Serum Hepatitis B Virus DNA as a Predictor of the Development of Cirrhosis and Hepatocellular Carcinoma

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Current Hepatitis Reports 2007, **6**:9–16
Current Medicine Group LLC ISSN 1540-3416

Chronic hepatitis B frequently progresses to cirrhosis and hepatocellular carcinoma. An elevated serum level of hepatitis B virus DNA is a major risk factor for cirrhosis and hepatocellular carcinoma, and there is a dose-response relationship between the serum hepatitis B virus DNA level and the risk of these complications. To lower the viral load to its lowest level is the method of choice to prevent major liver complications in chronic hepatitis B.

Introduction

More than 350 million people worldwide are chronically infected with hepatitis B virus (HBV) [1]. This infection is particularly prevalent in sub-Saharan Africa and the Asia-Pacific region [1,2]. Chronic HBV infection is a viral infection that results, if unabated, in organ-specific disease of the liver. Chronic hepatitis B (CHB) is the resulting state of liver injury from uncontrolled HBV infection. CHB frequently progresses to cirrhosis and hepatocellular carcinoma (HCC). It is the most common risk factor for the development of cirrhosis, decompensated liver disease, and HCC in the world [3–5].

There is significant individual variability in the natural history of chronic HBV infection. Age at infection determines the probability of chronic infection with HBV. The probability of becoming chronically infected is 80% to 100% with perinatal infection, 20% to 40% with infection in early childhood, and 0% to 10% with infection in adolescence and adult life [6,7]. The course of chronic infection acquired early in life is classically divided into three consecutive phases: immune tolerance, immune clearance, and residual phase [8,9]. The immune tolerance phase is characterized by active HBV replication, high serum levels of hepatitis B surface antigen (HBsAg), detectable hepatitis B e antigen (HBeAg) and HBV DNA, and normal serum alanine aminotransferase (ALT) levels. During the immune clearance phase (usually between ages 15 and 35 years), hepatitis flares may occur as the result of specific, cellular T-lymphocyte–mediated response against viral antigens and its downstream apoptotic mechanisms. This phase subsides with a decline in viral replication, a marked decrease in serum HBV DNA level, seroconversion of HBeAg to its antibody (anti-HBe), and clinical remission. Subsequently, patients enter the residual phase, during which they are HBsAg-seropositive and HBeAg-seronegative without active liver disease.

In its natural history, CHB frequently progresses to cirrhosis and HCC. The incidence of cirrhosis in chronic HBV carriers reported from tertiary care centers ranges from 2% to 7% annually [10–14]. In contrast, community-based studies of asymptomatic patients (so-called carriers) report lower rates. A community-based study of 1506 asymptomatic HBV carriers in Taiwan reported an annual rate of 0.7 % [15]. Patients with cirrhosis are estimated to progress to decompensated cirrhosis at a rate of 3% annually [16]. The 5-year survival is 84% among patients with compensated cirrhosis and 14% to 28% in those with decompensated cirrhosis [16,17]. The risk of HCC among HBsAg-seropositive patients is fivefold to 98-fold greater than among HBsAg-seronegative patients, with a population-attributable risk percentage ranging from 8% to 94% [18]. The lifetime risk that a patient with CHB will die from HCC has been estimated at 20% to 25% [19].

Factors reported to affect CHB disease progression include seropositivity of HBsAg and HBeAg, elevated serum level of ALT, age, sex, alcohol intake, cigarette smoking, aflatoxin exposure, male hormonal factor, familial tendency, genetic susceptibility, and serum HBV DNA level. [18,20••,21••,22•]. This article reviews published evidence on the role of markers of viral replication such as HBeAg and hepatitis B viral load as independent predictors of cirrhosis and HCC in people with CHB.

HBeAg Seropositivity as a Risk Factor for Disease Progression

HBeAg is a surrogate biomarker of active HBV replication. In the Risk Evaluation of Viral Load Elevation and

Associated Liver Disease/Cancer–HBV (REVEAL-HBV) study of 3653 patients with CHB in Taiwan, 523 (92.6%) of 565 HBeAg-seropositive patients and 453 (14.7%) of 3088 HBeAg-seronegative patients had a serum HBV DNA level of 100,000 copies/mL or higher [20••]. There is a striking age-dependent decrease in seroprevalence of HBeAg among HBsAg-seropositive patients, from 64.3% at ages 5 to 9 years to 5.5% at ages 60 to 69 years [23]. Meta-analysis of case-series studies of HBeAg seroprevalence showed a decreasing trend from CHB (57.2%) through cirrhosis (28.7%) to HCC (19.3%), indicating a significantly confounding effect by age [23]. Only age-matched case-control studies or cohort studies may correctly evaluate the importance of HBeAg seropositivity in the development of cirrhosis and HCC.

