MAJOR ARTICLE

Risk Stratification of Hepatocellular Carcinoma in Hepatitis B Virus e Antigen–Negative Carriers by Combining Viral Biomarkers

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Background. The serum hepatitis B virus (HBV) surface antigen (HBsAg) level can predict hepatocellular carcinoma (HCC) development in hepatitis B e antigen (HBeAg)–negative patients with an HBV DNA level of <2000 IU/mL. However, little is known regarding how well the combination of both viral biomarkers stratifies HCC risk.

Methods. A total of 2165 Taiwanese HBeAg-negative noncirrhotic patients were followed for 14.9 years. The predictive power of the HBsAg level for HCC was analyzed for different viral load ranges.

Results. In patients with HBV DNA levels of 2000–19 999 IU/mL (intermediate viral load), a positive correlation between HBsAg level and HCC development was identified after adjustment for other risk factors ($P = .002$). In contrast, no association was found between HBsAg level and HCC in patients with higher viral loads. HBsAg level was subsequently included to stratify HCC risk in patients with low and intermediate viral loads. Receiver operating characteristic curve analysis showed that combining HBV DNA and HBsAg level better predicts 10-year HCC development as compared to using HBV DNA level alone in the overall cohort ($P = .028$).

Conclusions. Serum HBsAg level helps stratify HCC risk in patients with intermediate viral loads. Combining HBV DNA and HBsAg levels better predicts HCC risk.

Keywords. HBsAg; HBV DNA; intermediate viral load; HCC; minimal risk; viral hepatitis.

Hepatitis B virus (HBV) infection is a global health problem, resulting in more than one million deaths per year [1]. Patients with chronic HBV infection are at risk of developing adverse outcomes, including cirrhosis and hepatocellular carcinoma (HCC), with an estimated lifetime risk of 25%–40% [1–4].

Currently, there are 2 viral factors that can be quantified by commercial assays: HBV DNA and HBV surface antigen (HBsAg). Several lines of evidence have indicated that both are important biomarkers for predicting long-term outcomes [5–10]. In adult HBV carriers, results from cohort studies have shown that a higher HBV DNA level is associated with a higher HCC risk [6, 9, 11]. However, in patients with an HBV DNA level <2000 IU/mL (low viral load), further categorized viral loads play an insignificant role in predicting HCC, and the HBsAg level becomes the only predictive biomarker [9]. More specifically, a higher HBsAg level (≥1000 IU/mL) is associated with a greater risk of HCC in HBV e antigen (HBeAg)–negative patients with low viral loads [9].

Whether the HBV DNA risk threshold for disease progression should be defined as 2000 IU/mL or 20 000 IU/mL has been actively debated [12]. When we had a closer look at the relationship between HCC risk and HBV DNA levels in different cohorts [6, 9, 11], we

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found that there was a moderate increase in risk in those with HBV DNA levels of 2000–19 999 IU/mL (intermediate viral load) when compared to patients with a low viral load. On the other hand, there was a dramatic increase in risk in those with HBV DNA levels ≥20 000 IU/mL (high viral load). Because HBsAg level is known to be a complementary marker for HCC risk in the low viral load group [9], we therefore hypothesized that categorized HBsAg levels may further stratify the risk in HBV-infected patients with intermediate viral loads [13]. If this hypothesis holds true in HBeAg-negative carriers, HBsAg level should be combined with HBV DNA level to allow for better prediction of HCC risk.

To address this important issue, we analyzed a large cohort of 2165 HBeAg-negative, treatment-naive patients who received a diagnosis of chronic HBV infection and underwent long-term follow-up at National Taiwan University Hospital [9]. The following 2 issues will be addressed. First, we determined whether the HBsAg level could stratify HCC risk for all HBV DNA levels, not just for individuals with low viral loads. Second, if HBsAg level does prove to play a role in predicting HCC development, we determined whether the combination of HBsAg level and HBV DNA level improves the risk stratification of HCC in HBeAg-negative patients overall.

METHODS

Patient Cohort

Figure 1 shows the inclusion and exclusion criteria of patients in the Elucidation of Risk Factors for Disease Control or Advancement in Taiwanese Hepatitis B carriers (ERADICATE-B) study [9]. In total, 3947 HBsAg-positive patients aged >28 years were consecutively enrolled between 1985 and 2000. All had been HBsAg positive for >6 months and underwent regular follow-up for >3 years at the National Taiwan University Hospital. After excluding patients with evidence of hepatitis C virus (HCV) or hepatitis D virus (HDV) coinfection and those with inadequate serum samples for analysis, 3489 patients remained. We further excluded 411 patients who received a diagnosis of cirrhosis at baseline, because they are recommended for antiviral therapy according to practice guidelines [14–16], and 390 patients who received antiviral therapy either before the HCC diagnosis or before the end of follow-up, as treatment may alter HCC risk [17]. A total of 2688 HBV carriers remained. Since HBeAg-positive HBV carriers may experience HBeAg seroconversion, which dramatically lowers HCC risk [1, 3], we decided to only enroll HBeAg-negative patients for analysis (n = 2165). All enrolled patients gave informed consent as required by the National Taiwan University Hospital ethics committee.

