High Levels of Hepatitis B Surface Antigen Increase Risk of Hepatocellular Carcinoma in Patients With Low HBV Load

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CLINICAL LIVER

This article has an accompanying continuing medical education activity on page e13. Learning Objective: Upon completion of this assessment, successful learners will be able to use HBsAg level to define different HCC risk in HBV carriers with low viral load.

See editorial on page 1057; see Covering the Cover synopsis on page 1048.

BACKGROUND & AIMS: Patients with chronic hepatitis B virus (HBV) infection have a high risk for developing hepatocellular carcinoma (HCC). Patients with lower levels of hepatitis B surface antigen (HBsAg) have higher chances of losing HBsAg than those with high levels. However, little is known about whether higher levels of HBsAg increase risk for HCC. **METHODS:** We followed 2688 Taiwanese HBsAg-positive patients without evidence of cirrhosis for a mean time period of 14.7 years. In addition to the known risk factors of HCC, we investigated the association between levels of HBsAg and development of HCC. **RESULTS:** Of the patients followed, 191 developed HCC, with an average annual incidence rate of 0.5%. Baseline levels of HBsAg and HBV were associated with development of HCC, and risk increased with level. Compared to HBsAg level, by receiver operating characteristic curve analysis, HBV DNA level better predicted the development of HCC during 10-year and 15-year periods (both, $P < .001$). However, when we evaluated hepatitis B e antigen−negative patients with levels of HBV DNA <2000 IU/mL, factors that determined HCC risk included sex, age, and levels of alanine aminotransferase and HBsAg (≥1000 IU/mL), but not level of HBV DNA. Multivariate analysis showed that the adjusted hazard ratio for HCC in patients with levels of HBsAg ≥1000 IU/mL versus <1000 IU/mL was 13.7 (95% confidence interval: 4.8−39.3). **CONCLUSIONS: Among patients infected with HBV genotype B or C, determinants of HCC risk include their sex, age, hepatitis B e antigen status, HBV genotype, and levels of alanine aminotransferase and HBV DNA, but not level of HBsAg. Among hepatitis B e antigen−negative patients with low viral loads, HCC risk is determined by levels of HBsAg and alanine aminotransferase and age, but not HBV DNA.**

Keywords: Chronic Hepatitis B; Liver Disease; Virology.

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Hepatitis B virus (HBV) infection is a global health problem resulting in >1 million deaths per year.[1] Patients with chronic HBV infection are at risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC), with an estimated lifetime risk of 25%−40% in carriers who acquire the virus early in life.[1–4]

The REVEAL-HBV (Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus) study from Taiwan indicated that HBV DNA is the major driver of disease progression in patients with chronic HBV infection.[5–7] In particular, patients with serum HBV DNA levels ≥2000 IU/mL at study entry have an increased risk of developing HCC.[5] In contrast, those with HBV DNA levels <2000 IU/mL are usually designated inactive or low-risk HBV carriers.[3,8,9] However, data from longitudinal studies indicated that these subjects still carry an annual incidence rate of 0.06% for HCC development.[5,10] Therefore, identification of factors predictive of HCC other than viral load in these low-risk patients remains mandatory and deserves additional studies.

Recently, hepatitis B surface antigen (HBsAg) quantification has become increasingly recognized as a marker for evaluating

Abbreviations used in this paper: ALT, alanine aminotransferase; CI, confidence interval; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; ROC, receiver operating characteristic; SD, standard deviation.

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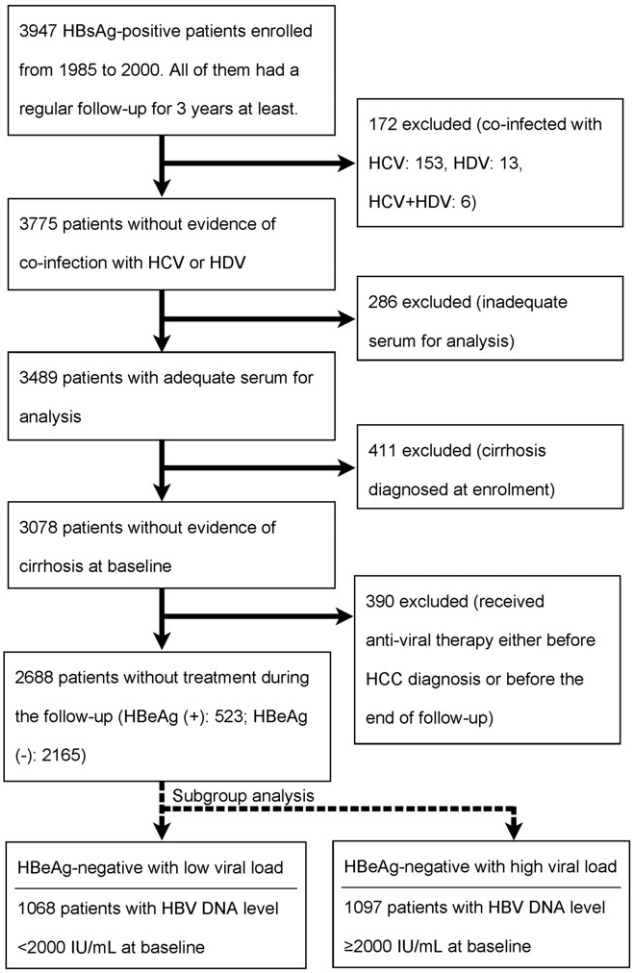


Figure 1. Flow of study participants.

viral replication and possible host immune control over HBV infection.[11–17] A lower HBsAg level is shown to be associated with a higher chance of HBsAg loss and lower risk of hepatitis activity in patients with HBV genotype B or C infection.[11,17,18] In addition, HBsAg level <1000 IU/mL was found to convincingly define inactive carrier state in Italian patients with HBV genotype D infection.[16] Because a lower HBsAg level usually signifies a better prognosis, it is of clinical interest to know whether a higher HBsAg level would be associated with a higher risk of HCC, especially in the special population of lowly viremic patients.

To address this interesting and important issue, we enrolled a large cohort of 2688 treatment-naïve patients who were diagnosed with chronic HBV infection and received long-term follow-up at the National Taiwan University Hospital. The primary aim of our study was to explore whether HBsAg level could complement HBV DNA level as a predictor of HCC development.

