Predictors of Nucleos(t)ide Analogues Discontinuation Relapse: Hepatitis B Virus RNA *versus* Hepatitis B Surface Antigen

Shuo Wu[1,2], Tao Li[1], Feng Liu[1], Dedong Yin[1], Lixin Zhang[1] and Lei Wang[1]

[1]*Department of Infectious Diseases and Hepatology, The Second Hospital of Shandong University, Jinan, China*
[2]*Department of Gastroenterology, Hospital of Traditional Chinese Medicine of Linyi City, Linyi, China*

ABSTRACT

Objective: To compare the predictive value of hepatitis B virus (HBV) RNA and HBsAg quantification upon discontinuation of nucleos(t)ide analogues (NAs) therapy for clinical and virological relapse in chronic hepatitis B (CHB).

Study Design: Observational study.

Place and Duration of the Study: Department of Infectious Diseases and Hepatology, The Second Hospital of Shandong University, Jinan, China, from July 2014 to December 2020.

Methodology: CHB patients received single NAs and discontinued treatment following appropriate standards. HBsAg quantification was conducted using the i2000 Chemiluminescent Immunoassay (CLIA) Analyser, while serum HBV RNA quantification was performed using specific RNA target capture and simultaneous amplification and testing. The main observational endpoints included virological relapse and clinical relapse.

Results: Eighty-one patients were recruited, with 15 patients achieving HBsAg loss at cessation. Twenty-nine individuals encountered virological relapse, while 13 patients experienced clinical relapse. Thirty-one patients achieved HBsAg <100 IU/ml at NAs cessation, among whom 26 achieved undetectable HBV RNA, while four patients suffered virological relapse (15.4%). Serum HBV RNA emerged as an independent determinant of virological relapse (HR 1.850), clinical relapse (HR 2.020), and HBsAg loss after NAs cessation (HR 0.138). The presence of HBsAg <100 IU/ml at cessation did not serve as a predictor for virological relapse and clinical relapse.

Conclusion: Lower HBV RNA levels predict a better off-treatment response. Discontinuation of prolonged NAs therapy appears as a viable and safe choice for patients with undetectable HBV RNA. In comparison to HBV RNA, HBsAg <100 IU/ml at cessation did not show sufficient predictive value for virological relapse and clinical relapse.

Key Words: *HBV RNA, Hepatitis B surface antigen, Chronic hepatitis B, Relapse.*

How to cite this article: Wu S, Li T, Liu F, Yin D, Zhang L, Wang L. Predictors of Nucleos(t)ide Analogues Discontinuation Relapse: Hepatitis B Virus RNA *versus* Hepatitis B Surface Antigen. *J Coll Physicians Surg Pak* 2024; **34(05)**:545-550.

INTRODUCTION

The prognosis for patients suffering from chronic hepatitis B (CHB) has markedly enhanced owing to continuous progress in potent treatments. Nucleos(t)ide analogues (NAs), the central therapeutic strategies extensively utilised in modern medical practice to tackle hepatitis B virus (HBV), efficiently curb viral replication and diminish the occurrence of liver-associated complications.[1,2] Nonetheless, the persistent presence of intrahepatic covalently closed circular DNA (cccDNA) poses a significant challenge in attaining complete viral elimination.[3] Achieving a complete cure is unlikely;[4] hence, the primary obstacle influencing patient compliance remains the prolonged and indefinite treatment course in CHB.

