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Title:
Serum HBV RNA as a predictive biomarker for HBeAg seroconversion during entecavir and tenofovir disoproxil fumarate therapy in chronic hepatitis B patients

Authors:
Shu Wu, Huiling Kang, Er Huang, Wennan Wu, Lijuan Liu, Ronghua Chen

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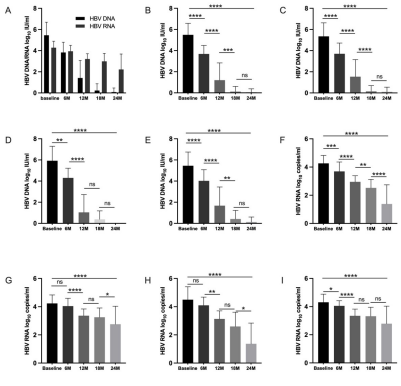
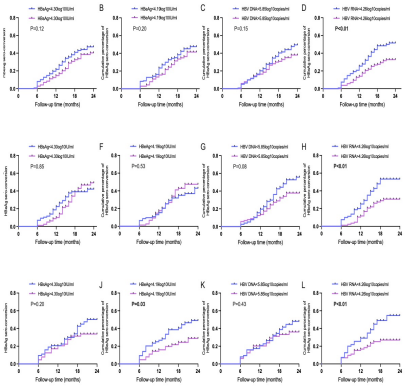
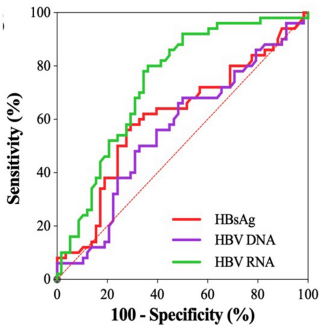
HBV DNA and HBV RNA levels declined significantly both in seroconversion group and non-seroconversion group.



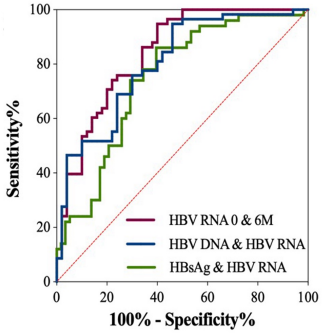
Lower baseline HBV RNA levels were linked to higher HBeAg seroconversion rates in CHB patients.



HBV RNA showed better predictive performance for HBeAg seroconversion at both baseline and 6 months.



The combination of baseline and 6-month HBV RNA levels achieved the highest predictive accuracy.



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Serum HBV RNA as a Predictive Biomarker for HBeAg Seroconversion During Entecavir and Tenofovir Disoproxil Fumarate Therapy in Chronic Hepatitis B Patients

Shu Wu[a,#], Huiling Kang[b,#], Er Huang[a], Wennan Wu[a],Lijuan Liu[b], Ronghua Chen[b,*]

[a]Department of Laboratory Medicine, The First Affiliated Hospital of Fujian Medical University, Fuzhou, China

[b]Department of Laboratory Medicine, Mengchao Hepatobiliary Hospital of Fujian Medical University

[#]These authors contributed equally to this work.

***Corresponding author:** Ronghua Chen

Mengchao Hepatobiliary Hospital of Fujian Medical University, No. 66 Jintang Road, Fuzhou, CN.

E-mail address: chenronghua3310@163.com

Abbreviations list: CHB: Chronic Hepatitis B, NA: Nucleos(t)ide Analogue, TDF: Tenofovir Disoproxil Fumarate, ETV: Entecavir, HBV: Hepatitis B Virus, HBsAg: Hepatitis B Surface Antigen, HBeAg: Hepatitis B e Antigen, cccDNA: Covalently Closed Circular DNA, pgRNA: Pre-genomic RNA, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, qRT-PCR: Quantitative Real-Time Polymerase Chain Reaction, ROC: Receiver Operating Characteristic, AUC: Area Under the Curve, COI: Cut-Off Index, HR: Hazard Ratio, CI: Confidence Interval.

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Abstract

Background and Aim: Chronic hepatitis B (CHB) affects millions globally and is a leading cause of liver cirrhosis and hepatocellular carcinoma. Nucleos(t)ide analogues (NAs) such as entecavir (ETV) and tenofovir disoproxil fumarate (TDF) are effective in suppressing HBV replication. However, functional cure, defined by HBsAg clearance, remains challenging. Reliable biomarkers are needed to predict treatment outcomes.

Methods: This study retrospectively analyzed 293 HBeAg-positive CHB patients undergoing TDF or ETV treatment. Serum HBsAg, HBeAg, HBV DNA, and HBV RNA levels were measured at baseline and every six months over 24 months. Kaplan-Meier and Cox regression analyses were performed to evaluate the relationship between HBV RNA levels and HBeAg seroconversion.

Results: HBeAg seroconversion occurred in 129 patients, with a median time of 14 months. Lower baseline and 6-month HBV RNA levels were associated with higher seroconversion rates. Multivariate analysis confirmed HBV RNA as an independent predictor of seroconversion. ROC analysis showed that HBV RNA levels outperformed other markers in predicting outcomes, with the combined use of baseline and 6-month levels achieving the highest predictive accuracy.