Materials and Methods

Patient Cohort

Figure 1 shows the inclusion and exclusion process of patients in the Elucidation of Risk Factors for Disease Control or Advancement in Taiwanese Hepatitis B Carriers (ERADICATE-B) study. Part of this cohort (patients enrolled from 1985 to 1995) had been used to investigate the issue of HBsAg loss.[17] The enrollment time frame was extended to 2000 in this study. In total, 3947 HBsAg-positive patients aged older than 28 years were consecutively enrolled between 1985 and 2000. All of them had been HBsAg-positive for longer than 6 months and received more than 3 years of regular follow-up at the National Taiwan University Hospital. After excluding patients with evidence of hepatitis C virus (HCV) or hepatitis D virus co-infection, and those without adequate serum samples for analysis, 3489 patients remained. We further excluded 411 patients who were diagnosed with cirrhosis at baseline because this is an indication for antiviral therapy in practice guidelines,[19–21] and 390 patients who received antiviral therapy either before HCC diagnosis or before the end of follow-up because of the possible modification of HCC risk by treatment.[22] Finally, a total of 2688 HBV carriers were included into analysis. A subgroup analysis was also performed on hepatitis B e antigen (HBeAg)–positive patients (n = 523) and HBeAg-negative patients (n = 2165), who were divided into high viral load group (1097 with HBV DNA level ≥2000 IU/mL) and low viral load group (1068 with HBV DNA <2000 IU/mL). All enrolled patients gave informed consent as approved by the National Taiwan University Hospital Ethical Committee.

Data Collection

Patients were tested for serological markers (HBsAg, HBeAg, anti-HBe, antibodies against hepatitis C virus [anti-HCV], and antibodies against hepatitis D virus), and had liver function tests and α-fetoprotein levels at baseline. Throughout the follow-up period, if alanine aminotransferase (ALT) levels were within normal limits, liver function tests and α-fetoprotein were assayed every 6 months, and at least every 3 months if ALT levels were elevated. Serum samples collected at each visit were stored at −20°C until analysis. Serum α-fetoprotein and abdominal ultrasonography using a high-resolution and real-time scanner were performed for HCC surveillance every 3 to 6 months from enrollment.

Diagnosis of Cirrhosis and HCC

Cirrhosis was diagnosed by histology or ultrasonographic findings, together with clinical features such as thrombocytopenia, gastroesophageal varices, or ascites.[23] For the diagnosis of cirrhosis made via abdominal ultrasound, the findings had to be consistent on at least 2 occasions 6 months apart.[6] HCC was diagnosed either by histology/cytology or by typical image findings (arterial enhancement and venous wash-out by contrast-enhanced computed tomography or magnetic resonance imaging scanning) in hepatic nodules >1 cm.[24]

Serological Assays

Serum HBsAg, HBeAg, anti-HBe, anti-HCV, and anti–hepatitis D virus were tested by commercial assays (Abbott Laboratories, Abbott Park, IL).

Quantification of HBV DNA and HBsAg Levels

Serum samples at enrollment were tested for both HBV DNA and HBsAg levels. HBV DNA level was quantified using the Abbott RealTime HBV assay, 0.2 mL protocol (Abbott Laboratories) with a low detection limit of 15 IU/mL. HBsAg level was quantified using the Architect HBsAg QT (Abbott Laboratories) according to manufacturer's instructions.[11,15] The detection range of Architect assay is 0.05 to 250 IU/mL. If the HBsAg level

was found to be >250 IU/mL, samples were diluted to 1:100 to 1:1000 to obtain a reading within the calibration curve range.

Extraction of Viral DNA

Viral DNA in the serum was extracted using commercial kits (QIAamp DNA Blood and Tissue Mini Kit; QIAGEN Inc, Valencia, CA, USA). The extracted DNA was used for HBV genotype determination.

Determination of HBV Genotype

HBV genotype was determined by real-time polymerase chain reaction–based single-tube assay as described previously.[25] This method consists of 2 consecutive steps. The first step uses polymerase chain reaction to amplify the region (nt 1261−1600), and the second step uses melting curve analysis to genotype HBV.

Statistical Analysis

Mean and standard deviation (SD) were calculated for continuous variables and percentages were used for categorical variables. Both HBV DNA levels (IU/mL) and HBsAg levels (IU/mL) were logarithmically transformed for Pearson's correlation analysis. HBV DNA levels were assigned as 15 IU/mL for those with undetectable values.

The clinical follow-up started at the time of enrollment. Person-years were censored on the date of identifying HCC, death, the last date of follow-up, or December 31, 2010, whichever came first. The cumulative incidence of HCC by different variables was derived using the Kaplan–Meier curve analysis, and log-rank test was used to test for the statistical difference.

Both HBV DNA and HBsAg levels were categorized into a log10 scale, according to earlier reports.[5,9,11,17] HBV DNA levels were categorized into <200 IU/mL (682 copies/mL, close to the <500 copies/mL as adopted by the REVEAL-HBV study), 200−1999, 2000−19,999, 20,000−199,999, and ≥200,000 IU/mL. HBsAg levels were categorized into <10, 10−99, 100−999, 1000−9999, and ≥10,000 IU/mL.

In order to compare the predictive values of different factors for HCC, we restricted the study population to patients who were followed for at least 10 and 15 years. Receiver operating characteristic (ROC) curve analysis was used to compute the area under the ROC curves for different factors. Their performance in predicting 10-year and 15-year HCC was compared. In addition, we evaluated the relationship between HBsAg level and risk of HCC in HBeAg-negative patients with HBV DNA levels <2000 IU/mL using the restricted cubic spline regression with different number of knots.[26] The reference level of HBsAg was 10 IU/mL (1 log10 IU/mL). The best-fitting cubic spline model was determined according to the values of Akaike information criterion.[27]

Cox proportional hazards regression model was used to calculate the crude and multivariate-adjusted hazard ratios (HR) of HCC. Age, sex, serum HBV DNA, HBsAg, and ALT levels were included as adjusting variables as they are known to be associated with HCC development.[5,28–30]

Statistical significance of all tests was defined as $P < .05$ by 2-tailed tests. All analyses were performed using Stata statistical software (version 10.0; Stata Corp, College Station, TX).

Results

Baseline Characteristics

Table 1 shows the demographics of the 2688 patients. Of these patients, 1634 (60.8%) were males; 783 (29.1%) had ALT level ≥40 U/L; 523 (19.5%) had HBeAg positivity; 754 (28.1%) had HBV DNA level ≥200,000 IU/mL; and 373 (13.9%) had HBsAg level ≥10,000 IU/mL. In patients with HBV DNA level ≥2000 IU/mL or positive HBeAg, 1308 (80.7%) patients were infected with genotype B virus.

Table 1. Demographic Data of 2688 HBV Carriers

	Patients, n (%)
Sex	
Female	1054 (39.2)
Male	1634 (60.8)
Age at enrollment	
28–39	1407 (52.3)
40–49	763 (28.4)
50–59	369 (13.7)
≥60	149 (5.5)
Serum ALT level, *U/L*	
<20	1051(39.1)
20–39	854 (31.8)
≥40	783 (29.1)
HBeAg	
Negative	2165 (80.5)
Positive	523 (19.5)
Serum HBV DNA level, *IU/mL*	
<15	150 (5.6)
15–199	288 (10.7)
200–1999	649 (24.1)
2000–19,999	555 (20.7)
20,000–199,999	292 (10.9)
≥200,000	754 (28.1)
Serum HBsAg level, *IU/mL*	
<10	129 (4.8)
10–99	268 (10.0)
100–999	703 (26.2)
1000–9999	1215 (45.2)
≥10,000	373 (13.9)
HBV genotype[a]	
B	1308 (80.7)
C	312 (19.3)

[a]Only determined in patients with either positive HBeAg or HBV DNA level ≥2000 IU/mL.

