Chapter 11
Natural History of Hepatitis B Virus Infection

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Introduction

Hepatitis B virus (HBV) infection is a global public health problem. Despite the development of highly effective vaccines against the disease since the early 1980s and the implementation of universal newborn vaccination programs in more than 168 countries, there is still a huge burden of liver disease due to chronic hepatitis B [1]. An estimated 240 million people in the world, representing over 3.7 % of the global population, are chronically infected with HBV and 75 % of them reside in Asia-Pacific region [2]. Between one-quarter and one-third are expected to develop progressive liver disease, including cirrhosis and hepatocellular carcinoma (HCC) and 15–25 % will die from HBV-related liver disease. Worldwide, HBV infection accounts for 30 % of patients with cirrhosis and 53 % of those with HCC, and over 200,000 and 300,000 hepatitis B surface antigen (HBsAg) carriers die each year from cirrhosis and HCC, respectively [3]. In Taiwan, HBsAg carriers are at 5.4- and 25.4-fold increased risk of mortality from cirrhosis and HCC, respectively [4].

The natural course of HBV infection is complex and variable. Substantial improvement during the past decades in the understanding of HBV virology and host immune response to HBV, combined with the recent availability of highly sensitive HBV DNA assays and quantitative HBsAg assays, has led to new insights into the natural history of HBV infection.

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Epidemiology

Worldwide, an estimated two billion people have been infected with HBV, and some patients with acute HBV infection develop chronic HBV infection. The global prevalence of chronic HBV infection varies greatly among different geographical areas, and was classified into high-prevalence (Southeast Asia, China, sub-Saharan Africa, and Alaska), intermediate-prevalence (Mediterranean countries, Eastern Europe, Central Asia, Japan, Latin, and South America), and low-prevalence areas (USA, Western Europe, Australia, and New Zealand) based on the prevalence of HBsAg carriers of >8, 2–8, and <2 %, respectively [1]. The corresponding lifetime risk of being exposed to HBV infection is approximately 60–80, 20–60, and 10–20 %, respectively.

Associated with a wide range in prevalence of chronic HBV infection are differences in the predominant mode of transmission and age at infection. In high-prevalence areas, the mode of HBV transmission differs remarkably between Southeast Asia and sub-Saharan Africa. In Southeast Asia, perinatal transmission is common and accounts for 40–50 % of chronic infection. In contrast, in sub-Saharan Africa, perinatal transmission does not play a major role and inapparent horizontal transmission by HBsAg positive family members and playmates or by unsafe therapeutic injections is the major route of HBV transmission, with most children being infected by the age of 5 years. This difference is related to the higher prevalence of hepatitis B e antigen (HBeAg) in Southeast Asia female carriers of childbearing age (40–50 %) than in sub-Saharan Africa (10–18 %) [5, 6], as 80–90 % of HBeAg positive mothers will transmit the disease to their off springs, compared with only 15–20 % of those seronegative for HBeAg [7]. In low-prevalence areas, hepatitis B is a disease of young adults, typically those who have high-risk behavior such as sexual promiscuity or drug abuse or are in high-risk occupations.

The worldwide incidence of HBV infection is decreasing as a result of vaccination and public health education. For instance, in Taiwan, after the implementation of universal vaccination programs in newborns in 1984, the HBsAg carrier rate among children <15 years of age decreases from 10 % in pre-vaccination era to 0.6 % in 2004 [8] and 0.5 % in 2009 [9]. The prevalence of HBsAg in the population ≤25 years of age born after the vaccination program is 0.9 % [9]. In the USA, the incidence of reported acute hepatitis B declines by 81 % from 8.5 to 1.6 cases/100,000 during the period 1990–2006. However, immigrants from high-prevalence areas are now responsible for an increasing burden of chronic HBV infection in many developed countries.

Acute HBV Infection

Clinical Manifestations

Acute HBV infection in neonates is clinically asymptomatic in most cases. Symptomatic hepatitis occurs in only 10 % of children less than 4 years old [10]. In rare instances, mothers seropositve for antibody against HBeAg (anti-HBe) may

transmit HBV to their babies, resulting in severe or fulminant hepatitis within the first year of life [11]. HBV with mutations of basal core promoter (BCP) and precore regions, which reduce or abrogate HBeAg production, may be one of the factors in the pathogenesis of fulminant hepatitis in children [12].

On the contrary, approximately 30 % of immunocompetent adults with acute HBV infection develop icteric hepatitis [10], and 0.1–1 % develop fulminant hepatitis [13]. Among patients hospitalized for acute hepatitis B, the fatality rate is 1 %. HBV infection accounts for >50 % of fulminant cases of viral hepatitis. The reason that HBV has a fulminant course in some patients remains unclear. Both viral and host factors may be involved in the pathogenesis of fulminant hepatitis B. However, the association of precore and BCP mutations with fulminant hepatitis B in adults is controversial [14, 15].

Chronic Evolution

The risk of chronicity after primary HBV infection varies and depends on the age and immune status at the time of infection. Among infants born to HBeAg positive mothers, hence infected in the perinatal period, the probability of chronic infection approaches 90 %. When infected at 1–5 years of age, 20–30 % of the children become chronically infected, while among older children the probability falls to 5–10 % [10]. The extremely high chronicity after perinatally acquired infection is presumably related to the immature immune system of the neonates. Another possible mechanism is that the fetus is tolerated in utero to HBV following transplacental passage of viral proteins [16]. The risk of chronicity among normal, healthy, immunocompetent adults is ≤5 %, but varies considerably (<1–12 %) among diverse populations [17], being extremely low (0.2 %) in Greece [18] and appreciably high (12.1 %) in Germany [19]. The risk of chronicity is greatly increased in immune compromised patients, such as patients on chronic hemodialysis, those on immunosuppression following solid organ transplantation, and those receive cancer chemotherapy. Patients with concomitant human immunodeficiency virus (HIV) infection are also at significant risk of developing chronic infection, with 20–30 % remain HBsAg positive after acute infection.

Classically, persistence of serum HBsAg for more than 6 months is considered to represent a progression to chronic infection. However, a recent study from Japan showed that 90.2 % of patients cleared serum HBsAg within 6 months, 7 % between 7 and 12 months after the onset of acute hepatitis B and 2.8 % had persistence of HBsAg for more than 12 months [20]. Another study showed that the rate of persistence of HBsAg was 23.4 % at 6 months and 7.5 % at 12 months in genotype A HBV infection; while the corresponding figure for genotype non-A HBV infection was 8.6 and 0.9 %, respectively [21]. These results further indicate that genotype A HBV is an independent risk factor for progression to chronic infection. The different risk of chronicity in adults from diverse geographical areas may be in part attributed to HBV genotype difference. It is also possible that the longer persistence of

serum HBsAg reflects the higher sensitivity of the most up-to-date assays for HBsAg as compared to previous assays. Persistence of HBsAg for more than 12 months, as measured with a highly sensitive method, may be suitable for redefining the progression of acute hepatitis B to chronicity.

Chronic HBV Infection

Clinical Presentation

In low- or intermediate-prevalence areas, approximately 30–50 % of patients with chronic HBV infection have a history of classical acute hepatitis that progressed to chronic infection. In patients from high-prevalence areas, most patients are incidentally identified to be HBsAg carriers, almost none had evidence of progression from overt acute hepatitis.

Patients with chronic HBV infection may experience acute hepatitis flare that may be asymptomatic or mimic acute hepatitis with fatigue, anorexia, nausea and, in rare instances, jaundice or even hepatic decompensation. In Taiwan, as many as 40 % of HBsAg positive patients with clinical diagnosis of acute hepatitis are actually chronic HBsAg carriers who remained unrecognized until they present the episode of overt acute hepatitis. They are positive for HBsAg but negative for immunoglobulin class M antibody against hepatitis B core antigen (IgM anti-HBc), so-called "previously unrecognized HBsAg carriers with acute hepatitis flares or superimposed other forms of acute hepatitis" [22]. In these high-prevalence areas, an episode of acute hepatitis in HBsAg positive patients is more likely an acute hepatitis flare of chronic HBV infection rather than acute hepatitis B.

Phases of Chronic HBV Infection

The patients with chronic HBV infection may present one of the following four biochemical and serological profiles: (1) HBeAg positive with normal alanine aminotransferase (ALT) levels; (2) HBeAg positive with abnormal ALT levels; (3) HBeAg negative with normal ALT levels; and (4) HBeAg negative with abnormal ALT levels. These four patterns of presentation actually represent different phases of chronic HBV infection.

As a result of the dynamic interplay of complex interactions involving HBV, the hepatocyte and the host immune response, the natural course of chronic HBV infection consists of distinct phases, characterized and diagnosed on the basis of HBeAg/anti-HBe serology, serum HBV DNA levels, ALT levels and liver histology. Typically, chronic infection acquired perinatally or during infancy consists of three chronological phases: the initial immune tolerance phase, followed by immune clearance phase, and finally the low replicative inactive phase [23–25]. In a subset

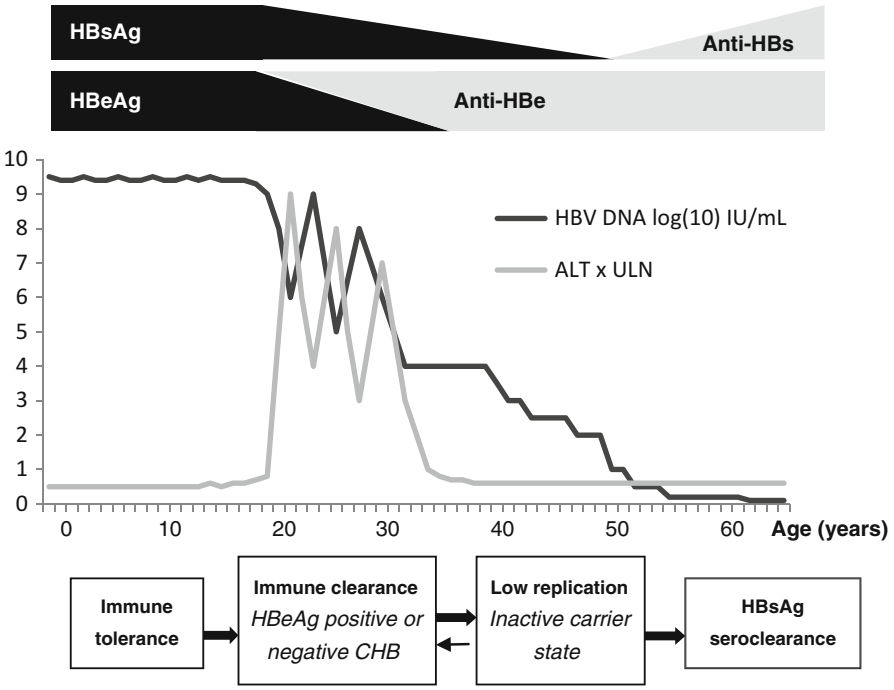

Fig. 11.1 Serological course of chronic hepatitis B virus infection. The initial immune tolerant phase is characterized by a high level of HBV replication, HBeAg positivity, and a normal ALT. This period can last up to 20–25 years following perinatal infection, but is short or absent in adult acquired infection. During the immune clearance phase, there is a reduction in HBV DNA levels associated with raised ALT levels (HBeAg positive chronic hepatitis B). This phase can last for years until HBeAg seroconversion. After HBeAg seroconversion, the patient enters the low replicative phase, characterized by low or undetectable HBV replication and a normal ALT (inactive carrier state). Some inactive carriers can develop HBV reactivation with either wild-type HBV and reversion to HBeAg positivity or more frequently with HBV variants with mutations limiting HBeAg production (HBeAg negative chronic hepatitis). Reactivation of HBV can be viewed as a variant of immune clearance phase. Serum levels of HBsAg decrease gradually during the natural course of chronic HBV infection. In a subset of inactive carriers, serum HBsAg even disappears spontaneously, followed by anti-HBs seroconversion in up to 75 % at 10 years following HBsAg seroclearance. *ULN* upper limit of normal

of inactive carriers, HBV may reactivate and trigger immune mediated liver injuries. This reactivation phase can be viewed as a variant of immune clearance phase [1]. Among the minority of adult patients who progress to chronic hepatitis B, there is usually no or very short initial immune tolerance phase. Successful immune clearance occurs more readily. Otherwise, the clinical course is the same as seen in perinatally acquired infection.