Conclusion: HBV RNA is a promising biomarker for predicting HBeAg seroconversion during NAs therapy. Its use could enhance personalized treatment strategies for CHB patients.

Keywords: CHB. HBV RNA. TDF. ETV. HBeAg seroconversion.

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Lay Abstract

Chronic hepatitis B (CHB) is a serious liver disease affecting millions of people worldwide, often leading to liver damage and even liver cancer. Current antiviral treatments, such as entecavir (ETV) and tenofovir disoproxil fumarate (TDF), help control the virus but rarely lead to a complete cure. Therefore, doctors need better ways to predict how well a patient will respond to treatment.

In this study, we analyzed data from 293 patients with CHB who were receiving ETV or TDF treatment. We focused on a specific blood marker called HBV RNA and examined its relationship with a key milestone in treatment—HBeAg seroconversion, which indicates a favorable immune response. Our findings show that lower levels of HBV RNA at the start of treatment and after six months were linked to a higher chance of HBeAg seroconversion.

This means that measuring HBV RNA levels can help doctors predict which patients are more likely to respond well to treatment. By using this information, treatment plans can be better tailored to individual patients, potentially improving long-term outcomes for people living with CHB.

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Authorship Declaration

The authors have contributed to this study as follows:

- Wu Shu: Writing-Original Draft
- Kang Huiling: Methodology
- Huang Er: Data curation
- Wu Wennan: Formal Analysis
- Liu Lijuan: Writing – Conceptualization
- Chen Ronghua: Writing – Review & Editing

All authors have reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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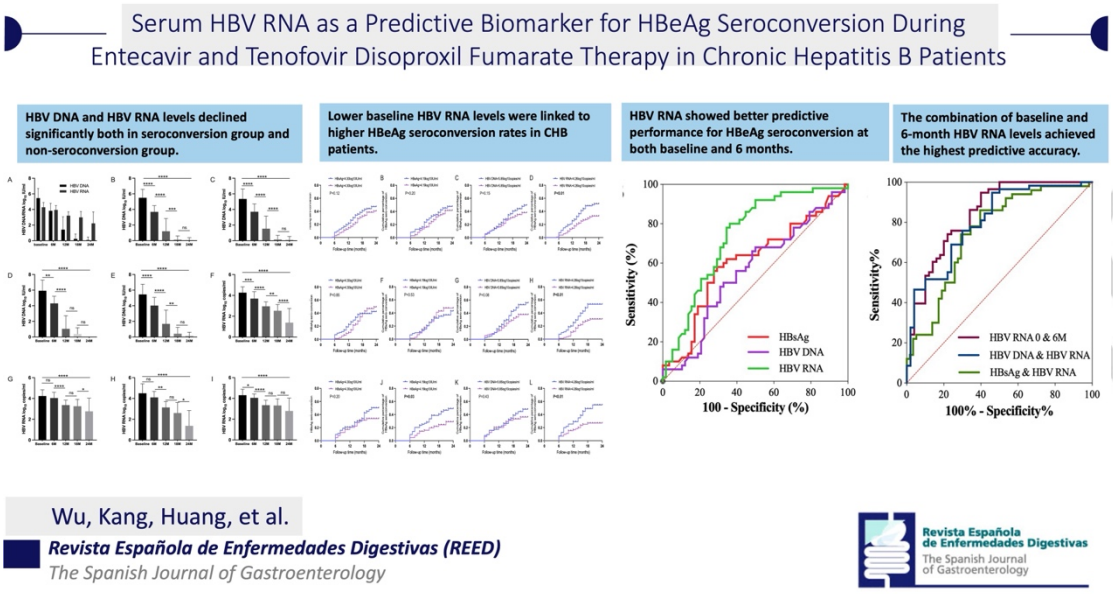
Visual Abstract



Key points

(1) Functional cure for CHB remains challenging despite effective antiviral therapy.

(2) In 293 HBeAg-positive patients, lower HBV RNA levels predicted higher seroconversion rates.

(3) HBV RNA was an independent predictor, outperforming other serological markers.

(4) Combining baseline and 6-month HBV RNA levels improved prediction accuracy. HBV RNA shows promise as a biomarker for personalized CHB treatment.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest related to this study. The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the writing, editing, data analysis, or figure preparation of this manuscript. All content was generated and reviewed solely by the authors.

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Introduction

Approximately 296 million people worldwide are infected with the hepatitis B virus (HBV), and chronic hepatitis B (CHB) caused by HBV infection is strongly associated with the development of cirrhosis and hepatocellular carcinoma[1]. Nucleos(t)ide analogues (NAs) are considered the first-line treatment for CHB, acting by inhibiting the reverse transcription of pre-genomic RNA (pgRNA) into HBV DNA. Among commonly used antiviral drugs, Entecavir (ETV) and Tenofovir disoproxil fumarate (TDF) are effective at suppressing viral replication, improving liver inflammation, and reducing the risk of cirrhosis and hepatocellular carcinoma[2]. However, in most patients, HBV DNA levels rebound after discontinuation of these drugs[3].

The ultimate goal of anti-HBV treatment, often referred to as functional cure, is the clearance of HBsAg[4]. For HBeAg-positive CHB patients, HBeAg seroconversion during treatment represents a critical step toward achieving functional cure and is often used to gauge treatment response. Therefore, predicting HBeAg seroconversion during ETV or TDF therapy plays a vital role in evaluating clinical efficacy and prognosis[5].

