Title

High accuracy model for HBsAg loss based on longitudinal trajectories of serum qHBsAg throughout long-term antiviral therapy

Authors

Rong Fan[1#*], M.D., Siru Zhao[1#], M.D., Junqi Niu[2], M.D., Hong Ma[3], M.D., Qing Xie[4], M.D., Song Yang[5], M.D., Jianping Xie[6], M.D., Xiaoguang Dou[7], M.D., Jia Shang[8], M.D., Huiying Rao[9], M.D., Qi Xia[10], M.D., Yali Liu[11], M.D., Yongfeng Yang[12], M.D., Hongbo Gao[13], M.D., Aimin Sun[14], M.D., Xieer Liang[1], M.D., Xueru Yin[1], M.D., Yongfang Jiang[15], M.D., Yanyan Yu[16], M.D., Jian Sun[1], M.D., Nikolai V. Naoumov[17], M.D., Ph.D., Jinlin Hou[1*], M.D., Chronic Hepatitis B Study Consortium

Affiliations

[1]Guangdong Provincial Key Laboratory of Viral Hepatitis Research; Guangdong Provincial Clinical Research Center for Viral Hepatitis; Key Laboratory of Infectious Diseases Research in South China, Ministry of Education; Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China;

[2]Hepatology Unit, No. 1 Hospital affiliated to Jilin University, Changchun, China;

[3]Liver Research Center, Beijing Friendship Hospital, Capital Medical University, Beijing, China;

[4]Department of Infectious Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China;

[5]Beijing Ditan Hospital, Capital Medical University, Beijing, China;

[6]Department of Infectious Diseases, Xiangya Hospital, Central South University, Changsha, China;

[7]Department of Infectious Diseases, Shengjing Hospital of China Medical University, Shenyang, China;

[8]Henan Provincial People's Hospital, Zhengzhou, China;

[9]Peking University Hepatology Institute, Peking University People's Hospital, Beijing, China;

[10]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China;

[11]Beijing Youan Hospital, Capital Medical University, Beijing, China;

[12]The Second Hospital of Nanjing, Nanjing, China;

[13]8th People's Hospital, Guangzhou, China;

[14]Henan Institute of Medical and Pharmaceutical Sciences, Zhengzhou University, Zhengzhou, China;

[15]Liver Disease Research Center, the Second Xiangya Hospital, Central South University, Changsha, China;

[16]Department of Infectious Diseases, First Hospital of Peking University, Beijing, China;

[17] Royal College of Physicians, London, United Kingdom

# These authors contributed equally to this work as the first authors.

Keywords

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Corresponding authors

Jinlin Hou

Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China

Tel: 86-20-61641941

Fax: 86-20-62786534

Email: jlhousmu@163.com

Rong Fan

Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China

Tel: 86-20-62787432

Fax: 86-20-62786534

Email: rongfansmu@163.com

List of Abbreviations

HBsAg, Hepatitis B surface antigen; CHB, chronic hepatitis B; qHBsAg, quantitative HBsAg; HBV, hepatitis B virus; cccDNA, covalently closed circular DNA; HCC, hepatocellular carcinoma; NAs, nucleos(t)ide analogues; siRNA, small interfering RNA; CAMs, core

assembly modulators; NAPs, nucleic acid polymers; LoDA, longitudinal discriminant analysis; MLT, mean-lead time; IQR, interquartile range.

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Abstract

Objective: Hepatitis B surface antigen (HBsAg) loss is the optimal outcome for patients with chronic hepatitis B (CHB) but this rarely occurs with currently approved therapies. We aimed to develop and validate a prognostic model for HBsAg loss on treatment using longitudinal data from a large, prospectively followed, nationwide cohort.

Design: CHB patients receiving antiviral treatment were enrolled from 50 centers in China. Quantitative HBsAg (qHBsAg) testing was prospectively performed biannually per protocol. Longitudinal Discriminant Analysis algorithm was used to estimate the incidence of HBsAg loss, by integrating clinical data of each patient collected during follow-up.

Results: In total, 6792 CHB patients who had initiated antiviral treatment 41.3 (IQR: 7.6, 107.6) months before enrollment and had median qHBsAg 2.9 (IQR: 2.3, 3.3) $\log_{10}$IU/mL at entry were analyzed. With a median follow-up of 65.6 (IQR: 51.5, 84.7) months, the 5-year cumulative incidence of HBsAg loss was 2.4%. A prediction model integrating all qHBsAg values of each patient during follow-up, designated GOLDEN model, was developed and validated. The AUCs of GOLDEN model were 0.981 (95% CI: 0.974-0.987) and 0.979 (95% CI: 0.974-0.983) in the training and external validation sets, respectively, and were significantly better than those of a single qHBsAg measurement. GOLDEN model identified 8.5-10.4% of patients with a high probability of HBsAg loss (5-year cumulative incidence: 17.0-29.1%), and was able to exclude 89.6-91.5% of patients whose incidence of HBsAg loss is 0. Moreover, the GOLDEN model consistently showed excellent performance among various subgroups.

Conclusion: The novel GOLDEN model, based on longitudinal qHBsAg data, accurately predicts HBsAg clearance, provides reliable estimates of functional HBV cure and may have the potential to stratify different subsets of patients for novel anti-HBV therapies.

Summary Box

What is already known about this subject:

- Hepatitis B surface antigen (HBsAg) loss is the optimal treatment outcome in chronic hepatitis B (CHB) but this is rarely achieved at present.
- A growing body of literature have investigated the factors associated with HBsAg loss, including traditional virological biomarkers like HBsAg and hepatitis B virus (HBV) RNA, and novel biomarkers like HBV haplotype number, transforming growth factor-β, etc.
- Prospective studies combining the information of multiple timepoints to determine the real longitudinal trajectory of probability of HBsAg loss based on a large real-life cohort were limited.

What are the new findings?

- We developed and validated a model to predict HBsAg loss on treatment based on longitudinal data from a prospectively followed, nationwide CHB cohort.
- The prediction tool, designated the GOLDEN model, integrated all qHBsAg values of each patient during follow-up and showed excellent performance both in the training and in the validation sets.
- The new model outperformed the qHBsAg data at enrolment in predicting HBsAg loss with greater AUC, sensitivity, and specificity among the overall population and in different subgroups.

How might it impact on clinical practice in the foreseeable future?

