
		T cell	ASC22 (Anti-PDL1)	Ascletis Pharma, PR China	II
			Cemiplimab (Anti-PD1)	Regeneron, USA	I/II
			Nivolumab (Anti-PD1)	Bristol Myers Squibb, USA	I
			APG-1387 (apoptosis inducer)	Ascentage, China	II
			IMC-I109V (Tcell receptor-based)	Immunocore Ltd. (UK)	I/II
		Therapeutic vaccine	HeberNasvac (ABX-203)	CIGB, Cuba	III
			GS-4774	GobeImmune with Gilead, USA	II
			HepTcell	Altimmune, USA	II
			AIC649	Aicuris, Germany	I
			HB-110	Ichor Medical with Genexine, USA	I
			VTP-300	Vaccitech, USA	I
			JNJ 64300535	Janssen, Ireland	I
			BRII-179 (VBI-2601)	VBI Vaccines, USA	I/II
			TG-1050	Transgene, France	I
			INO-1800	Inovio, USA	I
6	Other mechanisms	Monoclonal antibody	GC1102	Green Cross, South Korea	II
			Vir-3434	Vir Biotech, USA	I

ASO antisense oligonucleotide, *CpAM/ CAM* core protein allosteric modulator, *HBsAg* hepatitis B surface antigen, *NTCP* sodium taurocholate, *siRNA* small interfering RNA, *TLR* toll-like receptor

superiority in HBsAg reduction, HBV DNA was significantly reduced compared to PEG-IFN monotherapy. Furthermore, continuation of combination therapy till 48 weeks was associated with a higher proportion of patients with HBsAg reduction >1 log IU/mL and/or with negative HBsAg compared to PEG-IFN monotherapy group (Wedemeyer et al. 2020). Phase 2 trials for patients with CHB (without HDV) are in progress.

3.2.2 Interference of RNA

RNA interference (RNAi) is a naturally occurring and biologically conserved mechanism for specific posttranscriptional gene silencing (Agrawal et al. 2003; Mello and Conte Jr. 2004). This mechanism is initiated by double-stranded RNA (dsRNA) in the cytoplasm that is cleaved by ribonuclease protein Dicer into shorter fragments (usually 20–30 nucleotide length). These are called small interfering RNAs (siRNAs), which contain a "guide" strand that will subsequently be degraded, and a "passenger" strand that binds to the Argonaute 2 protein (Ago2) that assembles with the RNA-induced silencing complex (RISC/Ago2), which allows binding to the complementary sequence to the target RNA by the guide strand. In the context of novel therapy for CHB patients, siRNAs are being developed to target viral transcripts and induce their degradation by the RISC/Ago2 complex. Another class of agents that silences genes is called the antisense oligonucleotide (ASO), which induces cleavage of HBV RNAs inside the nucleus and cytoplasm via RNase H1. Gene silencing will thereby occur by inhibition of protein expression.

The host RNA polymerase II uses cccDNA as a template, which contains four open reading frames (ORF) for transcribing viral RNAs that encode precore/core, polymerase, surface, and X protein. As all four viral transcripts are encoded in ORFs with a common 3′ end, a single-target RNAi can conveniently lead to degradation of all four viral transcripts from both cccDNA and integrated DNA. Apart from gene silencing property leading to reduction in viral protein synthesis, there is a consequential immunity-enhancing effect by immune restoration through rescuing from chronic viral antigen exposure (see below). Many RNAi therapies for CHB are currently evaluated in phase II clinical trials. In NRTI-treated patients, a combination of NRTIs with RNAi therapies resulted in sustained reduction in HBsAg, HBV DNA, HBV RNA, HBeAg, and hepatitis B core-related protein (HBcrAg). To enhancing hepatic uptake, all early siRNAs developed today are tagged with N-acetylgalactosamine (GalNAc), which preferentially binds to asialoglycoprotein receptor which is enriched in hepatocytes.

In the phase IIa study AROHBV1001, JNJ-3989 (siRNA) was given at three monthly doses of subcutaneous injections (100 mg, 200 mg, 300 mg, and 400 mg) in 40 patients with chronic HBV who were treated with NRTI. At the HBsAg nadir, 39/40 (97.5%) patients achieved a $> 1\log_{10}$ IU/mL (log) reduction from Day 1 HBsAg values, and 22 (56%) of these had sustained HBsAg reductions (>1 log) approximately 9 months after the last dose of JNJ-3989 (Gane et al. 2020a). Similar results were seen in the phase II study of VIR-2218 (another siRNA) in 24 patients with CHB who receive a combination of NRTI plus 2 doses (4 weeks apart) of subcutaneous VIR-2218 injection. In this study, all patients who received the 200 mg dose achieved ≥1 log reduction in HBsAg, which was maintained through week 24 (Gane et al. 2020c), suggesting prolonged suppression in HBsAg production after finite doses of RNAi in HBeAg-positive or negative CHB patients. RG-6346 is an S-targeting synthetic siRNA with unique "tetraloop" folded design that inhibits HBsAg from both integrated HBV DNA and cccDNA. In treatment-naïve noncirrhotic CHB patients, a single dose of 3 mg/kg RG-6346 induced a mean HBsAg reduction of 1 log, which was associated with transient ALT flares in some patients.

Monthly injections of total four doses in 12 NRTI-treated CHB patients, whose baseline HBsAg were 3.4–3.7 logs, led to sustained HBsAg reduction as much as 2.66 logs. Seven out of these 12 patients (58%) achieved HBsAg <100 IU/mL regardless of HBeAg status. Other viral markers including HBV DNA, RNA, HBeAg, and HBcrAg were significantly reduced (Yuen et al. 2020f). AB-729 is another synthetic siRNA. While a single dose of subcutaneous AB-729 in NRTI-treated CHB patients led to mean HBsAg decline of about 1 log maintained till week 12, six-monthly doses resulted in a progressive reduction in HBsAg (1.44 logs at week 16, and 1.73 logs at week 20). Other viral markers were suppressed—92.3% and 28.6% subjects had unquantifiable or undetectable HBV RNA and HBcrAg, respectively at week 12 (Yuen et al. 2020c).

ISIS505358/GSK3228836 (GSK836) is a type of ASO. In the phase IIa trial, 300 mg GSK836 was administered by subcutaneous injection on days 1, 4, 8, 11, 15, and 22 to NRTI-naive patients as well as patients receiving NRTIs (n = 17). Significant reductions in HBsAg were observed in both patient groups from baseline to Day 29. In the NRTI-naive group (n = 12), average reduction reached 1.56 logs (p = 0.001 vs placebo). Greater average reduction of 2.51 logs was observed in the group of NRTI-treated patients (n = 5). Across the treatment groups, 6 patients had HBsAg reductions >3.0 logs, with levels falling below the limit of quantification (0.05 IU/mL) in 4 patients (Yuen et al. 2020e). The magnitude of HBsAg reduction within a short time period suggests that ASO plus NRTI leads to a higher likelihood than NRTI alone to achieve early functional cure. Other viral markers including HBV RNA and HBcrAg were also suppressed in a dose–response manner and maintained on posttreatment follow-up phase. Other novel RNAi-based agents are shown in Table 19.2.