Seropositivity of HBeAg has been found to be associated with an increased risk of cirrhosis in three cohort follow-up studies in Taiwan showing relative risks ranging from 1.7 to 3.0 [11,13,15]. Treatment-associated clearance of HBeAg was associated with a decreased risk of cirrhosis in a clinical trial of interferon-α therapy in HBeAg-seropositive patients [24]. In cirrhotic patients, the presence of HBeAg increases the risk of liver decompensation [25], and data from the Alaskan Natives Study indicates that frequent re-emergence of HBeAg in serum was a risk factor for disease progression [26].

Seropositivity of HBeAg has also been documented to be an important risk factor for HCC in patients chronically infected by HBV. In a meta-analysis of five age-matched case-control studies of HBeAg and HCC, the odds ratio (OR) of developing HCC was 3.65 for study subjects who were HBeAg-seropositive compared with an HBeAg-seronegative reference group [23]. In a community-based cohort study with an average follow-up of 7.8 years, seropositivity of HBeAg at entry examination was associated with an increased risk of HCC: the cumulative incidence per 100,000 person-years was 324.3 for men seropositive for HBsAg only and 1169.4 for men seropositive for both HBsAg and HBeAg [27]. The multivariate-adjusted relative risk was 6.3 for HBeAg-seropositive subjects compared with those who were HBeAg-seronegative. Furthermore, the association between HBeAg seropositivity and HCC risk remained significant in stratification analyses by age, cigarette smoking, and alcohol use. The importance of HBeAg seropositivity in the development of HCC also remained significant in stratification analyses by serum ALT level [28] and cirrhosis status [23]. In other words, HBeAg seropositivity is an important HCC risk predictor independent of elevated serum ALT level and cirrhosis status.

Serum HBV DNA Level as a Risk Factor for Disease Progression

Highly sensitive testing methods based on polymerase chain reaction (PCR) have become available for measuring serum level of HBV DNA. Some methods are qualitative (positive vs negative) and some are quantitative, with various detection limits. These newer, more precise ways of measuring HBV DNA have made it possible to more closely examine the role of HBV DNA level in CHB disease progression. This section summarizes some of the recent studies that have examined this question.

Serum HBV DNA level and cirrhosis

Few studies have examined the association between serum HBV DNA level and the risk of cirrhosis. As shown in Table 1, in a hospital-based case-control study of 79 patients with CHB who had cirrhosis-related complications and 158 patients without complications, matched for age, sex, and HBeAg status, the HBV DNA level was significantly higher in patients with cirrhotic complications ($P = 0.02$) [29]. Compared with the reference group of those who had a serum HBV DNA level below 1000 copies/mL, the risk of developing cirrhotic complications was 3.05 times higher for those who had a serum HBV DNA level higher than 100,000 copies/mL.

In a community-based prospective cohort study of mortality and morbidity of chronic liver disease and HCC in 2763 HBsAg-seropositive residents of Haimen City, China [30•], a significant increase in mortality from chronic liver disease, mostly cirrhosis, was observed across serum HBV DNA levels measured by real-time PCR (P for trend < 0.001). As shown in Table 1, the age- and sex-adjusted relative risk of dying from chronic liver disease was 1.5 for serum HBV DNA level of 1600 to 99,999 copies/mL and 15.2 for levels of 100,000 copies/mL or more, compared with a reference group with an undetectable level. The odds of severe liver disease also increased with increasing serum HBV DNA (P for trend < 0.001).

In a population-based prospective cohort study (REVEAL-HBV) of 3582 untreated HBsAg-seropositive participants without cirrhosis at recruitment from seven townships in Taiwan, a dose-response relationship between HBV DNA level and risk of cirrhosis was observed after a mean follow-up period of 11 years [21••] (Table 1). The hazard ratio (HR) for those with the highest HBV DNA level was 9.8 (95% CI, 6.7–14.4) after adjustment for age, sex, cigarette smoking, and alcohol consumption. The biological gradient remained statistically significant in the stratification analyses. Furthermore, the dose-response relationship remained significant in HBeAg-seronegative participants with a normal ALT level at cohort entry examination.

