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RESEARCH ARTICLE

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Predictive role of early treatment dynamics of HBV RNA and HBcrAg for HBeAg seroconversion in children with chronic hepatitis B

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Abstract

This study aimed to assess the predictive capacity of emerging serological markers, serum HBV RNA and HBcrAg, for HBeAg seroconversion in children with HBeAg-positive chronic hepatitis B (CHB). Treatment-naïve HBeAg-positive CHB children who admitted to the Liver Disease Center of Hunan Children's Hospital between April 2021 and September 2022 and received treatment with the combined entecavir and interferon-alpha treatment were recruited. Serum HBV RNA and HBcrAg were measured at baseline and Weeks 12, 24, and 48 of treatment. Our study showed that serum HBV RNA (HR = 0.71, 95% CI: 0.56–0.91, $p = 0.006$), HBcrAg (HR = 0.60, 95% CI: 0.43–0.84, $p = 0.003$), and HBsAg (HR = 0.49, 95%CI: 0.36–0.69, $p < 0.001$) at Week 12 were independent predictors of HBeAg seroconversion. ROC curve analysis presented that serum HBV RNA decline value (ΔHBV RNA) at Week 36 and HBcrAg decline value (ΔHBcrAg) at Week 12 (AUC = 0.871, $p = 0.003$ and AUC = 0.810, $p = 0.003$, respectively) could effectively predict HBeAg seroconversion. Furthermore, the optimal critical values were determined and the children with ΔHBV RNA > 3.759 log10 copies/mL at Week 36 or ΔHBcrAg >0.350 log10 U/mL at Week 12 more likely to achieve HBeAg seroconversion. The serum HBV RNA and HBcrAg provide new insights into the treatment of CHB in children. Early assessment of serum HBV RNA and HBcrAg during treatment can assist clinical decision-making and optimize individualized therapeutic approaches.

KEYWORDS

children with chronic hepatitis B, HBcrAg, HBeAg seroconversion, HBV RNA, individualized therapy

Abbreviations: ALT, alanine aminotransferase; anti-HBe, antibody to hepatitis B e antigen; AUC, aera under ROC curve; BMI, body mass index; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; C-index, Harrell's concordance index; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAg SC, HBeAg seroconversion; HBeAg, hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HBV RNA, hepatitis B virus Ribonucleic Acid; HBV, hepatitis B virus; IFN, interferon; NAs, nucleos(t)ide analogs; NPV, negative predictive value; Peg-IFN, peginterferon; PPV, positive predictive value; SE, sensitivity; SP, specificity.

Xin Lai and Wenxian OuYang are co-first authors. The first two authors contributed equally to this paper.

1 | INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health issue.[1] Despite the availability of effective HBV vaccines, about 1%–3% of children aged 5 and below are still infected with HBV, primarily through mother-to-child transmission or early childhood horizontal transmission, which may progress to chronic hepatitis B (CHB).[2,3] In untreated CHB patients, 15%–40% will develop into liver cirrhosis or even hepatocellular carcinoma.[4] For HBeAg-positive CHB children receiving antiviral treatment, HBeAg seroconversion (HBeAg SC) is a crucial treatment goal,[5] defined as HBeAg clearance with the appearance of anti-HBe.[6] However, the response rate of CHB children to antiviral treatment is low, with only approximately one-fourth of patients achieving HBeAg SC.[7,8] Moreover, prolonged antiviral treatment poses potential risks to children's health of growth and development.[6,9] Therefore, there is an urgent need to explore promising biomarkers that can effectively predict HBeAg SC in children with CHB.

A major challenge of antiviral therapy in CHB patients is the inability to effectively suppress or eliminate cccDNA within the hepatocytes,[10] which serves as the primary template for HBV replication. Direct assessment of therapeutic effects by detecting cccDNA is hampered by invasive testing methods and the lack of standardized quantification techniques.[11] However, increasing evidence suggests that serum hepatitis B virus ribonucleic acid (HBV RNA)[12–14] and hepatitis B core-related antigen (HBcrAg)[15–17] can accurately reflect cccDNA concentration and transcriptional activity in the liver of CHB patients.[13,14] In addition, a large number of studies conducted on adult CHB patients have suggested that serum HBV RNA and HBcrAg serve as reliable predictive markers for treatment response, surpassing traditional indicators in terms of their predictive accuracy.[18,19] However, there remains a paucity of study exploring the potential efficacy of serum HBV RNA and HBcrAg as predictive markers for antiviral treatment response in CHB children. Due to disparities in the natural history, progression of HBV infection and immune system functionality between CHB adult and children,[20,21] the generalizability of the findings from studies conducted on adult CHB patients to the children population is limited. These unresolved issues and potential associations prompt further exploration of the potential utility of serum HBV RNA and HBcrAg as reliable predictive markers for treatment response in CHB children.

In this study, we aimed to investigate the predictive value of serum HBV RNA and HBcrAg for HBeAg seroconversion in HBeAg-positive CHB children, which may provide new insights into the treatment of CHB in children.

