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MAJOR ARTICLE

Trajectories and Decline of Serum Hepatitis B Surface Antigen Predict Outcomes in Patients With Chronic Hepatitis B

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Background. The kinetics of serum hepatitis B surface antigen (HBsAg) levels during long-term nucleos(t)ide analogue (NA) therapy remains unclear. We delineated the kinetics of HBsAg and analyzed its association with long-term treatment outcomes.

Methods. We enrolled 912 treatment-naïve patients with chronic hepatitis B (CHB) who had received NA therapy for >12 months and analyzed the kinetic patterns through group-based trajectory models (GBTMs).

Results. The median treatment duration for the entire cohort was 60.3 months. GBTMs revealed 4 patterns in patients achieving HBsAg loss (groups 1–4) in the study population and in patients achieving HBsAg <100 IU/mL among those with HBeAg-negative CHB with baseline HBsAg ≥100 IU/mL (groups A–D). Patients in groups 1 and A had the highest rates of HBsAg loss (22.2%, 6/27) and of achieving HBsAg <100 IU/mL (47.5%, 56/118), respectively. HBsAg <40 IU/mL and <400 IU/mL at 12 months of treatment predicted group 1 and group A membership among all patients and those with HBeAg-negative CHB, respectively. Multivariable Cox regression analysis identified HBsAg trajectory group (group 1 vs groups 3 and 4: hazard ratio [HR], 179.46; $P < .001$; group 2 vs groups 3 and 4: HR, 24.34; $P < .001$) and HBsAg decline (HR, 82.14; $P < .001$) as independent predictors of both HBsAg loss and achieving HBsAg <100 IU/mL.

Conclusions. Serum HBsAg trajectories and decline can predict HBsAg loss and the achievement of HBsAg <100 IU/mL in patients with CHB receiving long-term NA therapy.

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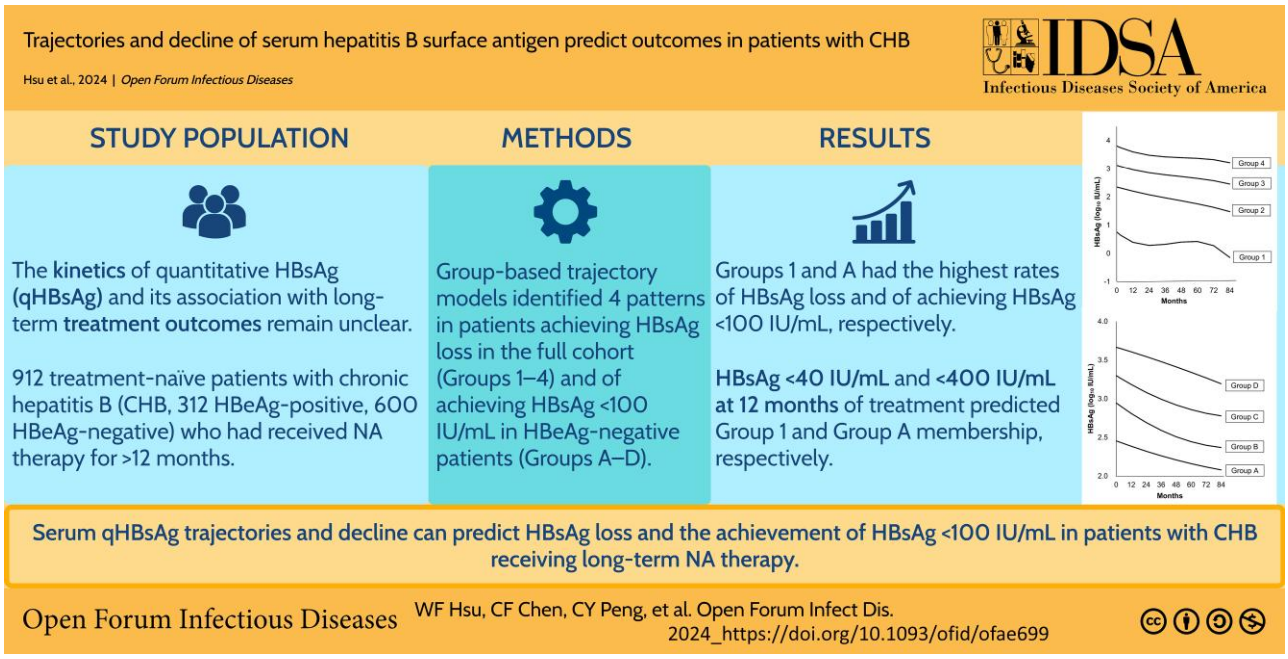
Graphical Abstract



This graphical abstract is also available at Tidbit: https://tidbitapp.io/tidbits/trajectories-and-decline-of-serum-hepatitis-b-surface-antigen-predict-outcomes-in-patients-with-chronic-hepatitis-b/update

Keywords. group-based trajectory models; HBsAg loss; hepatitis B surface antigen; kinetics of quantitative HBsAg; nucleos(t)ide analogue.

Chronic hepatitis B (CHB) is a global health concern. Nucleos(t)ide analogues (NAs) with a high genetic barrier are currently the first-line treatment for hepatitis B virus (HBV) [1–3]. The main disadvantage of NA therapy is its indefinite treatment duration because it does not directly affect nuclear covalently closed circular DNA (cccDNA) [4]. Moreover, on-treatment hepatitis B surface antigen (HBsAg) loss rarely occurs during NA therapy [5]. The annual HBsAg loss rate was estimated to be 0.33% [6], which is similar to that of untreated patients [7].