RNAi-based therapy is in general safe and well tolerated. More frequently observed adverse events are mild and include headache, nausea, and injection site reactions. ALT elevations were transient and mild especially in patients on NRTI, with no dose–response relationships with RNAi dosing. However, the trial of ARC-520—the first RNAi that entered the clinical phase of trial for CHB—was prematurely terminated due to death of nonhuman primates attributed to toxicity caused by ARC-520 excipient (not the siRNA product) despite encouraging results (Yuen et al. 2020a). This highlights the importance of prolonged duration of safety monitoring in drug development for CHB.

3.2.3 Inhibition of Capsid Assembly or Encapsidation

The core protein is essential for capsid formation, encapsidation, reverse transcription of pre-genomic RNA, virion formation, and cccDNA amplification (Mak et al. 2017). Core protein allosteric modulators (CpAMs or CAMs) are novel agents that target the step of capsid formation. Class 1 CpAMs lead to formation of aberrant capsids (inhibit proper shaping), and class 2 CpAMs lead to formation of empty capsids (inhibit encapsidation).

GLS4 is a class 1 CpAM, and ritonavir was added to increase plasma exposure level of GLS4 by inhibition of CYP3A4 metabolic enzymes and has been shown to enhance suppression of HBV RNA and hepatitis B core protein in the phase Ib trial

(Zhang et al. 2020b). In the phase II trial, when comparing GLS4 /ritonavir plus entecavir (ETV) with ETV alone, more patients receiving the combination therapy experienced ≥1.5 log HBsAg decline at week 24 compared to none receiving ETV monotherapy (12.5% vs. 0%, respectively). Moreover, the combination therapy was more effective than ETV monotherapy in suppressing other viral products, including HBV RNA (previously treatment-naïve: 3.53 vs. 0.73 log reduction, respectively; previously NRTI-treated: 1.55 vs. 0.16 log reduction, respectively) and HBcrAg (previously treatment-naïve: 1.32 vs. 0.65 log reduction, respectively) at week 24 (Zhang et al. 2020a).

JNJ-6379 (a class 2 CpAM) was investigated in the JADE study (phase IIa) in both treatment-naïve and treatment-experienced CHB patients. In the interim analysis, JNJ-6379 in combination with NRTI resulted in substantial HBV DNA and RNA reductions at week 24, whereas the degree of HBsAg reduction was modest (0.4 logs in HBeAg-positive and previously treatment-naïve patients). Those who had HBsAg decline mostly had HBeAg decline and frequently early on-treatment transient and isolated ALT flares. Due to cases of virological breakthroughs, JNJ-6379 will not be developed as a monotherapy, but will be developed in combination with NRTI (Janssen et al. 2020). ABI-H0731 (a class 2 CpAM), now called Vebicorvir, was investigated in phase IIa studies 202 and 201 in treatment-naive HBeAg-positive and NRTI-treated HBeAg-positive and HBeAg-negative patients for 24 weeks. The combination of Vebicorvir with NRTI was well tolerated and demonstrated faster and greater reductions in HBV DNA and HBV RNA than NRTI alone. After completion of the studies, eligible subjects would enter an open-label extension study 211 to receive Verbicorvir + NRTI for up to an additional 76 weeks. Apart from the known effects of strengthened suppression of HBV DNA and RNA, it was encouraging to observe that HBsAg and HBcrAg were also suppressed at continuation of combination therapy (Yuen et al. 2020d). Other CpAMs in clinical phase of evaluation are shown in Table 19.2.

With the efficacy and safety data of individual novel agents mentioned above, the triple combination of RNAi (monthly injections for 3 doses of JNJ-3989) + CpAM (daily oral doses of JNJ-6379 for 85 days) + NRTI (daily oral doses beyond the end of CpAM dosing) was explored in 12 CHB patients. The mean HBsAg reductions were 1.4 logs on day 85 and 1.8 logs in 7 patients with day 113 data. This combination therapy was in general well tolerated with no serious or severe adverse events reported. Grade 1 isolated ALT elevations were observed in 5 patients and were attributed to therapeutic flares (Yuen et al. 2019a). Longer follow-up data is awaited to prove the efficacy and safety of this strategy.

3.2.4 Inhibition of Viral Protein Export

Nucleic acid polymers (NAPs) block HBsAg (in the form of subviral particles) release from infected hepatocytes. REP 2139 or REP 2165 are NAPs and were investigated in combination with PEG-IFN and tenofovir disoproxil fumarate (TDF). In the phase 2 study, 40 HBeAg-negative patients were assigned to groups that received 48 weeks of experimental therapy (REP 2139/REP 2165 + TDF + PEG-IFN) or control (TDF + PEG-IFN) after initial 24 weeks of TDF. HBsAg below

detectable range (0.05 IU/mL) were observed in 60% patients overall, and 35% patients achieved functional cure upon 48 weeks of treatment-free follow-up. Serum ALT elevations were more frequently observed in the experimental group and correlated with initial decrease in HBsAg (Bazinet et al. 2020).

3.3 Enhancement of Host Immunity

As mentioned above, chronic viral antigen exposure weakens HBV-specific humoral immunity and leads to virus-specific T cell anergy, thereby causing immune exhaustion in the host. In addition to evidence of attenuated toll-like receptor (TLR)-mediated immune response and inhibitory cytokines (Jiang et al. 2014; Shin et al. 2016), recent studies have demonstrated that multiple inhibitory receptors, including programmed cell death (PD-1), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), T-cell immunoglobulin and mucin domain containing-3 (TIM-3), lymphocyte activation gene-3 (LAG-3), are overexpressed and play important roles in T cell exhaustion in patients with CHB (Boni et al. 2007; Schurich et al. 2011; Wu et al. 2012; Li et al. 2013). Novel therapies aiming at restoration or enhancement of host immunity are being actively developed. Table 19.2 summarizes those that are currently in clinical phase of development.

3.3.1 Toll-Like Receptor Agonist

TLRs are pattern-recognition receptors (PRRs) that recognize pathogen-associated molecular patterns, and TLR agonists stimulate various leukocytes in the innate and adaptive system. GS-9620 (TLR7 agonist), now named Vesatolimod, was shown to induce ISG15 in a dose-dependent manner in the phase II study that involved 162 CHB patients who were given various doses of Vesatolimod or placebo. However, no significant serum interferon (IFN) alpha expression and no HBsAg decline were demonstrated (Janssen et al. 2018). GS-9688 (TLR8 agonist), now named Selgantolimod, was given for 24 weeks with NRTI in a phase II study. This treatment regimen was shown to achieve a modest decline in HBsAg from baseline and 5% (1 out of 20) patients achieved functional cure. Selgantolimod induced dose-dependent cytokine responses (IL-12p40, IL-1RA, IFNγ) and shifts in peripheral immune cell subsets (Gane et al. 2020b). It is planned that further evaluation of Selgantolimod in combination with other antiviral agents with complementary immune and viral effects will be performed.

