Wu *et al. Virology Journal* (2025) 22:132
https://doi.org/10.1186/s12985-025-02712-y

Virology Journal

RESEARCH Open Access

Serum hepatitis B virus RNA in low-level viremia of chronic hepatitis B: clinical features and association with virological response

Yongbin Wu[1*], Guifang Tang[2], Jian Wen[3], Ying Wan[1], Yufei Wang[4] and Ling Li[5]

Abstract

Background The role of hepatitis B virus (HBV) RNA in the management of patients with chronic hepatitis B (CHB) experienced with low-level viremia (LLV) remains poorly defined. This study was designed to evaluate the prognostic utility of serum HBV RNA as a biomarker for predicting treatment outcomes in this population.

Methods A retrospective cohort analysis was conducted on 117 pediatric patients with LLV (mean age: 13.14 years; 34% female) treated with continuous entecavir (ConT) or modified regimens (switching to or combining with tenofovir disoproxil fumarate) for ≥ 120 weeks. Virological response was defined as HBV DNA < 10 IU/mL at week 120.

Results No significant baseline differences existed between ConT and modified regimen groups. Compared to ConT, modified regimens achieved greater reductions in serum HBV DNA, HBV RNA, and quantitative HBsAg, with higher cumulative undetectable rates at week 120 (HBV DNA: ≥ 80.0%; HBV RNA: ≥ 54.8%; $P < 0.05$). Notably, qHBsAg levels remained elevated in most patients, with only 3 individuals achieving undetectable levels (< 0.05 IU/mL). Multivariate analysis identified higher HBV RNA levels at week 48 as an independent risk factor for non-virological response (adjusted odds ratio: 5.86; 95% confidence interval: 1.40-24.62; $P = 0.016$). Although HBV RNA alone was less predictive than HBV DNA (area under the receiver operating characteristic curve [AUC]: 0.76 vs. 0.80; $P = 0.459$), combining both markers improved prediction accuracy (AUC: 0.82; $P < 0.05$ vs. single markers).

Conclusions In children with LLV, serum HBV RNA level is an independent risk factor for non-virological response and may serve as a complementary biomarker to HBV DNA for guiding antiviral therapy adjustments.

Keywords Low-level viremia, Serum HBV RNA, Chronic hepatitis B, Potential biomarker

*Correspondence:
Yongbin Wu
yongbinwu@163.com
[1]Department of Laboratory Medicine, Nanxishan Hospital of Guangxi Zhuang Autonomous Region, Guilin, China
[2]Department of Infectious Diseases, Nanxishan Hospital of Guangxi Zhuang Autonomous Region, Guilin, China
[3]Department of Hematology, Nanxishan Hospital of Guangxi Zhuang Autonomous Region, Guilin, China
[4]Department of Clinical Diagnosis, Laboratory of Beijing Tiantan Hospital, Capital Medical University, Beijing, China
[5]School of Basic Medical Science, Southern Medical University, Guangzhou, China

Introduction

Low-level viremia (LLV) represents a special case of suboptimal virological response (VR) in clinical practice, which is closely associated with poor prognosis and may lead to disease progression and terminal events [1, 2]. Globally, oral nucleos(t)ide analogs (NUCs) - such as entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide fumarate (TAF) - remain first-line antiviral therapies for chronic hepatitis B (CHB). However, persistent or intermittent LLV occurs in over 20% of patients despite continuous NUC therapy [3], raising concerns about its clinical significance. Current evidence is insufficient to conclusively determine the impact of LLV on treatment efficacy and long-term prognosis, and clinical guidelines lack consensus on its management [4]. To address this gap, identifying reliable biomarkers to evaluate LLV-associated risks and therapeutic outcomes is critical for guiding timely clinical interventions.

The ineffectual clearance or silencing of covalently closed circular DNA (cccDNA) may be associated with the development of LLV in CHB patients [5]. Hepatitis B virus (HBV) pregenomic RNA (pgRNA) is derived from cccDNA within the nuclei of infected hepatocytes, and its biosynthesis is not suppressed by NUCs, providing a true reflection of intra-patient cccDNA levels and transcriptional activity. Despite the clinical promise of serum HBV RNA quantification, evidence supporting its utility in LLV management remains limited, particularly in pediatric populations. For patients receiving NUC therapy, the degree of on-treatment viral suppression is a critical determinant of clinical outcomes. In this study, we employed non-virological response (NVR) as the primary endpoint and conducted a retrospective cohort analysis of pediatric CHB patients with LLV treated with NUCs. Longitudinal measurements of serum HBV RNA, and other markers were analyzed to (1) assess the prognostic implications of LLV in children, (2) identify risk factors for NVR, and (3) evaluate HBV RNA as a complementary biomarker to optimize therapeutic strategies.

Methods

Study design, setting, and patients

The study cohort consisted of real-world outpatients who received ETV as initial treatment between February 2009 and September 2021 at Nanxishan Hospital of Guangxi Zhuang Autonomous Region (Guilin, China). Participants were divided into two sequential cohorts: (1) treatment-naïve patients initially enrolled ($n = 559$) who received ETV monotherapy for 48 weeks, and (2) patients diagnosed with LLV at week 48 ($n = 142$), who were subsequently treated with either continued ETV (ConT), switched to TDF (SwiT), or combined ETV and TDF (ComT) until week 120 based on clinical decision and patient preference ($n = 117$). The inclusion criteria for the first cohort were as follows: (1) age < 18 years; (2) diagnosis of CHB under the guidelines [6] with hepatitis B surface antigen (HBsAg) present for ≥ 6 months; (3) serum alanine aminotransferase (ALT) > upper limit of normal (30 U/L) in 4 separate measurements taken 3 months apart; (4) baseline HBV DNA ≥ 2,000 IU/mL; and (5) treatment naïve and started on ETV monotherapy. Exclusion criteria for the first cohort were as follows: (1) co-infection with other hepatitis viruses or human immunodeficiency virus; (2) the presence of liver diseases from other etiologies (e.g., autoimmune liver disease, drug-induced liver injury); (3) incomplete related data; (4) poor clinical adherence; (5) treatment with other antiviral medications; (6) HBV DNA levels ≥ 2,000 IU/mL after 48 weeks of ETV treatment; and (7) achieving a VR in the first 48 weeks. After applying exclusion criteria, patients with LLV at week 48 were eligible for the second cohort, with additional exclusion criteria including poor clinical adherence (e.g., loss to follow-up or spontaneous withdrawal). These individuals were classified as missing data during screening and excluded prior to cohort assignment, as detailed in the flowchart (Fig. 1).

