Research article

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Prediction of long-term HBsAg seroclearance in patients with HBeAg-negative chronic hepatitis B

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Background & Aims: Predicting the long-term HBsAg seroclearance, an ideal endpoint, is relevant for decision-making regarding antiviral therapy for patients with chronic hepatitis B (CHB). This study aimed to identify predictors and develop a prediction model for HBsAg seroclearance in patients with HBeAg-negative CHB.

Methods: A total of 2,032 untreated HBeAg-negative patients who underwent a 2-year baseline observation period were enrolled. Prediction models were developed using independent predictors of seroclearance, and their performance was evaluated through internal and external validation using an independent cohort of 753 patients, along with sensitivity analyses.

Results: The estimated annual incidence of HBsAg seroclearance was 2.22% (15,508 person-years). **Hep**atitis **B** virus DNA **L**evel (Low-to-intermittently high-level viremia), **O**ld age, male **S**ex, and hepatitis B **S**urface antigen level <250 IU/ml independently predicted seroclearance. Subsequently, two prediction models were developed: HepBLOSS-1 and a simplified version, HepBLOSS-2. These models demonstrated excellent performance in predicting seroclearance at 5, 10, and 15 years, with C-indices and time-dependent area under the receiver operating characteristics curve (AUROC) values of 0.81–0.89. The 10-year cumulative incidence rate in patients with scores of ≥13 in HepBLOSS-1 and those with scores of 8 in HepBLOSS-2 was over 50%. Both models underwent rigorous internal and external validation, demonstrating good predictability with time-dependent AUROCs exceeding 0.80. The predicted seroclearance rate closely aligned with the observed rate in both models.

Conclusions: The HepBLOSS models for HBsAg seroclearance exhibited an outstanding ability to stratify the probability of seroclearance over a 15-year period. These models hold promising potential to guide treatment decisions, aiming to achieve a functional cure in patients with CHB.

Introduction

The World Health Organization has endorsed the 2030 elimination target of HBV infection, which affects approximately 296 million people worldwide. A functional cure, characterized by the loss of HBsAg, represents a currently achievable treatment endpoint for chronic hepatitis B (CHB). Patients with HBsAg seroclearance have a significantly lower risk of hepatocellular carcinoma (HCC), liver decompensation, death, or liver transplantation compared with those who remain HBsAg-positive.[1,2]

HBsAg seroclearance is a rare event, with an estimated annual incidence of approximately 1% among treatment-naïve patients with CHB and even lower in those receiving antiviral therapy (AVT).[3,4] Previously, Liu *et al.*[5] proposed a predictive scoring system for seroclearance in HBeAg-negative untreated patients. However, their study had several limitations, including a limited number of covariates analyzed, exclusion of liver cirrhosis (LC), and a focus only on treatment-naïve patients during follow-up. Moreover, they assessed only the baseline HBV DNA level,[5] which may not fully represent an individual's HBV replication activity given the dynamic nature of the disease. Another scoring system introduced by Terrault *et al.*[6] aimed to predict seroclearance over a 3-year period in patients with HBeAg-negative CHB. However, beyond short-term predictions, anticipating remote seroclearance is crucial in guiding decisions about antiviral strategy, with the ultimate goal of achieving a functional cure, especially in patients with low to minimal viral activity.

Therefore, to better facilitate antiviral strategies tailored to the individual likelihood of seroclearance, this study aimed to evaluate the incidence and predictors of HBsAg seroclearance in a large cohort during a long-term follow-up. By incorporating the predictors of seroclearance, we developed new prediction models. To accurately determine viral replication status, we assessed the patients for 2 years after enrollment, considering it as a baseline evaluation period.

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Patients and methods

Patients

A total of 2,762 consecutive patients diagnosed with CHB between 2008 and 2019 from the four medical centers of The Catholic University of Korea were reviewed for eligibility. Eligible patients were those who were HBsAg-positive for >6 months, were HBeAg-negative, had not received AVT, and had undergone more than four HBV DNA tests at intervals of 3–6 months over a 2-year period. Patients were excluded if they met any of the following criteria: (i) follow-up duration <2 years (n = 163); (ii) HCC or LC decompensation developed (n = 45), (iii) started AVT (n = 423), or (iv) HBsAg seroclearance occurred (n = 33) within 1 year of follow-up; (v) co-infection with HCV or HIV (n = 47); or (vi) another malignancy (n = 19). With the same study protocol, 1,046 patients were additionally evaluated at an independent tertiary center in Korea to determine eligibility for external validation (Fig. 1). The index date was when the first HBV DNA was measured. This study was approved by the Institutional Review Board of The Catholic University of Korea (XC22RADI0017).

Antiviral therapy

During the follow-up period, AVT was initiated in accordance with either the guidelines for CHB or the national insurance policy, following standard recommendations.[7] In South Korea, national insurance policies governing AVT initiation for CHB have traditionally been strict, with progressive expansions of coverage criteria over time. For patients with HBeAg-negative CHB, insurance has approved AVT for those with HBV DNA levels ≥2,000 IU/ml and alanine aminotransferase (ALT) levels ≥80 U/L. For patients with LC, initial criteria limited AVT to those with HBV DNA levels ≥20,000 IU/ml and ALT levels ≥80 U/L until 2010. In 2011, the criteria were broadened to include HBV DNA levels ≥2,000 IU/ml and ALT levels ≥40 U/L, and in 2015, the ALT criterion was removed. Most recently, in 2023, coverage expanded to all patients with detectable HBV DNA. For patients outside these criteria, AVT initiation was delayed until they met the requirements because of the high cost of treatment or was offered only to patients who could afford it.

Assessment

Laboratory tests, including virologic markers (HBsAg/anti-HBs/HBeAg/anti-HBe/HBV DNA) were conducted at regular intervals of ≥3–6 months. Serum HBV DNA was tested using a real-time PCR assay (>10 IU/ml; Abbott, Chicago, IL, USA). Quantitative HBsAg measurement was performed using the ARCHITECT assay, standardized to the World Health Organization international standard (0.05–250 IU/ml; Abbott, Chicago, IL, USA). In cases where HBsAg levels were >250 IU/ml, retesting was performed on samples diluted 1:500 and 1:1,000. As indicated by prior research highlighting the predominance of genotype C (>95%) in Korea,[8] routine examinations of HBV genotype were not conducted in clinical practice. Furthermore, we previously revealed that all assessed patients had genotype C disease.[8,9]