3.3.2 Enhancing T Cell Function

Recent cancer therapies have revealed the potential to restore T cell function by autoantibodies that block the inhibitory receptors. Among those discussed above, anti-PD-1 has been explored in CHB. Nivolumab is a monoclonal antibody against PD-1 that is approved for treatment of various solid organ tumors and lymphomas. In the phase Ib study using a reduced dose of nivolumab (0.1–0.3 mg/kg) in combination with GS-4774 (a therapeutic vaccine; see below) in NRTI-treated HBeAg-negative CHB patients, a modest reduction in HBsAg level (0.16 to 0.3 log reduction

at 12 weeks) was achieved and the treatment was well tolerated. In a patient who had baseline HBsAg of 3 logs IU/mL and developed functional cure at week 20, there was ALT flare between weeks 4 and 8 which was accompanied by an increase in peripheral HBsAg-specific T cells at week 24 (Gane et al. 2019). This finding supports the notion that suppression of viral burden precedes restoration of HBV-specific T cell function.

IMC-I109V is an immune-mobilizing monoclonal T cell receptor against virus (ImmTAV), a first-in-class bi-specific protein comprising a soluble affinity-enhanced T cell receptor (TCR, targeting domain) fused to an antibody single-chain variable fragment (effector domain). IMC-I109V TCR recognizes HBsAg presented by the human leukocyte antigen (HLA)-A*02:01 on the surface of infected hepatocytes. Upon engagement of TCR with HBsAg, the effector domain will bind to CD3 on any surrounding T cell, which is redirected to produce effector cytokines to destroy the hepatocyte that contains viral proteins and genomic materials including cccDNA and integrated DNA. In vitro study has confirmed that ImmTAV-Env can redirect T cells from healthy and HBV-infected donors toward HCC cells containing integrated DNA resulting in cytokine release that was suppressible by corticosteroid. The redirected T cells induced cytolysis of antigen-positive HCC cells and infected cells with HBV, causing a reduction of HBeAg and specific loss of cells expressing viral RNA (Fergusson et al. 2020). The phase I trial of IMC-I109V is currently underway. It is worth noting that in the initial phase of clinical trial, only CHB patients that are confirmed to be HLA-A*02:01 positive can be enrolled. The reported frequencies of this specific allele are highly variable among different ethnicities—Asians: 11–20%; Caucasians: 23–60% (Allele frequency net database (AFND) 2020). This is an example of adopting the approach of personalized medicine in the field of CHB. However, a HLA nonrestrictive approach (HLA-E) has been developed and this would allow drug target engagement in all patients (Leonard et al. 2020).

3.3.3 Therapeutic Vaccine

Unlike preventive vaccines that are highly immunogenic, therapeutic vaccines for CHB are only modestly effective in the context of established chronic infection. For instance, GS-4774 is a heat-inactivated, yeast-based, T cell vaccine that consists of highly immunogenic recombinant HBcAg, HBsAg, and HBx epitopes. In the phase II trial of 178 NRTI-treated CHB patients who received subcutaneous GS-4774 every 4 weeks until week 20, only 3 out of 50 patients receiving the highest dose of GS-4774 had HBsAg decline $\geq$0.5 logs at week 24, and no patients experienced HBsAg seroclearance (Lok et al. 2016). ABX-203, now named as HeberNasvac, is also a yeast-based vaccine that comprises HBsAg and HBcAg virus-like particles. In the long-term follow-up (5 years) of 6 patients with prior PEG-IFN treatment who were administered with ABX-203 intranasally every 2 weeks, 2 out of 6 developed HBsAg seroclearance. This study was limited by the small number of patients and the fact that all patients were given PEG-IFN beforehand, leading to ambiguity in interpretation of the treatment effect (Fernandez 2018). TG-1050 is an adenovirus 5-based vaccine that expresses HBV polymerase and domains of core and surface

antigen. In the phase I trial involving 48 NRTI-treated CHB patients, TG-1050 was shown to induce specific IFNγ-producing T cells. While minor decreases of HBsAg were observed, a number of subjects reached unquantifiable HBcrAg by the end of study (Zoulim et al. 2020). Although these results confirm target engagement of the therapeutic vaccines, efficacy data on enhancing functional cure of CHB is still being awaited.

4 Other Mechanisms

Monoclonal antibodies that neutralize HBsAg can potentially reduce viral burden in the serum. GC1102 is a recombinant monoclonal hepatitis B immunoglobulin (HBIg) with high affinity to HBsAg compared to HBIg derived from blood plasma of human donors. It has been shown to induce functional cure in 22.2% CHB patients whose baseline HBsAg were ≤ 1000 IU/mL after 7 weeks of treatment (Lee et al. 2018). VIR-3434 is another recombinant HBIg that is currently being evaluated in a phase I clinical trial.

Inarigrivir (SB9200) was an oral dual agonist of retinoic acid-inducible gene-1 (RIG-1) and nucleotide-binding oligomerization domain (NOD2) that are host PRRs that induce IFN-mediated antiviral immune response. It was demonstrated to induce HBsAg decline ≥0.5 logs from baseline in 22% patients who were associated with ALT flare (Yuen et al. 2019b). However, in January 2020, the developing pharmaceutical company prematurely terminated the phase IIb trial after the occurrence of unexpected serious adverse events, including one patient death in the Phase IIb CATALYST trial. This again highlights that safety is of utmost importance for a novel agent to move forward in the clinical stages of development.

5 Conclusion

The treatment landscape for CHB is evolving rapidly. Cessation of long-term NRTI is an "old-dog-new-trick" approach, which gives heterogeneous results in patients with different baseline characteristics. Careful patient selection is required with efforts to identify patients with favorable factors including Caucasian ethnicity and low HBsAg level. For novel agents, reduction of viral burden and enhancement/restoration of host immunity are interlinked and are equally important (Fig. 19.2). Most agents currently in clinical phase of development demonstrated favorable results in suppression of viral proteins and genomic materials, and some agents will enter phase 3 clinical trials for further evaluation. Safety data is of paramount importance. The future treatment regime will likely entail a combination of NRTI, virus-directing agent, and immune-boosting agent. The best cocktail therapy is still unknown, and will need to be revealed by well-designed randomized controlled trials.

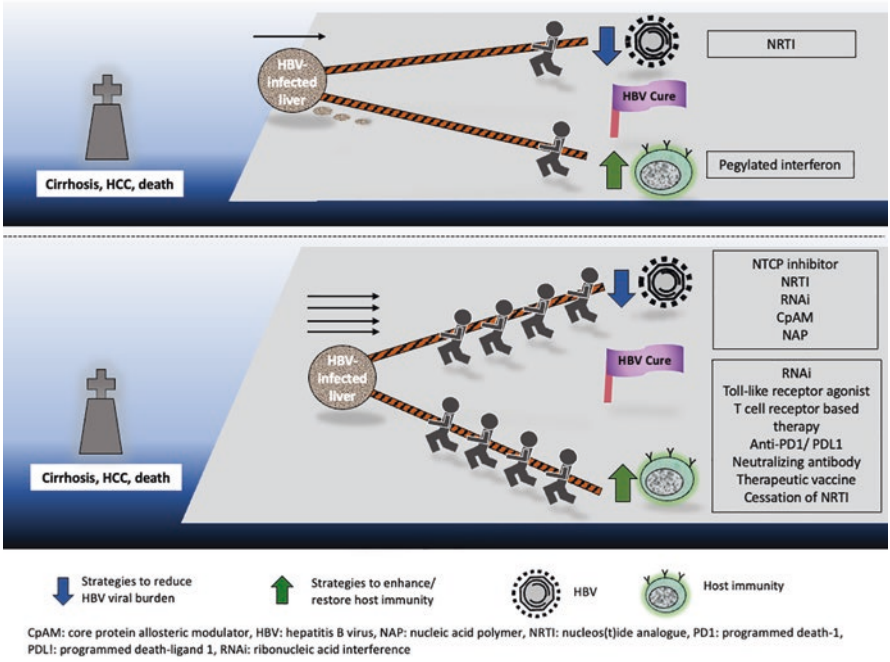

Fig. 19.2 Schematic diagram showing the strategies to enhance functional cure in chronic hepatitis B infection. *Upper panel:* Currently available therapeutic strategies; *Lower panel:* Novel therapeutic strategies, aiming at either reducing viral burden and/or enhancing or restoration of host immunity

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Is Cure of Hepatitis B Infection a Mission Possible?