Data Collection

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

Diagnosis of Cirrhosis and HCC

Cirrhosis was diagnosed by histologic or ultrasonographic findings together with clinical features such as thrombocytopenia, gastroesophageal varices, or ascites [18–20]. For the diagnosis of cirrhosis made via abdominal ultrasound, the findings had to be consistent on at least 2 occasions 6 months apart [5]. HCC was diagnosed on the basis of either histologic/cytologic findings or typical image findings (arterial enhancement and venous washout by contrast-enhanced computed tomography or magnetic resonance imaging) involving a hepatic nodule >1 cm [21].

Serological Assays

Tests for detection of HBsAg, HBeAg, anti-HBe, anti-HCV, and anti-HDV in serum were performed using 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, Abbott Park, IL), with a lower detection limit of 15 IU/mL. HBsAg level was quantified using the Architect HBsAg QT (Abbott Laboratories, Abbott Park, IL) according to the manufacturer's instructions [7, 22]. The detection range of the Architect assay is 0.05–250 IU/mL. If the HBsAg level was found to be >250 IU/mL, the samples were diluted to 1:100 or 1:1000 to obtain a reading within the calibration curve range.

HBV DNA Extraction and Genotype Determination

Viral DNA in the serum was extracted using a commercial kit (QIAamp DNA Blood and Tissue Mini Kit; Qiagen, Valencia, CA). HBV genotype was determined by a real-time polymerase chain reaction (PCR)–based single-tube assay as previously described [23]. This method consists of 2 consecutive steps. The first step uses PCR to amplify the region (nucleotides 1261–1600), and the second step uses melting curve analysis to genotype HBV. The detection limit of this assay is an HBV DNA level of around 200 IU/mL. For patients with viral loads lower than the detection limit, we used the Immunis HBV genotype enzyme immunoassay kit (Institute of Immunology, Tokyo, Japan), which detects genotype-specific epitopes in the preS2

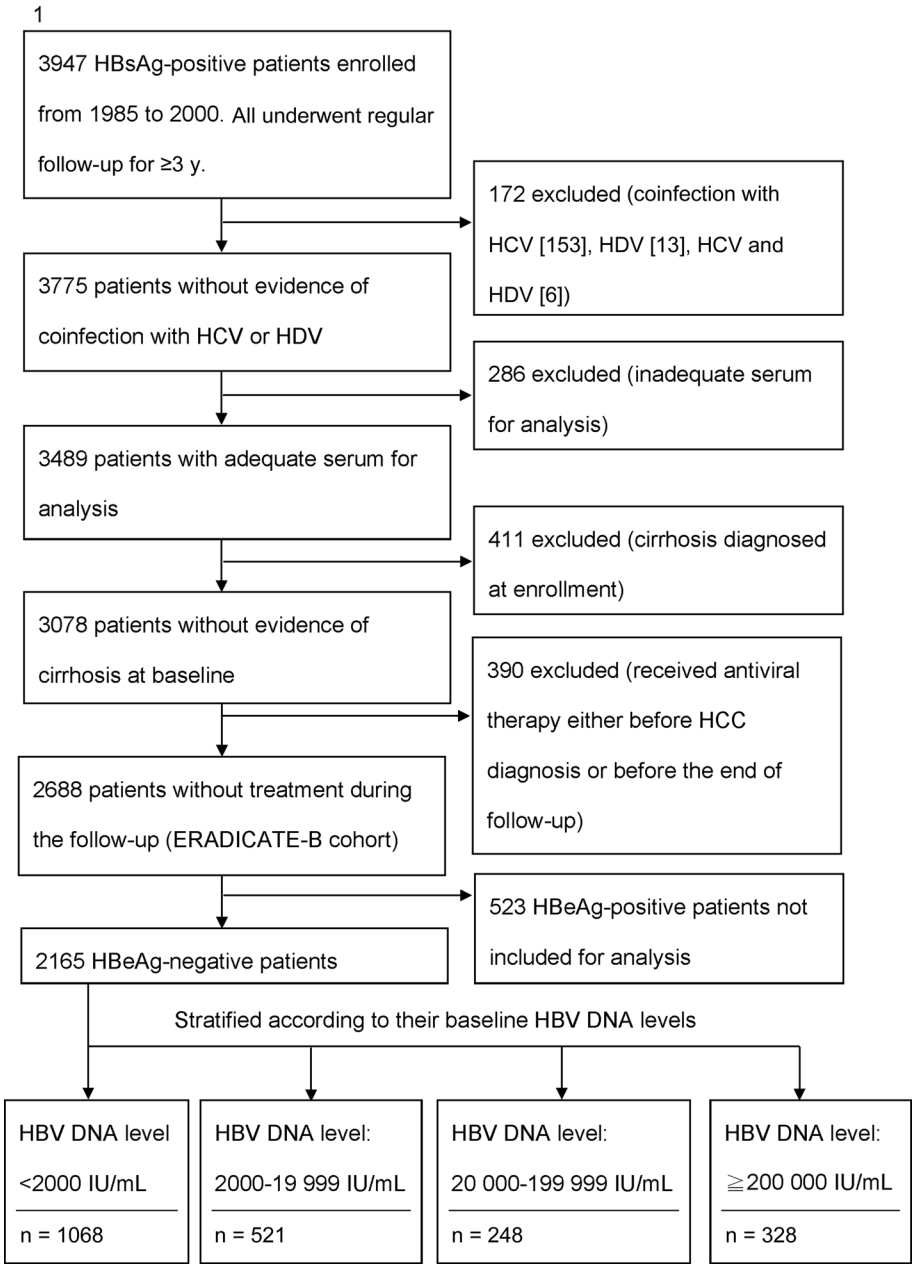

Figure 1. Flow of participants through the study. Abbreviations: HCC, hepatocellular carcinoma; HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus.

region [24]. The detection limit of this assay is an HBsAg level of around 100 IU/mL.

