Original Article

The decline of HBV RNA associated with HBeAg seroconversion and double-negative HBV DNA and RNA in chronic hepatitis B patients who received entecavir therapy: a 10-year retrospective cohort study

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Background: Whether the decline of hepatitis B virus (HBV) RNA was associated with antiviral efficacy in chronic hepatitis B (CHB) patients receiving long-term nucleos(t)ide analogues (NAs) therapy remains unclear. We observed the levels of serum HBV RNA in CHB patients treated with entecavir (ETV) for 10 years and explored the clinical significance of HBV RNA during long-term antiviral treatment.

Methods: A total of 33 hepatitis B surface antigen (HBsAg)-positive CHB patients treated with ETV for up to 10 years were recruited for this study. Liver function, HBsAg, hepatitis B envelope antigen (HBeAg), HBV DNA, and HBV RNA were measured at the baseline and each follow-up points. Antiviral efficacy was defined as negative HBV DNA (<20 IU/mL) and HBV RNA (<300 Copies/mL).

Results: (I) Serum HBV DNA and HBV RNA declined with the duration of antiviral treatment over 10 years (P<0.001). (II) There were positive correlations between serum HBV DNA and HBV RNA at each follow-up point (r=0.62 and P<0.001 at baseline, r=0.77 and P<0.001 at week 24, r=0.71 and P<0.001 at week 48, r=0.81 and P<0.001 at week 96, r=0.60 and P<0.01 at year 5 and r=0.77 and P<0.001 at year 10). (III) HBeAg and HBsAg levels at baseline and 10th year after ETV treatment have significant difference (P<0.05 and P<0.01). (IV) The decline of HBV RNA after ETV treatment was associated with HBeAg seroconversion, the area under the ROC curves (AUROCs) of the declines of HBV RNA were 0.25 at the baseline, 0.62 at week 24, 0.78 at week 48 and 0.86 at week 96, respectively. (V) The decline of HBV RNA after ETV treatment was associated with antiviral efficacy, the AUROCs of the declines of HBV RNA were 0.33 at the baseline, 0.74 at week 24, 0.83 at week 48 and 0.86 at week 96, respectively.

Conclusions: Serum HBV DNA and HBV RNA declined with the duration of antiviral treatment over 10 years. The decline of HBV RNA was associated with HBeAg seroconversion and antiviral efficacy in CHB patients receiving long-term ETV therapy, and the earliest prediction point was week 24.

Keywords: Chronic hepatitis B (CHB); HBV RNA; HBV DNA; hepatitis B envelope antigen (HBeAg) seroconversion; antiviral treatment

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Introduction

Hepatitis B virus (HBV) infection is a global public health problem threatening human health. It is estimated that about 257 million people in the world suffer from chronic HBV infection, and more than 800,000 people die each year from liver failure, cirrhosis and liver cancer related to HBV infection (1). To cure chronic hepatitis B (CHB), covalently closed circular DNA (cccDNA) needs to be cleared (2), but the 2 classes of existing anti-HBV drugs, interferons, and NAs, do not target cccDNA directly (3,4).

HBV DNA decline is a direct indicator of antiviral efficacy in patients undergoing antiviral therapy. The seroconversion of hepatitis B envelope antigen (HBeAg) and loss of hepatitis B surface antigen (HBsAg) are also widely accepted as treatment endpoints. However, these indicators cannot fully reflect the level of cccDNA. Thus, novel markers reflecting cccDNA to evaluate HBV replication after antiviral therapy urgently need to be identified.

Serum HBV RNA is mainly the pregenomic RNA (pgRNA) of HBV, which plays an important role in the life cycle of HBV replication. Serum HBV RNA is derived from cccDNA but cannot be affected by NAs, and it is used to reflect the transcriptional activity of cccDNA in the liver (5-8), monitor antiviral efficacy (9,10), evaluate the risk of drug withdrawal and recurrence (11-13), monitor HBV reactivity in HBeAg-negative patients (14), and predict HBeAg seroconversion (5,15). Research has shown that after NA treatment, there is a significant correlation between serum HBV RNA and HBV RNA levels in liver tissue, the grade of inflammation in liver tissue, and fibrosis stage (8) .

As a new biomarker of HBV replication and antiviral therapy, a growing research suggest that serum HBV RNA may serve as a serum biomarker for HBV infection, treatment, and may be helpful for predicting off-therapy in patients with long-term NA treatment (8,9,12). However, the clinical significance of HBV RNA during long-term antiviral treatment remains unclear. In this study, we observed the dynamic changes of HBV DNA and HBV RNA in 33 CHB patients treated with entecavir (ETV) for 10 years and assessed the clinical significance of HBV RNA in long-term antiviral treatment. We present the following article in accordance with the STARD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3265/rc).

