Hepatitis B Virus DNA Levels and Outcomes in Chronic Hepatitis B

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Serum hepatitis B virus (HBV) DNA levels can fluctuate markedly during the course of chronic HBV infection. Both case-control and cohort studies have shown a significant, dose-response association between serum HBV DNA levels measured at the time of initial evaluation and the subsequent risk of cirrhosis. A similar direct relationship has been shown for the risk of hepatocellular carcinoma (HCC) in cross-sectional, case-control, and cohort studies. Interventional studies have shown a strong correlation between the indices of disease activity seen on liver biopsy and levels of serum HBV DNA. These studies have also shown that reduction in HBV DNA levels correlate strongly with improvements in liver histology. For patients with HCC, prognosis (including risk of death, metastasis, and recurrence following surgery) is worse with higher serum HBV DNA levels. The preponderance of the evidence in the published literature demonstrates that serum HBV DNA level is an important and independent risk factor for disease progression in chronic hepatitis B. The relative importance of serial HBV DNA measurements, the loss of hepatitis B e and surface antigens, as well as the emergence of HBV mutants in the progression of chronic hepatitis B, especially in young patients, is an important need for future research. (HEPATOLOGY 2009;49:S72-S84.)

Introduction

Chronic hepatitis B virus (HBV) infection is a global public health concern. There are an estimated 350 million carriers of hepatitis B surface antigen (HBsAg) in the world among whom there are more than 500,000 deaths annually attributable to cirrhosis and liver cancer (World Health Organization and Centers for Disease Control and Prevention fact sheets are available at www.who.int and www.cdc.gov). Despite the success of HBV vaccination in reducing the prevalence of chronic HBV infection as well as the incidence of acute hepatitis B, fulminant hepatitis, and hepatocellular carcinoma (HCC) in vaccine recipients,1,2 there remains a large group of chronically infected persons at continued risk of developing cirrhosis or HCC.

A central pathway in the pathogenesis of liver diseases from viral and nonviral etiologies is the transformational change seen in the function of the hepatic stellate cells from vitamin A storage cells to activated, profibrogenic cells.3 The process of hepatic fibrogenesis is a dynamic one, and removal of the insult (viral and nonviral) may lead to reversal of fibrosis.3,5 In chronic hepatitis B, presence of circulating virus is a marker of active infection and signifies the potential for persistent insult to the liver. The importance of serum HBV DNA levels as a predictor of the development of cirrhosis and HCC has been extensively reviewed recently.4 Several hospital-based and community-based case-control and cohort studies have consistently found significant associations between elevated HBV DNA levels and risk of liver cirrhosis and HCC. However, many of these studies were limited by small number of cases and controls, inadequate matching

Abbreviations: ALT, alanine aminotransferase; HAI, histology activity index; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; REVEAL-HBV, Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-HBV study.

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Role of the Sponsors: Dr. Iloeje (employed by Bristol-Myers Squibb) was involved in the study design, development of the analysis plan, and interpretation of the data and writing of the report. All data handling was done by the staff at the Academia Sinica and National Taiwan University. At no time did the funding sources have access to the data. The corresponding author had final responsibility for the decision to submit for publication.
or adjustment of confounding factors, lack of causal temporality, and analysis of risk predictors at study entry only.

The progression of chronic hepatitis B to HCC is a multistage process and nonviral risk factors may include aflatoxin exposure, alcohol drinking, and host susceptibility factors. A population-based, long-term follow-up study with repeated measurements of key risk factors would be the best approach to address the complex questions around the dynamics of HBV infection markers and their impact on disease progression risk. The variation in HBV DNA level by various risk predictors of chronic hepatitis B progression and the importance of HBV DNA level in the determination of clinical outcomes will be reviewed in this article.

