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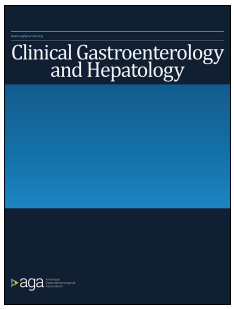

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Role of early on-treatment serum HBV RNA declines in predicting hepatocellular carcinoma risk in patients with chronic hepatitis B

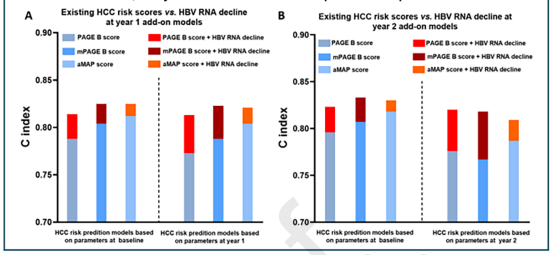
Serum HBV RNA declines at year 1 and 2 were independent predictors of HCC development

- Patients with less HBV RNA decline at year 1 (=< 0.4 log_{10} copies/mL) or 2 (=<0.6 log_{10} copies/mL) had 2.22- and 2.09-folds higher HCC risk, respectively, than those with more declines.

Early on-treatment serum HBV RNA declines	Adjusted HR	95%CI	*P*
Serum HBV RNA decline at year 1 of NA treatment			
Serum HBV RNA decline, per log_{10} copies/mL	0.70	0.53-0.91	.009
Serum HBV RNA decline=<0.4 vs. >0.4 log_{10} copies/mL	2.22	1.27-3.89	.005
Serum HBV RNA decline at year 2 of NA treatment			
Serum HBV RNA decline, per log_{10} copies/mL	0.71	0.54-0.94	.016
Serum HBV RNA decline=<0.6 vs. >0.6 log_{10} copies/mL	2.09	1.13-3.87	.019

Early on-treatment serum HBV RNA declines add-on models showed better predictive performance for HCC risk

- When incorporating these early on-treatment HBV RNA declines into existing HCC risk scores, including PAGE B, mPAGE B and aMAP score, they could enhance their predictive performance.



Clinical Gastroenterology and Hepatology

Role of early on-treatment serum HBV RNA declines in predicting hepatocellular carcinoma risk in patients with chronic hepatitis B

Short title: serum HBV RNA kinetics predict liver cancer

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Abbreviations

AUROC, area under the receiver operating characteristic curve; CHB, chronic hepatitis B; ETV, entecavir; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; IQR, interquartile range; LLOD, K-M, Kaplan-Meier; lower limit of detection; NA, nucleoside analogue; mPAGE B, modified PAGE B; qHBsAg, quantitative hepatitis B surface antigen; SAT, simultaneous amplification testing method; TDF, tenofovir disoproxil fumarate.

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Disclosures

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Abstract

Background and Aims: Hepatocellular carcinoma (HCC) risk prediction models established in patients with chronic hepatitis B (CHB) receiving nucleoside analogue (NA) rarely included viral factors because of mediocre predictability of traditional viral markers. Here, we investigate the role of serum hepatitis B virus (HBV) RNA, a novel biomarker, in predicting HCC risk in NA-treated patients.

Methods: A total of 1374 NA-treated patients were enrolled from two prospective CHB cohorts. Serum HBV RNA was detected at baseline, year 1, 2 and 3 of treatment. Cox proportional-hazard model was used to investigate the association of HBV RNA kinetics with HCC risk.

Results: After a median follow-up of 5.4 years, 76 patients developed HCC. HBV RNA declines at year 1 (adjusted hazard ratio (aHR) = 0.70, P = .009) and 2 (aHR = 0.71, P = .016) were independently associated with HCC risk. Patients with less HBV RNA decline at year 1 (=< 0.4 $\log_{10}$ copies/mL) or 2 (=<0.6 $\log_{10}$ copies/mL) had 2.22- and 2.09-folds higher HCC risk, respectively, than those with more declines. When incorporating these early on-treatment HBV RNA declines into existing HCC risk scores, including PAGE B, mPAGE B and aMAP score, they could enhance their predictive performance [i.e. C-index, 0.814 *vs.* 0.788 (Model (PAGE B + year-1 HBV RNA decline) *vs.* PAGE B score based on baseline parameters)].

Conclusions: Serum HBV RNA declines at year 1 and 2 were significantly associated with on-treatment HCC risk. Incorporating early on-treatment HBV RNA declines into HCC risk prediction models can be useful tools to guide appropriate surveillance

strategies in NA-treated patients.

Keywords: liver cancer, anti-HBV treatment, novel viral marker, predictability

Introduction

Chronic hepatitis B virus (HBV) infection is a global health problem, affecting approximately 296 million individuals worldwide and resulting in approximately 820,000 deaths annually, with hepatocellular carcinoma (HCC) as the primary cause of mortality [1]. Long-term nucleos(t)ide analogue (NA) treatment can significantly decrease but cannot eliminate the HCC risk, with an annual on-treatment HCC incidence remaining at 0.5% ~ 2.0% [2]. Identifying the risk factors and predictors for HCC development in NA-treated patients with chronic hepatitis B (CHB) and establishing HCC risk prediction models to accurately identify high-risk patients have important implications for the development of an optimized strategy for HCC surveillance in this population.

Previous studies have shown that age, male sex, cirrhosis, platelet and diabetes mellitus are risk factors of HCC in NA-treated patients with CHB [3, 4]. However, traditional viral markers, such as serum HBV DNA, hepatitis B e antigen (HBeAg) and quantitative hepatitis B surface antigen (qHBsAg), often showed mediocre predictive ability for HCC development in treated patients [5-7]. Existing HCC risk prediction scores established in NA-treated patients, such as PAGE B, modified PAGE B (mPAGE B) and aMAP score, only included commonly measured clinical parameters, such as age, sex, platelet and albumin [5-7]. However, our previous study found that serum HBV RNA level was significantly associated with HCC risk in NA-treated patients [8], suggesting the need to consider this novel viral marker when evaluating the HCC risk in the

treated population.

In the present study, we aimed to investigate the role of serum HBV RNA kinetics in the prediction of HCC risk in patients with CHB under entecavir (ETV) or tenofovir disoproxil fumarate (TDF) treatment enrolled from two prospective CHB cohorts, and explore whether serum HBV RNA kinetics can improve the predictive performance of existing HCC risk prediction scores, including PAGE B, mPAGE B and aMAP score.

Patients and Methods

Patients

The present study enrolled patientsfrom two prospective NA-treated CHB cohorts conducted in the Hepatology Unit, Nanfang Hospital (Search-B cohort: clinicaltrials. gov: NCT02167503, Guangzhou, China) since May 2014 and the Prince of Wales Hospital (HK Cohort, Hong Kong, China) since December 2005. The detailed information of the study designs and inclusion criteria of the two cohorts had been previously described [7, 9, 10]. Patients enrolled in these two cohorts were undergoing or starting antiviral treatment during screening. To investigate the association between early on-treatment serum HBV RNA kinetics and HCC risk, only patients receiving ETV or TDF treatment for no more than 6 months at enrollment were included in the present study, and the distribution of enrolled patients' treatment duration before enrollment were shown in Supplemental figure 1. The exclusion criteria were showed in Supplementary Materials. Finally, 1374 patients were enrolled in this study,

including 1266 and 108 patients from Nanfang Hospital and Prince of Wales Hospital, respectively (Figure 1). The data included in the analysis from Search-B and HK cohort were both as of May 2023.

Informed consent for clinical data and serum samples collection were obtained from each patient during recruitment. The protocols of Search-B and HK cohort were approved by the Ethics Committee of Nanfang Hospital (Guangzhou, China) and the Joint Chinese University of Hong Kong—New Territories East Cluster Clinical Research Ethics Committee respectively.

Clinical evaluation and follow-up

Clinical and laboratory data at cohort enrollment (defined as baseline in the present study) and during follow-up were collected from a physical examination, questionnaire, routine blood tests and virologic tests. All patients were monitored regularly for clinical and laboratory parameters every 3 to 6 months. The primary outcome in this study was HCC development. Patients underwent regular HCC surveillance by serum alpha-fetoprotein and/or liver ultrasonography every 6 months. The diagnoses of cirrhosis and HCC were based on standard histological and/or compatible radiological findings [11, 12]. For detailed information, please see the supplementary materials.

Detection of Serum HBV Markers

Serum HBV DNA and serologic markers, including HBsAg, anti-HBs, HBeAg and

anti-HBe were detected at baseline and during follow up. Serum HBV DNA was measured with the COBAS TaqMan platform with a lower limit of detection [LLOD] of 20 IU/mL in Nanfang Hospital and 10 IU/mL in Prince of Wales Hospital respectively. HBV serologic markers were measured with Elecsys immunoassay (both from Roche, Basel, Switzerland). Undetectable serum HBV DNA was defined as HBV DNA level below the LLOD.

Serum HBV RNA quantification

Serum HBV RNA levels were detected at baseline and year 1, 2 and 3 of treatment by RNA simultaneous amplification testing method (HBV-SAT) based on real-time fluorescence detection of isothermal RNA amplification using HBV-SAT kit (Shanghai Rendu Biotechnology Co., Ltd. China) according to the manufacturer's procedures. The kit was registered with China National Medical Products Administration (NMPA) on March 15th of 2021 and officially approved for clinical use. Detailed information of this serum HBV RNA assay and its performance, including linearity (Supplemental figure 2), sensitivity (Supplemental table 1), specificity, repeatability (Supplemental table 2), and accuracy (Supplemental table 3), were described in Supplementary material. Undetectable serum HBV RNA was defined as HBV RNA level below the LLOD (50 copies/ml).

Statistical Analysis

All statistical analyses were conducted using R statistical package (ver. 4.2.1,

http://www. r-project.org) and SPSS (ver. 20.0, Chicago, IL). P value < .05 was considered statistically significant. For more information, see Supplementary material.

Result

Patient characteristics

Table 1 shows the baseline characteristics of the enrolled patients. The median baseline levels of serum HBV DNA, qHBsAg and HBV RNA were 2.6 (IQR, 1.6-4.3) log_{10} IU/mL, 3.1 (IQR, 2.7-3.5) log_{10} IU/mL and 4.3 (IQR, 2.9-6.0) log_{10} copies/mL, respectively. After a median follow-up of 5.4 (IQR, 4.4-7.0) years, 76 patients developed HCC, with a 5-year cumulative incidence of 4.0% (Supplementary figure 3A). Starting from the landmark times of year 1, 2 and 3 of treatment, there were 58, 47 and 41 patients developing HCC, with 5-year cumulative incidence of 5.1%, 6.6% and 6.6% respectively (Supplementary figure 3B-D).

Association of serum HBV RNA kinetics during NA treatment with HCC risk

The kinetics of serum HBV DNA, RNA and qHBsAg during NA treatment were showed in Supplementary figure 4. Multivariable-adjusted restricted cubic spline analyses showed that there was a significant non-linear parabolic association of serum HBV RNA levels at baseline and year 1 with HCC risk (all P for non-linear trend < 0.050; Supplementary figure 5A-B). Similar trend was also observed between serum HBV RNA levels at year 2 and HCC risk with marginal statistical significance (pPfor non-linear trend = 0.114; Supplementary figure 5C), but was not observed between

serum HBV RNA levels at year 3 and HCC risk (P for non-linear trend = 0.866; Supplementary figure 5D). In addition, the association of serum qHBsAg at different time points with HCC risk were also investigated (Supplementary figure 6A-D), largely showing marginal significant non-linear associations.

Interestingly, there was an independent linear association between serum HBV RNA decline at year 1 of NA treatment and HCC risk (P for linear trend = 0.006, Figure 2A). Similar trend was observed between HCC risk and serum HBV RNA decline at year 2 (p for linear trend = 0.012, Figure 2B), but was not observed between HCC risk and serum HBV RNA decline at year 3 (Figure 2C). In addition, there was no evidence of associations between HBsAg declines after NA treatment and HCC risk (Supplementary figure 7A-C).