The serological course of chronic HBV infection is shown in Fig. 11.1. The clinical, serological, histopathological and virological characteristics of immune tolerance phase, immune clearance phase, inactive carrier state and reactivation of HBV are summarized in Table 11.1.

Table 11.1 Phases of chronic HBV infection: clinical, serological, histopathological and virological characteristics

Characteristics	Immune tolerance phase	Immune clearance phase	Inactive carrier state	Reactivation of hepatitis B
Age (years)	<20–25	20–40	>35-–40	>35–40
Serology				
HBsAg (log_{10} IU/mL)	4.5–5.0	3.5–4.5	2.0–2.5	3.0–3.5
HBeAg	Positive	Positive	Negative	Negative
HBV DNA levels (log_{10} copies/mL)	Very high 8–12	High 6–10	Low or undetectable <4	Moderate, fluctuating 4–8
ALT	Normal	Elevated	Normal	Elevated
Precore/basal core promoter	Wild type	Mixed (Wild type > mutant)	Mixed (mutant > Wild type)	Mutant
Histopathology				
Inflammation	Absence	presence	Absence	Presence
Hepatocyte HBsAg	Positive (membrane/ cytoplasm)	Positive (membrane/ cytoplasm)	Positive (cytoplasm)	Positive (membrane/ cytoplasm)
Hepatocyte HBcAg	Positive (nucleus)	Positive (nucleus/ cytoplasm)	Negative	Positive (cytoplasm)
Disease progression	No/minimal	Yes	No	Yes
HBsAg seroclearance	No	No	1–2 %/year	No

HBV hepatitis B virus, *HBsAg* hepatitis B surface antigen, *HBeAg* hepatitis B e antigen, *ALT* alanine aminotransferase, *HBcAg* hepatitis B core antigen

Immune Tolerance Phase

The immune tolerance phase is characterized by the presence of HBeAg, very high serum level of HBV DNA (>2×10^7 IU/mL) and HBsAg (4.5–5.0 log_{10}IU/mL) [26–28], normal ALT level, normal liver or only minimal necroinflammatory activity and scant fibrosis. Immunostaining of HBV antigens in liver shows that HBsAg is distributed diffusely on the hepatocyte membrane and focally in the cytoplasm, and hepatitis B core antigen (HBcAg) is distributed predominantly in nuclei [29]. There is usually little or no disease progression as long as serum ALT levels remain normal and the immune tolerance is maintained [30].

The exact mechanisms for immune tolerance are unknown. Even though HBV virus does not cross the placenta, HBeAg secreted by the virus does. Experiments in mice suggest that a transplacental transfer of maternal HBeAg may induce a specific unresponsiveness of helper T cells to HBeAg in neonates. Because HBeAg and HBcAg are highly cross-reactive at the T-cell level, deletion of the helper T-cell response to HBeAg results in an ineffective cytotoxic T-Iymphocyte (CTL) response

to HBcAg, the major target of the immune response [16]. The viral population identified during the immune tolerance phase usually consists of exclusively wild type HBeAg-positive HBV with little or no mutant type HBeAg-negative HBV [15, 16].

Immune Clearance Phase

The transition from immune tolerance to immune clearance phase usually occurs between age 20 and 40, but may sometimes start earlier and even occur in pediatric patients. During this phase, serum HBeAg is still positive but ALT levels become abnormal, accompanied by declining levels of serum HBV DNA and HBsAg. Serum HBV DNA levels generally exceed 20,000 IU/mL and HBsAg levels are usually in the range of 3.5–4.5 $\log_{10}$ IU/mL [26–28]. There is a positive correlation between serum HBsAg levels and serum HBV DNA or intrahepatic covalently closed circular DNA (cccDNA) levels. Liver biopsy demonstrates moderate or severe necroinflammation with variable amounts of fibrosis. HBsAg is distributed diffusely on the hepatocyte membrane and focally in the cytoplasm, as seen in immune tolerance phase, but intrahepatic nuclear HBcAg expression decreases with concomitant increase in cytoplasmic/membranous HBcAg expression [29]. These results suggest that membranous expression of HBsAg is closely related to active viral replication but is probably not responsible for liver cell damage, and that hepatocytes with cytoplasmic/membranous HBcAg expression might be possible targets for immune hepatocytolysis [29].

Little is known about the mechanisms that regulate the loss of immune tolerance in chronic HBV infection. The finding that immune clearance phase is accompanied by a change in the intrahepatic distribution of HBcAg from nuclear to cytoplasmic localization suggests that it may be triggered by a change in the presentation of viral antigens. However, a more recent study suggests that the shift of hepatocyte HBcAg from nucleus to cytoplasm during the immune clearance phase may be secondary to liver cell damage and regeneration [31].

Hepatitis Activity and Acute Hepatitis Flare During Immune Clearance Phase (HBeAg Positive Chronic Hepatitis)

Most patients in the immune clearance phase are asymptomatic and have mild to moderate elevation in ALT levels and hepatitis activity, so called HBeAg positive chronic hepatitis B (CHB). However, the clinical course may be punctuated by spontaneous acute hepatitis flare, defined as an abrupt elevation of ALT >5 times the upper limit of normal (ULN). These acute hepatitis flares are considered to be the results of HLA-class I antigen-restricted, CTL mediated immune response against HBV antigen(s) and its downstream apoptotic mechanisms [32]. The reasons for spontaneous acute hepatitis flares are not clear but are likely explained by subtle changes in immunological controls of viral replication. Several studies have found that acute hepatitis flares are often preceded by a sudden increase in serum levels of

HBV DNA [33, 34], HBeAg and HBeAg-specific immune complexes [34], and enhanced T-cell response to HBcAg and HBeAg [35]. These results suggest that increases in viral replication, accumulation of nucleocapsid proteins in serum and hepatocytes, and the subsequent immune response play an important role in initiating acute hepatitis flares in chronic HBV infection [34]. Histological evidence of lobular hepatitis superimposed upon the changes of chronic viral hepatitis is frequently observed [36]. IgM anti-HBc is positive in 14.4 % of patients during acute flares, but generally in lower titers than in acute HBV infection [37]. As HBeAg seroconversion is often preceded or accompanied by a transient ALT flare, it is believed that hepatitis flares are the results of the host attempt to clear the virus by the immune response. However, not all acute hepatitis flares lead to HBeAg seroconversion and HBV DNA clearance from serum, a phenomenon termed as "ineffective or abortive immune clearance" [32]. In this context, the patients may experience repeated episodes of acute hepatitis flares, which can account for increased risk of HBV-related cirrhosis.

The annual rate of acute hepatitis flare in patients with HBeAg positive CHB was as high as 28.6 % in an early hospital-based study from Taiwan [38]. However, in another study that followed up asymptomatic patients beginning at the immune tolerance phase through HBeAg seroconversion, the overall incidence of acute hepatitis flare was 28.8 % during immune clearance phase (mean 3.7 years), with an annual rate of 7.8 % only [39]. Most acute hepatitis flares are asymptomatic, but around 20 % of patients present with symptoms of overt acute hepatitis [37], and approximately 2–3 % may be complicated with hepatic decompensation [40]. One recent report from Taiwan found that a serum HBV DNA level $\geq 1.55 \times 10^9$ copies/mL at the onset of acute flare can predict hepatic decompensation [41]. In HBV high-prevalence areas, acute hepatitis flares of chronic HBV infection is the most important etiology of acute hepatitis and fulminant hepatitis in adults [42, 43].

HBeAg to Anti-HBe Seroconversion

The immune clearance phase has a variable duration and often lasts for many years until HBeAg seroconversion occurs. HBeAg seroconversion is frequently preceded by ALT elevation, followed by a marked reduction of serum HBV DNA levels that can only be detected by sensitive polymerase chain reaction (PCR) assay, decline of serum HBsAg level, ALT normalization and resolution of liver necroinflammation [32, 36]. However, abnormal ALT levels and high-level HBV DNA persist at the time of HBeAg seroconversion in about 5 % of patients [44]. These patients progress directly from HBeAg positive chronic hepatitis to HBeAg negative chronic hepatitis.

The average annual incidence of HBeAg seroconversion is 10 % (range, 2–15 %), depending on factors such as ethnicity, mode of transmission, age, ALT levels, histological activities and HBV genotype. HBeAg seroconversion is much more delayed in children with HBeAg positive carrier mothers than in children with HBeAg negative carrier mothers or children with non-carrier mothers [45]. Different

mode of HBV transmission accounts for the much lower HBeAg positivity rates in black Africans of childbearing age than in women in the Far East [5, 6]. A higher HBeAg seroconversion rate has been reported in non-Asian children with horizontal transmission than Asian children with vertical transmission [46]. In Taiwan, the annual rate of HBeAg seroconversion is <2 % in children ≤3 years of age and 4–5 % in older children, so that around 85 % of children still remain HBeAg positive by age 15 [47]. The likelihood of HBeAg seroclearance correlates positively with ALT levels: HBeAg seroclearance rates at 18-months of follow-up are 0, 3–8, 17, and 59–70 %, respectively, if baseline ALT levels increase over <1, 1–2.5, 2.5–5, and >5 times ULN [32]. In patients with acute hepatitis flare, 72 % undergo HBeAg seroclearance within 3 months if serum α-fetoprotein (AFP) levels >100 ng/mL, compared to only 18 % of those with AFP <100 ng/ml [48]. Serum HBV DNA levels ≤7 $\log_{10}$ copies/mL during acute hepatitis flare also can predict HBeAg seroconversion within 6 months [49]. The likelihood of HBeAg seroconversion also correlates with histological activities: the 5-year cumulative probabilities of HBeAg seroconversion is >65 % in patients with high necroinflammatory (interface or lobular) activities, compared to <25 % in those with low necroinflammatory activities [48]. HBeAg seroclearance may occur within 3 months in two-thirds of the patients with bridging hepatic necrosis [48]. In Eastern countries, patients infected with genotype B HBV seroconvert earlier and more frequently than those with genotype C HBV [50–52]. In Western countries, HBeAg seroconversion is similar in genotypes A, B, D, and F HBV infection but much slower in genotype C HBV infection [53, 54]. In Alaska native carriers, the median age of HBeAg seroclearance is <20 years in patients with genotypes A, B, D, and F HBV, but >40 years in patients with genotype C HBV [53]. Interestingly, HBeAg seroconversion is more frequently preceded by ALT flares >5 times ULN in genotype C HBV infection than in genotype B HBV infection, suggesting that a more vigorous immune-mediated hepatocytolysis may be needed to achieve HBeAg seroconversion in genotype C HBV infection [52].

In Taiwan, HBeAg seroconversion occurs at a median (interquartile range) age of 32 (26–36) years, with 90 % before age of 40 [55]. In accordance with these data, the prevalence of serum HBeAg declines remarkably from 85 % in children (age < 15 years) [46] to 5–10 % in adults over 40 years of age [5]. These findings suggest that HBeAg seroconversion most often occurs between 15 and 40 years of age in perinatally acquired chronic HBV infection. Persistence of HBeAg over 40 years of age is rare and can be considered as "delayed" HBeAg seroconversion [55].