2 | METHODS

2.1 | Study design and population

This study was conducted at the Liver Disease Center of Hunan Children's Hospital. We recruited treatment-naive children with HBeAg-positive CHB between April 2021 and September 2022 as study subjects. Inclusion criteria were: (1) meeting the diagnostic criteria for CHB in the "Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2022 Edition)"[6]; (2) positive for HBeAg; (3) not previously treated with HBV antiviral therapy; (4) aged 1–17 years; (5) starting to treatment. The exclusion criteria included: (1) major comorbidities (e.g., mental illness, malignancies); (2) mixed infections with other hepatitis viruses, cytomegalovirus, or human immunodeficiency virus; (3) concurrent liver diseases (e.g., autoimmune hepatitis, drug-induced liver injury, hepatic steatosis); (4) liver transplantation, liver cirrhosis, or liver cancer; (5) immunosuppressive therapy. This study was approved by the Ethics Committee of Hunan Children's Hospital, and all legal guardians or their representatives of the study subjects signed an informed consent form.

All patients received the combined entecavir (ETV) and interferon-alpha (IFN-α)/peginterferon-alpha-2a (Peg-IFN-α−2a) treatment. The recommended dose of common IFN-α for pediatric patients is 6 million units/m^2 of body surface area 3 times per week, and the course of treatment is 24–48 weeks. Peg-IFN-α−2a was administered at 104 μg/m^2 of body surface area once a week, and the course of treatment is 48 weeks. Entecavir dosing for this study was weight based at 0.015 mg/kg once-daily, up to a maximum of 0.5 mg/day. Add-on therapy (24 weeks of IFN with add-on of ETV) and Concurrent use of ETV and IFN-α were recommended.

The study participants received regular 12-week interval follow-up assessments in accordance with the clinical protocol, with the study follow-up scheduled to conclude in January 2024. To mitigate loss to follow-up, investigators proactively informed family members 1 week before each scheduled visit, ensuring the attendance of participants. The investigators longitudinally collected serum samples and laboratory test results from the participants (Supplementary Figure 1).

2.2 | Data collection

This study employed a self-designed questionnaire to collect baseline information, including gender, age, duration of infection, parental HBV carrier status, previous antiviral treatment, and medical history of study subjects. Baseline and follow-up laboratory test results of the participants were extracted from the electronic medical record system of the hospital. Given that the follow-up visits of the participants were not strictly aligned with the clinical follow-up time points, laboratory test results obtained within a 15-day before and after the scheduled follow-up time points were consolidated to ensure comprehensive and accurate follow-up data.

2.3 | Laboratory assessments

2.3.1 | Serum HBV RNA

Serum HBV RNA was quantitatively measured using a hepatitis B virus nucleic acid detection test kit (RNA capture probe method) provided by Shanghai Rendu Biotechnology Co., Ltd. The assay exhibited a linear detection range of $10^2 \sim 10^8$ copies/mL.

2.3.2 | Serum HBcrAg

Serum HBcrAg concentration was quantitatively determined using the Lumipulse G®HBcrAg assay kit provided by KingMed Diagnostics. The assay was conducted on a LUMIPULSE G1200 fully automated immunoassay analyzer. The chemiluminescent enzyme immunoassay technique employing a two-step sandwich immunoassay was utilized for the quantitative detection of HBcrAg in serum samples. The HBcrAg concentration was automatically calculated based on a calibration curve. The linear measurement range of the assay was 3 ~ 7 log10 U/mL. In cases where the serum samples exceeded a concentration of 7.0 log10 U/mL, dilution was performed before retesting.

2.3.3 | HBsAg, HBeAg, anti-HBe and HBV DNA

HBsAg, HBeAg, anti-HBe were detected using the electrochemiluminescence immunoassay method (reagents purchased from Roche Diagnostics GmbH, cobas e 601 immunoassay analyzer). Serum HBV DNA was quantitatively detected using a hepatitis B virus nucleic acid detection kit with PCR-fluorescent probe method (Hunan Shengxiang Biotechnology Co., Ltd., ABI 7500 fluorescent PCR instrument).

2.4 | Primary outcome

The primary outcome of this study was HBeAg seroconversion, defined as HBeAg clearance and the appearance of anti-HBe at Week 72 of treatment.

2.5 | Statistical analyzes

Statistical analysis was conducted using IBM SPSS Statistics 20.0, GraphPad Prism version 9.0.0 for Windows, and R version 4.2.3 software. Normality of continuous variables was assessed using the one-sample Kolmogorov-Smirnov test. Normally distributed continuous variables were presented as mean ± standard deviation (mean ± SD), while non-normally distributed continuous variables were presented as median (P_{25}, P_{75}) (median [P_{25}, P_{75}]). Categorical variables were described using frequency and percentage (*n*, [%]). Serum HBV RNA, HBcrAg, HBV DNA, and HBsAg levels were logarithmically transformed using log10. Spearman correlation analysis was performed to assess the correlation between HBV RNA, HBcrAg, and other HBV-related markers. Generalized estimating equation with repeated measurements was employed to analyze dynamic changes. Mann-Whitney U test was used for between-group comparisons of absolute values and change values in HBV RNA and HBcrAg. Multivariate Cox proportional hazards analyzes were conducted to the associations of HBV RNA and HBcrAg with HBeAg seroconversion. In addition, ROC curve analysis, calibration curve analysis, and Bootstrap internal validation were adopted to evaluate the predictive efficacy of serum HBV RNA and HBcrAg for HBeAg seroconversion. Statistical significance was defined as a two-sided $p < 0.05$.