Methods

Patient cohort

A total of 39 patients with chronic HBV infection were consecutively enrolled from April 2007 to March 2009. Six patients with poor treatment adherence were excluded from this study. The other 33 patients with available serum samples were included in the analysis. All of the patients had been HBsAg-positive for more than 6 months and were followed-up for 10 years at The First Affiliated Hospital of Xi'an Jiaotong University. The enrolled patients were considered eligible for monotherapy with ETV and provided informed consent. The study was approved by the Research Ethics Committee of Xi'an Jiaotong University (No. XJTUIAF2016LSK-16-1). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

To be eligible for inclusion in the study, patients had to meet the following inclusion criteria: (I) have CHB and have been HBsAg positive for more than 6 months; (II) be aged 18–65 years; (III) have a HBV DNA level $\geq 2\times10^4$ IU/mL; (IV) have an ALT >1 × ULN (upper limit of normal) (ULN reference ≤40 U/L); (V) agree to voluntarily enter the study and sign the related informed consent form.

Patients were excluded from the study if they met the following exclusion criteria: (I) had alcoholic liver disease, cholestatic liver disease, or autoimmune liver disease; (II) had hepatitis A, C, D, E, or other viral hepatitis; (III) had decompensated cirrhosis (Child Pugh C); (IV) were pregnant, or had an autoimmune disease, severe infection, severe cardiopulmonary, renal insufficiency, or a tumor, etc.; (V) were using anti-tuberculosis and other liver-injury drugs or had been taking a large dose of immunosuppressants for more than half a year.

Data collection

All the patients were given ETV, 0.5 mg/d, and followed-up for 10 years. Routine demographic data (e.g., age, gender, and a family history of hepatitis B) were collected from all patients in the study. Quantitative values for HBV DNA, HBsAg, HBeAg, liver function and blood count were obtained, and an upper abdominal ultrasound was carried out at the baseline, and then every 12 weeks at the 1st year,

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Table 1 Demographics and baseline characteristics of ETV-treated patients with CHB

Characteristics	n=33
Male gender, n (%)	24 (72.7)
Age (years), mean ± SD	33.39±9.01
Length of liver disease (years), mean ± SD	19.76±6.32
Family history, n (%)	12 (36.4)
HBeAg-positive rate, n (%)	25 (75.8)
HBeAg value (S/CO), mean ± SD	529.03±348.98
HBsAg value (IU/mL), mean ± SD	4,122.59±2,573.47
ALT (U/L), median [range]	77 [45–120]
HBV DNA (log10 IU/mL), mean ± SD	6.34±1.21
HBV RNA (log10 Copies/mL), mean ± SD	5.66±1.35

ETV, entecavir; CHB, chronic hepatitis B; SD, standard deviation; CO, Cut off; ALT, Alanine aminotransferase; HBV, hepatitis B virus; IU, International Unit.

and 24 weeks from the 2nd year to the end of the study. HBV RNA was collected before treatment, and at week 24, week 48, week 96, year 5, and year 10. Serum samples were collected at each visit and were stored at –80 ℃ awaiting analysis.

Serum HBV DNA and HBV RNA evaluation

HBV DNA was measured with the Roche COBAS® TaqMan® HBV test in the central laboratory as established by the research group (Lower limit of detection, 20 IU/mL). HBV RNA was measured using the Diagnostic Kit for HBV pgRNA [Polymease chain reaction (PCR) fluorescence probing, lower limit of detection, 300 Copies/mL] in accordance with the manufacturer's instruction (Beijing Hotgen Biotech Co. Ltd., Beijing, China).

Serum HBsAg and HBeAg evaluation

Serum HBsAg, HBeAg, and anti-HBe levels were measured by an Elecsys 2010 immunoanalyzer (Roche). The results were calculated based on the ratio of the sample (S) to the cut-off (CO) values for each sample and control. Samples with a value <0.05 IU/mL were considered negative for HBsAg, samples with a S/CO value <1.0 were considered negative for HBeAg, and those with a S/CO value >1.0 were considered negative for HBeAb.

Statistical analysis

The continuous measurements of normal distribution are represented as the mean (SD), the non-normal distributions are represented as the median value [interquartile range (IQR)], and the categorical variables are represented as the subject number (percentage). Repeated ANOVA was used to compare the levels of HBV DNA and HBV RNA at the different time points. The Pearson's test was used to analyze the correlations between HBV DNA and HBV RNA at different time points. The software of Graphpad 6 and OriginPro 9.1 was used to generate graphs. The statistical analysis was performed using SPSS 20 software. All statistical tests were two-tailed, and a P value <0.05 was considered statistically significant.

Results

Demographics and baseline characteristics

A total of 39 CHB patients were consecutively enrolled in this study. Of these patients, 33 with available serial samples were included in the analysis. The baseline characteristics of the 33 patients are set out in *Table 1*.

Dynamic changes in HBV DNA and HBV RNA

Patients' serum HBV DNA and HBV RNA levels were measured at the baseline, and after ETV treatment at week 24, week 48, week 96, year 5, and year 10 (see *Figure 1A-1C*). With the prolonged time of antiviral therapy, the HBV DNA and HBV RNA levels showed an overall down trend, and the differences were statistically significant at each time point (P<0.001).