20

Wen-Juei Jeng and Anna Suk-Fong Lok

Abstract

Chronic hepatitis B virus (HBV) infection is a major global health problem. Current antiviral therapy including pegylated interferon and nucleos(t)ide analogue is effective in reducing progression to cirrhosis, hepatic decompensation, and hepatocellular carcinoma but the rate of HBsAg seroclearance is low. In this chapter, we review the barriers to eradicate HBV and how they may be overcome. We also discuss how cure of chronic HBV infection should be defined and measured and the likelihood that new antiviral and immune-modulatory drugs in development can achieve the goal of HBV cure.

Keywords

Functional cure · Immune modulatory therapies · Direct-acting antiviral agents Hepatitis B surface antigen · Covalently closed circular DNA

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1 Introduction

The short-term goal of antiviral therapy of chronic hepatitis B virus (HBV) infection is to suppress HBV replication, thereby decreasing liver inflammation. Durable suppression of HBV replication has been shown to reverse liver fibrosis and to prevent cirrhosis, hepatic decompensation, hepatocellular carcinoma (HCC), and liver-related mortality (Liaw 2013; Lok et al. 2016a). Currently available antiviral therapies, including interferon (IFN) and nucleos(t)ide analogues (NA), are effective in suppressing HBV replication but they do not eradicate HBV and they do not completely eliminate the risk of HCC even in patients with complete virus suppression (Lin et al. 2007; Su et al. 2016; Papatheodoridis et al. 2017).

2 Definition of HBV Cure

In contrast to the recent scientific progress in hepatitis C where a short (8–12 week) course of well tolerated, orally administered direct-acting antiviral drugs (DAAs) can eradicate hepatitis C virus in more than 95% of patients with chronic hepatitis C (Pawlotsky 2020), with no evidence of reactivation even when these patients are subsequently immunosuppressed, cure for hepatitis B is more challenging.

To discuss whether the mission to cure hepatitis B is possible, consensus on the definition of HBV cure is needed. A *sterilizing cure* akin to that of hepatitis C, with complete eradication of HBV as reflected by sustained clearance of hepatitis B surface antigen (HBsAg) and undetectable HBV DNA, and elimination of covalently closed circular DNA (cccDNA) as well as integrated HBV DNA, is unlikely to be feasible. Indeed, even in persons who "recovered" from acute HBV infection with seroconversion from HBsAg to hepatitis B surface antibody (anti-HBs), cccDNA, and integrated HBV DNA are still present in the liver (Michalak et al. 1994; Torii et al. 2003; Murakami et al. 2004), and reactivation of HBV replication can occur when these persons are immunosuppressed (Yeo et al. 2009; Shi and Zheng 2020). Thus, experts have accepted a less ambitious goal—*functional cure*—sustained HBsAg clearance with or without seroconversion to anti-HBs and undetectable HBV DNA but continued presence of integrated HBV DNA and transcriptionally inactive cccDNA, after a finite course of therapy (Lok et al. 2017; Revill et al. 2019). This is comparable to patients with chronic HBV infection who spontaneously cleared HBsAg. These patients would still have residual liver disease but in the absence of other liver injuries, liver fibrosis will regress and risk of HCC will decrease over time. Functional HBV cure can be accomplished with currently available therapies—IFN or NA, but the rate of success is very low. Others have suggested a more pragmatic goal—*partial cure*. Patients with a partial HBV cure would remain HBsAg positive but hepatitis B e antigen (HBeAg) negative with undetectable serum HBV DNA after discontinuation of a finite course of treatment. Integrated HBV DNA and cccDNA with markedly decreased transcriptional activity, and inactive liver disease would still be present and the risk of HCC remains albeit at a lower

rate. This is akin to inactive carriers or patients with complete virus suppression on NA therapy (Table 20.1).

Given that the key measures of HBV cure rely on undetectable circulating HBsAg and HBV DNA, standardized assays with universally accepted lower limit of detection or quantification must be used for the detection of these markers. The lower limit of detection and the lower limit of quantification of most commercially available HBsAg assays is 0.05 IU/mL. However, new assays with improved sensitivity and lower limit of quantification of 0.0005 IU/mL are available (Lou et al. 2018; Matsubara et al. 2009; Takeda et al. 2013). Using these assays, studies have shown that as many as 50% of patients with HBsAg seroclearance after acute hepatitis B, and 48.2–94% of those with chronic HBV infection and spontaneous or treatment-related HBsAg seroclearance, based on current assays still have detectable HBsAg level by ultrasensitive assay (Shinkai et al. 2013; Ozeki et al. 2018). Similarly, the lower limits of detection and quantification of most commercially available real-time PCR assays for serum HBV DNA are 10–20 IU/mL but some

Table 20.1 Definitions of HBV cure

Serostatus/ clinical scenario	Sterilizing cure (Complete cure)	Idealistic functional cure	Realistic functional cure	Partial cure
Serology				
HBsAg/ anti-HBs	−/−	−/+	−/− or −/+	+/−
Serum HBV DNA	Undetectable	Undetectable	Undetectable	<2000 IU/ml or undetectable
HBeAg	Negative	Negative	Negative	Negative
Intrahepatic				
cccDNA concentration and transcription	Undetectable	Detectable but not transcriptionally active	Detectable but not transcriptionally active	Detected at lower concentration and decreased transcriptional activity
Integrated HBV DNA	Undetectable	May be Detected	Detected	Detected
Clinical outcome				
Comparable scenario	Never infected	Recovery after acute infection	Spontaneous HBsAg clearance after chronic infection	Inactive carrier state
Liver disease	None	None	Inactive, fibrosis regresses with time	Inactive
HCC risk	Similar to uninfected persons	Similar to uninfected persons	Declines with time	Lower risk than active hepatitis

assays such as in-house droplet-digital PCR can detect as little as 0.15–1.2 IU/mL (Liu et al. 2017; Yang et al. 2018). Using these more sensitive assays, studies have shown that as many as 27% of HBsAg positive persons and 3.6% of HBsAg negative persons and prior history of HBV infection, with undetectable serum HBV DNA using current assays still have detectable HBV DNA (Liu et al. 2017). It is not clear whether more sensitive assays should be used to assess HBV cure because data on the clinical significance (e.g., risk of viral relapse, HBV reactivation, and HCC) of residual low levels of HBsAg and/or HBV DNA detected by these ultrasensitive assays are limited.