Given the invasive and unconventional nature of cccDNA detection, various surrogate markers have been proposed for assessing efficacy and predicting prognosis in CHB patients.[5,6] The achievement of a functional cure is considered a viable objective.[4,7] Nevertheless, it is important to note that HBsAg is also encoded by integrated viral genomes.[8] The occurrence of HBsAg loss remains rare, leading to life-long treatment in the majority of CHB patients.[9] Prior research suggested that reduced levels of HBsAg were indicative of a more favourable off-treatment response.[10-12] HBsAg quantity below 100 IU/mL at the end of treatment (EOT) could act as an indicator for potential discontinuation of NAs. Nevertheless, the suboptimal virological relapse rates post-treatment, ranging from 9.1 to 19.6%, remain a persistent challenge.[11]

Recently, HBV RNA has been adopted as an alternate marker for cccDNA.[5,6,8] Essentially, serum HBV RNA is pregenomic RNA

Correspondence to: Dr. Lei Wang, Department of Infectious Diseases and Hepatology, The Second Hospital of Shandong University, Jinan, China
E-mail: wlsdeygbk@163.com

Received: January 04, 2024; Revised: March 23, 2024; Accepted: April 23, 2024
DOI: https://doi.org/10.29271/jcpsp.2024.05.545

(pgRNA) which remains unprocessed by reverse transcription.[5,8] Undetectable serum HBV RNA indicates disappearance or transcriptional silence of cccDNA in hepatocytes.[8] Previous studies have assessed the prognostic significance of serum HBV RNA for virological/clinical relapse following NAs discontinuation.[6,13,14] However, the relatively short follow-up period (primarily 12 months)[6,13] and low HBsAg levels at cessation[6] may impact the precision and representativeness of the aforementioned studies. Moreover, considering the diverse quantitative polymerase chain reaction (PCR) methods or RNA simultaneous amplification testing (SAT) techniques employed for the assay, the quantification of HBV RNA with reliable sensitivity and specificity should be of paramount importance.[13-15]

In this study, the aim was to compare the predictive value of HBV RNA and HBsAg quantification upon NAs discontinuation for clinical and virological relapse in CHB patients.

METHODOLOGY

Patients with CHB were enrolled at the Second Hospital of Shandong University during the period from July 2014 to December 2020. Inclusion criteria were CHB patients who underwent treatment with single NAs such as lamivudine, adefovir dipivoxil, telbivudine, entecavir, or tenofovir; the criteria for cessation conformed to the guidelines set by APASL.[16,17]These criteria are outlined as follows: A requisite consolidation therapy of no less than 12 months post hepatitis B e-antigen (HBeAg) seroconversion accompanied by undetectable HBV DNA and a normal ALT level in HBeAg-positive patients; a minimum guarantee of 18 months of consolidation therapy subsequent to the undetectability of HBV DNA and a normal ALT level, coupled with an overall treatment duration of at least 24 months in HBeAg-negative patients. Exclusion criteria were subjects exhibiting symptoms of decompensated liver disease, cirrhosis, or hepatocellular carcinoma; patients with other concurrent liver conditions; patients resistance to NAs. After discontinuation of NAs, patients underwent monthly follow-ups during the initial 4 months, followed by assessments at months 6, 9, and 12. Subsequently, a follow-up schedule at six-month intervals was implemented after the first year of cessation. The information of patient follow-up was updated to August 2021. Each participant provided written informed consent, and the research protocol obtained approval from the Ethical Committee of the hospital, in accordance with the Helsinki Declaration's principles.

In this post-hoc analysis, 137 patients were preliminary screened, while 56 patients were excluded because of the lack of serum samples at EOT (40 patients), lack of hypersensitive HBV DNA detection during the follow-up (15 patients), and HBsAg loss due to the usage of interferon (1 patient). The 81 recruited patients were divided into cohorts A and B. Cohort A comprised 66 individuals without HBsAg loss at cessation, whereas Cohort B consisted of 15 individuals who achieved HBsAg loss at cessation.

The principal observational endpoints of this investigation included virological relapse, defined as serum HBV DNA levels exceeding 2000 IU/mL following the discontinuation of NAs, verified by successive tests conducted at 2-week intervals; and clinical relapse, identified by the presence of virological relapse in conjunction with ALT levels exceeding twice the upper limit of normal (ULN). The secondary observational endpoint was the loss of HBsAg.