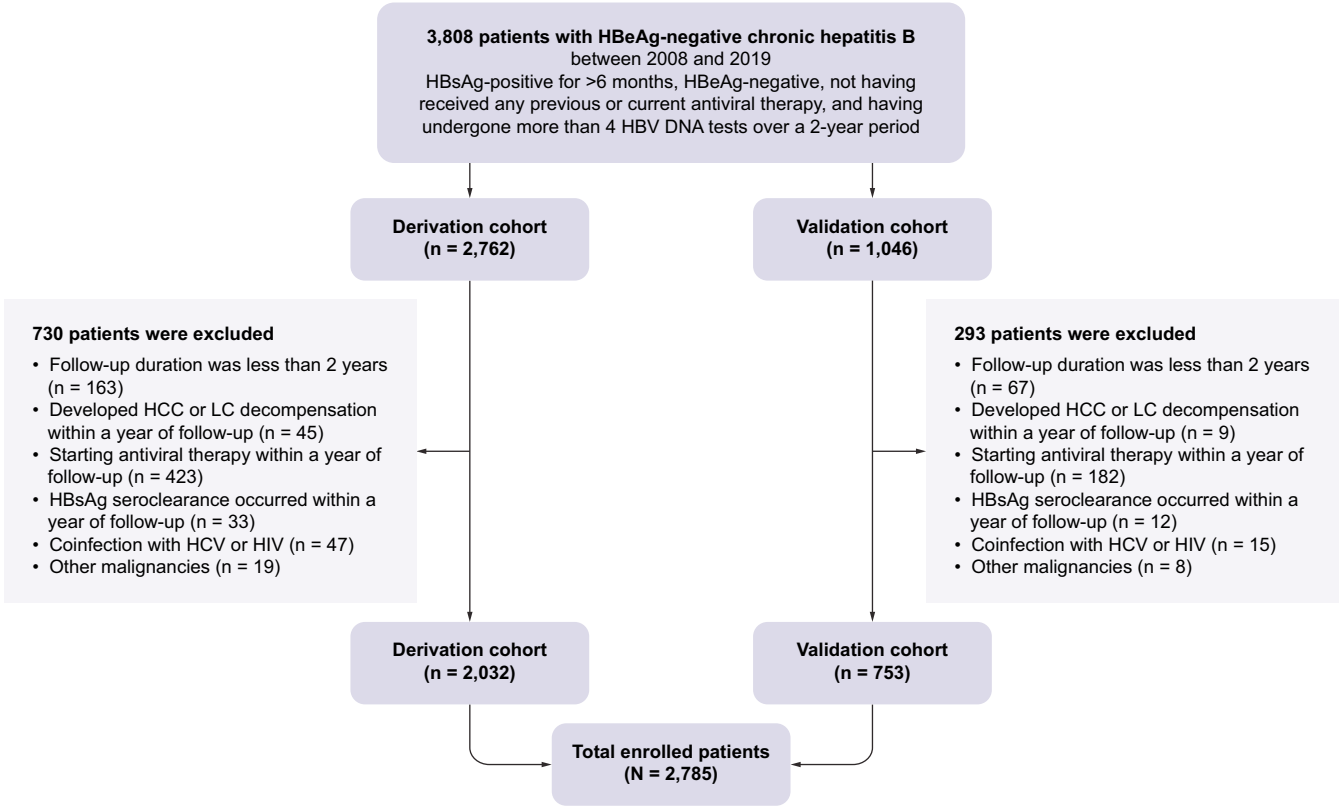


Fig. 1. Flowchart of patient enrollment. HCC, hepatocellular carcinoma; LC, liver cirrhosis.

Endpoints and definitions

The primary outcome was HBsAg seroclearance, defined as HBsAg-negative on two consecutive tests at least 6 months apart.[10] The patients were followed up until the date of seroclearance, January 2023, or their last visit—whichever occurred first—including those resulting from death, liver transplantation, or loss to follow-up.

We implemented a baseline evaluation period of 2 years after study enrollment to delicately assess the viral replication status. The patients were classified into three groups based on the pattern of HBV DNA levels during the 2-year period: (1) the low-level viremia (LLV) group included patients whose HBV DNA levels remained <2,000 IU/ml, (2) the high-level viremia (HLV) group included patients whose HBV DNA levels were ≥2,000 IU/ml during most (>75%) of the tests, and (3) the intermittently high-level viremia (IHV) group included the remaining patients. The presence of LC was defined by the following criteria: surface nodularity on sonography or computed tomography and evidence of portal hypertension, such as thrombocytopenia, splenomegaly, or variceal change.

Statistical analyses

Patient characteristics are described using mean ± SD, median (IQR), or number (%). Categorial data were compared using the Chi-square test or Fisher's exact test, and continuous data were compared using an independent *t* test or the Mann–Whitney *U* test, as appropriate. The Kaplan–Meier method was used to calculate the incidence of seroclearance, and the log-rank test was performed to compare incidence rates among different groups. A Cox proportional hazards model was used to identify independent factors.

Independent predictors identified through multivariate analysis were used to develop prediction models for HBsAg seroclearance. To calculate the score for each factor included in the model, the β coefficient was divided by the smallest β coefficient obtained from the final multivariate analysis. The resulting value was rounded to the nearest integer. The predictive accuracy of the model was assessed using time-dependent area under the receiver operating characteristic curve (AUROC) values as well as Harrell's C-index. Its performance was rigorously evaluated using calibration plots, with internal validation consisting of 1,000 bootstrap resampling iterations and external validation conducted on an independent cohort. A *p* value of <0.05 was considered statistically significant. All statistical analyses were performed using the R statistical package (version 4.2.3; R Foundation for Statistical Computing, Vienna, Austria) (http://www.r-project.org) and SAS (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

The clinical characteristics of the 2,032 enrolled patients are shown in Table 1. The mean age was 48 ± 11 years, and 52.8% were male. During the 2-year baseline period, the HBV DNA level was persistently low in 53.0% of the patients (the LLV group), fluctuated in 31.8% (the IHV group), and was high in 15.2% (the HLV group).

Comparing patients with and without seroclearance, several significant differences were observed. The proportion of patients who achieved seroclearance was greater in the LLV group, and these patients had lower HBsAg levels during the baseline period compared with those without seroclearance.

Table 1. Patient characteristics.