3 | RESULTS

3.1 | Baseline characteristics

In total, 111 HBeAg-positive CHB children were enrolled (Flowchart shown in Supplementary Figure 2). The study population exhibited a mean age of 6.02 ± 3.24 years, with a majority of 62.2% being male children, and a predominance of HBV genotype B (81.1%). A liver tissue inflammation grade of G < 2 was observed in over half of the children (57.7%) (Table 1). At the end of follow-up, 27 cases (24.3%) demonstrated HBeAg seroconversion and 29 cases (26.13%) achieved HBsAg loss.

3.2 | Correlation and dynamic changes of serum HBV RNA and HBcrAg with other HBV-related markers

Spearman correlation analysis revealed significant positive associations between baseline serum HBV RNA, HBcrAg and HBsAg, HBV DNA levels (All $p < 0.01$), and negative correlations with ALT ($p < 0.05$) (Supplementary Table 1).

During the treatment period, both serum HBV RNA and HBcrAg exhibited similar dynamic changes, demonstrating an overall declining pattern in concordance with HBsAg and HBV DNA. The levels of HBV RNA, HBcrAg, HBsAg, and HBV DNA at Weeks 24, 36, and 48 showed a statistically significant reduction compared to the baseline levels ($p < 0.001$) (Figure 1).

3.3 | Dynamic changes of serum HBV RNA and HBcrAg according to HBeAg seroconversion

Figure 2 shows comparison of the serum HBV RNA and HBcrAg levels between the groups based on HBeAg seroconversion. The seroconversion group exhibited significantly lower HBV RNA levels at Weeks 12, 24, 36, and 48 compared to the non-seroconversion group ($p < 0.05$). Similar results were found in HBcrAg levels. Serum HBV RNA decline (ΔHBV RNA) and HBcrAg decline (ΔHBcrAg) from baseline to Weeks 12, 24, 36, and 48 of treatment were also analyzed (Supplementary Figure 3). Significant differences in ΔHBV RNA were observed between the two groups at Weeks 12, 24, 36, and 48 (Week 12: 2.078 vs. 0.630, $p < 0.01$; Week 24: 3.567 vs. 0.882, $p < 0.01$; Week 36: 5.410 vs. 1.665, $p < 0.01$; Week 48: 4.690 vs. 1.677, $p < 0.001$). Additionally, significant differences were also observed in ΔHBcrAg

TABLE 1 Demographic and baseline characteristics of participants.

Characteristics	Number	mean ± SD/median (P_{25}, P_{75})/*n*, (%)
Gender	111	
Boys		69 (62.2)
Girls		42 (37.8)
Age, years	111	6.02 ± 3.24
BMI, kg/m^2[a]	102	16.27 ± 2.60
Estimated duration of HBV infection, years[b]	106	2.50 (0.25,5.00)
Parental HBV infection	111	
Yes		89 (80.2)
No		22 (19.8)
HBV genotype	111	
B		90 (81.1)
C		21 (18.9)
Liver inflammation activity grade[c]	105	
<G2		64 (57.7)
≥G2		41 (36.9)
Liver fibrosis stage[c]	105	
<S2		100 (90.1)
≥S2		5 (4.5)
ALT, IU/L[d]	106	30.90 (20.73,56.05)
HBsAg, log10 IU/mL[e]	110	4.49 (3.70,4.72)
HBV DNA, log10 IU/mL[f]	109	7.86 (6.80,8.43)
HBV RNA, log10 copies/mL[g]	109	6.89 (6.24,7.33)
HBcrAg, log10 U/mL[h]	106	8.40 (8.00,8.70)

Abbreviation: ALT, alanine aminotransferase; BMI, body mass index, weight (kg)/the square of height (m^2).

[a]Missing nine cases.

[b]Missing five cases.

[c]Missing six cases, Liver histopathological diagnosis was based on the Sheuer scoring system, Liver inflammation activity grade was classified into G0–G4, and Liver fibrosis stage was classified into S0–S4.

[d]Missing five cases.

[e]Missing one case.

[f]Missing two cases.

[g]Missing two cases.

[h]Missing five cases.

between the two groups at Weeks 12, 36, and 48 (Week 12: 0.700 vs. 0.300, $p < 0.01$; Week 24: 1.250 vs. 0.600, $p = 0.058$; Week 36: 2.200 vs. 0.800, $p < 0.05$; Week 48: 2.250 vs. 1.100, $p < 0.01$).