Quantitative serum HBsAg levels are associated with the transcriptional activity of cccDNA and reflect immune control over HBV infection [8]. Studies have demonstrated that HBsAg ≤100 IU/mL predicts spontaneous HBsAg loss in hepatitis B e antigen (HBeAg) spontaneous seroconverters and in patients with HBeAg-negative CHB [9, 10]. Other studies have revealed that HBsAg <100 IU/mL at the end of treatment predicts off-treatment HBsAg loss in patients with HBeAg-negative CHB undergoing NA therapy [11, 12]. Because on-treatment HBsAg loss is rare, HBsAg <100 IU/mL could be regarded as an alternative end point for NA therapy in patients with HBeAg-negative CHB [13].

Studies have reported that although HBsAg decline is modest during the first year of NA treatment, it is greater than during the remainder of the treatment duration; in addition, HBsAg decline can predict HBeAg seroconversion as well as achieving HBsAg <100 IU/mL and HBsAg loss during treatment [14, 15]. Different thresholds for HBsAg decline during the first year of NA therapy have been proposed [15–17]. However, the association between treatment outcomes and serum HBsAg decline after the first year of therapy remains unclear.

Group-based trajectory models (GBTMs) have been applied in clinical practice to identify clusters of individuals exhibiting similar dynamic changes in a single indicator over time [18]. In our previous study, GBTMs revealed 3 patterns in patients with HBeAg-positive and HBeAg-negative CHB; HBsAg levels at baseline and 12 months combined with HBsAg decline during this period were predictive of the trajectory associated with optimal therapeutic response [19]. In this study, we increased the cohort size and follow-up duration to delineate the kinetics of HBsAg, analyze its association with long-term treatment outcomes, and identify other potential predictors by using GBTMs.

METHODS

Patients

A consecutive cohort of 912 prior treatment-naïve patients with CHB receiving NA therapy from January 2003 to August 2018 was enrolled into this study. The inclusion criteria included seropositivity for HBsAg for >6 months, baseline HBV DNA >20 000 IU/mL for HBeAg-positive patients and >2000 IU/mL for HBeAg-negative or cirrhotic patients, and NA therapy for >12 months [3]. The exclusion criteria are shown in Supplementary Figure 1.

Patient Consent

This study was conducted in accordance with the 1975 Declaration of Helsinki. All patients provided written informed consent before enrollment, and this study was approved by the Research Ethics Committee of China Medical University Hospital (CMUH102-REC1-113), Taichung, Taiwan.

Laboratory Tests

Complete blood count analyses and blood biochemical parameters were measured at baseline. HBsAg levels were quantified using Abbott Architect HBsAg QT assays (dynamic range, 0.05–250 IU/mL) at baseline; at 3, 6, and 12 months; and annually thereafter. Serum HBV DNA levels were also measured at baseline; at 3, 6, and 12 months; and every 6 months thereafter using the COBAS AmpliPrep-COBAS TaqMan HBV test (detection limit, 12 IU/mL).

Therapeutic End Points

We assessed the achievement of HBsAg <100 IU/mL in patients who had HBeAg-negative CHB and HBsAg loss in the study population. HBsAg loss was defined as serum HBsAg <0.05 IU/mL, confirmed 6 months apart [15].

Statistical Analysis

Continuous variables are presented as median (interquartile range [IQR]). Comparisons of continuous variables between 2 groups and among >2 groups were performed using the Wilcoxon rank-sum test and the Kruskal-Wallis test, respectively. Categorical variables were analyzed using the chi-square test or Fisher exact test, as appropriate. The GBTM in the PROC TRAJ macro (SAS software, version 9.4, SAS Institute, Cary, NC, USA) was used to identify distinct groups of patients with long-term changes in serum HBsAg levels over 7 years of follow-up [20, 21]. Repeated measurements of HBsAg before the achievement of therapeutic end points were $\log_{10}$-transformed and modeled using a censored normal distribution. To evaluate the fit of models with distinct numbers of groups or trajectory shapes, we considered whether the sample size in each group was adequate, as well as the log Bayes factor, which indicates the strength of evidence for improvement in the model fit; this approach promoted model parsimony (Supplementary Table 1) [21]. Patients were categorized into groups according to long-term HBsAg changes based on the highest posterior probabilities of belonging to each group [20]. An average posterior probability of assignment (APPA) >0.7 and an odds of correct classification (OCC) >5 for each group were used as the criteria for a good model fit [22]. The long-term HBsAg change group to which each patient belonged was treated as a categorical variable in subsequent analyses. The receiver operating characteristic (ROC) curve and the corresponding area under the curve (AUC) were calculated to indicate the accuracy with which serum HBsAg decline ($\log_{10}$ IU/mL/year) could predict therapeutic outcomes. The following formula was adopted to calculate the magnitude of HBsAg decline and incidence rates of achieving the 2 therapeutic end points:

$$\text{Magnitude of HBsAg decline} = \frac{\text{baseline HBsAg (log10 IU/mL) - last HBsAg (log10 IU/mL)}}{\text{follow-up (year)}}$$

$$\text{Incidence rate of HBsAg loss} = \frac{\text{number of incident HBsAg losses}}{\text{follow-up (person-years)}} \times 100\,000$$

$$\text{Incidence rate of an ultimate HBsAg level of } <100\,\text{IU/mL} = \frac{\text{number of patients achieving HBsAg} <100\,\text{IU/mL}}{\text{follow-up (person-years)}} \times 100\,000$$