The key strength of the GOLDEN model is the comprehensive integration of all qHBsAg

values for each patient over time, which accurately defines the long-term dynamics in qHBsAg. This prediction model enables reliable and accurate estimate of achieving HBV functional cure and will be useful in clinical practice for personalized management of CHB patients.

Introduction

Chronic hepatitis B virus (HBV) infection remains a global public health challenge that affects nearly 300 million persons worldwide.[1] Hepatitis B surface antigen (HBsAg) loss is now generally agreed as the optimal goal for treatment of patients with chronic hepatitis B (CHB).[2-4] The importance of HBsAg loss was emphasized by marked suppression of HBV replication and covalently closed circular DNA (cccDNA) transcription, along with a further reduction in the risk of hepatocellular carcinoma (HCC) and liver complications.[5,6]

Currently, two approved treatment strategies are available with pegylated interferons or nucleos(t)ide analogues (NAs), which suppress HBV replication and slow the disease progression. However, both approaches offer limited efficacy in achieving HBsAg loss,[7,8] which emphasizes the urgent need for novel therapies to achieve sustained HBsAg loss, regarded as functional HBV cure.[2] Recently, there has been an encouraging progress in the development of novel antiviral agents, such as antisense oligonucleotides, small interfering RNA (siRNA), core assembly modulators (CAMs), nucleic acid polymers (NAPs), etc, which may be used in combination with NAs and possibly immunomodulatory therapies to achieve HBsAg clearance in a significant proportion of CHB patients.[9,10] Moreover, the new therapies are usually added to the background of a long-standing NA treatment.

The clinical trial evaluation of these novel approaches, alone or in combination, further enhanced the need of a prognostic model to better estimate the likelihood of HBsAg clearance. The key parameter most frequently associated with loss of HBsAg is low serum HBsAg levels as determined by quantitation of HBsAg (qHBsAg), as well as other characteristics such as older age, non-Asian ethnicity, serum HBV RNA or HBcrAg levels.[11,12] Several

prediction models have recently been proposed based on the above parameters at pretreatment, or using the qHBsAg reduction after treatment initiation.[13,14] However, these models do not consider that the chance of HBsAg clearance is consistently changing over a long-term period in relation to the response to treatment. Therefore, it is necessary to develop a new, comprehensive model with greater accuracy that uses longitudinal data and multiple qHBsAg measurements over time, depending on the trajectory of qHBsAg.

Therefore, we conducted the present study aiming to 1) develop and validate an accurate prediction model for HBsAg loss by using patients' longitudinal data during long-term follow-up, and 2) create a user-friendly web calculator in a prospective multicenter observational CHB cohort.

Methods

Study population

Search-B cohort is a prospective multicenter observational cohort of CHB patients in China (ClinicalTrials.gov NCT02167503). The detailed information of Search-B cohort had been published elsewhere in detail.[15-18] Briefly, it includes 10,175 adult CHB patients with or without cirrhosis, who were recruited from May 2014 to January 2018 from 50 centres in 16 provinces in China, among which 3,539 patients were the participants of China subgroup who completed the REALM study (NCT00388674) in 2016. The REALM study is a global randomized controlled trial to prospectively assess the long-term outcomes associated with entecavir (ETV) therapy as compared to other antivirals approved for the treatment of chronic HBV infection.[19] Patients with decompensated cirrhosis, HCC; liver transplantation; or coinfection(s) with hepatitis D, hepatitis C or human immunodeficiency virus were excluded

at enrollment. The diagnostic criteria of cirrhosis are shown in the Supplementary Material. The cut-off for data included in this analysis was as of July 2023.

Follow-up

All patients in the Search-B cohort received antiviral therapy at the discretion of their physicians according to the recommendations of the national and/or international guidelines before or at enrollment, and subsequently were followed biannually per protocol. Biochemical, hematological and HBV-related virological assessments were performed for all patients at each visit. HBV-related virological markers were measured in the central laboratory located in Nanfang Hospital, the detection platform of which had been reported previously.[11,20] qHBsAg levels were quantified using the platform of Abbott ARCHITECT or Roche COBAS E601 (dynamic range from 0.05 to 250 IU/mL), with a high correlation and close agreement.[21,22] Samples with qHBsAg >250 IU/mL were retested after a dilution of 1:500.

Longitudinal Discriminant Analysis (LoDA) algorithm for predicting HBsAg loss

The endpoint for the analysis in the current study was HBsAg loss, which was defined as having persistently negative results (< 0.05 IU/ml) at two timepoints with at least 6 months apart. The LoDA algorithm based on multivariate linear mixed effect models (MLMM), which has previously been described,[17,23-25] was used to develop the prediction model for HBsAg loss using the longitudinal profiles of patient biomarkers. For patients who achieved or did not achieve HBsAg loss, two distinctive MLMMs were fitted, separately. The LoDA approach then calculated a probability that a new patient would achieve HBsAg loss by assessing which of the 2 average profiles (HBsAg loss /non- HBsAg loss) the patient was closest to. The probability, or predicted value, changed consequently when a new follow-up

observation was recorded for the same patient. In our study, a patient was identified as likely to achieve HBsAg loss (expected to realize HBsAg clearance by the end of follow-up) once their probability exceeded a fixed and pre-specified threshold once, which was defined as favorable group. The other patients were allocated as unfavorable group **(Figure S1)**. The performance of LoDA was also evaluated with mean-lead time (MLT), measured as the average time at which the LoDA model assign favorable result before achieving real HBsAg loss. Full details are provided in the Supplementary material.

Statistical analyses

The statistical analysis was performed using R (Version 4.2.0, Vienna, Austria). All patients included in the analysis had two or more qHBsAg evaluations at least six-month apart. The training set to derive the longitudinal HBsAg model included patients from Nanfang Hospital with the largest sample size in the Search-B cohort. The patients from the other 49 centres of Search-B cohort were used as the external validation set. Five-fold cross-validation were used to evaluated the impact of data clusters on the model performance. To visualize changes in qHBsAg levels over time, the generalized additive model for longitudinal trajectory of qHBsAg was fitted with the mgcv package (Version 1.8-40). The LoDA algorithm was implemented using the mixAK package (Version 5.4). The cumulative incidences of HBsAg loss at year 5 were estimated by the Kaplan-Meier (K-M) method and compared using the log-rank test. The receiver operating characteristic (ROC) curve was used to evaluate the prediction accuracy of the model. Model calibration was evaluated by calibration curve. Based on a sample size estimation method developed specifically for clinical predictive models by Richard et al.,[26]we use the pmsampsize package in the R language to estimate a

minimum sample size **(Figure S2)**.