	Total	HBsAg loss	No HBsAg loss	
	(n = 2,032)	(n = 345)	(n = 1,687)	*p*
Age (years)	48 ± 11	51 ± 10	47 ± 11	<0.001
Sex (male)	1,073 (52.8)	217 (62.9)	856 (50.7)	<0.001
HBV DNA group*				<0.001
HLV	309 (15.2)	16 (4.6)	293 (17.4)	
IHV	646 (31.8)	79 (22.9)	567 (33.6)	
LLV	1,077 (53.0)	250 (72.5)	827 (49.0)	
ALT level (≤40 U/L)*	1,137 (56.0)	202 (58.6)	935 (55.4)	0.314
HBsAg level (IU/ml)*	486 (84–3,103)	172 (18–1,034)	582 (112–3,715)	<0.001
Cirrhosis	347 (17.1)	71 (20.6)	276 (16.4)	0.069
Diabetes	171 (8.4)	44 (12.8)	127 (7.5)	0.002
Hypertension	343 (16.9)	77 (22.3)	266 (15.8)	0.004
Fatty liver	434 (21.4)	90 (26.1)	344 (20.4)	0.025
HBV DNA level (IU/ml) at the initial assessment	486 (84 to 3.1 × 10^3)	172 (18 to 1.0 × 10^3)	582 (112 to 3.7 × 10^3)	<0.001
Platelet (10^3/μl)	195 ± 62	186 ± 63	196 ± 61	0.005
Prothrombin time (INR)	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.166
AST (U/L)	29 ± 16	28 ± 16	29 ± 16	0.414
ALT (U/L)	32 ± 25	31 ± 25	32 ± 25	0.348
Total bilirubin (mg/dl)	0.9 ± 0.5	0.9 ± 0.4	0.9 ± 0.5	0.236
Albumin (g/dl)	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	0.216
APRI	0.4 ± 0.4	0.5 ± 0.5	0.4 ± 0.4	0.244
APRI >1.5	49 (2.4)	11 (3.2)	38 (2.3)	0.401
FIB-4	1.6 ± 1.5	1.8 ± 1.6	1.6 ± 1.4	0.003
FIB-4 >3.25	155 (7.6)	31 (9.0)	124 (7.4)	0.352
Initiation of AVT during follow-up	384 (18.9)	12 (3.5)	372 (22.1)	<0.001
Duration from enrollment to AVT initiation (months)	73.0 (43.4–109.0)	70.5 (46.1–101.3)	73.4 (43.0–110.5)	

Data are expressed as mean ± standard deviation, median (interquartile range), or numbers (%). Categorial data were compared using the Chi-square, and continuous data were compared with independent t or Mann–Whitney U tests, as appropriate. *The HBV DNA group was determined based on observations during the 2-year baseline period. The numbers (%) for ALT level represent patients who remained ≤40 IU/L during the 2-year baseline. The HBsAg level represents the value checked within the 2-year baseline period. ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; AVT, antiviral therapy; FIB-4, Fibrosis-4; HBV, hepatitis B virus; HLV, high-level viremia; HR, hazard ratio; IHV, intermittently high-level viremia; LLV, low-level viremia.

The proportion of LC was similar between groups; however, patients who achieved seroclearance had higher proportions of diabetes, hypertension, and fatty liver compared with those who did not. AVT was initiated in 384 patients (18.9%) at a median of 73.0 (IQR 43.4–109.0) months after enrollment.

Changes in HBV DNA and ALT levels

Among patients with detectable HBV DNA levels <2,000 IU/ml at enrollment, 27.3% were classified into the IHV group and 0.8% were classified into the HLV group during the 2-year baseline period. Of the patients with HBV DNA levels ≥2,000 IU/ml at enrollment, 50.2% experienced an episodic decrease to <2,000 IU/ml, whereas the remaining patients persisted in the HLV group (Fig. 2A). During the baseline period, ALT levels remained ≤40 U/L in 65.3%, 51.7%, and 32.4% of the patients in the LLV, IHV, and HLV groups, respectively (Fig. 2B). AVT was initiated in 5.2% of the LLV group, 24.1% of the IHV group, and 55.7% of the HLV group during the follow-up period (Fig. 2C). These findings are also shown in Table S1. Among patients who initiated AVT, 3.1% achieved seroclearance at a median of 140.0 (IQR 95.0–162.6) months; among those without AVT, 20.2% achieved seroclearance at a median of 71.9 (IQR 48.5–104.8) months (Fig. 2D).

Incidence of HBsAg seroclearance

Overall, 345 patients (17.0%) achieved seroclearance at the age of 58 ± 10 years during a mean follow-up of 79.6 ± 44.7 months. The estimated annual incidence rate of seroclearance was 2.22% (15,508 person-years; 95% confidence interval [CI] 2.00–2.47). The cumulative incidence rates of seroclearance at 5, 10, and 15 years were 6.8%, 21.3%, and 36.4%, respectively (Fig. S1).

When stratified by HBV DNA group, the estimated annual incidence rates were 3.14% (95% CI 2.78–3.56) in the LLV group, 1.58% (95% CI 1.26–1.97) in the IHV group, and 0.63% (95% CI 0.37–1.04) in the HLV group (Fig. 3A). Comparing patient groups, the cumulative incidence rate was significantly higher in men than in women, in the older age group than in the younger age group, in patients with a low HBsAg level (<250 IU/ml) than in those with a high HBsAg level (≥250 IU/ml) during baseline, and in patients without AVT than in those with AVT during follow-up (Fig. 3B–E). We also compared the incidence of seroclearance among five groups stratified by HBsAg level during the baseline period. As a result, a significantly different incidence was observed according to the biological gradient of HBsAg levels (p <0.001) (Fig. 3F). The cumulative incidence rates at 10 years were 62.5% for those with an HBsAg level <100 IU/ml and 57.3% for those with a level of 100–250 IU/ml.

Factors associated with HBsAg seroclearance

In the multivariate analysis, the following factors were independently predictive of seroclearance: HBV DNA group, age group, HBsAg level <250 IU/ml, and AVT (Table S2). Male sex was a marginal predictor (p = 0.050). When censoring patients

