Chronic hepatitis B virus (HBV) infection has a complicated course. Three phases are identified: an immune tolerant phase with high HBV DNA and normal alanine aminotransferase (ALT) levels associated with minimal liver disease; an immune active phase with high HBV DNA and elevated ALT levels with active liver inflammation; and an inactive phase with HBV DNA levels < 2000 IU/mL and normal ALT levels with minimal inflammation and fibrosis on liver biopsy. Affected persons can move progressively from one phase to the next and may revert backward. The primary adverse outcomes of chronic HBV infection are hepatocellular carcinoma (HCC) and cirrhosis. Published natural history studies were reviewed and ranked by the strength of evidence regarding the study design. Factors with the highest evidence of risk for development of HCC or cirrhosis from population-based prospective cohort studies include male sex, family history of HCC, HBV DNA level above 2000 IU/mL in persons above age 40, HBV genotypes C and F, and basal core promoter mutation. Those with the next highest level of evidence include aflatoxin exposure, and heavy alcohol and tobacco use. Improved methods to identify persons at highest risk of developing HCC or cirrhosis are needed to allow intervention earlier with antiviral therapy in appropriate patients. Future studies should include prospective follow-up of established population-based cohorts as well as new cohorts recruited from multiple centers stratified by HBV genotypes/subgenotypes and clinical phase to determine the incidence of the various HBV phases, HCC, and cirrhosis. Also, nested case-control studies assessing immunological and host genetic factors among persons with active and inactive disease phases, HCC, and cirrhosis could be conducted using these types of cohorts. (HEPATOLOGY 2009;49:S45-S55.)

Introduction

Between 350 million and 400 million persons worldwide are chronically infected with hepatitis B virus (HBV). The two primary adverse outcomes of chronic infection are hepatocellular carcinoma (HCC) and cirrhosis, either of which can lead to a liver-related death. The natural history of chronic HBV infection in individuals is complex, and infected persons can pass through several phases. Patients can move from a state of high viral load and no liver disease to one of active liver disease, followed by inactive disease, and then revert back to active liver disease years later. Progression to advanced fibrosis can be rapid, slow, or sporadic. During the inactive periods, hepatic inflammation, fibrosis, and even early cirrhosis can be reversed over time only to reappear again if the disease reactivates. Thus, chronic hepatitis B is a dynamic condition and it is difficult to predict what will happen over time to an individual with this chronic infection.

Understanding the natural history of chronic hepatitis B is important because it can guide the clinician in deciding on the need and optimal timing for initiating antiviral therapy. The purpose of this article is to review the published literature on the natural history of chronic HBV infection to extract the best available evidence in regard to conclusions on management. In addition, because there are many gaps in the understanding of the natural history
of chronic HBV infection, this article will conclude with suggestions for future studies that could help resolve some of these questions.

### Evaluating Studies on the Natural History of Chronic HBV Infection

Many studies pertaining to the natural history of HBV have been published in the past three decades. However, these studies vary in quality of design and conduct. Few are prospective and fewer of those are population-based. Many are clinic-based case-control studies or case series. For these reasons, in reviewing the literature on this topic, a score was assigned to each study ranking the strength of the evidence that led to the conclusions (Table 1). Because natural history studies involve observation and not intervention, they are not “randomized” in the same way as prospective clinical trials which are typically assigned the highest scores for strength of medical evidence. Therefore, the strongest evidence-based rating score was assigned to longitudinal, prospective, population-based studies that followed cohorts of both HBV-infected and HBV-uninfected individuals to determine outcome over time. Outcomes could include HCC, cirrhosis, liver inflammation and/or fibrosis, or even resolution of liver disease. The weakest evidence scores were attributed to clinic-based studies involving infected patients evaluated at one time point, comparing characteristics of those who had developed an adverse outcome to those who had not. The level of available evidence, based on the scoring system shown in Table 1, is listed in parentheses after the association of interest.

### Adverse Outcomes of HBV Infection: HCC and Decompensated Cirrhosis

Longitudinal prospective outcome studies have clearly shown that patients with chronic HBV infection have a considerable risk of developing HCC during their lifetime (1a, 1b). Most compelling was the classical study by Beasley and colleagues conducted in Taiwan in the early 1970s. They screened 22,707 male railway workers for HBV markers, identified 3454 hepatitis B surface antigen (HBsAg)-positive carriers, and followed both carriers and noncarriers for a total of 75,000 person-years. The relative risk of developing HCC was 223 for carriers versus noncarriers, HCC accounting for 54% of the deaths among carriers compared to 1.5% among noncarriers. Since these findings were published, a high incidence of HCC has been reported from every country with high rates of HBsAg, including most recently Greenland. Although HCC occurs more frequently in HBV-infected men than HBV-infected women with a ratio of 3:1 to 4:1 (1b), HBV-infected women have also been shown to have a higher risk of HCC (1b). Other risk factors for HCC include older age (1a), family history of HCC (2c), presence of cirrhosis (1a, 2a), and hepatitis C virus (HCV) coinfection (2c).