LLV was defined as persistent or intermittent detectable HBV DNA levels (≥ 10 IU/mL but < 2,000 IU/mL), with first confirmation at week 48 in this study. VR was defined as undetectable HBV DNA (< 10 IU/mL) at week 120, while NVR was defined as detectable HBV DNA (≥ 10 IU/mL) at the same endpoint. Antiviral therapy was maintained until week 120, with routine laboratory assessments (including serum HBV RNA quantification) conducted at 3–6 month intervals. The primary outcome focused on NVR occurring at week 120 after the initial detection of LLV. This retrospective analysis of de-identified clinical data and archived samples was conducted under ethical approval (2020NXSYYEC-003) with a waiver of informed consent, in accordance with the Declaration of Helsinki guidelines for minimal-risk research.

Laboratory assessment

Baseline demographic and clinical characteristics, including sex, age, body mass index (BMI), historical use of NUCs, family history of hepatitis B, and serum biomarkers including ALT, hepatitis B e antigen (HBeAg), HBsAg, HBV DNA, and HBV RNA, were systematically documented at study enrollment. Serum HBV DNA levels were quantified via a TaqMan HBV diagnostic kit (Sansure Bio, Changsha, China), with 10 IU/mL as the lower limit of detection (LLOD). Serum HBV RNA levels were measured via an RNA capture probe assay kit (Rendu Bio, Shanghai, China), with 50 copies/mL as the LLOD. Detailed procedures have been described previously [7]. Serum quantitative HBsAg (qHBsAg) was measured via an Elecsys HBsAg II assay kit (LLOD: 0.05 IU/mL; Roche, USA). Serum HBeAg was measured via

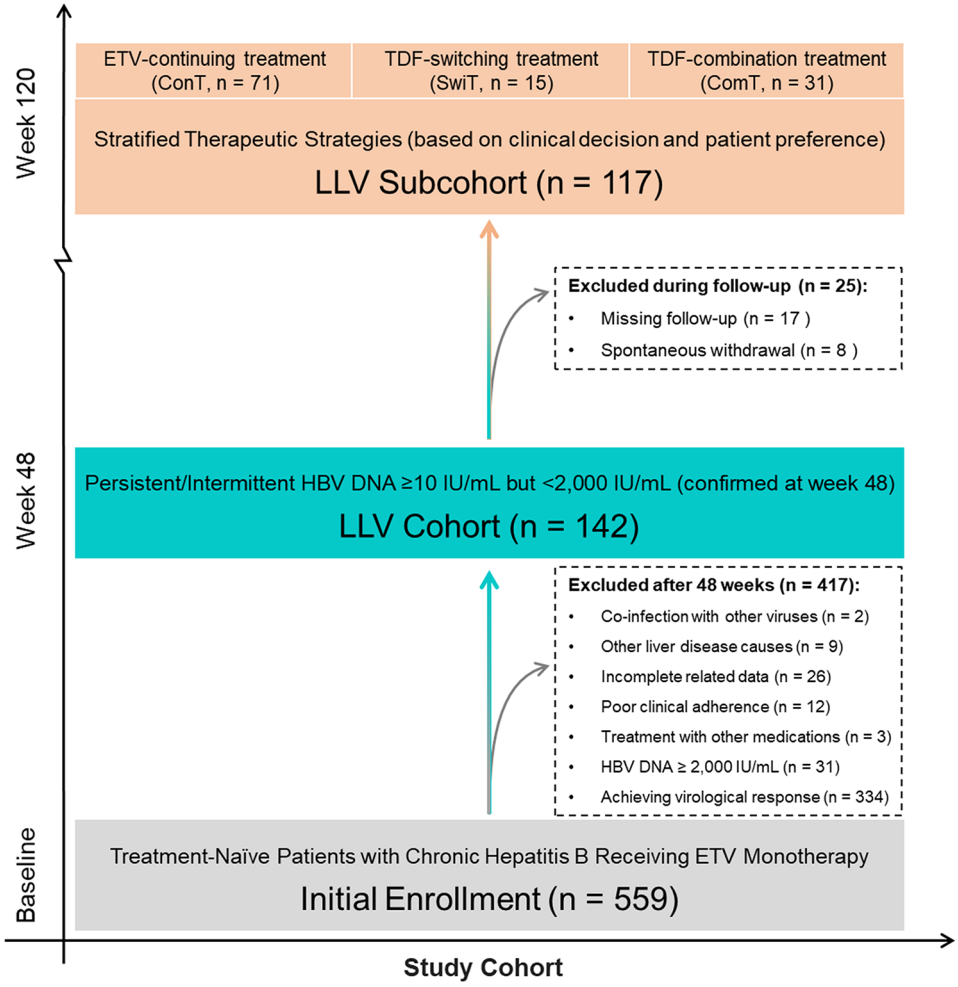

Fig. 1 Schematic diagram of the cohort study on pediatric patients with low-level viremia

a chemiluminescent immunoassay (Mindray Medical, Shenzhen, China). Residual serum samples were stored at −80°C for subsequent supplementary assays. The values derived from these assays in scientific notation were log-transformed prior to statistical analysis, and if the values were below the detection limits of the assays, thresholds were substituted.