3.4 | Independent predictors of HBeAg seroconversion

The multivariate Cox proportional hazards analysis demonstrated that baseline HBcrAg (HR = 0.66, 95% CI: 0.46–0.94, $p = 0.023$) and HBsAg (HR = 0.67, 95% CI: 0.47–0.97, $p = 0.034$) were independent predictors for HBeAg SC. Additionally, HBV RNA(HR = 0.71, 95% CI: 0.56–0.91, $p = 0.006$), HBcrAg (HR = 0.60, 95% CI: 0.43–0.84, $p = 0.003$) and HBsAg (HR = 0.49, 95% CI: 0.36–0.69, $p < 0.001$) at Week 12 of treatment were associated with HBeAg SC (Table 2).

To further explore the stability of our results, we conducted a sensitivity analysis focusing solely on the Peg-IFN treatment group. The results persistently indicated that serum HBV RNA (HR = 0.61, 95% CI: 0.44–0.82, $p = 0.001$), HBcrAg (HR = 0.55, 95% CI: 0.37–0.81, $p = 0.002$), and HBsAg (HR = 0.50, 95% CI: 0.36–0.70, $p < 0.001$) at Week 12 serve as independent predictors of HBeAg seroconversion (Supplementary Table 2).

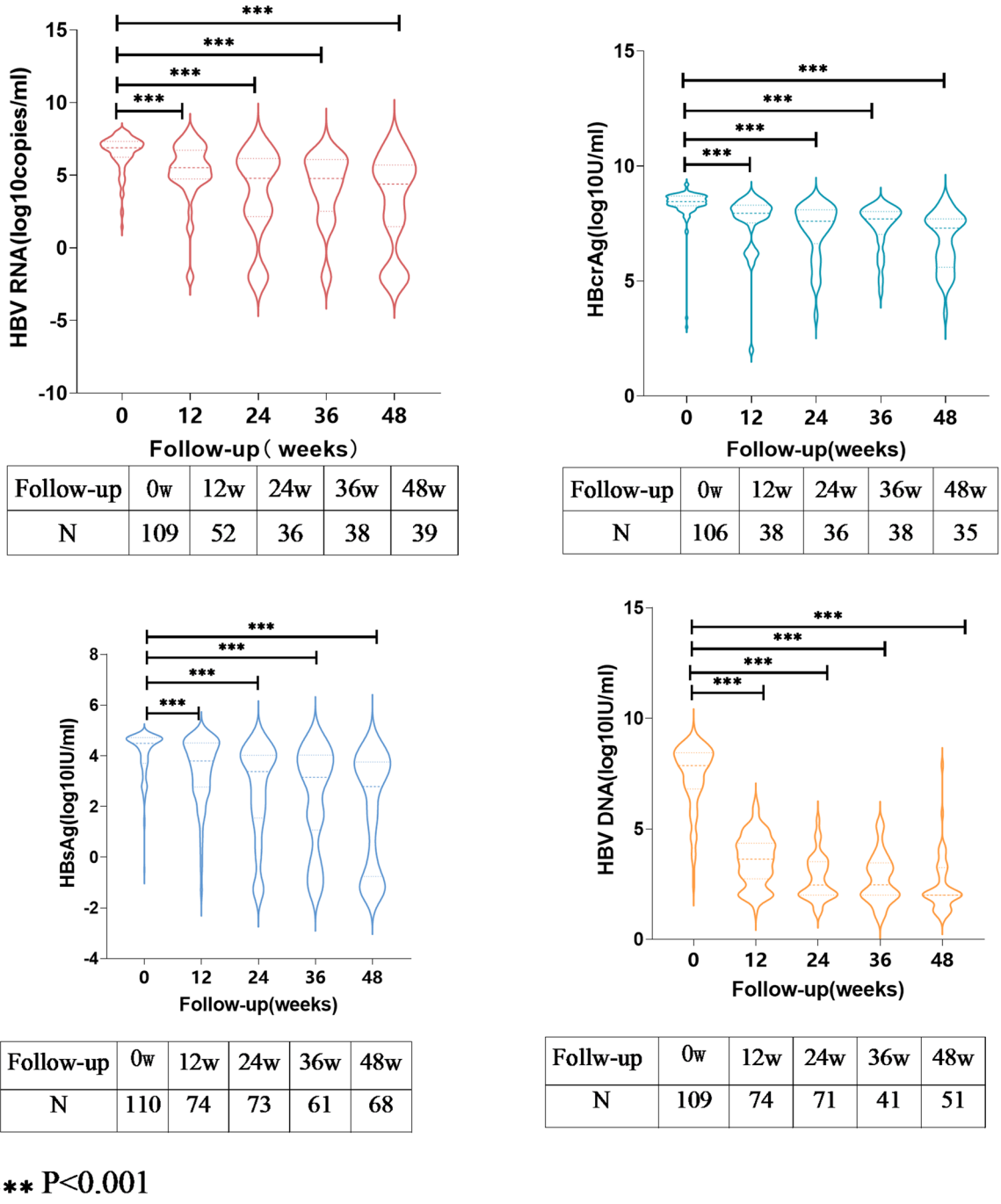
3.5 | Predictive value of serum HBV RNA and HBcrAg for HBeAg seroconversion

Baseline serum HBV RNA, HBcrAg, HBsAg, and HBV DNA exhibited limited predictive efficacy for HBeAgSC, with AUC values consistently below 0.7 and no significant differences were observed ($p > 0.05$) (Supplementary Figure 4). Notably, early treatment dynamics of serum HBV RNA and HBcrAg performed accepted predictive accuracy. Specifically, the AUC values for ΔHBV RNA at Week 36 and ΔHBcrAg at Week 12 were 0.871 ($p = 0.003$) and 0.810 ($p = 0.003$), respectively (Figure 3).