Early on-treatment serum HBV RNA declines as independent predictors of HCC development

To further evaluate the association of early on-treatment (within 2 years of NA treatment) serum HBV RNA declines with HCC risk, Cox proportional hazard regression analysis was conducted. Results showed that serum HBV RNA declines at year 1 (aHR= 0.70, 95%CI: 0.53-0.91, P = .009) and 2 (aHR= 0.71, 95%CI: 0.54-0.94, P = .016) were independent predictors for HCC risk, after adjusting baseline aMAP HCC risk score (an index reflecting the underlying HCC development risk calculated by age, sex, albumin, total bilirubin and platelet), cirrhosis, diabetes, HBeAg status,

drinking history and smoking history (Table 2).

Time-dependent ROC analyses showed that the AUROCs of serum HBV RNA declines at year 1 and 2 in predicting HCC development within 3-years were 0.635 and 0.659, respectively; within 5-years were 0.622 and 0.569, respectively (Supplementary figure 8). According to the highest sum of sensitivity and specificity, we then used 0.4 log_{10} copies/mL and 0.6 log_{10} copies/mL as the cut-off values of serum HBV RNA declines at year 1 and 2 for predicting on-treatment HCC risk. The 5-year HCC cumulative incidences were significantly lower in patients with more decline of early on-treatment HBV RNA than that in those with less declines (at year 1, 3.4% vs. 7.3%%, P = .003; at year 2, 4.9% vs. 9.2%, P = .021) (Figure 3A-B). Multivariate analyses showed that HCC risks in patients with less HBV RNA decline at year 1 and 2 were 2.22- and 2.09-folds higher than that in patients with more declines of HBV RNA (P = .005 and P = .019 respectively, Table 2).

Subgroup and sensitivity analyses for associations of early on-treatment serum HBV RNA declines with HCC risk

Subgroup analyses showed that the inverse association between serum HBV RNA decline at year 1 and HCC risk was largely consistent in most subgroups, including age < 45 years old, age >= 45 years old, baseline HBeAg positive, baseline HBeAg negative, and cirrhosis (Table 2). However, more decline of serum HBV RNA at year 2 was only significantly associated with decreased HCC risk in subgroups with age >= 45 and

cirrhosis (Table 2).

Our results were consistent across all sensitivity analyses, including those performed when we adjusted for the 1-year's or 2-year's values of HCC potential risk factors (Supplementary Table 4); when we limited the analysis to patients who had received =<2 months of NA before enrollment (Supplementary Table 5); after we excluded patients with undetectable baseline serum HBV RNA (Supplementary Table 6); and after we excluded patients without early HBV DNA response (defined as achieving HBV DNA undetectable within 1 year or 2 years after NA treatment) (Supplementary Table 7).

Early on-treatment serum HBV RNA declines add-on models showed better predictive performance for HCC risk

To further evaluate the clinical significance of early on-treatment serum HBV RNA declines, we established novel HCC prediction models by incorporating serum HBV RNA decline at year 1 or 2 into the existing HCC risk scores—PAGE B, mPAGE B and aMAP score, which were established based on NA-treated population with CHB, to investigated whether they could improve their predictive performance. As shown in Figure 4, regardless of baseline or on-treatment (year 1 and year 2) parameters, the C-indexes of early on-treatment HBV RNA declines add-on models were higher than those of the original HCC risk scores. Besides, time-dependent ROC analyses showed that these early on-treatment HBV RNA declines add-on models obtained higher

AUROCs in dynamic trends than the original HCC risk scores within 3 years (Supplementary figure 9).

Internal validation of early on-treatment serum HBV RNA declines add-on models

The predictive performance of early on-treatment serum HBV RNA declines add-on models were validated in the internal training and test sets, of which generated by repeated K-folds cross-validation method. As shown in Supplementary table 8 and Supplementary Figure 10, time-dependent AUROCs of the HBV RNA declines add-on models for 3-year HCC risk prediction were higher than those of original HCC risk scores. Besides, regardless of based on parameters at baseline or year 1 or 2, the early on-treatment HBV RNA declines add-on models also showed better discrimination ability than the original HCC risk scores in the internal validation set.

Discussion

In this multicenter retro-prospective cohort study of ETV/TDF-treated patients with CHB, we found nonlinear parabolic associations of serum HBV RNA levels at baseline and year 1 with HCC risk. Besides, early on-treatment serum HBV RNA declines (at year 1 and 2) showed linear associations with HCC risk and could serve as independent predictors of HCC development. When incorporating serum HBV RNA decline at year 1 or 2 into the existing HCC risk prediction scores, including PAGE B, mPAGE B and aMAP Score, they could enhance their predictive performance for the risk of HCC development.

Similar with the relationship between baseline serum HBV DNA and on-treatment HCC risk, reported by Choi et al [13], baseline HBV RNA level showed a nonlinear, parabolic association with HCC risk in long-term NA-treated patients. Because of high correlation between serum HBV RNA and HBV DNA at NA initiation [14, 15], it's reasonable to believe that the underlying mechanisms of this association were similar with that of baseline serum HBV DNA and HCC risk, which had been discussed by Choi et al [13], that is higher viremia levels (i.e HBV DNA ≥8 $\log_{10}$ IU/mL) indicate early stage of CHB (immune tolerant phase) thus exhibiting lower HCC risk, while moderate viremia (i.e HBV DNA of 5–7 $\log_{10}$ IU/mL) is associated with hepatic inflammation and the expansion of clonal hepatocytes resistant to HBV, both of which were risk factors for HCC, therefore increasing in HCC risk. Interestingly, the non-linear, parabolic association of serum HBV RNA levels and on-treatment HCC risk lasted at year 1 of NA treatment. This phenomenon may result from the indirect influence of NA treatment on serum HBV RNA levels, thus short-term NA treatment exert small impact for this non-linear association. When antiviral treatment lasted for the year 2, this non-linear association was attenuated and disappeared at year 3.

Unlike the levels of serum HBV RNA at one time point, serum HBV RNA declines at year 1 or 2 of NA treatment showed linear associations with HCC risk. Patients with less serum HBV RNA declines (=< 0.4 $\log_{10}$ copies/mL at year 1 and =<0.6 $\log_{10}$ copies/mL at year 2) had significantly higher HCC risk. We speculated firstly that the

higher early on-treatment HBV RNA declines associated with lower HCC risk contributed from early HBV DNA response as previous study reported [16], but we observed no significant association between early HBV DNA response (defined as serum HBV DNA achieving undetectable within 1 or 2 years after NA treatment) and HCC risk in the present study (data not shown). Furthermore, to further confirming that the relationship between early on-treatment serum HBV RNA declines and HCC risk was independent of effective HBV DNA suppression, we added a sensitivity analysis that only including those achieving early HBV DNA response, and still found a significant association of early on-treatment HBV RNA declines with HCC risk. These findings suggested that early on-treatment serum HBV RNA declines are still significantly associated with HCC risk among patients with similar extent of viral suppression. The underlying mechanism for this association may be related to the relationship between serum HBV RNA and intrahepatic cccDNA. Several studies had confirmed that serum HBV RNA, not serum HBV DNA, can reflect the transcriptional activity of intrahepatic cccDNA in NA-treated patients [17, 18]. It is reasonable to speculate that serum HBV RNA decline implicates the reduction of cccDNA transcriptional activity, and subsequent reduction of HBsAg and HBeAg production, thus possibly contributed to the alleviation of liver inflammation, fibrosis/cirrhosis and the reduction of HCC risk by attenuating the host immune response [19, 20].

The extent of which early on-treatment serum HBV RNA declines may impact clinical practice deserves to be further investigated. In this regard, we explored whether early

on-treatment serum HBV RNA declines could enhance the predictive performance of the existing HCC risk prediction scores, including PAGE B, mPAGE B, and aMAP score. When incorporating serum HBV RNA declines at year 1 or 2 into these existing HCC risk scores to construct HBV RNA add-on models, they showed better predictability than the original scores, which were internally validated. To verify the robustness of results, we established HBV RNA add-on models and assessed existing HCC risk scores based on parameters not only at baseline, but also at year 1 or 2 correspondingly, consistent results were observed regardless of the time point in which parameters were used. Moreover, we believed that for patients receiving NA treatment, the predictive power of novel established and optimized HCC risk prediction models can be further augmented if early on-treatment serum HBV RNA declines were incorporated. Certainly, large NA-treated CHB cohorts and external validation cohorts are needed to comprehensively confirm the predicative value of early on-treatment serum HBV RNA declines for HCC development. Taken together, these observations provide the strongest clinical arguments to date for the predictive value of serum HBV RNA declines in HCC development.

Our study has a few limitations. First, because of lacking international standard for serum HBV RNA, the performance of HBV-SAT automated serum HBV RNA assay cannot be compared with that of other automated HBV RNA assay, such as the assay based on m2000 system (Abbott Diagnostics, Abbott Park, IL), thus hampering the comparison of results in different studies. For example, the undetectable rates of on-

treatment HBV RNA in our study was different from that in Carey et al.'s report [21]. However, in addition to the possible different sensitivity of the HBV RNA assay adopted, different patients' clinical characteristics were also an important reason. Second, some of patients enrolled in the baseline were not treatment-naive but had received =< 6 months of antiviral treatment. To minimize the impact of short-term treatment on serum HBV RNA levels, we did a sensitivity analysis that only included patients with more narrow treatment duration (=<2 months) at baseline in the corresponding analysis datasets and still found a significantly relationship between early on-treatment serum HBV RNA declines and the risk of HCC development. Third, there was no external cohort to further validate the performance of early on-treatment serum HBV RNA declines add-on models in predicting HCC risk. To further validate the predictive performance, we did an internal validation by repeated K-folds cross-validations method and found similar results in the validation sets. Last, because our study only included Chinese patients with the most prevalent HBV genotypes being B and C, the generalizability of our findings to other HBV genotypes and ethnicities needs to be assessed.

In conclusion, serum HBV RNA declines at year 1 and 2 showed linear associations with HCC risk respectively and could serve as independent predictors of HCC development. Incorporating the early on-treatment HBV RNA declines into the existing HCC risk prediction scores could enhance their predictive performance for HCC development, and can be useful tools to guide appropriate HCC surveillance strategies

in NA-treated patients with CHB.

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Figure legends

Figure 1. Flow diagram of participant selection. CHB, chronic hepatitis B; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogues.

Figure 2. Adjusted HRs for the HCC risk by early on-treatment serum HBV RNA declines in NA-treated patients with CHB. Adjusted HRs for the HCC risk by serum HBV RNA decline at year 1 (A), year 2 (B) and year 3 (C) of NA treatment. The HR plots were adjusted for baseline aMAP Score, HBeAg status, cirrhosis, diabetes, smoking history and drinking history. HBV RNA level below 50 copies/mL was used as a reference. The blue line represents the point estimates, and the blue zone indicates 95% CI. CI, confidence interval; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratios.