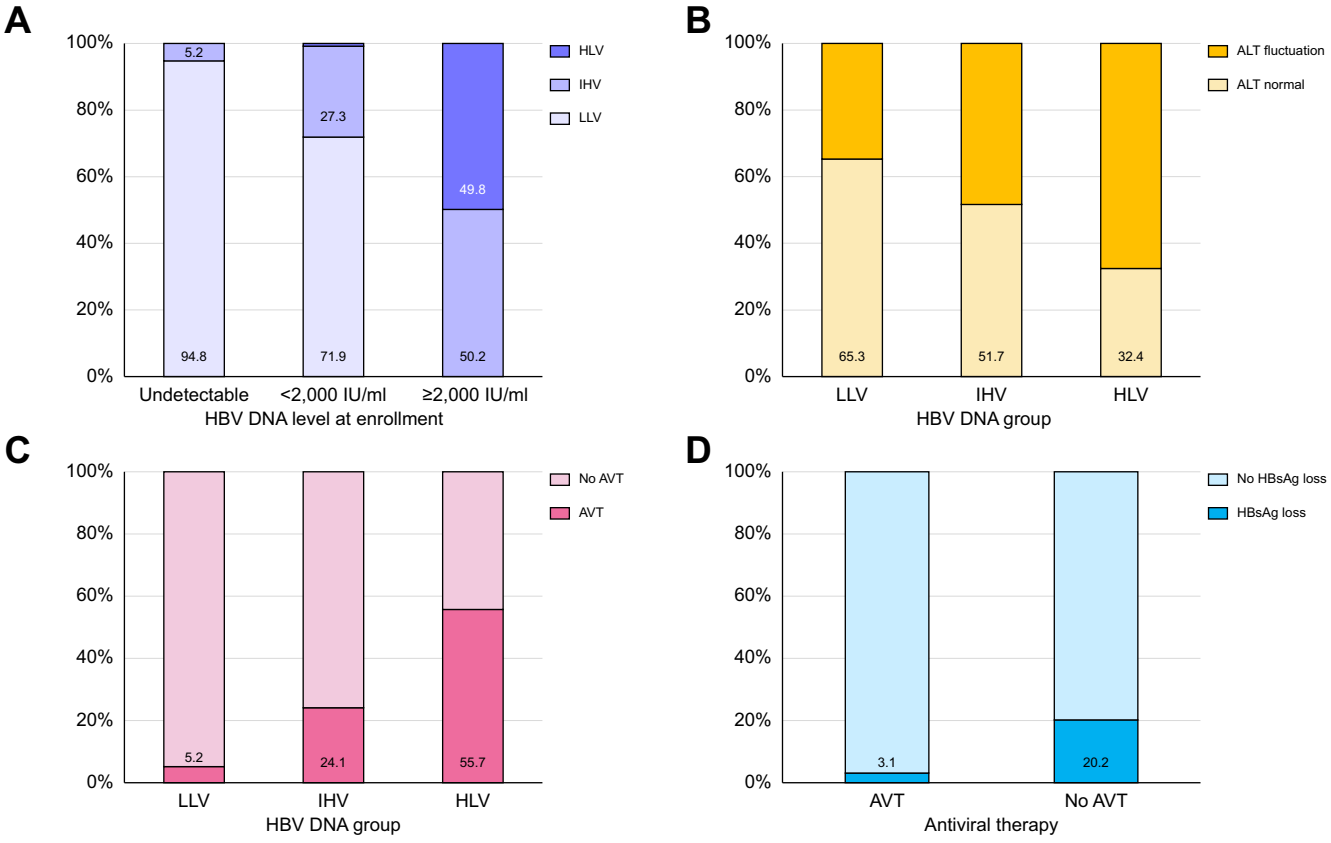
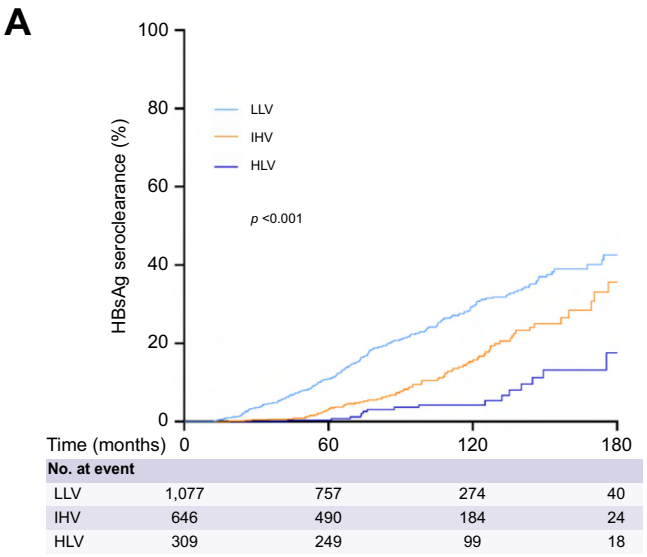
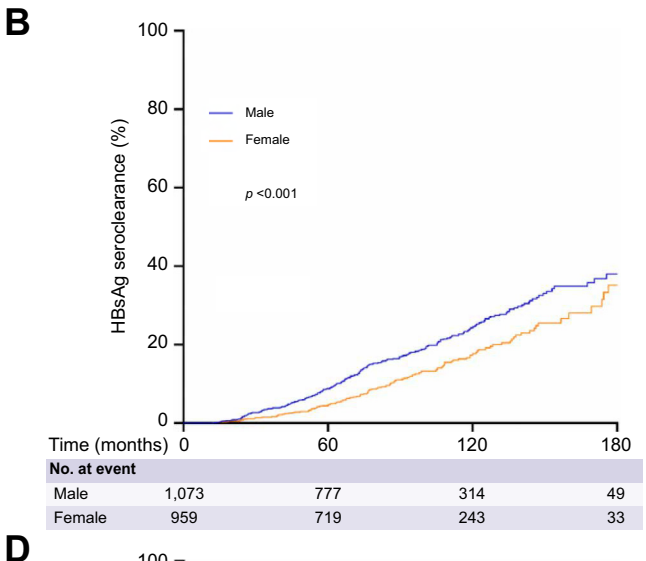


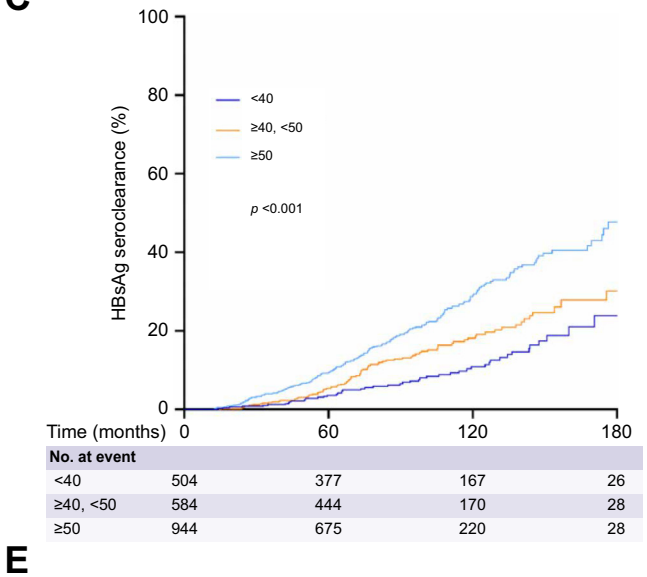
Fig. 2. Proportions of different patient groups. (A) HBV DNA groups according to HBV DNA level at enrollment, (B) patients whose ALT levels remained ≤40 U/L during the baseline period in each HBV DNA group, (C) patients initiating AVT in each HBV DNA group, and (D) patients achieving seroclearance in each group, divided by AVT initiation during the follow-up. ALT, alanine aminotransferase; AVT, antiviral therapy; HLV, high-level viremia; IHV, intermittently high-level viremia; LLV, low-level viremia.