HBeAg Persistence and Its Outcome

In some patients, the immune clearance phase may last for many years without HBeAg seroconversion. A prolonged HBeAg positive phase is associated with increased risk of disease progression. A recent cohort study from Taiwan demonstrated that the risk of progression to cirrhosis increased with increasing age of HBeAg seroconversion, with a hazard ratio of 3.8 per decade increase in age of HBeAg seroconversion [39]. In particular, patients with HBeAg seroconversion

after 40 years of ages were associated with a remarkably high risk of progression to cirrhosis [55, 56].

Several other studies also showed that persistence of serum HBeAg was associated with an increased risk for progression to cirrhosis, HCC development and liver related mortality [57–59]. For instance, in one study from Taiwan that followed up 233 untreated patients with HBeAg positive CHB for a median of 6.8 years, the annual incidence of cirrhosis and HCC development was significantly higher in 147 patients with persistent HBeAg (3.7 and 1.6 %, respectively) than in 86 patients who underwent HBeAg seroconversion (1.8 and 0.4 %, respectively) [58].

Low Replicative Inactive Phase

After successful immune clearance, serum HBeAg is seroconverted to anti-HBe. The patients are still positive for HBsAg, but there is usually a >1 $\log_{10}$ IU/mL reduction in HBsAg levels, compared to preceding immune clearance phase, and HBsAg levels rarely exceed 1000 IU/mL during this phase [26–28]. The hallmark event of HBeAg seroconversion usually signals a transition from CHB to an inactive carrier state. HBV DNA is usually undetectable by hybridization techniques but often detectable by PCR assays. The patients are asymptomatic and have normal ALT. Liver biopsy shows no or mild necroinflammatory activity with variable degrees of fibrosis, including inactive cirrhosis. HBsAg is distributed exclusively in hepatocyte cytoplasm and intrahepatic HBcAg is absent [29].

The majority of inactive carriers had levels of HBV DNA less than 2000 IU/mL, a level that has been used to discriminate inactive carrier state from HBeAg negative chronic hepatitis [60]. However, a recent study from Taiwan in 250 inactive carriers with persistently normal ALT for more than 10 years showed that only 64 % had levels of HBV DNA $<10^4$ copies/mL, and 26 and 10 % had levels of HBV DNA in the range of 10^4–10^5 and 10^5–10^6 copies/mL, respectively [61]. It thus should be more appropriate to adopt HBV DNA levels of 20,000 IU/mL, instead of 2000 IU/mL, as a cut-off value to discriminate active from inactive HBV infection [62].

Most inactive carriers remain in this phase with sustained remission and a lifelong inactive state, particularly if this phase is reached early in the disease course. In a Taiwan study of 283 HBeAg seroconverters, 189 (67 %) remained HBeAg negative with persistently normal ALT levels over a 9-year follow-up. Of these, only one progressed to cirrhosis and two developed HCC, with estimated annual rate of cirrhosis and HCC being 0.1 and 0.2 %, respectively [44]. The prognosis of inactive carriers from intermediate- or low-prevalence areas is even better, possibly due to the shorter duration of the infection [63, 64].

Reactivation of Hepatitis B

Following HBeAg seroconversion, a subset of patients ultimately undergo spontaneous reactivation of HBV replication, with reappearance of high levels of HBV DNA (>2000 or 20,000 IU/mL) and a rise in ALT levels. Only a small proportion of

carriers with HBV reactivation is associated with reappearance of serum HBeAg (HBeAg reversion) and the remainders are persistently HBeAg negative [39, 44], suggesting that reactivation of hepatitis B usually results from HBV variants with precore or BCP mutations. In addition, HBV replication can reactivate as a result of immunosuppression or cancer chemotherapy [65].

HBeAg Reversion

In a study from Alaska, 109 (20 %) of 541 seroconverters developed HBeAg reversion, which was frequently accompanied by hepatitis flare, and HBeAg tended to fluctuate between seroconversion and reversion [66]. HBeAg reversion, however, is much uncommon in other studies. In two studies from Taiwan, one involving 283 patients with HBeAg positive hepatitis and another involving 240 HBeAg positive carriers with normal baseline ALT, HBeAg reversion following initial HBeAg seroconversion occurred in 12 (4.2 %) and 7 (2.9 %) patients during a mean follow-up of 8.6 years and 6.8 years, respectively [39, 44]. In another study from Italy, only one (1.6 %) of 61 seroconverters had HBeAg reversion during a mean follow-up of 22.8 years [59]. Despite the low frequency, HBeAg reversion is significantly associated with increased risk of progression to cirrhosis as well as development of HCC [44, 66].

HBeAg Negative Chronic Hepatitis

The majority of patients with reactivation of hepatitis B are negative for HBeAg [39, 44] and have "HBeAg negative CHB." Patients with HBeAg negative CHB are usually older than patients with HBeAg positive CHB and are more likely to have advanced fibrosis and cirrhosis at the time of their first presentation. Serum levels of HBsAg are lower in HBeAg negative CHB than in HBeAg positive CHB by about 0.5–1 $\log_{10}$ IU/mL (3.0–3.5 vs. 3.5–4.5) [26–28]. Serum HBV DNA levels also tend to be lower (4–8 $\log_{10}$ copies/mL), compared to HBeAg positive CHB (6–10 $\log_{10}$ copies/mL). However, many patients with HBeAg negative CHB have wide fluctuations in both HBV DNA and serum ALT levels. Episodes of hepatitis flare are frequently seen, with a rate of about 1/3–1/2 of that in HBeAg positive counterparts [38]. Spontaneous sustained remission of disease activity is rare [67].

The prevalence of HBeAg negative case in patients with CHB varies widely in different geographical areas: 80–90 % in the Mediterranean basin, 30–50 % in Taiwan and Hong Kong, but less than 10 % in the USA and Northern Europe [68]. This difference may be in part attributed to the different HBV genotype distribution: precore mutant is frequently detected in genotype D (the main genotype in the Mediterranean basin) and genotypes B and C infection (the predominant genotypes in East Asia), but rarely detected in genotype A infection (the main genotype in the USA and Northern Europe). The prevalence of HBeAg negative CHB has been increasing over the last few decades as a result of aging of the HBV-infected population

and the effective prevention measures restricting new HBV infections. HBeAg negative CHB has become much more common than HBeAg positive CHB in many countries of the world nowadays.

However, the incidence of HBeAg negative CHB among HBeAg negative carriers remains largely unknown. This issue has been addressed in a few prospective studies that followed up the natural course following spontaneous HBeAg seroconversion. In two studies from Taiwan, the annual rate of HBeAg negative CHB was 2–3 % with a cumulative incidence of 25 % at 16 years, but hepatitis B reactivation typically occurred within the first 5–10 years [39, 44]. However, in another study from Italy, only 9 (14.8 %) of 61 seroconverters developed HBeAg negative CHB during a mean follow-up of 22.8 years (annual rate of 0.6 %) [59]. In another Italian study involving pediatric patients, the rate is even lower: only 4 (6.3 %) of 64 patients developed HBeAg negative CHB during a mean period of 15 years [69]. These differences can be explained by the finding that age of HBeAg seroconversion is an important factor for HBV reactivation [56].

The incidence of hepatitis B reactivation among incidentally identified inactive carriers also varies in different geographical areas. In a study of 1241 inactive carriers from Taiwan, 211 (17.0 %) developed HBeAg negative CHB during a mean follow-up of 12.3 years, with the annual incidence of 1.4 % and the cumulative incidence of 20.2 % at 20 years [70]. Reactivation of hepatitis B occurred much more commonly during the first 5–10 years and became extremely rare after 20 years [70]. In other studies that enrolled a relatively small number of inactive carriers, the annual incidence of reactivation of hepatitis B varied from 0.4 % in Italy [71] and Greece [72] to 2.1 % in Japan [73]. However, in a more recent study of 85 inactive carriers from Greece, the cumulative incidence of HBeAg negative CHB was 24 % at 4 years [74]. The reason for such a high rate of HBV reactivation remains unclear.

Factors predictive for hepatitis B reactivation following HBeAg seroconversion include male gender [75], genotype C HBV (>genotype B) [75], genotype D HBV (>genotype A) [54], HBV-DNA levels >2000 IU/mL [76] or $\geq 10^5$ copies/mL [73] and HBV DNA $>10^4$ copies/mL at 1 year after HBeAg seroconversion [77]. Age of HBeAg seroconversion <30 years is associated with a particularly low incidence of HBV reactivation [75]. In addition, ALT levels >5 × ULN during the immune clearance phase and age of HBeAg seroconversion >40 years are also associated with increased risk of hepatitis B reactivation [56, 75]. The latter findings suggest that HBV is more likely to reactivate if more vigorous immune-mediated hepatocytolysis or a more prolonged immune clearance phase is needed to clear the virus.

Recent studies have shown improved diagnostic accuracy by combined HBsAg and HBV DNA measurements to predict hepatitis B reactivation in inactive carriers: HBsAg >1000 IU/mL and HBV-DNA >200 IU/mL [78], HBsAg levels >850 IU/mL and HBV DNA >850 IU/mL [79], or HBsAg levels >1000 IU/mL in HBeAg negative carriers with HBV DNA <2000 IU/mL [80]. The latter finding reported from Taiwan of genotypes B and C patients is in keeping with the results of an earlier study from Italy of genotype D patients, in which the combined single point quantification of HBsAg <1000 IU/mL and HBV-DNA ≤2000 IU/mL allows the identification of inactive carriers with a very high diagnostic accuracy (94.3 %) [81].

Patients with hepatitis B reactivation have a 20-fold increased risk of progression to cirrhosis as compared with those without [82]. The annual rates of progression to cirrhosis and HCC were 2–3 % and 0.5 %, respectively, in patients with hepatitis B reactivation, significantly higher than 0.1 % and 0.2 %, respectively, in those with sustained remission of hepatitis [44, 82]. Notably, among patients with hepatitis B reactivation, the incidence of cirrhosis is significantly higher in males and in those with age of reactivation older than 40 years [82]

Spontaneous HBsAg Seroclearance

Rates and Predictive Factors

During the low replicative inactive phase, serum HBsAg may disappear (HBsAg seroclearance) spontaneously. Short-term studies showed that the annual incidence of HBsAg seroclearance was 1–2 % in Caucasian carriers, and even lower (0.1–0.8 %) in carriers from the high-prevalence areas [83, 84]. However, a recent long-term follow-up study from Taiwan showed that the incidence of HBsAg seroclearance was appreciably high with an overall annual incidence of 1.2 %, being higher (1.8 %) in those >50 years than in those <30 years (0.8 %), and a cumulative incidence of 8 % at 10 years, increasing disproportionately to 25 % at 20 years, and 45 % at 25 years of follow-up [85].