The optimal cutoff value of ΔHBV RNA at Week 36 and ΔHBcrAg at Week 12 was determined by the maximum Youden index (Supplementary Table 3). Kaplan-Meier survival curves were generated, grouped according to the determined optimal cutoff (Supplementary Figure 5). The results revealed that participants exhibiting a decline in HBV RNA exceeding 3.759 log10 copies/mL at Week 36, or a reduction in HBcrAg surpassing 0.350 log10 U/mL at Week 12, were associated with a higher likelihood of HBeAg seroconversion ($p < 0.05$).

3.6 | Accuracy and internal validation of serum HBV RNA and HBcrAg early dynamic changes in predicting HBeAg seroconversion

The calibration curve analysis demonstrated a robust consistency between the predicted and observed HBeAg SC. Brier scores for ΔHBV RNA at Week 36 and ΔHBcrAg at Week 12 were 0.101 and 0.177, respectively. Internal validation, employing 100 bootstrap resamples, showed C-index values (95% CI) of 0.842 (0.703, 1.010) for ΔHBV RNA at Week 36, and 0.761 (0.644, 0.912) for ΔHBcrAg at Week 12.



Follow-up	0w	12w	24w	36w	48w
N	109	52	36	38	39

Follow-up	0w	12w	24w	36w	48w
N	106	38	36	38	35

Follow-up	0w	12w	24w	36w	48w
N	110	74	73	61	68

Follw-up	0w	12w	24w	36w	48w
N	109	74	71	41	51

FIGURE 1 Dynamic changes of serum HBV RNA and HBcrAg with other HBV-related markers.

4 | DISCUSSION

Our study firstly revealed the predictive value of early treatment dynamics in serum HBV RNA and HBcrAg for HBeAg seroconversion in children with CHB. In current study, the cumulative incidence of HBeAg SC was 24.3%. This finding is similar with the reported rate by Gao et al. (21%).[22] During the treatment period, we observed a uniform decline trend in the serum HBV RNA, HBcrAg, HBsAg, and HBV DNA, indicating a significant correlation between the dynamic changes of HBV RNA and HBcrAg and virological and serological parameters associated with HBV infection. Additionally, our study revealed the potential of early treatment dynamics of serum HBV RNA and HBcrAg as predictive markers for HBeAg SC. From the 12th week of treatment onwards, patients who achieved HBeAg seroconversion had significantly lower levels of HBV RNA and HBcrAg compared to non-seroconversion participates. These findings are consistent with a study by Wu et al.[8] Among CHB children undergoing entecavir therapy, patients achieving HBeAg seroconversion exhibited lower HBV RNA levels starting from the 12th week of