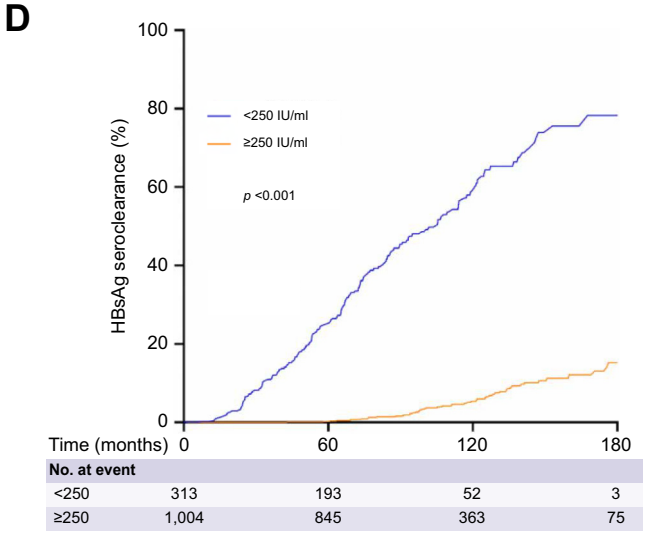
No. at event				
LLV	1,077	757	274	40
IHV	646	490	184	24
HLV	309	249	99	18



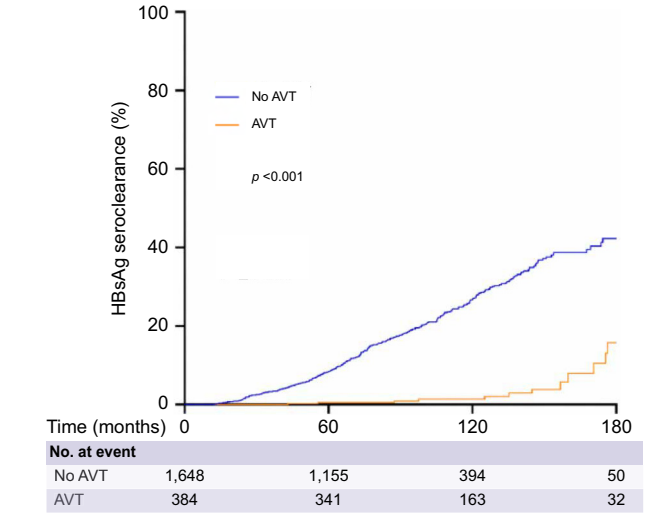
No. at event				
Male	1,073	777	314	49
Female	959	719	243	33



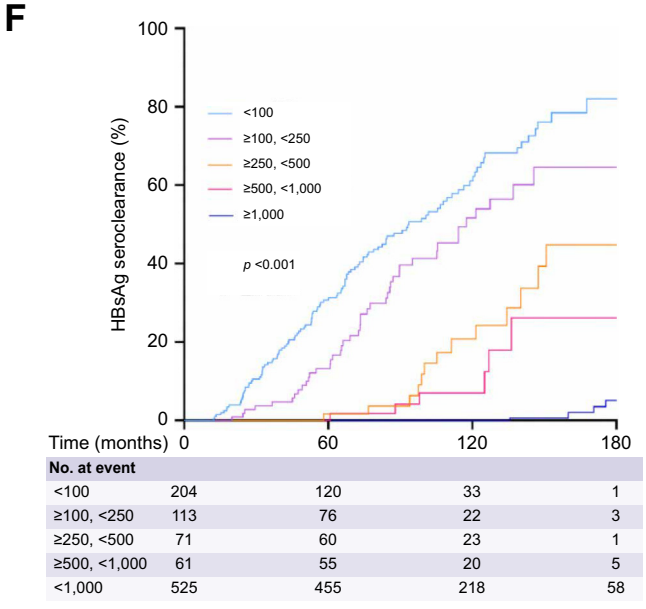
No. at event				
<40	504	377	167	26
≥40, <50	584	444	170	28
≥50	944	675	220	28



No. at event				
<250	313	193	52	3
≥250	1,004	845	363	75



No. at event				
No AVT	1,648	1,155	394	50
AVT	384	341	163	32



No. at event				
<100	204	120	33	1
≥100, <250	113	76	22	3
≥250, <500	71	60	23	1
≥500, <1,000	61	55	20	5
<1,000	525	455	218	58

Fig. 3. Kaplan–Meier curves for the cumulative incidence of HBsAg seroclearance. Data are stratified by (A) HBV DNA group, (B) sex, (C) age group, (D) HBsAg level cut-off of 250 IU/ml, (E) use of AVT, and (F) various HBsAg level cut-offs during the 2-year baseline period. The Kaplan–Meier method was used to calculate the incidence of seroclearance, and the log-rank test was performed to compare incidence rates among different groups. AVT, antiviral therapy; HLV, high-level viremia; IHV, intermittently high-level viremia; LLV, low-level viremia.

Table 2. Factors associated with HBsAg seroclearance.

	Univariate analysis		Multivariate analysis		Univariate analysis*		Multivariate analysis*	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
HBV DNA group†								
HLV	1		1		1		1	
IHV	2.58 (1.29–5.15)	0.007	2.49 (1.24–4.99)	0.010	2.85 (1.13–7.22)	0.027	2.69 (1.06–6.84)	0.038
LLV	4.53 (2.33–8.81)	<0.001	3.45 (1.77–6.74)	<0.001	4.89 (2.01–11.94)	<0.001	3.00 (1.23–7.35)	0.016
Sex (male *vs.* female)	1.47 (1.17–1.83)	<0.001	1.29 (1.03–1.62)	0.026	1.41 (1.05–1.88)	0.020		0.135
Age (years)								
<40	1		1		1		1	
40–49	1.86 (1.29–2.69)	0.001	1.69 (1.16–2.45)	0.006	1.99 (1.24–3.21)	0.005	1.74 (1.07–2.82)	0.025
≥50	3.43 (2.47–4.77)	<0.001	2.23 (1.59–3.13)	<0.001	3.99 (2.61–6.10)	<0.001	1.97 (1.27–3.06)	0.002
Cirrhosis (yes *vs.* no)	1.62 (1.24–2.12)	<0.001		0.098	1.99 (1.40–2.81)	<0.001		0.054
Fatty liver (yes *vs.* no)		0.254				0.330		
Diabetes	1.83 (1.33–2.52)	<0.001		0.219	1.91 (1.23–2.94)	0.004		0.286
Hypertension	1.60 (1.24–2.07)	<0.001		0.175	1.76 (1.28–2.43)	0.001		0.316
ALT (≥40 *vs.* <40 U/L)		0.793				0.606		
Platelet (≥150 *vs.* <150 × 10^3/μl)	0.70 (0.55–0.89)	0.004		0.407	0.72 (0.52–1.00)	0.049		0.996
Total bilirubin (≥1 *vs.* <1 mg/dl)		0.666				0.551		
Albumin (≥4 *vs.* <4 g/dl)		0.946				0.284		
HBsAg level† (<250 *vs.* ≥250 IU/ml)	14.85 (10.62–20.78)	<0.001	11.92 (8.41–16.88)	<0.001	14.91 (10.65–20.86)	<0.001	13.49 (9.46–19.25)	<0.001

A Cox proportional hazards model was used to identify independent factors.
ALT, alanine aminotransferase; HLV, high-level viremia; HR, hazard ratio; IHV, intermittently high-level viremia; LLV, low-level viremia.
*A Cox proportional hazards model was conducted among patients who underwent multiple tests of HBsAg levels during the 2-year baseline period (n = 1,317).
†HBV DNA group and HBsAg level were determined based on observations during the 2-year baseline period.

at the start of AVT, significant predictors of seroclearance were as follows: HBV DNA group, age group, male sex, and HBsAg level <250 IU/ml (Table 2).