Traditional viral markers, including HBsAg, HBeAg and HBV DNA, are currently used to predict HBeAg seroconversion during NAs therapy[6]. Numerous studies indicate that HBsAg levels can help predict HBeAg clearance at the 48th or 96th week of NAs treatment[7, 8], while HBV DNA levels useful for predicting HBeAg seroconversion by the 24th or 48th week[9, 10]. However, Fung et al. reported that an early decline in HBsAg levels was not associated with HBV DNA suppression or HBeAg seroconversion, underscoring the limitations of current HBV markers in predicting treatment outcomes[11].

In recent years, emerging molecular markers have been investigated to improve the prediction of outcomes in CHB patients. Liver covalently closed circular DNA (cccDNA) is regarded as the gold standard for monitoring HBV replication[12]. However, measuring cccDNA requires a liver biopsy, an invasive procedure with technical limitations, including potential sampling errors. HBV RNA, a transcript derived from cccDNA that plays a role in viral replication, has been detected in liver biopsies, patient serum, and cultured hepatocytes[13]. Studies have shown that serum HBV RNA levels correlate with several HBV markers, including HBcrAg, HBsAg, HBV

DNA, HBeAg status, HBV genotypes, and patient age[14, 15].

Given the strong association of serum HBV RNA with the HBeAg-positive phase of infection, this biomarker shows significant potential for predicting HBeAg seroconversion during NA therapy. In this study, we evaluated the predictive value of serum HBV RNA for HBeAg seroconversion in CHB patients undergoing ETV and TDF therapy.

Materials and Methods

Patients and study design

This study was conducted at the Mengchao Hepatobiliary Hospital of Fujian Medical University and the first Affiliated Hospital of Fujian Medical University between January 1, 2018, and December 31, 2024. Eligible patients were diagnosed with CHB according to the 2022 version of the Guidelines for the Prevention and Treatment of Chronic Hepatitis B. The inclusion criteria were as follows: (i) CHB patients aged ≥ 18; (ii) HBeAg positive patients; (iii) Patients who received monotherapy with ETV or TDF. The criteria for exclusion were: (i) Co-infection with other viruses, including hepatitis C or D virus and human immunodeficiency; (ii) Patients with decompensated liver function (ascites, hepatic encephalopathy or upper gastrointestinal bleeding); (iii) Patients with any diseases of other major organs, such as severe heart disease or kidney disease; (iv) Poor compliance; (v) History of a malignancy, including hepatocellular carcinoma, carcinoma in situ or atypical hyperplastic nodules; (vi) Patients with mental illness; (vii) patients who had received corticosteroids, immunosuppressants or chemotherapeutic drugs ≤ 6 months prior to enrolment; and (viii) pregnant or breast-feeding women.

Patients were followed up every 6 months during NAs therapy. Serum specimens were collected at each follow-up. HBsAg, HBeAg, anti-HBeAg, HBV DNA and HBV RNA were measured separately. We retrospectively quantified these tests at baseline, 6, 12, 18 and 24 months. HBeAg seroconversion was defined as occurring when the COI of HBeAg was less than 1 and the COI of anti-HBeAg was greater than 1.

The study was conducted in compliance with the Declaration of Helsinki. Use of the research samples was approved by the Medical Ethics Review Committee of the First

Affiliated Hospital of Fujian Medical University and Menchao Hepatobiliary Hospital of Fujian Medical University. Written informed consent was obtained from all subjects before enrollment.

Assay for ALT, AST, HBsAg, HBeAg, HBV DNA and HBV RNA

Aspartate transaminase (ALT) and alanine aminotransferase (AST) were measured using a Cobas8000 automatic biochemical analyzer (Roche Diagnostics, Switzerland). Serum HBsAg and HBeAg were detected using the Architect ci4100 automatic biochemical immunoassay system (Abbott Laboratories, USA).

Serum HBV DNA was quantified on the Roche LightCycler 480 system (Roche Corporation, Switzerland) using a quantitative real-time PCR (qRT-PCR) assay (Sansure Biotech, China).

HBV RNA was detected 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) following the manufacturer's recommendations.

Statistical analysis

To evaluate the impact of baseline levels of various serological markers on HBeAg seroconversion, patients were stratified based on baseline levels of serological markers, including HBV RNA, HBsAg, HBeAg, and HBV DNA. Each variable was dichotomized according to its median value, and the cumulative HBeAg seroconversion rates were compared between the two groups for each marker. Kaplan-Meier survival curves were generated accordingly.

To further clarify the independent associations between serological markers and HBeAg seroconversion, a multivariate Cox proportional hazards regression model was performed. HBeAg seroconversion was used as the outcome variable, and baseline and 6-month on-treatment levels of serological markers, including HBV RNA, HBV DNA, HBsAg, and HBeAg, as well as their declines, were included as covariates. ALT and AST levels were also incorporated into the model. Continuous variables were included directly or dichotomized based on their median values,

depending on their distribution.

