Received: 1 February 2021 | First decision: 4 March 2021 | Accepted: 5 July 2021

DOI: 10.1111/apt.16538

AP&T Alimentary Pharmacology & Therapeutics WILEY

Serum hepatitis B virus RNA level is associated with biochemical relapse in patients with chronic hepatitis B infection who discontinue nucleos(t)ide analogue treatment

Muye Xia[1] | Heng Chi[2] | Yaobo Wu[1] | Bettina E. Hansen[3] | Zhandong Li[1] | Shi Liu[1] | GuiChan Liao[1] | Xiaoyong Zhang[1] | Bin Zhou[1] | Jinlin Hou[1] | Jian Sun[1] | Harry L. A. Janssen[3] | Jie Peng[1]

[1]State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China

[2]Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

[3]Toronto Centre of Liver Disease, University Health Network, Toronto General Hospital, University of Toronto, Toronto, ON, Canada

Correspondence
Jie Peng, Hepatology Unit, Nanfang Hospital, Southern Medical University, 1838 Guangzhou Avenue North, Guangzhou, Guangdong Province, China.
Email: pjie138@163.com

Harry L.A. Janssen, Francis Family Chair in Liver Research, Director Toronto Centre for Liver Disease, Professor of Medicine, University of Toronto, University Health Network, Room 234 (9EB234), 9th floor, Eaton Building, Toronto General Hospital, 200 Elizabeth Street, Toronto, ON, M5G 2C4, Canada.
Email: harry.janssen@uhn.ca

Funding information
This work was supported by grants from the Major Science and Technology Special Project of China (2017ZX09304016, 2017ZX10302201004008), National Natural Science Foundation of China (81971949) and the Clinical Research Startup Program of Southern Medical University through High-level University Construction Funding of Guangdong Provincial Department of Education (LC2016PY003).

Summary

Background: Nucleos(t)ide analogue (NA) discontinuation may be attempted in carefully selected patients with chronic hepatitis B (CHB) infection.

Aim: To investigate whether a novel serum marker of quantitative hepatitis B virus (HBV) RNA levels could predict biochemical relapse after NA discontinuation.

Methods: We prospepctively followed non-cirrhotic Asian patients with CHB who stopped NA according to pre-specified stopping criteria. The primary endpoint was biochemical relapse (HBV DNA >2000 IU/mL and alanine transaminase >2x upper limit of normal), which were also the re-treatment criteria.

Results: Biochemical relapse occurred in 50 patients (48.3% at year 6). Multivariable analysis showed that higher HBV RNA levels (HR 1.34; $P < 0.001$) at the time of NA discontinuation were associated with increased biochemical relapse risk. The area under the curve of HBV RNA at the time of NA discontinuation for the incidence of biochemical relapse was 0.760 at 6 years. Six years after treatment discontinuation, all patients with HBV RNA levels ≥20 000 copies/mL at the end of treatment developed a biochemical relapse compared with 23.8% of patients with HBV RNA levels<1000 copies/mL ($P < 0.001$). More patients with HBV RNA levels <1000 copies/mL at end of treatment achieved loss of hepatitis B surface antigen than patients with higher levels (30.9% vs 1.6%; $P = 0.027$).

Conclusions: The HBV RNA level at end of treatment predicted biochemical relapse after treatment discontinuation and may be used to guide decisions on treatment discontinuation.

Muye Xia, Heng Chi and Yaobo Wu contributed equally as the corresponding authors.

The Handling Editor for this article was Professor Grace Wong, and it was accepted for publication after full peer-review.