Figure 3. Cumulative incidences of HCC development according to serum HBV RNA decline at year 1 (A) and year 2 (B). HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

Figure 4. Comparisons of the C-indexes in early on-treatment HBV RNA declines add-on models and the existing HCC risk scores. (A) Existing HCC risk scores vs. HBV RNA decline at year 1 add-on models; (B) Existing HCC risk scores vs. HBV RNA decline at year 2 add-on models. HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

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*Author names in bold designate shared co-first authorship

Table 1. Baseline characteristics of enrolled patients

Variables	N=1374
Age, year	40.4 (33.2-49.6)
Male, n/N (%)	1058/1374 (77.0)
Cirrhosis, n/N (%)	333/1374 (24.2)
HBeAg positive, n/N (%)	599/1369 (43.8)
Serum HBV RNA, log_{10} copies/mL	4.3 (2.9-6.0)
Serum HBV DNA, log_{10} IU/mL	2.6 (1.6-4.3)
Quantitative HBsAg, log_{10} IU/mL	3.1 (2.7-3.5)
Alanine aminotransferase, U/L	38 (25-63)
Platelet, $\times 10^3/mm^3$	173.0 (124.0-213.0)
Albumin, g/L	43.6 (40.8-45.8)
Total bilirubin, μmol/L	13.4 (9.9-19.1)
Alpha fetoprotein, ng/mL	4.2 (2.9-8.8)
Diabetes, n/N (%)	95/1366 (7.0)
Smoking history, n/N (%)	374/1258 (29.7)
Drinking history, n/N (%)	349/1258 (27.7)
Follow-up, year	5.4 (4.4-7.0)
HCC cases, n/N (%)	76/1374 (5.5)

Data are shown as median (interquartile range) or n/N (%). HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

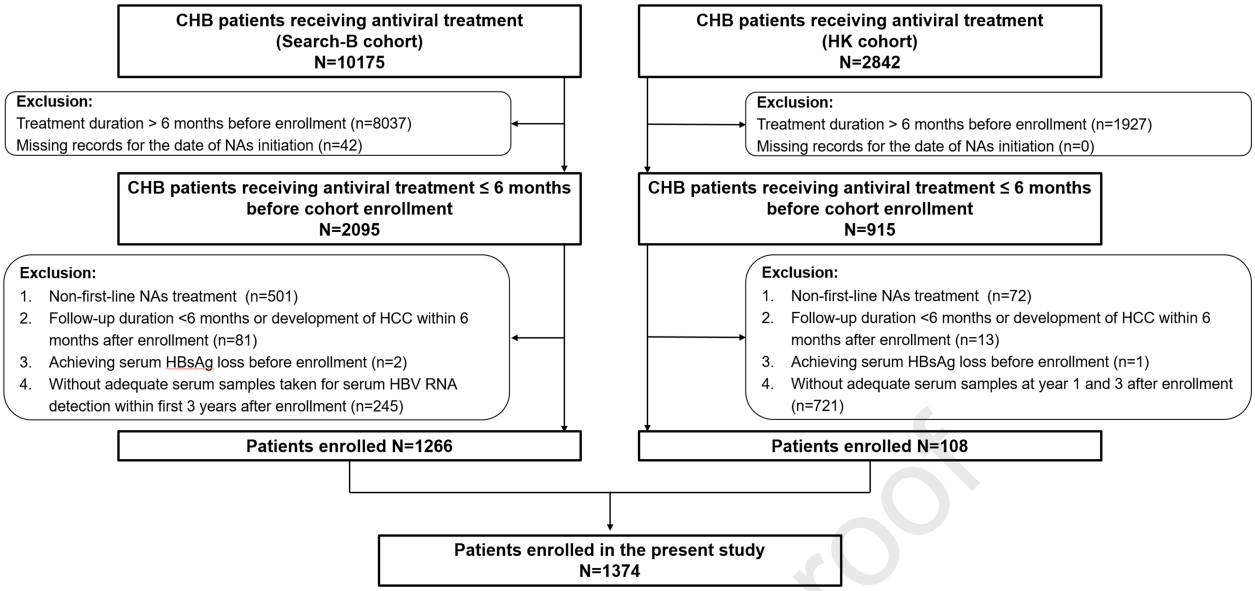
Table 2. Multivariate analysis of early on-treatment serum HBV RNA declines associated with HCC by Cox proportional hazard regression model

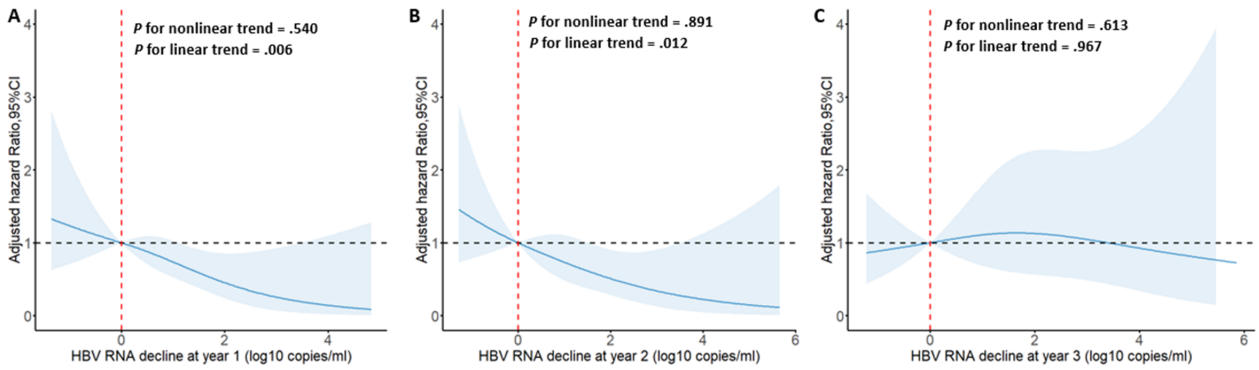
Early on-treatment serum HBV RNA declines	No. of Patients	HCC cases	aHR [a]	95%CI	P
Serum HBV RNA decline at year 1 of NA treatment					
All patients					
Serum HBV RNA decline, per log_{10} copies/mL	1217 [b]	58	0.70	0.53-0.91	.009
Serum HBV RNA decline =< 0.4 *vs.* > 0.4 log_{10} copies/mL			2.22	1.27-3.89	.005
Age < 45 years old					
Serum HBV RNA decline =< 0.4 *vs.* > 0.4 log_{10} copies/mL	808	16	3.07	1.03-9.13	.043
Age >= 45 years old					
Serum HBV RNA decline =< 0.4 *vs.* > 0.4 log_{10} copies/mL	409	42	1.88	0.97-3.65	.063
Baseline HBeAg Positive					
Serum HBV RNA decline =< 0.4 *vs.* > 0.4 log_{10} copies/mL	536	15	3.44	1.18-10.06	.024
Baseline HBeAg Negative					
Serum HBV RNA decline =< 0.4 *vs.* > 0.4 log_{10} copies/mL	681	43	1.96	1.02-3.77	.043

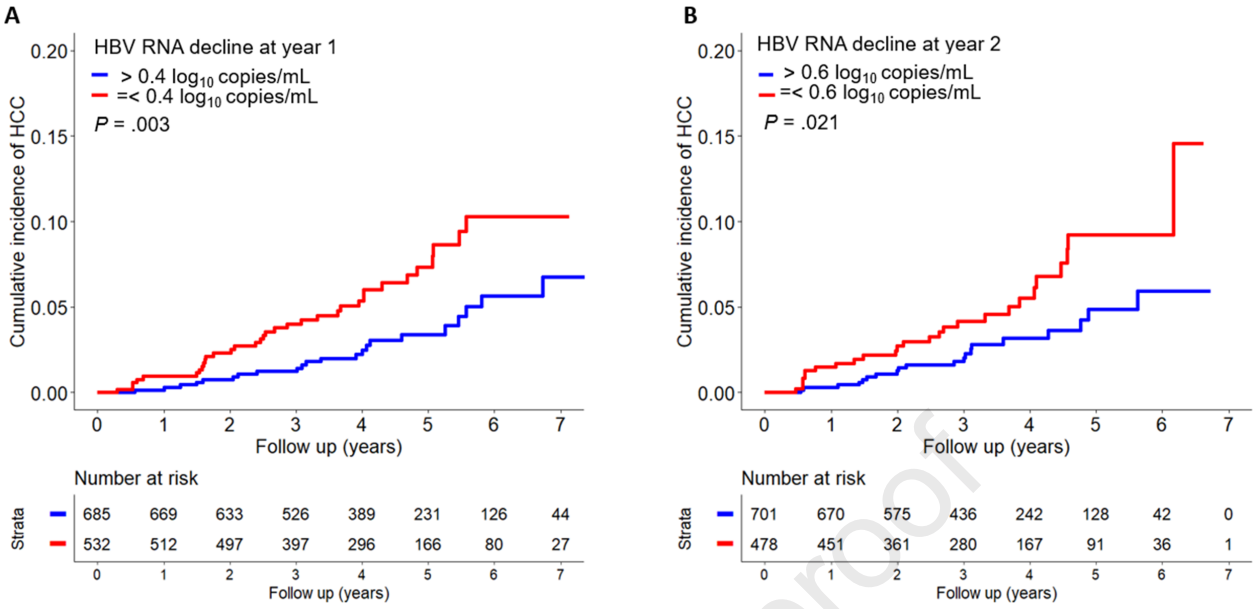
Cirrhotic					
Serum HBV RNA decline =< 0.4 *vs.* > 0.4 log_{10} copies/mL	301	40	2.58	1.29-5.13	.007
Non-cirrhotic					
Serum HBV RNA decline =< 0.4 *vs.* > 0.4 log_{10} copies/mL	916	18	1.57	0.59-4.13	.366
Serum HBV RNA decline at year 2 of NA treatment					
All patients					
Serum HBV RNA decline, per log10 copies/mL	1179 [c]	47	0.71	0.54-0.94	.016
Serum HBV RNA decline =< 0.6 vs. > 0.6 log_{10} copies/mL			2.09	1.13-3.87	.019
Age < 45 years old					
Serum HBV RNA decline =< 0.6 vs. > 0.6 log_{10} copies/mL	789	12	1.21	0.37-3.94	.753
Age >= 45 years old					
Serum HBV RNA decline =< 0.6 vs. > 0.6 log_{10} copies/mL	390	35	2.48	1.17-5.25	.018
Baseline HBeAg Positive					
Serum HBV RNA decline =< 0.6 vs. > 0.6 log_{10} copies/mL	531	15	1.97	0.66-5.94	.228

Baseline HBeAg Negative					
Serum HBV RNA decline =< 0.6 vs. > 0.6 log_{10} copies/mL	648	32	2.12	0.98-4.57	.056
Cirrhotic					
Serum HBV RNA decline =< 0.6 vs. > 0.6 log_{10} copies/mL	287	32	2.20	1.03-4.70	.041
Non-cirrhotic					
Serum HBV RNA decline =< 0.6 vs. > 0.6 log_{10} copies/mL	892	15	1.78	0.61-5.25	.295

[a] aHR, adjusted hazard ratio, obtained after adjusting baseline aMAP score (an index reflecting the underlying HCC development risk calculated by age, sex, albumin, total bilirubin and platelet), cirrhosis, diabetes, HBeAg status, drinking history and smoking history. [b] Patients with adequate serum samples at baseline and year 1 simultaneously were enrolled in this analysis. [c] Patients with adequate serum samples at baseline and year 2 simultaneously were enrolled in this analysis. HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma







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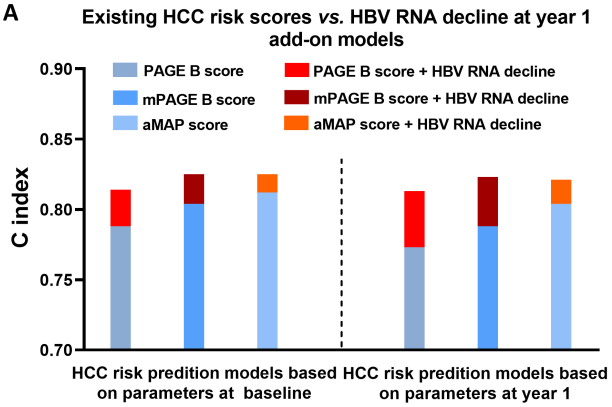
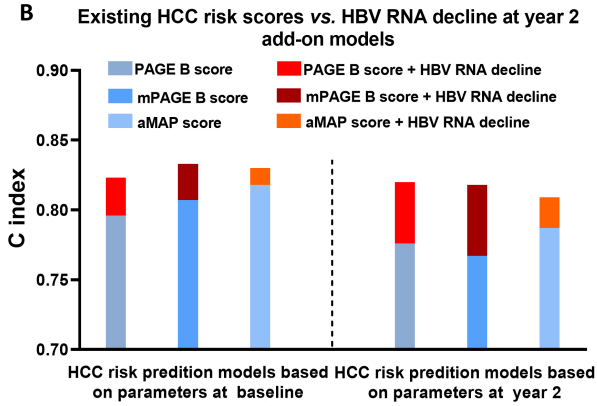