Statistical analysis

Descriptive statistics for continuous variables are expressed as the means ± standard deviations or medians plus interquartile ranges (IQRs), while categorical variables are expressed as numbers and percentages. Correlations were compared using Mann-Whitney U, Chi-Squared, Pearson's, or other non-parametric tests as required. Generalized estimating equations were employed to analyze longitudinal repeated-measures data, and Kaplan-Meier curves were used to plot differences in the cumulative incidence of undetectable HBV DNA and RNA between cohorts and to compare them with the log-rank test. Multivariate logistic regression was performed using variables with $P<0.05$ in the univariate analysis to evaluate factors associated with VR at 120 weeks, and the predictive power of HBV DNA and RNA was assessed by receiver operating characteristic (ROC) curves. Statistical significance was defined as $P<0.05$. SPSS version 26.0 (IBM Corporation, Armonk, USA) and Origin Pro 2023 (OriginLab Corporation, Northampton, USA) were used for statistical analysis and graphical display.

Results

Baseline characteristics and virological outcomes by treatment regimens

The baseline characteristics of the eligible children with CHB are detailed in Table 1. The differences between SwiT, ComT, and ConT at baseline levels of serum HBV DNA, HBV RNA, and qHBsAg were not statistically significant ($P>0.05$). At week 48, the serum HBV RNA levels in the SwiT and ComT groups were higher than those in the ConT group (3.95 vs. 3.54 $\log_{10}$ IU/mL, $P=0.008$; 3.79 vs. 3.54 $\log_{10}$ IU/mL, $P=0.08$), and essentially similar to

Table 1 Clinical characteristics of patients with CHB at baseline and on-treatment emergent low-level viremia

Characteristics	All patients (*n* = 117)	ConT[a] (*n* = 71)	SwiT[b] (*n* = 15)	*P* value (a vs. b)	ComT[c] (*n* = 31)	*P* value (a vs. c)	VR[†] (*n* = 74)	NVR[†] (*n* = 43)	*P* value
Age, years	13.14 ± 2.70	12.92 ± 2.74	13.61 ± 2.54	0.37	13.39 ± 2.71	0.42	12.98 ± 2.84	13.40 ± 2.44	0.38
Sex, male/female	77/40	45/26	10/5	0.83	22/9	0.44	49/25	28/15	0.90
BMI, kg/m ^{2}	18.53 ± 3.71	18.25 ± 3.73	19.76 ± 3.18	0.15	18.58 ± 3.88	0.69	19.12 ± 3.83	17.53 ± 3.29	0.025
HBV genotype, B/C/other[‡]	41/74/2	25/45/1	5/10/0	0.93	11/19/1	0.91	34/38/2	7/36/0	0.002
ALT, U/L	100(82–121)	98(84–120)	92(67–108)	0.57	109(83–125)	0.28	102(82–122)	95(84–121)	0.74
HBeAg, positive/negative	98/19	60/11	12/3	0.67	26/5	0.93	58/16	40/3	0.038
Baseline									
qHBsAg, $\log_{10}$ IU/mL	4.27(4.09–4.51)	4.25(4.07–4.43)	4.27(4.02–4.46)	0.65	4.39(4.10–4.66)	0.19	4.26(4.07–4.43)	4.27(4.02–4.46)	0.62
HBV DNA, $\log_{10}$ IU/mL	7.48(6.89–7.76)	7.39(6.88–7.66)	7.52(6.75–7.60)	0.63	7.69(6.93–7.94)	0.12	7.39(6.88–7.66)	7.52(6.75–7.60)	0.007
HBV RNA, $\log_{10}$ copies/mL	6.55(6.20–6.86)	6.56(6.21–6.89)	6.54(6.20–6.82)	0.65	6.55(6.14–6.87)	0.84	6.54(6.21–6.89)	6.56(6.20–6.82)	0.06
Week 48									
qHBsAg, $\log_{10}$ IU/mL	3.90(3.51–4.12)	3.89(3.52–4.04)	3.86(3.73–4.02)	0.71	3.99(3.45–4.28)	0.43	3.88(3.46–4.08)	3.90(3.67–4.19)	0.22
HBV DNA, $\log_{10}$ IU/mL	2.61(2.13–2.95)	2.51(2.02–2.87)	2.78(2.48–2.96)	0.08	2.77(2.23-3.00)	0.038	2.23(2.01–2.73)	2.89(2.62–3.09)	< 0.001
HBV RNA, $\log_{10}$ copies/mL	3.77(3.04–4.03)	3.54(2.95–3.97)	3.95(3.75–4.16)	0.008	3.79(3.34–4.30)	0.08	3.48(2.98–3.90)	4.06(3.54–4.33)	< 0.001

[†] VR, virological response: HBV DNA < 10 IU/mL at week 120

[†] NVR, non-virological response: HBV DNA ≥ 10 IU/mL at week 120

[‡] Unknown or undetected types

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; ComT, TDF-combination treatment; ConT, ETV-continuing treatment; ETV, entecavir; HBeAg, hepatitis B e antigen; qHBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; SwiT, TDF-switching treatment; TDF, tenofovir disoproxil fumarate

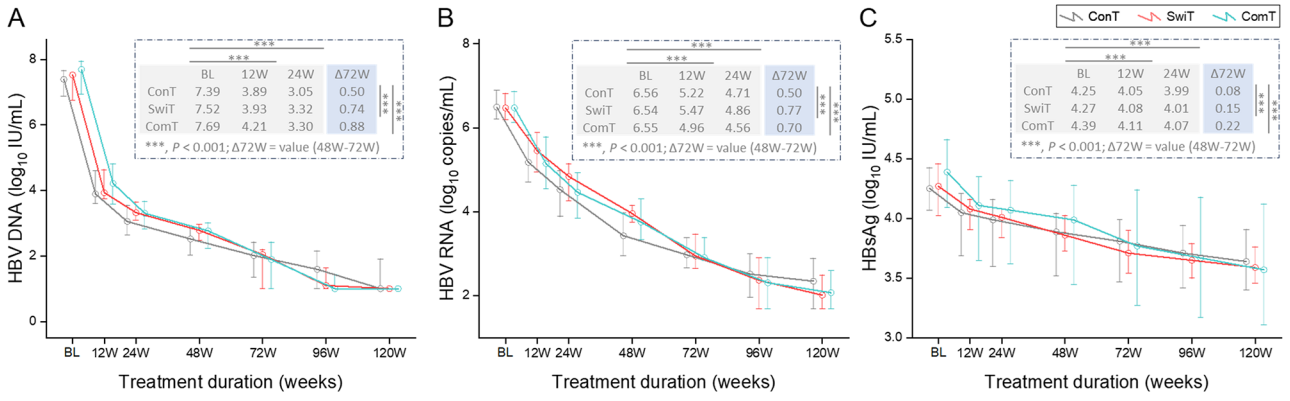

Fig. 2 Longitudinal trends in serum biomarker levels during antiviral therapy. (**A**) HBV DNA, (**B**) HBV RNA, and (**C**) HBsAg. Line plots represent median values at each time point, with interquartile ranges (25th-75th percentiles) indicated by error bars