1 | INTRODUCTION

Current treatment options for chronic hepatitis B virus (HBV) infection are efficacious for suppression of viral activity, but cannot eliminate the virus from patients. During infection, the virus delivers its partially double-stranded, relaxed circular DNA (rcDNA) genome to the nucleus of the hepatocytes and forms a covalently closed circular DNA mini-chromosome (cccDNA).[1,2] cccDNA together with integrated HBV genomic material provides transcriptional material for the virus. Nucleos(t)ide analogues (NA) treatment can suppress replication of HBV DNA through inhibition of the HBV polymerase, while exerting no direct effect on cccDNA or integrated HBV genomic material. Hence, although HBV is an enveloped DNA virus, the viral population in serum is now known to contain virion-like particles containing HBV RNA, and an empty viral envelope containing capsid without genomes. When suppressed adequately with NAs, HBV DNA is not detectable in the serum, whereas serum HBV RNA can still be found.[3,4] HBV RNAs are direct transcriptional products of cccDNA and the integrated HBV genome, and consist of different subtypes. Recently, it has been shown that HBV RNA in serum is encapsidated pregenomic RNA, which is used for the transcription of HBV DNA, core protein and polymerase.[3,5] HBV RNA is also correlated with the amount of intrahepatic cccDNA.[6-8] If serum HBV DNA is undetectable during NA treatment, then the HBV RNA level may serve as a marker to reflect the viral transcriptional activity. Subsequent studies have shown that serum HBV RNA level was associated with response to anti-viral treatment.[3,5,9-11]

NA discontinuation is an upcoming topic. Discontinuation after hepatitis B surface antigen (HBsAg) seroclearance is considered safe,[12] whereas discontinuation in HBsAg-positive patients usually leads to relapse.[13,14] However, a subset of patients may achieve a sustained off-treatment response. In this prospective study, we aimed to investigate the role of serum levels of HBV RNA in the prediction of sustained response after discontinuation of NA treatment.

2 | METHODS

2.1 | Patients and follow-up

The study protocol was approved by the Ethics Committee of Nanfang Hospital (Southern Medical University, Guangzhou, China) (NFEC-201209-k3). All persons gave their informed consent prior to their inclusion in the study. The study is part of an ongoing effort to prospectively investigate the discontinuation of NA therapy.

This was a prospective cohort study of chronic hepatitis B (CHB) patients who discontinued NA between November 2012 and October 2018 at Nanfang Hospital (Southern Medical University, Guangzhou, China). All follow-up data until October 2019 were analysed. Details on design and methods have been described extensively.[15-17] Briefly, start-of-treatment hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients who fulfilled the pre-defined cessation criteria were eligible. Start-of-treatment HBeAg-positive patients were eligible if they achieved HBeAg seroconversion and had undetectable serum HBV DNA followed by ≥12 months of treatment. HBeAg-negative patients were required to have undetectable HBV DNA followed by ≥18 months of treatment. 'Consolidation therapy' was defined as continued NA therapy after the achievement of HBeAg seroconversion of an undetectable HBV DNA level, and normalisation of the alanine transaminase (ALT) level in HBeAg-positive patients after the achievement of an undetectable HBV DNA level in HBeAg-negative patients. 'Liver stiffness' was measured by transient elastography (Fibroscan; Echosens). All patients had undetectable HBV DNA at least three times before NA discontinuation. The exclusion criteria were patients: (a) also infected with the hepatitis C virus or hepatitis D virus; (b) with a history of alcoholism, autoimmune hepatitis, or other severe or active disease; (c) with a history or presence of decompensated liver disease, cirrhosis or malignancy.

Patients were closely followed after discontinuation according to the study protocol. After discontinuation of NA therapy, patients were followed up every month during the initial 3 months. Thereafter, patients were followed up every 3 months. After 2 years of follow-up, patients were evaluated every 6 months. The primary endpoint was 'biochemical relapse', which was defined as HBV DNA >2000 IU/mL and ALT increase >2× the upper limit of normal. Patients who experienced biochemical relapse were retreated and withdrawn from the study.

