MAJOR ARTICLE

# Viral Determinants of Hepatitis B Surface Antigen Seroclearance in Hepatitis B e Antigen–Negative Chronic Hepatitis B Patients

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***Background.*** We studied whether quantification of serum HBsAg and HBV DNA levels could predict spontaneous HBsAg clearance in patients with negative hepatitis B e antigen (HBeAg).

***Methods.*** Serum HBsAg and HBV DNA levels were measured at baseline among a longitudinal cohort of 103 HBeAg-negative patients recruited since 1997.

***Results.*** Twelve (12%) patients developed HBsAg seroclearance after 88 ± 26 months (range, 21–139) of follow-up. At baseline, the serum HBsAg level among patients who cleared HBsAg (1.30 ± 1.27 log IU/mL) was significantly lower than those who did not clear HBsAg (2.96 ± 0.84 log IU/mL; $P < .001$). The area under receiver operating characteristics (ROC) curve for serum HBsAg to predict HBsAg seroclearance was 0.90 (95% confidence interval [CI], 0.83–0.97; $P < .001$). Nine (75%) of 12 patients who had HBsAg seroclearance versus 8 (9%) of 91 who remained HBsAg-positive had serum HBsAg ≤100 IU/mL at the baseline ($P < .001$). An HBsAg cutoff of ≤100 IU/mL had 75% sensitivity and 91% specificity to predict HBsAg seroclearance. Baseline serum HBV DNA could not predict HBsAg seroclearance; the area under ROC curve was 0.64 (95% CI, 0.46–0.81; $P = .13$).

***Conclusions.*** Single-point serum HBsAg level can predict the chance of HBsAg seroclearance in chronic hepatitis B patients with negative HBeAg.

Chronic hepatitis B virus (HBV) infection is the major cause of liver cirrhosis and hepatocellular carcinoma (HCC) in Asia [1]. Asian patients usually acquire HBV infection perinatally. Most patients have 2–3 decades of immune tolerance characterized by positive hepatitis B e antigen (HBeAg), very high HBV DNA, and normal alanine aminotransferase (ALT) [2]. Immune clearance will lead to HBeAg seroconversion with disappearance of HBeAg and appearance of antibodies to HBeAg (anti-HBe). However, ~20%–30% of patients with negative HBeAg still have active hepatitis [3–5]. Even among HBeAg-negative patients with normal ALT levels, those who have higher HBV DNA levels have a higher risk of developing liver cirrhosis and HCC in the subsequent 8–10 years [6–9].

Much effort has been spent to identify risk factors for cirrhotic complications in HBeAg-negative patients. In the regional guidelines, HBV DNA >2000 IU/mL with elevated ALT level is usually taken as the indication for antiviral therapy, while patients with lower HBV DNA and normal ALT are advised for observation [10–12]. As HBeAg-negative chronic hepatitis B is characterized by fluctuating HBV DNA and ALT levels, numerous studies have failed to identify a single HBV DNA cutoff that can accurately predict disease activity and progression [13–15]. A recent report including 1932 inactive HBV carriers who had HBV DNA levels <2,000 IU/mL followed up for 13 years showed an increased risk of HCC and liver-related death compared with the non–HBV-infected controls [16].

Hepatitis B surface antigen (HBsAg) seroclearance has been regarded as the ultimate goal of immune clearance. Spontaneous HBsAg seroclearance occurs at

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an annual incidence of ~1%–2% among chronic hepatitis B patients [17, 18]. Long-term follow-up studies showed excellent intrahepatic viral clearance and low viral replicative activity among patients who have cleared HBsAg [19]. The risk of developing HCC was also dramatically reduced if HBsAg seroclearance occurred before the age of 50 years and the development of liver cirrhosis [19, 20].

In the Taiwanese REVEAL cohort including 3087 HBV-infected patients, older age, cigarette smoking, lower HBV DNA, higher body mass index, seronegative for HBeAg, and being mainland Chinese were independent factors predicting HBsAg clearance after an average follow-up of 8 years [17]. Recently, serum HBsAg levels were shown to correlate with intrahepatic covalently closed circular DNA (ccc DNA) levels [21, 22] and decline with immune clearance [23]. As HBsAg seroclearance is an indicator of immune clearance of intrahepatic HBV, quantification of serum HBsAg might help to predict viral clearance in HBeAg-negative patients. We therefore studied the role played by serum HBV DNA and HBsAg levels in prediction of spontaneous HBsAg seroclearance in a longitudinal cohort of untreated chronic hepatitis B patients with negative HBeAg.

