

Early Serum HBV RNA Level in Combination With the HBeAg Response Can Effectively Predict the HBeAg Response in Patients on Nucleos(t)ide Analogue Therapy (ClinicalTrials.gov (NCT03909191))

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Research Article

Keywords: pre-genomic RNA (pgRNA), prediction, antiviral therapy, HBeAg seroconversion, hepatitis B virus (HBV)

Posted Date: June 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-602096/v1>

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Abstract

Background: Serum HBV RNA level has the potential to monitor antiviral therapy in patients with chronic hepatitis B. This study aimed to explore serum HBV RNA dynamic change pattern and its predict value on the efficacy of 96 weeks nucleos(t)ide analogue (NA) treatment in HBeAg-positive and HBeAg-negative patients with chronic hepatitis B (CHB).

Methods: A real-life cohort study of 78 patients with CHB on NA treatment was conducted. Dynamic change patterns of serum HBV RNA and correlation with other HBV markers in the early treatment period of 96 weeks of NA treatment in patients with CHB were determined and compared. The performance of serum HBV RNA on treatment efficacy was analyzed by receiver operating characteristic (ROC) analyses.

Results: HBeAg-positive and HBeAg-negative patients with CHB had similar viral change patterns during NA treatment. Serum HBV RNA level was consistently correlated with HBeAg and HBsAg titers in HBeAg-positive patients during NA treatment, but serum HBV RNA was only moderately correlated with serum HBV DNA level in HBeAg-negative patients before treatment. Serum HBV RNA decreased more rapidly in patients with the HBeAg seroconversion (SC) response than in patients without the HBeAg SC response; it had good early discriminatory ability for the HBeAg response with area under the ROC curve (AUROC) of 0.70 and 0.730 at 12 and 24 weeks of treatment, respectively. The cutoff value of serum HBV RNA of 4.31 log cps/mL in combination with the HBeAg decrease degree of 1.55 at 24 weeks of treatment had a good two-way predictive capability for the HBeAg response (PPV%: 83.33% and NPV%: 81.25%), and the specificity was 96.30%.

Conclusion: Serum HBV RNA level had early discriminatory ability for the HBeAg response. Early HBeAg response can improve the discriminatory ability of serum HBV RNA.

Introduction

Long-term nucleos(t)ide analogue (NA) treatment can improve liver inflammation, ameliorate the development of liver fibrosis, and reduce the risk of hepatocellular carcinoma (HCC) through efficient suppression of hepatitis B virus (HBV) replication. However, given the limited effect on covalently closed circular DNA (cccDNA), NA therapy rarely leads to a functional cure (defined as hepatitis B surface antigen [HBsAg] loss with undetectable HBV DNA)[1–3]. HBeAg loss and seroconversion (SC) are associated with immune restoration and can be the impetus for HBsAg loss and SC[1, 2, 4, 5]. Therefore, to improve treatment responses, pretreatment and on-treatment viral factors must be assessed.

Several HBV biomarkers, including HBV DNA, HBsAg titer, and hepatitis B core-related antigen (HBcrAg), have been used to monitor HBV chronic infection and antiviral therapy[6–10]. Unfortunately, these indicators are currently unable to effectively predict or guide NA therapy alone. Serum HBV RNA, a new surrogate for intrahepatic cccDNA transcription, has the potential to monitor chronic HBV infection, and a growing body of research suggested that serum HBV RNA may be a useful marker for monitoring chronic HBV infection including natural history, treatment, and prognosis[11–19]. However, previous research yielded inconsistent results, such as the correlation between serum HBV RNA and other viral markers in HBeAg-positive and HBeAg-negative patients with or without antiviral treatment[19–20], early serum HBV RNA kinetics in patients on NA therapy[21], and the potential use of serum HBV RNA and the cutoff value of serum HBV RNA level for prediction[22].

In this work, we aimed to determine the early kinetics of serum HBV RNA in HBeAg-positive and HBeAg-negative patients and evaluate the performance of early-stage serum HBV RNA for monitoring NA therapy patients with chronic hepatitis B (CHB).

Methods And Materials

Study design

A real-life cohort study was conducted in Shanghai Changhai Hospital between February 12, 2016 and February 21, 2018. Dynamic change patterns of serum HBV RNA in different patients with CHB undergoing long-term antiviral treatment were determined and compared. Furthermore, the role of serum HBV RNA alone or in combination with other HBV biomarkers on the treatment efficacy of HBeAg-positive patients with CHB was explored. The study was approved by the ethics research committee (CHEC2019-056) of Shanghai Changhai Hospital and registered on ClinicalTrials.gov (NCT03909191). Written informed consent was obtained from each patient.

Patients

Patients enrolled met the following criteria: positive for HBsAg more than 6 months before enrollment; alanineamino transferase (ALT) level higher than the upper limit of detection (14 days interval); no decompensated cirrhosis; no hepatitis C, hepatitis D, or human immunodeficiency virus co-infection; no HCC and other tumors or history of severe hepatitis; and no other systemic diseases, such as a history of cardiopulmonary diseases, thyroid disorders, immune system disorders, epilepsy, or mental illness (such as severe depression). The patients enrolled in this study were treated with first-line NAs or Peg interferon (IFN), followed by a switch to NAs for 96 weeks.