TABLE 1 Cohort characteristics (n = 135)

Characteristics	Sustained response (N = 85)	Biochemical relapse (N = 50)
Baseline (end-of-treatment)		
Age (years)[a]	35 ± 9.0	38 ± 7.5
Male sex (%)	69 (81)	41 (82)
NA therapy[b]	46(54%)	28(56%)
Consolidation therapy duration, months[c]	30.0 (17.0-43.0)	28.0 (17.5-48.0)
Lab (serum)		
ALT (U/L)[c]	21.0(16.6-31.0)	22.0 (17.0-29.5)
HBV DNA, log IU/mL[a]	UD	UD
HBsAg, log IU/mL[a]	2.3 ± 1.2	2.8 ± 0.8
HBV RNA, log copies/mL[a]	1.2(0.0-4.0)	4.2(2.8-4.5)
Start-of-treatment		
Lab (serum)		
HBeAg positivity (%)	64(75%)	32(64%)
ALT (U/L)[c]	181.0 (107.0-297.0)	245.0(109.5-438.0)
HBV DNA, log IU/mL[a]	6.4 ± 1.5	6.6 ± 1.4

Abbreviations: ALT, alanine transaminase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NA, Nucleos(t)ide analogue; UD, undetectable (<20 IU/mL).

[a]Mean ± standard deviation.

[b]First-line: entecavir, tenofovir. Second-line: lamivudine, adefovir, telbivudine.

[c]Median (interquartile range).

2.2 | Biochemistry and laboratory methods

Before treatment, the HBV DNA level was measured using a polymerase chain reaction HBV assay with a lower limit of detection of 1000 copies/mL (Daan Gene Co, Ltd.; Sun Yat-sen University; Guangzhou, China). After NA discontinuation and during the off-treatment follow-up, HBV DNA levels were assessed using the Cobas TaqMan polymerase chain reaction HBV assay with a lower limit of detection of 20 IU/mL (Roche Diagnostics). HBsAg, HBeAg and anti-HBe levels were determined using ARCHITECT (Abbott Laboratories). The HBsAg test had a lower limit of detection of 0.05 IU/mL.

Testing of serum HBV RNA was performed by real-time transcription-quantitative polymerase chain reaction (RT-qPCR) using a LightCycler480 II (Roche) with a TaqMan probe as described by Rong et al.[8,18] Two different pairs of primers were designed to determine the HBV RNA levels independently in this study (PreC-forward: 5′-CAC CTC TGC CTA ATC TCA TG-3′; and PreC -reverse: 3′-CAA ATT CTT TAT ACG GGT CAA TGT CC-5′; S-F: 5′-GCGGGG TTT TTC TTG AC-3′ and S-R: 3′-GCG ATA ACC AGG ACA AAT TG-5′). Negative HBV RNA was defined as both targets not detected by RT-qPCR with two different pairs of primers.

2.3 | Statistical analysis

The duration of follow-up was calculated from treatment discontinuation to the last follow-up or loss to follow-up. Data are presented as the mean ± SD or median (interquartile range). Characteristics of patients with biochemical relapse or a sustained response were compared using the χ^2 test, for categorical variables. The Student's *t*-test or Mann-Whitney test for continuous variables was used if appropriate. Cox proportional hazards regression analysis was used to assess the association between variables and study endpoints. The predictive accuracy of the risk score model was assessed by discrimination measured by the concordance index, and the time-dependent receiver operating characteristic (ROC) curve. The Kaplan-Meier method was used to estimate time-dependent ROC curves.

All data were analysed using the statistical package SPSS for Windows (version 22.0; SPSS Inc.). The analysis used the 'survival ROC' package, written using R 3.6.1(R Institute for Statistical Computing; www.r-project.org/), for performance assessment in time-dependent estimation ROC curves. A two-tailed $P < 0.05$ was considered statistically significant.