Variation in Serum HBV DNA Levels Among Patients with Chronic Hepatitis B

The natural course of chronic HBV infection is highly variable from patient to patient as well as over time and with increasing age. Early life or perinatal infection is characterized by a period of “immune tolerance” where the host coexists with the virus without apparent injury to the host. During this period, serum HBV DNA levels are persistently high. Following the immune tolerance phase, infected patients can progress through an immune active phase or immune clearance phase, where hepatitis becomes active and it appears that the host immune system tries to clear infected hepatocytes. This phase is marked by hepatic inflammation, serum alanine aminotransferase (ALT) elevations, and a modest to moderate reduction of the circulating HBV DNA levels. The immune clearance phase is highly variable in duration and frequency, but a prolonged phase or recurrent episodes of acute liver inflammation may result in repeated cycles of injury and regeneration resulting in necroinflammation/fibrosis and an increased risk of progression to cirrhosis and HCC. The progression to a state of detectable liver injury is characterized by the presence of HBsAg and hepatitis B e antigen (HBeAg) in serum, moderate to high levels of circulating HBV DNA, elevation of serum ALT levels, and absence of antibody against HBeAg (anti-HBe).

In some patients, seroconversion to anti-HBe-sero-positive status occurs with ongoing viral replication (the precore or basal core promoter mutant virus infections). These HBeAg-negative and anti-HBe-positive patients have serum HBV DNA levels usually lower than those in patients who are HBeAg-positive with chronic hepatitis B. Another group of infected persons is able to inactivate the infection and go into the “nonreplicative phase” or “inactive carrier state.” Patients in this phase are characterized by the continued presence of HBsAg in serum, absence of HBeAg and presence of anti-HBe, low levels of serum HBV DNA and normal serum ALT values. These patients usually do not progress to worsening liver disease, cirrhosis, or end-stage liver diseases, but these endpoints may have occurred during the immune clearance phase and the patient with the inactive carrier state may have residual liver fibrosis. In a recent nested case-control study of 58 HBeAg-seroconverted children with chronic HBV infection enrolled in community-based and clinic-based cohorts, the mean age (± standard deviation) at HBeAg seroconversion was 17.2 ± 5.8 years; mean peak HBV DNA levels were $10^{9.0±0.9}$ copies/mL before seroconversion and $10^{5.0±1.7}$ copies/mL after seroconversion.

Among patients infected with HBV as adults, the majority resolve the infection spontaneously, clearing HBV DNA and HBsAg and developing anti-HBs. In a small proportion (~5%), HBV infection becomes chronic. These adult-acquired infections usually progress directly into an immune active or immune clearance phase without experiencing a period of immune tolerance.

The REVEAL-HBV Study

In the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-HBV study (REVEAL-HBV study) on 4155 HBsAg-seropositive adults aged 30-64 years at enrollment in 1991-1992 and followed through 2004, serum samples collected and frozen at study entry and during follow-up were retrieved for analyses of HBV DNA level, genotype, and HBV mutant types. In the analyses of HBV viral levels and chronic hepatitis B outcomes, only subjects with adequate serum samples for HBV DNA quantification at study entry and who were negative for antibody to hepatitis C virus (anti-HCV) by immunoassay technique were included (n = 3653). As shown in Fig. 1, elevated baseline HBV DNA levels were associated with HBeAg-seropositivity ($P < 0.001$), liver cirrhosis at study entry ($P < 0.001$), male sex ($P < 0.001$), younger ages ($P < 0.001$), increasing serum ALT levels ($P < 0.001$), and habitual cigarette smoking ($P = 0.002$). There was no significant association between habitual alcohol drinking and serum HBV DNA level at study entry ($P = 0.15$). Participants infected with HBV genotype C had higher average HBV DNA levels at study entry than those infected with genotype B ($P < 0.001$).