Another complicating factor is the recent revelation that cccDNA is not the only source of circulating HBsAg. In fact, integrated HBV DNA may be a more important source of HBsAg in HBeAg-negative patients (Wooddell et al. 2017). Thus, a treatment could have rendered cccDNA transcriptionally inactive and no longer producing HBsAg but the key criterion of *functional cure* is not met because HBsAg continues to be translated from integrated HBV DNA. On the other hand, another treatment might appear to have resulted in HBsAg clearance when in fact HBsAg continues to be produced from cccDNA and failure to detect HBsAg in serum is merely due to it being bound with anti-HBs in immune complexes or selection of HBV S variants that produce altered HBsAg epitopes leading to false-negative results. The latter is more likely to occur if monoclonal and not polyclonal antibodies are used for capture and/or detection in serology assays for HBsAg.

In view of the fluctuating nature of chronic HBV infection, sustainability of HBsAg clearance must be defined. Studies of patients with HBsAg clearance either spontaneously or after IFN or NA treatment showed that the vast majority (82–92%) of patients who have at least two negative HBsAg test results more than 6 months apart have sustained clearance of HBsAg during follow-up to 2–5 years (Yip et al. 2017; Lok et al. 2020; Wu et al. 2020). The durability of HBsAg seroclearance is comparable whether it occurred spontaneously or after NA or Peg-IFN treatment (Yip et al. 2017; Kim et al. 2014; Stelma et al. 2017). Another important consideration is risk of viral relapse if treatment is discontinued after confirmed HBsAg clearance (2 negative test results ≥6 months apart). Limited data suggest that this is the case with rates of viral relapse (redetection of HBV DNA by PCR) of ~2% in patients who discontinued NA therapy after HBsAg clearance compared to >70% in patients who discontinued NA therapy after >2 years of HBV DNA suppression without HBsAg clearance (Lok et al. 2020; Papatheodoridis et al. 2016, 2018; Kim et al. 2020).

Another point that needs consensus agreement is whether seroconversion to anti-HBs must be accomplished to meet the definition of *functional cure*. Several studies showed that only 8.7%–44% of patients with spontaneous, IFN or NA related HBsAg clearance have detectable anti-HBs when HBsAg first become undetectable (Yip et al. 2017; Lok et al. 2020; Roushan et al. 2016). Indeed, even on follow-up, only 56–78% have detectable anti-HBs up to 1–2 years after HBsAg

clearance (Yip et al. 2017; Lok et al. 2020). Thus, a requirement for simultaneous anti-HBs seroconversion might result in lower rates of functional cure or would require continuation of treatment for several years to meet the definition of a functional cure. At issue is whether seroconversion to anti-HBs is necessary to maintain durable HBsAg clearance after treatment is stopped. Limited data suggest that seroconversion to anti-HBs is not critical for durable HBsAg clearance (Yip et al. 2017; Kim et al. 2014). Thus, the current consensus is that, confirmed HBsAg clearance with repeat test at least 6 months apart but not seroconversion to anti-HBs is required to meet the definition of HBV *functional cure*, and undetectable HBsAg and HBV DNA based on testing with currently available assays with limits of quantification/detection of 0.05 IU/mL and 10–20 IU/mL, respectively, would suffice (Cornberg et al. 2019).

A more fundamental issue is whether HBsAg clearance confers an additional benefit in improving clinical outcomes compared to suppression of HBV DNA to undetectable levels. Several studies have shown that durable HBV DNA suppression in the absence of HBsAg clearance is sufficient in decreasing the risk of cirrhosis, hepatic decompensation, HCC, and liver-related mortality; however, these risks, particularly risk of HCC, are even lower in patients who additionally cleared HBsAg (Yip et al. 2019, 2020), spontaneously or after treatment.

A basic premise of HBV functional cure is that it reflects a decrease in cccDNA concentration and transcriptional activity. Assessment of cccDNA concentration would require liver tissue that is not readily available and techniques that are not standardized. This has led to the development of serum markers that might serve as surrogates for cccDNA transcriptional activity. Earlier studies showed that quantitative HBsAg level reflects the transcriptional activity of cccDNA (Chan et al. 2011), and correlates better with cccDNA concentration than serum HBV DNA level though the correlation in HBeAg negative patients is not as strong as in HBeAg positive patients (Thompson et al. 2010; Lin et al. 2010), likely because a higher proportion of circulating HBsAg in HBeAg negative patients may be derived from integrated HBV DNA and not cccDNA. Recent studies found that serum HBV RNA level may be a better surrogate for cccDNA concentration (Wang et al. 2016; Giersch et al. 2017; Huang et al. 2018). Circulating HBV RNA is believed to represent partially reverse transcribed encapsidated pregenomic RNA (pgRNA) in virus-like particles (Hu and Liu 2017). However, current assays for HBV RNA levels are not standardized and may measure not only pgRNA but also messenger RNA and spliced RNA (Shen et al. 2020; Charre et al. 2019). Another marker is hepatitis B core-related antigen (HBcrAg), a composite of several viral antigens expressed from the pre-Core/Core gene: the hepatitis B core antigen, HBeAg, and p22 core-related antigen (Kimura et al. 2002, 2005). Several studies have shown that HBcrAg levels correlate better with cccDNA concentration than serum HBV DNA or HBsAg level (Suzuki et al. 2009; Testoni et al. 2019; Wang et al. 2019; Chen et al. 2019; Carey et al. 2020). However, current assays for HBcrAg levels have limited

sensitivity and because they also measure HBeAg, the results are less informative in HBeAg-positive patients.

3 HBV Life Cycle and Barriers to Cure

Recent advances in molecular techniques have provided more details on the HBV life cycle providing multiple targets for antiviral drug development. HBV enters the hepatocyte via binding of aa 2–48 of the pre-S1 region to a receptor - sodium taurocholate co-transporting peptide (NTCP/SLC10A1), leading to NTCP oligomerization, endocytosis, and viral internalization (Barrera et al. 2005; Glebe et al. 2005; Yan et al. 2012; Ni et al. 2014; Fukano et al. 2018). After uncoating, the nucleocapsid is imported into the hepatocyte nucleus where the second strand of the relaxed circular DNA (rcDNA) is completed and converted to cccDNA (Nassal 2015; Tsukuda and Watashi 2020). The cccDNA serves as a template for transcription into pgRNA and messenger RNAs, a process mediated by host RNA polymerase II (Karayiannis 2017) and modulated by epigenetic factors including histones, transcription factors, HBV core, and X proteins as well as chromatin-modifying enzymes (Karayiannis 2017; Pollicino et al. 2006; Levrero et al. 2009; Koumbi and Karayiannis 2015; Guo et al. 2017). The mRNAs are exported to hepatocyte cytoplasm and translated to viral proteins. The pgRNA serves as the template for reverse transcription to HBV DNA and translation to HBV core and polymerase proteins (Wu et al. 2019). The pgRNA is packaged with core protein inside the newly formed nucleocapsids where reverse transcription into the first and then the second strand HBV DNA occurs (Guo and Guo 2015). The nucleocapsids with partially double-stranded rcDNA are then enveloped and secreted as virions. Studies in duck and mouse models showed that cccDNA turnover occurs mainly through dilution during cell division (Reaiche et al. 2010; Lutgehetmann et al. 2010; Reaiche-Miller et al. 2013). The normal lifespan of hepatocytes is longer than 6 months (Macdonald 1961); thus, the half-life of cccDNA is long. The existence of an internal recycling pathway whereby the nuclear pool of cccDNA can be replenished without entry of new virions and the long half-life of cccDNA makes eradication of HBV difficult (Fig. 20.1).