Antiviral response definition

HBeAg SC was defined as the loss of HBeAg and the appearance of anti-HBeAg on two occasions, at least one month apart in HBeAg-positive patients during 96 weeks of NA treatment.

For subsequent analyses, HBeAg-positive patients who achieved HBeAg SC and those who did not achieve HBeAg seroconversion (NSC) were divided into the SC and NSC groups, respectively.

Serological and virological detection

Serum HBV RNA quantification

Serum HBV RNA was detected by RNA simultaneous amplification (SAT) using the commercially available HBV-SAT kit (Rendu Biotechnology, Shanghai, China) as previously described[11, 12]. Primers and probes were designed to amplify an HBV pre-genomic RNA (pgRNA) conserved region[23]. RNA extraction, amplification, and detection were processed on an automated AutoSAT system (Rendu Biotechnology, Shanghai, China). The HBV RNA assay was calibrated using an armored HBV RNA standard traceable to the HBVRNA standard in vitro transcripts. The minimum detection limit (MDL) was 100 copies/mL.

The serum HBV RNA test results were classified as follows[12]: (1) "target not detected," (2) "<100 copies/mL," and (3) "a numeric titer of >100 copies/mL." Negative HBV RNA was strictly defined as HBV RNA "target not detected." Positive HBV RNA was defined as HBV RNA <100 copies/mL or a numeric titer of >100 copies/mL.

Serum HBV DNA quantification

Serum HBV DNA was quantified by the commercially available hepatitis B viral DNA Determination Kit (Sansure Biotech, China), with a detection range of 1×10^2 IU/mL to 5×10^8 IU/mL. The value below the detection limit was replaced with the low detection limits.

HBV serological test

Serum HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc were measured by chemiluminescence microparticle immunoassays using the Architect i2000SR platform and Abbott Architect reagents (Abbott Laboratories, North Chicago, IL, USA) in our clinical laboratory.

Laboratory assessments were obtained at baseline, week 12, week 24, week 48, and week 96.

Statistical analysis

The data are presented as the mean, median (interquartile range) or proportion based on data type and distribution. The two groups (SC vs. NSC and HBeAg-positive vs. HBeAg-negative CHB) were compared using the Mann–Whitney U test for continuous data and chi-squared test for categorical data. Spearman correlation coefficients ρ were used to compare the correlation between two variables. Logistic regressions were used to assess the odds ratio and 95% confidence interval (95% CI) of HBV markers. Receiver operating characteristic (ROC) curves were used to compare the relative sensitivity and specificity of HBV RNA, HBsAg, HBeAg, and HBV DNA as predictors of antiviral efficacy. The cutoff value was chosen based on the ROC curve when the sensitivity and specificity were both relatively high. A P-value (two-tailed) of 0.05 was considered statistically significant. All statistical analyses were carried out using SPSS software (version 21.0.0; Chicago, IL, USA) and GraphPad Prism9 software (GraphPad, NJ, USA)

Results

Patient characteristics and treatment outcome

A total of 110 patients with CHB were recruited for this study from February 2016 to December 2018 in Shanghai Changhai Hospital. Finally, 79 patients with CHB (54 HBeAg-positive) were enrolled in this study. All of them were treated for more than 96 weeks with first-line NAs (ETV or TDF).

At baseline, HBeAg-positive patients with CHB had significantly higher viral replication level compared with HBeAg-negative patients with CHB. However, during the 96 weeks of NA treatment, HBeAg-positive and HBeAg-negative patients achieved similar serum HBV DNA below the MDL ($<$ MDL), HBsAg loss rate, and ALT normalization rate (Table 1). Of these 79 patients with CHB, only one patient achieved HBsAg loss, whereas four patients (two HBeAg-positive and two HBeAg-negative patients with CHB) achieved HBsAg $<$ 100 IU/mL during the 96 weeks of antiviral treatment. Fourteen (25.93%) HBeAg-positive patients with CHB achieved SC response. HBeAg-positive patients with SC had significantly higher ALT normalization rate and serum HBV DNA $<$ MDL rate than NSC patients (Table 1).