HCC risk prediction models [a]	C-index
Based on parameters at baseline	
PAGE B score	0.786
PAGE B score + HBV RNA decline at year 1	0.814
mPAGE B score	0.804
mPAGE B score + HBV RNA decline at year 1	0.825
aMAP score	0.811
aMAP score + HBV RNA decline at year 1	0.825
Based on parameters at year 1	
PAGE B score	0.773
PAGE B score + HBV RNA decline at year 1	0.813
mPAGE B score	0.788
mPAGE B score + HBV RNA decline at year 1	0.823
aMAP score	0.804
aMAP score + HBV RNA decline at year 1	0.821

B



HCC risk prediction models [b]	C-index
Based on parameters at baseline	
PAGE B score	0.796
PAGE B score + HBV RNA decline at year 2	0.823
mPAGE B score	0.807
mPAGE B score + HBV RNA decline at year 2	0.833
aMAP score	0.818
aMAP score + HBV RNA decline at year 2	0.830
Based on parameters at year 2	
PAGE B score	0.776
PAGE B score + HBV RNA decline at year 2	0.820
mPAGE B score	0.767
mPAGE B score + HBV RNA decline at year 2	0.813
aMAP score	0.787
aMAP score + HBV RNA decline at year 2	0.809

Supplementary Materials

1 The exclusion criteria of patients from Search-B cohort and HK Cohort in the present study

1.1 Treatment duration > 6 months before enrollment

1.2 Missing records for the date of NA initiation

1.3 Non-ETV or -TDF treatment

1.4 Follow-up duration <6 months or development of HCC within 6 months after enrollment

1.5 Achieving serum HBsAg loss before enrollment

1.6 Without adequate serum samples taken for serum HBV RNA detection within the first 3 years after enrollment

2 Definitions of hepatocellular carcinoma (HCC) and cirrhosis in each cohort

2.1 Search-B cohort

2.1.1 HCC

The diagnosis of HCC should meet any 1 of the following criteria:

1) Histopathological confirmation by liver biopsy or surgically excised tissue

2) Detection of a lesion which meets criteria for HCC with at least two imaging techniques (trans-abdominal USG, triphasic abdominal CT scan, magnetic resonance imaging study of liver, or hepatic angiogram)

3) Detection with one imaging technique coupled with an AFP concentration greater

than 400 μg/L.

2.1.2 Cirrhosis

The criteria for hepatic cirrhosis are defined as follows (including compensated and decompensated):

1) Evidence of cirrhosis based on liver biopsy (evaluated by Ishak score system).

2) In the absence of liver biopsy results, the patient should meet 1 of the following criteria:

− Ascites

− Hepatic encephalopathy

− Upper gastro-intestinal (esophageal and/or gastroduodenal) bleeding

− Hepatorenal syndrome

The complication caused by non-cirrhotic portal hypertension (such as portal vein thrombosis, congenital hepatic fibrosis, idiopathic portal hypertension, etc) should be excluded.

3) If none of the criteria above are met, the patient should meet any 2 of the following 4 criteria:

− Liver imaging showing features of cirrhosis: nodular liver and/or splenomegaly

− Platelet count <150,000/mm3 in the absence of other explanation

− Liver stiffness measurement (by Fibroscan) >12 kPa

− Gastro-esophageal varices as visualized by upper endoscopy

2.2 HK cohort

2.2.1 HCC

The diagnosis of HCC was established based on tumor histology or detected by multiphasic computed tomography (CT), dynamic contrast-enhanced magnetic resonance imaging (MRI) or contrast-enhanced ultrasound for cirrhotic patients with nodular(s) >= 1 cm [1].

2.2.2 Cirrhosis

Liver cirrhosis was defined by shrunken small liver with nodular surface noted on liver imaging and clinical features of portal hypertension (e.g.ascites, splenomegaly, and varices) [2].

3 Detailed information and the performance of serum HBV RNA assay based on HBV-SAT kit

3.1 Detection procedure

Serum HBV RNA was detected by RNA simultaneous amplification testing method (HBV-SAT) based on real-time fluorescence detection of 42℃ isothermal RNA amplification using HBV-SAT kit (Shanghai Rendu Biotechnology Co., Ltd. China). Briefly, only RNA of specimens was extracted by magnetic microparticles with HBV specific RNA oligonucleotides excluding the interference of DNA molecules. The target RNA was reverse transcribed by MMLV enzyme, transcribed by T7 RNA polymerase and detected by RNA molecular beacon probe labeled by fluorescence and quencher. RNA extraction, amplification, and detection were processed on an automated AutoSAT system (Shanghai Rendu Biotechnology Co., Ltd. China) without any manual operations. An internal calibrator/internal control (IC) was added to every

single reaction. The concentration of a sample was determined using the HBV and IC signals for each reaction and compared them with calibration information.

3.2 Linearity

The linearity of the serum HBV RNA assay was evaluated by testing 10-fold dilutions of armored HBV RNA with concentration from 10^2 to 10^8 copies/mL with 5 replicates. The average R^2 value of linear equation is 0.9971 (Supplementary Figure 1). The variable coefficient (CV) of the detected results of 10^2 copies/mL, the lower limit of quantification, was 6.3%.

3.3 Sensitivity and specificity

Assay analytical sensitivity was determined by detection of low-range dilution series of armored HBV RNA (70 copies/mL, 60 copies/mL, 50 copies/mL and 30 copies/mL) with 20 replicates respectively, then the limit of detection (95% detection) was extrapolated via Probit analyses to be 50 copies/mL (Supplementary table 1). Assay analytical specificity was confirmed by observation of no HBV RNA detection in 90 HBV-negative blood donors (donors with HBsAg, HBeAg and HBeAb, as well as an-HBc were all negative), demonstrating 100% specificity.

3.4 Repeatability

Three serum samples (high, medium and low concentration) were assayed in 4 different assay runs with 4 replicates to evaluate the repeatability of the serum HBV RNA assay.

Low SDs and CVs were observed for replicates within and between runs (Supporting Table S2), demonstrating the assay repeatability.

3.5 Accuracy

To assess the accuracy of the assays, three serum samples with concentration 10^7, 10^5 and 10^3 copies/ml were assayed for four times. The differences between experimentally measured and actual concentrations were showed in supplementary table S3.

4 Statistical analysis

Patients' characteristics were expressed as counts and percentages for categorical variables and as median and interquartile range (IQR) for continuous variables. The cumulative probabilities of HCC development were estimated by the Kaplan-Meier (K-M) method and compared using the log-rank test. Firstly, restricted cubic spline analyses with 3 or 4 knots (depended on the lowest value of the Akaike Information Criterion) were used to explore the potential linear or nonlinear relationship between the serum HBV RNA kinetics and HCC development [3]. Tests for nonlinearity comparing a model with only the linear term to a model with the linear and restricted cubic spline terms were conducted using likelihood ratio tests. If a test for nonlinearity was not significant, a test for linearity was conducted comparing a model with the linear term to a model with only the covariates of interest [4]. Then Cox proportional hazard models were used to estimate the effect of early on-treatment serum HBV RNA declines (at year 1 and 2 of NA treatment) on HCC risk. For the 1-, 2- and 3-year landmark

analysis, the whole evaluated period was from the landmark to HCC/last follow-up visit. At each landmark, data from at-risk patients at that time (e.g., those who are alive without HCC) were included.

Series of early serum HBV RNA declines add-on HCC risk prediction models by incorporating serum HBV RNA decline at year 1 or 2 into the models that included the variables in the existing HCC risk scores , including PAGE B (age, gender and platelets), mPAGE B (age, gender, platelets and albumin) and aMAP Score (age, gender, platelets and ALBI Score), based on baseline, 1-year or 2-year values using Cox proportional hazard analysis (Detailed information of the models were shown in supplemental materials). To evaluate the predictive performance of these novel and existing HCC risk prediction models, time-dependent area under the receiver operating characteristic curve (AUROC) and Harrell's C-index were used. In addition, repeated k-fold Cross Validation (5-folds with 1000 repeats in the present study) was performed for models' internal validation. The average Harrell's C-index and time dependent AUROC of the existing and early serum HBV RNA declines add-on models were evaluated in the internal validation sets.

5 Construction of early on-treatment serum HBV RNA declines add-on models for HCC risk prediction

In the present study, early on-treatment serum HBV RNA declines add-on models were established by incorporating serum HBV RNA declines at year 1 or 2 of NA treatment

into the models with parameters that included in the existing HCC risk scores–PAGE B, mPAGE B and aMAP score using multivariate Cox proportional hazard regression model. The parameters included in the models and existing HCC risk prediction scores were based on their values at baseline, year 1 and year 2 of NA treatment respectively. The detailed information of PAGE B, mPAGE B, and aMAP score is reported at previous studies [5-7], which calculated by age, sex, platelet (PAGE B Score); age, sex, albumin, platelet (mPAGE B Score); and age, sex, platelet and ALBI Score (aMAP Score) respectively.

The formula of the series of early on-treatment serum HBV RNA declines add-on models based on parameters at baseline are as follows: Model (PAGE B + HBV RNA decline at year 1) = 0.062*age (in years) + 1.088*sex (Male = 1, Female = 0)+(-0.304)*HBV RNA decline at year 1 (log_{10} copies/mL) + 0.980*platelet (<100, 2; 100-199,1; >200,0); Model (PAGE B + HBV RNA decline at year 2) = 0.064*age (in years) + 1.328*sex (Male = 1, Female = 0)+(-0.185)*HBV RNA decline at year 2 (log_{10} copies/mL) + 0.985*platelet (<100, 2; 100-199,1; >200,0); Model (mPAGE B + HBV RNA decline at year 1) = 0.056*age (in years) + 1.158*sex (Male = 1, Female = 0)+(-0.336)*HBV RNA decline at year 1 (log_{10} copies/mL) + (-0.012)* platelet + (-0.038)*albumin; Model (mPAGE B + HBV RNA decline at year 2) = 0.056*age (in years) + 1.429*sex (Male = 1, Female = 0)+(-0.244)*HBV RNA decline at year 2 (log_{10} copies/mL) + (-0.010)* platelet + (-0.064)*albumin; Model (aMAP + HBV RNA decline at year 1) = 0.057*age (in years) + 1.134*sex (Male = 1, Female = 0)+(-

0.334)*HBV RNA decline at year 1 (log_{10} copies/mL) + (-0.011)*platelet + 0.332*ALBI Score; Model (aMAP + HBV RNA decline at year 2) = 0.058*age (in years) + 1.379*sex (Male = 1, Female = 0)+(-0.240)*HBV RNA decline at year 2 (log_{10} copies/mL) + (-0.011)*platelet + 0.507*AIBI Score.

The formula of the series of serum HBV RNA decline at year 1 add-on models based on parameters at year 1 of treatment are as follows: Model (PAGE B + HBV RNA decline at year 1) = 0.066*age (in years) + 1.155*sex (Male = 1, Female = 0) + (-0.290) * HBV RNA decline at year 1 (log_{10} copies/mL) + 0.851*platelet (<100, 2; 100-199,1; >200,0); Model (mPAGE B + HBV RNA decline at year 1) = 0.056*age (in years) + 1.257*sex (Male = 1, Female = 0) + (-0.322)*HBV RNA decline at year 1 (log_{10} copies/mL) + (-0.008)*platelet + (-0.092)*albumin; Model (aMAP + HBV RNA decline at year 1) = 0.057*age (in years) + 1.243*sex (Male = 1, Female = 0)+(-0.325)*HBV RNA decline at year 1 (log_{10} copies/mL) + (-0.007)*platelet + 0.975*ALBI Score.