this episode, the serum HBV DNA levels in the SwiT and ComT groups were higher than those in the ConT group, but not serum qHBsAg levels (Table 1). At week 120 of follow-up, 36.8% of the patients had still not achieved a VR. Those who developed NVR presented, at baseline, a lower BMI, predominantly genotype C, HBeAg positivity, and higher HBV DNA levels. At 48 weeks, these patients still presented higher HBV DNA and HBV RNA levels, with median values of 2.89 $\log_{10}$ IU/mL and 4.06 $\log_{10}$ copies/mL, respectively (Table 1).

Longitudinal trends of serum biomarkers during initial entecavir therapy

Figure 2 depicts longitudinal trends in serum biomarkers (HBV DNA, HBV RNA, and qHBsAg) from baseline to week 48 of antiviral therapy, prior to the occurrence of LLV. All three markers exhibited biphasic kinetics: a rapid initial decline during the first 12 weeks of treatment, followed by a slower reduction thereafter. The most pronounced decreases occurred within the first three months. At weeks 12 and 24, the median serum HBV

RNA levels in the ConT group decreased by 1.32 and 1.83 $\log_{10}$ copies/mL from baseline ($P<0.001$), respectively. Similarly, the median serum qHBsAg levels in ConT declined by 0.20 and 0.26 $\log_{10}$ IU/mL ($P<0.001$), while HBV DNA levels showed more pronounced reductions of 3.50 and 4.34 $\log_{10}$ IU/mL ($P<0.001$) at the same time points. Comparable trends were observed in the modified regimen groups (SwiT and ComT), with HBV RNA reductions ranging from 1.09 to 1.60 $\log_{10}$ copies/mL at week 12 and 1.70–1.99 $\log_{10}$ copies/mL at week 24. Notably, HBV DNA suppression was consistently greater than that of HBV RNA and qHBsAg within each cohort ($P<0.001$ for intragroup comparisons).

Impact of treatment adjustment on biomarker kinetics

Following treatment adjustment at week 48, the SwiT and ComT groups exhibited renewed declines in HBV DNA, HBV RNA, and qHBsAg levels, breaking the prior plateau observed during continuous ETV therapy (ConT group), as detailed in Fig. 2. Longitudinal analysis revealed significantly steeper reductions in these biomarkers for SwiT and ComT compared to ConT ($P<0.001$ for both intergroup comparisons). By week 72, the SwiT group achieved additional median reductions of 0.74 $\log_{10}$ IU/mL (HBV DNA), 0.77 $\log_{10}$ copies/mL (HBV RNA), and 0.15 $\log_{10}$ IU/mL (qHBsAg) from week 48 baseline values. Similarly, the ComT group showed reductions of 0.88, 0.70, and 0.22 $\log_{10}$ for the respective biomarkers. In contrast, the ConT group demonstrated minimal declines (0.50, 0.50, and 0.08 $\log_{10}$), underscoring the superiority of regimen modification.

Superior viral suppression with modified treatment regimens

The cumulative rates of undetectable serum HBV DNA (< 10 IU/mL) and HBV RNA (< 50 copies/mL) increased progressively across all treatment groups (ConT, SwiT, ComT) during the study period (Fig. 3). After regimen adjustment (switching to or combining with TDF), the SwiT and ComT groups demonstrated significantly higher virological suppression rates compared to the ConT group (HBV DNA: $P<0.05$; HBV RNA: $P<0.05$, Fig. 3). By week 120, undetectable HBV DNA rates reached 80.0% in SwiT and 83.9% in ComT, which were 1.6-fold higher than the ConT group (50.7%). Similarly, undetectable HBV RNA rates were 60.0% (SwiT) and 54.8% (ComT), compared to 32.5% in the ConT group. In contrast, qHBsAg levels remained elevated in most patients, with only 3 individuals achieving undetectable qHBsAg (< 0.05 IU/mL) across the entire cohort (Fig. 2).