Table 1
Clinical Characteristics of patients with CHB

Characteristics	Total (n = 79)	HBeAg pos. (n = 54)	HBeAg Neg. (n = 25)	P value	HBeAg Pos.		P value
					SC (n = 14)	NSC (n = 40)	
Sex (M: F)	55:24	40:14	15:10	0,206	10:4	30:10	1.000
Age(year)	36.0(30.0– 45.0)	32.0(29.0– 40.0)	45.0(37.5– 55.5)	0.001	31.5(27.00– 40.00)	32.00(30.00– 41.5)	0.553
Baseline characteristics							
HBsAg (log 10, IU/ml)	3.47(3.08– 4.14)	3.69(3.22– 4.39)	3.11(2.75– 3.55)	0.004	3.80(3.20– 4.19)	3.63(3.20– 4.46)	0.845
HBeAg(log 10)S/CO	2.81(1.69– 3.11)	2.81(1.69– 3.11)	/	/	2.75(1.80– 2.96)	2.81(1.57– 3.16)	0.612
HBV DNA(log10)IU/ml	6.11(4.41– 7.70)	7.01(5.51– 7.90)	4.34(3.51– 5.25)	0.001	7.05(5.32– 7.96)	7.01(5.64– 7.93)	0.942
HBV RNA(log 10)copies/ml	6.64(5.58– 8.08)	7.54(5.96– 8.30)	5.78(5.23– 6.42)	0.001	7.20(5.59– 7.97)	7.70(6.09– 8.68)	0.302
ALT(U/L)	105.5(51.5– 227.75)	105.50(53.75– 230.25)	101.0(33.25– 225.0)	0.561	255.00(205.5– 444.75)	73.5(44.5– 153.25)	0.000
AST(U/L)	66.5(36.0– 112.0)	68.00(38.75– 112.00)	63.0(27.0– 121.0)	0.663	119.5(73.75– 195.0)	44.5(31.75– 87.00)	0.000
Treatment outcome							
HBV DNA below MDL	63(78.48)	41(75.93)	22(88.00)	0.214	14/14(100)	27/40(67.5)	0.037
HBsAg loss rate,n(%)	1(1.27)	1(1.85)	0(0)	1.00	1/14(7.14)	0/40(0)	0.259
HBsAg < 100IU/mL rate,n(%)	4(5.06)	2(3.70)	2(8.0)	0.796	2/14(14.29)	0/40(0)	0.064
ALT normalization rate,n(%)	60(75.95)	39(72.22)	21(84.00)	0.255	14/14(100)	25/40(62.5)	0.000
Continuity data were shown as median (interquartile range). SC: seroconversion; NSC: no seroconversion; pos. positive; Neg. negative. MDL: minimum detection limit; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus							

Correlation between serum HBV RNA and other HBV biomarkers in patients with CHB on NA treatment by baseline HBeAg status

In HBeAg-positive patients with CHB, before NA treatment, strong correlation was found between serum HBV RNA and HBV DNA (Spearman's $r = 0.73$; Fig. 1A), and this strong correlation gradually decreased with treatment (Spearman's $r = 0.53$ and 0.41 ; Figs. 1B and 1C). Furthermore, sustained moderate correlation was noted between serum HBV RNA and HBeAg and HBsAg titer during treatment (Spearman's $r = 0.59–0.40$; Figs. 1A–1C). However, in HBeAg-negative patients with CHB on NA treatment, a significant correlation only existed between serum HBV RNA and HBV DNA level before treatment (Spearman's $r = 0.53$; Fig. 1D) and the relationship decreased and disappeared gradually with treatment (Spearman's $r = 0.42$ and 0.25 ; Figs. 1E and 1F).

Kinetics of serum HBV RNA in patients with CHB on NA treatment by HBeAg status

During NA treatment, HBeAg-positive and HBeAg-negative patients with CHB had similar dynamic change patterns of serum HBV biomarkers, including serum HBV RNA, HBV DNA, and HBsAg titers (Figs. 2A–2C). Serum HBV DNA level decreased more rapidly than serum HBV RNA level in total CHB patients and HBeAg-positive patients during the early stage of NA treatment (Figs. 2D and 2E). However, serum HBV RNA and DNA level had similar decrease degrees in HBeAg-negative patients (Fig. 2F).

Dynamic change patterns of serum HBV RNA in HBeAg-positive patients with CHB on NA treatment by serum HBeAg conversion response

Before initiation of NA treatment, there were no significance difference in the serum HBV RNA and HBV DNA levels between HBeAg-positive patients with or without the HBeAg SC response during the 96 weeks of treatment (Figs. 3A and 3B). However, from week 12 of treatment, absolute values of serum HBV RNA, HBsAg, and HBeAg in patients with the SC response were significantly lower than those in patients without the SC response (Figs. 3B–3D). Consistent with this result, serum HBsAg and HBeAg titers in patients with the HBeAg SC response decreased rapidly from baseline during the first 12 weeks of treatment than that in patients without the HBeAg SC response (Figs. 2G and 2H). However, no significant difference was found between serum HBV RNA and DNA decrease degree in patients with different HBeAg SC responses (Figs. 3E and 3F).

Value of serum HBV RNA in monitoring the HBeAg SC response of NA therapy

Considering the strong correlation between serum HBV RNA and HBeAg level in HBeAg positive CHB patients before initiation and during NA treatment (Figs. 1A-C), and the significant difference in serum HBV RNA, HBsAg, and HBeAg titers in patients who achieved different HBeAg SC responses on NA treatment (Figs. 3), we illustrated the potential utility of serum HBV RNA in monitoring the HBeAg response of NA therapy. The ROC curves and area under curve (AUROC) scores of absolute and change from baseline levels for all viral indicators are shown in Figs. 4A–4E. Based on the AUROC scores of the absolute values of serum HBV RNA, HBeAg, and HBsAg, the SC responders could be well identified as early as 12 weeks of treatment, with the AUROC scores of 0.703, 0.780, and 0.761, respectively (Fig. 4B). At 24 weeks of treatment, only the absolute values of HBeAg and HBsAg demonstrated good discriminatory ability, with AUROC scores of 0.789 and 0.717, respectively (Fig. 4C). The absolute value of serum HBV DNA consistently showed poor discriminatory ability, with AUROC score ≤ 0.631 (Figs. 4A–4C).