Statistical analyses were performed using IBM SPSS statistics, version 22.0 (SPSS, Chicago, IL, United States). Continuous variables were expressed as means ± SD, while categorical data were expressed as counts. Student's t test and Fisher's exact test were used to compare continuous and categorical variables between two groups, respectively. The Wilcoxon rank sum test was used to compare data before and after treatment. All statistical analyses were carried out using SPSS version 22.0 software (SPSS Inc., USA) and GraphPad Prism 7.0 software (GraphPad Software Inc., USA). A two-tailed P value of ,0.05 was considered statistically significant.

Results

Baseline characteristics of enrolled patients.

We retrospectively analyzed the demographic and laboratory test data of 536 chronic hepatitis B (CHB) patients who visited the First Affiliated Hospital of Fujian Medical University and Mengchao Hepatobiliary Hospital of Fujian Medical University between January 1, 2018, and December 31, 2024. A total of 293 HBeAg-positive naïve CHB patients were enrolled in this study (Figure 1). 166 patients were treated with ETV 0.5 mg daily, and 127 patients were treated with TDF 300 mg daily. Of them, 129 patients achieved HBeAg seroconversion for 24 months NAs therapy. The baseline characteristics of the included patients are summarized in Table 1-3.

The changes of HBV DNA and HBV RNA during NAs therapy.

The baseline of HBV DNA and HBV RNA were 5.71±1.37 and 4.40±0.57 respectively as shown in Figure 2A. After 24 months NAs therapy, HBV DNA and HBV RNA levels declined significantly both in seroconversion group (SC group) and non-seroconversion group (NSC group). In TDF treatment cohort, the HBV RNA level was 4.04 at 6 months, significantly declined compared with the level at baseline in NSC group ($p = 0.047$). In ETV treatment cohort, there was no significant difference between the HBV RNA level at baseline and 6 months in NSC group ($p = 0.148$). HBV DNA declined significantly after 6 months both in ETV and TDF treatment cohort

regardless of the occurrence of HBeAg seroconversion.

The relevance of serological markers to seroconversion of HBeAg during NAs treatment.

44.03% (129 of 293) patients achieved seroconversion of HBeAg after 24 months of NAs treatment, and the median seroconversion time was 14 months. 45.71% (76 of 166) patients achieved seroconversion of HBeAg after ETV treatment and 47.37% (53 of 127) patients achieved seroconversion after TDF treatment. The cumulative HBeAg seroconversion rate gradually increased with time as shown in Figure 3. In Figure 3A-3D, Kaplan-Meier survival analysis showed that CHB patients with lower baseline serum HBV RNA levels before treatment had a higher cumulative HBeAg seroconversion rate ($p<0.01$), while the differences in HBsAg, HBeAg, and HBV DNA levels were not significant (all $p>0.05$). In CHB patients treated with either ETV or TDF, low serum HBV RNA levels were closely associated with higher HBeAg seroconversion rates (Figure 3H & 3I). In CHB patients treated with TDF, in addition to HBV RNA levels($p<0.01$), a lower baseline HBeAg level was also associated with a higher cumulative HBeAg seroconversion rate ($p=0.03$).

Multivariate Cox regression analysis (Table 4) revealed that lower baseline HBV RNA levels ($P=0.003$, HR=0.317, 95% CI: 0.149–0.672) and lower HBV RNA levels at 6 months ($P<0.001$, HR=0.210, 95% CI: 0.085–0.518) were independently associated with higher cumulative HBeAg seroconversion rates. Additionally, lower HBsAg levels at 6 months ($P=0.004$, HR=0.300, 95% CI: 0.134–0.674) and lower HBV DNA levels at 6 months ($P=0.002$, HR=0.290, 95% CI: 0.132–0.635) were significant predictors of HBeAg seroconversion. In contrast, variables such as ALT, AST, HBV DNA, and the degree of HBsAg, HBV DNA, and HBV RNA decline did not show significant associations with HBeAg seroconversion in the multivariate model (all $P>0.05$).

The potential predictive ability of serological markers for HBeAg seroconversion in CHB patients was evaluated using ROC curves.

We compared the predictive ability of different serological markers for HBeAg seroconversion in CHB patients using ROC curves (Figure 4A) at baseline and found

that the AUCs for HBsAg, HBeAg, HBV RNA, and HBV DNA were 0.65, 0.63, 0.66, and 0.73, respectively. Additionally, at 6 months of treatment, the AUC values for HBsAg, HBV DNA, and HBV RNA were 0.61, 0.56, and 0.74, respectively (Figure 4B). We also evaluated the combined predictive ability of these markers for HBeAg seroconversion and found that at 6 months, the combination of HBV RNA levels at baseline and at 6 months achieved the highest AUC (Figure 4C). Data related to the ROC curves are presented in Table 5.

Discussion

This study evaluated the predictive value of serum HBV RNA for HBeAg seroconversion in CHB patients undergoing nucleos(t)ide analogue (NA) therapy, focusing on the two most widely used regimens, ETV and TDF. The results indicate that baseline and 6-month serum HBV RNA levels are independently associated with higher HBeAg seroconversion rates. Moreover, the combination of baseline and on-treatment HBV RNA levels demonstrated the highest predictive accuracy for HBeAg seroconversion compared to other serological markers such as HBsAg, HBeAg, and HBV DNA. These findings underscore the clinical utility of HBV RNA as a biomarker for assessing treatment response and predicting outcomes in CHB patients.