## METHODS

### Patients

A cohort of chronic hepatitis B patients recruited since 1997 with longitudinal follow-up in the outpatient clinic, Prince of Wales Hospital, were studied [24]. These patients were followed at an interval of 6 months, or more frequently as clinically indicated. HBeAg and anti-HBe, liver biochemistry, and α-fetoprotein were monitored at every visit. Serum HBsAg was monitored yearly for HBsAg seroclearance. Ultrasound was performed regularly for surveillance of HCC, which was confirmed by standard imaging and histology. The patient selection criteria for this study included (1) negative HBeAg and positive anti-HBe at the initial visit; (2) HBV DNA monitoring more frequent than once every 2 years and at least 3 HBV DNA testing available; (3) follow-up duration of longer than 1 year; (4) no antiviral therapy during the entire follow-up period; and (5) coinfection by hepatitis C virus excluded. Residual serum samples at the initial visits were stored at −80 °C freezer for HBsAg quantification.

Patients were classified into 3 groups for analysis of disease activity. Active disease was defined as HBV DNA intermittently or persistently >20,000 IU/mL with or without elevation of ALT levels. Inactive disease was defined as HBV DNA ≤2000 IU/mL and ALT normal throughout the entire follow-up period. Mildly active disease was defined as HBV DNA fluctuating between 2000 and 20,000 IU/mL and/or with elevated ALT levels. A few patients who had active disease at the initial visits but the disease became inactive on subsequent follow-up were also defined as having mildly active disease. ALT flare was defined as an abrupt elevation of ALT levels above 200 IU/mL or a >3-fold increase from the baseline level, whichever was higher [3].

### Laboratory Assays

***Quantitative HBsAg Assay.*** HBsAg was quantified by Architect HBsAg QT (Abbott Diagnostic) according to the manufacturer's instruction. The sensitivity of the Architect assay ranged from 0.05 to 250 IU/mL. Samples with HBsAg titer higher than 250 IU/mL were diluted to 1:500 to 1:1000 to bring the reading within the range of the calibration curve.

***HBV DNA Assay.*** HBV DNA was quantified by TaqMan real-time polymerase chain reaction assay as described elsewhere [25]. The range of HBV DNA detection was from $10^2$ to $10^9$ copies/mL. In this assay, 4.86 copies/mL equaled 1 IU/mL.

***HBV Genotyping.*** HBV genotyping was determined by restriction fragment length polymorphism and confirmed by direct sequencing in case of doubt in the residual serum sample at the baseline visit as described elsewhere [26]. Basal core promoter mutation was determined by direct sequencing [27].

### Statistical Analysis

Statistical analysis was performed by SPSS software (version 15.0; SPSS). Continuous variables were expressed as mean ± standard deviation or median (range) as appropriate. HBV DNA (IU/mL) and HBsAg (IU/mL) were logarithmically transformed for analysis. For patients with undetectable HBV DNA and negative HBsAg levels, the results were taken as the lower limit of detection (20.6 IU/mL for HBV DNA and 0.05 IU/mL for HBsAg) for calculation. Ratio of HBsAg to HBV DNA was determined to reflect the proportion of subviral particles to virions, which was an indirect measurement on the association of HBsAg production and HBV replication [23, 28, 29]. To investigate for factors associated with HBsAg seroclearance and HCC development, continuous variables including HBV DNA and HBsAg were compared by Mann–Whitney U test and categorical variables by Fisher exact test due to the relatively low event rates. Area under receiver operating characteristics (ROC) curve was used to analyze the prediction of HBsAg and HBV DNA levels for HBsAg seroclearance, and the best cutoff values were determined on the coordinates of the ROC curve. Cox proportional hazard ratio was used to analyze association of HBsAg, HBV DNA, and ALT flares for HBsAg clearance. Kaplan-Meier survival analysis was used to determine the cumulative probability of spontaneous HBsAg seroclearance. Data were censored at the time of HBsAg seroclearance or the last follow-up visit. All statistical tests were 2-sided. Statistical significance was taken as $P < .05$.