Serum HBV DNA level and hepatocellular carcinoma: case-control studies

As shown in Table 2, several case-control studies have examined the association between serum HBV DNA level and HCC risk. In a community-based, nested case-control study of 44 patients with HCC and 86 matched controls who were HBsAg-seropositive and HBeAg-seronegative,

Table 1. Serum HBV DNA level and cirrhosis: case-control and cohort studies

Study (year)	Study population	End point	Testing method	HBV DNA levels, *copies/mL*	Main findings		Adjustment variables
Case-control study							
					Cirrhosis cases, *n* (OR)	**Controls, *n***	
Yuan et al. [29] (2005)	79 pts with CHB and cirrhotic complications (including HCC); 158 controls from hepatitis clinic without cirrhotic complications, matched for age, sex, HBeAg status	Cirrhotic complications	Digene Hybrid Capture II assay*; Cobas Amplicor Monitor test†	< 1000 1000–9999 10,000–99,999 100,000–999,999 ≥ 1,000,000	7 (referent) 8 (2.15) 8 (1.46) 14 (3.05) 42 (3.05)	32 17 25 21 63	None
Cohort studies							
					Cumulative mortality / 100,000	**Adjusted RR (95% CI)**	
Chen et al. [30•] (2006)	2763 HBsAg-positive adults	85 deaths from chronic liver disease	Real-time PCR‡	< 1600 1600–99,999 ≥ 100,000	414.9 651.5 5873.3	1.0 (referent) 1.5 (0.2–12.1) 15.2 (2.1–109.8)	Age, sex
					Cases / survivors (prevalence)	**Adjusted OR (95% CI)**	
		367 current cases of severe liver disease (incl. HCC) of 1683 survivors		< 1600 1600–99,999 ≥ 100,000	20/151 (13.2%) 136/772 (17.6%) 211/716 (29.5%)	1.0 (referent) 1.3 (0.8–2.1) 2.7 (1.6–4.5)	Age, sex
					Incidence of cirrhosis§	**Adjusted HR (95% CI)**	
Iloeje et al. [21••] (2006)	3582 HBsAg-positive and anti-HCV–negative adults	Diagnosis of cirrhosis by ultrasound	Cobas Amplicor HBV Monitor test¶	< 300 300–9999 10,000–99,999 100,000–999,999 ≥ 1,000,000	338.8 429.9 774.0 1878.6 2498.3	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)	Age, sex, cigarette smoking, alcohol, HBeAg, serum ALT

*Detection limit: 140,000 copies/mL (Digene Diagnostics, Gaithersburg, MD).
†Detection limit: 200 copies/mL (Roche Diagnostics, Branchburg, NJ).
‡Detection limit: 1600 copies/mL.
§Incidence per 100,000 person-years.
¶Detection limit: 300 copies/mL (Roche Diagnostics, Indianapolis, IN).
ALT—alanine aminotransferase; anti-HCV—antibodies to hepatitis C virus; CHB—chronic hepatitis B; 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; pts—patients.

Table 2. Case-control studies of serum HBV DNA level and hepatocellular carcinoma

Study (year)	Study design	Study population	End point	Testing method	HBV DNA levels	HCC cases, *n*	Controls, *n*	OR (95% CI)	Adjustment variables
Yang et al. [27] (2002)	Community-based, nested	44 pts with HCC and 86 matched controls nested in a cohort of 1991 men (HBsAg positive and HBeAg negative)	HCC incident cases	Branched-chain DNA assay (Quantiplex)*	< 2.5 pg/mL (referent)	27	74	1.0	Age, anti-HCV, cigarette and alcohol use
					2.5–13.0 pg/mL	7	7	2.3 (0.7–7.3)	
					> 13.0 pg/mL	10	5	6.0 (1.7–21.4)	
Ikeda et al. [31] (2003)	Hospital-based, nested	48 pts with HCC and 48 age- and sex-matched controls nested in 160 consecutive pts with cirrhosis (HBsAg positive, anti-HCV negative) who received no antiviral therapy	HCC incident cases	TMA and hybridization protection assay†	Continuously low	0	9	—	None
					Decrease lasting ≥ 3 y	0	13	—	
					Intermittently high	9	9	1.0 (referent)	
					Persistently high	39	17	2.3	
Tang et al. [32] (2004)	Community-based, nested	14 pts with HCC and 28 matched controls in Senegal	HCC death	Real-time TaqMan PCR‡	< 83 copies/mL	2	20	1.0 (referent)	Age, sex, HBsAg status, year of cohort entry, military rank
					≥ 83 copies/mL	12	8	15.6 (2.0–124.3)	
		55 pts with HCC and 55 matched controls in China (undetectable HBV DNA by dot blot hybridization)			< 83 copies/mL	15	24	1.0 (referent)	Age, sex, HBsAg status, dot blot result, year of study entry, township of residence
					≥ 83 copies/mL	40	31	3.1 (1.1–9.2)	
Yu et al. [22•] (2005)	Hospital-based, nested	154 pts with HCC and 316 controls nested in 4841 Taiwanese men from clinical settings, followed for 14 y	HCC incident cases	Real-time PCR assay§	4.23–4.90 $\log_{10}$ copies/mL	26	63	2.54 (1.16–5.59)	Age, date of blood collection, ethnicity, history of cigarette and alcohol use
					4.91–5.90 $\log_{10}$ copies/mL	28	63	2.44 (1.12–5.28)	
					5.91–10.81 $\log_{10}$ copies/mL	76	64	7.26 (3.54–14.89)	
Liu et al. [33] (2006)	Hospital-based, cross-sectional	200 pts with HCC and 160 chronic HBV carriers	Non-cirrhotic HCC cases	Real-time PCR assay§	< 10^5 copies/mL	124	102	1.0 (referent)	Age, sex, HBV genotype, precore A1896 mutant, basal core promoter T1762/A1764 mutant
					≥ 10^5 copies/mL	76	58	2.5 (1.1–5.7)	