At the 10th year of treatment, a small number of patients were still positive for HBV DNA and HBV RNA, but the levels were both extremely low, and the values did not reflect the effects of antiviral treatment. To better understand the antiviral efficacy, we observed the positive rates of HBV DNA and HBV RNA during antiviral therapy (see *Figure 1D*). The results showed that the positive rate of HBV DNA and HBV RNA at the baseline was 100%. With the extension of antiviral therapy, the positive rate of HBV DNA and HBV RNA decreased gradually. The HBV DNA positive rates at week 24, week 48, week 96, year 5, and year 10 were 94%, 76%, 48%, 24%, and 18%, respectively, and the HBV RNA positive rates were 79%, 67%, 48%, 33%, and 21%, respectively. At the 10th year of treatment, there were 7 HBV RNA positive patients, of whom 5 were both

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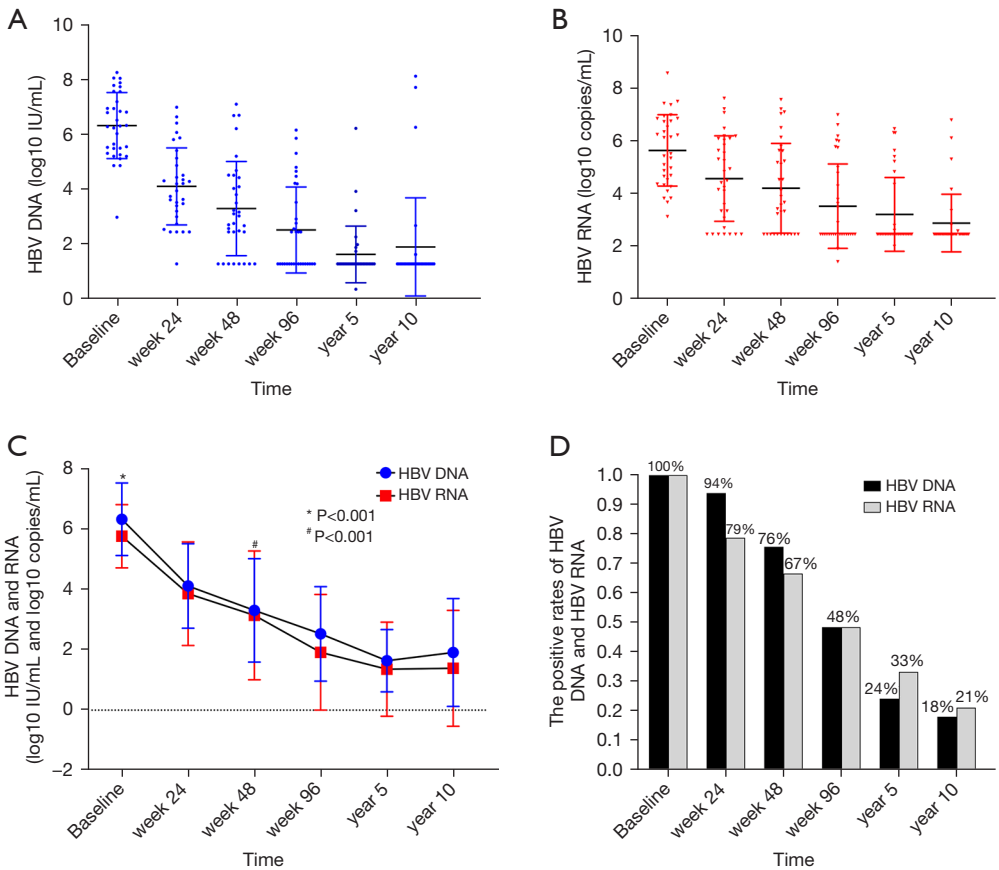

Figure 1 Changes in HBV DNA and HBV RNA following ETV treatment over 10 years. (A) Scatter plot of HBV DNA. (B) Scatter plot of HBV RNA. (C) Line chart of HBV DNA and HBV RNA. (D) Percentage of positive HBV DNA and HBV RNA. * represents the P value of HBV DNA and # represents the P value of HBV RNA. HBV, hepatitis B virus.

HBV RNA and HBV DNA positive, and 2 were HBV RNA positive, but HBV DNA. Additionally, at the 10th year of treatment, 6 were HBV DNA positive, of whom 5 were both HBV RNA and HBV DNA positive, and 1 was HBV DNA positive, but negative HBV RNA. Notably, HBV RNA was detected in some HBV DNA-negative patients, and HBV DNA was also detected in some HBV RNA-negative patients during the ETV treatment.

HBV RNA and HBV DNA correlation analysis

We detected the levels of serum HBV DNA and HBV RNA at different time points and performed a correlation analysis. We found that HBV DNA and HBV RNA were always positively correlated over the 10-year observation period (see *Figure 2*). The data were compared at (A) the baseline, r=0.62 and P<0.001, (B) week 24, r=0.77 and P<0.001, (C) week 48, r=0.71 and P<0.001, (D) week 96, r=0.81 and P<0.001, (E) year 5, r=0.60 and P<0.01, and (F) year 10, r=0.77 and P<0.001.