Relationships between levels of HBsAg and HBV DNA were further investigated in patients with HBV DNA levels <2000 and ≥2000 IU/mL, respectively. A positive correlation did exist between HBsAg and HBV DNA levels in both groups (Supplementary Figure 1). The correlation was better in the higher HBV DNA group ($r^2 = 0.44$; $P < .001$) than the lower HBV DNA group ($r^2 = 0.07$; $P < .001$).

Follow-Up Results

Our study had 39427.2 person-years of follow-up, with a mean (±SD) follow-up duration of 14.7 ± 4.3 years (median, 13.9 years; range, 2.5–25.8 years). Throughout the follow-up period, 191 patients developed HCC, with an incidence rate of 4.8 cases per 1000 person-years. The mean (±SD) duration from enrollment to HCC development was 11.0 ± 4.5 years (median, 10.2 years; range, 2.5–24.5 years). In addition, there were 294 patients who developed liver cirrhosis during the follow-up and 151 (79.1%) HCC patients had recognized liver cirrhosis before or when HCC was diagnosed.

Cumulative Incidence of HCC by HBeAg Status, ALT, HBV DNA, and HBsAg Levels and Other Risk Factors

We first correlated the cumulative incidence of HCC with HBeAg status and levels of ALT, HBV DNA, and HBsAg (Figure 2*A*−*D*). We found that HBeAg positivity and higher levels of ALT and HBV DNA were associated with a higher cumulative incidence of HCC. The risk of HCC was shown to increase when HBV DNA level was ≥2000 IU/mL (Table 2). Regarding HBsAg level, a higher HBsAg level was also associated with an increased HCC risk with a dose–response manner ($P = .001$). In addition, other common risk factors, including older age, male sex, and genotype C infection, were found to be associated with HCC development (Table 2).

Comparing ALT, HBV DNA, and HBsAg Levels for Predicting HCC

Because ALT, HBV DNA, and HBsAg levels were shown to be associated with HCC development, we evaluated which one was a better predictor. The performance of these 3 factors in predicting HCC development was compared by the subcohorts of 10 years of follow-up (n = 2491; 90 developed HCC) and 15 years of follow-up (n = 1219; 154 developed HCC) because the mean time to HCC was 11.0 years and mean follow-up period of this cohort was 14.7 years. In terms of predicting 10-year HCC, the area under the ROC were 0.70 (95% confidence interval [CI]: 0.65–0.75) for HBV DNA level, 0.75 (95% CI: 0.70–0.79) for ALT level, and 0.58 (95% CI: 0.52–0.64) for HBsAg level. The ROC curve analysis showed that both ALT and HBV DNA levels were superior to HBsAg level in predicting HCC development within 10 years (Figure 2*E*) and within 15 years (Figure 2*F*).

In HBeAg-positive patients, we evaluated these 3 factors in predicting 10-year and 15-year HCC development (Supplementary Table 1). Again, both ALT and HBV DNA levels were shown to be superior to HBsAg level in predicting HCC development.

We also evaluated the predictive accuracy of HBV genotype in patients with positive HBeAg or HBV DNA level ≥2000 IU/mL (Supplementary Table 1). The area under the ROC was 0.66 (95% CI: 0.60–0.72) and 0.63 (95% CI: 0.59–0.67) for the prediction of 10-year and 15-year HCC risk, respectively, and were both superior to HBsAg level. When patients were stratified by different HBV genotypes, the predictive accuracy of HBsAg level for HCC development did not significantly differ between genotype B and C patients (Supplementary Table 2).

Different Impact of HBsAg Level on HCC Development Between HBeAg-Negative Patients With Low and High Viral Loads

The risk of HCC among patients with HBV DNA level <2000 IU/mL was comparable at different ranges of viral load (Table 2). We explored whether HBsAg level could be another risk factor for HCC development in HBeAg-negative patients with a low viral load. The relationship between HBsAg level and HCC risk was first evaluated using the restricted cubic spline regression. The shape of the best-fitting regression spline for HBsAg levels, which was based on the smallest Akaike information criterion value (Supplementary Figure 2), is presented in Figure 3*A*. This model was derived using the restricted cubic spline regression with 3 knots placing on the 25th, 50th, and 75th percentiles of HBsAg distribution. We found that HCC risk appeared to be similar in those with HBsAg levels <1000 IU/mL, but gradually increased when the level was >1000 IU/mL.

To validate this finding, we categorized these patients with HBV DNA level <2000 IU/mL by the following 3 cutoff levels of HBsAg: 10 IU/mL (Figure 3*B*), 100 IU/mL (Figure 3*C*), and 1000 IU/mL (Figure 3*D*), and analyzed the data using the Kaplan–Meier curve analysis. Compatible with the finding in Figure 3*A*, the cumulative incidence of HCC was different only when patients were categorized by HBsAg level of 1000 IU/mL ($P < .001$), but not 10 or 100 IU/mL. The 10-year cumulative incidence of HCC was 0.2% and 2.2% for HBsAg <1000 and ≥1000 IU/mL, respectively. In contrast, in HBeAg-negative patients with HBV DNA level ≥2000 IU/mL, the HCC risk was not related to serum HBsAg levels ($P = .247$; Figure 3*E*). As we aimed to determine the impact of HBsAg level on disease progression, we focused on the subcohort of HBeAg-negative patients with HBV DNA level <2000 IU/mL.

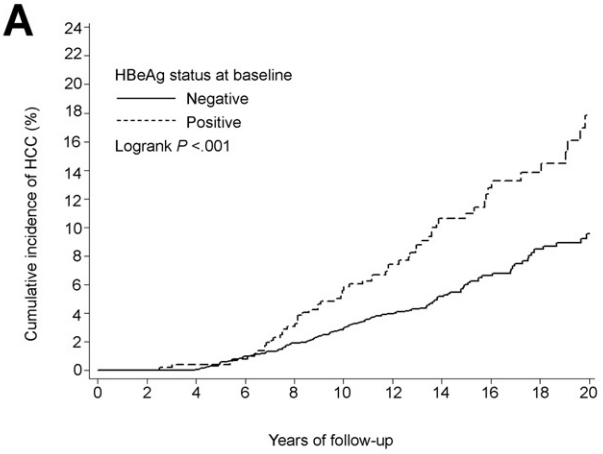
Factors Affecting HCC Risk in HBeAg-Negative Patients With Low Viral Load

In HBeAg-negative patients with HBV DNA level <2000 IU/mL, advanced age, male sex, and elevated ALT level, but not HBV DNA level, were found to be independent risk factors for HCC development (Table 3). As for HBsAg level, compared to patients with HBsAg level <1000 IU/mL, the HR of HCC was 5.4 (95% CI: 2.1–14.2) for patients with HBsAg level ≥1000 IU/mL using univariate analysis. Further multivariate analysis showed that HBsAg level ≥1000 IU/mL remained as an independent risk factor of HCC with an HR of 13.7 (95% CI: 4.8–39.3).