## RESULTS

### Patient Characteristics at Initial Visit

One hundred three HBeAg-negative patients in the longitudinal cohort fulfilled the criteria for this study. The clinical

**Table 1. Baseline Clinical Characteristics of Patients According to HBsAg Seroclearance**

| | Overall | HBsAg seroclearance | No HBsAg seroclearance | *P* |
|---|---|---|---|---|
| *N* | 103 | 12 | 91 | |
| Age, y | 46 ± 12 | 44 ± 10 | 46 ± 12 | .67 |
| Male gender | 70 (68%) | 11 (92%) | 59 (65%) | .097 |
| Platelet, $\times 10^9$/L | 176 ± 57 | 196 ± 44 | 173 ± 58 | .14 |
| Albumin, g/L | 39 ± 4 | 40 ± 3 | 39 ± 4 | .70 |
| Total bilirubin, μmol/L | 11 ± 11 | 8 ± 2 | 11 ± 11 | .31 |
| Alanine aminotransferase, IU/L | 63 ± 54 | 58 ± 48 | 64 ± 54 | .71 |
| HBV DNA, log IU/mL | 4.17 ± 1.67 | 3.58 ± 1.74 | 4.25 ± 1.65 | .13 |
| HBsAg, log IU/mL | 2.77 ± 1.04 | 1.30 ± 1.27 | 2.96 ± 0.84 | .001 |
| HBsAg/HBV DNA | 0.76 ± 0.50 | 0.33 ± 0.55 | 0.81 ± 0.47 | .002 |
| HBV genotype C[a] | 54 (53%) | 4 (33%) | 50 (56%) | .22 |
| BCP mutation[b] | 54 (61%) | 4 (57%) | 50 (62%) | 1.00 |

**NOTE.** HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; BCP basal core promoter.

[a] One patient with mixed genotype B and C HBV was regarded as genotype C HBV infection on analysis; one patient with no HBsAg seroclearance had negative polymerase chain reaction result on HBV genotyping.

[b] Five patients with HBsAg seroclearance and 10 patients without HBsAg seroclearance had negative polymerase chain reaction on BCP mutation detection.

characteristics of these patients are presented in Table 1. At baseline, 15 (15%) patients had ultrasound features of liver cirrhosis; 63 (61%) patients had HBV DNA levels >2000 IU/mL; and 37 (36%) patients had elevated ALT level (>58 IU/L, the reference range of the laboratory). Twenty-two patients had ALT flare during follow-up (9 patients had >1 episode of ALT flare). The majority of patients were infected with genotype B (47 patients) and C (53 patients) HBV. One patient was infected with genotype A HBV, one with a mixed genotype B and C HBV, and one had failed HBV genotyping due to undetectable HBV DNA.

### HBsAg Seroclearance on Follow-up

There were a total of 748 person-years of follow-up with an average follow-up period of 88 ± 26 months (median, 95; range, 21–139). Twelve (12%) patients underwent spontaneous HBsAg seroclearance, resulting in a calculated annual HBsAg seroclearance rate of 1.6%. At the time of HBsAg seroclearance, 11 patients had undetectable HBV DNA, and one patient had HBV DNA level of 83 IU/mL. These patients cleared HBsAg at 86 ± 29 months (range, 26–115) after the initial visit, and none of them had reappearance of HBsAg at the subsequent follow-up. Patients who cleared HBsAg had significantly lower baseline serum HBsAg level and lower HBsAg/HBV DNA ratio than those who did not clear HBsAg (Table 1). Otherwise, HBsAg seroclearance had no association with age, gender, serum biochemistry, HBV DNA level, the distribution of HBV genotypes, and the presence of basal core promoter mutation.