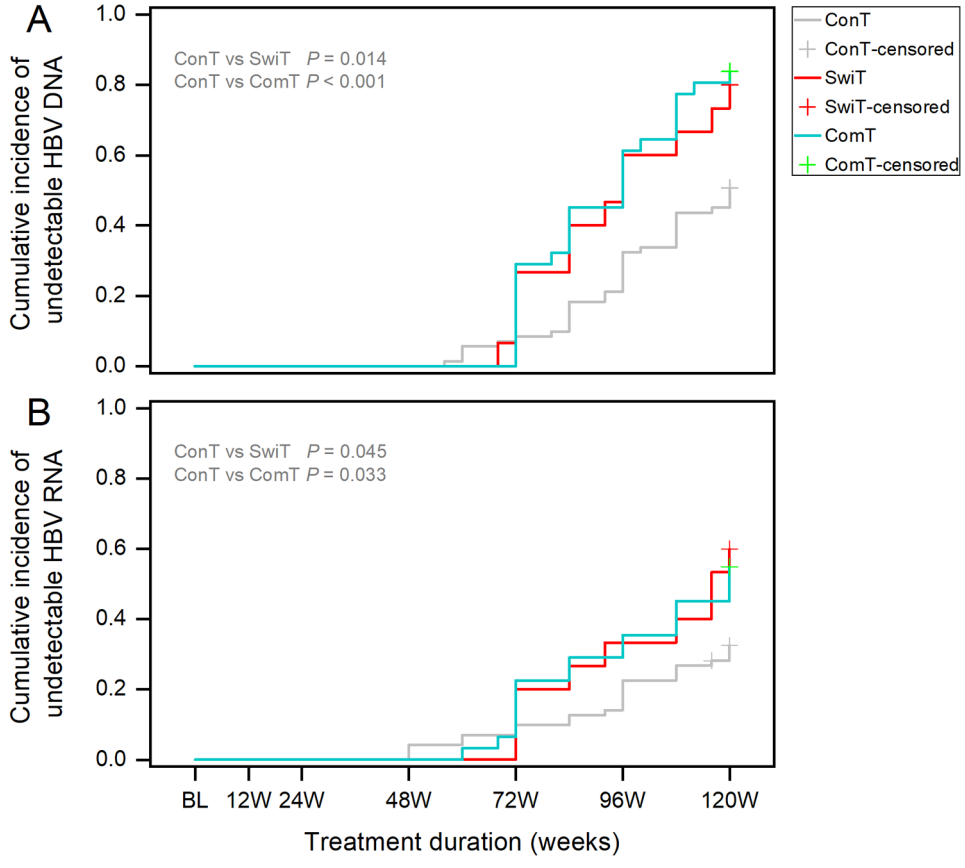

Fig. 3 Cumulative incidence of undetectable HBV DNA (**A**) and HBV RNA (**B**) from week 48 onward

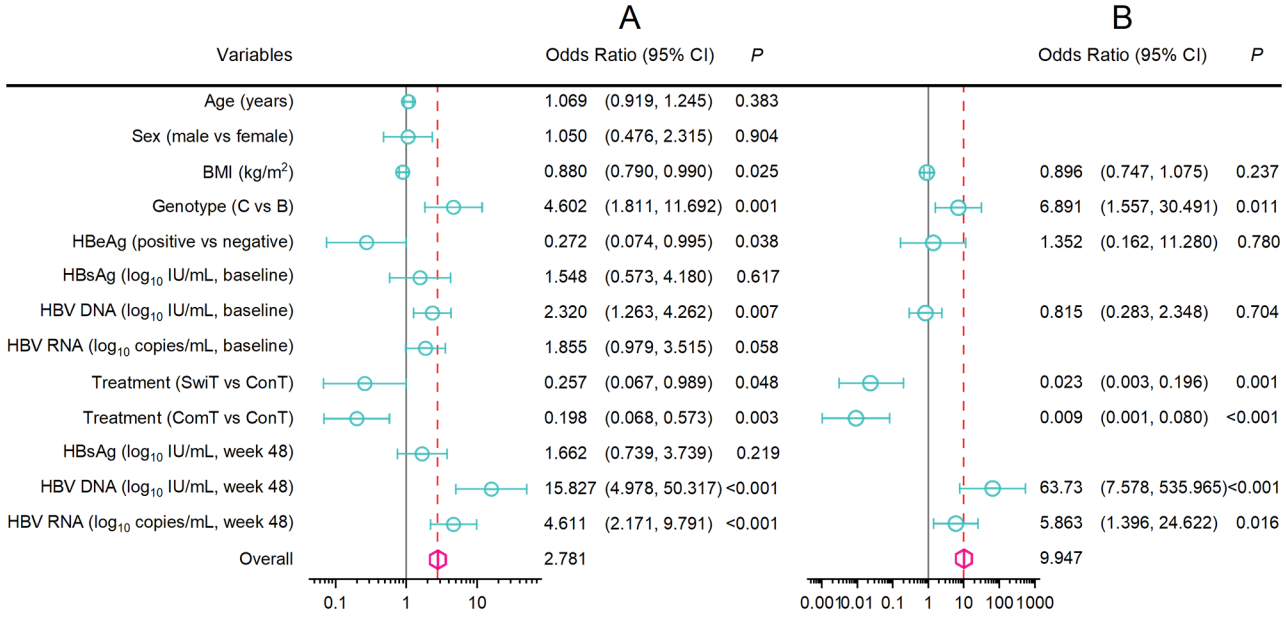

Fig. 4 Risk of non-virological response at week 120 according to different treatment regimens for low-level viremia. (**A**) Univariate analysis; (**B**) Multivariate analysis

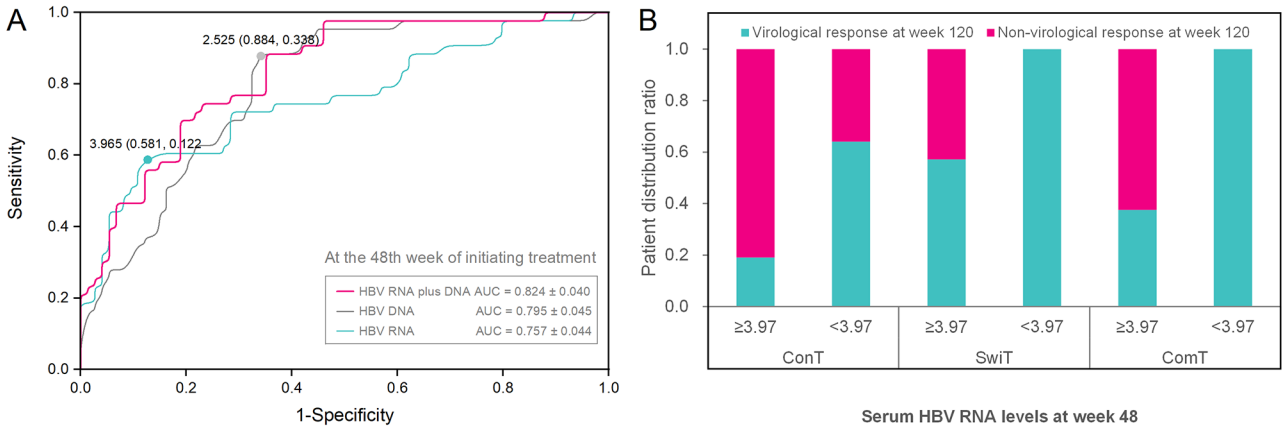

Fig. 5 Diagnostic value of serum HBV RNA in patients with low-level viremia. (**A**) Receiver operating characteristic curves for biomarkers at week 48 predicting virological response at week 120 in patients with low-level viraemia. (**B**) Distribution of virological responses at week 120 to the initial and modified treatment regimens according to the cut-off value of the HBV RNA level at week 48