This study aligns with previous research suggesting that early virological responses, including reductions in HBV RNA, are critical for achieving HBeAg seroconversion[16-18]. However, unlike studies that primarily focus on HBsAg and HBV DNA, we found that baseline and early reductions in HBV RNA levels had stronger predictive value for HBeAg seroconversion. Notably, Fung et al. reported limitations in the predictive power of early HBsAg decline, which this study corroborates, as HBsAg levels at baseline and 6 months were less predictive than HBV RNA[11]. Additionally, while HBV DNA suppression was significant during treatment, its predictive capacity was inferior to that of HBV RNA.

In contrast to studies emphasizing the role of HBsAg in predicting functional cure, this research highlights the importance of dynamic HBV RNA monitoring, especially in patients treated with TDF. Previous studies have reported that lower HBeAg levels at baseline are associated with higher seroconversion rates[19], consistent with our

findings. However, this study uniquely identifies that lower HBV RNA levels at baseline and 6 months independently predict seroconversion, suggesting its value as a complementary biomarker alongside traditional markers.

The detection of HBV RNA has been approached using various methodologies[15]. Traditional real-time PCR-based methods are widely utilized due to their high sensitivity and specificity; however, these methods can be labor-intensive and often require sophisticated laboratory setups[20, 21]. Droplet digital PCR, another advanced approach, provides greater quantification accuracy and sensitivity for low viral RNA levels but is costlier and less accessible in routine clinical settings[22].In this study, we employed the simultaneous amplification testing (SAT) method for HBV RNA detection, which offers significant advantages over these traditional approaches. SAT is an isothermal nucleic acid amplification technique that combines rapid amplification with real-time fluorescence detection[23]. Compared to PCR-based methods, SAT is less dependent on thermal cycling equipment, making it faster and more cost-effective. The SAT method's streamlined workflow allows for consistent and reliable measurement of HBV RNA, which is critical for accurately evaluating its predictive value for HBeAg seroconversion[24]. By leveraging this method, our study not only highlights the clinical utility of HBV RNA as a biomarker but also demonstrates the feasibility of integrating SAT technology into routine hepatitis B management protocols.

This study showed no significant differences in the cumulative HBeAg seroconversion rates between patients treated with ETV and those treated with TDF. Both NAs effectively suppressed HBV DNA and HBV RNA levels, but the dynamics of HBV RNA decline differed. TDF treatment led to a significant reduction in HBV RNA levels as early as 6 months in the non-seroconversion group, while ETV-treated patients did not show significant reductions during the same period. These findings suggest that TDF may have a more pronounced effect on viral transcriptional activity during the early treatment phase.

Previous studies have reported variable HBeAg seroconversion rates with different NAs, with TDF generally associated with slightly higher rates than ETV. These differences may relate to the distinct mechanisms of action of these drugs[25]. TDF, a

nucleotide analogue, has been shown to be more effective in reducing intrahepatic HBV cccDNA transcriptional activity, potentially explaining the earlier decline in HBV RNA levels observed in this study. However, further research is needed to confirm these observations and elucidate the mechanisms underlying these differences.

This study has several limitations. First, the sample size was relatively small, which may limit the generalizability of our findings. Larger, multicenter studies are needed to validate the predictive value of serum HBV RNA for HBeAg seroconversion in diverse populations. Second, the follow-up duration was limited, and longer-term outcomes, such as HBsAg loss and virological relapse, were not evaluated. This restricts the ability to assess the long-term prognostic implications of HBV RNA levels. Additionally, although we employed a highly sensitive SAT-based method for HBV RNA quantification, variations in methodologies across different studies could influence results, highlighting the need for standardized assays. Lastly, as this study focused on patients treated with TDF or ETV, the findings may not be directly applicable to other treatment regimens, such as pegylated interferon or newer antiviral agents. These limitations should be addressed in future research to better elucidate the role of HBV RNA as a biomarker in chronic hepatitis B management.

In conclusion, this study highlights the potential of HBV RNA as a novel biomarker for predicting HBeAg seroconversion during NA therapy. Its use in clinical practice, alongside traditional markers, may improve the management and prognosis of CHB patients. However, further validation and standardization are required to establish its role in treatment algorithms.

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Acknowledgements

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Tables

Table 1. Baseline characteristics of CHB patients

	Total (n=293)	Seroconversion Group (n=129)	Non-seroconversion Group (n=164)	p value
Gender (M/F)	179/114	77/52	102/62	0.662
Age	43.55±14.21	42.05±14.53	44.72±14.53	0.319
HBV Gene type （B/C）	195/98	92/55	103/43	0.149
Drug (ETV/TDF)	166/127	76/53	90/74	0.489
ALT （U/L）	52.51±22.33	50.35±23.37	54.21±23.56	0.053
AST (U/L)	31.12±13.36	31.42±11.54	30.89±15.03	0.514
HBsAg （log10IU/ml)	4.17±0.55	4.20±0.38	4.14±0.75	0.207
HBeAg （log10 S/CO）	4.10±0.35	4.11±0.24	4.09±0.31	0.682
HBV DNA （log10copies/ml）	5.71±1.37	5.73±1.38	5.69±1.16	0.144
HBV RNA （log10copies/ml）	4.40±0.57	4.44±0.45	4.36±0.81	0.732