The ideal study to establish the risk of developing cirrhosis would be a longitudinal population-based study where all participants have serial liver biopsies to determine whether they have cirrhosis as well as to identify the factors predictive of its development. However, such a study has not been done, largely because of the discomfort and invasiveness of liver biopsy and the current lack of dependable surrogate markers for detecting cirrhosis. Cirrhosis becomes clinically apparent once decompensation occurs, so that a more clinically measurable outcome for assessing the natural history of HBV is establishing the incidence of decompensated cirrhosis. One population-based study found the incidence of decompensation to be 0.5% per 1000 person-years (1b). In clinic-based longitudinal studies, the overall incidence of development of cirrhosis is 2%-3% per year (2a). Risk factors for developing cirrhosis include older age, presence of hepatitis B e antigen (HBeAg), and elevated alanine aminotransferase (ALT) levels (2a). The survival rate for untreated persons with compensated cirrhosis is 84% and 68% at 5 and 10 years, respectively, but the survival rate is only 14% at 5 years in persons who present with decompensated cirrhosis (2a). In persons with compensated cirrhosis, long-term survival is negatively associated with HBeAg positivity in that clearance of HBeAg improves survival and decreases the risk of liver decompensation (2a).

### The Phases of Chronic Hepatitis B Infection

In 2000 and 2006, the National Institutes of Health (NIH) sponsored two research workshops on the management of chronic hepatitis B. The conference participants defined three phases of chronic HBV infection that are now widely accepted: the immune tolerant phase, the immune active phase, and the inactive hepatitis B phase.
In addition, a fourth phase, the recovery phase, was proposed (Table 2, Fig. 1).

In persons who develop chronic hepatitis B infection, HBeAg is initially positive, accompanied by high levels of HBV DNA and may remain so for a few years to several decades. Most patients eventually lose HBeAg and develop antibody to HBeAg (anti-HBe). The observed rate of clearance of HBeAg in persons with or without elevated ALT levels averages between 8% and 12% per year (1b,2a),4,20,21 but this rate is much lower in persons who are in the immune tolerant phase.22,23 The rate and average age of seroconversion from HBeAg to anti-HBe varies by HBV genotype, because persons infected with genotype C remain HBeAg-positive for many years longer than those infected with genotypes A, B, D, or F (1b).24

The Immune Tolerant Phase. HBV-infected persons in the immune tolerant phase are HBeAg-positive, have normal ALT levels, and elevated levels of HBV DNA that are >20,000 IU/mL and commonly well above 1 million IU/mL. The immune tolerant phase is thought to occur most frequently in persons who are infected via perinatal transmission from HBeAg-positive mothers.24 HBeAg may act as an immune tolerant protein that aids the virus in avoiding detection by the immune system. In immune competent persons, HBV is not cytopathic and hepatocellular damage is induced by the host immune system’s efforts to eliminate HBV. The immune tolerant phase can last for a few years to more than 30 years (2b).25 During this phase, there is either no or minimal liver inflammation or fibrosis. However, because the HBV polymerase gene has reverse transcriptase properties,

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**Table 2. Phases of Chronic Hepatitis B Infection**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune Tolerant Phase</td>
<td>- Occurs primarily after perinatal infection from HBsAg/HBeAg-positive mother</td>
</tr>
<tr>
<td></td>
<td>- ALT levels are normal</td>
</tr>
<tr>
<td></td>
<td>- HBV DNA &gt; 200,000 IU/mL (&gt;1 million copies), often above 10^7-8 IU/mL</td>
</tr>
<tr>
<td></td>
<td>- Liver biopsy is normal or shows only minimal inflammation with no or minimal fibrosis</td>
</tr>
<tr>
<td></td>
<td>- Occurs most frequently in HBV genotype C infection</td>
</tr>
<tr>
<td>Immune Active (Clearance) Phase</td>
<td>- Elevated ALT levels</td>
</tr>
<tr>
<td></td>
<td>- HBV DNA &gt; 20,000 IU/mL</td>
</tr>
<tr>
<td>Inactive Phase</td>
<td>- Elevated ALT</td>
</tr>
<tr>
<td></td>
<td>- HBV DNA &gt; 2000 IU/mL</td>
</tr>
<tr>
<td></td>
<td>- Hepatic inflammation with or without fibrosis on biopsy often present in both HBeAg-positive and HBeAg-negative immune active phase</td>
</tr>
</tbody>
</table>

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**Fig. 1. Algorithm to display the natural history of chronic hepatitis B virus infection.**
HBV integrates randomly into the host’s hepatocyte DNA and during the immune tolerant phase, persistently high levels of HBV DNA over many years would likely mean an accumulation of integration sites, increasing the risk of HCC over time even in the absence of active liver inflammation and fibrosis.