TABLE 2 Cox proportional hazards regression analysis of biochemical relapse

	Biochemical relapse (n = 50)					
	Univariable			Multivariable		
	HR	95% CI	*P*	HR	95% CI	*P*
End of treatment						
Age, per year	1.04	1.00-1.07	0.035*	1.05	1.01-1.08	0.012*
Age ≥35 years	1.95	1.08-3.53	0.028*			
Female sex	1.08	0.52-2.22	0.837			
First-line NA[a]	1.15	0.66-2.02	0.619			
Peg-interferon experienced	1.07	0.54-2.15	0.840			
Consolidation duration, per month	1.00	0.98-1.01	0.496	0.99	0.97-1.01	0.214
ALT (U/L)	0.99	0.97-1.02	0.550			
HBsAg level, per log IU/mL	1.39	1.01-1.90	0.043*	1.26	0.88-1.81	0.205
HBV RNA level, per log copies/mL	1.32	1.13-1.54	<0.001***	1.34	1.15-1.57	<0.001***
HBV RNA level						
<1000 copies/mL	Reference					
1000-19 999 copies/mL	3.16	1.55-6.45	0.002			
≥20 000 copies/mL	2.14	1.48-3.07	<0.001***			
Start of treatment						
HBeAg positivity	0.60	0.33-1.07	0.082	0.62	0.33-1.17	0.138
HBV DNA, log IU/mL	1.10	0.88-1.37	0.420			
ALT (U/L)	1.00	1.00-1.00	0.639			

NA: Nucleos(t)ide analogue; ALT: alanine transaminase; HBV hepatitis B virus; HBsAg hepatitis B s antigen;

[a] First-line: entecavir, tenofovir. Second-line: lamivudine, adefovir, telbivudine.

* $p < 0.05$, *** $p < 0.001$.

3 | RESULTS

3.1 | Patient population

At the time of treatment discontinuation, 12 patients were tested as HBsAg negative and were excluded from the primary analysis. Seven patients were lost to follow-up (at weeks 8, 24, 36, 36, 48, 72 and 96 respectively). In total, 135 patients were included, with a median off-treatment follow-up of 2.6 (interquartile range 1.0-3.5) years. Cases of hepatic decompensation or hepatocellular carcinoma were not observed after treatment discontinuation. Fifty patients developed a biochemical relapse resulting in a cumulative rate of 48.3% at year 6 (Figure S1). HBsAg loss occurred in 13 patients, which resulted in a cumulative rate of 17.3% at year 6 (Figure S1). The characteristics of the patients are shown in Table 1.

At the end of treatment, 77% of patients had detectable HBV RNA, despite all being HBV DNA negative.

3.2 | Factors associated with biochemical relapse

In univariate analysis, age (hazard ratio [HR] 1.04, $P = 0.035$), the end-of-treatment HBsAg level (HR 1.39, $P = 0.043$) and end-of-treatment HBV RNA level (HR 1.32, $P < 0.001$) were significantly associated with biochemical relapse (Table 2). Multivariable analysis confirmed the association between HBV RNA levels and biochemical relapse after adjustment for other cofactors (HR 1.34, $P < 0.001$). Next, time-dependent ROC curves were generated to assess the diagnostic ability of the end-of-treatment HBV RNA level. The area under the curve of HBV RNA level upon NA discontinuation for biochemical relapse was 0.760 at 6 years (Figure 1). For the ease of clinical practice, cutoffs of 1000 and 20 000 copies/mL were explored for subsequent analyses. End-of-treatment HBV RNA levels >20 000 copies/mL were associated with the highest risk of biochemical relapse (100% at year 6) as compared with the level between 1000 and 19999 copies/mL (61% at year 6), whereas patients with HBV RNA level <1000 copies/mL (24% at year 6) had the lowest risk of biochemical relapse ($P < 0.001$, Figure 2A). During 6 years of follow-up, 20 of 34 patients with end-of-treatment HBV-RNA ≥20 000 copies/mL, 14 of 34 patients with end-of-treatment HBV-RNA 1000–20 000 copies/mL, and 16 of 67 patients with end-of-treatment HBV-RNA <10 000 copies/mL experienced biochemical relapses. More patients with HBV RNA<1000 copies/mL at end-off-treatment achieved HBsAg loss than other patients after treatment cessation (30.9% vs 1.6%, $P = 0.007$; Figure 2B).