Among the 12 patients who had spontaneous HBsAg seroclearance, 7 (57%) had inactive disease with persistently low HBV DNA and normal ALT levels. Four other patients had elevated HBV DNA (3.55–5.45 log IU/mL) and elevated ALT (65–112 IU/l) at the initial visits, but their disease became inactive and HBV DNA became undetectable during the subsequent follow-up before HBsAg seroclearance developed. The remaining patient had active disease with high HBV DNA (7.55 log IU/mL) at baseline and repeated ALT flares (peak ALT 211 IU/L) before the eventual HBsAg seroclearance. Overall, patients who had HBsAg seroclearance tend to have less active disease as compared with those who remained HBsAg-positive (Table 2). There was no difference in the number of HBV DNA testing, average time interval of HBV DNA testing, and the duration of follow-up between patients with and without HBsAg seroclearance.

### Serum Predictors of HBsAg Seroclearance

The serum HBsAg levels were lower among patients who cleared HBsAg on follow-up than those who remained HBsAg-positive (Table 1; Figure 1). The hazard ratio of serum HBsAg for HBsAg

**Table 2. Association of Disease Activity During Follow-up and HBsAg Seroclearance**

| | HBsAg seroclearance | No HBsAg seroclearance | *P* |
|---|---|---|---|
| *N* | 12 | 91 | |
| Disease activity | | | <.001 |
| Active | 1 (8%) | 60 (66%) | |
| Mildly active | 4 (33%) | 17 (19%) | |
| Inactive | 7 (58%) | 14 (15%) | |
| No. of HBV DNA testing | 8 (5–11) | 7 (3–13) | .95 |
| Average interval between HBV DNA testing, mo | 14 ± 5 | 13 ± 5 | .081 |
| Follow-up duration, mo | 94 ± 12 | 87 ± 27 | .99 |
| HCC development | 0 (0%) | 7 (8%) | 1.00 |

**NOTE.** HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma.

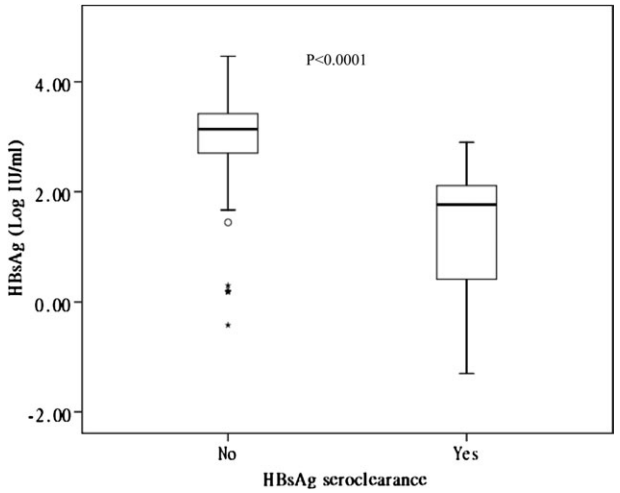


**Figure 1.** Box plot of baseline serum HBsAg levels among patients who did and did not achieve HBsAg seroclearance. The median (interquartile range) HBsAg level among patients who did and did not undergo HBsAg seroclearance was 1.76 (0.39–2.22) log IU/mL and 3.14 (2.68–3.43) log IU/mL, respectively.

seroclearance was 0.40 (95% confidence interval [CI], 0.26–0.61; $P < .001$). On ROC analysis, the best HBsAg cutoff value, which has the maximum sum of sensitivity and specificity to predict HBsAg seroclearance, was 100 IU/mL (Figure 2, Table 3). Nine of 12 (75%) patients who had HBsAg seroclearance vs 8 (9%) of 91 patients who remained HBsAg-positive had baseline serum