**The Immune Active Phase.** The immune active phase, also sometimes referred to as the “chronic hepatitis B phase” or the “immune clearance phase”, is characterized by elevated ALT levels and an elevated HBV DNA level above at least 2000 IU/mL. Active liver inflammation is usually present with or without liver fibrosis. Patients may be either HBeAg-positive or HBeAg-negative/anti-HBe–positive. Persons infected after birth who develop chronic HBV infection may advance to the immune active phase shortly after the time of infection, whereas those infected via the perinatal route may transition into this phase several years after experiencing the immune tolerant phase of HBV. In this phase, the host’s immune system recognizes HBV as being foreign and initiates an immune response that results in hepatocyte damage. In persons who are HBeAg-positive, HBV DNA levels may progressively fall, eventually resulting in seroconversion from HBeAg to anti-HBe. Seroconversion to anti-HBe can be preceded by a flare of hepatitis. Clearance of HBeAg that occurs spontaneously or as a result of antiviral therapy reduces the risk of hepatic decompensation and improves survival.

**Anti-HBe–Positive Chronic Hepatitis B.** Persons with anti-HBe–positive chronic hepatitis B can present in one of two ways. A small proportion, 10%-20%, will remain in the immune active phase after seroconverting from HBeAg to anti-HBe. Others will transition into the inactive hepatitis B phase only to experience one or more episodes of reactivation to the immune active phase. These patients usually have lower levels of HBV DNA (2000 IU/mL to 2 million IU/mL) than persons in the HBeAg-positive immune active phase. There are reports in some studies that the precore (PC) mutation, a G→A mutation at codon 1896 that results in the occurrence of a stop codon that renders the virus unable to encode for HBeAg, is associated with HBeAg-negative active hepatitis B; however, because PC mutations are also found in persons in the anti-HBe–inactive carrier phase, it is uncertain whether the occurrence of these mutations actually promote or are surrogate markers for liver inflammation. Anti-HBe–positive hepatitis B phase is characterized by a rise in HBV DNA to >2000 IU/mL, and improvement in liver fibrosis and inflammation over time. Prospective studies conducted for up to 10 years of persons in the inactive hepatitis B phase have shown that in most of them, HBeAg remains negative, ALT levels remain normal, and HBV DNA levels remain <2000 IU/mL or even negative (2a). Moreover, liver fibrosis is either absent or minimal in degree and shows no evidence of progression over time in those who remain in the inactive hepatitis B phase.

However, a few clinic-based cross-sectional studies have demonstrated that a minority of persons in the inactive HBV phase have had moderate, or occasionally even severe, fibrosis present on liver biopsy (2c). In these studies, the inactive HBV phase was defined as normal ALT levels and HBV DNA <2000 IU/mL for 3-12 months only. There are two conceivable explanations for the finding of more than mild fibrosis in a proportion of patients in these studies. First, some patients may have entered the inactive phase with already moderate to severe hepatic fibrosis before the observation period began and liver fibrosis may be in the process of slow regression. Second, some persons may be having recurrent flares of anti-HBe–positive hepatitis interspersed with prolonged periods of having normal ALT levels and may have been mislabeled as being in the inactive phase. Thus, these persons must be followed indefinitely to be sure that they remain in this phase.

### Possible Events After Seroconversion from HBeAg to Anti-HBe

Four possible scenarios can develop in HBV-infected patients after HBeAg seroconversion (Fig. 1). First, approximately 20% of patients will experience one or more reversions back to HBeAg positivity. These reversion/seroconversion events are usually associated with flares of hepatitis. Recently, it was found that the proportion of patients experiencing HBeAg reversion differs by HBV genotype, with the highest risk (~40%) occurring in persons infected with genotypes C and F (1b). Second, most patients (70%-80%) will go into the inactive hepatitis B phase where most will remain for life (1b, 2a). Third, after HBeAg/anti-HBe seroconversion, 10%-30% of patients will remain in the immune active phase manifested by the continued presence of elevated ALT values and HBV DNA levels above 2000 IU/mL (2a). Finally, 10%-30% of persons who initially go into the inactive phase will later experience one or more reactivations of anti-HBe–positive hepatitis, characterized by a rise in HBV DNA to >2000 IU/mL accompanied or followed by a rise in ALT levels. Persons in whom HBeAg reversions occur or who have
reactivation of anti-HBe–positive chronic hepatitis appear to be at higher risk of developing HCC or cirrhosis.4