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Table 2. Baseline characteristics of CHB patients with ETV therapy

	Total (n=166)	Seroconversion Group (n=76)	Non-seroconversion Group (n=90)	p value
Gender (M/F)	102/64	43/33	59/31	0.238
Age	44.68 ±13.48	45.69±13.54	43.36±13.37	0.193
HBV Gene type（B/C）	110/56	45/31	65/25	0.099
ALT（U/L）	52.77 ±19.56	50.21±17.35	54.19±22.16	0.522
AST (U/L)	31.09 ±15.02	30.60±15.20	31.50±14.33	0.376
HBsAg（log10IU/ml)	4.19 ±0.30	4.18±0.33	4.20±0.28	0.311
HBeAg（log10 S/CO）	4.12 ±0.51	3.99±0.37	4.23±0.71	0.117
HBV DNA（log10copies/ml）	5.64 ±1.19	5.79±1.09	5.51±1.28	0.932
HBV RNA（log10copies/ml）	4.45 ±0.74	4.36±0.44	4.52±0.51	0.263

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Table 3. Baseline characteristics of CHB patients with TDF therapy

	Total (n=127)	Seroconversion Group (n=53)	Non-seroconversion Group (n=74)	p value
Gender (M/F)	77/50	34/19	43/31	0.581
Age	42.07 ±15.04	40.38±14.07	43.42±15.74	0.519
HBV Gene type（B/C）	85/42	47/24	38/18	0.843
ALT（U/L）	52.13 ±17.37	50.55±12.60	53.46±20.71	0.579
AST (U/L)	31.16±12.54	32.60±13.43	30.00±15.19	0.929
HBsAg（log10IU/ml)	4.14 ±0.61	4.22±0.50	4.05±0.64	0.083
HBeAg（log10 S/CO）	4.07 ±0.45	4.28±0.69	3.88±0.61	0.077
HBV DNA（log10copies/ml）	5.81 ±1.32	5.64±1.37	5.94±1.30	0.213
HBV RNA（log10copies/ml）	4.32 ±0.57	4.55±0.78	4.12±0.34	0.742

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Table 4. Multivariate Cox regression analysis of factors associated with HBeAg seroconversion in CHB patients.

Variable	Exp(B)	95.0% Exp(B) CI	P
Gender (Male)	1.986	1.049-3.762	0.035
Age (Below 37 years)	0.997	0.971-1.023	0.791
ALT (Greater than 70 U/L)	0.984	0.968-1.000	**0.050**
AST (Greater than 60 U/L)	0.996	0.970-1.022	0/757
Genotype (Type B)	0.778	0.391-1.549	0.476
NAs (ETV)	1.252	0.654-2.397	0.497
HBsAg (Less than 4.3 $\log_{10}$ IU/mL)	1.202	0.627-2.304	0.579
HBeAg (Less than 4.19 $\log_{10}$ IU/mL)	1.384	0.720-2.662	0.330
HBV DNA (Less than 5.85 $\log_{10}$ IU/mL)	0.921	0.488-1.738	0.800
HBV RNA (Less than 4.26 $\log_{10}$ IU/mL)	0.317	0.149-0.672	**0.003**
At 6 months			
HBsAg (Less than 3.57 $\log_{10}$ IU/mL)	0.300	0.134-0.674	**0.004**
Degree of HBsAg decline (Less than 0.03 log10 IU/mL)	1.708	0.788-3.703	0.175
HBV DNA (Less than 2.64 $\log_{10}$ IU/mL)	0.290	0.132-0.635	**0.002**
Degree of HBV DNA decline (Less than 2.64 log10 IU/mL)	1.114	0.561-2.211	0.758
HBV RNA (Less than 3.68 $\log_{10}$ IU/mL)	0.210	0.085-0.518	**<0.001**
Degree of HBV RNA decline (Less than 1.36 log10 IU/mL)	0.870	0.479-1.580	0.647

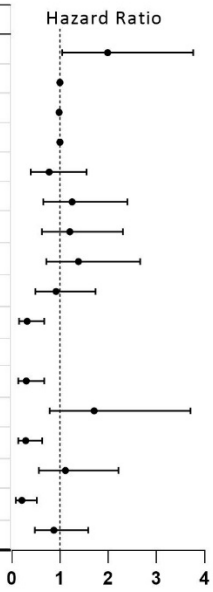

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Table 5. Predictive Performance of Serological Markers and Their Combinations for HBeAg Seroconversion in CHB Patients

	AUC	Optimal cutoff value	SE, %	SP, %	SE+SP, %
Baseline					
HBsAg	0.65	3.59	81	46	127
HBeAg	0.63	3.28	81	44	125
HBV DNA	0.66	6.65	31	98	129
HBV RNA	0.73	5.09	41	98	139
After 6 months					
HBsAg	0.61	3.33	70	58	128
HBV DNA	0.56	2.73	50	68	118
HBV RNA	0.74	3.71	64	80	144
Combined indicators					
HBV RNA 0 & 6M	0.84	0.33	94	60	154
HBV DNA & HBV RNA	0.78	0.28	94	50	144
HBsAg & HBV RNA	0.74	0.35	86	60	146