The optimal cutoff value of serum HBsAg decline for predicting therapeutic outcomes was determined using Youden's index. The person-years of follow-up for each patient were calculated from the date of treatment initiation until the date of therapeutic end point achievement, loss to follow-up, or end of follow-up. A Cox regression model was used to calculate adjusted hazard ratios with 95% CIs for achieving therapeutic end points in the long-term HBsAg change groups after adjustment of confounding factors. Kaplan-Meier analysis with a log-rank test was used to compare the therapeutic end points among patient subgroups. A 2-sided P value of <.05 was considered significant. SAS, version 9.4, was used for all statistical analyses.

RESULTS

Baseline Characteristics

Baseline characteristics of the 912 patients are shown in Table 1. Relative to patients with HBeAg-negative CHB, those with HBeAg-positive CHB were younger; had a higher platelet count; had higher aspartate aminotransferase (AST), alanine aminotransferase (ALT), HBV DNA, and HBsAg levels; and had a lower proportion of genotype B infection and liver cirrhosis (LC) (Table 1). The NA regimens used in this study are presented in Supplementary Table 2.

Trajectory Patterns and HBsAg Loss

GBTMs for predicting HBsAg loss revealed 4 patterns of HBsAg changes over the 7-year follow-up. The rates of HBsAg loss were 22.2% (6/27), 5.7% (11/194), 0.43% (2/460), and 0.43% (1/231) in groups 1, 2, 3, and 4, respectively (Supplementary Table 3). The serum HBsAg kinetics of the 4 groups are depicted in Figure 1*A*. GBTMs revealed 4 cubic groups of long-term HBsAg changes in the full cohort

Table 1. Baseline Characteristics and Treatment Outcomes

Variable	Total (n = 912)	HBeAg-Positive (n = 312)	HBeAg-Negative (n = 600)	*P* Value[a]
Age, y	49 (40–57)	41 (34–49)	53 (46–60)	<.001
Sex (male), No. (%)	657 (72.0)	218 (69.9)	439 (73.2)	.293
AST, U/L	67 (42–162)	80 (46–280)	63 (41–130)	<.001
ALT, U/L	100 (53–262)	127 (63–455)	88 (48–217)	<.001
Albumin, g/dL	4.2 (3.8–4.4)	4.2 (3.9–4.4)	4.2 (3.8–4.5)	.932
Total bilirubin, mg/dL	1.00 (0.76–1.43)	0.98 (0.73–1.45)	1.01 (0.77–1.42)	.581
INR	1.09 (1.04–1.17)	1.09 (1.04–1.16)	1.09 (1.04–1.17)	.646
Platelet, $\times 10^3/\mu L$	167 (121–203)	180 (136–213)	158 (115–195)	<.001
Liver cirrhosis, No. (%)	290 (31.80)	70 (22.44)	220 (36.67)	<.001
Genotype, No. (%)				<.001
B	561 (66.0)	156 (51.0)	405 (74.5)	
C	289 (34.0)	150 (49.0)	139 (25.6)	
HBV DNA, $\log_{10}$ IU/mL	5.85 (4.58–7.30)	7.32 (5.97–8.10)	5.25 (4.07–6.38)	<.001
HBsAg, $\log_{10}$ IU/mL	3.18 (2.74–3.61)	3.61 (3.23–4.25)	2.98 (2.50–3.36)	<.001
Treatment outcomes, No. (%)				
HBsAg <100 IU/mL	194 (21.3)	35 (11.2)	159 (26.7)	<.001
HBsAg loss	20 (2.2)	10 (3.2)	10 (1.7)	.132

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; INR, international normalized ratio.

[a]Difference between patients with HBeAg-positive and HBeAg-negative chronic hepatitis B.

(Figure 1*B*). The APPAs for being in groups 1, 2, 3, and 4 were 0.9683, 0.9417, 0.9337, and 0.9197, respectively, indicating that the model assigned patients to trajectory groups with high clarity. Furthermore, the lowest OCC was 13.84, demonstrating high assignment accuracy across all groups. The incidence rates of HBsAg loss in groups 1, 2, 3, and 4 were 427.7, 90.2, 6.2, and 5.9 cases per 100 000 person-years, respectively.

At 12 months of NA treatment, an absolute HBsAg level of <40 IU/mL was the strongest predictor of group 1 membership in the study population (AUC, 0.9976), with a sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 100.00% (27/27), 97.97% (867/885), 60.00% (27/45), and 100.00% (867/867), respectively.

GBTMs for predicting HBsAg loss also revealed 4 patterns of HBsAg changes each in HBeAg-positive (Supplementary Table 4) and HBeAg-negative patients (Supplementary Table 5). The HBsAg kinetics of the 4 groups are depicted in Supplementary Figure 2*A*–*D*.