Relationships Between HCC Risk and Dynamic Changes of HBV DNA, HBsAg, and ALT Levels in HBeAg-Negative Patients With Low Viral Loads at Baseline

In 1068 HBeAg-negative patients with HBV DNA level <2000 IU/mL, 980 (91.8%) had available stored serum samples at the third year of follow-up for the determination of HBV DNA and HBsAg levels. Compared to patients with persistently low levels of HBV DNA, HBsAg, or ALT, those with persistently high levels of these 3 factors were at a higher risk of HCC using univariate analysis (Table 4). For example, compared to patients with HBsAg level <1000 IU/mL at baseline and year 3, the HR of HCC was 8.0 (95% CI: 2.2–27.3) for those with HBsAg level ≥1000 IU/mL at baseline and year 3. In addition,

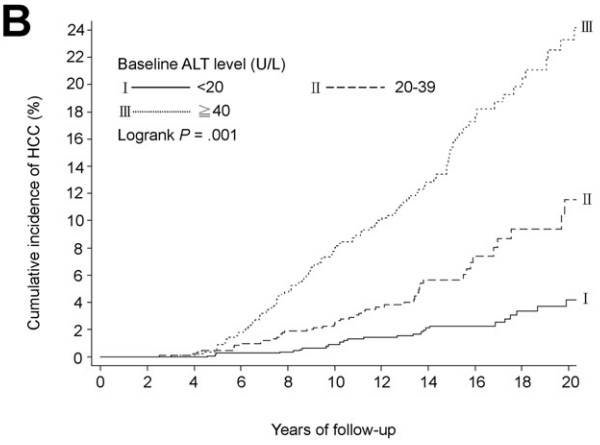
A



Number at risk

Serum HBeAg status at baseline											
Negative	2165	2165	2164	2142	2106	1938	1516	1050	649	421	291
Positive	523	523	521	519	504	463	376	274	187	132	91

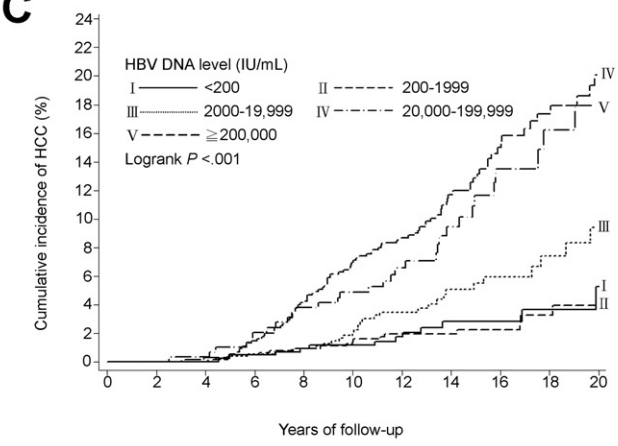
B



Number at risk

Serum ALT level at baseline (U/L)											
<20	1051	1051	1051	1047	1040	974	813	646	453	307	212
20-39	854	854	852	846	826	755	563	357	198	114	79
≧40	783	783	782	768	744	672	516	321	185	132	91

C



Number at risk

Serum HBV-DNA level at baseline (IU/mL)											
<200	438	438	438	435	428	394	307	221	137	91	60
200-1999	649	649	649	645	638	595	487	354	228	146	98
2000-19999	555	555	555	552	546	500	386	265	173	114	80
20000-199999	292	292	291	286	278	255	190	141	90	59	40
≧200000	754	754	752	743	720	657	522	343	208	143	104

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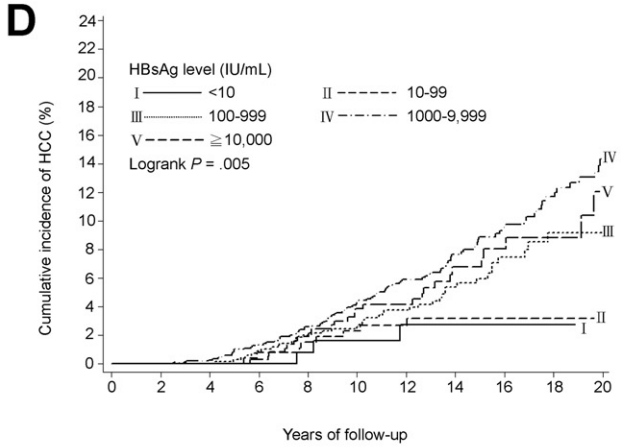
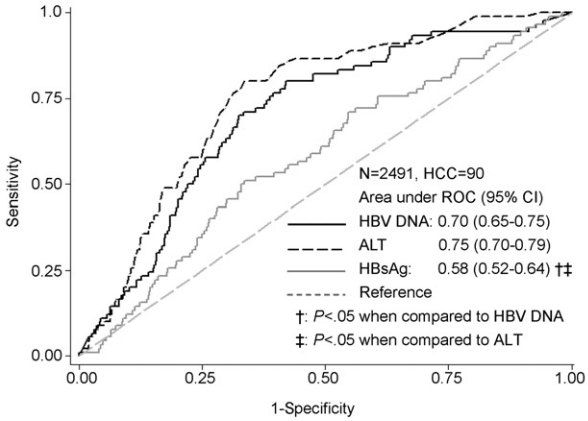


Number at risk

Serum HBsAg level at baseline (IU/mL)											
<10	129	129	129	129	126	113	85	56	21	9	7
10-99	268	268	268	265	262	248	192	128	76	54	37
100-999	703	703	703	697	684	631	484	353	220	135	92
1000-9999	1215	1215	1212	1198	1175	1080	868	603	400	281	196
≧10000	373	373	373	372	363	329	263	184	119	74	50

E



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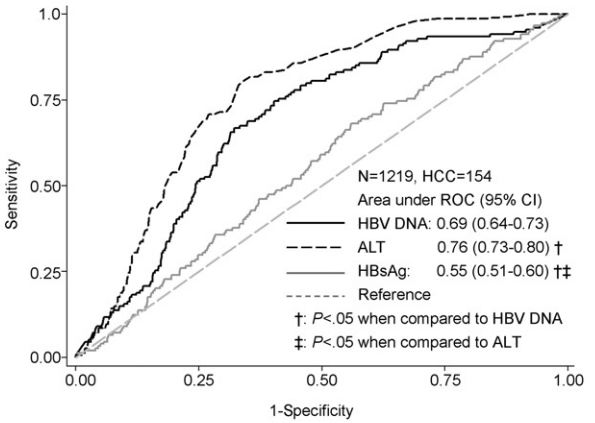


Table 2. Univariate Analysis of Factors Associated With HCC in 2688 HBV Carriers by Cox Proportional Hazards Regression Model