Factors significantly associated with HBsAg seroclearance include older age [54, 64, 66, 83, 85–87], normal ALT levels [85, 86], HBeAg negativity [66, 83, 86], low viral load (<300 copies/mL) [87], genotype A HBV (> genotype D) [54], or genotype B HBV (> genotype C) infection [88], sustained remission of hepatitis [85], presence of cirrhosis [83] and HCV superinfection [89]. Among these, advanced age is the most constant and important predictor for HBsAg seroclearance [90]. The annual incidence of HBsAg seroclearance varies among different series, but correlates significantly with the mean or median age of patients at enrollment of each cohort [90]. The median age of HBsAg seroclearce in three large cohorts from Taiwan [85], Hong Kong [91] and Japan [92] is approximately 50 years (range, 48–51). Given that the mean or median age of HBeAg seroconversion in Asian adult carriers ranges from 30 to 35 years, it can be expected that sustained remission of hepatitis for a mean of 15 years after HBeAg seroconversion is required to achieve subsequent HBsAg seroclearance. Of note, HBsAg seroclearance can occur sometimes in carrier children, albeit at a relative low rate (0.58 % per year during a mean follow-up of 20.6 years), usually after age 15 (mean, 17.7 ± 7.8; range, 4.1–33.0) and is more common in those with non-carrier mother [93]. Interestingly, in one case–control study, carriers with HBsAg seroclearance had significantly higher body mass index and higher degrees of fatty liver than those without [94]. Furthermore, the mean age of HBsAg seroclearance is significantly younger in patients with fatty liver than in those without (48.7 years vs. 53.4 years) [95]. Notably, in two large cohort studies, fatty liver [96] and obesity [87] were independent factors significantly associated with HBsAg seroclearance. The underlying mechanism by which fatty liver enhances HBsAg seroclearance remains unclear.

Table 11.2 HBsAg quantitation in predicting HBsAg seroclearance

A single point HBsAg level <100 IU/mL in HBeAg negative patients predict HBsAg seroclearance over time [97]
HBsAg level <100 IU/mL and HBV DNA <200 IU/mL at 1 year after HBeAg seroconversion correlate HBsAg seroclearance within 6 years [98]
HBsAg level <10 IU/mL in HBeAg negative carriers with HBV DNA <2000 IU/mL correlates both 5–year and 10–year HBsAg seroclearance [99]
HBsAg levels <200 IU/mL plus >1 $\log_{10}$ IU/mL decrease in preceding 2 years predicts HBsAg seroclearance at 1 and 3 years [100]
HBsAg levels <200 IU/mL or annual decrease of >0.5 $\log_{10}$ IU/mL predict HBsAg seroclearance within 3 years [101]
HBsAg <1000 IU/mL and annual decrease of ≥ 0.3 $\log_{10}$ IU/mL predict HBsAg seroclearance over time [78]

HBeAg hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBV* hepatitis B virus

More recent studies have investigated both absolute and serial changes of serum HBsAg levels in predicting HBsAg seroclearance [78, 97–101], as summarized in Table 11.2. Of these, two Asian studies used an HBsAg level <100 IU/mL as a remote (6–10 years) predictor of HBsAg seroclearance [97, 98]. For short-term prediction, a study from Taiwan has shown that HBsAg level <200 IU/mL plus >1 $\log_{10}$ IU/mL reduction in preceding 2 years can predict HBsAg seroclearance at 1 and 3 years [100]. Another study from Hong Kong has also shown that HBsAg <200 IU/mL or an annual reduction of >0.5 $\log_{10}$IU/mL is predictive for HBsAg seroclearance within 3 years [101].

Virological, Clinical and Histological Profiles After HBsAg Seroclearance

Only 17 % have detectable antibody against HBsAg (anti-HBs) within 1 year after HBsAg seroclearance, but the rate of anti-HBs seroconversion increases to 56 % after 5 years and 76 % after 10 years. Virtually all test negative for HBV DNA by hybridization assays after HBsAg seroclearance, but in some HBV DNA still can be detected by PCR-based assays. The persistence of low-level viremia after HBsAg seroclearance might be a potential source of HBV transmission through blood transfusion or transplantation and account for HBV reactivation with chemotherapy or immunosuppression. In a recent investigation using commercially available, ultrasensitive real-time PCR assay, HBV viremia was detectable in 24 % within 1 year after HBsAg seroclearance, and low-level HBV viremia persisted in ~15 % up to >10 years after HBsAg seroclearance [102]. Serum levels of HBV DNA all are below the sensitivity of hybridization assays (<100 IU/mL in 86 % and 121–2770 IU/mL in 14 %).

Despite the extremely low viremic states, 5–18 % of patients have abnormal ALT levels after HBsAg seroclearance. Non-HBV-related etiologies of abnormal ALT levels can be identified in 75–100 % of such cases, with fatty liver, alcoholism

and herbal medicine administration being the most common [90]. In addition, HCV might have displaced HBV to cause continuing ALT elevation and hepatitis activities [103].

Most patients with liver histological assessment after HBsAg seroclearance have only mild necroinflammation and no significant fibrosis. Immunostaining for HBsAg and HBcAg in liver is negative in all patients; however, all patients tested still harbor HBV inside the liver, mainly in the form of cccDNA, up to 4 years after HBsAg seroclearance [88], albeit at a very low replicative level and in a transcriptionally inactive phase.

Long-term Outcome After HBsAg Seroclearance

In an early study in 55 patients with spontaneous HBsAg seroclearance from Taiwan by Huo et al. [104], 32.7 % developed serious complications, including HCC, cirrhosis, and hepatic failure during a mean follow-up of 23 months. This study probably overestimated the frequency with which complication occurs, as it included 20 patients who had hepatitis C virus (HCV) or hepatitis D virus (HDV) coinfection. In subsequent studies that enrolled a large series of patients from Taiwan and Japan, virtually none of non-cirrhotic patients without HCV or HDV superinfection developed HCC, hepatic decompensation, or liver related death during a mean follow-up of 5 years [92, 105], as summarized in Table 11.3. HBsAg seroclearance usually confers excellent long-term prognosis, provided that HBsAg loss occurred in the absence of concurrent viral infections, and preceded the development of cirrhosis. However, in patients who have preexisting cirrhosis or HCV or HDV superinfection, clinical outcomes of disease progression may still occur [92, 105–108]. A recent report from Hong Kong suggested that cumulative risk for HCC was higher in patients with HBsAg seroclearance at age ≥50 years compared with those with HBsAg seroclearance at age <50 [91]. However, the majority of their patients who developed HCC (6 out of 7) after HBsAg seroclearance had ultrasonographic evidence of cirrhosis before or at the time of HBsAg seroclearance. Of note, the mean age of HBsAg seroclearance in the series of Huo et al. [104] is also appreciably high (see Table 11.3). It is highly suspected that patients who achieved HBsAg seroclearance at older age may be more likely to have undiagnosed cirrhosis and hence remain at risk for HCC. Older age of HBsAg seroclearance per se cannot be considered as an independent risk factor for HCC development after HBsAg seroclearance.

A more recent prospective population-based cohort study in 1271 Alaska native persons with chronic HBV infection followed for an average of 19.6 years showed that the incidence of HCC after HBsAg seroclearance was 36.8 per 100,000 per year (95 % CI 13.5–80.0), which was significantly lower than that in those who remained HBsAg positive (195.7 per 100,000 per year [95 % CI 141.1–264.5]; $P<0.001$) [109]. This study is the first to show a significant reduction in the risk of developing HCC after HBsAg seroclearance.

Table 11.3 Long-term outcome following HBsAg seroclearance in chronic HBV infection

Status at seroclearance	Authors/countries	No. of patients	Mean age (years)	Follow-up (months)	Outcome		
					Cirrhosis	Decompensation	HCC
Non-cirrhosis	Huo [104]/Taiwan	55 (20)[a]	55	23	6 (?)[a]	1 (?)[a]	11 (?)[a]
Non-cirrhosis	Chen [105]/Taiwan	189 (43)[a]	43	65	3 (3)[a]	2 (2)[a]	1 (1)[a]
Non-cirrhosis	Arase [92]/Japan	164 (0)[a]	51	61	0	0	0
Non-cirrhosis	Ahn [107]/Korea	32 (0)[a]	NA	NA	0	NA	1
Non-cirrhosis	Tong [108]/USA	22 (?)[a]	NA	NA	NA	NA	0
Cirrhosis	Fattovich [106]/Italy	32 (5)[a]	NA	55		2 (?)[a]	1 (1)[a]
Cirrhosis	Chen [105]/Taiwan	29 (12)[a]	54	51		4 (2)[a]	1 (1)[a]
Cirrhosis	Arase [92]/Japan	67 (0)[a]	53	74		0	2
Cirrhosis	Ahn [107]/Korea	17 (0)[a]	NA	NA		NA	4
Cirrhosis	Tong [108]/USA	13 (?)[a]	NA	NA		NA	4
Non-cirrhosis and cirrhosis	Yuen [91]/Hong Kong	298 (0)[a]	50	36	NA	5	7 (6 had cirrhosis)
Non-cirrhosis and cirrhosis	Simonnetti [109]/USA	158 (0)[a]	NA	109	NA	NA	6 (2 had cirrhosis)

HBsAg hepatitis B surface antigen, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *NA* not available

[a]Number in ( ) indicates number of patients with HCV or HDV superinfection

Concurrent Viral Infection as Part of Natural Course

In high-prevalence areas such as Taiwan, 50–60 % of adult patients hospitalized for overt acute hepatitis are previously unrecognized HBsAg carriers with reactivation of hepatitis B or non-B viral superinfection, as they are HBsAg positive but IgM anti-HBc negative [22]. Viral superinfection is demonstrated in as high as 30–40 % of these patients, with HCV and HDV being the most common [43]. Non-B viral superinfection in HBsAg carriers tends to increase the severity and case fatality rate during the acute phase. The incidence of fulminant hepatitis ranges from 10 to 20 %, without difference between HCV and HDV superinfection [43, 110].

Hepatitic C Virus

Worldwide, approximately 5–20 % of HBsAg carriers are found to be anti-HCV positive. In HBV endemic areas, HCV superinfection in the setting of chronic HBV infection is the most common scenario of HBV and HCV dual infection. Acute HCV infection in HBsAg carriers with serum HBeAg and HBV DNA may result in only transient HCV infection. In contrast, most acute HCV superinfection in HBsAg carriers without serum HBeAg and HBV DNA progress to persistent HCV infection [111]. These findings suggest that the presence of underlying active HBV replication may interfere with HCV replication and thereby inhibit the persistence of HCV infection.

Two studies from Taiwan showed that a substantial proportion of fulminant hepatitis in HBsAg carriers could be attributed to HCV superinfection [112, 113]. Another study showed that in patients admitted with acute HCV infection, the incidence of fulminant hepatitis was significantly higher among those with underlying HBV infection than those without (23 % versus 3 %, $P<0.01$) [114].

Most patients with HBV and HCV dual infection have detectable serum HCV RNA but not HBeAg or HBV DNA, suggesting that HCV is the predominant cause of liver disease in such cases [115]. More importantly, HCV superinfection is associated with earlier and more frequent progression to cirrhosis. In long-term follow-up analyses from the onset of acute HCV infection, patients with HCV superinfection had higher cumulative rates of cirrhosis (29 % at 5 years, 48 % at 10 years) and HCC (14 % at 10 years, 32 % at 20 years) than those with acute HDV superinfection or HBV mono-infection [110].

Finally, de novo HCV superinfection in HBsAg carriers may lead to a decrease in serum and liver HBV DNA levels and can result in HBeAg seroconversion and, in some cases, HBsAg seroclearance [103, 116]. Such patients had persistence of chronic hepatitis C after successful clearance of HBV [103].

Hepatitis D Virus

Hepatitis HDV is highly prevalent in the Mediterranean countries. The prevalence of HDV infection has significantly declined in some endemic areas, largely because of the HBV vaccination campaigns and the increased awareness on bloodborne infections following the HIV scare.