*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; OR—odds ratio; PCR—polymerase chain reaction; pts—patients; TMA—transcription-mediated amplification; y—years.

there was a significant dose-response relationship (P for trend = 0.005) between HCC risk and the serum HBV DNA levels detected by branched-chain DNA assay in entry samples [27].

In a nested case-control study in Japan of the association between serum HBV DNA level and HCC, 48 patients with HCC and 48 matched controls were selected from 217 patients diagnosed with cirrhosis between 1976 and 1989 [31]. Continuously or intermittently high serum HBV DNA levels (> 5000 copies/mL) in the preceding 3 years were observed in all cases of HCC and 26 matched controls (54%, $P < 0.001$).

In a nested case-control study carried out in Senegal and China, the detection of HBV DNA in serum samples by TaqMan PCR assay (Applied Biosystems, Foster City, CA) was significantly associated with the development of HCC [32]. In Senegal, 12 (85.7%) of 14 patients with HCC and 8 (28.6%) of 28 controls were HBV DNA–positive (OR, 15.6). In China, where only dot blot HBV DNA–negatives were studied, 40 (72.7%) of 55 patients with HCC and 31 (56.4%) of 55 matched controls were HBV DNA–positive (OR, 3.1).

In a hospital-based, nested case-control study in Taiwan, 154 patients with HCC and 316 matched controls were selected after 14 years of follow-up [22•]. Serum HBV DNA level at entry examination was tested by real-time PCR-based single tube assay. A dose-response relationship between serum HBV DNA level and HCC risk was observed (Table 2).

In another hospital-based, cross-sectional case-control study from Taiwan, 200 patients with HCC and 160 HBsAg-positive controls were studied [33]. Elevated serum HBV DNA level (≥ 100,000 copies/mL) was associated with an increased risk of HCC (OR, 2.5; 95% CI, 1.1–5.7) after adjustment for age, sex, HBV genotype, precore A1896 mutant, and basal core promoter T1762/A1764 mutant.

Serum HBV DNA level and hepatocellular carcinoma: cohort studies

As shown in Table 3, we found and reviewed five cohort studies on the association between serum HBV DNA level and risk of HCC. In a small, hospital-based follow-up study from 1980 to 1999 in Nagasaki, Japan, of 73 patients with CHB, 21 cases of HCC were diagnosed during the follow-up period [34]. Elevated HBV DNA level (> 1,000,000 copies/mL) was associated with an increased HCC risk (HR 3.08; 95% CI, 1.03–9.17) after adjustment for age, sex, habitual heavy alcohol drinking, elevated ALT level, interferon therapy, fibrosis stage, and inflammation grade, using Cox proportional hazard regression analysis.

In another hospital-based follow-up study from 1996 to 2003 in Okayama, Japan, among 91 patients with cirrhosis there were 23 cases of HCC over 7 years of follow-up [35]. Elevated HBV DNA level (> 100,000 copies/mL) was the only risk predictor of HCC, with an odds ratio of 2.33 (95% CI, 1.14–5.60).

In a retrospective cohort study of 57 patients with CHB receiving interferon therapy in Tokyo [36], HCC developed in two (8%) of 25 patients with loss in HBV DNA (< 5,000 copies/mL) and 11 (34.4%) of 32 patients without HBV DNA loss ($P = 0.026$).