The distributions of serum HBV DNA levels at study entry for participants who were HBeAg-seronegative and with normal ALT levels (<45 U/L) without cirrhosis are shown in Fig. 2. As in the analysis of patients with HBeAg, serum HBV DNA levels at study entry were significantly associated with male sex, younger age, higher (normal) ALT levels, and cigarette smokers (compared to nonsmokers). Again, HBV DNA levels were not associ-
ated with a history of alcohol use. Interestingly, participants infected with HBV genotype C had lower serum HBV DNA levels than those infected with genotype B (P < 0.001).

**Serum HBV DNA Level and Liver Cirrhosis**

The findings of case-control and cohort studies on the association between serum HBV DNA levels and risk of cirrhosis are summarized in Table 1. In a case-control study of 79 patients with cirrhosis and 158 controls matched on age, sex, and HBeAg serostatus, the presence of cirrhosis was associated with higher levels of serum HBV DNA. In a follow-up study of 2763 HBsAg-seropositive adults in China, elevated serum HBV DNA levels at study entry were associated with an increased mortality from chronic liver diseases and an increased morbidity of cirrhosis are summarized in Table 1.

![Fig. 1. Distributions of serum HBV DNA levels at study entry by various characteristics of all REVEAL-HBV Study participants (N = 3653).](image)
severe liver diseases among survivors. In the REVEAL-HBV study, the incidence of cirrhosis (per 100,000 person-years) increased with increasing serum HBV DNA levels (copies/mL) at study entry: ranging from 339 (≤10,000), 430 (10,000-99,999), 774 (100,000-999,999), and 1879 (1,000,000-9,999,999) to 2498 (≥10,000,000). The biological gradient remained significant in stratified analyses across a variety of baseline characteristics such as sex, age, and habits of cigarette smoking and alcohol drinking. In multivariate Cox regression analyses of risk factors predicting progression to liver cirrhosis, increasing HBV DNA level was the strongest independent predictor.

Serum HBV DNA Levels and HCC

The findings from case-control and case-cohort studies on the association between serum HBV DNA levels and risk for HCC are summarized in Table 2. A significant association between elevated serum HBV DNA levels and increased risk of HCC was observed in all studies despite differences in study design (cross-sectional versus longitudinal and community/hospital-based), health status of controls, method and detection limit for the determination of HBV DNA levels, HBV DNA levels selected as the referent group, and variables chosen for adjustment in the analyses. In a community-based, nested case-control study of 44 HBeAg-negative patients with newly developed HCC and 86 matched controls who were selected from a cohort of 1991 male HBeAg-negative HBV carriers in Taiwan, a significant dose-response relationship between serum HBV DNA levels at study entry and HCC risk was observed. Compared with serum HBV DNA levels <2.5 pg/mL as the referent group, the multivariate-adjusted odds ratio was 2.3 and 6.0, respectively, for se-
rum HBV DNA levels of 2.5–13.0 and >13.0 pg/mL. In a hospital-based nested case-control study on 48 patients with HCC and 48 age-matched and sex-matched controls with cirrhosis in Japan, patients with HCC had higher serum HBV DNA levels at study entry than patients with cirrhosis who remained HCC-free during follow-up. In three case-control studies in which the serum HBV DNA levels at study entry were dichotomized into two groups, the elevated HBV DNA level group in each study had an increased risk of developing HCC: the multivariate-adjusted odds ratio comparing high versus low HBV DNA levels being 15.6 (83 versus 83 copies/mL) in a study from Senegal; 83 versus 83 copies/mL) in a study from China, and 2.5 (83 versus 83 copies/mL) in a study from Taiwan.

Nested in a large-scale hospital-based long-term follow-up study of 4841 male HBV carriers in Taiwan, a case-control study of 154 newly developed HCC cases and 316 matched HBV carrier controls found a significantly increasing risk of HCC across the biological gradient of serum HBV DNA levels at study entry. In the further analysis of HBV DNA levels in serial serum samples collected during follow-up, a significant dose-response relationship was observed between the risk of HBV DNA levels and the development of HCC.