Serum HBV RNA level decrease degree from baseline at 12 and 24 weeks of NA treatment showed poor discriminatory ability, with low AUROC scores (Figs. 4D and 4E), but those for serum HBsAg and HBeAg titers showed similar discriminatory ability to their absolute value at the same time point, especially the HBeAg titer (Figs. 4D and 4E).

Cutoff value of HBV biomarkers to predict the HBeAg SC response of NA therapy

Considering the higher discriminatory ability of HBV biomarkers' absolute value and decrease degree from baseline at the early stage of treatment for the HBeAg response of NA treatment and the characteristics of HBV biomarkers' dynamics during NA treatment, the cutoff values of these HBV biomarkers at 12 and 24 weeks of treatment were identified. Moreover, their performance was compared.

At the early stage of treatment, serum HBV RNA level consistently showed good predictive ability for the HBeAg response during 96 weeks of NA treatment. The absolute values of serum HBV RNA of 4.94 (log cps/mL) at 12 weeks and 4.31 (log cps/mL) at 24 weeks of treatment both had good negative predictive value (NPV) for the HBeAg SC response (90.48% vs. 94.74%), and both possessed high sensitivity (83.33% vs. 90.0%, respectively). Classical viral indicators, HBsAg cutoff value of 3.46 \log_{10} IU/mL at 12 weeks of treatment, and HBeAg value of 2.87 \log_{10} S/CO at 12 weeks of treatment also

had good NPV% (> 80%) for the HBeAg SC response (Table 2). The 24-week HBeAg cutoff value of 0.306 log₁₀ S/CO showed a two-way predictive capability for the HBeAg response (PPV% and NPV% both > 80%; Table 2). In addition to the absolute cutoff value of HBeAg, the titer decreases from baseline with a cutoff value of 1.55 log IU/mL at 24 weeks of treatment had a good NPV% (86.47%) and high specificity (86.49%) (Table S1).

Table 2
Absolute cutoff value of HBV biomarkers at 12 and 24 weeks of treatment for predict HBeAg seroconversion.

characteristics	Cutoff values	Sensitivity, %	Specificity, %	PPV, %	NPV, %
12 weeks of treatment					
HBV RNA, log ₁₀ cps/mL	4.94	83.3	55.88	40	90.48
HBeAg, log S/CO	2.87	78.57	32.5	28.95	81.25
HBsAg, log ₁₀ (IU/ml)	3.46	84.62	59.46	42.31	91.67
24weeks of treatment					
HBV RNA, log ₁₀ cps/mL	4.31	90.0	62.07	45	94.74
HBeAg, log ₁₀ S/CO	0.306	57.15	95	80	86.36
HBsAg, log ₁₀ (IU/ml)	2.89	57.14	85	57.14	57.14
HBV: hepatitis B virus, HBsAg: Hepatitis B surface antigen, HBeAg: hepatitis B envelop antigen; PPV positive predictive value, NPV negative predictive value; cps/mL: copies/mL; cutoff value of HBV biomarkers was obtained from ROC analysis;					

Serum HBV RNA combined with a sharp decline in HBeAg can better predict the HBeAg response

To find better predictors for the HBeAg response, we explored the predictive value of serum HBV RNA in combination with other classical HBV biomarkers and found that serum HBV RNA combined with HBeAg had good predictive value and high AUROC scores (0.854, 95% CI: 0.754–0.954; Table S2). The absolute cutoff value of serum HBV RNA of 4.31 combined with HBeAg decrease degree of 1.55 at 24 weeks of treatment had a good two-way predictive capability for the HBeAg response (PPV%: 83.33% and NPV%: 81.25%), and the specificity was 96.30% (Table S2).

Discussion

Here, we longitudinally studied the dynamic pattern of serum HBV RNA profiles in HBeAg-positive or HBeAg-negative patients with CHB subjected to NA treatment. This work aimed to reveal whether serum HBV RNA has similar value in monitoring the antiviral response in HBeAg-positive and -negative patients. We found that the dynamic change pattern of serum HBV RNA in HBeAg-positive patients on long-term NA treatment mirrored that of serum HBV DNA, HBsAg, and HBeAg. Serum HBV RNA sustained moderate to strong correlation with these viral markers. However, the correlation between serum HBV RNA and other HBV biomarkers was not sustained in HBeAg-negative patients with CHB during NA treatment. These results indirectly illustrated that serum HBV RNA quantification may play a potential monitoring value on the NA treatment response in HBeAg-positive patients with CHB, other than in HBeAg-negative patients.