**Spontaneous Clearance of HBsAg**

Published studies have found that between 0.5% and 0.8% of chronically infected individuals will clear HBsAg per year (2a).4,48,49 Predictors of HBsAg clearance are older age and sustained presence of the inactive hepatitis stage.4,48 The clinical outcome after clearance of HBsAg is generally better than in persons who continue to be HBsAg-positive. Liver inflammation and fibrosis improve over time.50-52 In one study of 189 persons without cirrhosis at the time of HBsAg clearance, none developed cirrhosis and all had normal ALT levels an average of 62 months after seroclearance.53 Persons who clear HBsAg have been classified as being in the “recovery phase” of hepatitis B.18 However, this term may be a misnomer because several studies have clearly shown that HCC can develop in some of these individuals years after HBsAg clearance.4,50-53 In addition, HBV DNA can be found in the serum of up to 21% of persons as long as 5 years after HBsAg clearance. A higher proportion has detectable HBV DNA in liver. Thus, although while the risk of advancing liver disease and the development of cirrhosis may have waned, the risk of HCC is still present, albeit less likely. The ongoing risk of HCC may be due to two factors. First, the presence of integrated HBV DNA in hepatocytes that occurred over the years while HBV replication was high could conceivably trigger genomic mistakes during hepatocyte cell division that might result in HCC. Second, HBV may well be still present in low levels in chronically infected persons who cleared HBsAg. Thus, persons who were previously chronically HBsAg-positive and are later in the so-called “recovery phase” should still be followed for the development of HCC, as recommended in the current practice guidelines.54

**Risk Factors for the Development of HCC and Liver Fibrosis**

Risk factors identified to be associated with an increased risk of developing HCC, progressive liver disease and cirrhosis are listed in Table 3. Demographic risk factors have been discussed above. Heavy alcohol use is a risk factor for more severe liver disease, and aflatoxin exposure has been shown to be a cofactor in increasing the risk of HCC in HBV-infected persons.

**Viral Risk Factors for HCC and Cirrhosis**

**HBV Genotype.** Eight genotypes (A through H) of HBV have been identified that differ from each other in whole-genome sequencing by at least 8%.55 In addition, multiple subgenotypes (1, 2, 3, etc.) have been identified within the HBV genotypes, these differing by 4%-8%.56 Genotype A is found in Northern Europe and frequently among Caucasians in the United States. Genotypes B and C are common in Asian populations, including immigrants from Asia in the United States as well as first-generation and second-generation Asian Americans. Genotype D is the most common genotype found in Southern and Eastern Europe and is also common in the Middle East. Genotype F is found in native populations in North and South America. Genotype E, G, and H infections are uncommon and their epidemiology is not well characterized (Table 4).

A growing number of published studies have provided evidence that the natural history of chronic HBV infection differs according to the specific infecting HBV genotype and subgenotype. Shown in Table 4 are the geographic areas where specific HBV genotypes and subgenotypes have been found and includes clinical associations that have been made and the strength of evidence for these associations. Genotype A1 is associated with HCC in young men who are usually HBeAg-negative and anti-HBe–positive, have low levels of HBV DNA, and rarely have cirrhosis. Exposure to aflatoxin may be an important cofactor for the occurrence of this outcome. Genotype A2 is associated with HCC in older persons. Compared to HBV genotype D, genotype A2 is associated with a lower risk of HCC and a greater likelihood of resolution of active hepatitis, and clearance of HBV DNA and HBsAg.57

Genotype B is divided into two major groups: Bj found in Japan and Ba found in the rest of Asia. Bj (B1 and B6) is a “pure” strain of genotype B, while Ba (B2-5) contains