The study was approved by the Ethics Committee of Nanfang Hospital and was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of good clinical practice. All patients provided written informed consent to have their data used (anonymously) for research purposes.

Results

In total, 6792 patients with 72,423 visits were included in the analysis, after excluding 615 patients who were lost to follow up or achieved HBsAg loss within 6 months after enrollment, 123 patients who already were HBsAg negative, 1235 patients treated with two or more NAs at enrollment, and 1410 patients treated with interferon therapy before or after enrollment in this study **(Figure S3)**. At enrollment, the median age of patients was 43.5 (interquartile range [IQR]: 36.2, 51.9) years, with 78.7% (5345/6792) of males; 17.0% (1152/6792) of patients were diagnosed with cirrhosis; the median duration of treatment before enrollment was 41.3 (IQR: 7.6, 107.6) months, with qHBsAg of 2.9 (IQR: 2.3, 3.3) $\log_{10}$ IU/mL **(Table 1)**. During a median prospective follow-up of 65.6 (IQR: 51.5, 84.7) months, 173 patients achieved sustained HBsAg loss with a 5-year cumulative incidence of 2.4% **(Figure S4)**.

	Overall (n=6792)	Training set			Validation set		
		non-HBsAg loss (n=2774)	HBsAg loss (n=61)	*p* value	non-HBsAg loss (n=3845)	HBsAg loss (n=112)	*p* value
Age, years	43.5 (36.2, 51.9)	39.9 (33.2, 47.7)	41.3 (37.3, 47.6)	0.159	45.9 (38.9, 54.5)	45.9 (40.4, 52.8)	0.800
Males, n (%)	5345 (78.7)	2237 (80.6)	48 (78.7)	0.703	2969 (77.2)	91 (81.3)	0.315
Cirrhosis, n (%)	1152 (17.0)	607 (21.9)	8 (13.1)	0.100	528 (13.7)	9 (8.0)	0.083
PLT, $\times 10^3/mm^3$	179.0 (137.0, 219.0)	186.0 (142.0, 225.0)	192.0 (150.0, 219.0)	0.501	174.0 (134.0, 214.0)	167.0 (138.0, 212.0)	0.980
ALT, IU/L	26.0 (19.0, 39.0)	28.0 (20.0, 41.0)	27.0 (19.8, 36.8)	0.397	25.2 (18.0, 37.0)	22.1 (16.4, 34.8)	0.046
qHBsAg, log_{10} IU/mL	2.9 (2.3, 3.3)	3.0 (2.6, 3.4)	1.6 (0.4, 2.3)	<0.001	2.8 (2.3, 3.2)	0.8 (-0.4, 1.8)	<0.001
0.05-100 IU/mL, n (%)	955/6765 (14.1)	213/2773 (7.7)	39/60 (65.0)		615/3821 (16.1)	88/111 (79.3)	
100-1000 IU/mL, n (%)	3012/6765 (44.5)	1155/2773 (41.6)	14/60 (23.3)		1821/3821 (47.7)	22/111 (19.8)	
≥1000 IU/mL, n (%)	2798/6765 (41.4)	1405/2773 (50.7)	7/60 (11.7)		1385/3821 (36.2)	1/111 (0.9)	
Undetectable HBV DNA, n (%)	4338/6772 (64.1)	1463/2773 (52.8)	49 (80.3)	<0.001	2728/3827 (71.3)	98/111 (88.3)	<0.001
HBeAg positivity, n (%)	2162/6773 (31.9)	1039/2773 (37.5)	7 (11.5)	<0.001	1108/3828 (28.9)	8/111 (7.2)	<0.001

HBV: hepatitis B virus; ALT: alanine aminotransferase; PLT: Platelet; qHBsAg: Quantitative hepatitis B surface antigen; HBeAg: hepatitis B e antigen; IFN: interferon; NA: nucleos(t)ide analogues; ETV: Entecavir; TDF: Tenofovir disoproxil fumarate. * Participants who were just started antiviral therapy at enrollment; #: including participants treated with other NAs or two NAs.

Table 1: Clinical characteristics of patients at enrollment

	Overall (n=6792)	Training set			Validation set		
		non-HBsAg loss (n=2774)	HBsAg loss (n=61)	*p* value	non-HBsAg loss (n=3845)	HBsAg loss (n=112)	*p* value
Treatment naïve*	449 (6.6)	327 (11.8)	3 (4.9)	0.004	118.0 (3.1)	1.0 (0.9)	0.047
Antiviral therapy at enrollment, n (%)				0.046			0.673
ETV	5592 (82.3)	2181 (78.6)	43 (70.5)		3275 (85.2)	93 (83.0)	
TDF	339 (5.0)	257 (9.3)	8 (13.1)		73 (1.9)	1 (0.9)	
Other NAs#	861 (12.7)	336 (12.1)	10 (16.1)		497 (12.9)	18 (16.1)	
Treatment duration prior to enrollment, months	41.3 (7.6, 107.6)	10.2 (3.0, 32.2)	24.8 (9.9, 64.2)	<0.001	104.1 (30.3, 112.0)	106.4 (64.5, 112.9)	0.038

HBV: hepatitis B virus; ALT: alanine aminotransferase; PLT: Platelet; qHBsAg: Quantitative hepatitis B surface antigen; HBeAg: hepatitis B e antigen; IFN: interferon; NA: nucleos(t)ide analogues; ETV: Entecavir; TDF: Tenofovir disoproxil fumarate. * participants who were just started antiviral therapy at enrollment; # including participants treated with other NAs or two NAs.

Table 1 (Continued.)

Clinical characteristics of patients achieving HBsAg loss

At enrollment, patients who achieved HBsAg loss in the training (n=2835) and validation sets (n=3957) had significantly lower levels of qHBsAg, a lower proportion were HBeAg positive, a higher proportion of patients had undetectable serum HBV DNA. There was no difference between patients with or without HBsAg loss with respect to median age, proportion of male, as well as the levels of platelets **(**p >0.05, **Table 1)**. The clinical characteristics of patients in Search-B cohort over time were shown in the Table S1.