HBsAg quantification and HBV RNA were performed at the time of NAs therapy cessation. HBsAg quantification was also conducted before the discontinuation of NAs, if deemed necessary. HBsAg quantification was executed utilising the i2000 Chemical Luminescent Immunoanalyser (Abbott, USA, lower limit of quantification [LLQ] 0.05 IU/mL) with Abbott reagents. HBV DNA levels were assessed using the Roche COBAS TaqMan system (Basel, Switzerland, LLQ 20 IU/mL). In some cases, HBV DNA levels before the cessation of NAs were determined using a domestic reagent (PG Biotech, Shenzhen, China, LLQ 1×10^3 copies/mL). The serum HBV RNA was measured by specific RNA target capture and SAT method (Rendu Biotechnology, Shanghai, China, LLQ 50 copies/mL), which is a new quantitative method avoiding interference of mixed DNA in RNA extraction procedure. The term undetectable serum HBV RNA was characterised as target not detected. The detailed procedures for the HBV RNA quantification have been explicated in a prior study.[15]

Continuous variables were depicted as mean ± standard deviation (SD) or median with interquartile range (IQR). Categorical variables were expressed as numbers and percentages. Cumulative relapse rates were compared using the log-rank test. To identify predictors linked with virological/clinical relapse post NAs discontinuation, both univariate and multivariate Cox analyses were performed. HBV RNA was segmented as a variable based on levels below detection and LLQ. HBsAg at cessation was categorised following a prior meta-analysis (<100 IU/mL *vs.* ≥100 IU/mL).[11] Regardless of their results in univariate analysis, serum HBV RNA and HBsAg levels at cessation were incorporated into multivariate models. A p-value below 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS software version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

For the 81 included patients, the median patient age and HBsAg levels at NAs cessation were 35 (IQR 31-44) years and 735.5 (IQR 7.3-3307.4) IU/mL, respectively. Most patients were males (50 patients, 61.7%). Patients underwent a median of 65 (IQR 40-107) months' treatment duration and a median of 48 (IQR 28-86) months' consolidation treatment. The median follow-up duration for patients exhibiting a sustained response was 32 (IQR 24-48) months. Nearly half of the above patients (23/52, 44.2%) were followed up for more than or equal to 36 months. Single NAs were administered, with lamivudine prescribed to 11 patients (13.6%), adefovir to 15 patients (18.5%), telbivudine to 26 patients (32.1%), entecavir to 26 patients (32.1%), and tenofovir to 3 patients (3.7%). The patients' demographic characteristics are displayed in Table I.

Table I: Characteristics of the included 81 patients.

Characteristics	Values
Age at cessation (years)	35 (31-44)
Male/Female (n)	50/31 (61.7%/38.3%)
Pretreatment HBV DNA (log10copies/mL)	6.87 (5.88-7.78)
Pretreatment HBeAg (positive/negative)	61/20 (75.3%/24.7%)
Pretreatment ALT (U/L)	202 (134-378)
Pretreatment AST (U/L)	112 (63-220)
Total treatment periods (months)	65 (40-107)
Consolidation periods (months)	48 (28-86)
Serum HBVRNA (n, %)	
Undetectable	45 (55.6%)
<50 copies/mL	16 (19.8%)
≥50 copies/mL	20 (24.7%)
HBsAg at cessation (n, %)	
<100 IU/mL	31 (38.3%)
≥100 IU/mL	50 (61.7%)

HBV, Hepatitis B virus; HBeAg, Hepatitis B e antigen; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; HBsAg, Hepatitis B surface antigen.