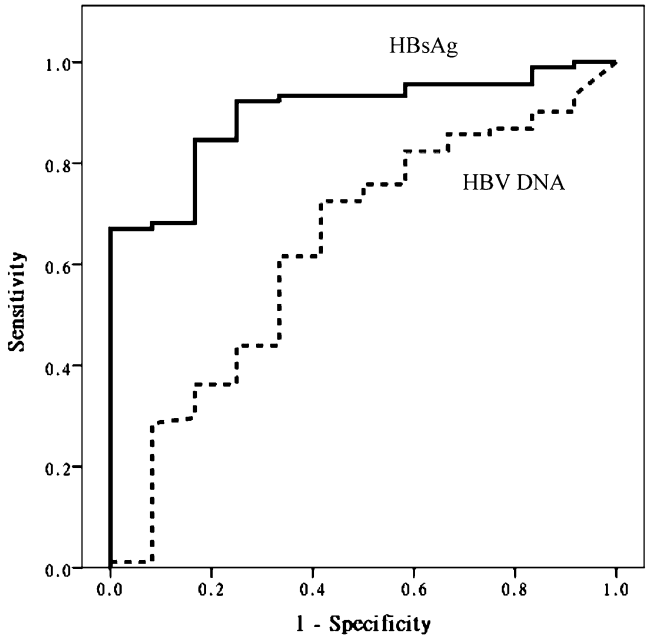


**Figure 2.** Receiver operating characteristic (ROC) curves of baseline serum HBsAg and HBV DNA for HBsAg seroclearance. The area under ROC curve for serum HBsAg to predict HBsAg seroclearance was 0.90 (95% CI, 0.83–0.97; $P < .001$) and that for serum HBV DNA was 0.64 (95% CI, 0.46–0.81; $P = .13$).

**Table 3. Prediction of HBsAg Seroclearance by Different Cutoff Values of Baseline HBsAg and HBV DNA**

| | HBsAg (IU/mL) | | HBV DNA (IU/mL) | |
|---|---|---|---|---|
| Cutoff | ≤100 | ≤1000 | ≤200 | ≤2000 |
| Sensitivity | 0.75 | 1.00 | 0.25 | 0.58 |
| Specificity | 0.91 | 0.63 | 0.87 | 0.64 |
| PPV | 0.53 | 0.26 | 0.20 | 0.18 |
| NPV | 0.97 | 1.00 | 0.90 | 0.92 |
| Positive LR | 8.53 | 2.68 | 1.90 | 1.61 |
| Negative LR | 0.27 | 0.00 | 0.86 | 0.65 |
| *P* value | <.001 | <.001 | .38 | .14 |

**NOTE.** HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio.

HBsAg ≤100 IU/mL ($P < .001$). Among these 9 patients, 8 of them had HBsAg ≤20 IU/mL. The cumulative probability of HBsAg seroclearance at 5 and 8 years among patients with baseline serum HBsAg ≤100 IU/mL were 12% and 38% while those with HBsAg >100 IU/mL were 0% and 1.5%, respectively (Figure 3*A*). If baseline HBsAg was ≤20 IU/mL, the 5- and 8-year cumulative probability of HBsAg seroclearance was 21% and 33%, respectively. Among the 8 patients who had baseline serum HBsAg ≤100 IU/mL but did not develop HBsAg seroclearance, 4 of them had inactive disease; 2 had mildly active disease with slightly elevated HBV DNA (one ranged from 3.17 to 3.68 log IU/mL and another ranged from 1.94 to 4.51 log IU/mL) but persistently normal ALT; and the remaining 2 had active disease with fluctuating HBV DNA (up to >7 log IU/mL) and repeated ALT flares (peak ALT of one patient was 310 IU/L and that of another patient was 457 IU/L).

All patients who underwent HBsAg seroclearance had baseline HBsAg ≤1000 IU/mL, which has 100% sensitivity and 100% negative predictive value to predict HBsAg seroclearance (Table 3). However, 34 (37%) of 91 patients who did not clear HBsAg also had a baseline HBsAg ≤1000 IU/mL. The cumulative probability of HBsAg seroclearance at 5 and 8 years among patients with baseline serum HBsAg ≤1000 IU/mL were 5% and 14%, respectively (Figure 3*B*).

Serum HBV DNA could not predict HBsAg seroclearance (hazard ratio, 0.78; 95% CI, 0.53–1.15; $P = .21$) (Table 1, Figure 2). HBV DNA cutoff of 200 IU/mL or 2000 IU/mL could not predict the chance of HBsAg seroclearance (Table 3). The cumulative probability of HBsAg seroclearance at 5 and 8 years among patients with baseline serum HBV DNA ≤2000 IU/mL were 5% and 11% ,while those with HBV DNA >2000 IU/mL were 0% and 4%, respectively (Figure 3*C*).