Statistical Analysis

Means and SDs were calculated for continuous variables, and percentages were used for categorical variables. The clinical follow-up started at the time of enrollment. The person-years of follow-up were censored when HCC was identified, at death, on 31 December 2011, or on the last date of follow-up. Since HBV DNA level is the most important viral factor for HCC development, we decided to categorize patients into 4 groups on the basis of HBV DNA levels: <2000 IU/mL, 2000–19 999 IU/mL, 20 000–199 999 IU/mL, and ≥200 0000 IU/mL [6]. Following our prior study, which focused on patients with an HBV DNA level <2000 IU/mL [9], HBsAg level was tested in the latter 3 groups to see whether it could serve a complementary marker for HCC prediction. In terms of categorizing HBsAg levels, a $\log_{10}$ scale was adopted [8, 9, 20, 25], and the cutoffs were chosen when each category had ≥10% of patients.

Looking at different variables, the cumulative incidence of HCC was derived using the Kaplan-Meier curve analysis, and the log-rank test was used to test for the statistical difference. Cox proportional hazards regression modeling was used to calculate the crude and multivariate-adjusted hazard ratios (HRs) of HCC. In every analysis, 2 models were adopted to adjust for the HR for HCC. According to the HCC risk score derived from the REACH-B study [26], sex, age, ALT level, HBeAg level, and HBV DNA level are important risk factors for HCC. Since this study enrolled only HBeAg-negative patients and HBV DNA level was a stratification variable, we adjusted for age, sex, and ALT level in the first model. In the second model, we further adjusted for HBV genotype [27, 28]. Our data were analyzed using 2 models because HBV genotype data were not available in every patient, especially if the viral load and HBsAg level were lower than the detection limits.

To compare different HCC predictors' predictive values, we adopted receiver operating characteristic (ROC) curve analysis and Harrell's C index [29]. In terms of ROC curve analysis, the study population was restricted to patients who were followed for at least 10 years. The area under the ROC curves (AUROC) in predicting the 10-year HCC risk was computed and compared between different predictors.

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

RESULTS

Baseline Characteristics and Follow-up Results

Table 1 shows the baseline characteristics of the 2165 HBeAg-negative patients categorized by HBV DNA levels. There were 1068 (49.3%) patients with HBV DNA level <2000 IU/mL, 521 (24.1%) with HBV DNA levels of 2000–19 999 IU/mL, and 328 (15.2%) with HBV DNA levels ≥200 000 IU/mL. All groups consisted of predominantly males and genotype B patients. The overall mean follow-up period (± SD) was 14.9 ± 4.3 years (median, 14.3 years; range, 3.9–26.7 years). A total of 128 patients developed HCC during the follow-up period with an incidence rate of 4.0 cases per 1000 person-years. Since the patients at enrollment did not have liver cirrhosis, none of the HBeAg-negative patients developed HCC within 3 years of follow-up. The median time to HCC development was 10.1 years (range, 3.9–22.8 years).

The Relationship Between HBsAg Level and HCC in Patients With an Intermediate Viral Load

To investigate the relationship between HBsAg level and HCC development in patients with HBV DNA levels of 2000–19 999 IU/mL, patients were divided into categories on the basis of HBsAg level. A total of 77 (14.8%) had an HBsAg level <100 IU/mL, 191 (36.7%) had HBsAg level of 100–999 IU/mL, and 253 (48.6%) had an HBsAg level ≥1000 IU/mL. Upon correlation with cumulative incidence of HCC, a trend was noted between HBsAg level and HCC development (P = .075; Figure 2*A*). After adjustment for sex, age, and ALT level and by use of an HBsAg level <100 IU/mL as a reference, the HRs were 6.2 (95% confidence interval [CI], .8–48.9; P = .082) for HBsAg levels of 100–999 IU/mL and 13.1 (95% CI, 1.7–100.9; P = .013) for HBsAg levels ≥1000 IU/mL (Figure 2*B*). The P value for trend was .002 (Figure 2*B*). In the second model, which adjusted for sex, age, ALT level, and HBV genotype, the result consistently showed that the adjusted HR for HCC development in patients with HBsAg levels ≥1000 versus <100 IU/mL was 8.9 (95% CI, 1.2–68.9; Table 2).