The outcome of ETV therapy

Virological response (VR) and serological response (SR) were defined as undetectable serum HBV DNA and HBeAg seroconversion, respectively. VR and SR during therapy increased from 24.2% and 4.0% at week 48 to 84.9% and 80.0% at year 10, respectively (see *Figure 3A*). The dynamic changes of HBeAg during ETV treatment are shown in *Figure 3B* (P<0.05). HBsAg levels at baseline and 10th year after ETV treatment are shown in *Figure 3C* (P<0.01). Of all the 33 patients, 29 (87.9%) and 1 (3.0%) achieved normal ALT and HBsAg loss, respectively, after 10 years of antiviral therapy. At year 10, the incidence of HCC, decompensated cirrhosis, and death rates were 3.0% (1/33), 0 and 0, respectively. All the 5 patients who were HBeAg

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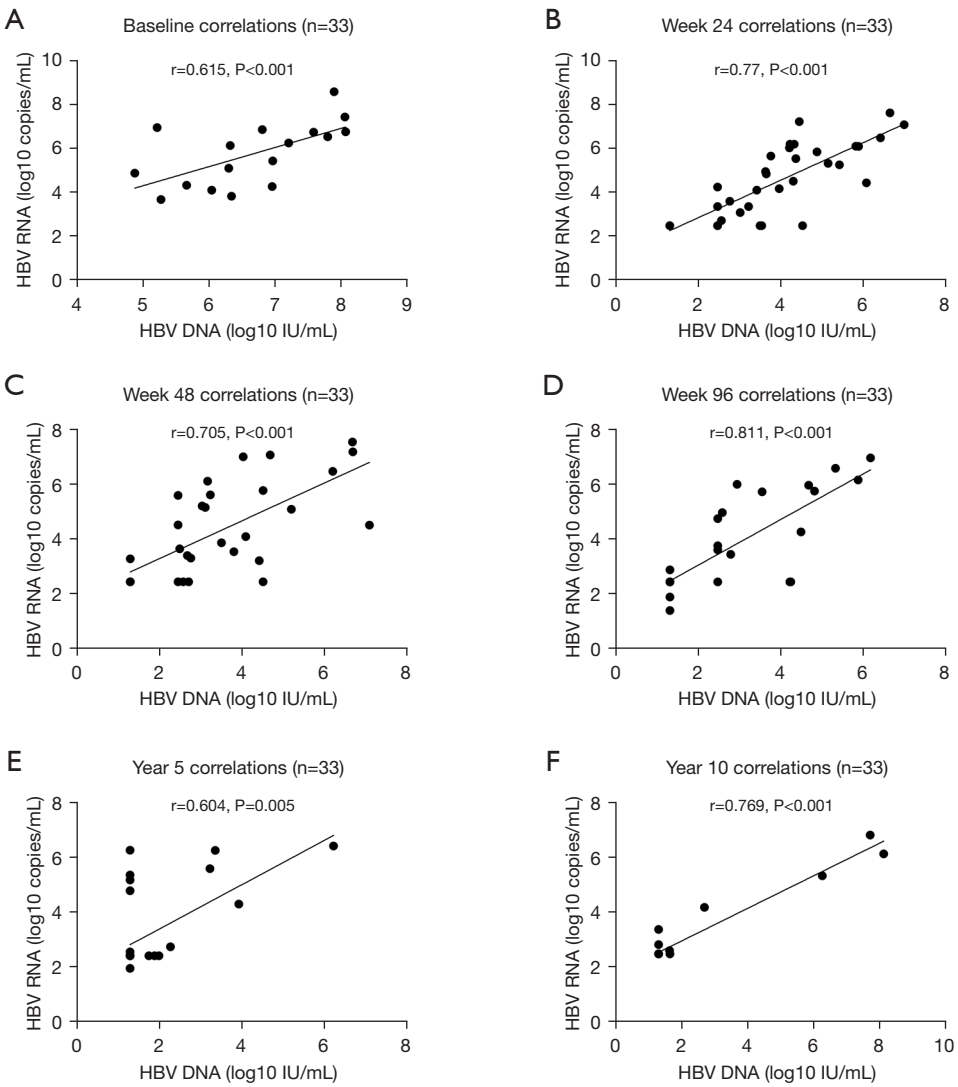

Figure 2 HBV DNA and HBV RNA correlation analysis. Scatter plots showing the corresponding levels of HBV DNA and HBV DNA. (A) At the baseline, (B) week 24, (C) week 48, (D) week 96, (E) year 5, and (F) year 10. n=33 in A-F; some of the dots are completely overlapping. r, Pearson's correlation coefficient; P value, the correlation *t* test.

positive at year 10 were both HBV DNA and HBV RNA positive.