<table>
<thead>
<tr>
<th>Table 3. Factors Associated with the Increased Risk of Progression of Liver Disease and Risk of HCC and Cirrhosis in Persons with Chronic HBV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
</tr>
<tr>
<td>• Male Sex: Increased risk of HCC (1a)</td>
</tr>
<tr>
<td>• Age: Increase risk HCC with advancing age (1a,1b)</td>
</tr>
<tr>
<td><strong>Social and Environmental</strong></td>
</tr>
<tr>
<td>• Alcohol: Increased risk for HCC and cirrhosis (2c)</td>
</tr>
<tr>
<td>• NAFLD: insufficient data</td>
</tr>
<tr>
<td>• Aflatoxin exposure: increased risk of HCC (2c)</td>
</tr>
<tr>
<td><strong>Viral</strong></td>
</tr>
<tr>
<td>• HBV Genotype/sub-genotype risk for HCC and cirrhosis (1b, 2a, 2b)</td>
</tr>
<tr>
<td>• HBV DNA level over age 40 years for HCC and cirrhosis(1b)</td>
</tr>
<tr>
<td>• Viral coinfection</td>
</tr>
<tr>
<td>• HBV + HIV</td>
</tr>
<tr>
<td>• Increase HBV DNA levels (2c)</td>
</tr>
<tr>
<td>• Increased risk of cirrhosis/HCC (3)</td>
</tr>
<tr>
<td>• HBV + HCV</td>
</tr>
<tr>
<td>• Increase risk of HCC (2a)</td>
</tr>
<tr>
<td>• HBV + HDV</td>
</tr>
<tr>
<td>• Increase risk of cirrhosis (2c)</td>
</tr>
</tbody>
</table>
a portion of the genome of genotype C recombined into the core region of genotype B. Ba is associated with older age at the time of HBeAg seroconversion, a higher risk for HCC, and a higher frequency of BCP mutations than genotype Bj.58

Numerous studies have reported the clinical outcome of chronic hepatitis in patients with HBV genotype C infection compared to other HBV genotypes, especially genotype B. There are compelling data (1b, 2a) from multiple population-based and clinic-based prospective trials which show that HBV genotype C is independently associated with a higher risk of HCC than genotypes A2, Ba and Bj, and D.59-62 Thus, HBV genotype C may be the most treacherous of the HBV genotypes. A population-based study from Alaska that included 1152 Alaska Natives with chronic HBV infection who were followed for 21 years found that 50% of those infected with HBV genotypes A2, B6, D, and F1 cleared HBeAg before reaching 20 years of age. In contrast, the average age of HBeAg seroconversion in persons with genotype C was 47 years.24

HBV genotype D has been associated with HBeAg-negative chronic hepatitis and frequently harbors the PC variant.40 However, persons infected with genotype D and found to be in the inactive hepatitis B phase are likely to remain in this phase without developing complications of liver disease or HCC; in one study, 97% of those with minimal or no fibrosis or inflammation on liver biopsy had no progression of histology on repeat liver biopsy after 4 years of follow-up.44 A possible reason for these conflicting findings could be that persons infected with genotype D either go into the inactive hepatitis B phase and stay there or develop chronic hepatitis (the immune active phase of HBV) at the time of or shortly after HBV seroconversion.

Studies are not available to assess the influence of HBV genotypes E, G, and H on disease outcome. HBV genotype F1 has recently been shown to be associated with a high risk of HCC in Alaska compared to HBV genotypes A2, B1, and D, particularly in children and young adults <30 years of age.62

### Level of HBV DNA Associated with Active Liver Disease

A few cross-section studies that examined the association of active liver inflammation and fibrosis and HBV DNA level found that approximately 90% of those with active liver disease at the time of liver biopsy who are HBeAg-negative/anti-HBe–positive and have an elevated ALT level have an HBV DNA level of >10^5 genomic copies/mL (20,000 IU/mL), 10% have levels between 10^4 and 10^5 copies/mL (2000-20,000 IU/mL), and 1% have <10^4 copies/mL.52,63 However, many persons in these studies with HBV DNA levels above 10^4 or even 10^5 copies/mL have minimal or no fibrosis or inflammation on biopsy. Thus, while HBV DNA >2000 IU/mL appears to be a reasonable level at which to evaluate persons with chronic HBV infection for the extent of liver disease, a liver biopsy may be necessary to identify those with the significant findings of moderate or severe inflammation and fibrosis. Thus, not all patients with HBV DNA levels >2000 IU/mL have active liver disease or fibrosis, but most persons with levels <2000 IU/mL have inactive disease.