The clinical features of acute HDV coinfection are indistinguishable from acute hepatitis B [117], although it may be more severe and biphasic ALT peaks may be observed. The rate of progression to chronicity is the same as that of acute hepatitis B. On the contrary, HDV superinfection in HBsAg carriers more likely causes severe acute hepatitis, which progresses to chronicity in up to 80 % [117]. HBV replication is usually suppressed to low levels during acute HDV superinfection and this suppression becomes persistent when progresses to chronicity [118, 119]. Once chronic HDV infection is established, it usually exacerbates the preexisting liver disease due to HBV [120]. In Western studies as many as 70–80 % of chronic hepatitis D patients may develop cirrhosis within 5–10 years [121] and 15 % within 1–2 years [122]. Overall, the relative risk of developing cirrhosis in patients with chronic HDV infection is twofold that in patients with chronic HBV mono-infection [123]. In addition, among patients suffering from compensated HBV-related cirrhosis, there is a three- and twofold increase, respectively, of developing HCC and of death, compared with those with HBV mono-infection [124].

In Taiwan, the prevalence HDV infection is also decreasing [125]. HDV superinfection is associated with relatively milder disease, compared to the Western studies. In one longitudinal study, although HDV superinfection tended to accelerate the progression to cirrhosis relatively shortly after the onset of acute HDV superinfection (21 % at 5 years), the overall incidence of cirrhosis (21 % at 10 years) and HCC (7 % at 10 years) was similar to those with HBV mono-infection [110]. These apparent differences are probably related to the different geographic distribution of HDV genotypes, with genotype II being dominant in Taiwan and genotype I in the Western countries [126].

Human Immunodeficiency Virus

In HBV low-prevalence areas, the majority of the population is not protected by antibodies to natural HBV infection by the age of sexual maturity. Thus, HBV and HIV infections are confined to specific adolescent and adult risk groups, and exposure to both these viruses may occur at more or less the same time. About 10 % of HIV infected patients are coinfected with HBV. HBV tends to be more aggressive in HIV-positive individuals, with higher HBV carrier rates following acute exposure, higher levels of HBV viremia in chronic carriers, and diminished incidence of spontaneous seroclearance of HBeAg and/or HBsAg, more frequent episodes of activation, and faster progression to cirrhosis [127, 128]. HCC occurs more often, its onset is earlier, and its course is more aggressive in HBV and HIV coinfection than HBV mono-infection [129]. In a multicenter study involving 5293 homosexual men,

liver-related mortality was significantly higher in HIV and HBV coinfection (14.2 per 1000 person-years) than in HBV (0.8 per 1000 person-years) or HIV mono-infection (1.7 per 1000 person-years). In coinfected individuals, the liver related mortality rate was highest in those with lower nadir CD4+ cell counts and was twice as high after 1996, when highly active antiretroviral therapy (HAART) was introduced [130]. The development of effective antiretroviral regimen has led to immune reconstitution in many HIV-infected patients. The so-called immune reconstitution flare of hepatitis B has been observed in HBV and HIV coinfected patients following the initiation of HAART [131].

Conversely, most adolescents and adults in HBV high-prevalence areas are already protected from HBV infection or are chronic HBsAg carriers by the time of their first exposure to HIV infection. The rate of HBV coinfection in HIV positive individuals in Taiwan is 21.7 % [132], a little higher than the background HBsAg carrier rate (15–20 %) in the general population. The great majority of patients with HBV and HIV coinfection are presumed to be chronic HBsAg carriers with HIV superinfection. Interestingly, acute HIV superinfection in HBsAg carriers can suppress HBV replication and result in HBeAg seroclearance and, in some instances, HBsAg seroclearance [133]. It remained unclear whether this suppression is transient or persistent. The natural course difference between HBsAg carriers with and without HIV superinfection has rarely been addressed before. However, a higher risk of hepatitis flare, hepatic decompensation and liver-related death in HBV and HIV coinfection than in HIV mono-infection in the era of HAART was also reported [132].

Sequelae and Mortality

The long-term outcomes of chronic HBV infection vary considerably from an inactive carrier state to the development of cirrhosis, hepatic decompensation, and HCC. Contrary to patients in the immune tolerance phase and those in inactive carrier state, patients with active hepatitis either in the immune clearance phase or the reactivation phase are at high risk of disease progression. The estimated 5-year cumulative rates of progression from chronic hepatitis to cirrhosis, compensated cirrhosis to hepatic decompensation, and compensated cirrhosis to HCC are 8–20 %, 15–20 %, and 6–15 %, respectively (Fig. 11.2).

Cirrhosis and Contributing Factors

It is estimated that cirrhosis develops in approximately 20 % of patients with chronic HBV infection [1]. Whether this rate is higher in perinatally acquired infection because of the longer duration of infection than adult-acquired infection remains unknown. In one clinicopathologic study from Taiwan, cirrhosis was noted in 21 % of asymptomatic HBsAg carriers with age over 40 [134].

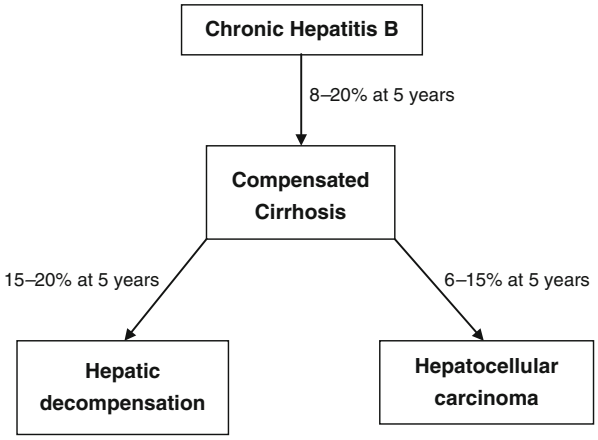

Fig. 11.2 Long-term sequelae of chronic hepatitis B virus infection

The annual incidence and cumulative probability of cirrhosis in patients with chronic HBV infection varied considerably in different reported series, possibly due to inclusion of patients in different phases of infection as well as the variable severity of liver injury in each phase. In two representative studies that enrolled 684 and 105 patients with chronic hepatitis B from Taiwan and Italy, the annual incidence of cirrhosis ranged from 2 to 6 % and the 5-year cumulative incidence ranged from 8 to 20 % [135, 136]. Factors identified to contribute to the development of cirrhosis include older age [135, 136], HBeAg positivity at recruitment [137, 138], persistent HBeAg seropositivity [57–59], persistence of HBV DNA by non-PCR assays [136, 139], HBeAg reversion [44, 66], delayed HBeAg seroconversion over age 40 [39, 55, 56], hepatitis B reactivation [39, 44], especially if reactivation at age over 40 [82], severe chronic active hepatitis with bridging hepatic necrosis [136], and ALT flares complicated with hepatic decompensation or recurrent AL T flares with high AFP or bridging hepatic necrosis [135]. HBV genotype is also a contributing factor [51, 52]. Many patients have developed cirrhosis during the HBeAg positive phase, as shown in a recent study that 28 (30 %) of 93 patients were HBeAg positive at the onset of cirrhosis [140] These data implies that the ultimate outcome of chronic HBV infection appears to depend on the duration of the immune clearance phase and reactivation phase, as well as on the severity of liver damage during these phases. Other factors significantly correlated with progression to cirrhosis include advanced age, longer duration of infection, male gender, and concurrent HCV, HDV or HIV superinfection, alcoholism and superimposed non-alcoholic fatty liver disease.

A recent population-based cohort study (the REVEAL-HBV) of more than 3500 untreated HBsAg carriers (median 45 years of age at enrolment, 85 % HBeAg negative, 94 % with normal ALT) in Taiwan found that the risk of cirrhosis increased significantly with increasing baseline serum HBV DNA levels at a dose-dependent manner [141]. The adjusted relative risks (RR) of progression to cirrhosis was 2.5,

5.6, and 6.5 when baseline HBV DNA levels were $\geq 10^4$, 10^5, and 10^6 copies/mL, respectively and HBV DNA levels $\geq 10^4$ copies/mL was the strongest predictor of future cirrhosis, regardless of HBeAg and ALT levels at baseline [141]. It seems likely that these patients are prone to have hepatitis B reactivation and progress to HBeAg negative CHB prior to cirrhosis development. A more recent study from Taiwan suggested that HBsAg levels greater than 1000 IU/mL in HBeAg negative carriers with low viral load (<2000 IU/mL) also tended to increase the risk of HBeAg negative CHB and cirrhosis [77].

Hepatic Decompensation

HBV replication and necroinflammation may have subsided at the onset of cirrhosis. However, at least 1/3–1/2 of patients with HBV-related cirrhosis still have a high level of HBV replication (positive HBeAg or HBV DNA by non-PCR assays, or HBV DNA $>10^5$ copies/mL) at presentation [140, 142]. About 3–4 % of the patients with compensated HBV-related cirrhosis developed decompensation (jaundice, ascites, hepatic encephalopathy) and/or gastroesophageal varices each year, with a 5-year cumulative incidence of 15–20 % [123, 142]. The risk of hepatic decompensation is fourfold higher in HBeAg or HBV DNA positive patients (4 % per year) than in HBeAg and HBV DNA negative patients (1 % per year) [142] . The average annual incidence of hepatic decompensation after the onset of cirrhosis is 1.5 %, but hepatic decompensation tends to occur later in the course, with the cumulative incidence of 5, 18 and 31 %, respectively, at 5, 10 and 20 years [140]. As the mean age at the onset of cirrhosis ranges from 41 to 44 years [140, 143] and that at the onset of decompensation ranges from 55 to 60 years, it is estimated that hepatic decompensation usually occurs 10–15 years after the onset of cirrhosis.

One form of hepatic decompensation in HBV-related cirrhosis is secondary to acute hepatitis flares. In two early studies from Taiwan, the annual incidence of acute hepatitis flare was 15–25 % in HBeAg positive patients and 5–10 % in HBeAg negative patients. Some 10–15 % of acute hepatitis flares were complicated with jaundice and 3–5 % with ascites [143, 144].

Hepatocellular Carcinoma and Contributing Factors

The incidence of HCC in chronic HBV infection correlates closely with the severity of the underlying liver diseases, as summarized by Fattovich et al. [123]. In the East Asian countries, the summary annual incidence of HCC ranges from 0.2 % among inactive carriers to 0.8 % in patients with CHB and 3.7 % in subjects with compensated cirrhosis; the corresponding 5-year cumulative incidences is 1, 3, and 17 %, respectively. In the Western countries, the summary annual incidence of HCC is 0.02 % in inactive carriers, 0.3 % in patients with CHB and 2.2 % in patients with compensated cirrhosis; the corresponding 5-year cumulative incidences is 0.1, 1, and 10 %, respectively. These data confirm that cirrhosis is a well documented risk

factor for HCC development and also suggest that perinatally acquired HBV infection is associated with a greater risk of HCC than infection acquired in adults, possibly because of the longer duration of infection.

Most cases of HCC are likely to have concomitant cirrhosis. Factors significantly predictive for progression to cirrhosis therefore also contribute to HCC development. Other factors significantly associated with HCC development in chronic HBV infection include race (Asians and Africans), a family history of HCC, HBV genotype, BCP mutations and pre-S deletion mutations, aflatoxin exposure and alcohol drinking [145–147]. In HBeAg negative carriers with low viral load, HBsAg levels >1000 IU/mL also is an independent risk factor for HCC development [148].

In the REVEAL-HBV study, the risk of HCC increased significantly starting at the level of 10^4 copies/mL and was highest for patients with the highest baseline HBV DNA level (>10^6 copies/mL) with a hazard risk of 2.3 and 6.6, respectively [149]. Unfortunately, this study did not report the prevalence of cirrhosis among HCC patients, so it is difficult to determine whether increased viral replication, known to encourage the development of cirrhosis, may have any additional impact on HCC development. The prognostic value of HBV replication for the development of HCC in patients with HBV-related cirrhosis remains controversial [140, 143, 150–152]. A recent case–control study did not show significant difference in serum levels of HBV DNA between HBV-related cirrhosis with and without HCC [153].