### Table 1. Case-Control and Cohort Studies of Serum HBV DNA Level and Liver Cirrhosis

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Study Population</th>
<th>Endpoint</th>
<th>Testing Method</th>
<th>HBV DNA Levels, (copies/mL)</th>
<th>Main Findings</th>
<th>Adjustment Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case-control study</strong></td>
<td></td>
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<tr>
<td>Yuan et al. (2005)</td>
<td>79 cases with chronic hepatitis B and cirrhotic complications (including HCC); 158 controls from hepatitis clinic without cirrhotic complications</td>
<td>Cirrhotic complications</td>
<td>Digene Hybrid Capture II assay*; Cobas Amplicor Monitor test†</td>
<td>&lt; 1000 1000–9999 10,000–99,999 100,000–999,999 1,000,000–999,999</td>
<td>Cirrhosis cases, n (OR) Controls, n</td>
<td>Matched for age, sex and HBeAg status</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7 (referent) 8 (2.15) 8 (1.46) 14 (3.05) 42 (3.05)</td>
<td>32 17 25 21 63</td>
</tr>
<tr>
<td><strong>Cohort studies</strong></td>
<td></td>
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</tr>
<tr>
<td>Chen et al. (2006)</td>
<td>2763 HBsAg-positive adults</td>
<td>85 deaths from chronic liver disease</td>
<td>Real-time PCR‡</td>
<td>&lt; 1600 1600–99,999 ≥ 100,000</td>
<td>Cumulative mortality/100,000</td>
<td>Adjusted RR (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>414.9 651.5 5873.3</td>
<td>1.0 (referent) 1.5 (0.2–12.1) 15.2 (2.1–109.8)</td>
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<td></td>
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<td>20/151 (13.2%) 136/772 (17.6%) 211/716 (29.5%)</td>
<td>Adjusted OR (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0 (referent) 1.3 (0.8–2.1) 2.7 (1.6–4.5)</td>
<td></td>
</tr>
<tr>
<td>Iloeje et al. (2006)</td>
<td>3582 HBsAg-positive, anti-HCV-negative adults</td>
<td>Diagnosis of cirrhosis by ultrasound</td>
<td>Cobas Amplicor HBV Monitor test§</td>
<td>&lt; 300 300–99,999 10,000–99,999 100,000–999,999 1,000,000–999,999</td>
<td>Incidence of cirrhosis per 100,000 person-years</td>
<td>Adjusted HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>338.8 429.9 774.0 1878.6 2498.3</td>
<td>1.0 (referent) 1.4 (0.9–2.2) 2.5 (1.6–3.8) 5.9 (3.0–9.0) 9.8 (6.7–14.4)</td>
</tr>
</tbody>
</table>

*Detection limit: 140,000 copies/mL (Digene Diagnostics, Gaithersburg, MD).
†Detection limit: 200 copies/mL (Roche Diagnostics, Branchburg, NJ).
‡Detection limit: 1600 copies/mL.
§Detection limit: 300 copies/mL (Roche Diagnostics, Indianapolis, IN).

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; OR, odds ratio; PCR, polymerase chain reaction.
Table 2. Case-Control and Case-Cohort Studies of Serum HBV DNA Level and Hepatocellular Carcinoma