### Table 4. Geographic Distribution of Specific HBV Genotypes/Subgenotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Geographic Region</th>
<th>Disease Association</th>
<th>Strength of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Sub-Saharan Africa</td>
<td>HCC in young males often without cirrhosis</td>
<td>2c</td>
</tr>
<tr>
<td>A2</td>
<td>Northern Europe</td>
<td>HCC and cirrhosis in older persons</td>
<td>2c</td>
</tr>
<tr>
<td>A3</td>
<td>West Africa</td>
<td>Unknown</td>
<td>2c</td>
</tr>
<tr>
<td>B1</td>
<td>Japan</td>
<td>HCC and cirrhosis in older persons</td>
<td>2c</td>
</tr>
<tr>
<td>B2-6</td>
<td>East Asia</td>
<td>HCC and cirrhosis occurs at younger age than B1</td>
<td>2c</td>
</tr>
<tr>
<td>C1-4</td>
<td>China, Korea, Southeast Asia, Japan, South Pacific Islands</td>
<td>Higher risk of HCC and cirrhosis compared with genotypes B</td>
<td>1b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HBeAg seroconversion occurs 1-3 decades later than in genotypes A, B, D, and F1</td>
<td>1b,2a</td>
</tr>
<tr>
<td>D1-4</td>
<td>Russia, Middle East, Mediterranean, North Africa, Eastern Europe, Indian Subcontinent</td>
<td>Anti-HBe chronic hepatitis B, HCC, and cirrhosis in older individuals</td>
<td>2c</td>
</tr>
<tr>
<td>E</td>
<td>West Africa</td>
<td>Unknown</td>
<td>NA</td>
</tr>
<tr>
<td>F1</td>
<td>Alaska, Central America, South America</td>
<td>HCC in children and young adults in Alaska only</td>
<td>2b</td>
</tr>
<tr>
<td>F2</td>
<td>Central America, Amazon region</td>
<td>Unknown</td>
<td>NA</td>
</tr>
<tr>
<td>G</td>
<td>Europe, United States, Australia</td>
<td>Almost exclusively found in persons coinfected with other HBV genotypes, mainly A1. Clinical significance unknown.</td>
<td>NA</td>
</tr>
<tr>
<td>H</td>
<td>Central America, Amazon region</td>
<td>Unknown</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not available.
HBV DNA Level and Risk of Subsequent Development of HCC and Cirrhosis

In the past few years, several prospective population-based studies have analyzed outcomes of HBV infection in relation to the level of HBV DNA at the beginning of the observation period. These studies have uniformly shown that HBV DNA levels >10^5 or 10^6 copies/mL (~2000 or 20,000 IU/mL) are associated with an increased risk of HCC, and one study has shown that there is an increased risk of developing cirrhosis.59-64 In all of these studies, the mean age at enrollment was in the mid-40s and follow-up was as long as 11 years. In the REVEAL-HBV (Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus) study, 28,870 persons from 10 towns in Taiwan were tested for HBV markers; 4155 were HBsAg-positive and 3653 had baseline HBV DNA levels tested. The median age at enrollment was 46 years and the average age at a median of 11.4 years follow-up was 57 years. Persons with HBV DNA levels above 10^4 copies/mL were again tested at end of follow-up and those whose levels remained above 10^4 copies/mL were at increased risk of developing HCC and cirrhosis.65,66 Other independent risk factors identified besides HBV DNA levels were older age and the presence of cirrhosis. This and the other prospective studies provide strong evidence that for persons over age 40, HBV DNA level above 2000 IU/mL is a risk factor for developing HCC even in persons with ALT levels between 0.5 and 1.0 times the upper limit of the normal range.

However, there is at this time no evidence that persons below the age of 40 years with HBV DNA levels above 2000 IU/mL are at increased risk of HCC until they reach their 40s. One small prospective study of persons in the immune tolerant phase whose mean age was 30 years showed that those who remained in this stage (HBsAg-positive with normal ALT levels) had mild disease on biopsy at entry, and on repeat biopsy 5 years later, had minimal progression of fibrosis. Those who developed ALT elevations and remained HBsAg-positive had evidence of progressive fibrosis and a marked increase in the histologic activity index score.67 Additional prospective studies of persons less than 40 years of age with elevated HBV DNA levels are needed to clarify this issue.

Specific HBV Mutations and the Risk of HCC and Cirrhosis

Specific mutations in the HBV genome, especially those that result in amino acid changes in the viral peptides expressed by HBV, may affect the subsequent risk of developing HCC or cirrhosis. Many studies have been reported on the consequences of a double substitution: A1762T and G1764A in the (BCP) region of HBV.39,41,62,69 The overwhelming evidence from both cross-sectional and prospective studies is that BCP is an independent risk factor for the development of active liver disease and HCC. Even after adjusting for HBV genotype, BCP has been found to be an independent risk factor for HCC in persons infected with genotypes A2, B, C, and D but not in genotype F1.62 Another mutation, the precore mutant, has been associated with liver inflammation, especially anti-HBe-positive immune active HBV, and the development of HCC in some but not all studies.39,40,62 Recently, the presence of the PC mutation was associated with a lower risk of developing HCC independent of HBV genotype and BCP mutation.61 Other small studies examining both the HBV core and the X regions, and the pre-S region of the HBV envelope, have shown that mutations in these areas might be important predictors of risk, but more definitive studies are needed before any conclusions can be made.70,71