Finally, although inactive carriers with HBV DNA <10^4 copies/mL and normal ALT are at lowest risk for HCC among chronic HBV infected individuals, they still have a substantial risk of HCC as compared with HBV and HCV negative controls. The multivariate-adjusted hazard ratio is 4.6 (95 % CI: 2.5–8.3). Older age and alcoholism are independent predictors of risk for inactive carriers [154].

Survival

The 5-year survival of compensated, Child-Pugh class A HBV-related cirrhosis is approximately 80–85 %, which correlates closely with the status of HBV replication. Survival probability is >95 % in patients negative for HBeAg and HBV DNA by non-PCR assays but only 60–72 % in HBeAg or HBV DNA positive patients [142]. Among the latter, HBeAg seroclearance is associated with a 2.2-fold decrease in mortality [155] and ALT normalization is a better predictor of improved survival than HBeAg seroclearance [156].

Once hepatic decompensation has developed, survival probability decreases remarkably. The reported 5-year survival rates of decompensated, Child-Pugh class B or C HBV-related cirrhosis vary considerably from 14 to 88 % (average, 30–50 %) [142]. In one study from Hong Kong, the 5-year survival rate was significantly lower in patients with serum HBeAg at presentation (57 %) than in HBeAg negative patients (88 %) [157]. In contrast, in another study from the Netherlands, the 5-year survival rate was extremely low (14 %) independent of serum HBeAg at enrollment

[155]. These data may suggest that survival probability correlates significantly with the status of HBV replication in patients with less severe hepatic decompensation but not in patients with more severe hepatic decompensation.

Conclusion

The natural history of HBV infection and the estimated overall and annual incidence of each event are summarized in Fig. 11.3. HBV replication with subsequent interactions between HBV, hepatocytes and immune cells during the immune clearance or reactivation phase may lead to hepatitis activity and disease progression. High HBV DNA levels and hepatitis activity at enrollment or during follow-up are the best predictors of adverse clinical outcomes. Sustained reduction of HBV replication before the onset of cirrhosis confers a favorable outcome. Sustained reduction of HBV replication in cirrhotic patients also reduces the risk of hepatic decompensation, HCC development and improves survival. The improvements in the knowledge of the natural history of HBV infection and a detailed understanding of predictors for disease progression will help in the management of patients with chronic HBV infection.

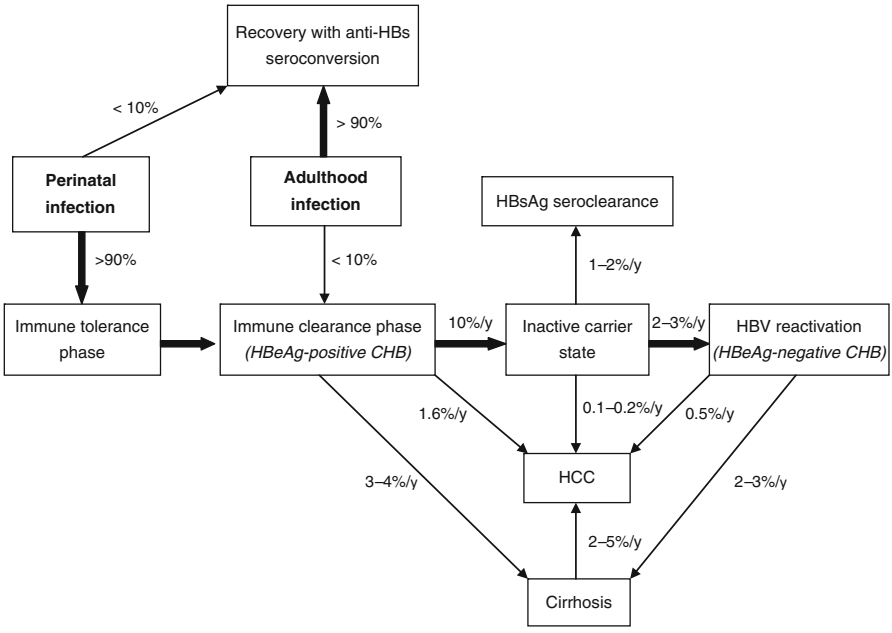

Fig. 11.3 Natural history of chronic hepatitis B virus infection. The overall rates as well as the annual rates of each event are included

References

1. Liaw YF, Chu CM. Hepatitis B virus infection. Lancet. 2009;373:582–92.
2. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine. 2012;30:2212–9.
3. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006;45:529–38.
4. Iloeje UH, Yang HI, Jen CL, Su J, Wang LY, You SL, Chen CJ. Risk evaluation of viral load elevation and associated liver disease/cancer-hepatitis B virus study group Risk and predictors of mortality associated with chronic hepatitis B infection. Clin Gastroenterol Hepatol. 2007;5:921–31.
5. Chu CM, Sheen IS, Lin SM, Liaw YF. Sex difference in chronic hepatitis B virus infection: studies of serum HBeAg and alanine aminotransferase levels in 10,431 asymptomatic Chinese HBsAg carriers. Clin Infect Dis. 1993;16:709–13.
6. Kew MC. Progress towards the comprehensive control of hepatitis B in Africa: a view from South Africa. Gut. 1996;38 Suppl 2:S31–6.
7. Shiraki K. Perinatal transmission of hepatitis B virus and its prevention. J Gastroenterol Hepatol. 2000;15(Suppl):E11–5.
8. Ni YH, Huang LM, Chang MH, Yen CJ, Lu CY, You SL, et al. Two decades of universal hepatitis B vaccination in Taiwan: impact and implication for future strategies. Gastroenterology. 2007;132:1287–93.
9. Ni YH, Chang MH, Wu JF, Hsu HY, Chen HL, Chen DS. Minimization of hepatitis B infection by a 25-year universal vaccination program. J Hepatol. 2012;57:730–5.
10. McMahon BJ, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, Maynard JE. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. J Infect Dis. 1985;151:599–603.
11. Chen HL, Chang CJ, Kong MS, Huang FC, Lee HC, Lin CC, et al. Pediatric fulminant hepatic failure in endemic areas of hepatitis B infection: 15 years after universal hepatitis B vaccination. Hepatology. 2004;39:58–63.
12. Friedt M, Gerner P, Lausch E, Trübel H, Zabel B, Wirth S. Mutations in the basic core promotor and the precore region of hepatitis B virus and their selection in children with fulminant and chronic hepatitis B. Hepatology. 1999;29:1252–8.
13. Lee WM. Hepatitis B, virus infection. N Engl J Med. 1997;337:1733–45.
14. Laskus T, Rakela J, Nowicki MJ, Persing DH. Hepatitis B virus core promoter sequence analysis in fulminant and chronic hepatitis B. Gastroenterology. 1995;109:1618–23.
15. Chu CM, Yeh CT, Lee CS, Sheen IS, Liaw YF. Precore stop mutant in HBeAg-positive patients with chronic hepatitis B: clinical characteristics and correlation with the course of HBeAg-to-anti-HBe seroconversion. J Clin Microbiol. 2002;40:16–21.
16. Milich D, Liang TJ. Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. Hepatology. 2003;38:1075–86.
17. Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. Clin Infect Dis. 1995;20:992–1000.
18. Tassopoulos NC, Papaevangelou GJ, Sjogren MH, Roumeliotou-Karayannis A, Gerin JL, Purcell RH. Natural history of acute hepatitis B surface antigen-positive hepatitis in Greek adults. Gastroenterology. 1987;92:1844–50.
19. Kaboth U, Adami B, Alexander M. Cooperative prospective study “acute viral hepatitis” (In German). Verh Dtsch Ges Inn Med. 1980;86:749–56.
20. Yotsuyanagi H, Ito K, Yamada N, Takahashi H, Okuse C, Yasuda K, et al. High levels of hepatitis B virus after the onset of disease lead to chronic infection in patients with acute hepatitis B. Clin Infect Dis. 2013;57:935–42.
21. Ito K, Yotsuyanagi H, Yatsuhashi H, Karino Y, Takikawa Y, Saito T, et al. Risk factors for long-term persistence of serum hepatitis B surface antigen following acute hepatitis B virus infection in Japanese adults. Hepatology. 2014;59:89–97.

22. Chu CM, Liaw YF, Pao CC, Huang MJ. The etiology of acute hepatitis superimposed upon previously unrecognized asymptomatic HBsAg carriers. Hepatology. 1989;9:452–6.
23. Chu CM, Karayiannis P, Fowler MJ, Monjardino J, Liaw YF, Thomas HC. Natural history of chronic hepatitis B virus infection in Taiwan: studies of hepatitis B virus DNA in serum. Hepatology. 1985;5:431–4.
24. Chu CM. Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol. 2000;15(Suppl):E25–30.
25. Chu CM, Liaw YF. Natural history differences in perinatally versus adult acquired disease. Curr Hepat Rep. 2004;3:123–31.
26. Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, et al. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. J Hepatol. 2010;52:508–13.
27. Jaroszewicz J, Calle Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R, et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. J Hepatol. 2010;52:514–22.
28. Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, Sung JJ. A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. Hepatology. 2010;52:1232–41.
29. Chu CM, Liaw YF. Intrahepatic distribution of hepatitis B surface and core antigens in chronic hepatitis B virus infection. Hepatocyte with cytoplasmic/membranous hepatitis B core antigen as a possible target for immune hepatocytolysis. Gastroenterology. 1987;92: 220–5.
30. Hui CK, Leung N, Yuen ST, Zhang HY, Leung KW, Lu L, et al. Natural history and disease progression in Chinese chronic hepatitis B patients in immune-tolerant phase. Hepatology. 2007;46:395–401.
31. Chu CM, Yeh CT, Sheen IS, Liaw YF. Subcellular localization of hepatitis B core antigen in relation to hepatocyte regeneration in chronic hepatitis B. Gastroenterology. 1995;109: 1926–32.
32. Chang ML, Liaw YF. Hepatitis B flares in chronic hepatitis B: pathogenesis, natural course and management. J Hepatol. 2014;61:1407–17.
33. Liaw YF, Pao CC, Chu CM. Changes of serum HBV-DNA in relation to serum transaminase level during acute exacerbation in patients with chronic type B hepatitis. Liver. 1988;8:231–5.
34. Maruyama T, Iino S, Koike K, Yasuda K, Milich DR. Serology of acute exacerbation in chronic hepatitis B virus infection. Gastroenterology. 1993;105:1141–51.
35. Tsai SL, Chen PJ, Lai MY, Yang PM, Sung JL, Huang JH, et al. Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. J Clin Invest. 1992;89:87–96.
36. Liaw YF, Chu CM, Su IJ, Huang MJ, Lin DY, Chang-Chien CS. Clinical and histological events preceding hepatitis B e antigen seroconversion in chronic type B hepatitis. Gastroenterology. 1983;84:216–9.
37. Liaw YF, Yang SS, Chen TJ, Chu CM. Acute exacerbation in hepatitis B e antigen positive chronic type B hepatitis. A clinicopathological study. J Hepatol. 1985;1:227–33.
38. Liaw YF, Tai DI, Chu CM, Pao CC, Chen TJ. Acute exacerbation in chronic type B hepatitis: comparison between HBeAg and antibody-positive patients. Hepatology. 1987;7:20–3.
39. Chu CM, Hung SJ, Lin J, Tai DI, Liaw YF. Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. Am J Med. 2004;116:829–34.
40. Sheen IS, Liaw YF, Tai DI, Chu CM. Hepatic decompensation associated with hepatitis B e antigen clearance in chronic type B hepatitis. Gastroenterology. 1985;89:732–5.
41. Jeng WJ, Sheen IS, Liaw YF. Hepatitis B virus DNA level predicts hepatic decompensation in patients with acute exacerbation of chronic hepatitis B. Clin Gastroenterol Hepatol. 2010;8:541–5.