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Study Design</th>
<th>Study Population</th>
<th>Endpoint</th>
<th>Testing Method</th>
<th>HBV DNA Levels</th>
<th>HCC Cases, n</th>
<th>Controls, n</th>
<th>OR (95% CI)</th>
<th>Adjustment Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu et al. (2002)†</td>
<td>Community-based, nested</td>
<td>44 cases with HCC and 86 matched controls nested in a cohort of 1991 men (HBsAg positive and HBeAg negative)</td>
<td>HCC incident cases</td>
<td>Branched-chain DNA assay (Quantiplex)*</td>
<td>&lt; 2.5 pg/mL, 2.5–13.0 pg/mL, &gt; 13.0 pg/mL</td>
<td>27</td>
<td>7</td>
<td>7</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Ikeda et al. (2003)‡</td>
<td>Hospital-based, nested</td>
<td>48 cases with HCC and 48 age-sex-matched controls nested in 160 consecutive pts with cirrhosis (HBsAg positive, anti-HCV negative) who received no antiviral therapy</td>
<td>HCC incident cases</td>
<td>TMA and hybridization protection assay†</td>
<td>Continuously low Decreasing in 3 years Persistently high</td>
<td>0</td>
<td>9</td>
<td>13</td>
<td>—</td>
</tr>
<tr>
<td>Tang et al. (2004)§</td>
<td>Community-based, nested</td>
<td>14 cases with HCC and 28 matched controls in Senegal</td>
<td>HCC death</td>
<td>Real-time TaqMan PCR §</td>
<td>&lt; 83 copies/mL, ≥ 83 copies/mL</td>
<td>2</td>
<td>12</td>
<td>8</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Yu et al. (2005)†‡</td>
<td>Hospital-based, nested</td>
<td>154 cases with HCC and 316 matched controls nested in 4841 Taiwanese men from clinical settings, followed for 14 years</td>
<td>HCC incident cases</td>
<td>Real-time PCR assay §</td>
<td>&lt; 3.62 log_{10} copies/mL, 3.62–4.22 log_{10} copies/mL, 4.23–4.90 log_{10} copies/mL, 4.91–5.90 log_{10} copies/mL, 5.91–10.81 log_{10} copies/mL</td>
<td>12</td>
<td>63</td>
<td>63</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Liu et al. (2006)†</td>
<td>Hospital-based, cross-sectional</td>
<td>200 cases with HCC and 160 chronic HBV carriers</td>
<td>Noncirrhotic HCC cases</td>
<td>Real-time PCR assay §</td>
<td>&lt; 10^{6} copies/mL, ≥ 10^{6} copies/mL</td>
<td>124</td>
<td>102</td>
<td>58</td>
<td>0.8 (0.6–1.1)</td>
</tr>
<tr>
<td>Tsai et al. (2007)‡</td>
<td>Hospital-based, cross-sectional</td>
<td>26 cases with HCC and 155 chronic HBV carriers (&lt;40 years of age)</td>
<td>HCC incident cases</td>
<td>Real-time PCR assay §</td>
<td>log_{10} titre</td>
<td>26</td>
<td>155</td>
<td>0.6 (0.5–1.5)</td>
<td>Age, sex, ALT level, and HBV genotype</td>
</tr>
<tr>
<td>Liu et al. (2008)‡</td>
<td>Community-based, nested</td>
<td>170 cases with HCC and 276 with chronic hepatitis B with normal ALT level at study entry</td>
<td>HCC incident cases</td>
<td>Fluorescein quantitative-PCR (LOQ: 500 copies/mL)</td>
<td>Undetectable, 2.69–3.99 log_{10} copies/mL, 4.00–4.99 log_{10} copies/mL, 5.00–5.99 log_{10} copies/mL, 6.00–6.99 log_{10} copies/mL, ≥ 7.00 log_{10} copies/mL</td>
<td>44</td>
<td>186</td>
<td>46</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Wu et al. (2008)‡</td>
<td>Hospital-based, case-cohort</td>
<td>112 HCC cases and 1031 HBV carriers</td>
<td>HCC incident cases</td>
<td>Real-time PCR assay (LOQ: 215 copies/mL)</td>
<td>No follow-up sample, ≥ 4.39 log_{10} copies/mL, One follow-up sample, ≥ 4.39 log_{10} copies/mL, Two or more follow-up samples but &lt; 50% of follow-up samples, ≥ 4.39 log_{10} copies/mL, ≥ 50% of follow-up samples, ≥ 4.39 log_{10} copies/mL</td>
<td>41</td>
<td>16</td>
<td>5</td>
<td>1.0 (referent)</td>
</tr>
</tbody>
</table>

*Detection limit: 2.5 pg/mL (Chiron Diagnostics, Emeryville, CA ).
†Detection limit: 3.7 log_{10} copies/mL.
‡Detection limit: 83 copies/mL (Applied Biosystems, Foster City, CA).
§Detection limit: 100 copies/mL.