Another barrier to HBV cure is the impaired immune response to HBV in patients with chronic HBV infection. HBV is a stealth, noncytotoxic virus. Impaired innate and adaptive immune response is a key contributor to chronicity of HBV infection. HBV escapes innate immune recognition by (i) its use of intra-nucleus cccDNA as transcriptional template, (ii) protection of newly transcribed viral genome within capsids, and (iii) interference/inhibition of innate immune response by releasing nonstructural proteins or suppression of toll-like receptor (TLR) expression by HBsAg and HBeAg (Wieland and Chisari 2005; Bertoletti et al. 2010; Ferrari 2015). Lack of protective T cell memory maturation and exhaustion of HBV-specific T cell response are attributed to the abundance of circulating HBsAg (Wherry and Ahmed 2004; Bertoletti and Ferrari 2016). The exhausted T cells express high levels of

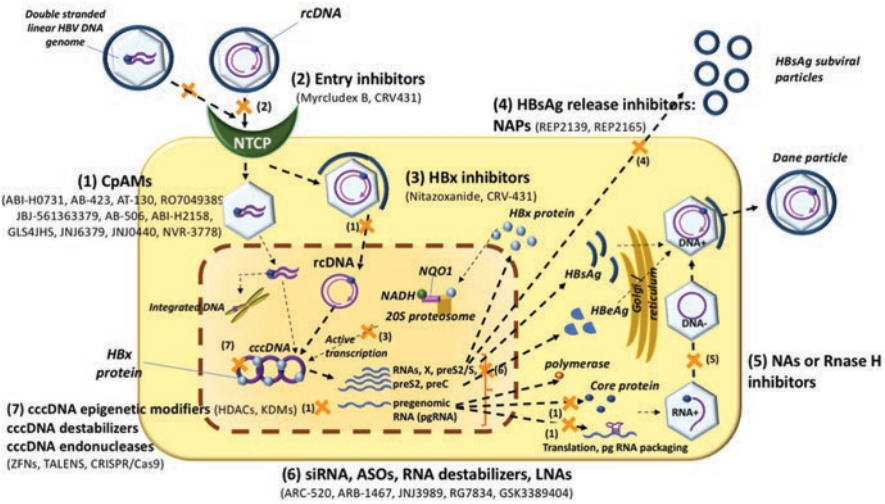

Fig. 20.1 HBV life cycle and target sites of novel direct-acting antiviral drugs. (1) Core particle assembly modulators (CpAMs) act through production of aberrant or empty core particles preventing pre-genomic RNA packaging, HBV DNA replication and virion production; (2) Entry receptor inhibitors prevent virions entering hepatocyte by blocking binding to NTCP receptor; (3) HBx inhibitors act by interfering with HBx protein, which regulates cccDNA expression; (4) nucleic acid polymers (NAP) prevent subviral particle release from hepatocytes; (5): NAs or HBV ribonuclease H(RNase H) inhibitors inhibit reverse transcriptase or interfere with the RNaseH activity required for RNA cleavage causing accumulation of long RNA and blocking HBV DNA synthesis, respectively; (6): RNA interference (siRNA) or antisense oligonucleotides (ASOs), RNA destabilizers, locked nucleic acids (LNAs), interfere with the transcription of viral RNA and in turn HBV DNA replication and production of HBV virions and proteins; (7): cccDNA epigenetic modifiers, destabilizers, endonucleases act by decreasing cccDNA concentration, stability or transcription. (*Adapted and modified from* Fanning et al. (2019))

co-inhibitory molecules including programmed cell death-1 (PD-1), lymphocyte-activation gene-3 (LAG-3), cytotoxic T lymphocyte-associated antigen (CTLA)-4, T-cell immunoglobulin and mucin domain-3 (TIM3), and cluster of differentiation (CD) 244 (Ferrari 2015; Ye et al. 2015; Fisicaro et al. 2020). Impaired Natural Killer (NK) cell function results in decreased non-cytolytic antiviral cytokine (IFN-γ and TNF-α) production (Oliviero et al. 2009; Peppa et al. 2010; Tjwa et al. 2011; Mondelli et al. 2012; Rehermann 2013; Maini and Peppa 2013; Schuch et al. 2014; Lunemann et al. 2014). The intrahepatic enrichment of IL10, TGF-β, and arginase may promote the tolerogenic immune response (Ferrari 2015). Restoration of both innate and adaptive immune responses will be necessary to achieve sustained immune control of HBV infection and functional cure (Fisicaro et al. 2020; Meng et al. 2019).

Many studies have shown that patients with chronic HBV infection who cleared HBeAg or HBsAg spontaneously or after IFN or NA treatment can regain immune

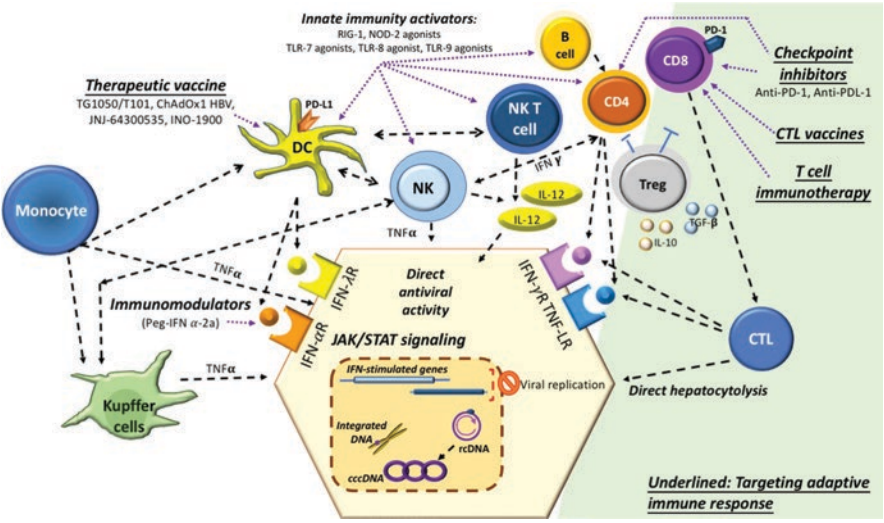

Fig. 20.2 Strategies to enhance adaptive and innate immune responses to HBV. Cytokines, Toll-like receptor 7 (TLR7), TLR8 or retinoic acid-inducible gene I protein (RIG-1), and pegylated IFNα target innate immunity. IFN-α and IFN-β trigger the expression of IFN-stimulated genes (ISGs) downstream of IFN-stimulating response elements and JAK-STAT pathway. RIG-1 triggers the secretion of IFN-α, IFN-β, IFN-γ, and activates NF-kB to produce inflammatory cytokines. TLR-7 and TLR-8 agonists stimulate antiviral cytokine production and activation of natural killer (NK) cells. Checkpoint inhibitors against programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) reverse HBV-specific T cell exhaustion and restore immunity. Therapeutic vaccines stimulate host immune responses to restore HBV-specific adaptive immune control. *(Adapted and modified from* Mouzannar and Liang (2020))