During additional analysis, when altering the definition of HLV from "mostly high viremia" to "persistently high viremia" (level remaining ≥2,000 IU/ml during the baseline period), the predictors remained the same, namely, HBV DNA group, age group, sex, and HBsAg level (Table S3). Similarly, when changing the HBsAg cut-off level from 250 to 100 IU/ml, the independent predictors were the HBV DNA group, age group, and HBsAg level (Table S4). In a subgroup analysis of patients who underwent multiple tests for HBsAg levels during the baseline period (n = 1,317), the HBV DNA group, age group, and HBsAg level consistently remained significant factors (Table 2).

Development of prediction models for HBsAg seroclearance

Considering that antiviral agents are typically recommended for patients with high HBV DNA levels, yet only a small proportion of them achieve seroclearance, prediction models were developed with patients censored at the initiation of AVT.

The first prediction model (Model 1) was developed based on independent predictors, including HBV DNA group, age group, sex, and HBsAg level. The total score in the model ranged from 0 to 19 points (Table 3), and the corresponding rates of seroclearance based on these scores are illustrated in Fig. 4A. Harrell's C-index was 0.82 (95% CI 0.80–0.84), and the time-dependent AUROCs were 0.86, 0.81, and 0.86 at 5, 10, and 15 years, respectively (Fig. 4B). The calibration plot of the model demonstrated excellent agreement between the predicted probabilities and observed incidence of seroclearance at 5, 10, and 15 years (Fig. 4C).

The second model (Model 2) was established using a subset of 1,317 patients who underwent multiple tests for HBsAg levels. Model 2 used the same factors, except for sex. The IHV and LLV subgroups were assigned the same scores, as were the age groups of 40–49 and ≥50 years, resulting in a simplified model with two groups for each factor. The total sum of scores ranged from 0 to 8 points (Table 3), and the corresponding rates are shown in Fig. 4D. Simplified Model 2 also exhibited remarkable performance, with a Harrell's C-index of 0.84 (95% CI 0.82–0.87) and time-dependent AUROCs of 0.89, 0.84, and 0.84 at 5, 10, and 15 years, respectively (Fig. 4E). The calibration plot showed excellent agreement at 5, 10, and 15 years (Fig. 4F).

Model-based cumulative seroclearance rates

We named the models **HepBLOSS**, representing **Hep**atitis **B** virus DNA **L**evel, **O**ld age, male **S**ex, and hepatitis B **S**urface antigen level. Patients were classified into three groups based on their seroclearance probability, as calculated by the HepBLOSS model: the low-probability group (annual incidence rate <0.5%), the high-probability group (annual incidence rate ≥5%), and an intermediate group for all other values. In Model 1 (HepBLOSS-1), the annual seroclearance incidence rates were 0.4%, 1.1%, and 6.1% for patients with scores of 0–4, 5–12, and ≥13, respectively. In Model 2 (HepBLOSS-2), the rates were 0.4%, 1.1%, and 8.8% for scores of 0–2, 3–7, and 8, respectively. The cumulative incidence rates in the low-

Table 3. Risk scores for HBsAg seroclearance.

	Model 1		Model 2	
	β	Risk score	β	Risk score
HBV DNA group*				
HLV	Reference	0	Reference	0
IHV	0.87	3	0.89	2
LLV	1.21	5	1.04	2
Age (years)				
<40	Reference	0	Reference	0
40–49	0.50	2	0.52	1
≥50	0.76	3	0.60	1
HBsAg level* (IU/ml)				
≥250	Reference	0	Reference	0
<250	2.43	10	2.54	5
Sex				
Female	Reference	0	–	–
Male	0.25	1	–	–

Independent predictors identified through multivariate analysis were used to develop prediction models for HBsAg seroclearance. To calculate the score for each factor included in the model, the β coefficient was divided by the smallest β coefficient obtained from the final multivariate analysis. The resulting value was rounded to the nearest integer.

HLV, high-level viremia; IHV, intermittently high-level viremia; LLV, low-level viremia.

*The HBV DNA group and HBsAg level were determined based on observations during the 2-year baseline period.

probability group at 5, 10, and 15 years were 0.6%, 2.1%, and 13.5% in HepBLOSS-1 (scores 0–4) and 0.5%, 3.2%, and 13.1% in HepBLOSS-2 (scores 0–2). Conversely, the cumulative incidence rates in the high-probability group of HepBLOSS-1 (score ≥13) were 19.4%, 53.1%, and 72.6%, whereas in HepBLOSS-2 (score of 8), they were 26.9%, 64.6%, and 89.4%, respectively (Fig. 5A, B).

Internal and external validation of prediction models

In the analyses of internal and external validation, both models demonstrated a strong predictive ability for seroclearance. The baseline characteristics of the external validation, which included 753 patients followed up for 84.8 ± 50.4 months, are shown in Table S5. The models exhibited robust discrimination performances, as reflected in the excellent Harrell's C-index and time-dependent AUROCs (Table S6). The calibration plot showed a strong correlation between the predicted probabilities and observed incidence of seroclearance in both validation models (Figs. S2A–D).

Sensitivity analyses

To assess the robustness of the HepBLOSS models, sensitivity analyses were conducted to evaluate their performance in patients, regardless of whether they received AVT during follow-up. The time-dependent AUROCs at 5, 10, and 15 years were 0.87, 0.83, and 0.85 in HepBLOSS-1 and 0.89, 0.86, and 0.81 in HepBLOSS-2, respectively. The models also demonstrated excellent performance in various subgroups of patients, including those enrolled between 2008 and 2012, those enrolled between 2013 and 2019, those without LC, and those with LC (Table S7).

Discussion

This study evaluated predictors of HBsAg seroclearance and developed prediction models for patients with HBeAg-negative CHB. Our HepBLOSS prediction models, incorporating independent predictors such as HBV DNA group, age, sex, and HBsAg level, demonstrated high accuracy in predicting seroclearance over a 15-year period and were rigorously validated internally and externally, along with sensitivity analyses. Notably, this study was conducted on a large cohort, exclusively involving patients with genotype C, which helped mitigate the potential confounding effects of HBV genotype on seroclearance. In addition, the extended mean follow-up period of >6.5 years, with one-quarter of the patients participating in follow-up for >10 years, allowed the identification of 345 patients who achieved seroclearance, surpassing the results of previous studies.[3,6] Consequently, the HepBLOSS models demonstrated robust and accurate prediction of seroclearance over the longest duration ever reported.