In the above-mentioned community-based prospective cohort study in Haimen City, China, a significant increase was observed in the age- and sex-adjusted relative risk of dying from HCC across serum HBV DNA levels measured by real-time PCR (P for trend < 0.001) (Table 3)[30•].

In the REVEAL-HBV study of 3653 patients with untreated CHB, a dose-response relationship between serum HBV DNA level at entry examination and risk of HCC (incidence and hazard ratio) was observed after a mean follow-up period of 11 years after adjustment for age, sex, habits of cigarette smoking and alcohol consumption, serostatus of HBeAg, serum ALT level, and cirrhosis status at entry examination [20••]. In stepwise analyses removing participants with study entry status of seropositivity of HBeAg, elevated ALT level, and cirrhosis sequentially, the dose-response relationship (P for trend < 0.001) between risk of HCC and serum HBV DNA level at entry examination became increasingly stronger.

The HBV DNA levels in serum samples collected at the follow-up examination just preceding the diagnosis of HCC (or at the last follow-up examination for those who had not developed HCC) were further examined for those who had a serum HBV DNA level greater than 10,000 copies/mL at entry examination. Among those who had serum HBV DNA level at study entry of 10,000 to 99,999 copies/mL, a statistically significant increase in HCC risk (adjusted HR, 3.5; 95% CI, 1.4–9.2) was observed for a follow-up level of at least 100,000 copies/mL after adjustment for age, sex, habits of cigarette smoking and alcohol consumption. Among those with serum HBV DNA levels at entry examination of at least 100,000 copies/mL, a significant biologic gradient of HCC risk by follow-up HBV DNA level was observed ($P < 0.01$) after adjustment for HBeAg serostatus, serum ALT level, and cirrhosis at entry examination.

Implications for the Management of CHB

Controlling the health impact of chronic HBV infection remains an important public health concern. As shown in Taiwan, a comprehensive, nationwide immunization program can reduce the incidence of acute HBV infection, CHB, and HCC among children [37]. The morbidity and mortality associated with CHB is likely to be reduced in the future through comprehensive vaccination programs that include a birth dose [38], but unfortunately not all countries have adopted universal vaccination policies, nor have they experienced the kind of success reported in Taiwan. Additionally, about 50 million new cases of HBV infection are diagnosed annually [39], and a significant number of these patients will become chronically infected. As vaccination does not prevent the ongoing risk

Table 3. Cohort studies of serum HBV DNA level and hepatocellular carcinoma

Study (year)	Study design	Study population	End point	Testing method	HBV DNA levels	Main findings		Adjustment variables
						Cumulative incidence	Adjusted RR (95% CI)	
Ohata et al. [34] (2004)	Hospital-based	73 pts with CHB, anti-HCV negative, followed > 6 mo	21 HCC incident cases	TMA*	< 10^6 copies/mL	6/35 (17%)	1.0 (referent)	Age, sex, habitual heavy drinking, ALT, IFN therapy, fibrosis stage, inflammation grade
					≥ 10^6 copies/mL	15/28 (54%)†	3.08 (1.03–9.17)	
Mahmood et al. [35] (2005)	Hospital-based	91 pts with cirrhosis	23 HCC incident cases	Amplicor Monitor kit‡	< 2.6 $\log_{10}$ copies/mL	1/20 (5%)	—	ALT
					2.6–5.0 $\log_{10}$ copies/mL	5/21 (23.8%)	1.0 (referent)	
					> 5.0 $\log_{10}$ copies/mL	17/47 (36.2%)	2.33 (1.14–5.60)	
Ikeda et al. [36] (2005)	Hospital-based, retrospective	57 pts with CHB receiving IFN therapy	13 HCC incident cases	TMA and hybridization protection assay*	HBV DNA loss (< 5000 copies/mL)	2/25 (8%)	1.0 (referent)	None
					Without HBV DNA loss	11/32 (34.4%)	4.3, not adjusted	
Chen et al. [30•] (2006)	Community-based	2763 HBsAg-positive adults	231 deaths from HCC	Real-time PCR§	< 1600	1.24%	1.0 (referent)	Age, sex
					1600–99,999	2.61%	1.7 (0.5–5.7)	
					≥ 100,000	15.1%	11.2 (3.6–35.0)	
						Incidence rate per 100,000 person-years	**Adjusted HR (95% CI)**	
Chen et al. [20••] (2006)	Community-based	3653 adults (HBsAg-positive and anti-HCV–negative)	164 HCC incident cases	Cobas Amplicor HBV Monitor test¶	< 300	108	1.0 (referent)	Sex, age, cigarette and alcohol use, HBeAg, ALT, cirrhosis at entry
					300–9999	111	1.1 (0.5–2.3)	
					10,000–99,999	297	2.3 (1.1–4.9)	
					100,000–999,999	962	6.6 (3.3–13.1)	
					≥ 1,000,000	1152	6.1 (2.9–12.7)	