ALT, alanine aminotransferase; anti-HCV, antibodies to hepatitis C virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LOQ, limit of quantification; OR, odds ratio; PCR, polymerase chain reaction; TMA, transcription-mediated amplification.
HCC and the proportion of follow-up serum samples with elevated HBV DNA levels. The significant dose-response relationship was also observed in another community-based nested case-control study of 170 newly diagnosed cases of HCC and 276 HBV carrier controls with normal ALT levels at study entry. In a hospital-based cross-sectional case-control study of 183 cases of HCC and 202 HBV carrier controls, serum HBV DNA levels were significantly associated with HCC risk in the older (>40 years) but not in the younger (≤40 years) age group.

The findings of cohort studies on the association between serum HBV DNA levels and HCC risk are summarized in Table 3. There was also consistency among these studies despite differences in study design and methodology. The number of cohort participants was <100 in three hospital-based cohort studies, and the adjustment for confounding factors was considered inadequate in one community-based study and two hospital-based studies. In a hospital-based cohort study of 1006 patients with chronic hepatitis B, serum HBV DNA levels at study entry were significantly associ-
lated with subsequent development of HCC in a dose-response relationship after adjustment for risk predictors including HBV genotype.27

In the REVEAL-HBV study, the incidence of new onset HCC (per 100,000 person-years) increased with serum HBV DNA levels (in copies/mL) at study entry ranging from 108 (<300), 111 (300-9.9 × 103), 297 (1.0 × 104-9.9 × 104), 962 (1.0 × 105-9.9 × 105), to 1152 (≥ 1 × 106).11 In multivariate Cox regression analyses of risk factors predicting progression to HCC, increasing HBV DNA category was the strongest independent predictor of HCC risk after cirrhosis. In the subset analysis of last follow-up serum HBV DNA levels for individuals who had serum HBV DNA levels ≥ 1 × 104 copies/mL at study entry, HCC risk was highest among those who had persistently high HBV DNA levels of ≥ 1 × 105 copies/mL. In a recent analysis, the independent effect of HBV viral levels on HCC was assessed after adjustment for HBV genotype and mutants.28 HBV genotype was identifiable only for participants with detectable serum HBV DNA levels at study entry (n = 2762), and HBV mutants were tested only for participants with serum HBV DNA levels ≥ 104 copies/mL (n = 1526). HBV genotype C infection was associated with a higher risk of HCC than HBV genotype B. The G1896A mutation in the precore region was associated with a lower risk of HCC compared to the wild-type virus, while the double mutation (A1762T/G1764A) in the basal core promoter region was associated with a higher risk than the wild-type virus. Elevated serum HBV DNA levels at study entry were associated with an increased risk of HCC after multivariate adjustment for risk predictors including HBV genotype and mutants (odds ratio 1.6 for HBV DNA levels ≥105 versus 104 to <105 copies/mL, P = 0.04).