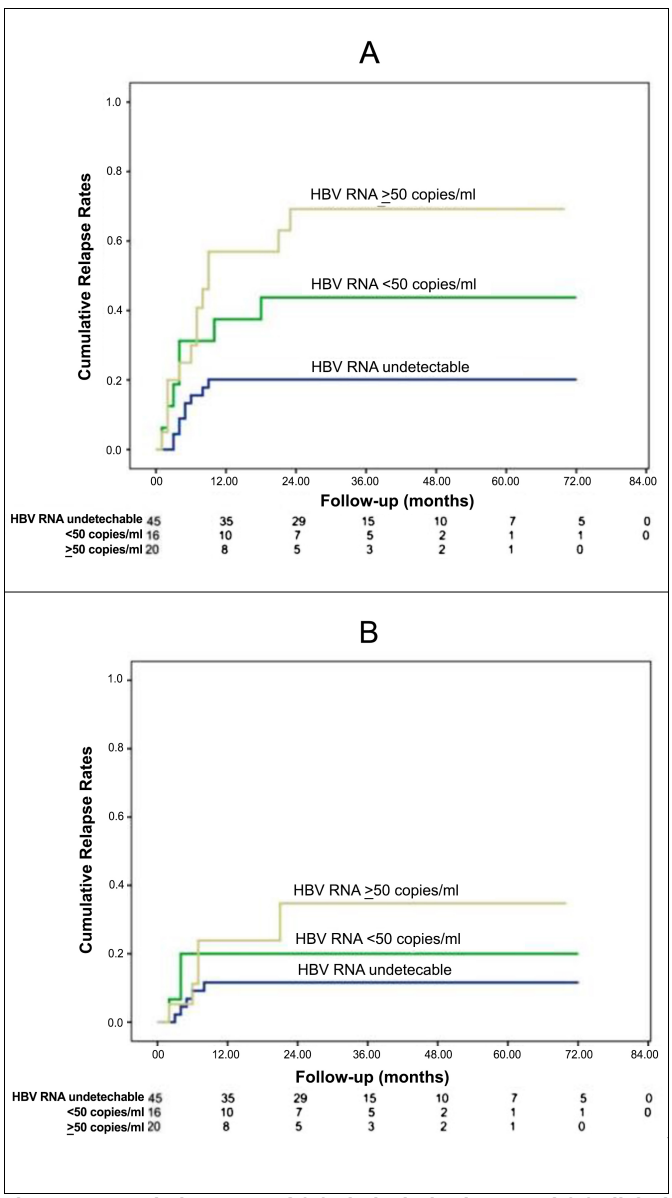

Figure 1: Cumulative rates of (A) virological relapse and (B) clinical relapse.

Twenty-nine patients experienced virological relapse, while 13 patients encountered clinical relapse. The cumulative relapse rates for virological and clinical relapses at years 1 and 2 were 32.4%, 36.9%, and 16.1%, 18.0%, respectively.

The undetectable serum HBV RNA was attained in 45 patients (55.6%), among whom nine patients (20.0%) experienced virological relapse, and five patients (11.1%) encountered clinical relapse.

Thirty-one patients achieved HBsAg <100 IU/ml at NAs cessation, of whom six patients (19.4%) suffered virological relapse, and five patients (16.1%) suffered clinical relapse. Out of the 31 patients with HBsAg levels <100 IU/ml at discontinuation, twenty-six achieved undetectable HBV RNA, while four patients experienced virological relapse (15.4%), and three patients encountered the clinical relapse (11.5%). Five of the 31 patients did not achieve undetectable HBV RNA while two patients suffered virological relapse and clinical relapse (40.0%).

Salvage interventions were initiated upon the occurrence of virological relapse, clinical relapse, or after several months, guided by patients' preferences, ALT levels, and treatment history. None of the patients experienced hepatic decompensation or liver failure.

Prognostic value of HBV RNA for virological relapse and clinical relapse in total population was analysed. Univariate Cox analysis revealed that both HBV RNA and HBsAg at cessation were predictors of virological relapse. However, only the serum HBV RNA (HR 1.850, p = 0.009) emerged as an independent factor for virological relapse in multivariate Cox analysis (Table II). In terms of predicting the clinical relapse, age emerged as the sole independent factor in the univariate Cox analysis, meanwhile, serum HBV RNA (HR 2.020) and age (HR 1.067) were independent factors identified for clinical relapse using multivariate Cox analysis (Table III).