*Detection limit: 3.7 $\log_{10}$ copies/mL.
†Approximate figure.
‡Roche Diagnostics, Tokyo, Japan.
§Detection limit: 1600 copies/mL.
¶Detection limit: 300 copies/mL (Roche Diagnostics, Indianapolis, IN).
ALT—alanine aminotransferase; anti-HCV—antibodies to hepatitis C virus; CHB—chronic hepatitis B; HBeAg—hepatitis B e antigen; HBsAg—hepatitis B surface antigen; HBV—hepatitis B virus; HCC—hepatocellular carcinoma; HR—hazard ratio; IFN—interferon; mo—months; PCR—polymerase chain reaction; pts—patients; TMA—transcription-mediated amplification.

of morbidity and mortality in the population currently infected, the burden of disease from CHB will remain a public health concern for the foreseeable future.

It is very difficult to totally eradicate HBV in chronically infected patients because the virus resides in human hepatocytes. Seroconversion from HBsAg-seropositive to HBsAg-seronegative is rare: the annual rate ranges from 0.1% to 2.0%, depending on age at infection [40–43]. According to current clinical guidelines for the treatment of CHB [44–46], the treatment population is generally defined as those with some evidence of hepatic injury indicated by either serum ALT levels higher than twice the upper limit of normal or by biopsy evidence as well as serum HBV DNA levels above 100,000 copies/mL. The endpoints of CHB treatment include the loss of HBeAg with or without seroconversion to anti-HBe, reduction of serum HBV DNA level (< 100,000 copies/mL), and normalization of ALT. Based on the findings of the studies reviewed above, however, it appears that these short-term goals might not prevent or delay the development of HCC and cirrhosis in patients with CHB.

According to the REVEAL-HBV study [20••,21••], the lower the serum HBV DNA level, the lower the risk of developing cirrhosis and HCC. A serum HBV DNA level of 10,000 to 99,999 copies/mL was associated with a significantly increased risk of cirrhosis (HR 2.5; 95% CI, 1.6–3.8) and HCC (HR, 2.3; 95% CI, 1.1–4.9), compared with undetectable serum HBV DNA level (< 300 copies/mL). Furthermore, the biologic gradient of cirrhosis and HCC risk across the serum HBV DNA levels was even more striking among chronic HBV carriers who were HBeAg-seropositive with normal serum ALT level. Without lowering the serum HBV DNA level, neither seroconversion of HBeAg nor normalization of ALT may be adequate to protect a patient with CHB from cirrhosis and HCC.

Lowering the serum HBV DNA level is beneficial, but it remains to be determined whether a "safe" level of HBV DNA exists. As shown on Table 4, when the serum samples of the subjects from the REVEAL-HBV study were re-analyzed using a more sensitive real-time PCR method, the hazard ratio of developing HCC increased sharply with increased levels of HBV DNA after adjustment for gender, age, cigarette smoking, and alcohol consumption (Unpublished data). These findings suggest that even relatively low levels of serum HBV DNA (< 10,000 copies/mL) increase risk when compared with very low levels of serum HBV DNA (< 100 copies/mL). It would appear therefore that maximal and durable suppression of viral replication should be the goal in managing CHB patients.

Table 4. Serum HBV DNA level and risk of hepatocellular carcinoma*

HBV DNA level, *copies/mL*	Hazard ratio (95% CI)
< 100 (reference group)	1.0
100–999	1.2 (0.3–4.4)
1000–9999	3.2 (1.5–7.1)
10,000–99,999	4.2 (2.0–9.0)
100,000–999,999	11.1 (5.3–23.2)
≥ 1,000,000	16.5 (8.2–33.1)

*Unpublished data from REVEAL-HBV study [20••,21••], analyzed using real-time polymerase chain reaction method. HBV—hepatitis B virus.