As a surrogate biomarker for intrahepatic cccDNA quantity and transcript activity, serum HBV RNA level has been confirmed to be a valuable marker for CHB management, including natural course management and antiviral management[10–16, 18–20, 24–27]. Our previous study and other researches revealed that serum HBV RNA alone or combined with other viral indicators such HBsAg titer or HBcrAg level can guild antiviral cessation in patients with CHB[12, 15, 16, 28]. In this study, we further studied the monitoring value of serum HBV RNA on the NA treatment response. We

found no difference in serum HBV RNA level at baseline between patients with the SC response or not, but serum HBV RNA level in patients with the SC response was obviously lower than that in patients without the SC response at the early stage of NA treatment. Moreover, the AUROC values of serum HBV RNA at 12 and 24 weeks of treatment exceeded 0.70, indicating that serum HBV RNA level had early predictability of the HBeAg SC response in patients on NA treatment. We then analyzed the absolute cutoff value of serum HBV RNA at 12 and 24 weeks of treatment separately and found that the cutoff of HBV RNA at 4.94 or 4.31 log₁₀ copies/mL at 12 and 24 weeks of treatment, respectively, had the highest NPV for the SC response in NA treatment. All these results confirmed that serum HBV RNA alone showed good discriminatory ability for the antiviral response on NA treatment. Compared with HBeAg level and HBsAg titer of the same time point, serum HBV RNA alone had no obvious advantage in predicting the antiviral response. Further analysis of the NA treatment response of HBV biomarkers demonstrated no obvious advantage of serum HBV RNA in predicting the SC response during 96 weeks of NA treatment. However, serum HBV RNA value combined with HBeAg decrease degree at 24 weeks of NA treatment showed a good predictive value for the SC response, with high specificity and AUROC value. These results were similar to the findings reported by Wang[18] and Luo[29], who both found that the combination of serum HBV RNA and HBeAg response shows better predicting value for NA treatment response than serum HBV RNA alone.

Although our results here and the findings of previous studies revealed that serum HBV RNA alone or in combination with HBeAg treatment response can monitor NA treatment early, the cutoff values of serum HBV RNA in the literature varied widely[16, 29]. This difference may be related to the methodological heterogeneity of serum HBV RNA detection. Given the complexity of serum HBV RNAs, serum HBV RNA levels detected by different primers targeting varying regions of the HBV genome were not only inconsistent[30] but also different in treatment outcomes[31]. Therefore, before serum RNA is applied to clinical practice in the future, further understanding of the biological significance of serum HBV pgRNA and standardization of testing methods is critical.

As a real-life cohort study, our results were more representative of the clinical reality than team-registered studies. However, our study had some shortcomings, such as a relatively small sample size, and the significance of serum HBV RNA prediction needs to be further verified in larger sample sizes. Moreover, in our study, HBeAg was measured using a chemiluminescence microparticle immunoassay, which is the most common assay method in China but not a total quantitative test. Despite these limitations, the HBeAg level still has meaningful reference value in clinical practice.

Conclusion

In conclusion, serum HBV RNA level change pattern in HBeAg positive and negative CHB patients on NA therapy was different, serum HBV RNA level had good correlation with other viral markers, such as HBV DNA level, HBeAg level and HBsAg titer, in HBeAg positive CHB patients during NA treatment. Serum HBV RNA level had early discriminatory ability for the HBeAg response, and early HBeAg decrease degree from baseline can improve the discriminatory ability of serum HBV RNA.

Abbreviations

NA: nucleos(t)ide analogue; HCC: hepatocellular carcinoma; cccDNA: hepatocellular carcinoma; HBsAg: hepatitis B surface antigen; SC: seroconversion; HBcrAg: hepatitis B core-related antigen; CHB: chronic hepatitis B; ALT: Alanine amino transferase; NSC: no seroconversion; SAT: simultaneous amplification; pg-RNA: pre-genomic RNA; MDL: minimum detection limit; ROC: Receiver operating characteristic; AUROC: Area under the ROC curve

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Changhai Hospital. (CHEC2019-056). Written informed consent was obtained from all the study participants.

Consent for publication

Not applicable

Availability of data and material

All data and analysis results are included in this article. The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by Beijing Medical Health Foundation-Medicine Science Study (No. YWJKJJHKYJJ-G17007), Grants from Shanghai Natural Science Fund (No.16ZR1400400, 20ZR1456900), and Wu Jieping Medical Foundation (LSWJPMF-102-17001). The fund units are not involved in the design, analysis, and conduct of this study.

Authors' contributions

LYY recruited the patients and collected specimens, edited the clinical data and performed the data analysis;

LW enrolled and followed the patients, edited the clinical data of enrolled patients;

XAJ, YW, YJ, FWH, XJY treated and followed the patients;

LXS conceived and designed the study; checked the data analysis and drafted the manuscript;

All authors have read and approved the final manuscript.

Acknowledgment

The authors thank Dr Qian He (Department of Health Statistics, Second Military Medical University, Shanghai) for giving helpful comments in data analysis and confirmation of statistical methods.