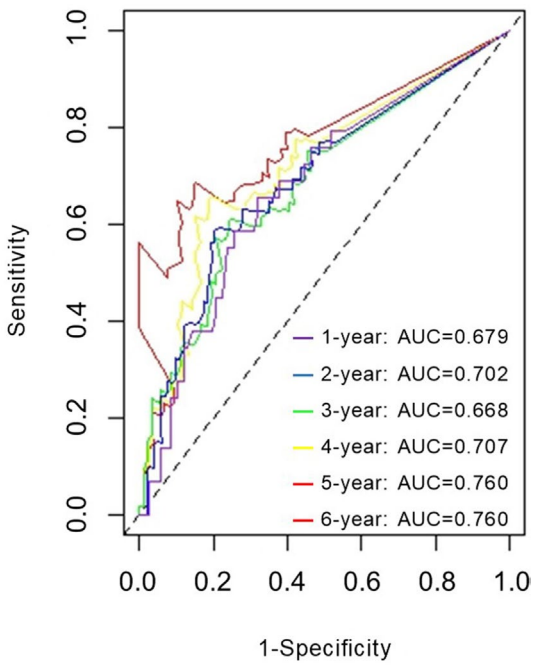

FIGURE 1 Time-dependent ROC curves for prediction of relapse after discontinuation of treatment of nucleos(t)ide analogs at 6 years according to the HBV RNA level at the end of treatment. ROC, receiver operating characteristic; AUC, area under the ROC curve

3.3 | Off-treatment HBV RNA kinetics

The HBV RNA level at all off-treatment time points was significantly higher in patients who developed biochemical relapse (Figure S2). In addition, HBV RNA <1000 copies/mL at off-treatment weeks 4, 8, 12 and 24 was associated with a lower risk of biochemical relapse, whereas a high HBV RNA level (≥20 000 copies/mL) at off-treatment weeks 12, 24, 36 and 48 was significantly associated with a higher risk of biochemical relapse (Figure S3A–D).

3.4 | Correlation between the serum level of HBV RNA and other factors

The end-of-treatment HBV RNA level was not significantly correlated with age, sex, start-of-treatment HBeAg-status, duration of consolidation therapy or liver stiffness ($P > 0.05$ for all). At most off-treatment time points, the HBV RNA level was significantly correlated with HBV DNA and HBsAg levels, whereas the correlation with ALT levels varied (Table S1).

4 | DISCUSSION

The serum level of HBV RNA is a promising marker of viral transcriptional activity, particularly during NA treatment because the serum level of HBV DNA is frequently undetectable due to inhibition of HBV polymerase. In a prospective cohort, we demonstrated that a high serum level of HBV RNA was associated with a high risk of biochemical relapse after discontinuation of NA treatment.

In accordance with our findings, other studies have shown a correlation between the serum level of HBV RNA with intrahepatic levels of RNA and cccDNA.[6,7,19] In addition, the HBV RNA level has been found to be associated with the likelihood of a serological response