Longitudinal qHBsAg dynamics

The longitudinal dynamics in the proportion of patients stratified according to qHBsAg during the 5-year follow-up, demonstrated a progressive decrease in the subgroup of patients with qHBsAg >1000 IU/ml (from 41.4% to 29.9%) with concomitant increase in those with qHBsAg <100 IU/ml (from 14.1% to 21.5%), and a smaller increase in the subgroup with qHBsAg 100 - 1000 IU/ml (from 44.5% to 48.6%, **Figure 1A**). To further analyze the correlation of the dynamic change of qHBsAg levels and HBsAg loss, we plotted time backward from the time of achieving HBsAg loss or the last follow-up and presented smoothed mean profiles with 95% confidence interval (CI) separately for patients who achieved HBsAg loss and those who did not in the training and validation sets, using the generalized additive models. The exploratory plot of qHBsAg over time showed a particularly clear separation in the average qHBsAg profiles over time between patients who achieved HBsAg loss and those who did not in both sets. The qHBsAg level showed a progressive decline among patients achieving HBsAg loss during up to 5 years of follow-up, while there was no reduction of qHBsAg level for patients in the non-HBsAg loss group over time

(Figure 1B and C).

Derivation and validation of the longitudinal prediction model for HBsAg loss

We evaluated the performance of various combinations of the clinical variables, which differentiated the groups of patients with or without HBsAg loss using the LoDA algorithm **(Table 2)**. Since the addition of other variables did not substantially improve the predictive power, the LoDA model was confined to longitudinal data of qHBsAg only, called the GOLDEN model, with an AUC of 0.981 (95% CI: 0.974-0.987) and 0.979 (95% CI: 0.975-0.997) in the training and validation sets, respectively **(Figure 2A)**.

A patient was classified as being in the favorable group of achieving HBsAg loss (as assigned by the GOLDEN model) if they had a score higher than a threshold of 0.95 (Youden Index) at any timepoint during their follow-up evaluation. In the training set, our model classified 91.5% (2594/2835) of CHB patients in the unfavorable group and 8.5% (241/2835) in the favorable group, with a 5-year cumulative HBsAg loss rate of 0 and 17.0%, respectively ($p<0.0001$). The significant difference between the unfavorable and favorable groups in the incidence of future HBsAg loss was also confirmed in the validation set (0 vs. 29.1%, $p<0.0001$; **Figure 2B**). Among the overall population, the GOLDEN model correctly identified 98.8% of patients who achieved HBsAg loss (sensitivity) and over 90% of patients who did not achieve HBsAg loss (specificity). 26.2% of patients predicted to achieve HBsAg loss actually achieved HBsAg loss (PPV), whereas 100% of patients whose probability of HBsAg loss never increased to greater than the optimal threshold of 0.95, did not achieve HBsAg loss (NPV) (**Table 3, Figure 2C).** The stable AUC values in data clusters generated by five-fold cross-validation were showed in **Table S2**. The calibration plots for the 5-year probability of

remaining HBsAg positive were performed well (**Figure S5**).

Combinations	Training Set			Validation Set		
	Overall (n=2835)	**Cirrhosis (n=615)**	**Non-cirrhosis (n=2220)**	**Overall (n=3957)**	**Cirrhosis (n=537)**	**Non-cirrhosis (n=3420)**
qHBsAg	0.981 (0.974-0.987)	0.989 (0.981-0.998)	0.979 (0.971-0.987)	0.979 (0.974-0.983)	0.986 (0.975-0.997)	0.978 (0.973-0.982)
qHBsAg + Treatment duration of NAs	0.980 (0.974-0.987)	0.989 (0.980-0.997)	0.978 (0.971-0.986)	0.977 (0.972-0.982)	0.986 (0.976-0.996)	0.976 (0.971-0.981)
qHBsAg + HBV DNA	0.980 (0.973-0.986)	0.986 (0.976-0.996)	0.978 (0.970-0.986)	0.977 (0.972-0.982)	0.984 (0.973-0.995)	0.976 (0.970-0.981)
qHBsAg + Treatment duration of NAs + HBV DNA	0.982 (0.976-0.987)	0.988 (0.980-0.996)	0.980 (0.974-0.987)	0.974 (0.969-0.979)	0.985 (0.975-0.996)	0.972 (0.966-0.977)

NA: nucleos(t)ide analogues; qHBsAg: Quantitative hepatitis B surface antigen

Note: The longitudinal serum biomarker qHBsAg and HBV DNA were log transformed for the analysis.

Table 2: The AUC (95% CI) of different candidate clinical variables combinations for predicting HBsAg loss by using LoDA algorithm

Subgroup analysis

We further analyzed the predictive accuracy of the GOLDEN model in different subgroups of patients (**Table 3).** Among all patients, our model performed well in most cases regardless of sex, age, with or without drug withdrawal, and treatment duration before enrollment, as well as qHBsAg levels at enrollment, with the AUCs ranging from 0.894 to 0.997.

Notably, among patients with qHBsAg levels of 0.05-100, 100-1000, and ⩾1000 IU/mL at enrollment, the K-M curves showed that the GOLDEN model could clearly further separate patients into two groups with significant differences of probability of achieving HBsAg loss **(Figure 3)**. We further evaluated the discrimination ability of the GOLDEN model among patients with measurement of qHBsAg levels of 0.05-10, 20, and 50 IU/mL at 2-year follow-up in predicting HBsAg loss afterward, by using the longitudinal qHBsAg data within 2-year follow-up backward. The results showed that the GOLDEN model exhibited consistently excellent performance in identifying the subgroup of patients who have a higher probability of achieving HBsAg loss among traditionally defined high HBsAg clearance probability patients **(Figure 4)**.

	N	HBsAg loss, n (%)	AUC (95% CI)	SEN	SPE	PPV	NPV	ACC	MLT, months
Overall	6792	173 (2.5)	0.980 (0.976-0.983)	0.988	0.927	0.262	1.000	0.929	18.936
Data set									
Training set	2835	61 (2.2)	0.979 (0.974-0.983)	0.967	0.934	0.245	0.999	0.935	21.319
Validation set	3957	112 (2.8)	0.981 (0.974-0.987)	1.000	0.922	0.272	1.000	0.924	17.680
Sex									
Male	5345	139 (2.6)	0.978 (0.974-0.983)	0.986	0.925	0.258	1.000	0.926	20.538
Female	1447	34 (2.3)	0.985 (0.979-0.991)	1.000	0.938	0.279	1.000	0.939	12.479
Age, years									
>45	4352	108 (2.5)	0.980 (0.975-0.984)	1.000	0.934	0.278	1.000	0.935	18.740
≤45	3761	93 (2.5)	0.963 (0.949-0.976)	0.839	0.940	0.261	0.996	0.937	17.951
Cirrhosis									
Yes	1152	17 (1.5)	0.987 (0.981-0.994)	1.000	0.944	0.210	1.000	0.944	19.646
No	5640	156 (2.8)	0.978 (0.974-0.982)	0.987	0.924	0.270	1.000	0.926	18.857
HBeAg status									
Positive	2400	17 (0.7)	0.894 (0.825-0.963)	0.588	0.941	0.066	0.997	0.938	33.186
Negative	5799	170 (2.9)	0.983 (0.980-0.987)	0.988	0.944	0.346	1.000	0.945	17.601

SEN: sensitivity; SPE: specificity; PPV: positive predictive value; NPV: negative predictive value; ACC: accuracy; MLT: mean-lead time; CI: confidence Interval; HBeAg: hepatitis B e antigen; qHBsAg: Quantitative hepatitis B surface antigen; * treated with interferon therapy before or after enrollment.