Serum HBV DNA and HBV RNA levels in relation to HBeAg seroconversion

We evaluated the levels of HBV DNA and HBV RNA in relation to HBeAg seroconversion. At the baseline, the mean serum HBV DNA level of patients with HBeAg seroconversion was 7.32 log10 IU/mL, which was higher than that of 6.86 log10 IU/mL in patients without HBeAg seroconversion and that of 5.79 log10 IU/mL in HBeAg-negative patients (see *Figure 4A*). For HBV RNA, the mean serum HBV RNA level of 7.55 log10 copies/mL in patients without HBeAg seroconversion was higher than that of 6.71 log10 copies/mL in patients with HBeAg seroconversion and that of 5.45 log10 copies/mL in HBeAg-negative patients at the baseline (see *Figure 4B*). At each subsequent follow-up time point, the HBV DNA and HBV RNA levels of the patients with HBeAg seroconversion and those who were HBeAg-negative decreased continuously, while the HBV DNA and HBV RNA level of patients without HBeAg

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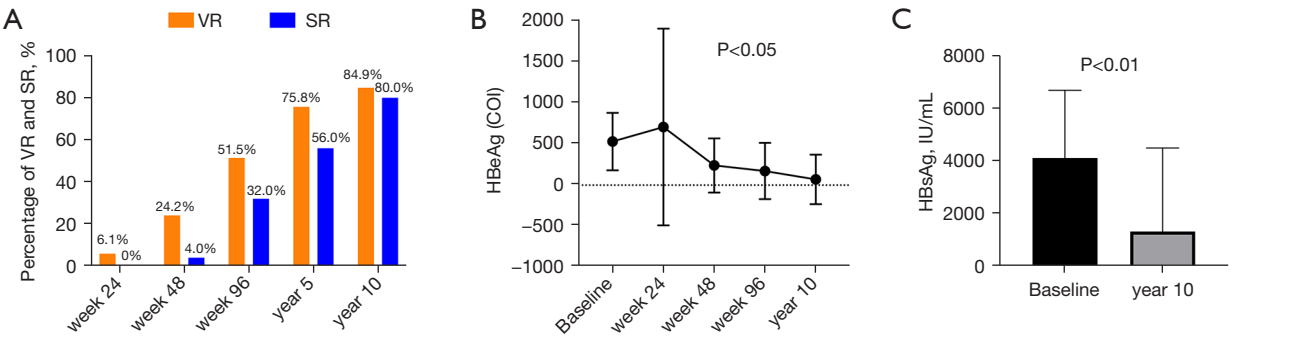

Figure 3 Therapy efficacy and dynamic changes of HBeAg during ETV treatment. (A) Percentages of patients who achieved SR and VR during ETV treatment. (B) Dynamic changes of HBeAg during ETV treatment. (C) HBsAg levels at baseline and 10th year after ETV treatment. VR, virological response; SR, serological response; ETV, entecavir.

seroconversion fluctuated. The HBV DNA and HBV RNA levels of patients without HBeAg seroconversion were higher than those of patients with HBeAg seroconversion and HBeAg-negative patients.

Serum HBV RNA predicts HBeAg seroconversion

We further performed ROC analysis to evaluate the prediction value of serum HBV DNA and HBV RNA associated with HBeAg seroconversion. The area under the ROC curves (AUROCs) of HBV DNA and HBV RNA were 0.45 and 0.25 at the baseline, respectively (see *Figure 4C*); thus, the baseline HBV DNA and HBV RNA levels had no association with HBeAg seroconversion. During treatment, the AUROCs of HBV DNA and HBV RNA decreased by 0.71 and 0.62 at week 24, respectively (see *Figure 4D*); 0.73 and 0.78 at week 48, respectively (see *Figure 4E*); and 0.93 and 0.86 at week 96, respectively (see *Figure 4F*). These results indicated that the decline of HBV DNA and HBV RNA was associated with HBeAg seroconversion, and the earliest prediction point was at week 24 after ETV treatment. The AUROCs of the HBV RNA levels were higher than those of the HBV DNA levels at predicting the HBeAg seroconversion at week 48.

Serum HBV RNA predicts the antiviral efficacy of ETV

We performed a ROC analysis to evaluate the prediction value of serum HBV DNA and HBV RNA associated with antiviral efficacy at the 10th year of treatment. Antiviral efficacy was defined as both HBV DNA and HBV RNA negative. The AUROCs of HBV DNA and HBV RNA were 0.36 and 0.33 at the baseline, respectively (see *Figure 5A*). The AUROCs of the declines of HBV DNA and HBV RNA were 0.55 and 0.74 at week 24, respectively (see *Figure 5B*), 0.69 and 0.83 at week 48, respectively (see *Figure 5C*), and 0.76 and 0.86 at week 96, respectively (see *Figure 5D*). These results indicate that the baseline HBV DNA and HBV RNA levels had no ability to predict antiviral efficacy. However, the decline of HBV DNA and HBV RNA was associated with antiviral efficacy at the 10th year, and the earliest prediction point was at week 24 after ETV treatment. During the treatment, the AUROCs of the HBV RNA levels were higher than those of the HBV DNA levels at predicting antiviral efficacy at week 24, week 48, and week 96 (8).