One patient who had ALT flare developed HBsAg seroclearance. This patient had ALT flare at initial visit (ALT 211 IU/l) but developed HBsAg seroclearance at month 76. Overall, ALT flare was not associated with HBsAg seroclearance (hazard ratio, 0.44; 95% CI, 0.06–3.44; $P = .44$).

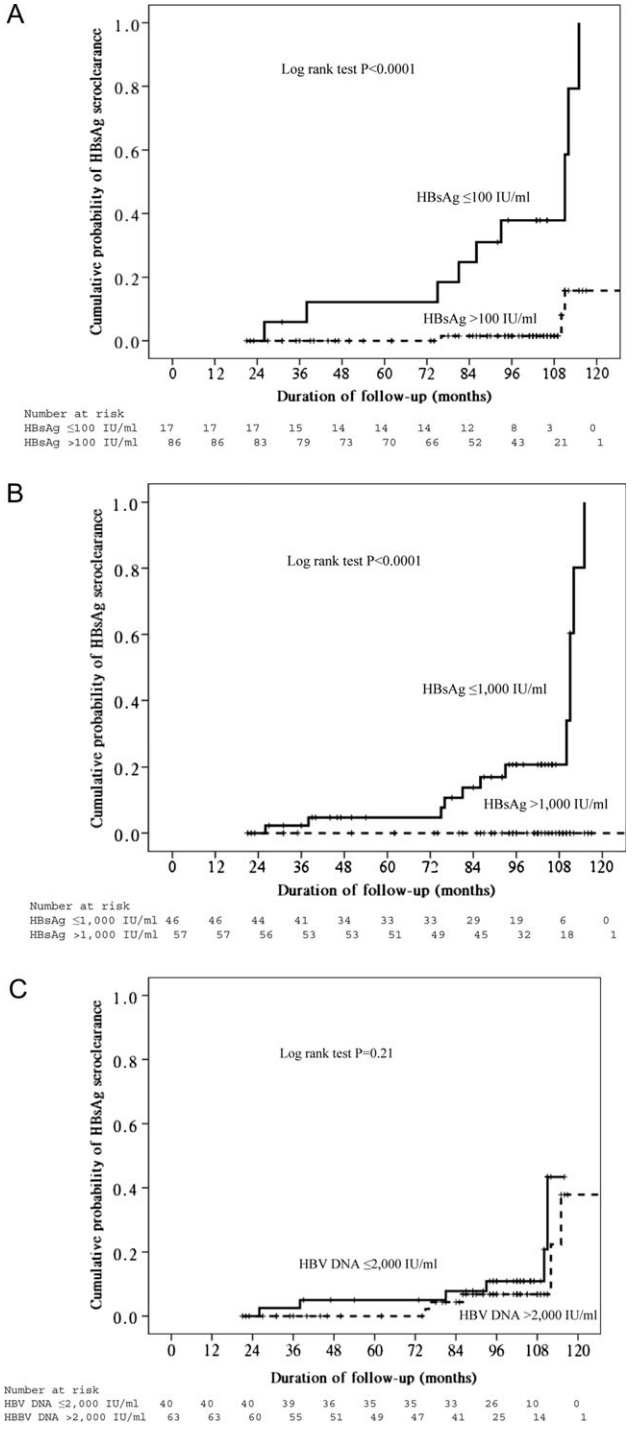


**Figure 3.** Cumulative probability of spontaneous HBsAg seroclearance among patients (*A*) with baseline serum HBsAg ≤100 IU/mL vs those with HBsAg >100 IU/mL; (*B*) with baseline serum HBsAg ≤1000 IU/mL vs those with HBsAg >1,000 IU/mL; and (*C*) with baseline serum HBV DNA ≤2000 IU/mL vs those with HBV DNA >2,000 IU/mL.

### Combination of HBV DNA and HBsAg to Predict HBsAg Seroclearance

Patients who had HBsAg ≤100 IU/mL usually had HBV DNA ≤2000 IU/mL, and they had the highest chance of clearing HBsAg in 5 years (Table 4). The hazard ratio of clearing HBsAg for the 17 patients with HBsAg ≤100 IU/mL was 13.84 (95% CI, 3.74–57.25; $P < .001$). One patient who cleared HBsAg had active disease as shown in Table 3; he had baseline HBsAg of 680 IU/mL and HBV DNA of 7.55 log IU/mL, but his HBsAg was reduced to 8.3 IU/mL with an HBV DNA of 4.68 log IU/mL in 52 months; he eventually cleared HBsAg at month 76. Among the 22 patients who had HBsAg ≤1000 IU/mL and HBV DNA ≤2000 IU/mL, the hazard ratio for HBsAg seroclearance was 9.69 (95% CI, 2.48–37.87; $P = .001$), and the 5- and 8-year cumulative probability of clearing serum HBsAg were 10% and 23%, respectively.