42. Chu CM, Lin SM, Hsieh SY, Yeh CT, Lin DY, Sheen IS, Liaw YF. Etiology of sporadic acute viral hepatitis in Taiwan: the role of hepatitis C virus, hepatitis E virus and GB virus-C/ hepatitis G virus in an endemic area of hepatitis A and B. J Med Virol. 1999;58:154–9.
43. Chu CM, Liaw YF. Increased incidence of fulminant hepatic failure in previously unrecognized HBsAg carriers with acute hepatitis independent of etiology. Infection. 2005;33:136–9.
44. Hsu YS, Chien RN, Yeh CT, Sheen IS, Chiou HY, Chu CM, Liaw YF. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology. 2002;35:1522–7.
45. Tseng YR, Wu JF, Ni YH, Chen HL, Chen CC, Wen WH, et al. Long-term effect of maternal HBeAg on delayed HBeAg seroconversion in offspring with chronic hepatitis B infection. Liver Int. 2011;31:1373–80.
46. Marx G, Martin SR, Chicoine JF, Alvarez F. Long-term follow-up of chronic hepatitis B virus infection in children of different ethnic origins. J Infect Dis. 2002;186:295–301.
47. Chang MH, Hsu HY, Hsu HC, Ni YH, Chen JS, Chen DS. The significance of spontaneous hepatitis B e antigen seroconversion in childhood: with special emphasis on the clearance of hepatitis B e antigen before 3 years of age. Hepatology. 1995;22:1387–92.
48. Liaw YF, Chu CM, Huang MJ, Sheen IS, Yang CY, Lin DY. Determinants for hepatitis B e antigen clearance in chronic type B hepatitis. Liver. 1984;4:301–6.
49. Kim HS, Kim HJ, Shin WG, Kim KH, Lee JH, Kim HY, Jang MK. Predictive factors for early HBeAg seroconversion in acute exacerbation of patients with HBeAg-positive chronic hepatitis B. Gastroenterology. 2009;136:505–12.
50. Chu CJ, Hussain M, Lok AS. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. Gastroenterology. 2002;122: 1756–62.
51. Kao JH. Role of viral factors in the natural course and therapy of chronic hepatitis B. Hepatol Int. 2007;1:415–30.
52. Chu CM, Liaw YF. Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B: a longitudinal study of hepatitis B e antigen-positive patients with normal aminotransferase levels at baseline. J Hepatol. 2005;143:411–7.
53. Livingston SE, Simonetti JP, Bulkow LR, Homan CE, Snowball MM, Cagle HH, et al. Clearance of hepatitis B e antigen in patients with chronic hepatitis B and genotypes A, B, C, D, and F. Gastroenterology. 2007;133:1452–7.
54. Sánchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodés J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. Gastroenterology. 2002;123:1848–56.
55. Chu CM, Liaw YF. Chronic hepatitis B virus infection acquired in childhood: special emphasis on prognostic and therapeutic implication of delayed HBeAg seroconversion. J Viral Hepat. 2007;14:147–52.
56. Chen YC, Chu CM, Liaw YF. Age-specific prognosis following spontaneous hepatitis B e antigen seroconversion in chronic hepatitis B. Hepatology. 2010;51:435–44.
57. Niederau C, Heintges T, Lange S, Goldmann G, Niederau CM, Mohr L, Häussinger D. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med. 1996;334:1422–7.
58. Lin SM, Yu ML, Lee CM, Chien RN, Sheen IS, Chu CM, Liaw YF. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. J Hepatol. 2007;46:45–52.
59. Fattovich G, Olivari N, Pasino M, D'Onofrio M, Martone E, Donato F. Long-term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years. Gut. 2008; 57:84–90.
60. Keeffe EB, Dieterich DT, Han SH, Jacobson IM, Martin P, Schiff ER, Tobias H. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: 2008 update. Clin Gastroenterol Hepatol. 2008;6:1315–41.

61. Chu CM, Chen YC, Tai DI, Liaw YF. Level of hepatitis B virus DNA in inactive carriers with persistently normal levels of alanine aminotransferase. Clin Gastroenterol Hepatol. 2010;8:535–40.
62. Chen YC, Huang SF, Chu CM, Liaw YF. Serial HBV DNA levels in patients with persistently normal transaminase over 10 years following spontaneous HBeAg seroconversion. J Viral Hepat. 2012;19:138–46.
63. Villeneuve JP, Desrochers M, Infante-Rivard C, Willems B, Raymond G, Bourcier M, et al. A long-term follow-up study of asymptomatic hepatitis B surface antigen-positive carriers in Montreal. Gastroenterology. 1994;106:1000–5.
64. Manno M, Cammà C, Schepis F, Bassi F, Gelmini R, Giannini F, et al. Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years. Gastroenterology. 2004;127:756–63.
65. Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated liver disease. Gastroenterology. 2001;120:1009–22.
66. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. Ann Intern Med. 2001;135:759–68.
67. Bonino F, Brunetto MR. Chronic hepatitis B e antigen (HBeAg) negative, anti-HBe positive hepatitis B: an overview. J Hepatol. 2003;39 Suppl 1:S160–3.
68. Funk ML, Rosenberg DM, Lok AS. World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants. J Viral Hepat. 2002;9:52–61.
69. Bortolotti F, Guido M, Bartolacci S, Cadrobbi P, Crivellaro C, Noventa F, et al. Chronic hepatitis B in children after e antigen seroclearance: final report of a 29-year longitudinal study. Hepatology. 2006;43:556–62.
70. Chu CM, Liaw YF. Spontaneous relapse of hepatitis in inactive HBsAg carrier. Hepatol Int. 2007;1:311–5.
71. de Franchis R, Meucci G, Vecchi M, Tatarella M, Colombo M, Del Ninno E, et al. The natural history of asymptomatic hepatitis B surface antigen carriers. Ann Intern Med. 1993;118:191–4.
72. Zacharakis GH, Koskinas J, Kotsiou S, Papoutselis M, Tzara F, Vafeiadis N, et al. Natural history of chronic HBV infection: a cohort study with up to 12 years follow-up in North Greece (part of the Interreg I-II/EC-project). J Med Virol. 2005;77:173–9.
73. Nakazawa T, Shibuya A, Takeuchi A, Shibata Y, Hidaka H, Okuwaki Y, et al. Viral level is an indicator of long-term outcome of hepatitis B virus e antigen-negative carriers with persistently normal serum alanine aminotransferase levels. J Viral Hepat. 2011;18:e191–9.
74. Papatheodoridis GV, Chrysanthos N, Hadziyannis E, Cholongitas E, Manesis EK. Longitudinal changes in serum HBV DNA levels and predictors of progression during the natural course of HBeAg-negative chronic hepatitis B virus infection. J Viral Hepat. 2008;15:434–41.
75. Chu CM, Liaw YF. Predictive factors for reactivation of hepatitis B following hepatitis B e antigen seroconversion in chronic hepatitis B. Gastroenterology. 2007;133:1458–65.
76. Feld JJ, Ayers M, El-Ashry D, Mazzulli T, Tellier R, Heathcote EJ. Hepatitis B virus DNA prediction rules for hepatitis B e antigen-negative chronic hepatitis B. Hepatology. 2007;46:1057–70.
77. Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. Hepatology. 2013;57:441–50.
78. Martinot-Peignoux M, Lapalus M, Laouénan C, Lada O, Netto-Cardoso AC, Boyer N, et al. Prediction of disease reactivation in asymptomatic hepatitis B e antigen-negative chronic hepatitis B patients using baseline serum measurements of HBsAg and HBV-DNA. J Clin Virol. 2013;58:401–7.
79. Park H, Lee JM, Seo JH, Kim HS, Ahn SH, Kim do Y, et al. Predictive value of HBsAg quantification for determining the clinical course of genotype C HBeAg-negative carriers. Liver Int. 2012;32:796–802.
80. Tseng TC, Liu CJ, Chen CL, Wang CC, Su TH, Kuo SF, et al. Serum hepatitis B virus-DNA levels correlate with long-term adverse outcomes in spontaneous hepatitis B e antigen seroconverters. J Infect Dis. 2012;205:54–63.

81. Brunetto MR, Oliveri F, Colombatto P, Moriconi F, Ciccorossi P, Coco B, et al. Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. Gastroenterology. 2010;139:483–90.
82. Chu CM, Liaw YF. Incidence and risk factors of progression to cirrhosis in inactive carriers of hepatitis B virus. Am J Gastroenterol. 2009;104:1693–9.
83. Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. Hepatology. 1991;13:627–31.
84. Wu TT, Hsu HC, Chen DS, Sheu JC, Su IJ, Chen SL, Chuang SM. Clearance of hepatitis B surface antigen (HBsAg) after surgical resection of hepatocellular carcinoma. J Hepatol. 1987;4:45–51.
85. Chu CM, Law YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology. 2007;45:1187–92.
86. Kim JH, Lee JH, Park SJ, Bae MH, Kim JH, Kim do Y, et al. Factors associated with natural seroclearance of hepatitis B surface antigen and prognosis after seroclearance: a prospective follow-up study. Hepatogastroenterology. 2008;55:578–81.
87. Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Wang LY, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology. 2010;139:474–82.
88. Yuen MF, Wong DK, Sablon E, Tse E, Ng IO, Yuan HJ, et al. HBsAg seroclearance in chronic hepatitis B in the Chinese: virological, histological, and clinical aspects. Hepatology. 2004;39:1694–701.
89. Sheen IS, Liaw YF, Lin DY, Chu CM. Role of hepatitis C and D viruses in the termination of chronic HBsAg carrier state: a multivariate analysis in a longitudinal follow-up study. J Infect Dis. 1994;170:358–61.
90. Chu CM, Liaw YF. Hepatitis B surface antigen seroclearance during chronic HBV infection. Antivir Ther. 2010;15:133–43.
91. Yuen MF, Wong DK, Fung J, Ip P, But D, Hung I, et al. HBsAg Seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology. 2008;135:1192–9.
92. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, et al. Long-term outcome after hepatitis B surface antigen seroclearance in patients with chronic hepatitis B. Am J Med. 2006;119:719.e-16.
93. Chiu YC, Liao SF, Wu JF, Lin CY, Lee WC, Chen HL, et al. Factors affecting the natural decay of hepatitis B surface antigen in children with chronic hepatitis B virus infection during long-term follow-up. J Pediatr. 2014;165:767–72.
94. Chu CM, Lin DY, Liaw YF. Does increased body mass index with hepatic steatosis contribute to seroclearance of hepatitis B virus (HBV) surface antigen in chronic HBV infection. Int J Obes (Lond). 2007;31:871–5.
95. Chu CM, Lin DY, Liaw YF. Clinical and virological characteristics post HBsAg seroclearance in hepatitis B virus carriers with hepatic steatosis versus those without. Dig Dis Sci. 2013;58:275–81.
96. Tai DI, Lin SM, Sheen IS, Chu CM, Lin DY, Liaw YF. Long-term outcome of hepatitis B e antigen-negative hepatitis B surface antigen carriers in relation to changes of alanine aminotransferase levels over time. Hepatology. 2009;49:1859–67.
97. Chan HL, Wong GL, Tse CH, Chan HY, Wong VW. Viral determinants of hepatitis B surface antigen seroclearance in hepatitis B e antigen-negative chronic hepatitis B patients. J Infect Dis. 2011;204:408–14.
98. Tseng TC, Liu CJ, Su TH, Wang CC, Chen CL, Chen PJ, et al. Serum hepatitis B surface antigen levels predict surface antigen loss in hepatitis B e antigen seroconverters. Gastroenterology. 2011;141:517–25.
99. Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. Determinants of spontaneous surface antigen loss in hepatitis B e antigen-negative patients with a low viral load. Hepatology. 2012;55:68–76.