The formula of the series of serum HBV RNA decline at year 2 add-on models based on parameters at year 2 of treatment are as follows: Model (PAGE B + HBV RNA decline at year 2) = 0.067*age (in years) + 1.316*sex (Male = 1, Female = 0)+(-0.183)*HBV RNA decline at year 2 (log_{10} copies/mL) + 0.784*platelet (<100, 2; 100-199,1; >200,0); Model (mPAGE B + HBV RNA decline at year 2) = 0.064*age (in years) + 1.480*sex (Male = 1, Female = 0)+(-0.206)*HBV RNA decline at year 2 (log_{10}

copies/mL) + (-0.006)*platelet + (-0.069)*albumin; Model (aMAP + HBV RNA decline at year 2) = 0.065*age (in years) + 1.485*sex (Male = 1, Female = 0)+(-0.202)*HBV RNA decline at year 2 (log_{10} copies/mL) + (-0.005)*platelet + 0.777*ALBI Score.

Reference

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Table S1. Assay sensitivity data

Copies/ml	Total number of detection	Number of positive results	Positive rate
70	20	20	100%
60	20	20	100%
50	20	19	95%
40	20	18	90%
30	20	15	75%

Table S2. Assay repeatability data

Sample	Concentration (log copies/ml)	Intra-run [b] SD [a]	Inter-run [c] SD [a]	Intra-run [b] CV	Inter-run [c] CV
1	7.00	0.062	0.08	0.88%	1.19%
2	5.00	0.105	0.12	2.12%	2.35%
3	3.00	0.057	0.09	1.88%	3.09%

[a] SD in log U/ml, [b] average SD within runs, [c] SD between runs. SD, standard deviation; CV, Coefficient of Variation

Table S3. Assay accuracy data

Actual concentration (log_{10} copies/ml)	Measured concentration (log_{10} copies/ml)				Difference (log_{10} copies/ml)	
	repeat1	repeat2	repeat3	repeat4	Mean	Fold changes
7.00	7.05	7.04	7.06	7.07	7.06	1.009
5.00	4.85	4.97	5.16	4.95	4.98	0.996
3.00	2.98	2.96	3.03	2.89	2.97	0.990

Table S4. Adjusted analyses for the association between early on-treatment HBV RNA declines and HCC risk in NA-treated patients with chronic hepatitis B

HBV RNA decline	aHR *	95% CI	P value
At year 1, log_{10} copies/mL			
> 0.4	Reference		
=< 0.4	2.12	1.22-3.68	0.008
At year 2, log_{10} copies/mL			
> 0.6	Reference		
=< 0.6	2.01	1.10-3.69	0.024

* aHR, adjusted hazard ratio, obtained after adjusting aMAP HCC risk score (an index reflecting the underlying HCC development risk calculated by age, sex, albumin, total bilirubin and platelet), cirrhosis, diabetes, drinking history and smoking history at year 1 of NA treatment and baseline HBeAg status. HBV, hepatitis B virus; NA, nucleos(t)ide analogue; HBeAg, hepatitis B e antigen.

Table S5. Association between early on-treatment HBV RNA declines and HCC risk in patients with NA treatment duration before enrollment =<2 months

HBV RNA decline	No. of Patients	HCC cases	aHR [a]	95% CI	*p* value
At year 1, log_{10} IU/mL					
> 0.4	574 [b]	29	Reference		
=< 0.4			2.36	1.09-5.12	0.029
At year 2, log_{10} IU/mL					
> 0.6	517 [b]	21	Reference		
=< 0.6			2.84	1.11-7.25	0.030

[a] aHR, adjusted hazard ratio, obtained after adjusting baseline aMAP HCC risk score (an index reflecting the underlying HCC development risk calculated by age, sex, album, total bilirubin and platelet), cirrhosis, diabetes, HBeAg status, drinking history and smoking history. b Patients with treatment duration before enrollment =<2 months were enrolled for the analyses. HBV, hepatitis B virus; NA, nucleos(t)ide analogue; HBeAg, hepatitis B e antigen.

Table S6. Association between early on-treatment HBV RNA declines and HCC risk in patients with detectable serum HBV RNA at baseline

HBV RNA decline	No. of Patients	HCC cases	aHR [a]	95% CI	*p* value
At year 1, log_{10} IU/mL					
> 0.4	1061 [b]	52	Reference		
=< 0.4			2.70	1.52-4.78	0.001
At year 2, log_{10} IU/mL					
> 0.6	1039 [b]	42	Reference		
=< 0.6			2.36	1.26-4.44	0.008

[a] aHR, adjusted hazard ratio, obtained after adjusting baseline aMAP HCC risk score (an index reflecting the underlying HCC development risk calculated by age, sex, album, total bilirubin and platelet), cirrhosis, diabetes, HBeAg status, drinking history and smoking history. b Patients with undetectable baseline HBV RNA were excluded for the analyses. HBV, hepatitis B virus; NA, nucleos(t)ide analogue; HBeAg, hepatitis B e antigen.

Table S7. Association between early on-treatment HBV RNA declines and HCC risk in patients with early HBV DNA response

HBV RNA decline	No. of Patients	HCC cases	aHR [a]	95% CI	*p* value
At year 1, log_{10} IU/mL					
> 0.4	879 [b]	47	Reference		
=< 0.4			2.49	1.29-4.78	0.006
At year 2, log_{10} IU/mL					
> 0.6	1027 [c]	43	Reference		
=< 0.6			2.14	1.11-4.12	0.023

[a] aHR, adjusted hazard ratio, obtained after adjusting baseline aMAP HCC risk score (an index reflecting the underlying HCC development risk calculated by age, sex, album, total bilirubin and platelet), cirrhosis, diabetes, HBeAg status, drinking history and smoking history. [b] Patients without early HBV DNA response (defined as achieving HBV DNA within 1 year of NA treatment) were excluded for the analyses. [c] Patients without early HBV DNA response (defined as achieving HBV DNA within 2 year of NA treatment) were excluded for the analyses HBV, hepatitis B virus; NA, nucleos(t)ide analogue; HBeAg, hepatitis B e antigen.

Table S8. The average values of Harrell's C-indexes and time-dependent AUROCs of the early on-treatment serum HBV RNA declines add-on models and existing HCC risk scores in the internal validation set.

HCC risk prediction models	C-index	Time-dependent AUROC	
		3-year	5-year
Training set			
Based on parameters at baseline			
PAGE B score	0.786	0.805	0.746
PAGE B score + HBV RNA decline at year 1	0.815	0.833	0.782
mPAGE B score	0.804	0.822	0.749
mPAGE B score + HBV RNA decline at year 1	0.827	0.840	0.787
aMAP score	0.811	0.819	0.759
aMAP score + HBV RNA decline at year 1	0.826	0.838	0.786
Based on parameters at year 1			
PAGE B Score	0.773	0.792	0.719
PAGE B Score + HBV RNA decline at year 1	0.815	0.831	0.776
mPAGE B Score	0.788	0.810	0.726
mPAGE B Score + HBV RNA decline at year 1	0.824	0.849	0.785
aMAP Score	0.804	0.822	0.752
aMAP Score + HBV RNA decline at year 1	0.822	0.840	0.783
Based on parameters at baseline			
PAGE B score	0.796	0.814	0.706

PAGE B score + HBV RNA decline at year 2	0.824	0.845	0.731
mPAGE B score	0.807	0.825	0.726
mPAGE B score + HBV RNA decline at year 2	0.834	0.855	0.747
aMAP score	0.818	0.830	0.738
aMAP score + HBV RNA decline at year 2	0.831	0.851	0.744
Based on parameters at year 2			
PAGE B score	0.778	0.789	0.672
PAGE B score + HBV RNA decline at year 2	0.820	0.840	0.725
mPAGE B score	0.767	0.777	0.656
mPAGE B score + HBV RNA decline at year 2	0.814	0.837	0.713
aMAP score	0.787	0.797	0.682
aMAP score + HBV RNA decline at year 2	0.810	0.836	0.710
Test set			
Based on parameters at baseline			
PAGE B score	0.786	0.804	0.746
PAGE B score + HBV RNA decline at year 1	0.802	0.820	0.766
mPAGE B score	0.803	0.822	0.750
mPAGE B score + HBV RNA decline at year 1	0.812	0.827	0.770
aMAP score	0.811	0.819	0.760
aMAP score + HBV RNA decline at year 1	0.812	0.825	0.769
Based on parameters at year 1			
PAGE B score	0.772	0.791	0.720
PAGE B score + HBV RNA decline at year 1	0.802	0.821	0.760

mPAGE B score	0.787	0.810	0.726
mPAGE B score + HBV RNA decline at year 1	0.810	0.836	0.767
aMAP score	0.803	0.822	0.752
aMAP score + HBV RNA decline at year 1	0.806	0.827	0.765
Based on parameters at baseline			
PAGE B score	0.794	0.815	0.709
PAGE B score + HBV RNA decline at year 2	0.809	0.836	0.709
mPAGE B score	0.805	0.825	0.727
mPAGE B score + HBV RNA decline at year 2	0.819	0.845	0.724
aMAP score	0.817	0.830	0.739
aMAP score + HBV RNA decline at year 2	0.816	0.842	0.721
Based on parameters at year 2			
PAGE B score	0.775	0.789	0.676
PAGE B score + HBV RNA decline at year 2	0.804	0.831	0.703
mPAGE B score	0.765	0.777	0.658
mPAGE B score + HBV RNA decline at year 2	0.793	0.825	0.684
aMAP score	0.785	0.797	0.685
aMAP score + HBV RNA decline at year 2	0.789	0.823	0.679

AUROC, area under the receiver operating characteristic curve; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

Fig.S1 The distribution of enrolled patients' treatment duration before enrollment

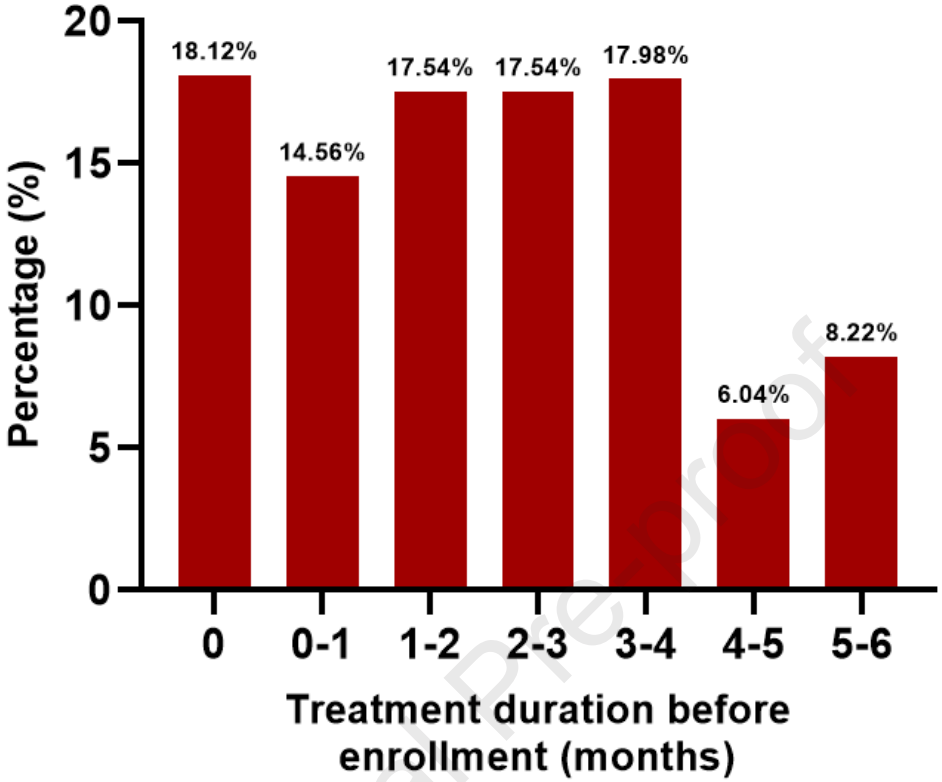


Fig. S2. Detection results for a 10-fold dilution of armored HBV RNA show linearity over tested range.