Trajectory Patterns and the Achievement of HBsAg <100 IU/mL in Patients With HBeAg-Negative CHB

GBTMs for predicting the achievement of HBsAg <100 IU/mL revealed 4 patterns of HBsAg changes over the 7-year follow-up in patients with HBeAg-negative CHB who had baseline HBsAg levels of ≥100 IU/mL (n = 515; median treatment duration [IQR], 59.5 [35.8–92.3] months). The rates of achieving HBsAg <100 IU/mL were 47.5% (56/118), 13.8% (25/181), 3.4% (6/178), and 0% (0/38) in groups A, B, C, and D, respectively (Supplementary Table 6). The serum HBsAg kinetics of the 4 groups are illustrated in Figure 2*A*. GBTMs revealed 4 quadric groups of long-term HBsAg changes among those patients (Figure 2*B*). For all 4 trajectory groups, the lowest APPA was 0.90 and the lowest OCC was 16.94, indicating a good model fit. The incidence rates of achieving HBsAg <100 IU/mL in groups A, B, C, and D were 1010.0, 211.3, 48.9, and 0 cases per 100 000 person-years, respectively.

At 12 months of NA treatment, an absolute HBsAg level of <400 IU/mL was the strongest predictor of group A membership (AUC, 0.9698) among those with HBeAg-negative CHB who had a baseline HBsAg level of ≥100 IU/mL; sensitivity, specificity, PPV, and NPV were 90.68% (107/118), 92.70% (368/397), 78.68% (107/136), and 97.10% (368/379), respectively.

Optimal HBsAg Decline for Predicting HBsAg Loss in All Patients and for Predicting HBsAg <100 IU/mL in Patients With HBeAg-Negative CHB

Because few patients in certain groups achieved the therapeutic end points, we combined groups 3 and 4, as well as groups C and D, into 1 group each for the following analyses. The median HBsAg decline in patients with HBsAg loss (IQR) was 0.74 (0.45–1.17) $\log_{10}$ IU/mL/year, which was significantly greater than that in patients without HBsAg loss (0.08 [0.02–0.19] $\log_{10}$ IU/mL/year; $P < .001$). A high AUC value (0.94; 95% CI, 0.91–0.97) was attained for HBsAg decline predicting HBsAg loss. The highest Youden's index was obtained for the HBsAg decline of 0.3437 $\log_{10}$ IU/mL/year for predicting HBsAg loss (Supplementary Figure 3*A*). The magnitude of HBsAg decline was 0.07 (−0.11 to 0.74), 0.11 (0.02–0.25), and 0.08 (0.02–0.19) in group 1, group 2, and groups 3 and 4, respectively (1.17 [0.52–1.89], 0.53 [0.34–0.97], and 0.67