Table 1. Baseline Characteristics of 2165 Hepatitis B Virus (HBV) e Antigen–Negative Patients

Characteristic	Serum HBV DNA level, IU/mL			
	<2000 (n = 1068)	2000–19 999 (n = 521)	20 000–199 999 (n = 248)	≥200 000 (n = 328)
Sex				
Female	468 (43.8)	193 (37.0)	84 (33.9)	97 (29.6)
Male	600 (56.2)	328 (63.0)	164 (66.1)	231 (70.4)
Age at enrollment, y				
28–39	565 (52.9)	243 (46.6)	97 (39.1)	115 (35.1)
40–49	317 (29.7)	147 (28.2)	86 (34.7)	113 (34.5)
50–59	132 (12.4)	91 (17.5)	47 (19.0)	76 (23.2)
≥60	54 (5.1)	40 (7.7)	18 (7.3)	24 (7.3)
Serum ALT level, U/L				
<20	582 (54.5)	256 (49.1)	84 (33.9)	40 (12.2)
20–39	328 (30.7)	193 (37.0)	106 (42.7)	97 (29.6)
≥40	158 (14.8)	72 (13.8)	58 (23.4)	191 (58.2)
Serum HBsAg level, IU/mL				
<10	117 (11.0)	7 (1.3)	3 (1.2)	1 (0.3)
10–99	167 (15.6)	70 (13.4)	16 (6.5)	9 (2.7)
100–999	301 (28.2)	191 (36.7)	85 (34.3)	86 (26.2)
1000–9999	453 (42.4)	233 (44.7)	140 (56.5)	196 (59.8)
≥10 000	30 (2.8)	20 (3.8)	4 (1.6)	36 (11.0)
HBV genotype[a]				
B	767 (71.8)	453 (87.0)	219 (88.3)	270 (82.3)
C	178 (16.7)	68 (13.1)	29 (11.7)	58 (17.7)
Undetermined[a]	123 (11.5)	. . .	. . .	. . .

Data are no. (%) of patients.

Abbreviations: ALT, alanine aminotransferase; HCC, hepatocellular carcinoma.

[a] In 630 patients, HBV DNA levels were 200–1999 IU/mL; 10 (1.6%) had no genotype data. In 438 patients, HBV DNA levels were <200 IU/mL; 113 (25.8%) had no genotype data.

The Relationships Between HBsAg Level and HCC in Patients With a High Viral Load

We then investigated the role of HBsAg levels in patients with HBV DNA levels of 20 000–199 999 IU/mL and ≥200 000 IU/mL. The HBsAg cutoffs were chosen when corresponding