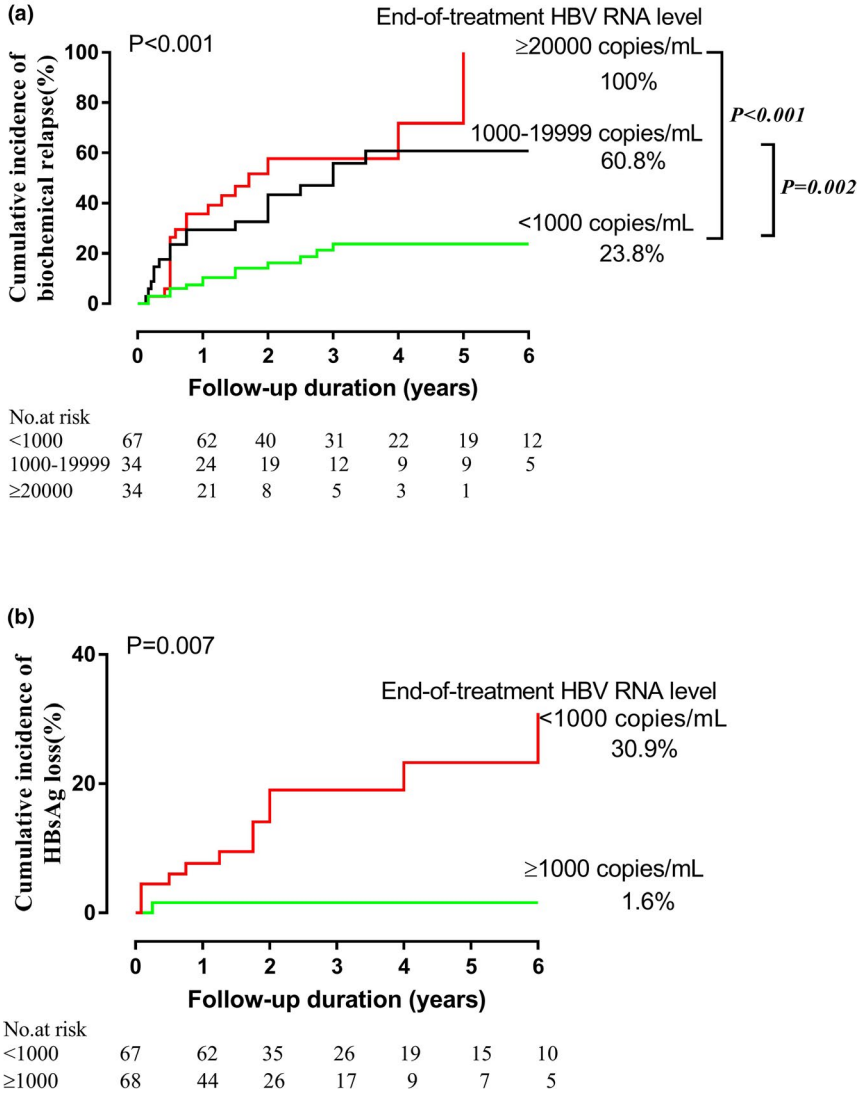

FIGURE 2 Cumulative prevalence of biochemical relapse (A) and HBsAg loss (B) after discontinuation of treatment with nucleos(t)ide analogs according to the end-of-treatment HBV RNA level. HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus

to treatment with NA and pegylated interferon.[5,9-11,20] Several small studies had previously revealed that HBV RNA might be a predictor of viral rebound after discontinuing treatment.[3,18,21] In a larger prospective cohort with longer follow-up, our study demonstrated the significant role of the serum level of HBV RNA in patients who discontinued NA treatment. Specifically, we showed that all patients with a high level of serum HBV RNA level (≥20 000 copies/mL) relapsed, whereas patients with a low level (<1000 copies/mL) were more likely to clear HBsAg after NA discontinuation. Furthermore, most patients (77%) had a detectable HBV RNA level at the end of treatment, despite being HBV DNA-negative. This is a reasonable finding since NAs suppress viral replication by functioning as chain terminators; thus, blocking HBV DNA synthesis while having no effect on the synthesis of HBV RNA.[3,4] Detection of HBV RNA virions in the serum may reflect the presence and active transcription of cccDNA in the liver of patients, either reflecting a significant amount of cccDNA in hepatocytes and/or an 'inadequate' HBV-directed immune response.