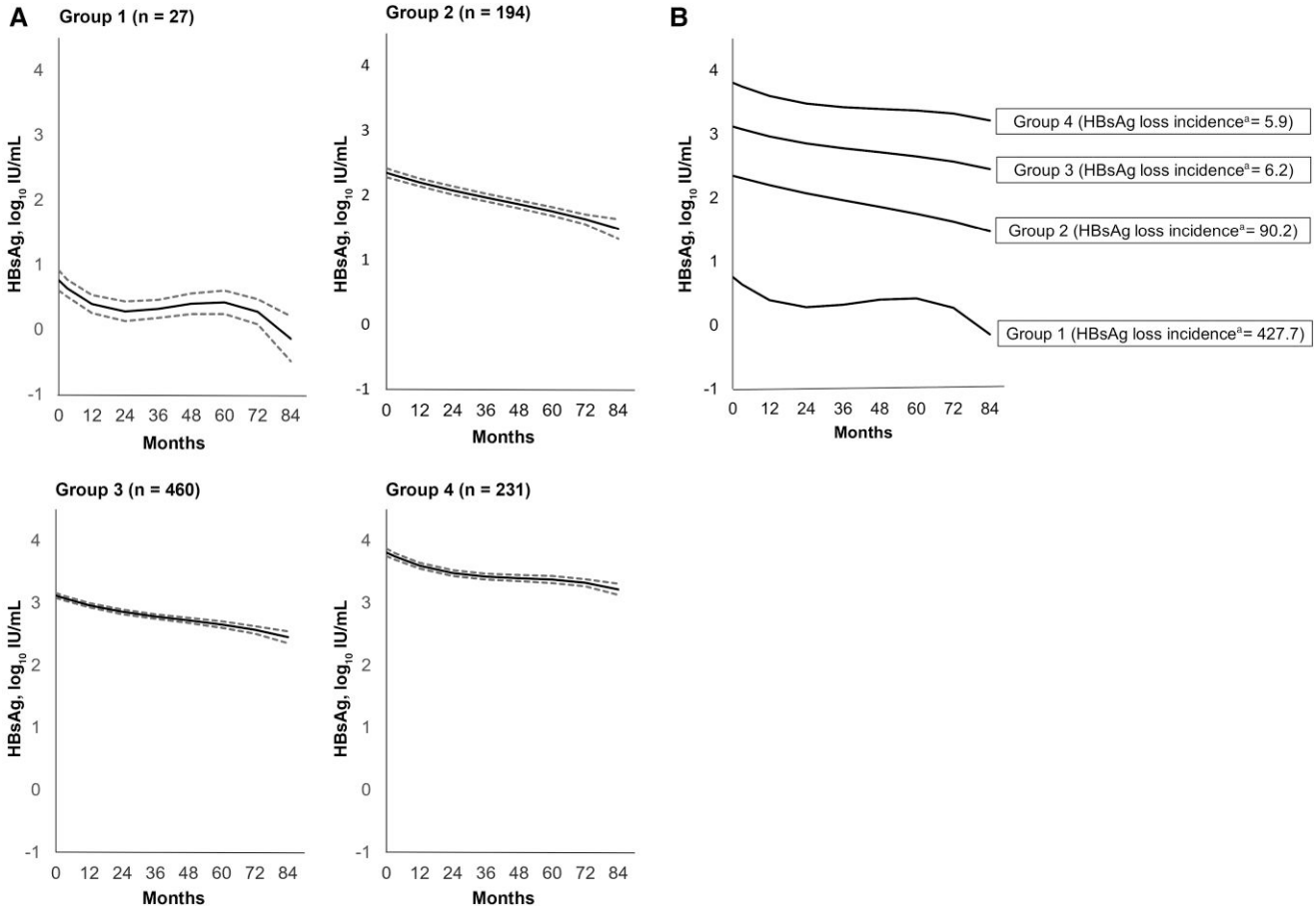


Figure 1. Four groups representing long-term changes in serum HBsAg levels over 7 years of follow-up in the full cohort and the incidence of HBsAg loss. *A*, The solid line represents predicted levels of long-term HBsAg change over the 7-year follow-up, and the dashed line represents the 95% CIs of predicted levels of long-term HBsAg change over the 7-year follow-up. All predicted levels and CIs of long-term HBsAg change were generated using the group-based trajectory model. *B*, Plots were generated using the group-based trajectory model. [a]HBsAg loss incidence rate was calculated as follows: [(number of patients achieving HBsAg loss)/follow-up (person-years)] × 100 000. Abbreviation: HBsAg, serum hepatitis B surface antigen.

[0.51–0.82], respectively, for patients with HBsAg loss in comparison with those without HBsAg loss: 0.01 [−0.15 to 0.24], 0.10 [0.02–0.22], and 0.08 [0.02–0.19], respectively; $P < .01$ in each subgroup comparison between patients with and without HBsAg loss). The AUC values and optimal cutoffs of HBsAg decline for predicting HBsAg loss were 0.91 (95% CI, 0.87–0.96) and 0.3896 $\log_{10}$ IU/mL/year in patients with HBeAg-positive CHB as well as 0.95 (95% CI, 0.91–0.99) and 0.1698 $\log_{10}$ IU/mL/year in patients with HBeAg-negative CHB, respectively (Supplementary Figure 3*B* and *C*). Among 312 HBeAg-positive patients, 182 and 113 patients achieved HBeAg loss and HBeAg seroconversion, respectively. Among them, the optimal cutoffs of HBsAg decline for predicting HBsAg loss were 0.3599 and 0.3408 $\log_{10}$ IU/mL/year, respectively (Supplementary Figure 3*D* and *E*).

The median HBsAg decline in patients with HBeAg-negative CHB who achieved HBsAg <100 IU/mL was 0.16 (0.07–0.29) $\log_{10}$ IU/mL/year, which was significantly greater than that in patients who did not achieve HBsAg <100 IU/mL (0.07 [0.02–0.04] $\log_{10}$ IU/mL/year; $P < .001$). HBsAg decline had an acceptable AUC value (0.69; 95% CI, 0.63–0.75) for predicting the achievement of HBsAg <100 IU/mL. An HBsAg decline of 0.1540 $\log_{10}$ IU/mL/year had the highest Youden's index for predicting the achievement of HBsAg <100 IU/mL (Supplementary Figure 4). The magnitude of HBsAg decline was 0.09 (0.02–0.18), 0.09 (0.04–0.16), and 0.07 (0.02–0.15) in group A, group B, and groups C and D, respectively ($P < .01$ in each subgroup comparison between patients with and without the achievement of HBsAg <100 IU/mL).

Overall Predictors of HBsAg Loss

A multivariable Cox regression analysis revealed the following independent predictors of HBsAg loss: HBeAg status, HBsAg trajectory group, and HBsAg decline (Table 2).

Another Cox regression analysis for predicting HBsAg loss by using the optimized dichotomous cutoff of 0.3437 $\log_{10}$