Table 3: Subgroup analysis of GOLDEN model in the overall patients

	N	HBsAg loss, n (%)	AUC (95% CI)	SEN	SPE	PPV	NPV	ACC	MLT, months
Treatment duration at enrollment*									
naive	449	4 (0.9)	0.987 (0.965-1.000)	1.000	0.903	0.085	1.000	0.904	30.275
<1 year	1607	22 (1.4)	0.991 (0.986-0.996)	1.000	0.948	0.212	1.000	0.949	11.269
1-5 years	1768	45 (2.5)	0.979 (0.971-0.987)	0.956	0.944	0.309	0.999	0.945	19.963
5-10 years	2428	87 (3.6)	0.975 (0.969-0.981)	1.000	0.909	0.291	1.000	0.913	18.936
>10 years	540	15 (2.8)	0.970 (0.956-0.985)	1.000	0.909	0.238	1.000	0.911	24.211
Drug withdrawal									
Yes	299	28 (9.4)	0.973 (0.955-0.992)	0.964	0.926	0.574	0.996	0.930	21.555
No	6493	145 (2.2)	0.981 (0.977-0.984)	0.993	0.927	0.238	1.000	0.929	18.444
qHBsAg level at enrollment, IU/mL									
0.05-100	955	127 (13.3)	0.946 (0.931-0.960)	0.992	0.757	0.385	0.998	0.788	21.032
100-1000	3012	36 (1.2)	0.985 (0.979-0.991)	0.972	0.954	0.202	1.000	0.954	9.746
≥1000	2798	8 (0.3)	0.997 (0.996-0.999)	1.000	0.952	0.057	1.000	0.952	25.195

SEN: sensitivity; SPE: specificity; PPV: positive predictive value; NPV: negative predictive value; ACC: accuracy; MLT: mean-lead time; CI: confidence Interval; HBeAg: hepatitis B e antigen; qHBsAg: Quantitative hepatitis B surface antigen.

Table 3 (Continued.)

Early identification of favorable patients by using the GOLDEN model

MLT is an assessment of the ability of early prediction, measured as the average time at which the LoDA model assign favorable result before achieving real HBsAg loss. The MLT of GOLDEN model could achieve 18.94 months **(Table 3)**, with 61.99% (106/171) patients predicted more than 1 year earlier and another 38.0% (65/171) patients predicted at a median of 6.07 months earlier. Moreover, to identify the favorable patients as earlier as possible, a comparison analysis of the clinical characteristics at enrollment was conducted between the favorable and unfavorable groups **(Table S3)**. The results revealed that the favorable patients identified by the GOLDEN model were among those who were non-cirrhotic with undetectable serum HBV DNA, longer duration of antiviral treatment and a lower level of qHBsAg.

Comparison between the GOLDEN model performance with the qHBsAg level alone at different timepoints in predicting future HBsAg loss

Next, we evaluated the predictive value of key clinical characteristics at enrollment with Cox regression analysis. The univariate analysis identified that the qHBsAg level (log_{10}IU/ml, Hazard ratio [HR]: 0.293, 95% CI: 0.251-0.341), HBeAg negativity (positive vs. negative, HR: 0.223, 95% CI: 0.102-0.491) and undetectable serum HBV DNA (undetectable vs. detectable, HR: 3.273, 95% CI: 1.740-6.156) were associated with a higher incidence of HBsAg loss (**Table S4).** In the multivariable Cox regression analysis, only the qHBsAg level at enrollment was significantly associated with HBsAg loss with an adjusted HR of 0.306 (95% CI 0.259-0.360) **(Table S4).** The qHBsAg level was the only associated predictor among patients with qHBsAg of 0.05-100 IU/mL at enrollment in the training set

(Table S5).

The predictive performance of the GOLDEN model was then compared with the qHBsAg level alone. The results showed that the GOLDEN model displayed significantly greater performance than using only qHBsAg level at enrollment in predicting future HBsAg clearance, as demonstrated by its consistently higher AUC **(Table 4),** sensitivity, and specificity **(Table S6)** among the overall population and in different subgroups. Additionally, it was found that the performance of the GOLDEN model was better than the qHBsAg level alone at 1 year (AUC: 0.969 vs. 0.980, p=0.021) or 2 years (AUC: 0.910 vs. 0.980, p<0.001) before HBsAg loss or the last follow-up, and continued to improve over time along with the increase of longitudinal data timepoints, in terms of values of AUC **(Figure S6)**.

	N	HBsAg loss, n (%)	GOLDEN model	qHBsAg at enrollment	*p* value
Overall	6792	173 (2.5)	0.980 (0.976-0.983)	0.882 (0.851-0.913)	<0.001
Data set					
Training set	2835	61 (2.2)	0.981 (0.974-0.987)	0.845 (0.777-0.914)	<0.001
Validation set	3957	112 (2.8)	0.979 (0.974-0.983)	0.901 (0.971-0.930)	<0.001
Sex					
Male	5345	139 (2.6)	0.978 (0.974-0.983)	0.887 (0.853-0.922)	<0.001
Female	1447	34 (2.3)	0.985 (0.979-0.991)	0.863 (0.793-0.933)	<0.001
Age, years					
>45	4352	108 (2.5)	0.980 (0.975-0.984)	0.899 (0.861-0.937)	<0.001
≤45	3761	93 (2.5)	0.963 (0.949-0.976)	0.875 (0.828-0.921)	<0.001
Cirrhosis					
Yes	1152	17 (1.5)	0.987 (0.981-0.994)	0.897 (0.794-1.000)	0.086
No	5640	156 (2.8)	0.978 (0.974-0.982)	0.882 (0.850-0.915)	<0.001
HBeAg status					
Positive	2400	17 (0.7)	0.894 (0.825-0.963)	0.689 (0.495-0.882)	0.050
Negative	5799	170 (2.9)	0.983 (0.980-0.987)	0.885 (0.857-0.914)	<0.001
Treatment duration at enrollment					
naive	449	4 (0.9)	0.987 (0.965-1.000)	0.603 (0.131-1.000)	0.112
<1 year	1607	22 (1.4)	0.991 (0.986-0.996)	0.851 (0.751-0.952)	0.007

HBeAg: hepatitis B e antigen; qHBsAg: Quantitative hepatitis B surface antigen; * treated with interferon therapy before or after enrollment.