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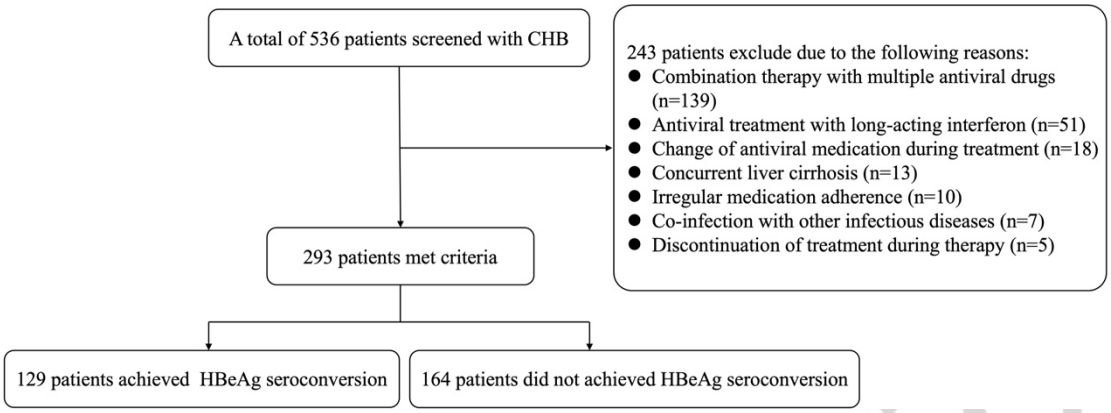

Figure 1. Flowchart of patient selection and HBeAg seroconversion outcomes in CHB patients. A total of 536 patients were screened, with 243 excluded based on predefined criteria. The final cohort consisted of 293 patients, of whom 129 achieved HBeAg seroconversion, while 164 did not.

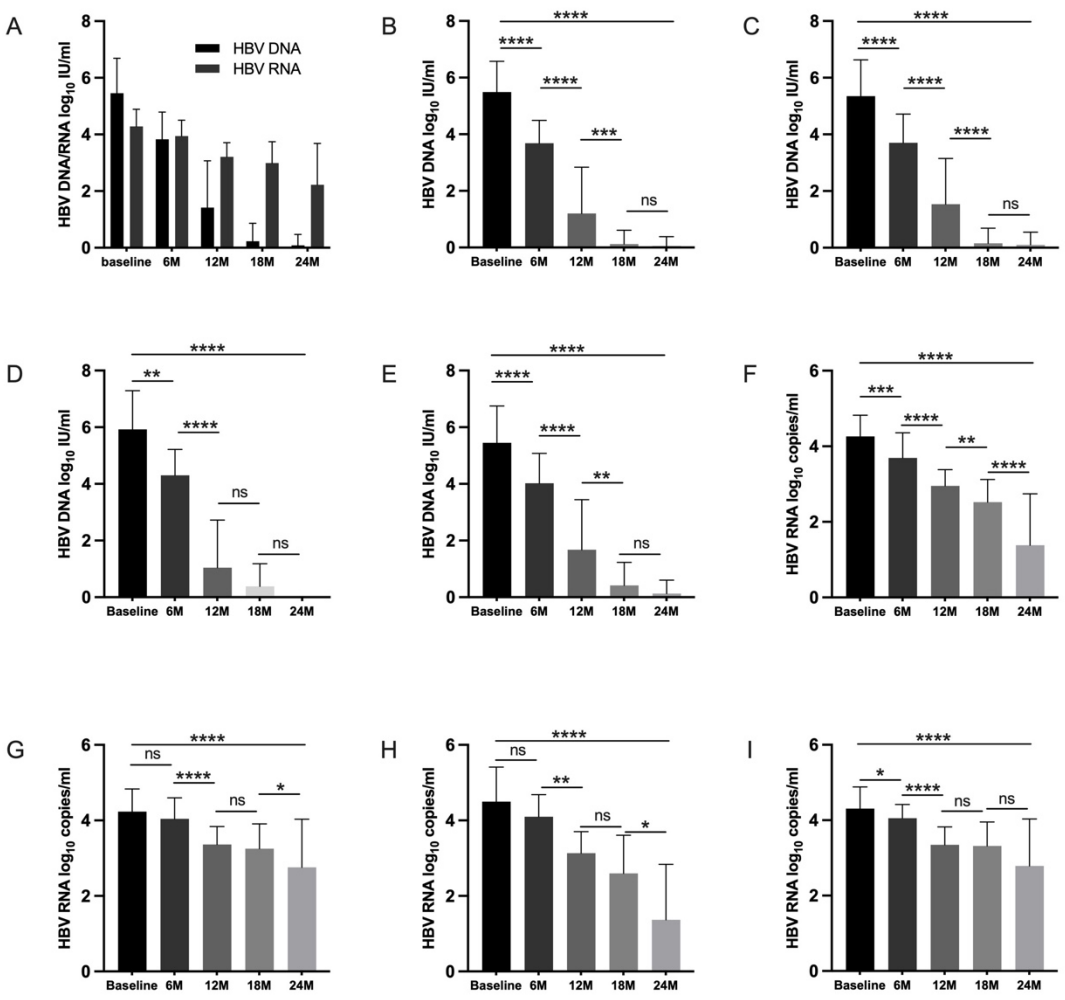

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Figure 2. The dynamic of HBV DNA and RNA levels during NAs therapy(A); HBV DNA changes in ETV-treated patients with and without HBeAg seroconversion(B-C); HBV DNA changes in TDF-treated patients with and without HBeAg seroconversion(D-E); HBV RNA changes in ETV-treated patients with and without HBeAg seroconversion(F-G); HBV RNA changes in TDF-treated patients with and without HBeAg seroconversion(H-I). *: $p<0.05$, **: $P<0.01$, ***: $P<0.001$, ****: $P<0.0001$.