The current investigation scrutinised the 66 patients in Cohort A to mitigate the confounding effects of functional cure. The serum HBV RNA was likewise established as an independent predictor for both virological relapse (HR 1.748, p = 0.015) and clinical relapse (HR 2.306, p = 0.032).

The authors also explored cumulative relapse rates of virological and clinical relapses stratified by the serum HBV RNA in total population. Regarding cumulative virological relapse rates, a statistically significant difference was observed between patients with the undetectable HBV RNA and those with HBV RNA levels <50 copies/ml (p = 0.052) or HBV RNA levels ≥50 copies/ml (p <0.001). However, there was no significant distinction between patients with HBV RNA levels <50 copies/ml and those with HBV RNA levels ≥50 copies/ml (p = 0.240). Cumulative clinical relapse rates did not exhibit a significant difference across patients with the undetectable HBV RNA, those with HBV RNA <50 copies/ml, and those with HBV RNA ≥50 copies/ml (p >0.05, Figure 1).

Table II: Univariate and multivariate analyses of predictors associated with virological relapse.

	Univariate			Multivariate		
	HR	**95% CI**	**p-values**	**HR**	**95% CI**	**p-values**
Age at cessation	1.011	0.977-1.047	0.517			
Male/Female	0.872	0.416-1.827	0.716			
Pretreatment ALT	1.000	0.999-1.001	0.633			
Pretreatment AST	1.000	0.997-1.002	0.655			
Consolidation periods	0.999	0.989-1.009	0.791			
Pretreatment HBeAg status	1.556	0.594-4.080	0.368			
HBV RNA	2.040	1.347-3.087	0.001	1.850	1.168-2.930	0.009
HBsAg at cessation (≥100 IU/mL *vs*. <100 IU/mL	2.625	1.068-6.453	0.035	1.586	0.590-4.260	0.361
Pretreatment HBVDNA (log10copies/mL)	1.022	0.820-1.274	0.845			

HR, Hazard ratio; CI, Confidence interval; HBeAg, Hepatitis B e antigen; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; HBV, Hepatitis B virus; HBsAg, Hepatitis B surface antigen.

Table III: Univariate and multivariate analyses of predictors associated with clinical relapse.

	Univariate			Multivariate		
	HR	**95% CI**	**p-values**	**HR**	**95% CI**	**p-values**
Age at cessation	1.049	0.995-1.106	0.073	1.067	1.004-1.135	0.038
Male/Female	1.407	0.433-4.572	0.570			
Pretreatment ALT	1.001	0.999-1.002	0.475			
Pretreatment AST	1.000	0.996-1.003	0.808			
Consolidation periods	1.008	0.995-1.021	0.219			
Pretreatment HBeAg status	0.517	0.169-1.580	0.247			
HBV RNA	1.637	0.891-3.008	0.112	2.020	0.997-4.089	0.051
HBsAg at cessation (100 IU/mL *vs*. <100 IU/mL)	1.061	0.346-3.247	0.918	0.873	0.224-3.409	0.845
Pretreatment HBVDNA (log10copies/mL)	1.060	0.748-1.501	0.744			

HR, Hazard ratio; CI, Confidence interval; HBeAg, Hepatitis B e antigen; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; HBV, Hepatitis B virus; HBsAg, Hepatitis B surface antigen.

Predictors for achieving HBsAg loss after NAs cessation in Cohort A were also explored. In Cohort A, HBsAg loss was not attained at NAs cessation; nonetheless, ten patients achieved functional cure during follow-up, with five experiencing HBsAg seroconversion. Multivariate Cox analysis unveiled that the serum HBV RNA (HR 0.138, p = 0.049) and HBsAg at the time of discontinuation (HR 0.015, p = 0.010) were independent factors linked to the loss of HBsAg following the cessation of NAs.

DISCUSSION

The current investigation delved into the superior prognostic value of the serum HBV RNA compared to lower HBsAg levels in CHB patients discontinuing NAs in accordance with prevailing guidelines. Multivariate analysis demonstrated that the serum HBV RNA served as a predictor for virological relapse, clinical relapse, and the loss of HBsAg following the discontinuation of NAs. NAs cessation in patients with undetectable HBV RNA may be generally feasible and enduring. Achieving undetectable serum HBV RNA could potentially emerge as a new pragmatic goal in NAs treatment.