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Figures

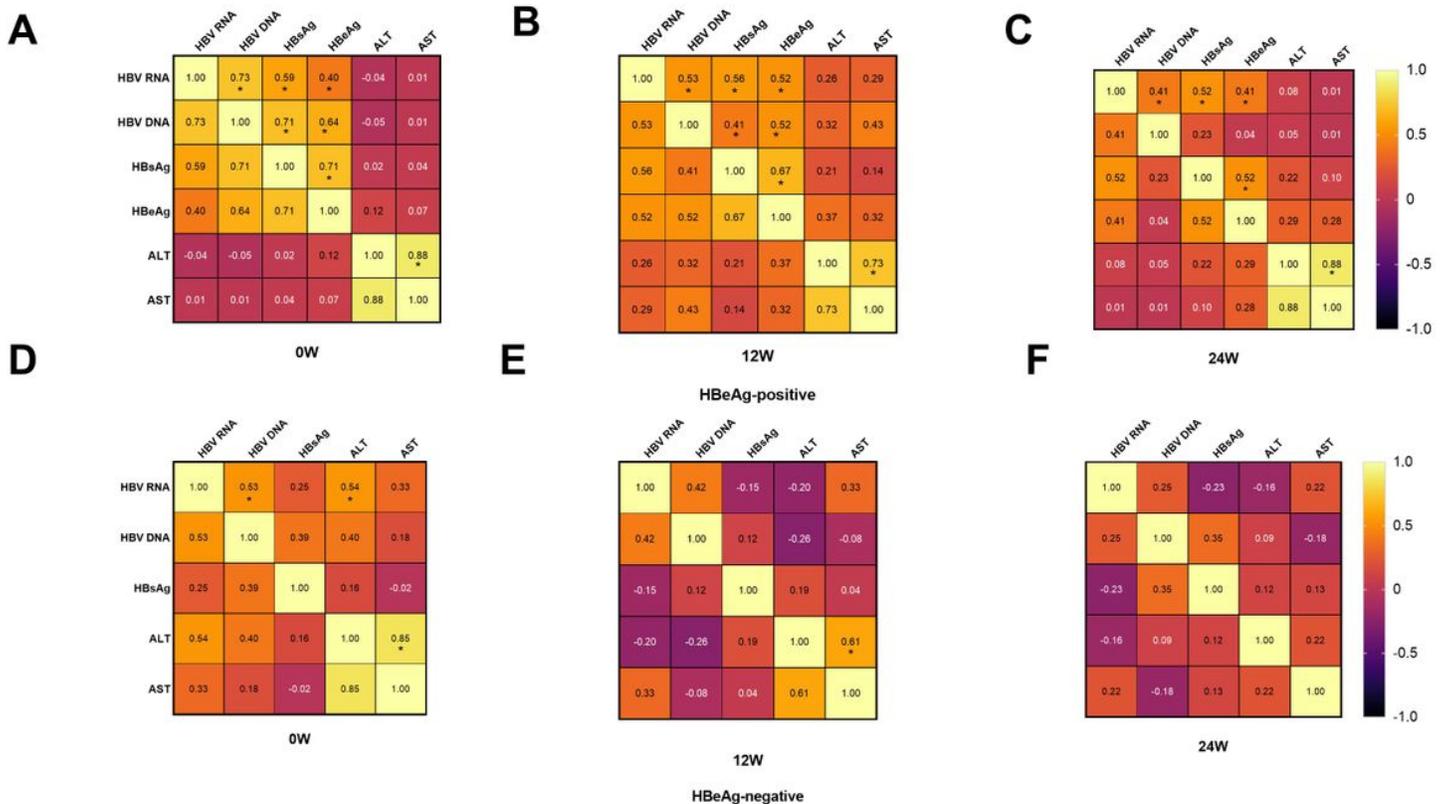


Figure 1

Correlation of serum HBV RNA with other HBV biomarkers in CHB patients on NAs therapy by HBeAg status. Correlation between serum HBV RNA and other HBV indicators were analyzed using Spearman analysis. Number means Spearman

correlation coefficients (r) and associated P values. * $P < 0.05$. A: At baseline in HBeAg-positive CHB; B: At 12-week in HBeAg-positive CHB; C: At 24-week in HBeAg-positive CHB; D-F: at different timepoint in HBeAg-negative CHB.