The international guideline states that the disease phase of CHB cannot be determined by a single assessment because of the continuous interaction between viral activity and the host immune response.[11] Thus, we implemented a 2-year baseline period to accurately evaluate the viral replication status of patients with CHB. During this period, a significant number of patients experienced a transition between phases of CHB. Notably, approximately 30% of the patients exhibited fluctuating HBV DNA levels below or above 2,000 IU/ml, and 48.3% of them showed ALT elevation, hindering the classification of chronic infection or chronic hepatitis based on a single evaluation. Stratification of distinct HBV DNA groups based on a 2-year baseline period revealed a substantial difference in seroclearance and transition to an active disease phase. These findings underscore the importance of prolonged observation to accurately determine viral replication status, resulting in distinct prognostic outcomes.

This study used a cut-off level of 250 IU/ml for HBsAg, whereas others have commonly used 100 or 1,000 IU/ml.[5,12] This choice was primarily based on the use of the ARCHITECT assay, which offers automated and standardized HBsAg measurements up to 250 IU/ml using an undiluted sample. When comparing the cumulative incidence of seroclearance, patient groups with HBsAg levels <100 and 100–250 IU/ml exhibited >50% incidence with a similar slope, indicating both the 100 and 250 IU/ml levels as excellent predictors. Setting the cut-off level at 250 IU/ml rather than 100 IU/ml provided the advantage of identifying patients with a high likelihood of remote seroclearance earlier, whereas applying lower HBsAg cut-offs may be limited to short-term prediction of seroclearance. When we applied the cut-off level of 100 IU/ml, the same factors were identified as independent predictors of seroclearance, except for sex. Considering these aspects, the selection of a cut-off level of 250 IU/ml for HBsAg was deemed reasonable in this study.

The long-term prediction models developed in this study provide valuable guidance to clinicians, particularly in determining the need for AVT. Patients who are unlikely to achieve seroclearance in our model can be recommended for AVT according to their ALT/HBV DNA levels, whereas those with a low HBsAg level and a high probability of seroclearance can be monitored without immediate initiation of AVT, provided advanced liver fibrosis is excluded. These patients are already experiencing restoration of host immunity toward HBsAg seroclearance, and AVT has a limited impact on seroclearance despite its potent viral suppression.[3,13] In addition, these patients may have a favorable prognosis regarding HCC

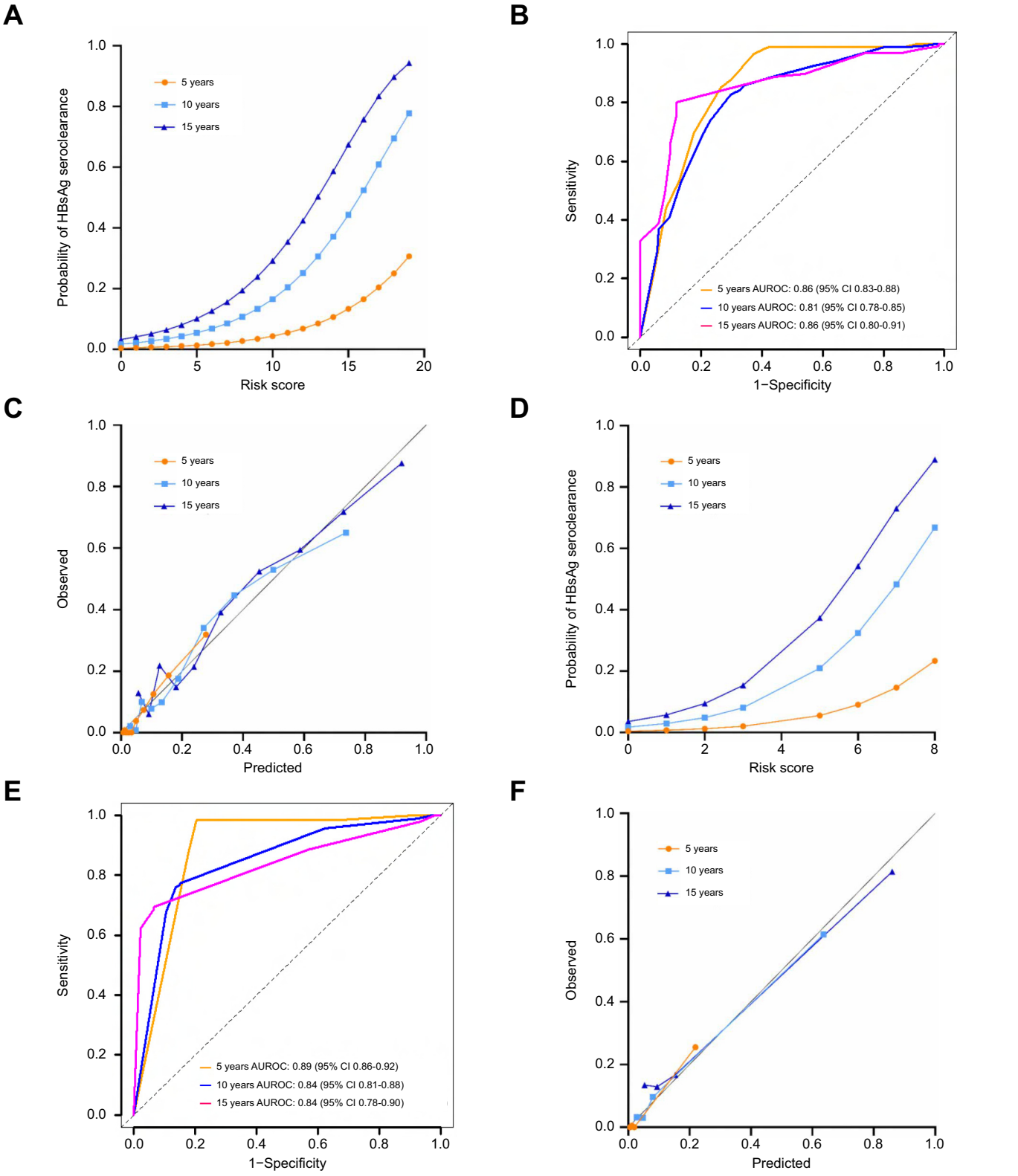


Fig. 4. Predictive performance of the HepBLOSS models. HepBLOSS-1: (A) Nomogram; (B) time-dependent AUROCs at 5, 10, and 15 years; and (C) calibration plots. HepBLOSS-2: (D) nomogram; (E) time-dependent AUROCs at 5, 10, and 15 years; and (F) calibration plots. AUROC, area under the receiver operating characteristic curve; CI, confidence interval.