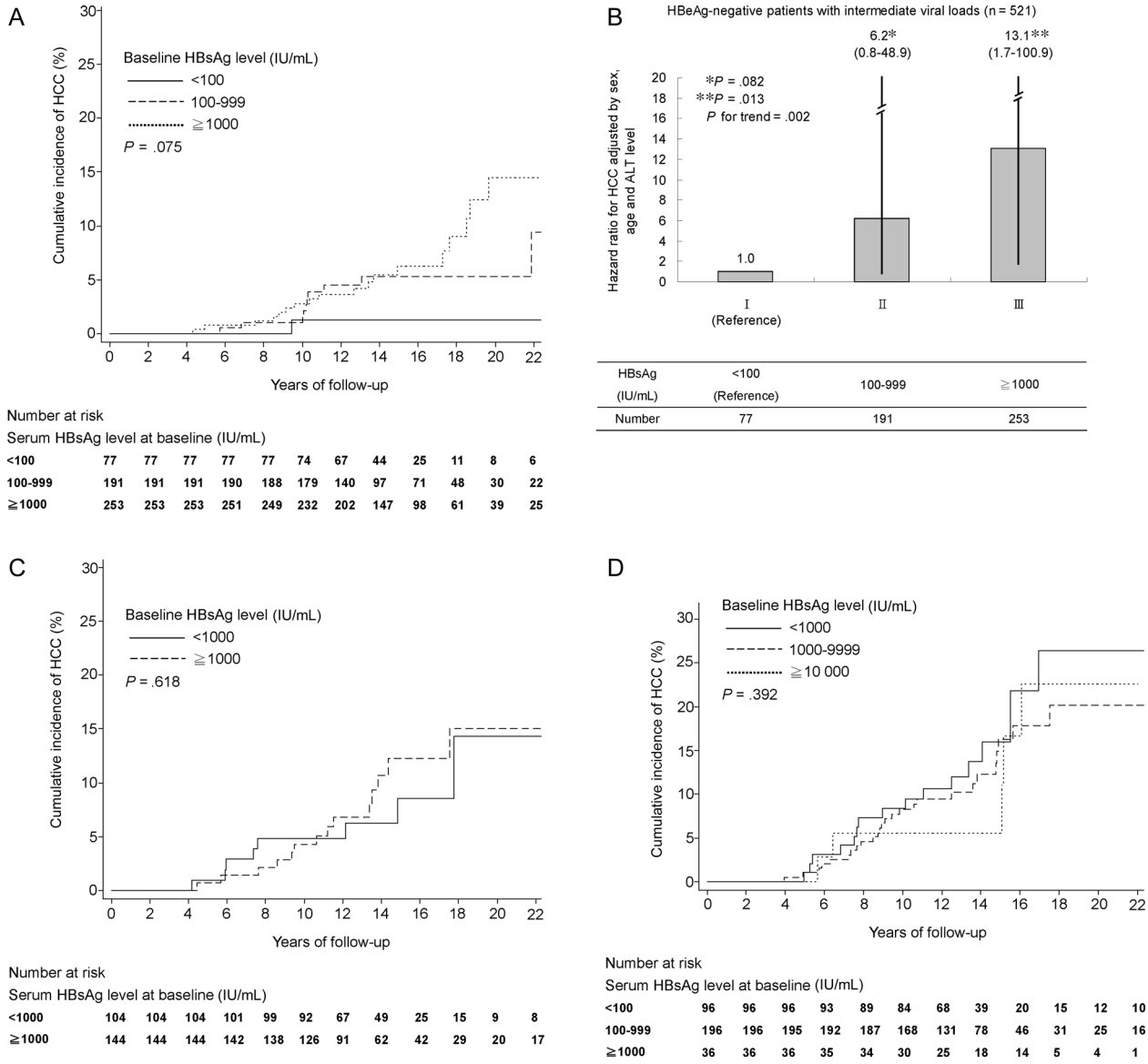

Figure 2. In 521 hepatitis B virus (HBV) e antigen (HBeAg)–negative patients with intermediate viral loads, the cumulative incidence of hepatocellular carcinoma (HCC) was positively associated with HBsAg levels (*A*), and the hazard ratio (HR) increased with their HBV surface antigen (HBsAg) levels (*B*; data are adjusted by sex, age, and alanine aminotransferase [ALT] level). In contrast, there was no correlation between HBsAg levels and HCC development in patients with HBV DNA levels of 20 000–199 999 IU/mL (*C*) and ≥200 000 IU/mL (*D*).

categories had ≥10% of patients in each group. No correlation was found between HBsAg and HCC development in either cohort ($P = .618$ and .392, respectively; Figure 2*C* and 2*D*). Similarly, neither univariate analysis nor either of the 2 multivariate analysis models showed any correlation (data not shown).

HBsAg Level as a New Biomarker for Predicting HCC Development in HBeAg-Negative Patients

In patients with low and intermediate viral loads, HBsAg level has been shown to be an important biomarker for stratifying HCC risk. We thus decided to investigate how to combine HBV DNA and HBsAg to better predict HCC development. Our data first illustrated that increasing HBV DNA levels were associated with the cumulative incidence of HCC, which was consistent with prior results (Figure 3*A*). In short, our data noted that a higher HBV DNA level was associated with increased HCC risk, and viral load could be divided into 4 categories with different risk levels. In addition, our findings showed that, after adjustment for sex, age, and ALT level, patients with intermediate viral loads only had a marginal increase in HCC risk (adjusted HR, 1.7; 95% CI, 1.0–2.9; $P = .038$), unlike patients with higher viral loads (Figure 3*B*).

Table 2. Univariate and Multivariate Analysis of Factors Associated With Hepatocellular Carcinoma in Patients With Hepatitis B Virus (HBV) DNA Levels of 2000–19 999 IU/mL

Characteristic	Follow-up Duration, PY	Cases, No.	Annual Incidence Rate[a]	Crude HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Sex							
Female	2886.5	5	173.2	1.0		1.0	
Male	5080.5	24	472.4	2.7 (1.0–7.0)	.047	3.3 (1.2–9.0)	.022
Age at enrollment, y							
28–39	3772.0	5	132.6	1.0		1.0	
40–49	2237.8	5	223.4	1.8 (.5–6.2)	.354	2.1 (.6–7.3)	.246
50–59	1427.9	10	700.3	5.3 (1.8–15.6)	.002	7.2 (2.4–21.2)	<.001
≥60	529.3	9	1700.3	15.7 (5.2–47.5)	<.001	18.5 (5.9–57.8)	<.001
Serum ALT level, U/L							
<20	4269.0	10	234.3	1.0		1.0	
20–39	2741.2	9	328.3	1.7 (.7–4.3)	.256	1.3 (.5–3.2)	.626
≥40	956.7	10	1045.2	5.9 (2.4–14.9)	<.001	3.6 (1.4–9.8)	.010
Serum HBsAg level, IU/mL							
<100	1170.4	1	85.4	1.0		1.0	
100–999	2916.4	10	342.9	3.8 (.5–29.9)	.201	5.6 (.7–44.1)	.103
≥1000	3880.1	18	463.9	5.2 (.7–39.2)	.108	8.9 (1.2–68.9)	.036
HBV genotype							
B	6907.6	24	347.5	1.0		1.0	
C	1059.4	5	472.0	1.4 (.5–3.6)	.532	1.3 (.5–3.5)	.627

Data were calculated using Cox proportional hazards regression modeling.

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; HBsAg, HBV surface antigen; HR, hazard ratio; PY, person-years.

[a] Per 100 000 PY.

To analyze HCC risk by use of HBsAg level, we categorized the patients with low viral loads into 2 groups, using an HBsAg level of 1000 IU/mL as a cutoff, as recommended by a previous study [9]. In addition, we divided the cohort with intermediate viral loads, using HBsAg levels of 100 and 1000 IU/mL as cutoffs (Figure 3*C*). Our analysis found a complex association pattern; thus, groups with similar cumulative incidences of HCC were merged to simplify the prediction system. The final 4 new categories based on HBV DNA and HBsAg levels were as follows: minimal risk, patients with low viral loads plus HBsAg levels <1000 IU/mL and those with intermediate viral loads plus HBsAg levels <100 IU/mL; medium risk, patients with low viral loads plus HBsAg levels ≥1000 IU/mL and those with intermediate viral loads plus HBsAg levels of 100–999 IU/mL; medium high risk, patients with intermediate viral loads plus HBsAg levels ≥1000 IU/mL and those with HBV DNA levels of 20 000–199 999 IU/mL; and high risk, patients with HBV DNA levels ≥200 000 IU/mL.