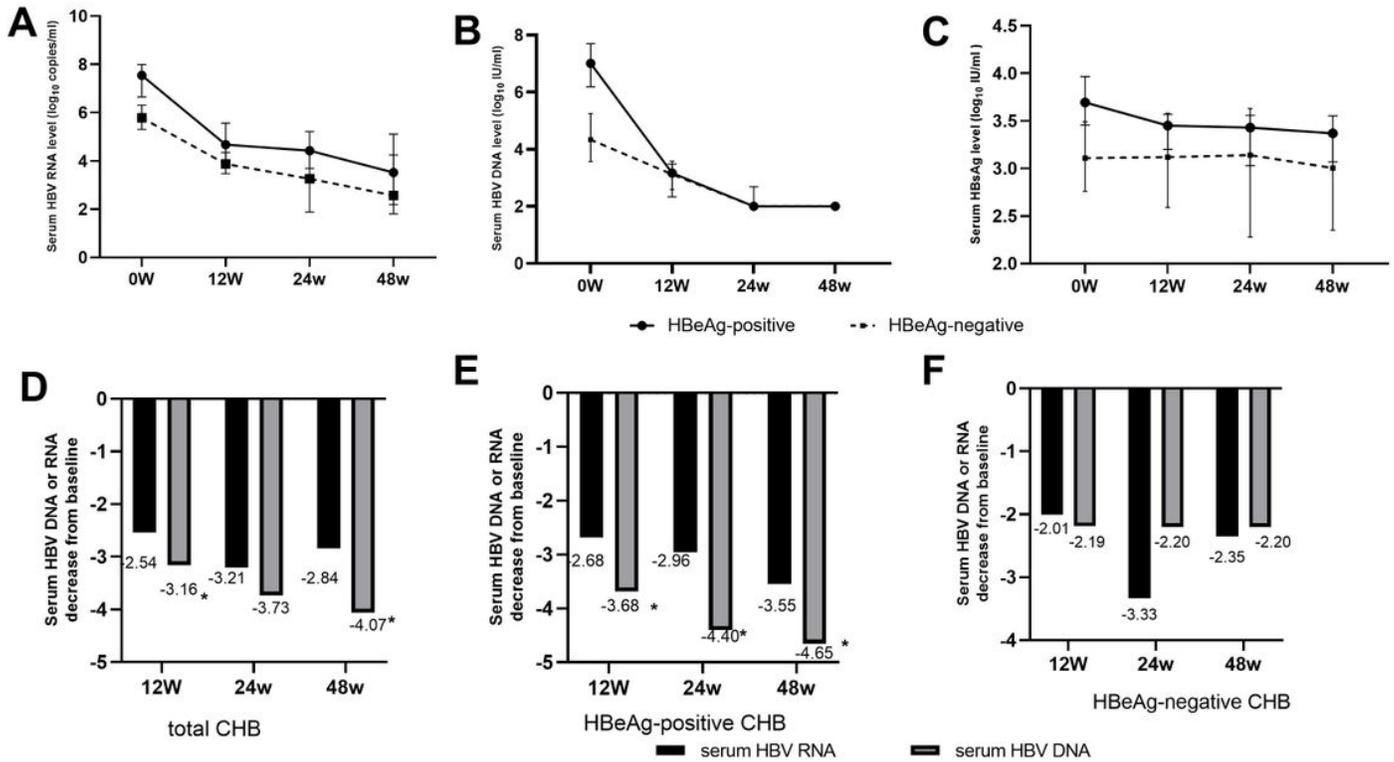


Figure 2

Kinetics and mean change from baseline of serum HBV RNA, HBV DNA level, and HBsAg titer in CHB patients on NA treatment by HBeAg status at baseline. Dot and error bar shows median (interquartile range, IQR). * $P < 0.05$, Mann-Whitney U test indicates statistical significance between HBV RNA and DNA decrease degree from baseline at same timepoint and indicated by stars. A: serum HBV RNA; B: serum HBV DNA; C: Serum HBsAg; D: serum HBV RNA and DNA decrease degree in total CHB patients; E: serum HBV RNA and DNA decrease degree in HBeAg-positive CHB patients; D: serum HBV RNA and DNA decrease degree in HBeAg-negative CHB patients.