Discussion

HBV RNA is a novel serum marker for cccDNA transcriptional activity and could be used to monitor the responses of antiviral treatment in CHB patients treated with interferon (IFN) or NAs (6,10). However, the clinical significance of HBV RNA during long-term antiviral treatment remains unclear. In this study, we investigated the dynamic changes of HBV RNA in CHB patients during 10 years of ETV therapy. We found a decline in the HBV RNA levels associated with HBeAg seroconversion and double-negative HBV DNA and RNA in CHB patients receiving long-term ETV therapy.

According to current research, serum HBV DNA and HBV RNA decline as the duration of antiviral treatment increases (16). In a cohort study of 66 HBeAg-negative HBV patients, after 3 years of antiviral treatment, HBV RNA was detected in 30% of the HBV DNA-negative patients who received long-term NA therapy. After 5 years of NA treatment, HBV RNA was detected in 14% of the

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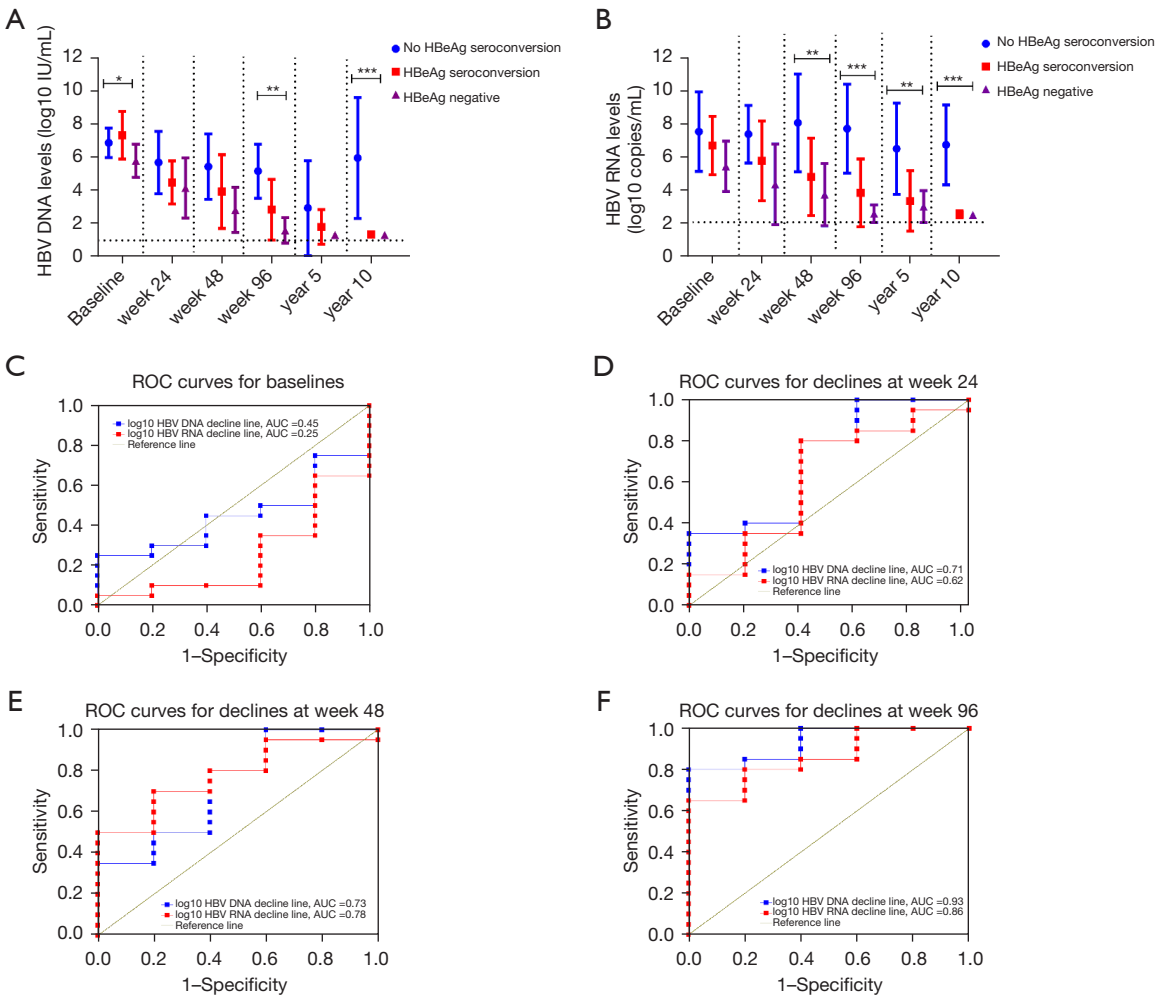

Figure 4 Levels of HBV DNA and HBV RNA in relation to HBeAg seroconversion. Plots of serum HBV DNA (A) and serum HBV RNA (B) levels in patients receiving ETV according to HBeAg seroconversion. The dotted horizontal lines represent the lower limit of detection (HBV DNA 1.3 log10 and HBV RNA 2.48 log10). The error bars show standard deviations. (C-F) The ROC curve describes the prediction of HBeAg seroconversion at the 10th year by serum HBV DNA and HBV RNA levels during ETV treatment. (C) HBV DNA and HBV RNA at the baseline. (D) HBV DNA and HBV RNA declines at week 24. (E) HBV DNA and HBV RNA declines at week 48. (F) HBV DNA and HBV RNA declines at week 96. *, P<0.05; **, P<0.01; ***, P<0.001. AUC, area under the curve; ROC, receiver operating characteristic; HBV, hepatitis B virus; ETV, entecavir.