We analyzed the relationships between these categories and cumulative incidence of HCC (Figure 3*D*). We found a significant difference in risk between the minimal risk and the medium risk groups (age-, sex-, and ALT-adjusted HR, 7.1; 95% CI, 3.0–17.1; $P < .001$), which was more pronounced than the risk difference between low and intermediate viral load groups (Figure 3*B* and 3*E*). In addition, the annual incidence rate of HCC for the minimal risk group (0.6 cases per 1000 person-years) was lower than that of the low viral load group (1.8 cases per 1000 person-years). We further analyzed whether the new categories stratified by HBV DNA and HBsAg levels remained as an independent risk factor after adjustment for age, sex, ALT level, and HBV genotype (Table 3). The multivariate analysis consistently showed this new categorizing system was independently associated with HCC development.

Comparison Between Combining HBV DNA and HBsAg Levels and HBV DNA Level Alone in Predicting HCC Development

To investigate whether combining HBsAg and HBV DNA levels as opposed to HBV DNA level alone was superior in predicting HCC development, we compared the combined predictor with the single predictor, using ROC curve analysis and Harrell C index in 2 clinical setting: HBeAg-negative patients with HBV DNA levels <20 000 IU/mL and overall HBeAg-negative patients (Table 4). This design was adopted because HBsAg level only served as an effective biomarker in patients with low or intermediate viral loads.

ROC curve analysis was used to compare the 10-year predictive values for HCC development. This time frame was chosen because approximately 50% of HCC cases developed within 10 years in this cohort (median time to HCC development, 10.1 years). ROC curve analysis found combining HBV DNA