response to HBV (Rehermann et al. 1996; Rossol et al. 1997; Carey et al. 2011) suggesting that the impaired response is mainly due to exhaustion. HBV is unique in that besides complete virions, it also produces subviral particles that contain only envelope proteins, which may be more than 100–100,000-fold more abundant than complete virions (Blumberg 1977; Luckenbaugh et al. 2015). Constant exposure to large amounts of circulating HBsAg has been postulated to be the main contributor to immune exhaustion (Tout et al. 2020). Thus, inhibition of HBsAg production is critical in restoring immune response to HBV (Fig. 20.2).

4 Efficacy of Current Therapy in Achieving Functional Cure

NA monotherapy is associated with very low rates of HBsAg clearance, annual incidence of 0.15–0.33%(Kim et al. 2014; Jeng et al. 2018), and only 2.5–4.9% after 7–10 years of continuous treatment even with entecavir or tenofovir, which have very low rates of antiviral drug resistance (Buti et al. 2015; Lam et al. 2017;

Marcellin et al. 2019; Hou et al. 2020). Paradoxically, higher rates of HBsAg clearance had been observed in retrospective studies of HBeAg negative patients who discontinued NA after at least 2–4 years of complete HBV DNA suppression, with annual incidence of 1.8% and cumulative incidence increasing to 13–41% by post-treatment year 5–6 compared to 0.08% and < 5%, respectively, in patients who continued NA, with substantially lower rates in Asians compared to Caucasians (Papatheodoridis et al. 2018; Jeng et al. 2018; Hadziyannis et al. 2012; Berg et al. 2017; Buti et al. 2019; Chen et al. 2020; Hirode et al. 2020). The exact mechanism for a higher rate of HBsAg clearance after discontinuing NA treatment is unclear. It has been suggested that viral relapse may trigger immune response to HBV though rates of HBsAg clearance do not seem to directly correlate with posttreatment hepatitis flares (Ghany et al. 2020). In vitro studies show that exhausted T cell function may be restored after years of virus suppression (Boni et al. 2012), and studies in patients with sustained response and subsequent HBsAg loss show a less exhausted phenotype with increased PD-1^{+}HBV-specific T cells (Rinker et al. 2018; Rivino et al. 2018; Garcia-Lopez et al. 2021). Several studies have shown that low HBsAg level and low HBV RNA level reflecting lower concentrations or decreased transcription activity of cccDNA at the time of NA discontinuation are better predictors of sustained viral suppression and HBsAg clearance after NA withdrawal.

IFN has both antiviral and immunomodulatory effects. Although IFN has weaker effects on suppressing HBV DNA replication than NA, pegylated IFNα monotherapy is associated with higher rates of HBsAg clearance than NA monotherapy, 3–7% after 1 year of treatment (Lau et al. 2005; Janssen et al. 2005; Marcellin et al. 2004, 2008), increasing to 8–14% after 3–5 years posttreatment follow-up (Buster et al. 2008; Marcellin et al. 2009, 2013). IFN's effects on HBsAg clearance are, however, genotype dependent with lower rates in non-A genotypes (Flink et al. 2006).

Various strategies combining NA and pegylated IFN have been evaluated in an attempt to increase the rate of HBsAg loss. One study found that de novo combination of tenofovir DF and pegylated IFNα for 48 weeks resulted in an overall HBsAg clearance rate of 5.7%, 8.1%, 10.4% at the end of treatment, and at 24 and 72 weeks posttreatment, respectively, compared to 2.3%, 2.9%, and 3.5% among those who received pegylated IFNα monotherapy (Marcellin et al. 2016; Ahn et al. 2018, 2019). Among the patients who received combination therapy for 48 weeks, HBsAg clearance rates were 37.5% and 7% ($P = 0.026$) for patients with A versus non-A HBV genotype at 24 weeks off-therapy (Marcellin et al. 2016). A recent meta-analysis included 33 studies with de novo combination of IFNα and NA therapy, 15 studies with IFNα added to NA and 12 studies where NA therapy was switched to IFNα. De novo combination therapy improved the probability of HBsAg clearance (relative risk [RR]: 15.59, 95% CI 3.22–75.49) compared to NA monotherapy but not to IFNα monotherapy. NA switch to IFNα appeared to have a greater effect in improving HBsAg clearance (RR: 12.15, 95% CI 3.99–37.01) compared to NA monotherapy, than adding IFNα to NA (RR: 4.52, 95% CI 1.95–10.47) (Liu et al. 2020). These studies suggest that HBsAg clearance may be achieved in a higher percentage of patients by using a combination of NA and pegylated-IFNα, or

Table 20.2 HBsAg clearance rates with currently available treatments

Treatment	HBsAg clearance			
	Annual incidence	Treatment duration (years)	Post-treatment follow-up (years)	Cumulative incidence
NA monotherapy	0.15–0.33% (25, 90)	7–10		2.5–3.7% (Lam et al. 2017; Marcellin et al. 2019; Hou et al. 2020; Suzuki et al. 2019)
Peg-IFNα monotherapy	N/A	1	3–5	11–14% (Buster et al. 2008; Marcellin et al. 2009, 2013)
NA + peg-IFNα*				
De novo	N/A	1	0.5–1.5	9.1–10.4% vs. 2.8–3.5% vs. 0% (combination vs. Peg-IFNα monotherapy vs. TDF monotherapy) (Marcellin et al. 2016; Ahn et al. 2018)
Add-on	N/A	>1 year NA with 48-week add-on peg-IFNα	2	10% vs. 4% (ITT, 48-week add-on IFNα vs. mono-NA) (Bourliere et al. 2017); pooled study compared to NA monotherapy, Peg-IFNα add-on NA therapy increase HBsAg loss rate (RR = 4.52, 95% CI: 1.95–10.47) (Liu et al. 2020)
Switch	N/A	NA for 9–36 months, then switch to 48-week peg-IFNα with 8-week overlapping in the beginning of switched arm	0	8.5% vs. 0% ($P = 0.0028$) (Ning et al. 2014); pooled study compared to NA monotherapy, switch to Peg-IFNα from NA therapy increase HBsAg loss rate (RR: 12.15, 95% CI 3.99–37.01) (Liu et al. 2020)
NA withdrawal	1.78% (90)	1.3– ~ 5	5.5–6	13–41% (Jeng et al. 2018; Hadziyannis et al. 2012; Berg et al. 2017; Hirode et al. 2020)

[a]Published randomized-controlled trial; Abbreviation: *NA* nucleos(t)ide analogue, *N/A* not available, *Peg-IFN*α pegylated interferon alfa

switching patients from NA to pegylated IFNα after HBV DNA has been suppressed; however, not all patients can tolerate IFN and the rate of functional cure remains low, particularly for patients with non-A HBV genotype. Furthermore, these strategies have not been compared to NA withdrawal (Table 20.2).