HBV DNA negative patients (9). Among 122 CHB patients treated with ETV, HBV DNA could not be detected in 90% of patients at week 48, and only 18% of the patients had HBV RNA below the detection limit (17). Our data showed that compared to the baseline, HBV DNA and HBV RNA both showed a downward trend at week 24, week 48, week 96, year 5, and year 10 after ETV treatment. Patients with double-negative HBV DNA and HBV RNA after ETV treatment were able to maintain undetectable HBV DNA and HBV RNA during the subsequent follow-ups (see Table S1). Notably, at week 24, while the decrease of HBV DNA was more rapid than that of HBV RNA, the negative conversion rate of HBV RNA (7/33, 21%) was much higher than that of HBV DNA (2/33, 6%).

The level of HBV RNA is related to host and virus factors. The baseline HBV RNA level is associated with ALT levels, basal core promoter (BCP) variants, HBV genotype, HBcrAg, and HBV DNA (18,19). In this study, we found a correlation between HBV DNA and HBV RNA. Butler *et al.* found that the serum level of HBV RNA was

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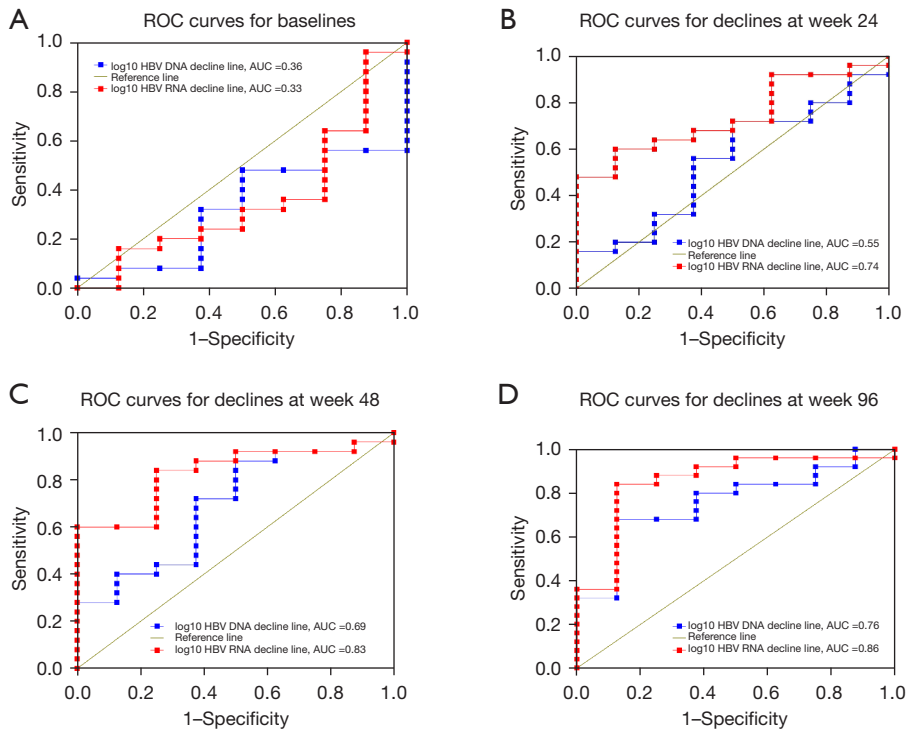

Figure 5 The ROC curve describes the ability of serum HBV DNA and HBV RNA levels during ETV treatment to predict double-negative HBV DNA and HBV RNA at the 10th year. (A) HBV DNA and HBV RNA at the baseline. (B) HBV DNA and HBV RNA declines at week 24. (C) HBV DNA and HBV RNA declines at week 48. (D) HBV DNA and HBV RNA declines at week 96 of ETV treatment. ROC, receiver operating characteristic; HBV, hepatitis B virus; ETV, entecavir.

approximately 2 log lower than that of HBV DNA (20). This is consistent with the findings of our study. They also found that serum HBV RNA was associated with HBV DNA at the baseline, but there was a low correlation between HBV RNA and HBV DNA during NA treatment (20). We found that HBV RNA was associated with HBV DNA at the baseline and at week 24, week 48, week 96, year 5, and year 10 after ETV treatment, indicating that there was a stable correlation between HBV DNA and HBV RNA.