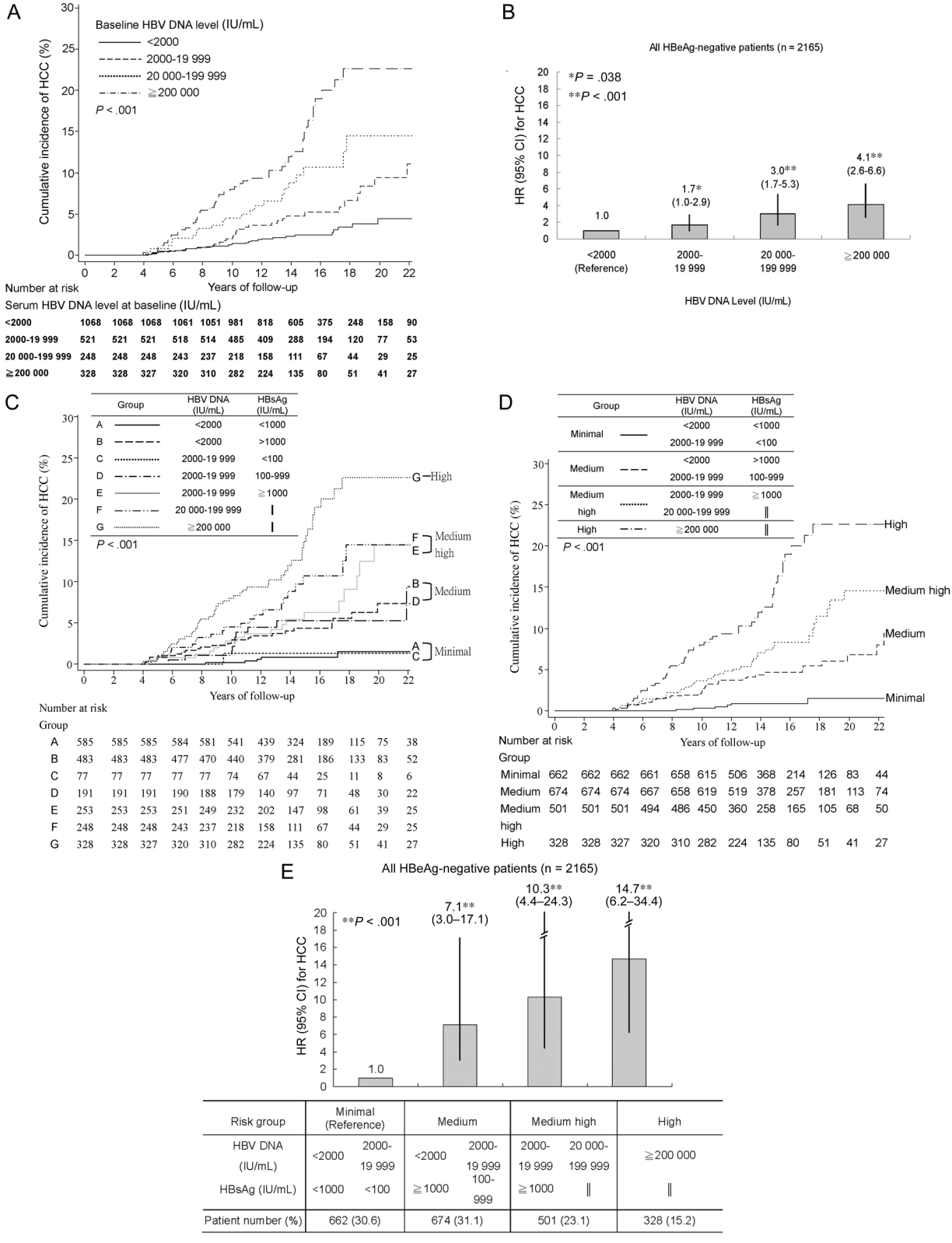

Figure 3. In 2165 hepatitis B virus (HBV) e antigen (HBeAg)–negative patients, the cumulative incidence of hepatocellular carcinoma (HCC) was positively associated with HBV DNA levels (*A*), and the hazard ratio (HR) increased with their HBV DNA levels (*B*). When combining HBV DNA and HBV surface antigen (HBsAg) levels as a variable, the patients could be divided into 4 risk levels (*C*; data are adjusted by sex, age, and alanine aminotransferase [ALT] level). Both cumulative incidence of HCC (*D*) and the HR (*E*; data are adjusted by sex, age, and ALT level) increased along with their risk levels.

Table 3. Univariate and Multivariate Analysis of Factors Associated With Hepatocellular Carcinoma in all Hepatitis B Virus (HBV) e Antigen–Negative Patients, by Combining HBV DNA and HBV Surface Antigen Levels

Characteristic	Follow-up Duration, PY	Cases, No.	Annual Incidence Rate[a]	Crude HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Sex							
Female	12563.3	22	175.1	1.0		1.0	
Male	19756.1	106	536.5	3.1 (1.9–4.9)	<.001	2.5 (1.6–4.0)	<.001
Age at enrollment, y							
28–39	15711.1	29	184.6	1.0		1.0	
40–49	9904.0	37	373.6	2.1 (1.3–3.4)	.003	2.4 (1.4–3.9)	.001
50–59	4955.3	36	726.5	4.2 (2.6–6.8)	<.001	4.3 (2.6–7.2)	<.001
≥60	1749.1	26	1486.5	9.2 (5.4–15.8)	<.001	10.0 (5.8–17.3)	<.001
Serum ALT level, U/L							
<20	15520.2	24	154.6	1.0		1.0	
20–39	10300.0	40	388.4	2.8 (1.7–4.7)	<.001	1.7 (1.0–2.8)	.060
≥40	6499.2	64	984.7	7.4 (4.6–11.9)	<.001	3.9 (2.3–6.7)	<.001
Risk group							
Minimal	9900.8	6	60.6	1.0		1.0	
Medium	10395.2	34	327.1	5.3 (2.2–12.6)	<.001	6.6 (2.7–16.6)	<.001
Medium high	7411.5	40	539.7	8.9 (3.8–21.1)	<.001	8.9 (3.6–22.0)	<.001
High	4612.0	48	1040.8	17.8 (7.6–41.6)	<.001	9.3 (3.7–23.1)	<.001
HBV genotype							
B	25492.2	95	372.7	1.0		1.0	
C	5000.1	32	640.0	1.7 (1.1–2.6)	.009	1.9 (1.3–2.9)	.002
Undetermined	1827.1	1	54.7	0.1 (.02–1.1)	.057	0.9 (.1–6.9)	.885

Data were calculated using Cox proportional hazards regression modeling.

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; HR, hazard ratio; PY, person-years.

[a] Per 100 000 PY.

and HBsAg levels was superior to using HBV DNA alone in predicting the 10-year HCC risk in the subcohort with HBV DNA levels <20 000 IU/mL (AUROC, 0.68 [95% CI, .60–.76] vs 0.54 [95% CI, .44–.64]; $P = .004$) and in the overall HBeAg-negative cohort (AUROC, 0.74 [95% CI, .68–.79] vs 0.70 [95% CI, .63–.77]; $P = .028$).

We also analyzed this issue using Harrell's C index. Again, combining the HBV DNA level and HBsAg level served as a better predictor than the HBV DNA level alone in predicting HCC either in the subcohort ($P = .016$) or the overall cohort ($P = .004$; Table 4).

DISCUSSION

HCC is a life-threatening complication for patients with chronic HBV infection. Prior studies have demonstrated that serum HBV DNA level is a major marker of disease progression in HBV carriers [5, 6, 10]. Our recent study further indicated that serum HBsAg level can complement the HBV DNA level for predicting HCC risk in HBeAg-negative patients with low viral loads but not in those with HBV DNA levels ≥2000 IU/mL [9]. In this study, which categorized HBsAg level into categories for analysis, we found that HBsAg level could serve as an independent predictor for HCC in patients with intermediate viral loads (2000–19 999 IU/mL) but not in those with higher viral loads (20 000–199 999 and ≥200 000 IU/mL). Our new predictor model combining HBV DNA level and HBsAg level could improve the categorization of HBeAg-negative patients into 4 different HCC risk levels. This measure could translate to a better prediction of HCC risk than using viral load alone.