Although great improvement has been achieved on the treatment of CHB, the disease still remains incurable. The primary objective of investigating NAs cessation in the management of chronic HBV infection is to identify the characteristics of patients who exhibit a relatively sustained response following withdrawal of the medication. HBsAg loss has been generally recognised as the endpoint of NAs treat-

ment.[4] However, the low incidence makes the vast majority of patients unable to achieve this goal. Previous studies have indicated that discontinuing NAs before HBsAg loss may be safe in carefully selected CHB patients.[10,18] Several serologic, virologic, or immunologic predictors have been explored to screen these CHB patients.[10] Relatively low HBsAg levels at EOT indicated certain immune control and its role for the decision of cessation have also been validated by previous studies.[6,11] Although the more stringent criteria (e.g., 10 IU/ml) ensure the safety of drug withdrawal, it is also more difficult to achieve. The significance of HBsAg at EOT <100 IU/ml has been explored by a previous meta-analysis,[11] however, the cumulative relapse rates after cessation remain relatively high. Meanwhile, in the current study, HBsAg <100 IU/ml at cessation did not show enough predictive value for virological relapse and clinical relapse when entering multivariate analysis with HBV RNA at the same time.

The pgRNA, which is transcript of cccDNA, serves as reverse transcription and translation templates for viral polymerase and core protein.[5] Although the mechanisms on the release of pgRNA from infected hepatocytes into the circulation remains unclear,[5] serum HBV RNA is closely related to the level of pgRNA in hepatocytes according to previous studies.[19,20] In view of this principle, this new biomarker may help identify the patients in whom NAs can safely be stopped.

In the current investigation, serum HBV RNA emerged as an independent predictor for virological relapse in both univariate and multivariate analyses, which was consistent with previous studies.[6,13,14] The relatively longer follow-up periods after NAs cessation (median 32 months in patients with sustained response) than previous studies[6,13] enhances the credibility of the research conclusion. Notably, serum HBV RNA undetectable, not below the LLQ, should be preferred according to this study's conclusions (Figure 1). For clinical relapse, the serum HBV RNA was only significant in multivariate Cox analysis. This phenomenon may be related to the early retreatment of relapse in this study. The criteria of rescue therapy after relapse are far from standardisation.[10,12,21,22] Late retreatment helps the immune clearance of HBsAg, but it may bring the risk of disease aggravation. Meanwhile, early retreatment is not conducive to host immune response to the virus. While earlier studies suggested that refraining from retreatment post-relapse predicted subsequent off-treatment HBsAg loss.[10,22] It is noteworthy that in the current cohort, the inclination was towards early retreatment for the safety of the patients. Nevertheless, a considerable number of included patients in this study achieved off-treatment HBsAg loss.

HBV infection suppresses host innate immunity to facilitate its persistence.[3] Immune exhaustion, marked by the impairment of natural killer cells, dendritic cells, and CD4+/CD8+ T cells, has been documented in CHB patients.[10] Discontinuation of NAs improved the performance of HBV-specific CD4+/CD8 + T cells, especially in individuals who encountered relapse.[10,23,24] According to conclusions of the present study and previous studies,[6,13] HBsAg loss after cessation tends to be achieved in patients with lower HBsAg levels. All individuals in the current study maintained a sustained virological response. Furthermore, the serum HBV RNA has emerged as a self-reliant indicator for the loss of HBsAg, emphasising the importance of achieving undetectable HBV RNA by the conclusion of treatment.