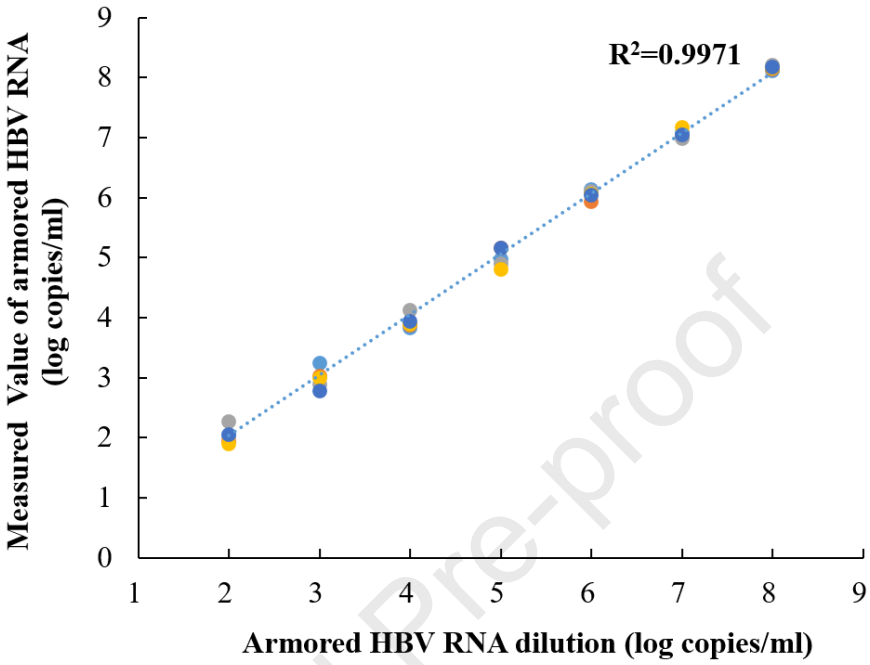


Fig. S3. Cumulative incidence of HCC in the datasets starting from baseline (A), year 1 (B), year 2 (C) and year 3 (D) of NA treatment respectively. HCC, hepatocellular carcinoma; NA, nucleot(s)ide analogue.

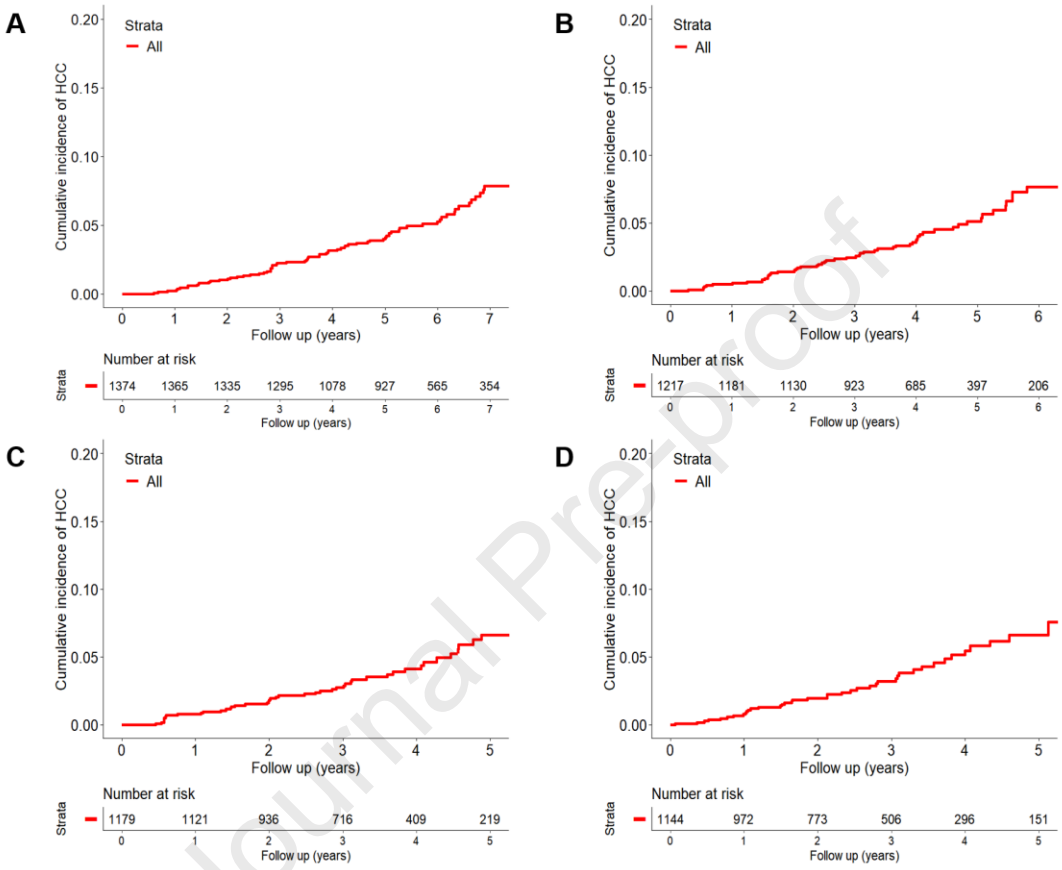


Fig.S4. The kinetics of serum viral markers within the first 3 years of NA treatment. (A) Serum HBV DNA kinetics; (B) Serum qHBsAg kinetics; (C) Serum HBV RNA kinetics; (D) Undetectable rates of serum HBV DNA and RNA. NA, nucleos(t)ide analogue; HBV, hepatitis B virus; qHBsAg, quantitative hepatitis B surface antigen; LLOD, lower limit of detection.

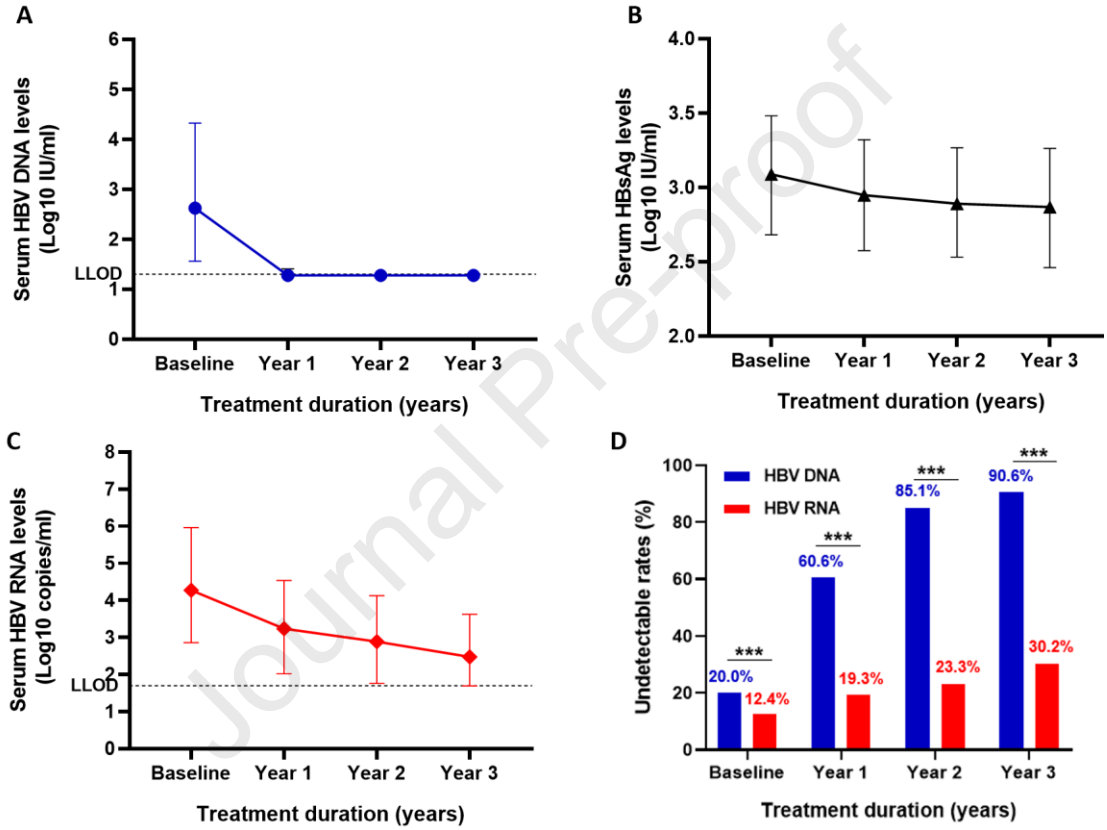


Fig.S5. Adjusted HRs for the HCC risk by serum HBV RNA levels at baseline (A), year 1 (B), year 2 (C) and year 3 (D) of NA treatment in patients with chronic hepatitis B. The HR plot was adjusted for baseline aMAP score, HBeAg status, cirrhosis, diabetes, smoking history and drinking history. HBV RNA level below 50 copies/mL was used as a reference. The blue line represents the point estimates, and the blue zone indicates 95% CI. HR, hazard ratio; HCC, hepatocellular carcinoma; NA, nucleoside analogue. aMAP score, an index reflecting the underlying HCC development risk calculated by age, sex, albumin, total bilirubin and platelet.

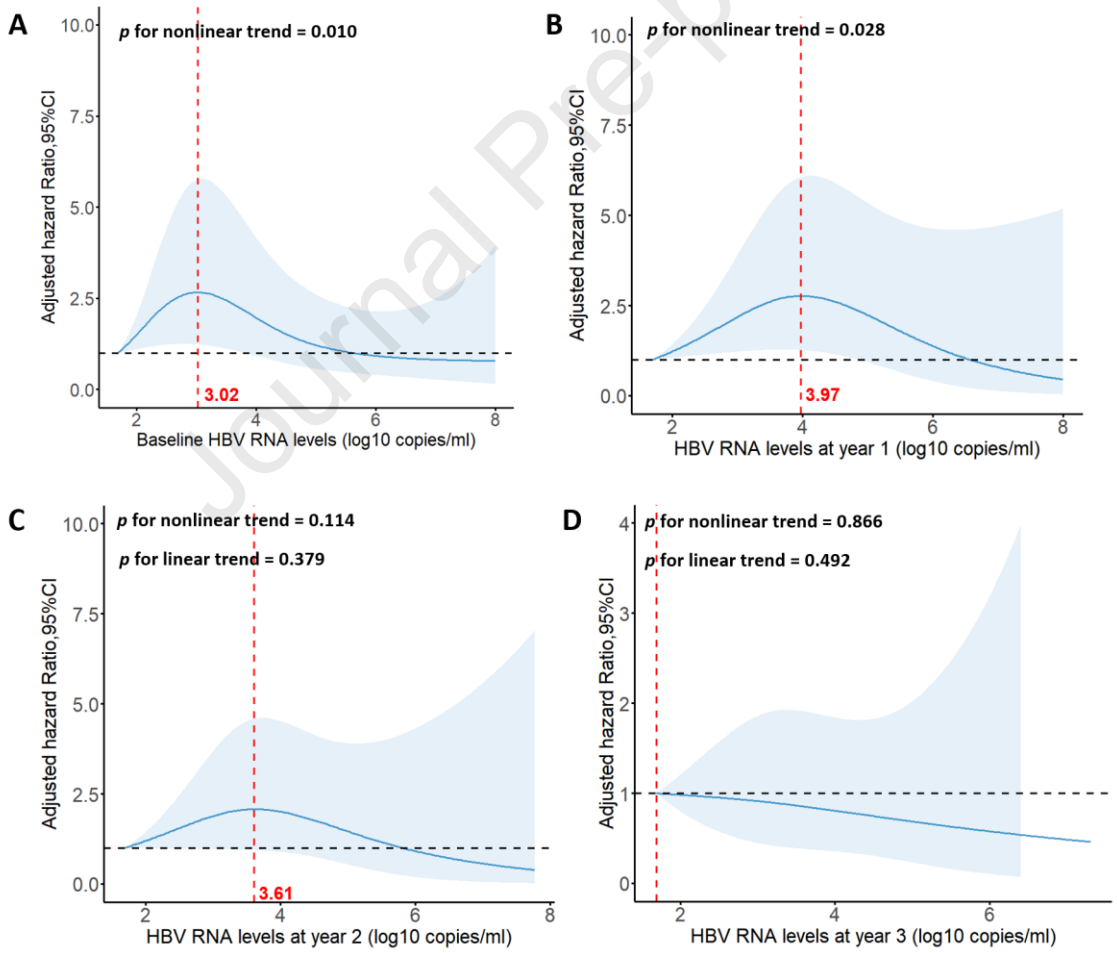


Fig.S6 Adjusted HRs for the HCC risk by qHBsAg levels at baseline (A), year 1 (B), year 2 (C) and year 3 (D) of NA treatment in patients with chronic hepatitis B.