	Patients, n	Patient-years of follow-up	HCC, n	Annual incidence rate (per 100,000 patient-years)	Crude HR (95% CI)	*P* value
Sex						
Female	1054	15,440.3	37	239.6	1.0	
Male	1634	23,986.8	154	642.0	2.7 (1.9–3.8)	<.001
Age, *y*						
28–39	1407	21,236.5	62	292.0	1.0	
40–49	763	11,152.4	54	484.2	1.7 (1.2–2.5)	.004
50–59	369	5164.7	43	832.6	3.0 (2.1–4.5)	<.001
≥60	149	1873.5	32	1708.0	6.9 (4.5–10.6)	<.001
Serum ALT level, *U/L*						
<20	1051	16,611.0	27	162.5	1.0	<.001
20–39	854	11,908.6	49	411.5	2.8 (1.8–4.5)	
≥40	783	10,907.6	115	1054.3	7.2 (4.7–511.0)	<.001
HBeAg status						
Negative	2165	31,588.6	127	402.0	1.0	
Positive	523	7838.6	64	816.5	2.0 (1.5–2.7)	<.001
Serum HBV DNA level, *IU/mL*						
<200	438	6454.6	12	185.9	1.0	
200–1999	649	9780.3	17	173.8	0.9 (0.4–1.9)	.824
2000–19,999	555	8141.4	30	368.5	2.0 (1.0–3.9)	.044
20,000–199,999	292	4223.6	32	757.6	4.1 (2.1–8.0)	<.001
≥200,000	754	10,827.1	100	923.6	5.1 (2.–9.2)	<.001
Serum HBsAg level, *IU/mL*						
<10	129	1735.8	3	172.8	1.0	
10−99	268	3916.0	8	204.3	1.1 (0.3–4.2)	.881
100−999	703	10,269.6	43	418.7	2.3 (0.7–7.3)	.171
1000−9999	1215	18,077.3	108	597.4	3.2 (1.0–10.0)	.048
≥10,000	373	5428.5	29	534.2	2.9 (0.9−9.5)	.080
HBV genotype[a]						
B	1308	19,154.7	93	485.5	1.0	
C	312	4327.1	69	1594.6	3.4 (2.5−4.6)	<.001

[a]Only determined in patients with either positive HBeAg or HBV DNA level ≥2000 IU/mL.

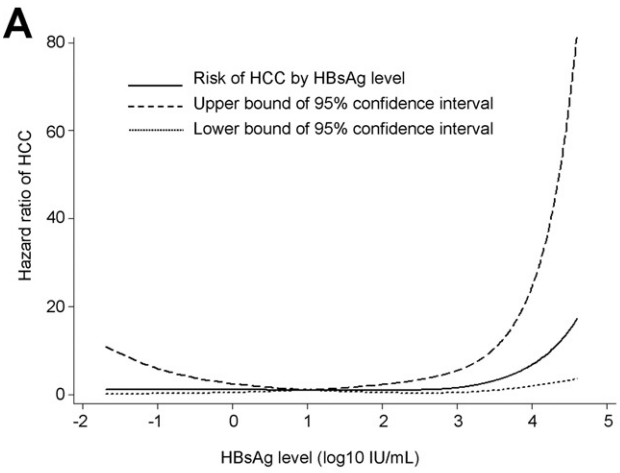
HCC risk increased when patients had increased HBV-DNA level (HR = 4.7; 95% CI, 2.2–10.0), increased HBsAg level (HR = 7.2; 95% CI, 1.8–28.6), and elevated ALT level (HR = 6.6; 95% CI, 2.2–19.8). Using multivariate analysis, persistently high levels of HBsAg or ALT and increased levels of HBsAg or ALT were still independent risk factors for HCC (Table 4).

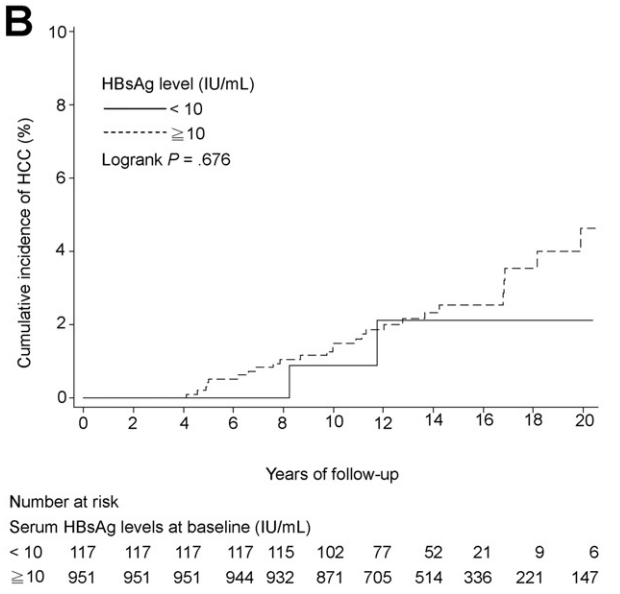
Discussion

For chronic hepatitis B patients, HCC is a devastating complication. It is important to identify risk factors of HCC in clinical practice.[3] In this hospital-based cohort study, we demonstrated that advanced age, male sex, elevated ALT level, genotype C, positive HBeAg, and higher HBV DNA level were associated with HCC development over time. The risk of HCC started to increase when HBV DNA level was >2000 IU/mL. All these results were consistent with earlier studies,[5,28,30–33] validating the accuracy of our findings in this large hospital-based cohort. Although both levels of HBV DNA and HBsAg were shown to be associated with HCC development, we found that HBV DNA level had better predictive accuracy than HBsAg level when investigating the overall cohort. However, in patients with HBV DNA level <2000 IU/mL who had a similar risk of HCC, HBsAg level ≥1000 IU/mL was identified as a new independent risk factor for HCC development. These data suggested that HBsAg level might complement HBV DNA level in predicting HCC development, especially in the lowly viremic HBV carriers.

It is known that there is a positive correlation between HBsAg and HBV DNA levels.[12,13,34] The correlation has been shown to be higher at HBeAg-positive phase, lower at HBeAg-negative phase, and lowest at the lowly replicative phase, which was consistent with our results. This discrepancy between levels of HBsAg and HBV DNA at the lowly replicative phase might be caused by accumulation of integrated viral envelope sequences in infected hepatocytes. The HBsAg is mainly derived from the integrated form of HBV DNA rather than the episomal form.[13,35] In other words, lowly viremic patients who have high HBsAg

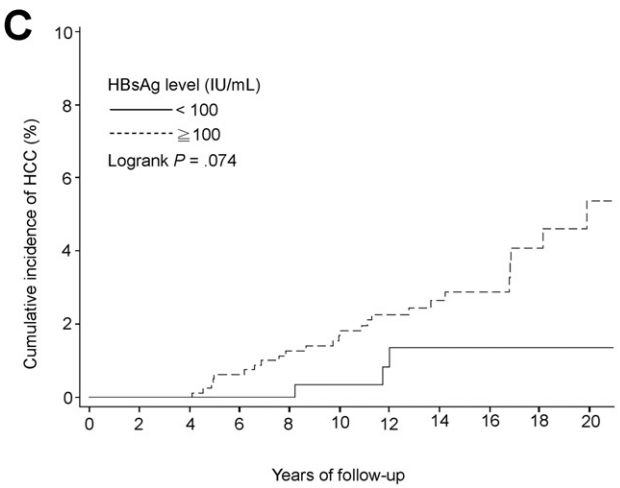
Figure 2. Cumulative incidence of HCC in a cohort of 2688 HBsAg-positive patients was associated with (*A*) serum HBeAg status, (*B*) ALT level, (*C*) HBV DNA level, and (*D*) HBsAg level at study entry. A better prediction of serum HBV and ALT levels than HBsAg level for HCC within (*E*) 10 years of follow-up (n = 2491) and (*F*) 15 years of follow-up (n = 1219) by ROC curve analysis.