Recently, a growing number of studies have shown that HBV RNA has predictive value for HBeAg seroconversion in CHB patients treated with NAs and pegylated interferon (Peg-IFN) (15,21-23). Zhang *et al.* showed HBV RNA at week 12 was an effective indicator of HBeAg seroconversion in HBeAg-positive patients treated with pegylated interferons (22). Jansen *et al.* found that a low baseline HBV RNA level was an independent predictor of the combined response of HBeAg-negative CHB patients after PEG-IFN combined with NA treatment (24). Wang *et al.* indicated the level of HBV RNA at week 24 was a powerful predictor of HBeAg seroconversion in HBeAg-positive patients after 144 weeks of ETV treatment (5). However, the ability of serum HBV RNA to predict the long-term outcome of HBeAg seroconversion during NA treatment remains unknown.

Our data showed that the baseline HBV RNA has no predictive value for HBeAg seroconversion. However, the decline in HBV RNA levels is associated with HBeAg seroconversion. The AUROCs of the HBV RNA levels were equivalent to those of HBV DNA at week 24 and higher than those of HBV DNA at week 48 and week 96, which indicates that the decline of serum HBV RNA showed a better prediction of HBeAg seroconversion than HBV DNA during ETV treatment, and the earliest prediction point was at week 24 after treatment.

HBeAg seroconversion and HBV DNA inhibition at the end of antiviral treatment are the 2 main endpoints related to the outcomes of HBeAg-positive patients. However, HBeAg seroconversion and HBV DNA inhibition do not reflect the clearance of HBV cccDNA in hepatocytes, and the clearance of HBV cccDNA is the aim of CHB cures. HBV RNA is the novel marker for reflecting the level of

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cccDNA in the liver. Recently, data showed that double-negative HBV DNA and RNA has been used as a safety measure to indicate when CHB patients can stop NA treatment.

In a previous study, we found that the baseline HBV DNA and HBV RNA had no prediction effect for HBV DNA and HBV RNA double-negative after long-term ETV treatment in CHB patients (25). However, the predictive value of HBV RNA levels after NA treatment remains unclear. In this study, we found that the decline of HBV RNA levels was associated with double-negative HBV DNA and RNA in CHB patients receiving long-term ETV therapy. The AUROCs of the HBV RNA levels were higher than those of the HBV DNA levels at week 24, week 48, and week 96, indicating that the decline in serum HBV RNA levels was a better predictor of double-negative HBV DNA and RNA than HBV DNA during ETV treatment, and the earliest prediction point was at week 24 after treatment.

The incidence of HCC has increased in many countries in recent years, and HBV and HCV remain the most important risk factors for HCC (26-28). The risk factors of HBV-related HCC have been divided into host and viral factors, and effective antiviral treatments for HBV have contributed to a decrease in the rates of viral-associated HCC (29,30). In this study, 1 of the 33 patients developed liver cancer at the 10th year. This patient had positive HBeAg, HBV DNA, and HBV RNA at the baseline, but these were all negative from the 48th week and continued to be negative to the 10th year after ETV treatment. The patient had liver cirrhosis at the baseline and his mother died of HBV-related HCC, which indicates that factors other than the virus, such as liver cirrhosis and host inheritance, are involved in the occurrence of HBV-related HCC, which requires further study in the future.

This study had several limitations. First, the number of cases was small, and HBV RNA could not be compared across the groups treated with different NAs. Second, 75.8% of the patients were HBeAg positive, and more HBeAg-negative cases would have been needed to observe the HBV RNA in the HBeAg-negative patients. In addition, this was a single center research; multicenter research should be carried out to further verify the role of HBV RNA in the process of antiviral therapy.

In conclusion, these findings will help us to better understand the clinical significance of serum HBV RNA in long-term NAs therapy. We propose that serum HBV RNA may serve as a potential biomarker for predicting HBeAg seroconversion and antiviral efficacy in CHB patients receiving long-term NAs therapy, which may help to evaluate the risk of drug withdrawal and recurrence in the future.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3265/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3265/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3265/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Ethics Review Committee of The First Affiliated Hospital of Xi'an Jiaotong University (No. XJTUIAF2016LSK-16-1). The patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Table S1 Outcomes of HBV DNA from HBV RNA positive or negative patients during the 10-year ETV treatment

Time	HBV RNA	Number of cases	HBV DNA	48 W	96 W	5 Y	10 Y
24 W	RNA–	7	RNA/DNA double–	4	7	7	7
			RNA– or DNA–	3	0	0	0
	RNA +	26	RNA/DNA double–	3	7	12	18
			RNA– or DNA–	23	19	14	8
48 W	RNA-	11	RNA/DNA double–		10	11	11
			RNA– or DNA–		1	0	0
	RNA +	22	RNA/DNA double–		4	8	14
			RNA– or DNA–		18	14	8
96 W	RNA–	17	RNA/DNA double–			17	17
			RNA– or DNA–			0	0
	RNA +	16	RNA/DNA double–			4	8
			RNA– or DNA–			12	8
5 Y	RNA–	22	RNA/DNA double–				21
			RNA– or DNA–				1
	RNA +	11	RNA/DNA double–				4
			RNA– or DNA–				7

HBV, Hepatitis B virus; ETV, entec

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