HBV RNA as a risk factor for non-virological response and ROC analysis

To identify risk factors of NVR at week 120, we first performed univariate analyses of baseline and on-treatment variables. In univariate analysis, BMI, genotype, treatment regimen, HBeAg status, baseline serum HBV DNA levels, and week 48 serum HBV DNA/RNA levels demonstrated associations with virological suppression at week 120 in NUC-treated children with LLV (Fig. 4A). Variables with $P<0.05$ were subsequently included in multivariate regression analysis. The results demonstrated that genotype C (adjusted odds ratio [aOR]: 6.89; 95% confidence interval [CI]: 1.56–30.49; $P=0.011$), continuous ETV therapy, and higher week 48 serum HBV DNA (aOR: 63.73; 95% CI: 7.58-535.97; $P<0.001$) and HBV RNA levels (aOR: 5.86; 95% CI: 1.40-24.62; $P=0.016$) were independent risk factors for NVR at week 120 (Fig. 4B).

To assess the predictive value of biomarkers, ROC curves were analyzed. At week 48, HBV DNA and HBV RNA showed area under the curve (AUC) values of 0.80 (sensitivity: 0.88, specificity: 0.66) and 0.76 (sensitivity: 0.58, specificity: 0.88), respectively, though the difference between them was not statistically significant ($P=0.459$; Fig. 5A). However, combining HBV DNA and RNA significantly improved predictive accuracy (AUC: 0.82; sensitivity: 0.88, specificity: 0.65; $P<0.05$ vs. single markers; Fig. 5A). Stratification by the optimal HBV RNA cut-off

(3.97 $\log_{10}$ copies/mL at week 48) revealed that patients with RNA levels below this threshold achieved higher virological suppression rates at week 120 across all treatment strategies (continuation, switch, or add-on strategies) compared to those above the threshold ($P < 0.05$; Fig. 5B).

Discussion

Whether it is necessary to initiate treatment or adjust the initial treatment strategy for children with LLV remains a point of controversy in current guidelines and expert opinions [8–11]. In the real world, the majority of pediatric patients are not subjected to antiviral treatment, primarily due to the limited efficacy of current therapies in eradicating HBV infection. A recent editorial questioned the rationale for prolonging antiviral therapy in all patients with CHB, particularly in cases where long-term treatment benefits remain uncertain [12]. However, untreated patients with compensated cirrhosis and LLV should also be closely monitored and selectively targeted [13, 14]. By acknowledging the above facts, and from the perspective of assisting in clinical decision-making, this study focuses more on evaluating potential biomarkers for pediatric patients who have chosen treatment, emphasizing the complexity of managing LLV in pediatric patients receiving NUC therapy.

The actual incidence of LLV may be grossly underestimated. As quantitative testing techniques continue to improve, the lower limit of detection of viral load continues to decrease, leading to an invisible increase in the detection rate of LLV. In our study, the detection rate of LLV at week 48 was as high as 25.4% in patients receiving standard ETV therapy (Fig. 1), a result that is generally consistent with previous studies [5, 15]. HBV DNA quantification remains the reference method for assessing the level of viral replication in HBV-infected patients and is an important indicator for the selection of indications for antiviral therapy and the judgment of its efficacy. For CHB patients receiving antiviral therapy, lower HBV DNA levels are associated with a greater likelihood of early VR and HBeAg seroconversion [16, 17]. Therefore, in clinical practice, serum HBV DNA levels in patients with CHB should be dynamically monitored for timely recognition of LLV, when economically and conditionally feasible.

In recent years, studies on LLV therapy have focused on the adult population and have transitioned from evaluating the therapeutic effects of traditional antiviral drugs such as ETV or TDF to newer agents such as TAF [18]. A multicenter study suggested that patients with LLV who switched to TAF treatment after being treated with ETV, TDF, or a combination of NUCs until week 144 achieved complete virological suppression in ≥ 80% of cases, regardless of their baseline HBV DNA levels [19]. Similarly, a study from China indicated that switching from ETV to TAF is beneficial for achieving HBV DNA levels < 20 IU/ml by week 24 in patients with LLV, regardless of sex, age, family history of CHB, HBV DNA, and liver cirrhosis status [20]. As a complement to these studies, our study filled a long-standing gap in assessment of the efficacy of NUCs on LLV in pediatric population. Specifically, children who developed LLV by week 48 of ETV monotherapy demonstrated significantly higher rates of virological suppression at week 120 when switching to TDF or adding TDF to their regimen, compared to those continuing EVT alone. Nevertheless, current data are limited in exploring which strategy is the better approach and the optimal time point for considering a change in therapy; decisions regarding whether to continue, switch, or add another NUC should be based on available evidence, precise follow-up, and a thorough assessment of risks and benefits.

Emerging evidence highlights the critical role of intrahepatic HBV persistence in driving the progression of liver pathology [21, 22]. In the present study, we extensively profiled the dynamic characteristics of HBV RNA, a novel noninvasive HBV biomarker, in patients with LLV and explored its application in monitoring the effectiveness of antiviral therapy. Longitudinal analysis revealed a more pronounced decline in HBV DNA levels compared to HBV RNA and qHBsAg during antiviral therapy. This observation underscores the unique value of HBV RNA as a surrogate marker of intrahepatic cccDNA transcriptional activity, as residual HBV RNA may reflect ongoing viral replication despite suppressed HBV DNA levels. Notably, serum HBV RNA levels remained significantly higher in non-virological responders than in responders, aligning with recent evidence [23]. In contrast, qHBsAg levels exhibited limited correlation with therapeutic outcomes. This discrepancy may be related to the characteristics of our study cohort and the fact that qHBsAg production depends not only on cccDNA activity but also on integrated HBV DNA fragments, which are unaffected by NUCs [24]. Our study provides the first evidence validating HBV RNA as a biomarker for monitoring NUC efficacy in pediatric LLV populations and highlight the need for complementary markers beyond qHBsAg to assess intrahepatic viral activity.