5 HBV New Drug Development

Several classes of DAAs targeting different steps of the HBV lifecycle are in clinical trials. They include (i) entry receptor inhibitors that prevent binding of HBV to the NTCP receptor; (ii) core particle assembly modulators (CpAM) that primarily act through production of aberrant or empty core particles preventing pre-genomic RNA packaging, HBV DNA replication, and virion production; (iii) secretion inhibitors such as nucleic acid polymers (NAP) that prevent subviral particle release from hepatocytes; and (iv) transcription inhibitors: RNA interference (siRNA) or antisense oligonucleotides that interfere with the transcription of viral RNA and in turn HBV DNA replication and production of HBV virions and proteins. Most of these DAAs have been evaluated in combination with either NA or pegylated IFNα with a few trials having tested triple combinations, e.g., siRNA (JNJ-3988), CpAM (JNJ-6379), and NA; and nucleic acid polymer (REP 2139 or REP 2165), pegylated IFNα and NA. To date, entry inhibitors notably Bulevirtide alone and multiple CpAMs have only produced a minimal reduction in HBsAg levels (Bogomolov et al. 2016; Sulkowski et al. 2019; Ma et al. 2019; Yuen et al. 2019a; Zoulim et al. 2020) though CpAMs consistently decrease serum pgRNA levels (Sulkowski et al. 2020) reflecting an inhibition on cccDNA transcription. By contrast, several siRNAs and antisense oligonucleotides have produced >1 $\log_{10}$ decrease in HBsAg levels after only a few doses (Yuen et al. 2019a, b) and small studies of NAPs have observed HBsAg clearance in up to 50% of patients which appeared to be sustained (Bazinet et al. 2020).

Various immune modulatory therapies have been evaluated including boosting of adaptive T cell immune response (Lok et al. 2016b; Kratzer et al. 2018; Lim et al. 2019), removal of immune inhibition (anti-PD-1) (Gane et al. 2019a; NIAID 2020), and restoration of innate immunity via toll-like receptor 7 or 8 agonist (Janssen et al. 2018; Boni et al. 2018; Niu et al. 2018; Gane et al. 2018; Mackman et al. 2020). These therapies have been tested alone and in combination with NA either as de novo or as add-on therapy to NA. To date, immune modulatory therapies have not produced marked reductions in HBsAg levels though they appear to enhance immune response to HBV (Lok et al. 2016b; Gane et al. 2019a, b; Janssen et al. 2018; Boni et al. 2018, 2019). It is possible that immune modulatory therapies have a greater chance of success if administered after not only suppression of HBV DNA replication but also inhibition of HBsAg production.

6 Path to HBV Functional Cure

The combination of new DAAs and NA with or without pegylated IFNα have shown promise but to date, few have resulted in HBsAg clearance. It has been suggested that a sequential approach may be needed starting with potent suppression of HBV replication using a combination of CpAM and NA which has been shown to produce more rapid as well as more marked decline in both HBV DNA and pgRNA levels (Yuen et al. 2019a; Sulkowski et al. 2020; Yuen et al. 2020; Yuen 2020). This

should be followed by addition of siRNA or NAP, with or without pegylated IFNα to block HBsAg production and/or secretion, and then initiation of immune modulatory therapies to restore immune control to ensure sustained virologic response after discontinuation of treatment. Whether this multi-pronged approach should be implemented step-wise or simultaneously and whether host immune response can be restored without the need for immune modulatory therapies if DAAs can produce sufficient inhibition of HBsAg production is unclear.

7 Challenges in Developing New HBV Treatments

In addition to the barriers to HBV cure discussed earlier, a major challenge in developing new HBV treatments is the excellent safety profile of NA. The safety of new treatments will need to be comparable to be approved by regulatory agencies and to be accepted by the medical community and by patients. Small trade-offs in safety—transient adverse effects that are not serious—may be considered if substantially higher rates of HBsAg loss can be accomplished with a finite course of therapy, e.g., 30% HBsAg loss after 1–2 years of treatment, but treatment-emergent adverse effects that are serious or long-lasting would not be acceptable. Similarly, treatments that may select for drug resistance or immune escape variants would also not be acceptable. A common event during HBV treatment is hepatitis flare which can result in hepatic decompensation and death. Hepatitis flares may be due to drug-induced liver injury or viral breakthrough due to drug resistance or immune-mediated lysis of infected hepatocytes. The latter is considered to be good flares as they can potentially aid in eliminating cccDNA and decrease HBsAg production. However, even good flares if out of control can cause liver failure. Unfortunately, several new DAAs in development had to be abandoned due to safety concerns (biopharma A 2019) and one immune modulatory therapy trial with Inarigivir had an unexpected fatality (Spring Bank Pharmaceuticals I 2019).

Another challenge is the complexity in choosing the right classes of drugs to combine that will have additive or synergistic antiviral and/or immune enhancing effects, but no added adverse events. In addition, the route of administration of these combination regimens has to be convenient as they will be compared against NA which requires only one pill once a day with infrequent monitoring needed.

A third challenge is the efficient and appropriate design of clinical trials to test these new therapies. Chronic HBV infection is a heterogeneous disease and responses to some treatments may differ in HBeAg-positive versus HBeAg-negative patients, patients in the immune tolerant versus immune active phase, and across HBV genotypes. Furthermore, responses and assessment of treatment response will be different in patients virally suppressed on NA versus those currently not on treatment. Selecting the most appropriate patient population for the initial trials is key to obtaining an accurate early indication of treatment efficacy. However, the ultimate trials must include a broad spectrum of patients with chronic HBV infection with stratified randomization to ensure balance between treatment arms.

Finally, new treatments must be affordable and accessible to the 250 million patients with chronic HBV infection worldwide. Generic NAs are available in most countries yet only a small percentage of patients who meet treatment criteria are receiving treatment. Thus, developing new drugs on its own will not help in curing HBV infection unless these drugs are affordable and parallel strategies to improve diagnosis and linkage to care are in place.

8 Future Perspective

Sterilizing HBV cure is likely not feasible but functional HBV cure is possible. In fact, functional HBV cure—undetectable HBsAg and serum HBV DNA—occurs spontaneously and with current NA or IFN therapy, albeit rarely. The development of DAAs targeting different steps in the HBV life cycle inhibiting not only HBV DNA replication but also virus entry, nucleocapsid assembly, cccDNA transcription, and HBsAg production and secretion; and immune modulatory therapies that can enhance innate and adaptive immune responses to HBV provide hope that functional cure can be achieved in a higher percentage of patients after a finite course of therapy. This will require collaborations not only between regulatory agencies, pharmaceutical industry, scientists, and clinicians but also between companies to facilitate testing of the best combination of drugs. Though there is still a long way before any of the new drug combinations in development will achieve the goal of a functional HBV cure in a high percentage of patients after a finite course of therapy, the renewal of interest in HBV treatment and the momentum in the last 5–6 years make the mission of HBV functional cure possible.

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