Serum HBV DNA Level and Liver Histology

The evidence for an association between serum HBV DNA levels and histopathological findings on liver biopsies are summarized in Table 4.29-33 In a cross-sectional study of 55 patients who were HBeAg-negative with chronic hepatitis B, patients with detectable HBV DNA levels (≥ 0.5 mEq/mL) had significantly higher necroinflammation scores in histology activity index (HAI) than those with undetectable HBV DNA levels.29 In a cross-sectional study of 94 chronic hepatitis B patients,31 serum HBV DNA levels were positively correlated with necro-inflammation scores of HAI (r = 0.31, P = 0.014), fibrosis scores (r = 0.33, P = 0.017), and total HAI scores (r = 0.37, P = 0.008) in patients who were positive for anti-HBe but not for those who were HBeAg-positive. In a cross-sectional study of 47 HBsAg-positive blood donors with detectable serum HBV DNA levels, serum HBV DNA levels significantly correlated with HAI-necroinflammation scores (r = 0.59; P < 0.001) and Ishak fibrosis stages (r = 0.50; P < 0.001).32 In another cross-sectional study of 213 patients with chronic hepatitis B with serum HBV DNA level >104 copies/mL, serum HBV DNA levels were not correlated with histological grade and stage of liver disease in either HBeAg-positive and HBeAg-negative patients.33 In a large meta-analysis of 26 intervention studies including 3428 treated patients, histological grades were significantly associated with serum HBV viral level at study entry (r = 0.78; P = 0.0001) and at the end of treatment (r = 0.71; P = 0.003).30 More importantly, improvement in histological grade was strongly correlated with a decrease in serum HBV DNA levels (r = 0.96; P < 0.001).

Serum HBV DNA Level and Prognosis of HCC

The findings of case-series and cohort studies of serum HBV DNA levels and metastasis, recurrence, and death from HCC are summarized in Table 5.14,34-41 In four case-series studies on the recurrence of HCC after surgical resection34,38,39 or transarterial chemolipidolization,37 patients with high serum HBV DNA levels at study entry had a significantly higher risk of HCC recurrence than those with low levels. In another three case-control studies on death from HCC, patients with high serum HBV DNA levels at study entry had a significantly higher risk of HCC death than those with low serum HBV DNA levels.35,36,41 A dose-response relationship between serum HBV DNA levels and the metastasis or recurrence of HCC was reported in another case-series study.40

In the REVEAL-HBV study,14 the mortality (per 100,000 person-years) increased with baseline HBV DNA level (in copies/mL) ranging from 9 (<300), 48 (300-9.9 × 103), 75 (1.0 × 104-9.9 × 104), 143 (1.0 × 105-9.9 × 105), to 267 (≥ 1 × 106) for chronic liver disease and cirrhosis; and 73, 48, 174, 692, and 816, respectively, for liver cancer. In multivariate Cox regression analyses of risk factors predicting progression to mortality, increasing HBV DNA level was the strongest independent predictor of death from chronic liver disease and cirrhosis, and was second to cirrhosis in predicting death from HCC. There was no association between serum HBV DNA levels and non–liver related mortality.

Treatment-Induced Viral Suppression and Liver Disease Progression

In what has become recognized as a landmark study, the impact of antiviral therapy on disease progression
was tested in a randomized, placebo-controlled study in patients with advanced fibrosis or cirrhosis due to hepatitis B. As shown in Table 6, 436 patients received lamivudine and 215 patients received placebo. The trial was terminated after 36 months on the recommendation of an independent data safety and monitoring board, based on a significant difference in clinical outcomes between lamivudine-treated and placebo-treated patients. Antiviral therapy significantly lowered the risk of liver disease progression (17.7% versus 7.8%), and led to a lower rate of progression in Child-Turcotte-Pugh score (8.8% versus 3.4%). Even after the relatively brief treatment period of 36 months, lamivudine treatment was associated with fewer cases of HCC than was placebo treatment (7.4% versus 3.9%), although this difference was just short of statis-
tional significance. The observed benefits of treatment was most likely driven by viral suppression, because disease progression among treated patients who developed lamivudine resistance (YMDD mutants) was 13%, which was higher than that in the group without drug resistance.