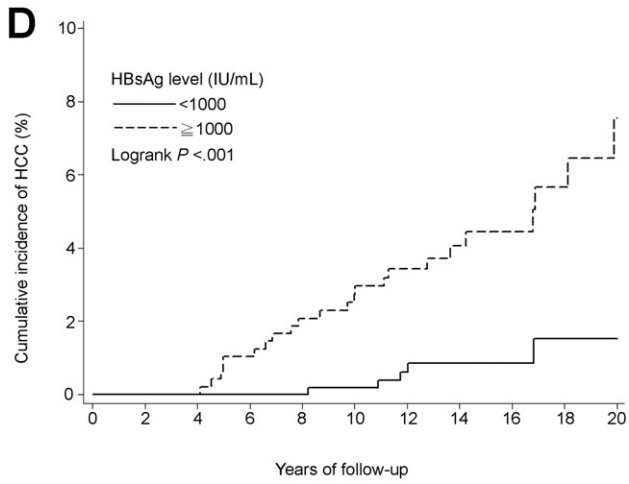
Number at risk

Serum HBsAg levels at baseline (IU/mL)											
< 10	117	117	117	117	115	102	77	52	21	9	6
≧10	951	951	951	944	932	871	705	514	336	221	147



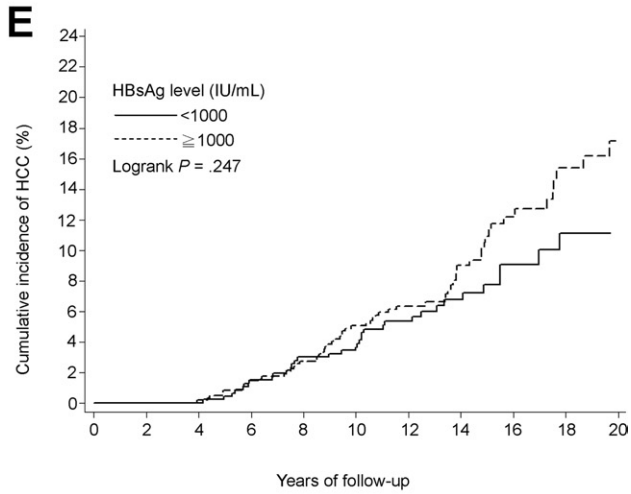
Number at risk

Serum HBsAg levels at baseline (IU/mL)											
< 100	284	284	284	284	280	260	199	135	71	46	32
≧100	784	784	784	778	767	713	583	431	286	184	122



Number at risk

Serum HBsAg levels at baseline (IU/mL)											
<1000	585	585	585	584	578	536	418	306	180	108	72
≧1000	483	483	483	477	469	437	364	260	177	122	82



Number at risk

Serum HBsAg levels at baseline (IU/mL)											
<1000	468	468	468	461	452	419	312	208	119	79	56
≧1000	629	629	628	620	607	546	422	276	173	112	81

Figure 3. In 1068 HBeAg-negative patients with HBV DNA level <2000 IU/mL. (*A*) HR of HCC in relation to HBsAg levels was analyzed using the restricted cubic spline regression with 3 knots on the 25th, 50th, and 75th percentiles. Cumulative incidence of HCC was analyzed by cutoff HBsAg levels of (*B*) 10 IU/mL, (*C*) 100 IU/mL, and (*D*) 1000 IU/mL in HBeAg-negative patients with HBV DNA level <2000 IU/mL and (*E*) by HBsAg level of 1000 IU/mL in HBeAg-negative patients with HBV DNA levels ≥2000 IU/mL.

Table 3. Univariate and Multivariate Analysis of Factors Associated With HCC in 1068 HBeAg-Negative Patients With HBV DNA Level <2000 IU/mL by Cox Proportional Hazards Regression Model

	Patients, n	Patient-years of follow-up	Annual incidence rate (per 100,000 patient-years)	Crude HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
Sex							
Female	468	6956.7	86.3	1.0		1.0	
Male	600	8988.6	255.9	3.0 (1.2−7.3)	.018	2.2 (0.9−5.9)	.099
Age at enrollment, *y*							
28−39	565	8590.2	81.5	1.0		1.0	
40−49	317	4819.3	186.8	2.3 (0.9−6.2)	.097	3.5 (1.3−9.4)	.014
50−59	132	1821.6	384.3	5.1 (1.8−14.8)	.002	11.8 (3.9−35.5)	<.001
≥60	54	714.2	840.1	11.7 (3.9−35.3)	<.001	38.5 (11.2−132.1)	<.001
Serum ALT level, *U/L*							
<20	582	9143.0	65.6	1.0		1.0	
20−39	328	4629.3	172.8	3.0 (1.0−8.6)	.046	2.2 (0.7−6.9)	.187
≥40	158	2173.1	690.3	11.7 (4.5−30.3)	<.001	11.8 (4.3−32.7)	<.001
Serum HBV DNA level, *IU/mL*							
<200	438	6454.6	185.9	1.0		1.0	
200−1999	630	9490.7	179.1	1.0 (0.5−2.0)	.898	0.9 (0.4−1.9)	.691
Serum HBsAg level, *IU/mL*							
<1000	585	8585.1	58.2	1.0		1.0	
≥1000	483	7360.2	326.1	5.4 (2.1–14.2)	.001	13.0 (4.6−37.0)	<.001

level might harbor more hepatocytes with HBV integration than those who have low HBsAg level. Therefore, the higher risk of HCC in former patients can be attributed to the increased genomic instability as a result of integrated viral sequences, which plays an important role in hepatocarcinogenesis.[36] Another possible explanation for the positive correlation between HCC risk and HBsAg level, but not HBV DNA level, is the narrow range in HBV DNA. In HBV carriers with HBV DNA level <2000 IU/mL, the dynamic range of HBsAg is wider than HBV DNA level (0.05 to >10,000 IU/mL vs 15 to 2000 IU/mL). A wide-range factor is prone to provide more power to differentiate patients at different risk.