The relatively long follow-up periods and representativeness of HBsAg at EOT (median 735.5 IU/ml) should be the strength of the present study. Nonetheless, it is crucial to acknowledge certain limitations in the current study. Firstly, patients who underwent standardised NAs cessation with extended follow-up typically exhibited good compliance, introducing potential bias. Secondly, low-genetic barrier NAs were administered to some patients due to early enrollment, although this decision facilitated sufficient follow-up. The exclusion of patients with NAs resistance aimed to mitigate potential impacts from such medications. Additionally, the limited number of patients who attained HBsAg loss might introduce bias.

CONCLUSION

Serum HBV RNA serves as a valuable predictor for virological relapse, clinical relapse, and the loss of HBsAg after discontinuation of NAs. Diminished HBV RNA levels forecast a more favourable off-treatment response. Discontinuation of prolonged NAs therapy appears to be viable and safe in individuals with undetectable HBV RNA. In contrast to HBV RNA, HBsAg <100 IU/ml at cessation lacked sufficient predictive value for virological relapse and clinical relapse.

ETHICAL APPROVAL:
The study protocol has been approved by the Ethical Committee of the Second Hospital of Shandong University and followed the provisions of the Helsinki Declaration.

PATIENTS' CONSENT:
Written informed consent has been obtained from all the patients.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
SW, TL, FL: Study design, data collection, literature review and manuscript preparation.
LW, LZ: Study conception, critical review and supervision.
DY: Statistical analysis and data interpretation.
All authors approved the final version of the manuscript to be published.