HBV Coinfections with HCV, Human Immunodeficiency Virus or Hepatitis B Delta Virus

Between 6% and 13% of persons infected with human immunodeficiency virus (HIV) are also chronically infected with HBV, with the highest prevalence of coinfection found in sub-Saharan Africa.72 Coinfection with HBV and HIV is associated with higher levels of HBV DNA, lower rates of spontaneous HBeAg seroconversion, and higher rates of liver-related mortality than monoinfection with HBV.73,74 Severe flares of hepatitis have been reported in patients coinfected with HBV/HIV with low CD4+ T cell counts who experience immune reconstitution after initiation of highly active antiretroviral therapy.73 In addition, some coinfected patients with high levels of HBV DNA and hepatic necroinflammation may be HBsAg-negative but anti-HBe-positive (latent HBV).73

In the United States and elsewhere, up to 15% of HBV-infected persons are also infected with HCV, the highest risk being found among injection drug users.75 In developing countries, HCV coinfection is usually due to use of poor sterile technique during vaccination and medical procedures.1 Acute HCV infection in persons with chronic HBV infection can increase the risk of developing severe, even fulminant, hepatitis.76 In HBV/HCV coinfected patients, HCV can become the dominant virus and suppress HBV DNA levels.77 Patients with HBV/HCV coinfection are at a much higher risk of developing HCC.78,79
Hepatitis B delta virus (HDV) is a satellite virus dependent on HBV for production of its envelope protein. Chronic HBV/HDV coinfection is primarily a result of an HDV superinfection occurring in an individual chronically infected with HBV. Coinfection with both viruses increases the risk of cirrhosis and hepatic decompensation.

**Combining Risk Factors to Better Characterize Those at the Highest Risk of Developing HCC and Decompensated Cirrhosis**

Recently, the authors of the REVEAL study used their cohort to detect other viral-related risk factors for HCC (Table 5). Independent risk factors identified in testing the samples obtained at study entry included an HBV DNA level of >10^4 copies/mL (approximately 2000 IU/mL), HBV genotype C (compared with genotype B), the presence of BCP mutations, and surprisingly, the absence of PC mutation. In those who had all three of these factors, the incidence of HCC was 2254 per 100,000 person-years of follow-up compared to those who were infected with HBV genotype B, who did not have BCP but did have PC mutation (174 per 100,000 person-years). To put this into perspective, the risk of developing HCC over the 11-year period of this study was almost 25% in those who had all three independent risk factors, versus less than 2% in those who had none. This type of information derived from well-designed natural history studies can be of great help to clinicians in making decisions regarding who should receive therapy for chronic HBV infection.

**Conclusions**

Natural history studies are crucial to understand not only the outcome of chronic HBV infection, but also what risk factors promote progression of disease and when the best time is to intervene with antiviral therapy to prevent the development of HCC and cirrhosis. To date, the strongest risk factors for the development of HCC and/or cirrhosis are male sex (1a), increasing age (1b), HBV DNA >2000 IU in persons over age 40, but not under 40 (1b), genotypes C and F1 (1b), family history of HCC (1b), BCP mutation (1b), and presence of cirrhosis (1b). Risk factors with lesser evidence include heavy alcohol or tobacco use and aflatoxin exposure (2c). Factors for which there is either insufficient evidence to determine risk or conflicting evidence include nonalcoholic fatty liver disease, HBV DNA > 2000 IU/mL in persons under 40 years, and the presence of PC mutation.

**Needs for Future Research**

At least 50%-60% of patients with chronic HBV infection will go through life without developing the life-threatening complications of HCC or decompensated cirrhosis. The goals of future natural history studies should include the identification of demographic, viral, immunologic, host genetic, and social and environmental factors that influence the outcome of HBV infection. This information could then be used to develop monitoring strategies, including how often and what tests to use to follow patients based on their risk profile. For example, from what is known from the REVEAL and other natural history studies, persons over the age of 40, with HBV DNA levels >2000 IU/mL infected with HBV genotype C, and who have BCP mutations, should undergo more rigorous surveillance for HCC than those without such risk factors. These patients might be recommended to have serum markers and imaging tests performed every 3 rather than every 6 months and to start antiviral therapy sooner. In contrast, persons over the age of 40 who are in the inactive phase of hepatitis B might be advised to undergo surveillance less frequently, perhaps every 12 months, and they may not need antiviral therapy.