The HR plot was adjusted for baseline aMAP Score, HBeAg status, cirrhosis, diabetes, smoking history and drinking history. HBV RNA level below 50 copies/mL was used as a reference. The blue line represents the point estimates, and the blue zone indicates 95% CI. HR, hazard ratio; HCC, hepatocellular carcinoma; NA, nucleoside analogue. qHBsAg, quantitative hepatitis B surface antigen. aMAP score, an index reflecting the underlying HCC development risk calculated by age, sex, albumin, total bilirubin and platelet.

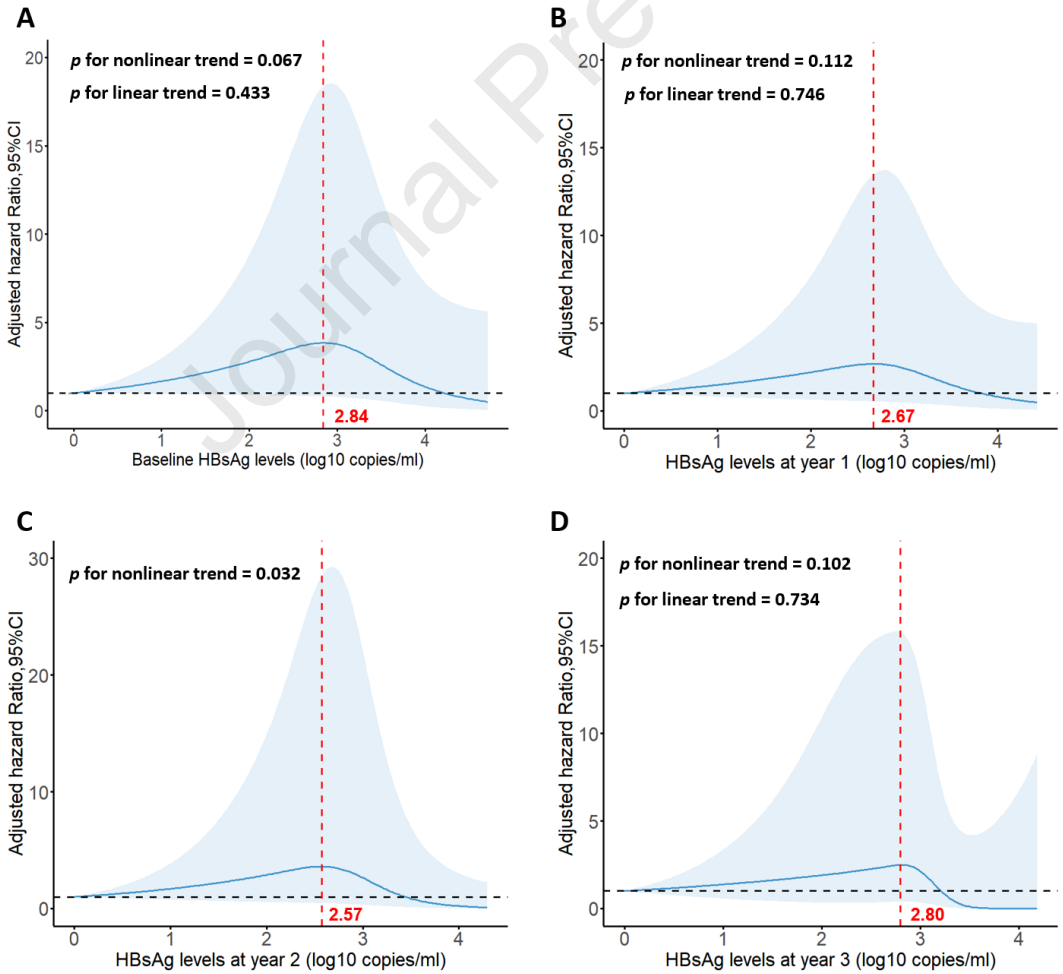


Fig. S7. Adjusted HRs for the HCC risk by qHBsAg decline at year 1 (A), year 2 (B) and year 3 (C) of NA treatment in patients with chronic hepatitis B. The HR plot was adjusted for baseline aMAP Score, HBeAg status, cirrhosis, diabetes, smoking history and drinking history. HBV RNA level below 50 copies/mL was used as a reference. The blue line represents the point estimates, and the blue zone indicates 95% CI. HR, hazard ratio; HCC, hepatocellular carcinoma; NA, nucleoside analogue. qHBsAg, quantitative hepatitis B surface antigen. aMAP score, an index reflecting the underlying HCC development risk calculated by age, sex, albumin, total bilirubin and platelet.

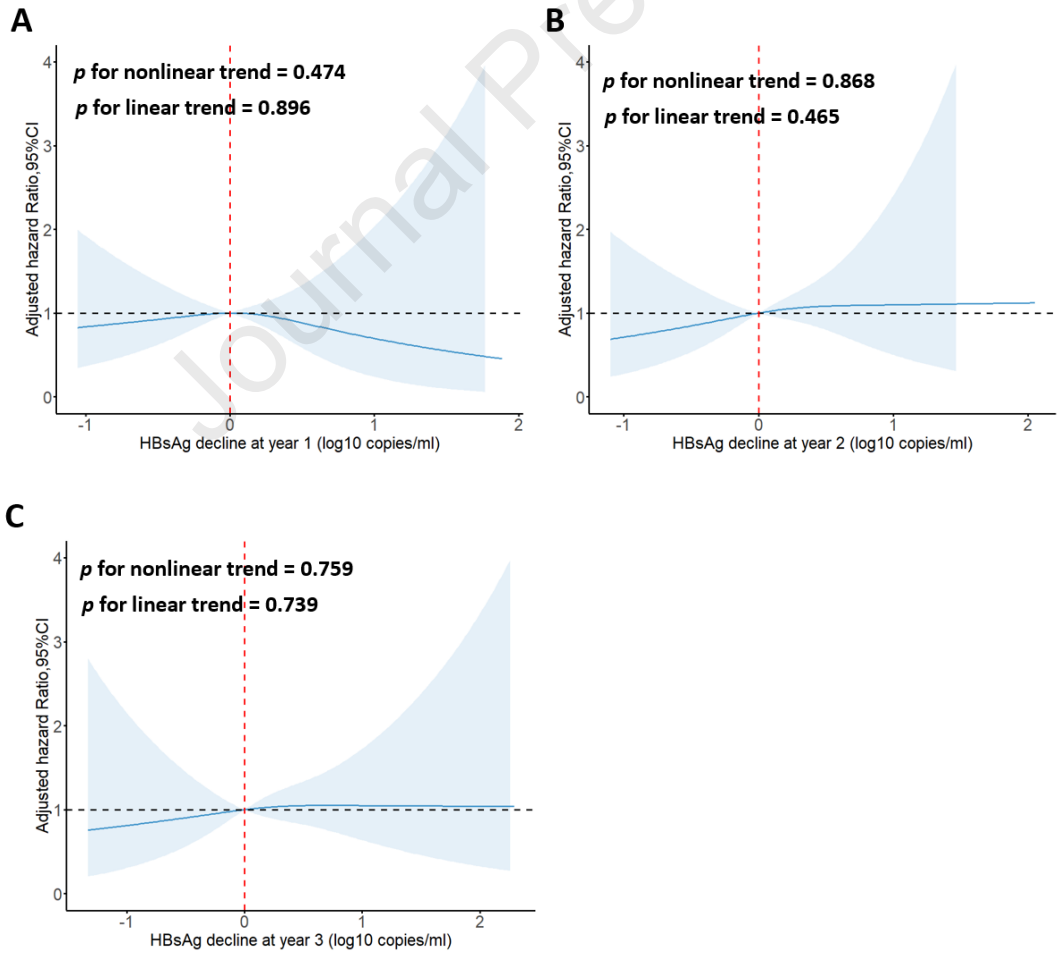


Fig. S8. Time-dependent AUROCs of serum HBV RNA decline at year 1 (A) and year 2 (B) in predicting on-treatment HCC risk. AUROC, area under the receiver operating characteristic curve; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

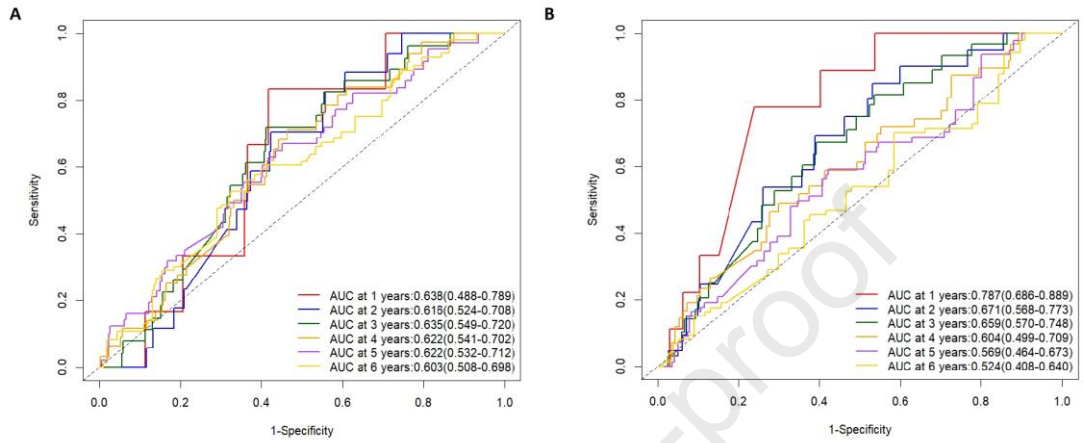


Fig.S9. Time-dependent AUROCs of existing HCC risk scores and early on-treatment serum HBV RNA declines add-on models based on parameters at different time points of NA treatment in predicting HCC risk. (A-B) HCC risk prediction models containing serum HBV RNA decline at year 1; (C-D) HCC risk prediction models containing serum HBV RNA decline at year 2. AUROCs, area under the receiver operating characteristic curve; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NA, nuclet(s)ide analogue.