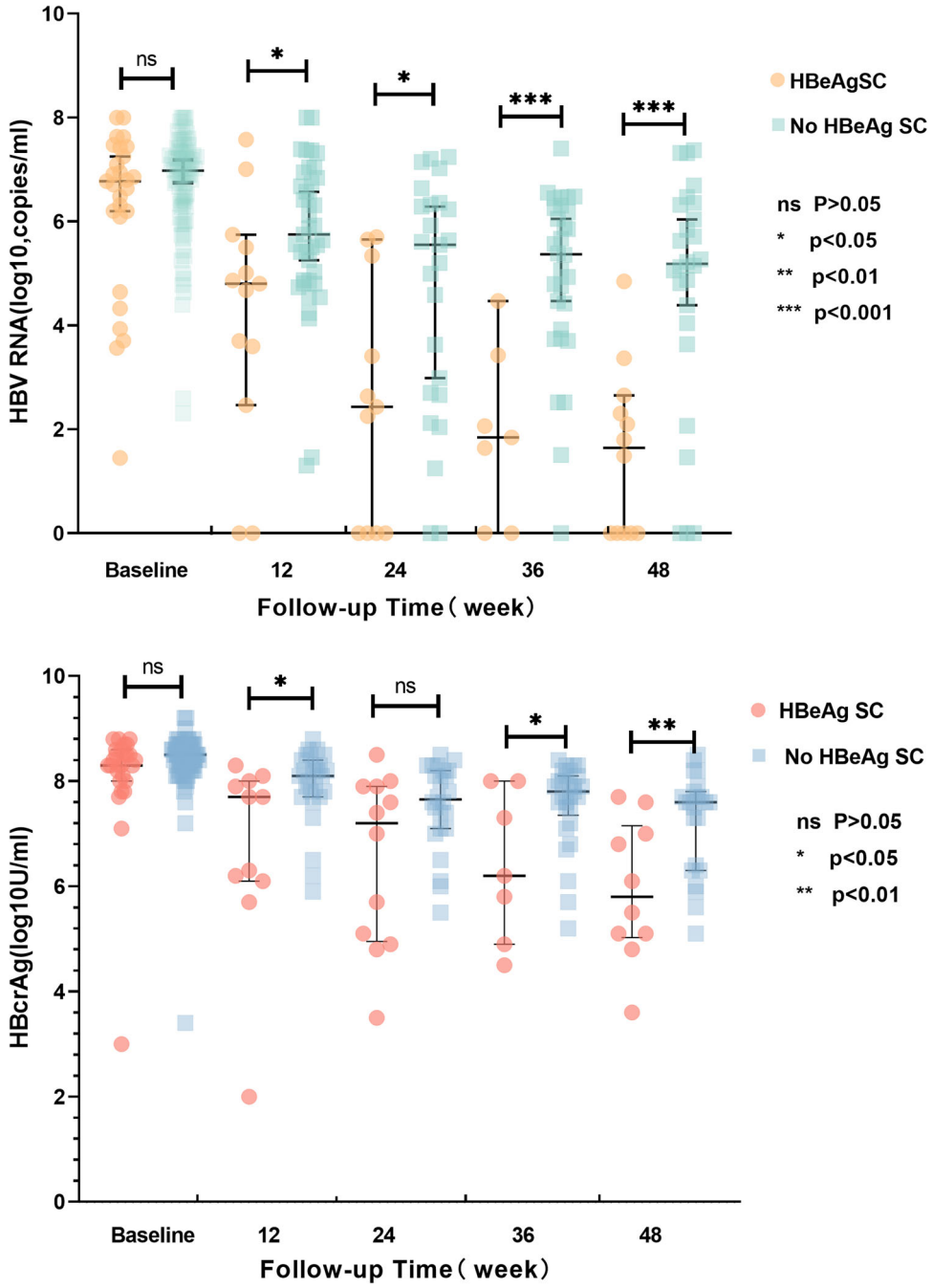


FIGURE 2 Comparison of the serum HBV RNA and HBcrAg levels between the groups based on HBeAg seroconversion.

treatment ($p < 0.001$).[8] Another study on adult CHB patients receiving pegylated interferon and adefovir combination therapy showed that responders consistently had lower average levels of HBV RNA compared to non-responders throughout the treatment period, with the significant difference becoming apparent after 30 weeks of treatment.[23] The earlier emergence of this significant discrepancy in our CHB children, as well as in the study by Wu et al.,[8] suggests that serum HBV RNA may provide an earlier predictive advantage for treatment response in CHB children compared to adults.

The observed kinetic patterns of serum HBcrAg changes in our study are consistent with those reported by Song et al.[24] They found that patients in the non-spontaneous HBeAg seroconversion group had significantly higher HBcrAg levels at Week 12 compared to those in the spontaneous HBeAg seroconversion group ($p = 0.004$). Furthermore, our study demonstrated a decline in HBV RNA and HBcrAg levels at the 12th week of treatment, with a more pronounced decline observed in children who subsequently achieved HBeAg seroconversion. Previous studies suggested that serum HBV RNA and HBcrAg are derived exclusively from cccDNA and the

TABLE 2 Association of HBV RNA, HBcrAg, HBsAg, and HBV DNA with HBeAg seroconversion.

		Unadjusted		Model 1		Model 2	
	HBV markers	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
Baseline	HBV RNA, log10 copies/mL						
		–	0.066	–	0.066	–	0.079
	HBcrAg, log10 U/mL						
		0.65 (0.48–0.89)	0.007**	0.65 (0.48–0.89)	0.007**	0.66 (0.46–0.94)	0.023*
	HBsAg, log10 IU/mL						
		–	0.050	–	0.050	0.67 (0.47–0.97)	0.034*
	HBV DNA, log10 IU/mL						
		–	0.125	–	0.125	–	0.283
12w	HBV RNA, log10copies/mL						
		0.76 (0.60–0.96)	0.024*	0.76 (0.60–0.96)	0.024*	0.71 (0.56–0.91)	0.006**
	HBcrAg, log10 U/mL						
		0.73 (0.56–0.95)	0.017*	0.73 (0.56–0.95)	0.017*	0.60 (0.43–0.84)	0.003**
	HBsAg, log10 IU/mL						
		0.63 (0.48–0.82)	0.001**	0.63 (0.48–0.82)	0.001**	0.49 (0.36–0.69)	<0.001***
	HBV DNA, log10 IU/mL						
		–	0.963	–	0.963	–	0.888

Note: Model 1: Adjusted for age and sex; Model 2: Adjusted for age, sex, BMI, estimated duration of infection, parental HBV infection, ALT, liver inflammation activity grade, liver fibrosis stage, and HBV genotype.