To explore more strategies that could guide the safe discontinuation of NAs, we further evaluated the predictive value of end of treatment HBV RNA level. The area under the curve for HBV RNA was 0.760 and therefore had good diagnostic ability for the prediction of biochemical relapse. Up to 6 years after treatment discontinuation, all patients with end-of-treatment HBV RNA levels ≥20 000 copies/mL developed biochemical relapse. Such patients should therefore not discontinue treatment.

Even though our study cohort was relatively large and the follow-up period was longer than that of previous studies evaluating discontinuation of NA treatment, an even larger sample size would have allowed computation of an accurate prediction model to safely select patients for discontinuation of NA treatment. Further research in this direction is warranted (ie creating and validating a prediction model for an off-treatment sustained response using demographic, biochemical and virological parameters). Moreover, an elevated HBV DNA and ALT were used as primary endpoint and retreatment criteria (similar to criteria for treatment initiation), but it should be noted that the optimal retreatment criteria are yet to be determined. In addition, the cut-off values for HBV RNA levels were not pre-determined, but served an exploratory purpose. Finally, all included patients were of Chinese origin. Confirmation of results in a non-Chinese population is needed.

5 | CONCLUSIONS

In conclusion, the end-of-treatment HBV RNA level predicted biochemical relapse after NA treatment discontinuation and may be used in a prediction model to safely select patients who could discontinue NA treatment.

ACKNOWLEDGEMENT

The authors thank all the patients in this study, and the nurses who assisted in patient care and the collection of serum samples.

Declaration of personal and funding interests: This study was supported by grants from the Major Science and Technology Special Project of China (2017ZX09304016, 2017ZX10302201004008), National Natural Science Foundation of China (81971949), and the Clinical Research Startup Program of Southern Medical University through High-level University Construction Funding of Guangdong Provincial Department of Education (LC2016PY003).

AUTHORSHIP

Guarantor of the article: Muye Xia.

Author contributions: M. Xia involved in analysis and interpretation of data, drafting and finalising the manuscript. H. Chi and B.E. Hansen involved in study concept and design, analysis and interpretation of data, drafting and finalising the manuscript. Y. Wu, S. Liu, Z. Li, G. Liao, X. Zhang, B. Zhou and J. Hou involved in patient recruitment, data collection and critical revision of the manuscript. J. Sun carried out study concept and design, analysis and interpretation of data. H.L.A. Janssen involved in study concept and design, analysis and interpretation of data, drafting and finalising the article. J. Peng involved in study concept and design, drafting, finalising and critical review of the final manuscript. All authors approved the final version of the manuscript.

ORCID

Jinlin Hou https://orcid.org/0000-0001-8230-8583
Jian Sun https://orcid.org/0000-0001-5320-227X
Jie Peng https://orcid.org/0000-0003-0928-3134