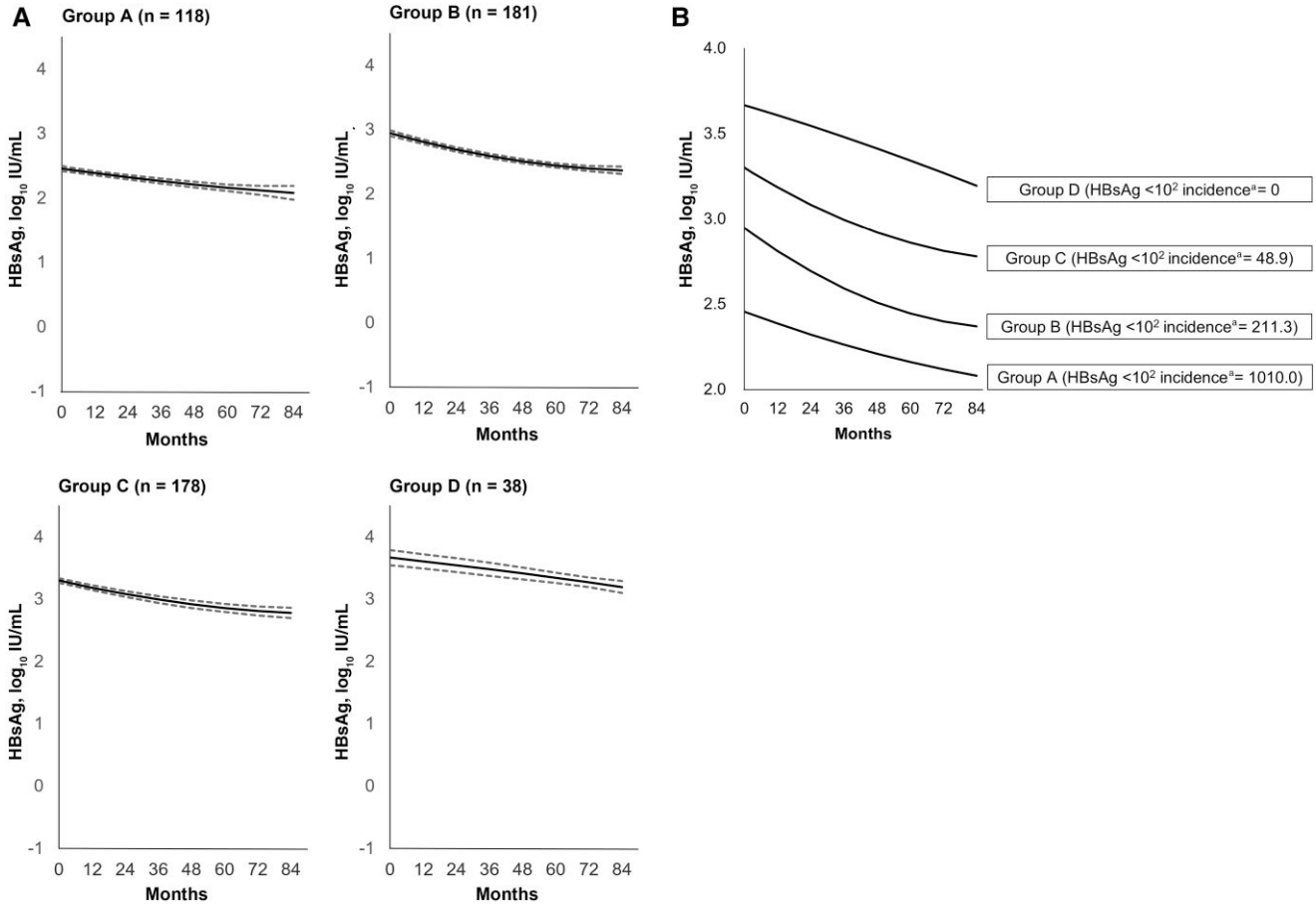


Figure 2. Four groups representing long-term changes in serum HBsAg levels over 7 years of follow-up among HBeAg-negative patients with baseline HBsAg >100 IU/mL and the incidence of achieving HBsAg <100 IU/mL. *A*, The solid line represents predicted levels of long-term HBsAg change over the 7-year follow-up, and the dashed line represents 95% CIs of predicted levels of long-term HBsAg change over the 7-year follow-up. All predicted levels and CIs of long-term HBsAg change were generated using the group-based trajectory model. *B*, Plots were generated using the group-based trajectory model. [a]HBsAg <100 IU/mL incidence rate was calculated as follows: [(number of patients achieving HBsAg <100 IU/mL)/follow-up (person-years)] × 100 000. Abbreviations: HBeAg, hepatitis B e antigen; HBsAg, serum hepatitis B surface antigen.

Table 2. Multivariable-Adjusted Hazard Ratios of Achieving HBsAg Loss

	Univariate Analysis		Multivariable Analysis	
Variable	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age	0.96 (0.93–1.00)	.037	0.95 (0.90–1.01)	.072
Sex (male vs female)	1.02 (0.37–2.81)	.974	0.70 (0.14–3.42)	.660
ALT (≥5× vs <5× ULN)	3.46 (1.41–8.5)	.007	0.65 (0.17–2.54)	.538
HBeAg (positive vs negative)	1.76 (0.73–4.24)	.206	7.36 (1.33–40.78)	.022
HBV DNA, $\log_{10}$ IU/mL	1.16 (0.89–1.51)	.287	0.80 (0.49–1.30)	.364
Genotype (B vs C)	3.04 (0.88–10.51)	.633	1.91 (0.35–10.37)	.452
Liver cirrhosis (yes vs no)	0.20 (0.05–0.87)	.032	0.10 (0.01–1.22)	.071
Group of serum HBsAg trajectory				
Groups 3 & 4	1.00 (referent)		1.00 (referent)	
Group 2	16.91 (4.7–60.88)	<.001	24.34 (4.80–123.39)	<.001
Group 1	101.87 (24.58–422.19)	<.001	179.46 (14.26–2258.61)	<.001
HBsAg decline, $\log_{10}$ IU/mL/y	178.84 (35.08–911.78)	<.001	82.14 (12.12–556.76)	<.001

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV hepatitis B virus; HR, hazard ratio; ULN, upper limit of normal.