Expanding on the clinical implications of HBV RNA, emerging studies suggest its potential applications in predicting virological relapse after treatment interruption, evaluating qHBsAg seroconversion, and assessing the efficacy of novel antiviral agents [25–27]. Our previous study suggested that on-treatment HBV RNA dynamic predicted ETV-induced HBeAg seroconversion in children with CHB [28]. However, evidence on HBV RNA in LLV populations remains sparse. In this study, after adjustment for baseline and on-treatment

variables—including BMI, genotype, treatment regimen, HBeAg status, baseline serum HBV DNA levels and week 48 serum HBV DNA/RNA levels—lower serum HBV RNA levels at week 48 were associated with a more favorable virologic response, irrespective of age and sex at baseline. Furthermore, HBV RNA emerged as an independent predictor of NVR at week 120. While HBV RNA alone showed suboptimal predictive performance compared to HBV DNA, combining both markers significantly improved accuracy. These results underscore the utility of HBV RNA as a complementary biomarker to refine therapeutic strategies in LLV management.

This study has several limitations inherent to its retrospective design. First, potential selection bias may arise from the non-randomized assignment of treatment regimens (ConT, SwiT, or ComT) at week 48, as clinical decisions and patient preferences influenced therapeutic choices. This non-randomized allocation could also explain the heterogeneous distribution of baseline characteristics across subgroups. Although we attempted to analyze the risk of NVR separately for ConT and modified regimens (SwiT/ComT), the limited sample size restricts subgroup analyses. Second, the single-center cohort predominantly comprised Han Chinese children, which may limit the generalizability of findings to other ethnicities or regions with differing HBV genotypes and healthcare practices. Third, the 120-week follow-up duration, while sufficient to assess short-term virological outcomes, precludes evaluation of long-term clinical endpoints such as cirrhosis or hepatocellular carcinoma. Future multicenter studies with larger, ethnically diverse cohorts and extended follow-up periods are warranted to validate these findings, refine patient selection criteria for regimen modification, and elucidate the long-term benefits of HBV RNA-guided therapy in pediatric LLV populations.

Conclusions

In children with LLV, serum HBV RNA level is an independent risk factor for NVR at week 120 and may serve as a complementary biomarker to HBV DNA for guiding antiviral therapy adjustments. Our findings advance the understanding of LLV dynamics in pediatric CHB and underscore the clinical utility of integrating HBV RNA monitoring into current management protocols. Future studies should focus on validating HBV RNA-based prognostic models and exploring its role in tailoring personalized treatment strategies for children with LLV.

Abbreviations

ALT	Alanine aminotransferase
AUC	Area under the ROC curve
BMI	Body mass index
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
ComT	TDF-combination treatment
ConT	ETV-continuing treatment
ETV	Entecavir
HBeAg	Hepatitis B e antigen
HBV	Hepatitis B virus
IQR	Interquartile range
LLV	Low-level viremia
LLOD	Lower limit of detection
NUC	Nucleos(t)ide analog
NVR	Non-virological response
qHBsAg	Quantitative hepatitis B surface antigen
ROC	Receiver operating characteristics
HBV	Hepatitis B virus
SwiT	TDF-switching treatment
TAF	Tenofovir alafenamide fumarate
TDF	Tenofovir disoproxil fumarate
VR	Virological response

Acknowledgements

The authors thank the Guangxi Healthcare Key Cultivation Discipline Construction Project for academic support.

Author contributions

YB.W. and J.W. conceived and designed the clinical study, analyzed the data, and wrote the manuscript. GF. T. and J.W. coordinated and conducted the clinical study. Y.W. and YB. W. coordinated the laboratory analysis and data management. YF.W. and L.L. critically revised the manuscript for important intellectual content. All authors have read, assisted with editing, and approved the final version of this manuscript.

Funding

This work was supported by the Scientific Research Project of Nanxishan Hospital of Guangxi Zhuang Autonomous Region (grant number NY202005).

Data availability

Data are available upon request from corresponding author.

Declarations

Ethics approval and consent to participate

This retrospective analysis of de-identified clinical data and archived samples was conducted under ethical approval (2020NXSYYEC-003) with a waiver of informed consent, in accordance with the Declaration of Helsinki guidelines for minimal-risk research.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 23 October 2024 / Accepted: 21 March 2025
Published online: 05 May 2025

References

1. Sinn DH, Lee J, Goo J, Kim K, Gwak GY, Paik YH, et al. Hepatocellular carcinoma risk in chronic hepatitis B virus-infected compensated cirrhosis patients with low viral load. Hepatology. 2015;62(3):694–701. https://doi.org/10.1002/hep.27889.
2. Kim JH, Sinn DH, Kang W, Gwak GY, Paik YH, Choi MS, et al. Low-level viremia and the increased risk of hepatocellular carcinoma in patients receiving Entecavir treatment. Hepatology. 2017;66(2):335–43. https://doi.org/10.1002/hep.28916.
3. Wang X, Cai D, Juan Z. Low-level viremia in patients with chronic hepatitis B receiving Entecavir, Tenofovir and Tenofovir Alafenamide. J Hepatol. 2022.
4. Zhang Q, Cai DC, Hu P, Ren H. Low-level viremia in nucleoside analog-treated chronic hepatitis B patients. Chin Med J (Engl). 2021;134(23):2810–7. https://doi.org/10.1097/CM9.0000000000001793.
5. Lai CL, Wong D, Ip P, Kopaniszen M, Seto WK, Fung J, et al. Reduction of covalently closed circular DNA with long-term nucleos(t)ide analogue treatment