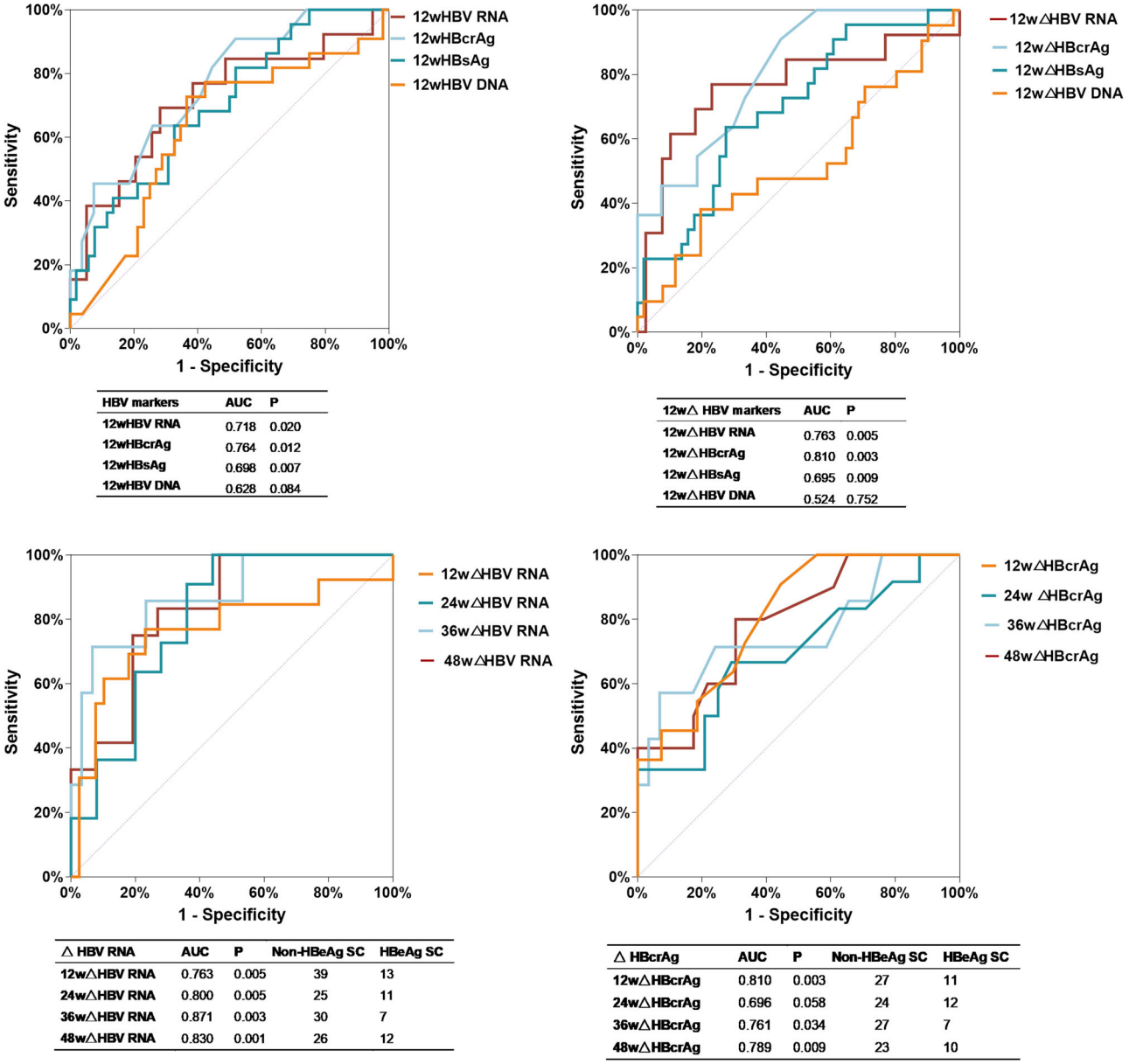
Abbreviations: HR, hazard ratio; 95% CI, 95% Confidence interval.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

dynamic decline can reflect the status of intrahepatic cccDNA during treatment. Compared to HBV DNA, serum HBV RNA and HBcrAg show superior performance in monitoring sustained viral response and even the elimination of intrahepatic cccDNA.[16,25] Therefore, it is reasonable to hypothesize that the sustained loss of serum HBV RNA and HBcrAg is associated with the elimination or transcriptional silencing of intrahepatic cccDNA.

Our study provides compelling evidence supporting the predictive role of serum HBV RNA and HBcrAg in HBeAg seroconversion. After adjusting for multiple confounding factors, HBV RNA and HBcrAg at Week 12 remained significantly associated with HBeAg seroconversion. Although the baseline serum HBV RNA (AUC = 0.565, $p = 0.312$) and HBV RNA at Week 12 (AUC = 0.718, $p = 0.020$) demonstrated only moderate predictive performance for HBeAg seroconversion, a significant improvement in predictive accuracy was observed when evaluating the HBV RNA decline from baseline to 36 weeks (AUC = 0.871, $p = 0.003$). Participants with ΔHBV RNA > 3.759 log10 copies/mL at Week 36 were more likely to achieve HBeAg seroconversion. Furthermore, the predictive performance of ΔHBV RNA at Week 36 (AUC = 0.871) surpassed that of HBV DNA (Week 12: AUC = 0.628; ΔHBV DNA at Week 12: AUC = 0.524) and HBsAg (Week 12: AUC = 0.698; ΔHBsAg at Week 12: AUC = 0.695). Possible mechanistic explanations for this observation may be related to the generation of HBsAg from cccDNA and integrated HBV DNA within the host genome, which can affect the utility of serum HBsAg in predicting HBeAg seroconversion.[26] Additionally, the rapid decline of serum HBV DNA following NAs therapy limits its predictive value for HBeAg seroconversion in the context of NAs therapy.[27] The strong association between HBV RNA and HBcrAg with HBeAg seroconversion, as compared to HBV DNA and HBsAg, can be attributed to their direct representation of cccDNA activity. In contrast, HBV DNA and HBsAg may be constrained in capturing the intricate dynamics of cccDNA during the treatment period.