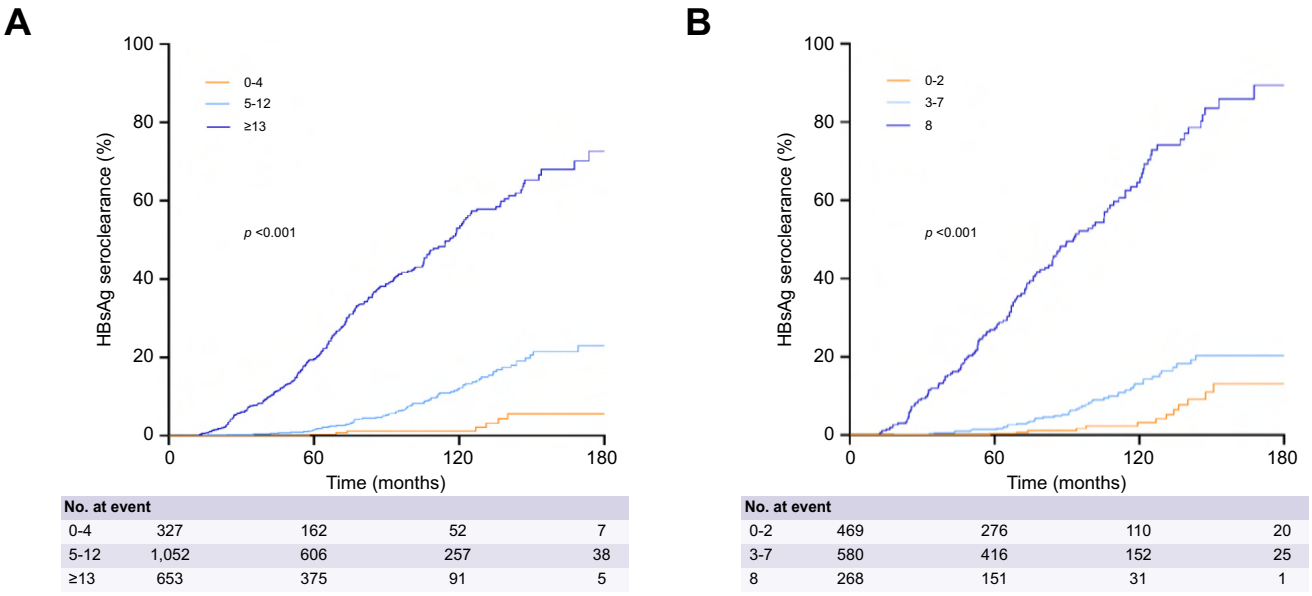


Fig. 5. Kaplan–Meier curves of the cumulative incidence of HBsAg seroclearance. Data are stratified into three score categories based on different probabilities of seroclearance (<0.5%, ≥0.5% to <5%, and ≥5% annual incidence) for (A) HepBLOSS-1 and (B) HepBLOSS-2. The Kaplan–Meier method was used to calculate the incidence of seroclearance, and the log-rank test was performed to compare incidence rates among different groups.

development. Svicher *et al.*[14] demonstrated that HBV integration, which can promote hepatocarcinogenesis, correlated with HBsAg level, whereas the distribution of covalently closed circular DNA (cccDNA) and intrahepatic total HBV DNA, as markers of intrahepatic HBV reservoir, did not significantly differ between persistently low viremia and moderate viremia (HBV DNA level <20,000 IU/ml) in patients with HBeAg-negative CHB. In addition, studies in Western populations showed that most patients with HBeAg-negative CHB and HBV DNA levels ≤10,000–20,000 IU/ml remained inactive and did not require AVT for several years, suggesting prioritization of close monitoring *vs*. AVT.[15,16]

Low levels of HBV DNA and HBsAg are consistently suggested as predictors of seroclearance.[5,17] As HBsAg production is derived from intrahepatic cccDNA and integrated HBV DNA,[18] seroclearance occurs when cccDNA levels or the transcription activity of intrahepatic HBV DNA significantly decreases. Consequently, low levels of HBV DNA and HBsAg observed during this process closely correlate with seroclearance. A close association between old age and HBsAg seroclearance observed in this study is in line with our previous finding of a gradual decrease in HBsAg level according to age.[9] The longer duration of infection in older patients might lead to a greater probability of seroclearance. However, even after achieving seroclearance, there remains a risk of HCC development, and age is an independent predictor of this outcome.[19,20] Therefore, novel therapeutic approaches such as treatments targeting HBV replication cycles, immunotherapeutic agents, or combination treatments with different targets[21] are anticipated for these patients, with the goal of promoting seroclearance or lowering the HBsAg level at an earlier age.

There are some limitations in this study. First, there were inconclusive results regarding the effect of sex on seroclearance, as male sex was not associated with the outcome in subgroup analysis. Previous studies have similarly reported heterogeneous results in this regard,[3,6] highlighting the need for further research to better understand this relationship. Second, because of insurance policy limitations in Korea, not all patients had regular access (<6-monthly intervals) to HBsAg level measurement. Although this might lead to an underestimation of seroclearance, our prediction models still demonstrated excellent predictability, offering a simple calculation using this straightforward cut-off level. Third, as the cohort in this study consisted of a homogeneous population with an exclusive HBV genotype, it is important to validate the generalizability of the prediction models in other populations. Lastly, novel virological markers, such as HBV RNA and HBcrAg, which are currently under active investigation, could not be evaluated as predictors of seroclearance because they are not yet globally available for routine use, and this study was retrospective in nature.

The treatment strategy for patients with CHB is shifting toward achieving HBsAg seroclearance to further reduce detrimental outcomes. In this context, HepBLOSS models incorporating HBV DNA group, age, sex, and HBsAg level serve as useful tools for estimating seroclearance for up to 15 years, with exceptional performance and guidance of clinicians in practical decision-making in opting for close monitoring or AVT. We anticipate these models to be further validated in other cohorts.

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Abbreviations

ALT, alanine aminotransferase; anti-HBe, antibody to HBeAg; anti-HBs, antibody to HBsAg; AUROC, area under the receiver operating characteristic curve; AVT, antiviral therapy; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CI, confidence interval; HCC, hepatocellular carcinoma; HLV, high-level viremia; IHV, intermittently high-level viremia; LC, liver cirrhosis; LLV, low-level viremia.

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Conflicts of interest

The authors of this study declare that they do not have any conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Guarantor of the article: JWJ. Conceptualization: HLL, SHB, JYC, SKY, JWJ. Data curation: HLL, JWJ. Formal analysis: HLL, JWJ. Funding acquisition: HLL, JWJ. Investigation: HLL, JWJ. Methodology: HLL, HY, HN, JWJ. Projection administration: HLL, JWJ. Resources: HLL, SKL, JWH, HY, HN, PSS, HYK, SWL, DSS, JHK, CWK, SHB, JYC, SKY, JWJ. Software: HLL, JWJ. Validation: HLL, JWJ. Visualization: HLL. Supervision: JWJ. Writing—original draft: HLL, JWJ. Writing—review and editing: HLL, JWJ.

Data availability statement

The data for this retrospective cohort study were derived from electronic medical record systems of multicenters in South Korea. The data used in this research are available from the corresponding author upon request, with details on how to request the data.

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Supplementary data

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Author names in bold designate shared co-first authorship

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