In another intervention study of lamivudine therapy in 353 patients with chronic hepatitis B and 303 pa-
Patients with cirrhosis, the risk of developing HCC or liver-related death was three-fold higher among patients with cirrhosis who had virological breakthrough than in those who achieved persistent viral suppression on treatment.43

Needs for Future Research

There is ample and strong evidence linking elevations in serum HBV DNA levels and liver disease progression in chronic hepatitis B. Elevations in serum HBV DNA is not only a major risk factor for disease progression, but the risk factor most amenable to modification. These findings raise the question if there is enough evidence that persistent elevations in HBV DNA levels over time (rather than a single elevated value) is a stronger risk predictor for disease progression. Most studies have been based on determinations of serum HBV DNA levels at a single time at study entry rather than at multiple time points. The REVEAL-HBV study demonstrated that persistently elevated serum HBV DNA levels documented at two different time points (at study entry and last follow-up examination) is associated with the highest risk of HCC. Recent unpublished data from this study, using data on serum HBV DNA and ALT levels at multiple time points and time-dependent regression modeling, confirm that elevated serum HBV DNA at multiple time points is indeed a very strong independent predictor of HCC risk.44 Because the REVEAL-HBV study population were infected in early life with HBV genotype B and C and recruited into this study at the age of at least 30, the results may not be directly applicable to other populations, especially younger adults and children and patients who were infected in adulthood. Other prospective non-interventional studies (especially in non-Asian populations) that measure serum HBV DNA levels as well as other key risk factors at repeated time points should contribute to answering this question.

The following studies are also needed to assess the importance of long-term change in serum HBV DNA levels in the development of liver cirrhosis and HCC: (1) The impact of long-term sequential changes in HBeAg serostatus, serum levels of HBV DNA and ALT, HBsAg serostatus, and HAI on the development of liver cirrhosis and HCC; (2) The importance of HBV genotypes and subtypes and long-term emergence of mutants in the development of liver cirrhosis and HCC; (3) The individual variation in the environmental and genetic susceptibility to disease progression of chronic hepatitis B; and (4) The assessment of development of end-stage liver diseases in patients with chronic hepatitis B who are treated with antivirals.

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### Table 6. Intervention Studies of Antiviral Treatment on Long-Term Progression of Chronic Hepatitis B

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Study Population</th>
<th>Endpoint</th>
<th>Study Variable</th>
<th>Main Findings</th>
<th>Adjustment Variables</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liaw et al. (2004)</td>
<td>436 patients with advanced fibrosis or cirrhosis receiving Lamivudine and 215 patients receiving placebo</td>
<td>Overall disease progression</td>
<td>Lamivudine Placebo</td>
<td>7.8 17.7 3.4 8.8</td>
<td>Country, sex, baseline ALT level, Child-Pugh score, and Ishak fibrosis score</td>
<td>1.0 (referent) 0.5 (0.3–0.7) 1.0 (referent) 0.5 (0.2–0.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase in Child-Pugh score</td>
<td>Lamivudine Placebo</td>
<td>7.8 17.7 3.4 8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HCC</td>
<td>Lamivudine Placebo</td>
<td>3.9 7.4</td>
<td></td>
<td>1.0 (referent) 0.5 (0.3–1.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response to lamivudine</td>
<td>HR (95% CI)</td>
<td></td>
<td></td>
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<tr>
<td>Di Marco et al. (2004)</td>
<td>353 patients with chronic hepatitis and 303 patients with cirrhosis receiving Lamivudine treatment</td>
<td>HCC</td>
<td>Maintained virological response</td>
<td>1.0 (referent) Above excluding age</td>
<td>Sex, age, initial diagnosis, baseline HBV DNA levels, baseline ALT levels, hepatic flare after virological breakthrough, and previous IFN therapy</td>
<td>3.0 (1.3–6.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virological breakthrough</td>
<td>HR (95% CI)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Liver-related death</td>
<td>Maintained virological response</td>
<td>2.9 (1.3–6.7)</td>
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<tr>
<td></td>
<td></td>
<td>Virological breakthrough</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN, interferon; HR, hazard ratio.
References


2. Chien YC, Jan CF, Kuo HS, Chen CJ. Nationwide hepatitis B vaccination program in Taiwan: effectiveness in the 20 years after it was launched. Epidemiol Rev 2006;28:126-135.