Previous studies have indicated that a lower HBsAg level is associated with better clinical outcomes, including a higher likelihood of HBsAg loss and lower risk of HBeAg-negative hepatitis.[11,17,18] In a recent study comparing the prognosis between inactive HBV carriers (viral load <2000 IU/mL) and non-HBV plus non-HCV controls, the 10-year cumulative incidence rates of HCC were 0.6% and 0.2%, respectively.[10] In our study, the 10-year cumulative incidence rate of HCC in patients with HBV DNA level <2000 IU/mL plus HBsAg level <1000 IU/mL was 0.2%, which was similar to the controls.[10] These data suggested that, in addition to HBV DNA level <2000 IU/mL, HBsAg level <1000 IU/mL

Table 4. HCC Risk in 980 Patients With Baseline Serum HBV DNA <2000 IU/mL: Categorized by Serum Levels of HBV DNA, HBsAg, and ALT at Baseline and 3-Year Follow-Up

		Patients, n (%)	HCC, n	Patient-years of follow-up	HR (95% CI)	*P* value	Adjusted HR[a] (95% CI)	*P* value
Serum HBV DNA level, *IU/mL*								
At baseline	At year 3							
<2000	<2000	842 (85.9)	15	12,619.3	1.0		1.0	
<2000	≥2000	138 (14.1)	12	2117.8	4.7 (2.2−10.0)	<.001	2.0 (0.9−4.4)	.104
Serum HBsAg level *IU/mL*								
At baseline	At year 3							
<1000	<1000	493 (50.3)	3	7284.3	1.0		1.0	
<1000	≥1000	129 (13.2)	6	1973.6	7.2 (1.8−28.6)	.005	14.4 (3.3−62.7)	<.001
≥1000	<1000	33 (3.4)	1	487.7	5.2 (0.5−49.7)	.155	5.5 (0.5−57.2)	.151
≥1000	≥1000	325 (33.2)	17	4991.6	8.0 (2.3−27.3)	.001	16.6 (4.4−63.6)	<.001
Serum ALT level, *U/L*								
At baseline	At year 3							
<40	<40	761 (77.7)	8	11,654.2	1.0		1.0	
<40	≥40	73 (7.5)	4	1056.4	6.1 (1.8−20.4)	.003	3.9 (1.1−13.6)	.035
≥40	<40	76 (7.8)	9	1060.4	12.9 (5.0−33.6)	<.001	14.0 (5.1−38.4)	<.001
≥40	≥40	70 (7.1)	6	966.1	10.0 (3.4−29.1)	<.001	6.6 (3.7−35.2)	<.001

[a]Adjusting variables include age, sex, and dynamics of HBV DNA, HBsAg, and ALT levels.

can be considered to define low-risk patients who are infected with HBV genotype B or C. Of particular note, a recent study on Italian patients with genotype D virus infection also indicated that HBsAg level <1000 IU/mL is associated with a sustained viral suppression within a 3-year follow-up.[16] These findings lend strong support to our hypothesis that a lower HBsAg level can signify adequate host immune control against HBV infection, leading to the decrease of HCC risk over time. Taking these lines of evidence together, HBsAg level <1000 IU/mL could be considered as a general criterion to define low-risk or inactive HBV carriers infected with different HBV genotypes. However, this speculation needs to be confirmed in HBV patients infected with genotypes other than B, C, and D.

The cutoff HBsAg level reported in this study would become clinically useful in different aspects. First, the recently introduced nomogram is based on the results derived from the REVEAL-HBV study,[30] and its usefulness has been confirmed by an external cohort including patients from Korea and Hong Kong.[37] Our data further illustrated that HBsAg ≥1000 IU/mL was another key factor associated with HCC development in patients with HBV DNA level <2000 IU/mL, and this easy-to-use marker should be integrated into the nomogram. Second, when treating HBeAg-negative patients with nucleos(t)ide analogues, there still lacks a reliable marker to predict sustained viral suppression after stopping therapy. HBV DNA level is not useful because most patients have an undetectable HBV DNA level at the end of therapy.[38] In addition, although clearance of HBsAg is considered as an ideal end point of HBV therapy, it is very difficult to achieve, especially in Asian HBV patients who acquire the infection early in life. Thus practicing physicians urgently require a good indicator to stop nucleos(t)ide analogue therapy in HBeAg-negative patients. If HBsAg level <1000 IU/mL could be reliably used to define low-risk or inactive HBV carriers, we can adopt this cutoff level as the intermediate treatment goal when its value is validated in future studies.

This study had several unique features. It is well known that HCC has a low occurrence rate, thus a large cohort with a reasonable follow-up period is mandatory to evaluate this rare event. To fulfill this requirement, we adopted a strict inclusion criterion that patients needed to have regular follow-up for at least 3 years. This allowed us to ensure that included patients adhered to our follow-up program, which is especially important, as we used a nonconcurrent prospective cohort. Consequently, we achieved a large hospital-based cohort with a mean follow-up period of 14.7 years. This cohort offered adequate statistical power to address the rare event of HCC development. Second, the ERADICATE-B study, a hospital-based cohort study, has different characteristics from the REVEAL-HBV study, which is a community-based cohort study. Compared to the REVEAL-HBV study, the ERADICATE-B study consisted of a higher proportion of patients with elevated ALT level (29% vs 6%) and a lower proportion of patients with HBV DNA levels <2000 IU/mL (35% vs 56%), just like the patient population in our clinical practice. Of particular note is that although these 2 cohorts have different composition, both reveal the importance of HBV DNA level in predicting HCC risk. These consistent findings validate the impact of HBV DNA on HCC development unequivocally. Third, serial changes in levels of ALT and HBV DNA have been shown to affect HCC development.[39] In our lowly viremic cohort, the impact of dynamics of HBV DNA, HBsAg, and ALT levels on HCC was also investigated. We found that in patients with low baseline levels of HBV DNA, HBsAg, or ALT, their HCC risk increased in parallel with increased levels of HBV DNA, HBsAg, or ALT at year 3. These data suggested that dynamic data could improve the predictive accuracy of baseline factors. Taking these lines of evidence together, even for patients traditionally regarded as the low-risk group based on baseline data, a regular follow-up with repeated measurements of these risk factors is required because the risk of HCC can vary over time.

Our study had a few limitations. First, we excluded patients who had cirrhosis at enrollment because these patients require antiviral therapy under current treatment guidelines.[19–21] Second, this study is a hospital-based cohort, and it is inevitable that patients had a higher chance to receive antiviral therapy, which has been shown to decrease HCC development.[22] To avoid this interference, we only enrolled patients before 2000, when nucleos(t)ide analogues were not widely available in Taiwan. In addition, we excluded patients who received antiviral therapy either before HCC diagnosis or before the end of follow-up. In other words, this is a homogenous cohort that is free from treatment and ensures that the derived results are not biased.

In summary, in a cohort of 2688 patients infected with HBV genotypes B or C, determinants of HCC risk include their sex, age, HBeAg status, HBV genotype, and levels of ALT and HBV DNA, but not level of HBsAg. Among HBeAg-negative patients with low viral loads, age, baseline levels, and dynamic changes of HBsAg and ALT predict HCC development. Therefore, combination of HBV DNA level <2000 IU/mL and HBsAg level <1000 IU/mL can be considered as essential criteria to define the minimal-risk HBV carriers.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2011.02.007.