Table 3. Multivariable-Adjusted Hazard Ratios of Achieving HBsAg <100 IU/mL in Patients With HBeAg-Negative CHB

	Univariate Analysis		Multivariable Analysis	
Variable	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age	1.01 (0.99–1.03)	.426	0.97 (0.95–1.00)	.028
Sex (male vs female)	1.12 (0.70–1.81)	.632	2.08 (1.16–3.72)	.013
ALT (≥5× vs <5× ULN)	1.81 (1.16–2.83)	.009	1.38 (0.75–2.52)	.301
HBV DNA, $\log_{10}$ IU/mL	0.91 (0.78–1.05)	.184	0.94 (0.79–1.13)	.538
Genotype (B vs C)	1.13 (0.68–1.89)	.629	0.97 (0.56–1.69)	.923
Liver cirrhosis (yes vs no)	1.34 (0.88–2.05)	.171	1.76 (1.06–2.93)	.028
Group of serum HBsAg trajectory				
Groups C & D	1.00 (referent)		1.00 (referent)	
Group B	6.06 (2.48–14.84)	<.001	5.76 (2.28–14.55)	<.001
Group A	40.41 (17.09–95.52)	<.001	54.44 (21.72–136.45)	<.001
HBsAg decline, $\log_{10}$ IU/mL/y	3.53 (2.51–4.97)	<.001	2.91 (2.06–4.10)	<.001

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV hepatitis B virus; HR, hazard ratio; ULN, upper limit of normal.

IU/mL/year for HBsAg decline revealed that HBsAg trajectory group and HBsAg decline (≥0.3437 vs <0.3437 $\log_{10}$ IU/mL/year; hazard ratio [HR], 40.19; 95% CI, 8.22–196.63) were independent predictors of HBsAg loss (Supplementary Table 7).

Predictors of Achieving HBsAg <100 IU/mL in Patients With HBeAg-Negative CHB

Univariate and multivariable Cox regression analyses identified the following independent predictors for achieving HBsAg <100 IU/mL: younger age, male sex, LC, HBsAg trajectory group, and HBsAg decline (Table 3).

A separate Cox regression analysis for predicting the achievement of HBsAg <100 IU/mL by using the optimized dichotomous cutoff of 0.1540 $\log_{10}$ IU/mL/year for HBsAg decline identified the following independent predictors for achieving HBsAg <100 IU/mL: LC, HBV DNA, HBsAg trajectory group, and HBsAg decline (≥0.1540 vs <0.1540 $\log_{10}$ IU/mL/year; HR, 13.51; 95% CI, 7.27–25.10) (Supplementary Table 8).

Kaplan-Meier Analysis

A Kaplan-Meier analysis revealed that patients with different patterns of HBsAg changes had different cumulative incidences of HBsAg loss (groups 1–4: $P < .001$) (Supplementary Figure 5*A*) and of achieving HBsAg <100 IU/mL (groups A–D: $P < .001$) (Supplementary Figure 5*B*). Patients with an HBsAg decline of ≥0.3437 $\log_{10}$ IU/mL/year in the entire cohort and those with an HBsAg decline of ≥0.1540 $\log_{10}$ in patients with HBeAg-negative CHB and baseline HBsAg levels of ≥100 IU/mL had higher cumulative incidences of achieving therapeutic end points than their counterparts (both $P < .001$) (Supplementary Figure 5*C* and *D*).

DISCUSSION

In this study, GBTMs revealed 4 patterns of long-term HBsAg changes for predicting HBsAg loss in the study population (groups 1–4) and achieving an ultimate HBsAg level of <100 IU/mL in patients with HBeAg-negative CHB (groups A–D). At 12 months of NA treatment, HBsAg level was the strongest predictor of group 1 (AUC, 0.9976) or group A (AUC, 0.9698) membership. Patients in group 1 had the highest likelihood of achieving HBsAg loss (22.2%), and HBsAg <40 IU/mL at 12 months of treatment predicted group 1 membership in the study population. Group A had the highest rate of achieving HBsAg <100 IU/mL (47.5%), and HBsAg <400 IU/mL at 12 months of treatment predicted group A membership among patients with HBeAg-negative CHB with baseline HBsAg ≥100 IU/mL.

Studies have indicated that patients with HBeAg-negative CHB who receive a finite duration of NA therapy with an end-of-treatment HBsAg level of <100 IU/mL have a high rate of sustained response with HBsAg loss (33% at 5 years off therapy) [11, 12]. In another study, patients who achieved low HBsAg levels and a sustained response after discontinuing NA therapy had a higher rate of HBsAg loss than those who had continued NA therapy [23]. The treatment strategy for patients with HBeAg-negative CHB is shifting from indefinite long-term NA therapy to finite therapy until a low HBsAg level is achieved, which is indicative of effective host immune control against HBV [13]. Hirode et al. revealed that HBsAg <1000 IU/mL in Caucasians and HBsAg <100 IU/mL in Asians at the end of NA therapy were predictors of HBsAg loss [24]. Furthermore, 2 studies on the natural history of CHB indicated that HBsAg <100 IU/mL was a predictor of spontaneous HBsAg loss [9, 10]. On the basis of this evidence, in the present study, we adopted HBsAg <100 IU/mL as an alternative therapeutic end point that suggests that host immune control against HBV is adequately restored. Therefore, a potential treatment approach is discontinuing NA therapy once effective immune control is achieved. However, additional immunological studies are required to confirm our hypothesis. Nonetheless, physicians should be aware that ~1.1% of patients

develop severe hepatic flare with liver decompensation 5 years after cessation of NA therapy [25].