Conclusions

From this review, it is evident that HBeAg seropositivity as a surrogate biomarker of active HBV replication is associated with an increased risk of cirrhosis. However, elevated serum level of HBV DNA is a major risk factor for cirrhosis and HCC independent of HBeAg seropositivity, elevated serum ALT level, and cirrhosis status. The dose-response relationship between the serum HBV DNA level and the risk of cirrhosis and HCC has been observed in both case-control and cohort studies. From the REVEAL-HBV study, the most comprehensive study of this subject to date, lower serum HBV DNA levels at entry and at follow-up examinations translate to a lower risk of liver complications. The biologic gradient was even more striking among chronic HBV carriers who were HBeAg-seronegative without elevated ALT level and cirrhosis at entry examination. Decreasing the viral load to its lowest level through antiviral therapy without resulting in drug resistance seems to be the method of choice to prevent major liver complications in patients with chronic HBV infection.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Goldstein ST, Zhou F, Hadler SC, et al.: **A mathematical model to estimate global hepatitis B disease burden and vaccination impact.** *Int J Epidemiol* 2005, 34:1329–1339.
2. Chen CJ, Wang LY, Yu MW: **Epidemiology of hepatitis B virus infection in the Asia-Pacific region.** *J Gastroenterol Hepatol* 2000, 15(suppl):E3–E6.
3. Beasley RP: **Hepatitis B virus. The major etiology of hepatocellular carcinoma.** *Cancer* 1988, 61:1942–1956.
4. Lok AS, McMahon BJ: **Chronic hepatitis B.** *Hepatology* 2001, 34:1225–1241.
5. Perz JF, Armstrong GL, Farrington LA, et al.: **The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide.** *J Hepatol* 2006, 45:529–538.
6. Beasley RP, Trepo C, Stevens CE, Szmuness W: **The e antigen and vertical transmission of hepatitis B surface antigen.** *Am J Epidemiol* 1977, 105:94–98.
7. McMahon BJ, Alward WL, Hall DB, et al.: **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.
8. Chen DS: **From hepatitis to hepatoma: lessons from type B viral hepatitis.** *Science* 1993, 262:369–370.

9. Chu CM, Liaw YF: **Natural history of chronic hepatitis B virus infection: an immunopathological study.** *J Gastroenterol Hepatol* 1997, 12:S218–S222.
10. Fattovich G, Brollo L, Giustina G, et al.: **Natural history and prognostic factors for chronic hepatitis type B.** *Gut* 1991, 32:294–298.
11. Hsu YS, Chien RN, Yeh CT, et al.: **Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B.** *Hepatology* 2002, 35:1522–1527.
12. Huo T, Wu JC, Hwang SJ, et al.: **Factors predictive of liver cirrhosis in patients with chronic hepatitis B: a multivariate analysis in a longitudinal study.** *Eur J Gastroenterol Hepatol* 2000, 12:687–693.
13. Liaw YF, Tai DI, Chu CM, Chen TJ: **The development of cirrhosis in patients with chronic type B hepatitis: a prospective study.** *Hepatology* 1988, 8:493–496.
14. Lin SM, Sheen IS, Chien RN, et al.: **Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection.** *Hepatology* 1999, 29:971–975.
15. Yu MW, Hsu FC, Sheen IS, et al.: **Prospective study of hepatocellular carcinoma and liver cirrhosis in asymptomatic chronic hepatitis B virus carriers.** *Am J Epidemiol* 1997, 145:1039–1047.
16. Fattovich G, Pantalena M, Zagni I, et al.: **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, 97:2886–2895.
17. de Jongh FE, Janssen HL, de Man RA, et al.: **Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver.** *Gastroenterology* 1992, 103:1630–1635.
18. Chen CJ, Yu MW, Liaw YF: **Epidemiological characteristics and risk factors of hepatocellular carcinoma.** *J Gastroenterol Hepatol* 1997, 12:S294–S308.
19. Beasley RP: **Hepatitis B virus as the etiological agent in hepatocellular carcinoma: epidemiological consideration.** *Hepatology* 1982, 2:21–26.
20.•• Chen CJ, Yang HI, Su J, et al.: **Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level.** *JAMA* 2006, 295:65–73.

This large-scale community-based cohort study showed a dose-response relationship between incidence of hepatocellular carcinoma and serum HBV DNA level at entry examination. The risk of hepatocellular carcinoma was highest for those who had persistent elevation of serum HBV DNA level.

21.•• Iloeje UH, Yang HI, Su J, et al.: **Predicting cirrhosis risk based on the level of circulating hepatitis B viral load.** *Gastroenterology* 2006, 130:678–686.

This large-scale community-based cohort study showed a dose-response relationship between incidence of cirrhosis and serum HBV DNA level at entry examination.

22.• Yu MW, Yeh SH, Chen PJ, et al.: **Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men.** *J Natl Cancer Inst* 2005, 97:265–272.