Two types of natural history studies would be useful. First, established prospective population-based cohorts, such as from the REVEAL or the Alaska cohort studies, could be recruited to conduct nested case-control studies examining viral, immunologic, and host genetic factors associated with the three different phases of HBV infection, HCC, and cirrhosis. Secondly, additional prospective cohort studies could be established. These studies would best be either multicentered national or international in scope. Because population-based studies are expensive, and because large numbers of persons need to be screened to identify those who are infected with HBV, future studies might have to be clinic-based. Medical cen-

Table 5. Stratifying Independent Risk Factors for Development of Hepatocellular Carcinoma in a Population-Based Prospective Cohort Study (REVEAL-HBV) in Persons with HBV DNA > 10^4 copies/mL (2000 IU/mL)*

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Incidence of HCC per 100,000 Person Years</th>
<th>% Who Would Develop HCC Over 10 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype C</td>
<td>786/100,000</td>
<td>8%</td>
</tr>
<tr>
<td>Genotype B</td>
<td>237/100,000</td>
<td>2%</td>
</tr>
<tr>
<td>BCP</td>
<td>1149/100,000</td>
<td>11%</td>
</tr>
<tr>
<td>No BCP</td>
<td>359/100,000</td>
<td>4%</td>
</tr>
<tr>
<td>PC</td>
<td>996/100,000</td>
<td>10%</td>
</tr>
<tr>
<td>No PC</td>
<td>269/100,000</td>
<td>3%</td>
</tr>
<tr>
<td>Genotype C + BCP + No PC</td>
<td>2254/100,000</td>
<td>23%</td>
</tr>
<tr>
<td>Genotype B + PC + No BCP</td>
<td>174/100,000</td>
<td>2%</td>
</tr>
</tbody>
</table>

*Modified from the REVEAL-HBV study.
Hepatocytes participating in these studies would be encouraged to recruit patients in all three phases of HBV infection.

The ideal future prospective cohort natural history study might include persons infected with each of the major HBV genotypes/subgenotypes that occur most commonly in the world. Those to date would include genotypes A1, A2, A3, B1, B2-5, B6, C, D, F1, and possibly E and H. Genotype G appears to be uncommon. Approximately 200-300 persons from each relevant genotype/subgenotype category could be recruited for a total of between 2000 and 3000 participants, and then stratified by HBV phase of infection at enrollment. At enrollment, a history would be taken to include the use of alcohol and tobacco, a physical examination would be conducted, and laboratory tests would be performed that include a complete liver panel; complete blood counts; routine serum chemistries; lipid panel; fasting glucose and insulin levels; HBV genotype; BCP and PC mutation testing; HIV, HCV, and HDV antibodies; and HBV DNA level. Sera would be stored and peripheral blood mononuclear cells collected and stored for immunological studies. Participants would be evaluated and blood drawn at least twice yearly for at least 5 years and liver biopsies performed if HBV DNA levels were >2000 IU/mL and ALT values were elevated, or regardless of the ALT value, if HBV DNA levels were >2000 IU/mL and age >40 years. The cohort would be observed for the incidence of developing the immune active phase as well as developing HCC and cirrhosis. It would be expected that the incidence of subsequently developing HCC and cirrhosis would be reduced, because patients in the study would be offered antiviral therapy if indicated by existing practice guidelines. The most important endpoint might be the incidence of developing the immune active phase, especially in those who have already undergone HBeAg seroconversion, because this would be a potential time to treat to prevent the risk of cirrhosis and HCC.

The questions asked of these prospective, as well as the established cohort studies, would include the following:

(1) Are there any specific single or multiply occurring mutations besides BCP that independently increase the risk of developing the immune active phase, HCC, or cirrhosis?

(2) What is the influence of alcohol, metabolic syndrome, and tobacco on progression of chronic hepatitis B and the development of HCC and cirrhosis?

(3) What are the differences in the immunological response to HBV infection that underlie each of the three phases?

(4) How might host genetics influence outcome?

Investigations using these cohorts might consist of a nested case-control design comparing age-matched, sex-matched, and HBV genotype–matched persons with an adverse event (HCC, cirrhosis, or the immune active phase) and persons in the immune tolerant and inactive phases:

(1) Full-genome sequencing of HBV isolates to search for significant mutations or patterns of mutations that are associated with each phase and outcome.

(2) Identification by HBV genotype–specific peptides that might elicit cytotoxic T cell responses to determine which viral peptides and specific T cell epitopes are responsible for hepatocyte damage in chronic hepatitis B.

(3) Using gene chip analysis, identification of genes that are over-expressed and under-expressed in each phase of HBV infection as well as in persons with HCC or cirrhosis.

References


55. Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology 2004;40:790-792.