Previous research has established that serum HBcrAg is a more accurate marker of intrahepatic cccDNA transcriptional activity compared to HBsAg.[28] Studies conducted by Huang et al.[29] and Teston et al.[16] have demonstrated a quantitative relationship between serum HBcrAg levels and the quantity and activity of cccDNA, highlighting its potential as a marker for monitoring disease progression. These findings support the potential value of HBcrAg as a predictive factor for treatment response. Our study further revealed that early treatment dynamics of serum HBcrAg can effectively predict HBeAg seroconversion. In current cohort of CHB children, we found that individuals withΔHBcrAg at Week 12 > 0.350 log10 U/mL



HBV markers	AUC	P
12wHBV RNA	0.718	0.020
12wHBcrAg	0.764	0.012
12wHBsAg	0.698	0.007
12wHBV DNA	0.628	0.084

12w△ HBV markers	AUC	P
12w△HBV RNA	0.763	0.005
12w△HBcrAg	0.810	0.003
12w△HBsAg	0.695	0.009
12w△HBV DNA	0.524	0.752

△ HBV RNA	AUC	P	Non-HBeAg SC	HBeAg SC
12w△HBV RNA	0.763	0.005	39	13
24w△HBV RNA	0.800	0.005	25	11
36w△HBV RNA	0.871	0.003	30	7
48w△HBV RNA	0.830	0.001	26	12

△ HBcrAg	AUC	P	Non-HBeAg SC	HBeAg SC
12w△HBcrAg	0.810	0.003	27	11
24w△HBcrAg	0.696	0.058	24	12
36w△HBcrAg	0.761	0.034	27	7
48w△HBcrAg	0.789	0.009	23	10

FIGURE 3 ROC curve analysis of early dynamic changes in serum HBV RNA, HBcrAg, HBsAg, and HBV DNA for Predicting HBeAg Seroconversion.

(AUC = 0.810, $p = 0.003$) have a distinct advantage in achieving HBeAg seroconversion. The superiority of HBV RNA and HBcrAg over HBV DNA and HBsAg in predicting HBeAg seroconversion stems from their direct reflection of cccDNA activity, dynamic changes during treatment, and clinical accessibility through noninvasive serum testing. Assessing early treatment dynamics of serum HBV RNA and HBcrAg holds promise for optimizing personalized therapy in CHB children and improving treatment outcomes.

Our study results underscore the predictive potential of early treatment dynamics of serum HBV RNA and HBcrAg for HBeAg seroconversion, providing valuable insights for optimizing individualized treatment strategies. Employing a prospective design involving treatment-naïve HBeAg-positive CHB children, thereby minimize the influence of confounding factors and previous treatment exposure. Nonetheless, certain limitations warrant acknowledgment. Firstly, the influence of mutations in the precore region of HBV and the basal core promoter on HBeAg seroconversion were not specifically controlled in the current study. However, we did adjust for HBV DNA associated with these factors in the multivariate analysis. Secondly, the study population consisted exclusively of HBeAg-positive CHB children from China with genotypes B or C, warranting caution when extrapolating the results to patients of different ethnicities or genotypes. Future studies should involve larger-scale multicenter investigations to further validate these findings.

In conclusion, our study confirmed the independent associations of serum HBV RNA and HBcrAg levels at Week 12 with HBeAg seroconversion in children with HBeAg-positive CHB. Early changes in serum HBV RNA at Week 36 and HBcrAg at Week 12 show strong predictive accuracy for HBeAg seroconversion. Monitoring these markers could inform clinical decision-making and optimize treatment strategies for pediatric CHB patients, contributing valuable insights into CHB management in children.

AUTHOR CONTRIBUTIONS

All authors made significant contributions to this work. Songxu Peng, Wenxian OuYang, Shuangjie Li, and Jun Qiu established the research site. Xinlai and Wenxian OuYang implemented the study, including participant enrollment, data collection, statistical analysis, and drafting the initial manuscript. Hui Zhang, Tao Jiang, Xiaomei Qin, Lian Tang, Yingping Gu, and Zhenzhen Yao assisted in participant enrollment and data collection. Songxu Peng reviewed the manuscript. All listed authors have reviewed and approved the content of the submitted manuscript.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available due to the research still being carried on but are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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