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Reprint requests

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Conflicts of Interest

These authors disclose the following: Jia-Horng Kao is a consultant for Abbott and Bristol-Myers Squibb, and is on the speaker's bureau for Abbott and Bristol-Myers Squibb. The remaining authors disclose no conflicts.

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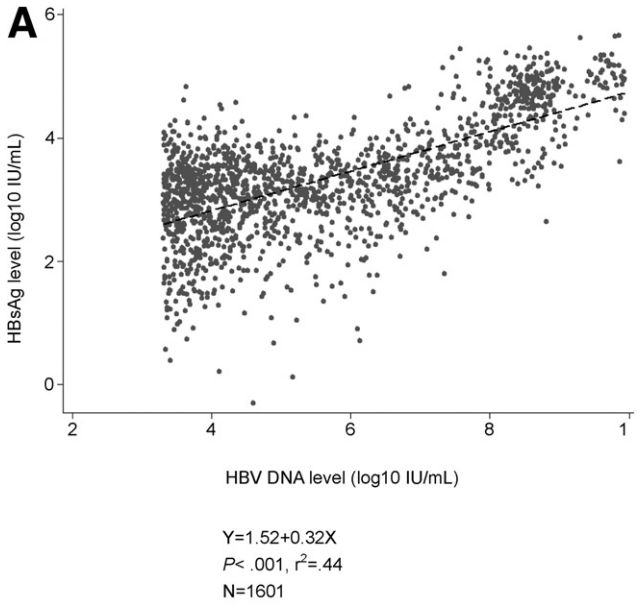
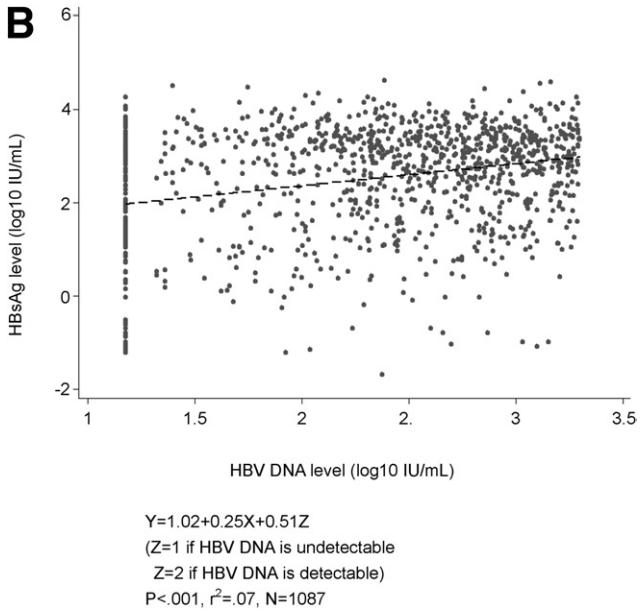




Supplementary Figure 1. Positive relationships between serum levels of HBV DNA and HBsAg in patients with HBV DNA levels (*A*) ≥2000 IU/mL ($r^2 = 0.44$, $P < .001$) and (*B*) <2000 IU/mL ($r^2 = 0.07$, $P < .001$).

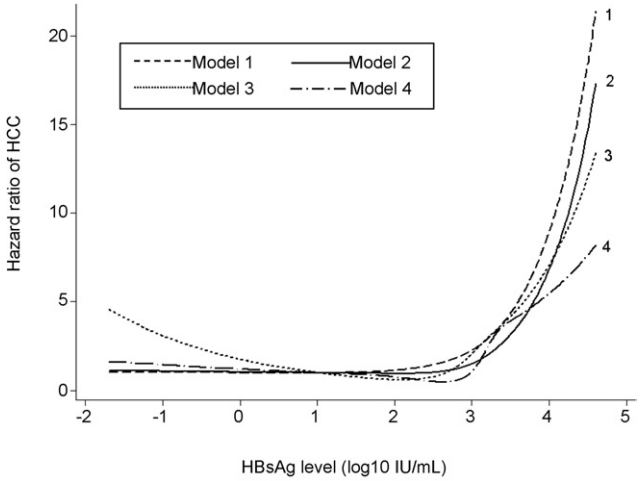
Supplementary Table 1. Receiver Operating Characteristic Curve Analysis of Baseline Risk Factors in Predicting 10-Year HCC and 15-Year HCC Development in Different Subcohorts

	10-Year HCC		15-Year HCC	
	Area under ROC curve (95% CI)	*P* value	Area under ROC curve (95% CI)	*P* value
Subcohort of patients with positive HBeAg	n = 492; HCC, n = 29		n = 278; HCC, n = 49	
HBsAg level	0.35 (0.24−0.46)		0.39 (0.31−0.48)	
HBV DNA level	0.48 (0.38−0.59)	.003[a]	0.52 (0.43−0.61)	<.001[a]
ALT level	0.59 (0.51−0.67)	<.001[a]	0.59 (0.52−0.66)	.002[a]
Subcohort of patients with available genotype data[b]	n = 1504; HCC, n = 76		n = 749; HCC, n = 131	
Genotype	0.66 (0.60−0.72)		0.63 (0.58−0.67)	
HBV DNA level	0.64 (0.59−0.70)	.609[c]	0.60 (0.55−0.65)	.327[c]
HBsAg level	0.49 (0.42−0.56)	<.001[c]	0.47 (0.41−0.52)	<.001[c]
ALT level	0.70 (0.65−0.75)	.325[c]	0.69 (0.65−0.74)	.027[c]

[a]*P* value derived from the comparison with HBsAg level.
[b]Including patients with positive HBeAg or HBV DNA level ≥2000 IU/mL.
[c]*P* value derived from the comparison with genotype.

Supplementary Table 2. Comparison of Predictive Accuracy of Baseline HBsAg Levels Between Different HBV Genotypes for 10-Year HCC and 15-Year HCC Development

	HBsAg level in predicting 10-year HCC (n = 1504; HCC, n = 76)			HBsAg level in predicting 15-year HCC (n = 749; HCC, n = 131)		
HBV genotype	n	Area under ROC curve (95% CI)	*P* value	n	Area under ROC curve (95% CI)	*P* value
B	1213	0.48 (0.39−0.58)		575	0.42 (0.36−0.49)	
C	291	0.40 (0.30−0.51)	.276	174	0.45 (0.36−0.54)	.674



Model	Knot number	Knot location (percentile)	Akaike information criterion (AIC)
1	3	10th, 50th, 90th	333.2
2	3	25th, 50th, 75th	333.1
3	4	20th, 40th, 60th, 80th	333.9
4	5	17th, 33rd, 50th, 67th, 83rd	334.9

Supplementary Figure 2. Estimated curves of hazard ratio of HCC and corresponding Akaike information criterion (AIC) values in HBeAg-negative patients with HBV DNA level <2000 IU/mL by different regression models.