Table 4: Comparison of AUC (95% CI) between the GOLDEN model and qHBsAg level at enrollment in predicting HBsAg loss

	N	HBsAg loss, n (%)	GOLDEN model	qHBsAg at enrollment	*p* value
1-5 years	1768	45 (2.5)	0.979 (0.971-0.987)	0.875 (0.809-0.942)	0.003
5-10 years	2428	87 (3.6)	0.975 (0.969-0.981)	0.896 (0.859-0.934)	<0.001
>10 years	540	15 (2.8)	0.970 (0.956-0.985)	0.900 (0.825-0.974)	0.070
Drug withdrawal					
Yes	299	28 (9.4)	0.973 (0.955-0.992)	0.814 (0.696-0.932)	0.009
No	6493	145 (2.2)	0.981 (0.977-0.984)	0.895 (0.866-0.925)	<0.001
qHBsAg level at enrollment, IU/mL					
0.05-100	955	127 (13.3)	0.946 (0.931-0.960)	0.779 (0.729-0.829)	<0.001
100-1000	3012	36 (1.2)	0.985 (0.979-0.991)	0.689 (0.602-0.775)	<0.001
≥1000	2798	8 (0.3)	0.997 (0.996-0.999)	0.735 (0.589-0.881)	<0.001

HBeAg: hepatitis B e antigen; qHBsAg: Quantitative hepatitis B surface antigen; * treated with interferon therapy before or after enrollment.

Table 4 (Continued.)

Discussion

Based on a large, prospective, multi-center study involving CHB patients receiving approved antiviral therapies, we serially measured qHBsAg during a median follow-up of 65.6 months and developed the GOLDEN model—a novel and precise tool for predicting HBsAg seroclearance. The GOLDEN model showed excellent performance and accurately identified patients with a high probability of achieving HBsAg loss, with a 5-year cumulative incidence of around 30%. Furthermore, the model determined the subset of patients whose incidence of HBsAg clearance is 0, with an impressive accuracy of almost 100%. To our knowledge, the present study is the first to develop and evaluate an accurate prediction model for serum HBsAg clearance by using the trajectory of qHBsAg over time. The key strength of our GOLDEN model is the comprehensive integration of qHBsAg values at all timepoints for each patient, capturing the long-term dynamic changes in qHBsAg which is a central element for accurate prediction.

HBV functional cure is now the goal of new therapies for CHB and HBsAg loss is widely considered as the most reliable indicator for functional cure.[2-4] qHBsAg and its changes during treatment have been suggested as the dominant biomarker in predicting HBsAg loss. In HBeAg (+) patients with effective suppression of HBV replication on prolonged NA treatment, a rapid decline of qHBsAg (>1 $\log_{10}$IU/ml) was associated with greater likelihood for HBsAg seroclearance.[27] Similarly, a study involving 329 CHB patients from Taiwan indicated that the pattern of serum HBsAg kinetics throughout NAs treatment identify different rates of HBsAg loss.[28] A North American study based on treatment-naïve patients suggested that a model incorporating qHBsAg change at 1- year of therapy, instead of

qHBsAg values at enrollment performed better, with significantly higher AUC in predicting the 1-year loss of HBsAg (0.95 vs. 0.99, p=0.01).[13] However, these findings were from selected subgroups of patients with specific characteristics, for example, only those without receiving anti-HBV treatment, or treated with a single treatment strategy. Furthermore, the change of qHBsAg values used in previous studies involved calculation based on two specific timepoints, which will limit the reflection of the real probability of HBsAg clearance in relation to treatment during long-term follow up. In this study, involving a large cohort with a full spectrum of CHB patients treated with different strategies, the GOLDEN model developed by the LoDA approach takes into account the correlation between qHBsAg values updated over time for the same patient, that is, the model could comprehensively reflect the absolute value of qHBsAg and qHBsAg change at different intervals for each patient, then allowing more accurate and patient-specific predictions of chance to be made for practical application. The information provided by the GOLDEN model could be used to reassess therapy for achieving HBsAg loss. Favorable patients predicted within 1 year will stay on the same therapy; Patients who are unlikely to achieve HBsAg loss will need change of therapy or adding a new agent, or a combination regimen to enhance the chance for HBsAg loss. The model could also be used for selection and stratification of patients enrolled into clinical studies testing new therapies to achieve HBsAg loss.

Given that 57.9% (3917/6765) of patients were with a qHBsAg level of 0.05-1000 IU/mL at enrollment in this study, the qHBsAg values became lower when patients were identified as the favorable group during the subsequent follow-up. This raises the question of whether the qHBsAg trajectory over time or low qHBsAg value at last follow-up play a more important

role in the predictive accuracy for identifying the favorable group. To answer this question, we further evaluated the performance of the GOLDEN model among patients with qHBsAg 0.05-10, 20, and 50 IU/mL at the 2-year follow-up in predicting HBsAg loss afterward, by using the qHBsAg data within 2-year follow-up backward. These results confirmed that the GOLDEN model consistently delivers excellent discrimination performance among this subgroup of patients. Furthermore, our study also demonstrated that the favorable patients were identified by the GOLDEN model about 18 months before occurrence of HBsAg loss, and the performance of GOLDEN model continues to improve over time along with the increased longitudinal data timepoints. The above findings further emphasize the benefit of using longitudinal data, instead of only the low qHBsAg level, or a rapid reduction of qHBsAg during a certain period, in identifying favorable/unfavorable patients of HBsAg loss.