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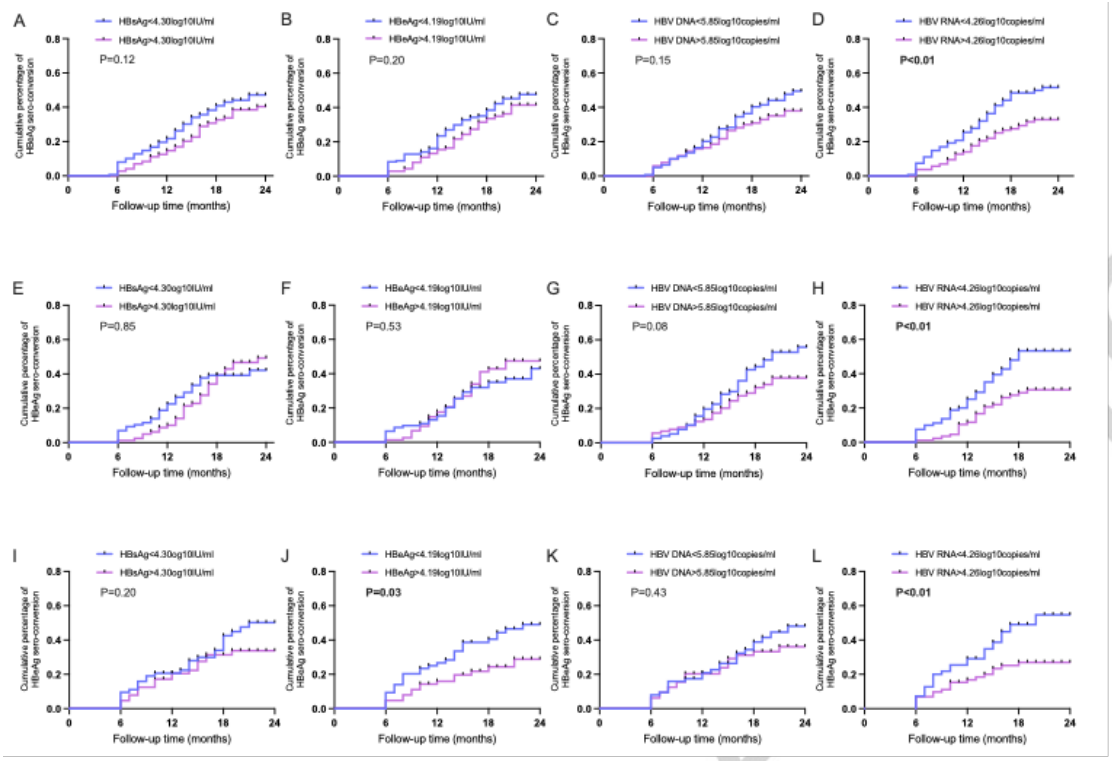

Figure 3. Kaplan-Meier survival analysis of the cumulative HBeAg seroconversion rates across different groups stratified by HBsAg, HBeAg, HBV DNA and HBV RNA levels(A-D). The cumulative HBeAg seroconversion rates in CHB patients treated with ETV(E-H) and in those treated with TDF(I-L).

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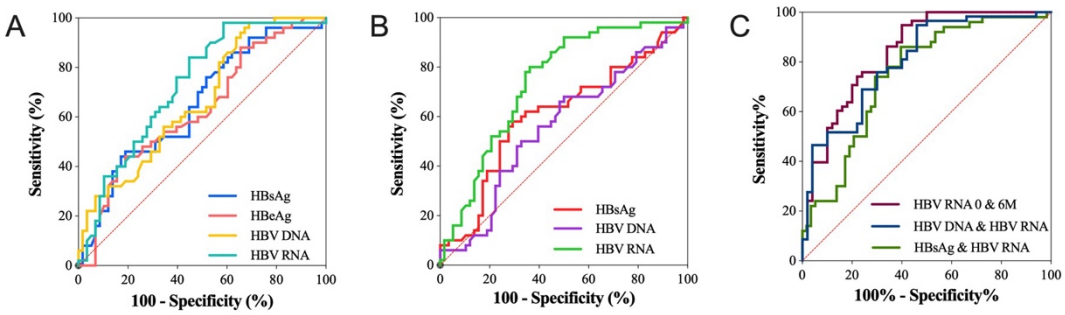

Figure 4. ROC curves for serological markers (HBsAg, HBeAg, HBV DNA, and HBV RNA) predicting HBeAg seroconversion at baseline(A) and at 6 months of treatment (B). ROC curves also demonstrate the combined predictive ability of the serological markers (C).