REFERENCES

1. Seto WK, Lau EH, Wu JT, Hung IF, Leung WK, Cheung KS, *et al.* Effects of nucleoside analogue prescription for hepatitis B on the incidence of liver cancer in Hong Kong: A territory-wide ecological study. *Aliment Pharmacol Ther* 2017; **45(4)**:501-9. doi: 10.1111/apt.13895.
2. Leoni S, Casabianca A, Biagioni B, Serio I. Viral hepatitis: Innovations and expectations. *World J Gastroenterol* 2022; **28(5)**:517-31. doi: 10.3748/wjg.v28.i5.517.
3. Dusheiko G, Agarwal K, Maini MK. New approaches to chronic hepatitis B. *N Engl J Med* 2023; **388(1)**: 55-69. doi: 10.1056/NEJMra2211764.
4. Lok AS, Zoulim F, Dusheiko G, Ghany MG. Hepatitis B cure: From discovery to regulatory approval. *Hepatology* 2017; **66(4)**:1296-313. doi: 10.1002/hep.29323.
5. Deng R, Liu S, Shen S, Guo H, Sun J. Circulating HBV RNA: From biology to clinical applications. *Hepatology* 2022; **76(5)**:1520-30. doi: 10.1002/hep.32479.
6. Seto WK, Liu KS, Mak LY, Cloherty G, Wong DK, Gersch J, *et al.* Role of serum HBV RNA and hepatitis B surface antigen levels in identifying Asian patients with chronic hepatitis B suitable for entecavir cessation. *Gut* 2021; **70(4)**:775-83. doi: 10.1136/gutjnl-2020-321116.
7. Papatheodoridi M, Papatheodoridis G. Emerging diagnostic tools to decide when to discontinue nucleos(t)ide analogues in chronic hepatitis B. *Cells* 2020; **9(2)**:493. doi: 10.3390/-cells9020493.
8. Wang J, Shen T, Huang X, Kumar GR, Chen X, Zeng Z, *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(4)**:700-10. doi: 10.1016/j.jhep.2016.05.029.
9. Chen H, Ding X, Liao G, Xia M, Ren Z, Fan R, *et al.* Hepatitis B surface antigen kinetics after discontinuation of and retreatment with oral antivirals in non-cirrhotic HBeAg-positive chronic hepatitis B. *J Viral Hepat* 2021; **28(8)**:1121-9. doi: 10.1111/jvh.13526.
10. Tout I, Lampertico P, Berg T, Asselah T. Perspectives on stopping nucleos(t)ide analogues therapy in patients with chronic hepatitis B. *Antiviral Res* 2021; **185**:104992. doi: 10.1016/j.antiviral.2020.104992.
11. Liu J, Li T, Zhang L, Xu A. The role of hepatitis B surface antigen in nucleos(t)ide analogues cessation among Asian patients with chronic hepatitis B: A systematic review. *Hepatology* 2019; **70(3)**:1045-55. doi: 10.1002/hep.30474.
12. Kao JH, Jeng WJ, Ning Q, Su TH, Tseng TC, Ueno Y, *et al*. APASL guidance on stopping nucleos(t)ide analogues in chronic hepatitis B patients. *Hepatol Int* 2021; **15(4)**: 833-51. doi: 10.1007/s12072-021-10223-5.
13. Kaewdech A, Tangkijvanich P, Sripongpun P, Witeerungrot T, Jandee S, Tanaka Y, *et al.* Hepatitis B surface antigen, core-related antigen and HBV RNA: Predicting clinical relapse after NA therapy discontinuation. *Liver Int* 2020; **40(12)**: 2961-71. doi: 10.1111/liv.14606.
14. Fan R, Zhou B, Xu M, Tan D, Niu J, Wang H, *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(3)**:719-27.e7. doi: 10.1016/j.cgh.2019.07.046.
15. Liu Y, Jiang M, Xue J, Yan H, Liang X. Serum HBV RNA quantification: Useful for monitoring natural history of chronic hepatitis B infection. *BMC Gastroenterol* 2019; **19(1)**:53. doi: 10.1186/s12876-019-0966-4.
16. Liaw YF, Kao JH, Piratvisuth T, Chan HLK, Chien RN, Liu CJ, *et al.* Asian-Pacific consensus statement on the management of chronic hepatitis B: A 2012 update. *Hepatol Int* 2012; **6(3)**:531-61. doi: 10.1007/s12072-012-9365-4.
17. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HLK, Chen CJ, *et al.* Asian-Pacific clinical practice guidelines on the management of hepatitis B: A 2015 update. *Hepatol Int* 2016; **10(1)**:1-98. doi: 10.1007/s12072-015-9675-4.
18. Papatheodoridi M, Papatheodoridis G. Can we stop nucleoside analogues before HBsAg loss? *J Viral Hepat* 2019; **26(8)**:936-41. doi: 10.1111/jvh.13091.
19. Wang J, Yu Y, Li G, Shen C, Meng Z, Zheng J, *et al.* Relationship between serum HBV-RNA levels and intrahepatic viral as well as histologic activity markers in entecavir-treated patients. *J Hepatol* 2017; **S0168-8278(17)**:32261-4. doi: 10.1016/j.jhep.2017.08.021.
20. Giersch K, Allweiss L, Volz T, Dandri M, Lutgehetmann M. Serum HBV pgRNA as a clinical marker for cccDNA activity. *J Hepatol* 2017; **66(2)**:460-2. doi: 10.1016/j.jhep.2016.09.028.
21. Papatheodoridis GV, Manolakopoulos S, Su TH, Siakavellas S, Liu CJ, Kourikou A, *et al*. Significance of definitions of relapse after discontinuation of oral antivirals in HBeAg-negative chronic hepatitis B. *Hepatology* 2018; **68(2)**:415-24. doi: 10.1002/hep.29497.
22. Liaw YF. Hepatitis B flare after cessation of nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B: To retreat or not to retreat. *Hepatology* 2021; **73(2)**:843-52. doi: 10.1002/hep.31525.
23. Hall SAL, Burns GS, Mooney BJ, Millen R, Morris R, Vogrin S, *et al.* Hepatitis B virus flares after nucleot(s)ide analogue cessation are associated with activation of toll-like receptor signaling pathways. *J Infect Dis* 2022; **227(1)**:123-32. doi: 10.1093/infdis/jiac375.
24. van Bommel F, Berg T. Risks and benefits of discontinuation of nucleos(t)ide analogue treatment: A treatment concept for patients with HBeAg-negative chronic hepatitis B. *Hepatol Commun* 2021; **5(10)**:1632-48. doi: 10.1002/hep-4.1708.