Over the past decades, several novel drugs for CHB have been developed, and showed promising efficacy in reducing qHBsAg levels.[9,29,30] One practical application of the prognostic model will be to improve the selection and/or stratification of CHB patients with greater chance for achieving HBsAg loss in future clinical trials with novel therapies. qHBsAg level at enrollment has traditionally been regarded as an independent predictor for future HBsAg loss, which was also observed in our study. We compared the predictive performance of the GOLDEN model and a single qHBsAg measurement at enrollment. This analysis showed that the GOLDEN model has markedly better performance for 5-year HBsAg loss chance prediction across all patient subsets than a single qHBsAg level at enrollment. The GOLDEN model was able to effectively stratify patients into two distinct

groups with significantly different probabilities of HBsAg clearance. Importantly, the GOLDEN model consistently exhibited excellent discrimination ability among patients who had different qHBsAg levels (0.05-100, 100-1000, and ⩾1000 IU/mL) at enrollment. Our results showed that approximately 10% of patients were classified into the favorable group, showing a higher likelihood of achieving HBsAg loss, demonstrated by the 5-year cumulative incidence of HBsAg loss reaching as high as 39.9%. Current ongoing clinical trials of novel drugs often take qHBsAg <3000 IU/mL as the common inclusion criteria due to these patients with lower qHBsAg level are more likely to benefit from novel drugs in achieving HBsAg loss. Collectively, we believe the GOLDEN model, would be useful as a valuable tool for identifying patients who may benefit much more from novel therapies beyond the traditional inclusion criteria.

Key strengths of our study are the large sample size, serial per-protocol qHBsAg measurements, and the long follow-up. The study population reflects the real-world CHB population in the current era of widely used NAs antiviral drugs, and includes both treatment-naïve and -experienced patients. More importantly, by considering both the comprehensive integration of qHBsAg values and the longitudinal trajectory, as well as its superior performance, the GOLDEN model represents a substantial advancement in HBsAg loss prediction and could contribute to developing more effective HBV treatment strategies.

In addition to outlining the strengths of our study, it is important to acknowledge its limitations. Firstly, due to a median treatment duration of 41.3 months (IQR: 7.6, 107.6) prior to enrollment, collecting comprehensive and precise data that accurately reflect the condition at the initiation of treatment presented significant challenges, which may influence

the model's sensitivity and PPV values. Although consistent wonderful performance of GOLDEN model across patients with different treatment duration was proved in the current study, further validation of the GOLDEN model in large-scale treatment-naive patients are also needed. Secondly, all patients enrolled in the study consisted solely of Chinese patients treated with NAs, and nearly 60% of the whole population had the qHBsAg level ⩽1000 IU/mL at enrollment, future evaluations in patients of other ethnicities (e.g., Caucasian, African), different qHBsAg categories, and interferon therapy or novel drugs (e.g., antisense oligonucleotides, siRNA) are needed. Thirdly, we should also acknowledge that the heterogeneity of the population may bring results bias as an observational study, although the stratified analysis showed consistent wonderful results. Another aspect to consider is the algorithm of the GOLDEN model, which, despite its complexity, has been made accessible through a web-based tool for broader dissemination (http://8.130.21.110:8001/). However, ongoing refinement and validation on data with different time intervals and lengths in order to predict HBsAg loss earlier, determine important specific time points and minimize the data involved, are crucial to decrease costs associated with detection for its successful real-world application.

In conclusion, based on a large cohort of prospectively followed CHB patients and using longitudinal data of qHBsAg, we generated a novel and precise model, called the GOLDEN model, for prediction of HBsAg seroclearance. The GOLDEN model enables to reliably determine the likelihood of achieving HBV functional cure, which currently is the recommended treatment endpoint in CHB. This model could be used for decision-making and patients' selection in clinical trials for HBV cure and holds promise of becoming a

useful tool for practicing clinicians in their quest for effective personalized management of CHB patients treated with NAs.

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Conflicts of Interest

Jinlin Hou received consulting fees from GlaxoSmithKline, Gilead Sciences, and a Grant from Roche. Nikolai V. Naoumov is an independent advisor to HistoIndex and a member of the scientific advisory board for InSphero. The other authors declare no conflicts of interest that pertain to this work.

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Author's Contribution

JLH, RF and NVN contributed to the conception and design of the study. JLH and RF coordinated the study. RF, JQN, HM, Qing Xie, SY, JPX, XGD, Jia Shang, HYR, Qi Xia, YLL, YFY, HBG, AMS, XEL, XRY, YFJ, YYY, Jian Sun, and the other members from chronic Hepatitis B Study Consortium (For detailed list, please see supplementary material) acquired the data. JLH, RF, and SRZ did the statistical analysis, interpreted the data and verified the underlying data. JLH, RF, NVN and SRZ prepared the manuscript. All authors

contributed to the discissions and interpretation of study results, approved the final manuscript and had final responsibility for the decision to submit for publication.

Data Availability Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Patient and public involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Patient consent for publication

Not applicable.

Ethics approval

The study protocol was approved by the Ethics Committee of Nanfang Hospital (NFEC-2014-017), and all patients gave their written informed consent.

References

1 Collaborators PO. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol* 2018; 3(6): 383-403.

2 Cornberg M, Lok AS, Terrault NA, Zoulim F. Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference(‡). *J Hepatol* 2020; 72(3): 539-57.

3 Lim SG, Baumert TF, Boni C, et al. The scientific basis of combination therapy for chronic hepatitis B functional cure. *Nat Rev Gastroenterol Hepatol* 2023; 20(4): 238-53.

4 Chang ML, Liaw YF. Emerging Therapies for Chronic Hepatitis B and the Potential for a Functional Cure. *Drugs* 2023; 83(5): 367-88.

5 Yip TC, Wong GL, Chan HL, et al. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. *J Hepatol* 2019; 70(3): 361-70.

6 Yip TC, Wong VW, Tse YK, et al. Similarly low risk of hepatocellular carcinoma after either spontaneous or nucleos(t)ide analogue-induced hepatitis B surface antigen loss. *Aliment Pharmacol Ther* 2021; 53(2): 321-31.

7 Tout I, Loureiro D, Mansouri A, Soumelis V, Boyer N, Asselah T. Hepatitis B surface antigen seroclearance: Immune mechanisms, clinical impact, importance for drug development. *J Hepatol* 2020; 73(2): 409-22.

8 Wong GLH, Gane E, Lok ASF. How to achieve functional cure of HBV: Stopping NUCs, adding interferon or new drug development? *J Hepatol* 2022; 76(6): 1249-62.

9 Yuen MF, Lim SG, Plesniak R, et al. Efficacy and Safety of Bepirovirsen in Chronic Hepatitis B Infection. *N Engl J Med* 2022; 387(21): 1957-68.