REFERENCES

1. EASL. Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol*. 2017;2017:370–398.
2. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology*. 2018;67:1560–1599.
3. Wang J, Shen T, Huang X, et al. Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound. *J Hepatol*. 2016;65:700–710.
4. Butler EK, Gersch J, McNamara A, et al. Hepatitis B virus and RNA Levels in nucleos(t)ide analog-treated or untreated patients during chronic and acute infection. *Hepatology*. 2018;68:2106–2117.
5. Jansen L, Kootstra NA, van Dort KA, Takkenberg RB, Reesink HW, Zaaijer HL. Hepatitis B virus pregenomic RNA is present in virions in plasma and is associated with a response to pegylated interferon alfa-2a and nucleos(t)ide analogues. *J Infect Dis*. 2016;213:224–232.
6. Giersch K, Allweiss L, Volz T, Dandri M, Lutgehetmann M. Serum HBV pgRNA as a clinical marker for cccDNA activity. *J Hepatol*. 2017;66:460–462.
7. Wang J, Yu Y, Li G, et al. Relationship between serum HBV RNA levels and intrahepatic viral as well as histologic activity markers in entecavir-treated patients. *J Hepatol*. 2018;68:16–24.
8. Huang Q, Zhou B, Cai D, et al. Rapid Turnover of HBV cccDNA indicated by monitoring emergence and reversion of signature-mutation in treated chronic hepatitis B patients. *Hepatology*. 2021;73:41–52.52.
9. van Bömmel F, Bartens A, Mysickova A, et al. Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors. *Hepatology*. 2015;61:66–76.
10. Ma G, Lou B, Lv FZhao DZhang Z, Chen Y. HBcrAg and pgRNA and the therapeutic effect in HBeAg-positive patients receiving antiviral therapy, baseline serum HBV-RNA is a powerful predictor of response. *J Viral Hepat*. 2020;27:837–846.
11. Luo H, Tan N, Kang Q, et al. HBV pgRNA status can reveal the long-term prognoses of chronic hepatitis B patients treated with nucleos(t)ide analogues. *J Viral Hepat*. 2020;27:323–328.
12. Chi H, Wong D, Peng J, et al. Durability of response after hepatitis B surface antigen seroclearance during nucleos(t)ide analogue treatment in a multiethnic cohort of chronic hepatitis B patients: results after treatment cessation. *Clin Infect Dis*. 2017;65:680–683.
13. Chi H, Hansen BE, Yim C, et al. Reduced risk of relapse after long-term nucleos(t)ide analogue consolidation therapy for chronic hepatitis B. *Aliment Pharmacol Ther*. 2015;41:867–876.
14. Liem KS, Fung S, Wong DK, et al. Limited sustained response after stopping nucleos(t)ide analogues in patients with chronic hepatitis B: results from a randomised controlled trial (Toronto STOP study). *Gut*. 2019;68:2206–2213.
15. Chi H, Li Z, Hansen BE, et al. Serum level of antibodies against hepatitis B core protein is associated with clinical relapse after discontinuation of nucleos(t)ide analogue therapy. *Clin Gastroenterol Hepatol*. 2019;17:182–191.e1.
16. Cao J, Chi H, Yu T, et al. Off-treatment hepatitis b virus (HBV) DNA levels and the prediction of relapse after discontinuation of nucleos(t)ide analogue therapy in patients with chronic hepatitis b: a prospective stop study. *J Infect Dis*. 2017;215:581–589.
17. Xia M, Liao G, Chen H, et al. Plasma CXCL13 is a predictive factor for HBsAg loss and clinical relapse after discontinuation of nucleos(t)ide analogue treatment. *Clin Immunol*. 2019;198:31–38.
18. Fan R, Zhou B, Xu M, et al. Association between negative results from tests for HBV DNA and RNA and durability of response after discontinuation of nucles(t)ide analogue therapy. *Clin Gastroenterol Hepatol*. 2020;18:719–727.e7.
19. Carey I, Gersch J, Wang B, et al. Pre-genomic HBV RNA and HBcrAg predict outcomes in HBeAg negative chronic hepatitis B patients suppressed on nucleos(t)ide analogue therapy. *Hepatology*. 2020;72:42–57.
20. Huang Y-W, Takahashi S, Tsuge M, et al. On-treatment low serum HBV RNA level predicts initial virological response in chronic hepatitis B patients receiving nucleoside analogue therapy. *Antivir Ther*. 2015;20:369–375.
21. Tsuge M, Murakami E, Imamura M, et al. Serum HBV RNA and HBeAg are useful markers for the safe discontinuation of nucleotide analogue treatments in chronic hepatitis B patients. *J Gastroenterol*. 2013;48:1188–1204.

SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

How to cite this article: Xia M, Chi H, Wu Y, et al. Serum hepatitis B virus RNA level is associated with biochemical relapse in chronic hepatitis B patients who discontinued nucleos(t)ide analogue treatment. *Aliment Pharmacol Ther*. 2021;00:1–6. https://doi.org/10.1111/apt.16538