in chronic hepatitis B. J Hepatol. 2017;66(2):275–81. https://doi.org/10.1016/j.jhep.2016.08.022.
6. [The guidelines of prevention and treatment for chronic hepatitis B. (2019 version)]. Zhonghua Gan Zang Bing Za Zhi. 2019;27(12):938–61. https://doi.org/10.3760/cma.j.issn.1007-3418.2019.12.007
7. Liu S, Wong GL, Fan R, Niu J, Ma H, Liang W, et al. Role of early On-Treatment serum HBV RNA declines in predicting hepatocellular carcinoma risk in patients with chronic hepatitis B. Clin Gastroenterol Hepatol. 2025;23(2):291–e915. https://doi.org/10.1016/j.cgh.2024.07.024.
8. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Clin Liver Dis (Hoboken). 2018;12(1):33–4. https://doi.org/10.1002/cld.728.
9. Terrault N. Is low level viremia acceptable during antiviral therapy of patients with HBV infection and decompensated cirrhosis?? Clin Gastroenterol Hepatol. 2018;16(12):1876–8. https://doi.org/10.1016/j.cgh.2018.09.010.
10. EASL. 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67(2):370–98. https://doi.org/10.1016/j.jhep.2017.03.021
11. Kao JH, Hu TH, Jia J, Kurosaki M, Lim YS, Lin HC, et al. East Asia expert opinion on treatment initiation for chronic hepatitis B. Aliment Pharmacol Ther. 2020;52(10):1540–50. https://doi.org/10.1111/apt.16097.
12. Di Dato F, Iorio R. Expanding indications for chronic hepatitis B treatment: is it really desirable to treat everyone? World J Gastroenterol. 2024;30(17):2294–7. https://doi.org/10.3748/wjg.v30.i17.2294.
13. Ghany MG, Saraswat VA. Patients with compensated hepatitis B Virus-Related cirrhosis and Low-Level viremia: treat or not to treat? Am J Gastroenterol. 2023;118(6):970–1. https://doi.org/10.14309/ajg.0000000000002224.
14. Yang J, Choi WM, Shim JH, Lee D, Kim KM, Lim YS, et al. Low level of hepatitis B viremia compared with undetectable viremia increases the risk of hepatocellular carcinoma in patients with untreated compensated cirrhosis. Am J Gastroenterol. 2023;118(6):1010–8. https://doi.org/10.14309/ajg.0000000000002181.
15. Lee SB, Jeong J, Park JH, Jung SW, Jeong ID, Bang SJ, et al. Low-level viremia and cirrhotic complications in patients with chronic hepatitis B according to adherence to Entecavir. Clin Mol Hepatol. 2020;26(3):364–75. https://doi.org/10.3350/cmh.2020.0012.
16. Yuen MF, Seto WK, Fung J, Wong DK, Yuen JC, Lai CL. Three years of continuous Entecavir therapy in treatment-naïve chronic hepatitis B patients: VIRAL suppression, viral resistance, and clinical safety. Am J Gastroenterol. 2011;106(7):1264–71. https://doi.org/10.1038/ajg.2011.45.
17. Ono A, Suzuki F, Kawamura Y, Sezaki H, Hosaka T, Akuta N, et al. Long-term continuous Entecavir therapy in nucleos(t)ide-naïve chronic hepatitis B patients. J Hepatol. 2012;57(3):508–14. https://doi.org/10.1016/j.jhep.2012.04.037.
18. Lim YS, Seto WK, Kurosaki M, Fung S, Kao JH, Hou J, et al. Review Article: switching patients with chronic hepatitis B to Tenofovir alafenamide-a review of current data. Aliment Pharmacol Ther. 2022;55(8):921–43. https://doi.org/10.1111/apt.16788.
19. Ogawa E, Nakamuta M, Koyanagi T, Ooho A, Furusyo N, Kajiwara E, et al. Switching to Tenofovir Alafenamide for nucleos(t)ide analogue-experienced patients with chronic hepatitis B: week 144 results from a real-world, multicentre cohort study. Aliment Pharmacol Ther. 2022;56(4):713–22. https://doi.org/10.1111/apt.17107.
20. Li ZB, Li L, Niu XX, Chen SH, Fu YM, Wang CY, et al. Switching from Entecavir to Tenofovir Alafenamide for chronic hepatitis B patients with low-level viraemia. Liver Int. 2021;41(6):1254–64. https://doi.org/10.1111/liv.14786.
21. Sun Y, Wu X, Zhou J, Meng T, Wang B, Chen S, et al. Persistent low level of hepatitis B virus promotes fibrosis progression during therapy. Clin Gastroenterol Hepatol. 2020;18(11):2582–91.e6.
22. Zhang Q, Peng H, Liu X, Wang H, Du J, Luo X, et al. Chronic hepatitis B infection with low level viremia correlates with the progression of the liver disease. J Clin Transl Hepatol. 2021;9(6):850–9. https://doi.org/10.14218/jcth.2021.00046.
23. Li T, Chen Y, Zhang Y, Wu S, Zhang L, Wang L. Elevated hepatitis B virus RNA levels in HBeAg-positive patients with low-level viraemia or previous low-level viraemia. J Viral Hepat. 2024;31(8):504–7. https://doi.org/10.1111/jvh.13957.
24. Lok J, Dusheiko G, Carey I, Agarwal K. Review Article: novel biomarkers in hepatitis B infection. Aliment Pharmacol Ther. 2022;56(5):760–76. https://doi.org/10.1111/apt.17105.
25. Ghany MG, King WC, Hinerman AS, Lok AS, Lisker-Melman M, Chung RT, et al. Use of HBV RNA and to predict change in serological status and disease activity in CHB. Hepatology. 2023;78(5):1542–57. https://doi.org/10.1097/hep.0000000000000413.
26. Thompson AJ, Jackson K, Bonanzinga S, Hall SAL, Hume S, Burns GS, et al. Baseline serum HBV RNA is associated with the risk of hepatitis flare after stopping nucleoside analog therapy in HBeAg-negative participants. Hepatol Commun. 2023;7(8). https://doi.org/10.1097/hc9.0000000000000188.
27. Shi L, Grace L-HW, Rong F, Junqi N, Hong M, Wanying L et al. Role of early on-treatment serum HBV RNA declines in predicting hepatocellular carcinoma risk in patients with chronic hepatitis B. Clin Gastroenterol Hepatol. 2024(0). https://doi.org/10.1016/j.cgh.2024.07.024
28. Wu Y, Wen J, Tang G, Zhang J, Xin J. On-treatment HBV RNA dynamic predicts entecavir-induced hbeag seroconversion in children with chronic hepatitis B. J Infect. 2021;83(5):594–600. https://doi.org/10.1016/j.jinf.2021.08.044.

Publisher's note