**Table 4. Cumulative Probability of HBsAg Seroclearance at 3, 5, and 8 Years at Different Cutoffs of HBV DNA and HBsAg**

| HBV DNA(IU/mL) | HBsAg (IU/mL) | No. of patients | 3 years | 5 years | 8 years |
|---|---|---|---|---|---|
| ≤2000 | ≤1000 | 22 | 5% | 9% | 21% |
| | >1000 | 18 | 0% | 0% | 0% |
| >2000 | ≤1000 | 24 | 0% | 0% | 21% |
| | >1000 | 39 | 0% | 0% | 0% |
| ≤2000 | ≤100 | 11 | 9.1% | 18.2% | 37.7% |
| | >100 | 29 | 0% | 0% | 0% |
| >2000 | ≤100 | 6 | 0% | 0% | 40% |
| | >100 | 57 | 0% | 0% | 2.4% |

**NOTE.** HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

### Predictors of HCC Development

Seven patients developed HCC. Patients who developed HCC tend to have lower platelet count, lower serum albumin, and higher ALT levels than those who did not develop HCC (Table 1). This was probably related to the higher prevalence of liver cirrhosis among patients who developed HCC. There was no association between HCC development and age, gender ratio, HBV DNA level, HBsAg level, HBV DNA/HBsAg ratio, HBV genotype distribution, or basal core promoter mutation. The hazard ratio among patients with HBV DNA >2000 IU/mL for HCC development was 4.90 (95% CI, 0.58–41.32; $P = .14$). All 7 patients who developed HCC had HBV DNA intermittently or persistently >20,000 IU/mL with or without elevation of ALT levels during the follow-up period. All 7 patients had baseline HBsAg >100 IU/mL, and all of them failed to clear HBsAg. However, serum HBsAg ≤100 IU/mL could not predict the risk of development of HCC (hazard ratio, 0.04; 95% CI, 0.00–168.24; $P = .44$).

## DISCUSSION

In this longitudinal study of untreated chronic hepatitis B patients with negative HBeAg, we found that a single serum HBsAg level could predict the chance of spontaneous HBsAg

seroclearance. An HBsAg level of ≤100 IU/mL provided good prediction for HBsAg seroclearance with high specificity (0.91) and negative predictive value (0.97). An HBsAg ≤1000 IU/mL was almost a prerequisite for HBsAg seroclearance in the subsequent years. Serum HBV DNA level alone, on the contrary, could not predict the chance of HBsAg seroclearance. Patients with serum HBV DNA ≤2000 IU/mL and HBsAg ≤1,000 IU/mL can also predict spontaneous HBsAg clearance in 5–8 years.

Good correlation between serum HBsAg level and intrahepatic cccDNA was found in HBeAg-positive patients [21, 22]. This correlation could not be demonstrated in HBeAg-negative patients regardless of their disease activities [30, 31]. In HBeAg-negative chronic hepatitis B, the immune control was predominantly on the clearance of cccDNA and reduction of viral replicative efficiency [30, 32, 33]. However, the efficiency of HBsAg transcription and translation was very similar between HBeAg-positive and HBeAg-negative patients [32]. These observations suggested an uncoupling of immune control on the process of HBsAg production and viral replication in patients with negative HBeAg. Furthermore, HBsAg might be produced by the integrated HBV surface gene sequence into the host genome, which cannot provide a template for viral replication. Nonetheless, a low serum HBsAg level might indicate a more complete immune clearance than a low HBV DNA level. It is therefore not surprising that serum HBsAg level provides a better prediction for spontaneous HBsAg seroclearance than serum HBV DNA.