10 Fung S, Choi HSJ, Gehring A, Janssen HLA. Getting to HBV cure: The promising paths forward. *Hepatology* 2022; 76(1): 233-50.

11 Fan R, Peng J, Xie Q, et al. Combining Hepatitis B Virus RNA and Hepatitis B Core-Related Antigen: Guidance for Safely Stopping Nucleos(t)ide Analogues in Hepatitis B e Antigen-Positive Patients With Chronic Hepatitis B. *J Infect Dis* 2020; 222(4): 611-8.

12 Sonneveld MJ, Chiu SM, Park JY, et al. Probability of HBsAg loss after nucleo(s)tide analogue withdrawal depends on HBV genotype and viral antigen levels. *J Hepatol* 2022; 76(5): 1042-50.

13 Terrault NA, Wahed AS, Feld JJ, et al. Incidence and prediction of HBsAg seroclearance in a prospective multi-ethnic HBeAg-negative chronic hepatitis B cohort. *Hepatology* 2022; 75(3): 709-23.

14 Sonneveld MJ, Park JY, Kaewdech A, et al. Prediction of Sustained Response After Nucleo(s)tide Analogue Cessation Using HBsAg and HBcrAg Levels: A Multicenter Study (CREATE). *Clin Gastroenterol Hepatol* 2022; 20(4): e784-e93.

15 Fan R, Papatheodoridis G, Sun J, et al. aMAP risk score predicts hepatocellular carcinoma development in patients with chronic hepatitis. *J Hepatol* 2020; 73(6): 1368-78.

16 Liu S, Deng R, Zhou B, et al. Association of Serum Hepatitis B Virus RNA With Hepatocellular Carcinoma Risk in Chronic Hepatitis B Patients Under Nucleos(t)ide Analogues Therapy. *J Infect Dis* 2022; 226(5): 881-90.

17 Fan R, Chen L, Zhao S, et al. Novel, high accuracy models for hepatocellular carcinoma prediction based on longitudinal data and cell-free DNA signatures. *J Hepatol* 2023; 79(4): 933-44.

18 Fan R, Niu J, Ma H, et al. Association of central obesity with hepatocellular carcinoma in patients with chronic hepatitis B receiving antiviral therapy. *Aliment Pharmacol Ther* 2021; 54(3):

329-38.

19 Hou JL, Zhao W, Lee C, et al. Outcomes of Long-term Treatment of Chronic HBV Infection With Entecavir or Other Agents From a Randomized Trial in 24 Countries. *Clin Gastroenterol Hepatol* 2020; 18(2): 457-67.e21.

20 Fan R, Zhou B, Xu M, et al. Association Between Negative Results From Tests for HBV DNA and RNA and Durability of Response After Discontinuation of Nucles(t)ide Analogue Therapy. *Clin Gastroenterol Hepatol* 2020; 18(3): 719-27.e7.

21 Liao CC, Hsu CW, Gu PW, Yeh CT, Lin SM, Chiu CT. Comparison of the elecsys HBsAg II assay and the architect assay for quantification of hepatitis B surface antigen in chronic hepatitis B patients. *Biomed J* 2015; 38(3): 250-6.

22 Sonneveld MJ, Rijckborst V, Boucher CA, et al. A comparison of two assays for quantification of Hepatitis B surface Antigen in patients with chronic hepatitis B. *J Clin Virol* 2011; 51(3): 175-8.

23 Hughes DM, Komárek A, Czanner G, Garcia-Fiñana M. Dynamic longitudinal discriminant analysis using multiple longitudinal markers of different types. *Stat Methods Med Res* 2018; 27(7): 2060-80.

24 Hughes DM, El Saeiti R, García-Fiñana M. A comparison of group prediction approaches in longitudinal discriminant analysis. *Biom J* 2018; 60(2): 307-22.

25 Hughes DM, Berhane S, Emily de Groot CA, et al. Serum Levels of α-Fetoprotein Increased More Than 10 Years Before Detection of Hepatocellular Carcinoma. *Clin Gastroenterol Hepatol* 2021; 19(1): 162-70.e4.

26 Riley RD, Ensor J, Snell KIE, et al. Calculating the sample size required for developing a clinical prediction model. *Bmj* 2020; 368: m441.

27 Wursthorn K, Jung M, Riva A, et al. Kinetics of hepatitis B surface antigen decline during 3 years of telbivudine treatment in hepatitis B e antigen-positive patients. *Hepatology* 2010; 52(5): 1611-20.

28 Hsu WF, Chen CF, Lai HC, et al. Trajectories of serum hepatitis B surface antigen kinetics in patients with chronic hepatitis B receiving long-term nucleos(t)ide analogue therapy. *Liver Int* 2018; 38(4): 627-35.

29 Lok AS, Pan CQ, Han SH, et al. Randomized phase II study of GS-4774 as a therapeutic vaccine in virally suppressed patients with chronic hepatitis B. *J Hepatol* 2016; 65(3): 509-16.

30 Bazinet M, Pântea V, Placinta G, et al. Safety and Efficacy of 48 Weeks REP 2139 or REP 2165, Tenofovir Disoproxil, and Pegylated Interferon Alfa-2a in Patients With Chronic HBV Infection Naïve to Nucleos(t)ide Therapy. *Gastroenterology* 2020; 158(8): 2180-94.

Figure Legends

Figure 1. Changes in the proportion of patients in different subgroups according to quantitative HBsAg levels and cumulative incidence of HBsAg loss during follow-up (A). Longitudinal trajectory plot of quantitative HBsAg in the training (B) and validation sets (C). The red line and black dots indicate cases achieving HBsAg loss; The blue line and transparent dots indicate cases not achieving HBsAg loss; the bands represent 95% CIs around the mean profile; "0" means the time of achieving HBsAg loss or the last follow-up for patients who achieved HBsAg loss and those who did not separately.

Figure 2. Performance of the GOLDEN model in the training and validation sets. (A) Receiver operating characteristic (ROC) curves to predict HBsAg loss. (B) Cumulative incidence of HBsAg loss. (C) Distribution of the predicted score and probability groups among the overall population.

Figure 3. Cumulative incidence of HBsAg loss in subgroups stratified by quantitative HBsAg level at enrollment among the overall patients

Figure 4. Cumulative incidence of HBsAg loss in subgroups stratified by quantitative HBsAg level at 2-year follow-up among the overall patients.