100. Chen YC, Jeng WJ, Chu CM, Liaw YF. Decreasing levels of HBsAg predict HBsAg seroclearance in patients with inactive chronic hepatitis B virus infection. Clin Gastroenterol Hepatol. 2012;10:297–302.
101. Seto WK, Wong DK, Fung J, Hung IF, Fong DY, Yuen JC, et al. A large case-control study on the predictability of hepatitis B surface antigen levels three years before hepatitis B surface antigen seroclearance. Hepatology. 2012;56:812–9.
102. Chu CM, Liaw YF. Prevalence of and risk factors for hepatitis B viremia after spontaneous hepatitis B surface antigen seroclearance in hepatitis B carriers. Clin Infect Dis. 2012;54:88–90.
103. Liaw YF, Tsai SL, Chang JJ, Sheen IS, Chien RN, Lin DY, Chu CM. Displacement of hepatitis B virus by hepatitis C virus as the cause of continuing chronic hepatitis. Gastroenterology. 1994;106:1048–53.
104. Huo TI, Wu JC, Lee PC, Chau GY, Lui WY, Tsay SH, et al. Sero-clearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. Hepatology. 1998;28:231–6.
105. Chen YC, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. Gastroenterology. 2002;123:1084–9.
106. Fattovich G, Giustina G, Sanchez-Tapias J, Quero C, Mas A, Olivotto PG, et al. Delayed clearance of serum HBsAg in compensated cirrhosis B: relation to interferon alpha therapy and disease prognosis. European Concerted Action on Viral Hepatitis (EUROHEP). Am J Gastroenterol. 1998;93:896–900.
107. Ahn SH, Park YN, Park JY, Chang HY, Lee JM, Shin JE, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. J Hepatol. 2005;42:188–94.
108. Tong MJ, Nguyen MO, Tong LT, Blatt LM. Development of hepatocellular carcinoma after seroclearance of hepatitis B surface antigen. Clin Gastroenterol Hepatol. 2009;7: 889–93.
109. Simonetti J, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S, et al. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. Hepatology. 2010;51:1531–7.
110. Liaw YF, Chen YC, Sheen IS, Chien RN, Yeh CT, Chu CM. Impact of acute hepatitis C virus super-infection in patients with chronic hepatitis B virus infection. Gastroenterology. 2004;126:1024–93.
111. Chu CM, Yeh CT, Sheen IS, Liaw YF. Acute hepatitis C virus (HCV) infection in chronic carriers of hepatitis B virus (HBV): the impact of underlying active HBV replication on persistence of HCV infection and antibody responses to HCV. Gut. 2002;51:95–9.
112. Wu JC, Chen CL, Hou MC, Chen TZ, Lee SD, Lo KJ. Multiple viral infection as the most common cause of fulminant and subfulminant viral hepatitis in an area endemic for hepatitis B: application and limitations of the polymerase chain reaction. Hepatology. 1994;19: 836–40.
113. Chu CM, Sheen IS, Liaw YF. The role of hepatitis C virus in fulminant viral hepatitis in an area with endemic hepatitis A and B. Gastroenterology. 1994;107:189–95.
114. Chu CM, Yeh CT, Liaw YF. Fulminant hepatic failure in acute hepatitis C: increased risk in chronic carriers of hepatitis B virus. Gut. 1999;45:613–7.
115. Liaw YF. Role of hepatitis C virus in dual and triple hepatitis virus infection. Hepatology. 1995;22:1101–8.
116. Liaw YF, Lin SM, Sheen IS, Chu CM. Acute hepatitis C virus superinfection followed by spontaneous HBeAg seroconversion and HBsAg elimination. Infection. 1991;19:250–1.
117. Smedile A, Farci P, Verme G, Caredda F, Cargnel A, Caporaso N, et al. Influence of delta infection on severity of hepatitis B. Lancet. 1982;2:945–7.
118. Krogsgaard K, Kryger P, Aldershvile J, Andersson P, Sørensen TI, Nielsen JO. Delta-infection and suppression of hepatitis B virus replication in chronic HBsAg carriers. Hepatology. 1987;7:42–5.

119. Farci P, Karayiannis P, Lai ME, Marongiu F, Orgiana G, Balestrieri A, Thomas HC. Acute and chronic hepatitis delta virus infection: direct or indirect effect on hepatitis B virus replication? J Med Virol. 1988;26:279–88.
120. Smedile A, Dentico P, Zanetti A, Sagnelli E, Nordenfelt E, Actis GC, Rizzetto M. Infection with the delta agent in chronic HBsAg carriers. Gastroenterology. 1981;81:992–7.
121. Govindarajan S, De Cock KM, Redeker AG. Natural course of delta superinfection in chronic hepatitis B virus-infected patients: histopathologic study with multiple liver biopsies. Hepatology. 1986;6:640–4.
122. Saracco G, Rosina F, Brunetto MR, Amoroso P, Caredda F, Farci P, et al. Rapidly progressive HBsAg-positive hepatitis in Italy. The role of hepatitis delta virus infection. J Hepatol. 1987;5:274–81.
123. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol. 2008;48:335–52.
124. Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Realdi G, Schalm SW. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). Gut. 2000;46:420–6.
125. Huo TI, Wu JC, Lin RY, Sheng WY, Chang FY, Lee SD. Decreasing hepatitis D virus infection in Taiwan: an analysis of contributory factors. J Gastroenterol Hepatol. 1997;12:747–51.
126. Wu JC, Choo KB, Chen CM, Chen TZ, Huo TI, Lee SD. Genotyping of hepatitis D virus by restriction-fragment length polymorphism and relation to outcome of hepatitis D. Lancet. 1995;346:939–41.
127. Soriano V, Puoti M, Peters M, Benhamou Y, Sulkowski M, Zoulim F, et al. Care of HIV patients with chronic hepatitis B: updated recommendations from the HIV-Hepatitis B Virus International Panel. AIDS. 2008;22:1399–410.
128. Puoti M, Torti C, Bruno R, Filice G, Carosi G. Natural history of chronic hepatitis B in co-infected patients. J Hepatol. 2006;44 suppl 1:S65–70.
129. Bräu N, Fox RK, Xiao P, Marks K, Naqvi Z, Taylor LE, et al. Presentation and outcome of hepatocellular carcinoma in HIV-infected patients: a US-Canadian multicenter study. J Hepatol. 2007;47:527–53.
130. Thio CL, Seaberg EC, Skolasky Jr R, Phair J, Visscher B, Muñoz A, Thomas DL. AIDS Multicenter Cohort Study. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). Lancet. 2002;360:1921–6.
131. Drake A, Mijch A, Sasadeusz J. Immune reconstitution hepatitis in HIV and hepatitis B co-infection, despite lamivudine therapy as part of HAART. Clin Infect Dis. 2004;39:129–32.
132. Sheng WH, Chen MY, Hsieh SM, Hsiao CF, Wang JT, Hung CC, Chang SC. Impact of chronic hepatitis B virus (HBV) infection on outcomes of patients infected with HIV in an area where HBV infection is hyperendemic. Clin Infect Dis. 2004;38:1471–7.
133. Jiao Y, Li N, Chen X, Zhang T, Li H, Li W, et al. Acute HIV infection is beneficial for controlling chronic hepatitis B. Clin Infect Dis. 2015;60:128–34.
134. Yang PM, Chen DS, Lai MY, Su IJ, Huang GT, Lin JT, et al. Clinicopathologic studies of asymptomatic HBsAg carriers: with special emphasis on carriers older than 40 years. Hepatogastroenterology. 1997;34:251–4.
135. Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. Hepatology. 1988;8:493–6.
136. Fattovich G, Brollo L, Giustina G, Noventa F, Pontisso P, Alberti A, et al. Natural history and prognostic factors for chronic hepatitis type B. Gut. 1991;32:294–8.
137. Yu MW, Hsu FC, Sheen IS, Chu CM, Lin DY, Chen CJ, Liaw YF. Prospective study of hepatocellular carcinoma and liver cirrhosis in asymptomatic chronic hepatitis virus carriers. Am J Epidemiol. 1997;145:1039–47.
138. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, et al. Hepatitis Be antigen and the risk of hepatocellular carcinoma. N Engl J Med. 2002;347:168–74.
139. Brunetto MR, Oliveri F, Coco B, Leandro G, Colombatto P, Gorin JM, Bonino F. Outcome of anti-HBe positive chronic hepatitis B in alpha-interferon treated and untreated patients: a long term cohort study. J Hepatol. 2002;36:263–70.

140. Chen YC, Chu CM, Yeh CT, Liaw YF. Natural course following the onset of cirrhosis in patients with chronic hepatitis B: a long-term follow-up study. Hepatol Int. 2007;1:267–73.
141. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ, Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-In HBV (the REVEAL-HBV) Study Group. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006;130:678–86.
142. Chu CM, Liaw YF. Hepatitis B virus-related cirrhosis: natural history and treatment. Semin Liver Dis. 2006;26:142–52.
143. Liaw YF, Lin DY, Chen TJ, Chu CM. Natural course after the development of cirrhosis in patients with chronic type B hepatitis: a prospective study. Liver. 1989;9:235–41.
144. Liaw YF, Chen JJ, Chen TJ. Acute exacerbation in patients with liver cirrhosis: a clinicopathological study. Liver. 1990;10:177–84.
145. Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. J Gastroenterol Hepatol. 1997;12:S294–308.
146. Liu S, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. J Natl Cancer Inst. 2009;101:1066–82.
147. Yang HI, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, et al. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. J Natl Cancer Inst. 2008;100:1134–43.
148. Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology. 2012;142:1140–9.
149. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295:65–73.
150. Fattovich G, Giustina G, Schalm SW, Hadziyannis S, Sanchez-Tapias J, Almasio P, et al. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B. The EUROHEP Study Group on Hepatitis B Virus and Cirrhosis. Hepatology. 1995;21:77–82.
151. Fattovich G, Pantalena M, Zagni I, Realdi G, Schalm SW, Christensen E, European Concerted Action on Viral Hepatitis (EUROHEP). Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. Am J Gastroenterol. 2002;97:2886–95.
152. Mahmood S, Niiyama G, Kamei A, Izumi A, Nakata K, Ikeda H, et al. Influence of viral load and genotype in the progression of Hepatitis B-associated liver cirrhosis to hepatocellular carcinoma. Liver Int. 2005;25:220–5.
153. Chu CM, Lin CC, Chen YC, Jeng WJ, Lin SM, Liaw YF. Basal core promoter mutation is associated with progression to cirrhosis rather than hepatocellular carcinoma in chronic hepatitis B virus infection. Br J Cancer. 2012;107:2010–5.
154. Chen JD, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, et al. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. Gastroenterology. 2010;138:1747–54.
155. de Jongh FE, Janssen HL, de Man RA, Hop WC, Schalm SW, van Blankenstein M. Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver. Gastroenterology. 1992;103:1630–5.
156. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EDROHEP). Hepatology. 1997;26:1338–42.
157. Chung HT, Lai CL, Lok AS. Pathogenic role of hepatitis B virus in hepatitis B surface antigen-negative decompensated cirrhosis. Hepatology. 1995;22:25–9.