A recent study indicated that the HBV DNA level fluctuates more readily than HBsAg levels [7]. Therefore, low viral load at a single time point does not guarantee limited viral replication persistently. Also, HBsAg level has been shown to be a better surrogate marker of intrahepatic covalently closed circular DNA (cccDNA) level [30], which is the replication template of HBV. Taking these lines of evidence, lower HBsAg levels in patients with low or intermediate viral loads may indicate even lower cccDNA levels and, thus, confer further decreased viral replication and even lower HCC risk.

Our study addressed the following issues. First, the optimal HBV DNA threshold level has been long debated upon. Multiple studies have demonstrated that, unlike HBV carriers with high viral loads, patients with intermediate viral loads only have a small increase in HCC risk [6, 11, 31]. In addition, another longitudinal study has suggested that patients with

Table 4. Comparison of Hepatocellular Carcinoma (HCC) Prediction Between the Single and Combined Predictors

Enrolled patients	Method	HBV DNA Level Alone	HBV DNA and HBsAg Levels	*P*
HBeAg-negative patients with HBV DNA level <20 000 IU/mL	AUROC curve (95% CI)[a]	.543 (.441–.644)	.680 (.600–.760)	.004
	Harrell's C-index	.581	.664	.016
All HBeAg-negative patients	AUROC curve (95% CI)[b]	.699 (.631– .768)	.735 (.681– .790)	.028
	Harrell's C-index	.689	.712	.004

Data were calculated using receiver operating characteristic curve analysis and Harrell's C index.

Abbreviations: AUROC curve, area under receiver operating characteristic curve; CI, confidence interval; HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus.

[a] Ten-year HCC development (n = 1490; HCC = 24).

[b] Zero-year HCC development (n = 2027; HCC = 61).

intermediate viral loads may have persistently normal ALT levels and are less likely to progress to cirrhosis or HCC [12]. Our study lent support to previous reports and clearly showed that HBsAg level could further stratify patients with intermediate viral loads into 3 different HCC risk levels: minimal risk, medium risk, and medium high risk. We believe that redefining risk levels by using a combination of HBsAg level and HBV DNA level may be the answer to the long debate.

Second, HCC surveillance program includes repeated diagnostic imaging with or without an AFP test in patients at risk for HCC. However, there are still debates about which level of HCC risk is cost-effective for initiating HCC surveillance [21, 32]. According to the American Association for the Study of Liver Diseases guideline, it is recommended to initiate HCC surveillance program in noncirrhotic HBV carriers with an HCC incidence rate >0.2% per year [21]. When we looked back at our data, the annual incidence rates were 0.06%, 0.3%, 0.5%, and 1.0%, in minimal, medium, medium high, and high risk groups, respectively (Table 3). Consideration should thus be given to implementation of HCC surveillance program only for noncirrhotic HBV carriers with a medium risk or higher if our findings can be validated. In other words, about 30.6% of HBeAg-negative patients (Figure 3*E*) might not need HCC surveillance, and the medical expenditure would be spared. For cirrhotic HBV carriers, we could not answer whether the criteria for minimal risk holds true, since our cohort excluded patients with liver cirrhosis at study entry.

Finally, the optimal HBV DNA level for defining the treatment end point, especially for patients receiving pegylated interferon, is yet to be decided [33, 34]. This is because the risk difference between HBV DNA levels of 2000 and 20 000 IU/mL is very limited. On the basis of our results, the minimal risk criteria could identify very-low-risk patients. Therefore, we proposed that treatment end point criteria should consider incorporating minimal risk criteria. Nonetheless, further studies are required to prove or disprove this speculation.

Our study had some limitations. First, in patients with intermediate viral loads, the HCC risk difference between HBsAg levels <100 IU/mL and 100–999 IU/mL was not found to be statistically significant. The lack of statistical power may be due to the small number of patients with HBsAg levels <100 IU/mL (n = 77). Second, PCR-based genotyping assays could only genotype HBV in patients with HBV DNA levels ≥200 IU/mL (around 680 copies/mL); therefore, we used an enzyme-linked immunosorbent assay (ELISA) to genotype samples with viral loads lower than the detection limit of the PCR-based assay. Nevertheless, we still had 123 samples (5.7% of the overall HBeAg-negative cohort) without genotype data, and all had HBV DNA levels <2000 IU/mL. Third, the clinical usefulness of a new biomarker needs to be validated in an independent cohort study, which is unavailable in our study. However, another Taiwanese community-based cohort study, the REVEAL-HBV study, has reported similar findings [35]. It has been shown that HBsAg levels could only stratify HCC risks in patients with low and intermediate viral loads. The HBsAg levels of 100 IU/mL and 1000 IU/mL were also recommended cutoffs to categorize HCC risks in patients with intermediate viral loads [35]. If the role of HBsAg level in predicting HBV prognosis could be further confirmed, this new biomarker should be incorporated into the risk calculator for HCC development [31].

In summary, HBsAg levels can stratify the HCC risk in HBeAg-negative patients with low or intermediate viral loads but not in those with higher viral loads. Combining HBV DNA and HBsAg levels can better categorize HBeAg-negative patients as having a minimal risk, medium risk, medium high risk, or high risk for HCC. This combined predictor offers a better risk prediction of HCC than use of viral load alone.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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