In case-control studies, the HBsAg level was generally lower in the low replicative phase than in active disease among patients with negative HBeAg in Asia and Europe [32, 33]. Similarly, in a longitudinal study with a mean follow-up of 8 years in Hong Kong, the serial HBsAg levels were persistently lower among patients in the low replicative phase than those with active HBeAg-negative disease [23]. However, no cutoff value of HBsAg could accurately differentiate these 2 groups of HBeAg-negative patients due to the significant overlap in their ranges of HBsAg levels. One potential bias would fall in the definition of disease status, because patients with negative HBeAg tend to have fluctuating disease course. As in the current study, 5 (42%) of the 12 patients who underwent spontaneous HBsAg seroclearance would have been classified as mildly active or active hepatitis based on the HBV DNA and ALT levels.

A recent Italian study has reported the use of serum HBsAg level to distinguish inactive from active carriers among 209 patients with negative HBeAg followed up for a median of 3 years [34]. Inactive carrier was stringently defined as HBV DNA level persistently <2000 IU/mL on monthly monitoring in the first 12 months follow-up. The investigators found that HBsAg <1000 IU/mL together with HBV DNA ≤2000 IU/mL could offer very accurate prediction for inactive carriers. One peculiar feature of this Italian cohort was the absence of patients who had elevated HBV DNA on recruitment but disease remission on subsequent follow-up (as in 4 of the 12 patients who underwent spontaneous HBsAg seroclearance in our study). Whether this phenomenon was related to HBV genotype (genotype D HBV in Italy and genotype B and C HBV in Hong Kong) or a relative short duration of follow-up was uncertain [35]. Overall, our study was in agreement with this Italian study that an HBV DNA level of ≤2000 IU/mL and HBsAg level of ≤1000 IU/mL was able to predict HBsAg seroclearance. Therefore, patients who have HBsAg between 100 IU/mL and 1000 IU/mL, particularly if the HBV DNA is low, should have HBsAg regularly monitored for HBsAg seroclearance.

Although a single HBV DNA could not offer good prediction on the occurrence of HBsAg seroclearance in our study, patients who cleared HBsAg had progressive reduction of HBV DNA levels as demonstrated in a recent Taiwanese report [17]. Four of the 5 patients who had HBV DNA >2000 IU/mL at baseline but cleared HBsAg had HBV DNA reduced to undetectable level well before HBsAg seroclearance occurred during the follow-up. Therefore, among patients who have high HBV DNA levels but low HBsAg level, one can consider monitoring for reduction in HBV DNA rather than have antiviral therapy started immediately.

Our study was limited by the relatively small number of patients who had spontaneous HBsAg seroclearance. The annual incidence of HBsAg seroclearance in our cohort was ~1.6%, which was comparable to that reported in the Taiwanese cohorts [17, 18]. As classification into different disease status based on HBV DNA and ALT levels was sometimes difficult and biased, we believed that HBsAg seroclearance was the most reliable serological marker for complete immune control. Our patients represented a unique cohort that was prospectively followed up since recruitment. Owing to our stringent criteria of patient selection, only 103 patients fulfilled the study criteria. Nonetheless, our results could clearly demonstrate the predictive ability of HBsAg level for spontaneous HBsAg seroclearance, but the cutoffs should be validated in other independent cohorts. As the follow-up duration of our cohort varied widely, we could not preclude the possibility that some patients who had shorter follow-up would develop HBsAg seroclearance if they were followed longer. Hence, the use of ROC curve might impose bias to the determination of the best HBsAg cutoff, although our data were supported by subsequent survival analysis. Last, we did not have data on the role of serial quantitative HBsAg monitoring to guide the use of this serum marker to predict HBsAg seroclearance. Based on our previous study, reduction of HBsAg by 1 log was associated with a favorable outcome [23].

In conclusion, we showed that a single serum HBsAg measurement could predict the chance of HBsAg seroclearance in the subsequent 8 years of follow-up. As HBsAg levels tend to decrease very slowly at ~0.04 log IU/mL/year in HBeAg-negative patients [23], frequent monitoring of HBsAg level may not be necessary. Determination of serum HBsAg level in supplementation to HBV DNA measurement may

represent a new angle for risk stratification and consideration for antiviral therapy in chronic hepatitis B patients with negative HBeAg.

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