Studies have demonstrated that HBsAg decline is more pronounced during the first year of NA therapy [14, 15]. Two studies have indicated that an HBsAg decline of ≥1.0 $\log_{10}$ IU/mL at months 12 and 6 of telbivudine and tenofovir therapy, respectively, predicted HBsAg loss at 3 and 5 years, respectively, of treatment in patients with HBeAg-positive CHB [16, 17]. We previously described the kinetics of HBsAg during the first year of entecavir therapy and demonstrated that early HBsAg decline, defined as an HBsAg decline of ≥75% from baseline at 3 and 12 months of treatment in patients with HBeAg-positive and HBeAg-negative CHB, respectively, predicted the achievement of HBsAg <100 IU/mL for entecavir therapy [15]. In addition to early HBsAg decline, baseline HBsAg <3000 IU/mL was an independent predictor for achieving HBsAg loss or HBsAg <100 IU/mL [15]. Two studies have reported that baseline HBsAg <1000 IU/mL combined with an HBsAg decline of >0.166 $\log_{10}$ IU/mL/year predicted HBsAg loss in patients with CHB receiving lamivudine therapy and achievement of HBsAg <200 IU/mL in patients receiving entecavir therapy [26, 27]. Our extensive characterization of the trajectory patterns and decline of HBsAg revealed that both the specific HBsAg trajectory and magnitude of HBsAg decline independently predicted treatment outcomes in patients receiving long-term NA therapy. Baseline HBsAg levels had a major impact on the trajectory pattern of HBsAg kinetics following the initiation of NA therapy. With long-term NA therapy, groups 3 and 4 and groups C and D had a low probability of achieving HBsAg loss (Supplementary Figure 5*A*) and HBsAg <100 IU/mL (Supplementary Figure 5*B*), respectively. Furthermore, patients in the same trajectory group who achieved therapeutic end points exhibited a significantly greater HBsAg decline than those who did not achieve those end points. Thus, our findings may assist physicians in predicting the probability of achieving therapeutic end points over the duration of NA therapy.

Few studies have investigated how HBeAg status affects HBsAg loss in patients with CHB receiving long-term NA therapy. Our results indicated that HBeAg positivity was a predictor of HBsAg loss. This finding might be explained by the transient restoration of T-cell function after NA therapy in patients with HBeAg-positive CHB [28], thus leading to greater HBsAg decline. However, the detailed immunological mechanisms remain unknown. In our cohort, patients with LC had a lower baseline HBsAg level than those without LC (3.03 [2.54–3.33] vs 3.31 [2.78–3.87] IU/mL; $P < .001$), which might partially explain why LC was a predictor of achieving an ultimate HBsAg level of <100 IU/mL in patients with HBeAg-negative CHB.

The immunological mechanisms underlying HBsAg decline or loss are unclear. Two studies have demonstrated that baseline serum interferon-γ-induced protein 10 (IP-10) levels, particularly levels of >350 pg/mL, were significantly associated with an on-treatment HBsAg decline of ≥0.5 $\log_{10}$ IU/mL in patients with HBeAg-negative CHB [29]. IP-10 is involved in the recruitment of activated T-cells and natural killer cells, which is implicated in the cytolytic elimination and noncytolytic immune control of HBV [30]. Whether the specific HBsAg trajectory and magnitude of HBsAg decline are regulated by IP-10 remains to be studied.

This study has several strengths. First, we identified 4 HBsAg trajectories for predicting therapeutic outcomes through GBTMs. Second, we demonstrated that the magnitude of HBsAg decline was a predictor for achieving therapeutic end points, and we also identified the optimal cutoffs for predicting HBsAg loss and achieving HBsAg <100 IU/mL. Third, given the increased research attention for the clinical development of novel direct-acting antivirals for curing HBV infection, our findings pertaining to on-treatment HBsAg kinetics provide valuable information for future trial design and patient selection.

Our study has some limitations. First, because this study was conducted in a single tertiary medical center, selection bias is possible. Second, although patients in group 1 represented <5% of the entire cohort (3.0%), their distinct trajectory and significantly higher HBsAg loss rate compared with group 2 justified their classification into 2 groups. A total of 4 groups among the entire cohort showed a better model fit than 3 groups. Third, a small proportion of our patients received weak NA therapy. However, NA potency does not affect overall HBsAg kinetics during treatment [19]. Fourth, whether our findings can be extrapolated to patients with genotype A or D CHB receiving long-term NA therapy remains to be investigated. Finally, the immunological mechanisms underlying distinct HBsAg trajectories or patterns of HBsAg decline remain unknown.

In conclusion, the trajectory of serum HBsAg levels and the magnitude of HBsAg decline can predict HBsAg loss and the achievement of HBsAg <100 IU/mL in patients with CHB receiving long-term NA therapy.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Author contributions. W.F.H. and C.Y.P. conceived and designed the study. W.F.H., H.C.L., W.P.S., H.W.W., S.H.C., and C.Y.P. acquired data. C.F.C. and C.Y.P. analyzed and interpreted the data. W.F.H. drafted the manuscript. C.Y.P. critically revised the manuscript. All authors approved the final version of the manuscript.

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