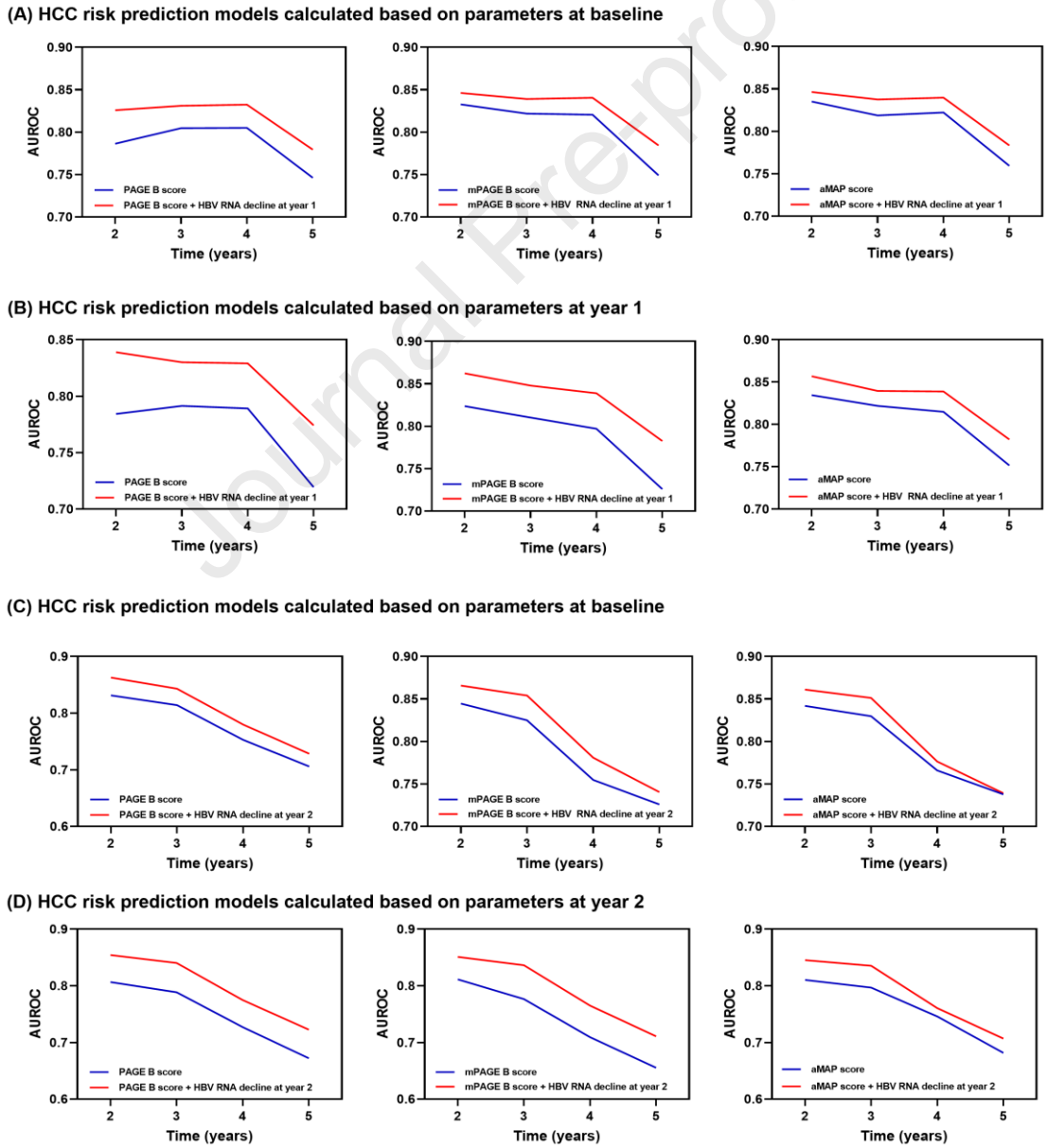
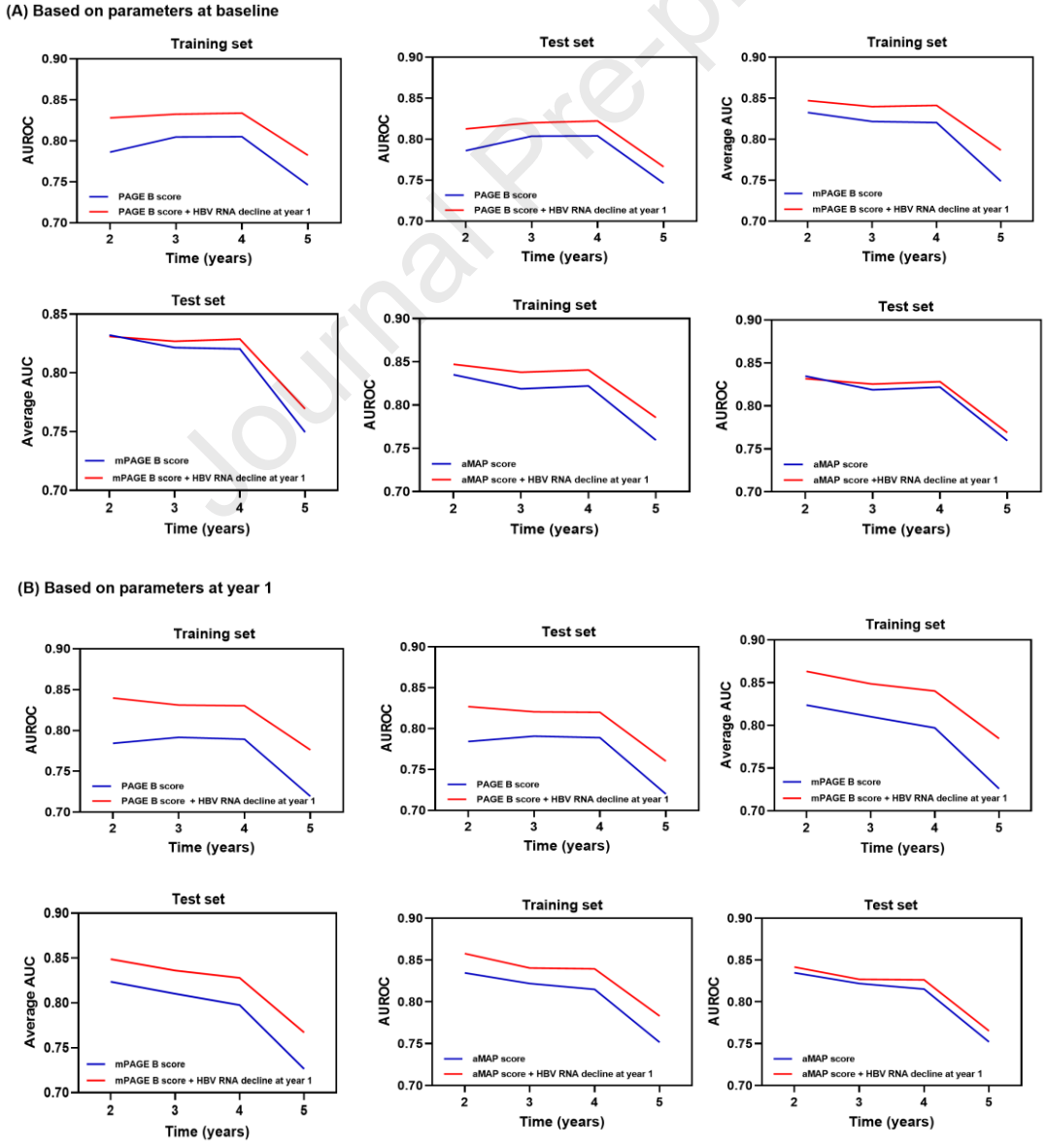
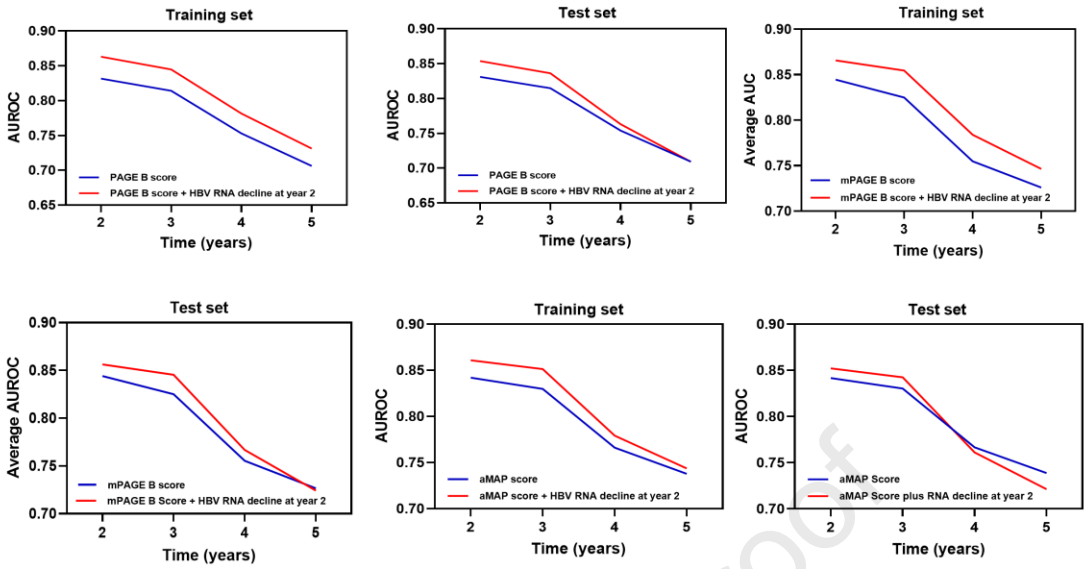


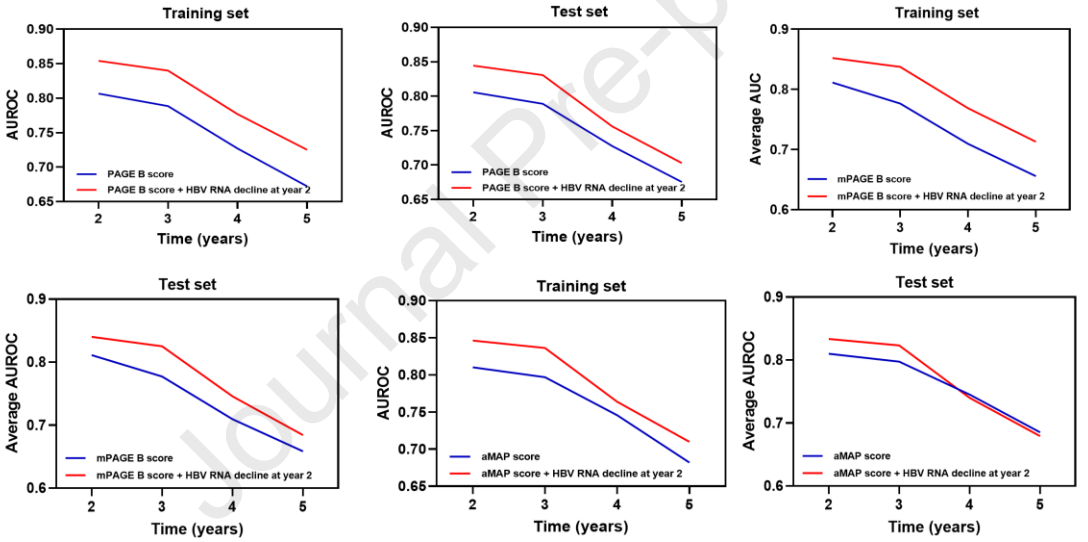
Fig.S10. Average time-dependent AUROCs of existing HCC risk scores and early on-treatment serum HBV RNA declines add-on models based on parameters at different time points of NA treatment in predicting HCC risk in the training and test sets obtained from K-folds cross-validation method. (A-B) HCC risk prediction models containing serum HBV RNA decline at year 1; (C-D) HCC risk prediction models containing serum HBV RNA decline at year 2. AUROCs, area under the receiver operating characteristic curve; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NA, nuclet(s)ide analogue.



(C) Based on parameters at baseline



(D) Based on parameters at year 2



What You Need to Know

Background

Viral markers were rarely included in hepatocellular carcinoma (HCC) risk prediction models in patients with chronic hepatitis B infection (CHB) under nucleoside analogue (NA) treatment.

Findings

Serum hepatitis B virus (HBV) RNA declines at year 1 and 2 of NA treatment serve as independent predictors of HCC development, which could enhance the predictive performance of the PAGE B, mPAGE B and aMAP score.

Implications for patient care

In the era of antiviral treatment, early on-treatment HBV RNA declines can be useful tools to guide appropriate HCC surveillance strategies in patients with CHB.