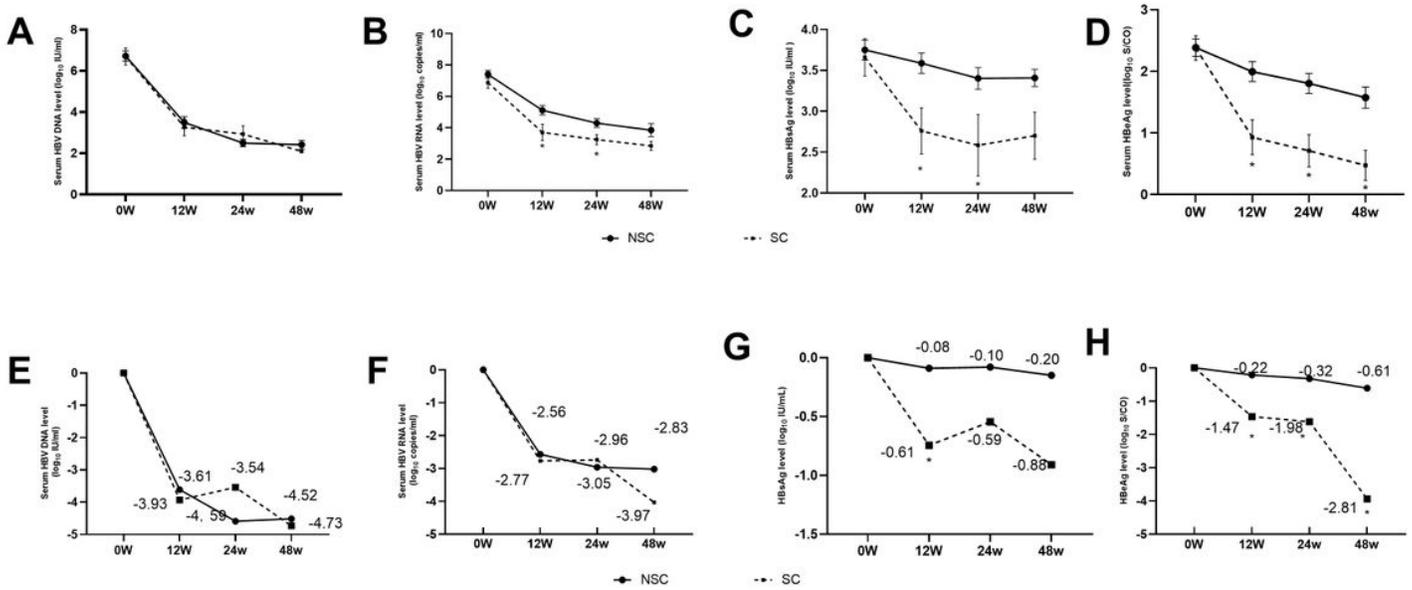


Figure 3

Kinetics and mean change from baseline level in HBeAg-positive CHB patients by serum HBeAg seroconversion response. The symbol represents mean level and median decrease from baseline level at each timepoint and with statistically significance between two groups $P < 0.05$, Mann-Whitney U test indicated by stars. A-D shows the median indicators level at different timepoint, A: Serum HBV DNA; B: HBV RNA; C: HBsAg; D: HBeAg; E-H shows the median decrease from baseline indicators level: E: Serum HBV DNA; F: HBV RNA; G: HBsAg; H: HBeAg. Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

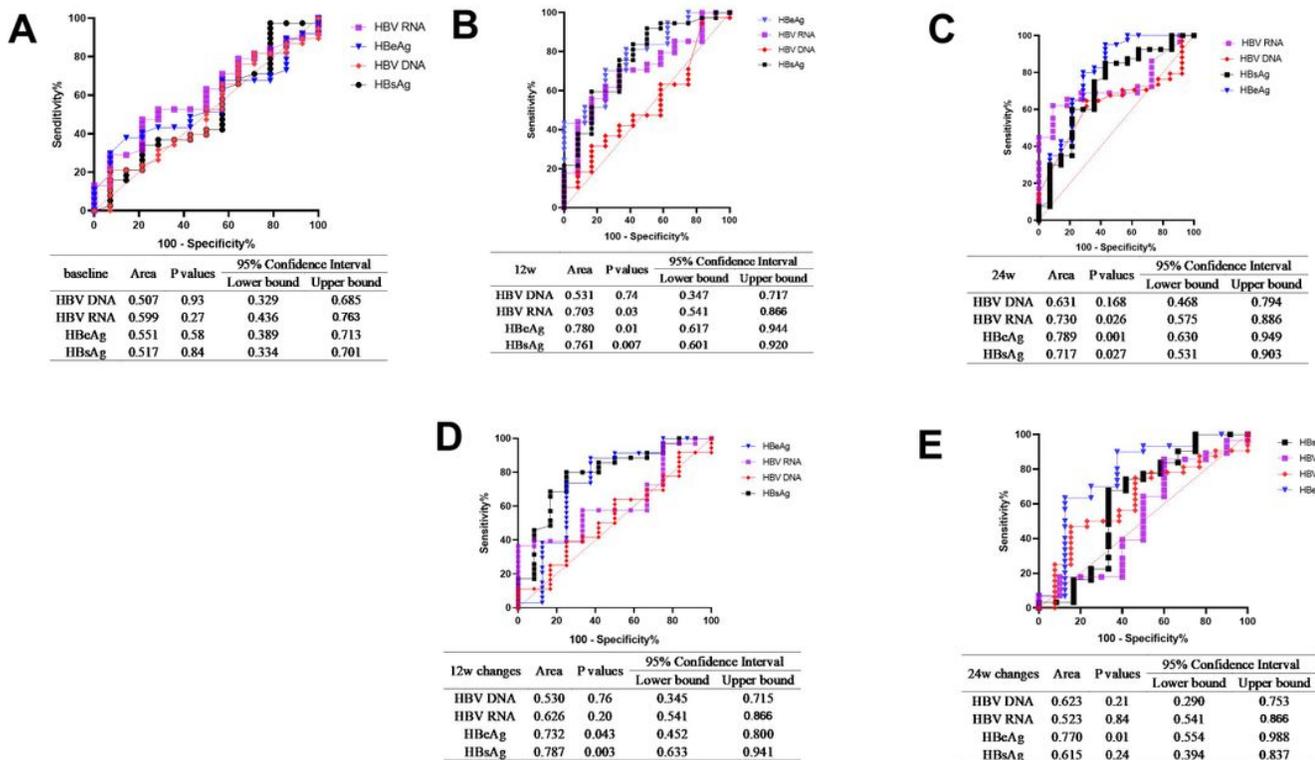


Figure 4

Predictive value of viral indicators at different timepoint for HBeAg seroconversion in patients on NAs therapy. Receiver operating characteristic curve (ROC) is used and $p < 0.05$ means statistical significance. The absolute value and change from baseline level at different timepoint were analyzed. A to C: absolute value at different timepoint of treatment; A: baseline; B: 12 weeks of treatment; C: 24 weeks of treatment; D: change from baseline at 12 weeks of treatment; E: change from baseline at 24 weeks of treatment.

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