This nested case-control study showed significant association between the risk of hepatocellular carcinoma and the HBV C genotype and DNA level.

23. You SL, Yang HI, Chen CJ: **Seropositivity of hepatitis B e antigen and hepatocellular carcinoma.** *Ann Med* 2004, 36:215–224.
24. Niederau C, Heintges T, Lange S, et al.: **Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B.** *N Engl J Med* 1996, 334:1422–1427.
25. Fattovich G, Giustina G, Schalm SW, 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.
26. 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, 135:759–768.
27. Yang HI, Lu SN, Liaw YF, et al.: **Hepatitis B e antigen and the risk of hepatocellular carcinoma.** *N Engl J Med* 2002, 347:168–174.
28. Chen CJ, Yang HI, You SL: **Hepatitis B e antigen and the risk of hepatocellular carcinoma [author reply].** *N Engl J Med* 2002, 347:1722.
29. Yuan HJ, Yuen MF, Ka-Ho Wong D, et al.: **The relationship between HBV-DNA levels and cirrhosis-related complications in Chinese with chronic hepatitis B.** *J Viral Hepat* 2005, 12:373–379.
30.• Chen G, Lin W, Shen F, et al.: **Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study.** *Am J Gastroenterol* 2006, 101:1797–1803.

This community-based cohort study showed the importance of serum HBV DNA level in the determination of mortality and morbidity of hepatocellular carcinoma and chronic liver disease, especially cirrhosis.

31. Ikeda K, Arase Y, Kobayashi M, et al.: **Consistently low hepatitis B virus DNA saves patients from hepatocellular carcinogenesis in HBV-related cirrhosis. A nested case-control study using 96 untreated patients.** *Intervirology* 2003, 46:96–104.
32. Tang B, Kruger WD, Chen G, et al.: **Hepatitis B viremia is associated with increased risk of hepatocellular carcinoma in chronic carriers.** *J Med Virol* 2004, 72:35–40.
33. Liu CJ, Chen BF, Chen PJ, et al.: **Role of hepatitis B viral load and basal core promoter mutation in hepatocellular carcinoma in hepatitis B carriers.** *J Infect Dis* 2006, 193:1258–1265.
34. Ohata K, Hamasaki K, Toriyama K, et al.: **High viral load is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis B virus infection.** *J Gastroenterol Hepatol* 2004, 19:670–675.
35. Mahmood S, Niiyama G, Kamei A, et al.: **Influence of viral load and genotype in the progression of hepatitis B-associated liver cirrhosis to hepatocellular carcinoma.** *Liver Int* 2005, 25:220–225.
36. Ikeda K, Arase Y, Kobayashi M, et al.: **Hepatitis B virus-related hepatocellular carcinogenesis and its prevention.** *Intervirology* 2005, 48:29–38.
37. 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.
38. Centers for Disease Control and Prevention (CDC): **Global progress towards universal childhood hepatitis B vaccination.** *MMWR* 2003, 52:868–870.
39. Merican I, Guan R, Amarapuka D, et al.: **Chronic hepatitis B virus infection in Asian countries.** *J Gastroenterol Hepatol* 2000, 15:1356–1361.
40. Da Silva LC, Madruga CL, Carrilho FJ, et al.: **Spontaneous hepatitis B surface antigen clearance in a long-term follow-up study of patients with chronic type B hepatitis. Lack of correlation with hepatitis C and D virus superinfection.** *J Gastroenterol* 1996, 31:696–701.
41. Liaw YF, Sheen IS, Chen TJ, et al.: **Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study.** *Hepatology* 1991, 13:627–631.
42. Sampliner RE, Hamilton FA, Iseri OA, et al.: **The liver histology and frequency of clearance of the hepatitis B surface antigen (HBsAg) in chronic carriers.** *Am J Med Sci* 1979, 277:17–22.
43. Wu TT, Hsu HC, Chen DS, et al.: **Clearance of hepatitis B surface antigen (HBsAg) after surgical resection of hepatocellular carcinoma.** *J Hepatol* 1987, 4:45–51.
44. de Franchis R, Hadengue A, Lau G, et al.: **EASL International Consensus Conference on Hepatitis B. 13–14 September, 2002 Geneva, Switzerland. Consensus statement (long version).** *J Hepatol* 2003, 39(suppl 1):S3–S25.
45. Liaw YF, Leung N, Guan R, et al.: **Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2005 update.** *Liver Int* 2005, 25:472–489.
46. Lok AS, McMahon BJ: **Chronic hepatitis B: update of recommendations.** *Hepatology* 2004, 39:857–861.