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ORIGINAL ARTICLE

The 96-week clinical outcomes after cessation of nucleos(t)ide analog treatment in chronic hepatitis B patients

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Abstract

Background: Chronic hepatitis B (CHB) patients have a high virological relapse rate after cessation of nucleos(t)ide analog (NA) treatment, but the clinical outcome remains unclear. This study aimed to investigate the 96-week clinical outcomes and the risk factors for relapse in CHB after cessation of NAs.

Methods: This study was a prospective trial; 74 eligible patients were enrolled. The patients underwent NA cessation and follow-up according to the 2012 Asian Pacific Association for the Study of the Liver Guideline. Symptoms, biochemical (aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, urea nitrogen, creatinine), virological data (hepatitis B surface antigen [HBsAg], hepatitis B e antigen [HBeAg], hepatitis B e antibody [HBeAb], hepatitis B virus [HBV] DNA levels), and color Doppler ultrasound examination results were recorded and analysed.

Results: After NA cessation, 19 cases were HBsAg-negative without relapse during the 96-week follow-up. Of the 55 cases of HBsAg-positive after cessation, four types of clinical outcomes were observed. Twelve patients had no relapse during the 96-week follow-up (type A, 21.8%), 7 patients underwent virological relapses but spontaneously had a non-virological relapse (type B, 12.7%), 10 patients maintained virological relapse (type C, 18.2%), and 26 patients turned to clinical relapse, received NA retreatment, and achieved ALT normalization and negative conversion of HBV DNA within 12 months (type D, 47.3%). The 2-year overall cumulative rates of virological and clinical relapses were 58.1% and 24.3%, respectively. Independent factors associated with virological relapse were duration of negative HBV DNA, EOT (end of treatment) HBsAg, and original status of HBeAg. The EOT HBsAg was also an independent factor for clinical relapse.

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Conclusions: There are four types of clinical outcomes in patients with CHB after cessation of NA treatment. Further research is needed to explore the mechanism of different clinical outcomes. The EOT HBsAg level is an independent factor associated with both virological and clinical relapse.

Key words: chronic hepatitis B; nucleos(t)ide analogs; cessation; relapse; hepatitis B virus

Introduction

Hepatitis B virus (HBV) infection is a global public health problem, which is estimated to affect >2 billion people worldwide [1]. The World Health Organization (WHO) estimated that 257 million people or 3.5% of the population had chronic HBV infection in 2015 [2]. In China, 78 million people are estimated to have chronic hepatitis B (CHB) [3], accounting for nearly one-third of all global cases [4].

Currently, the first-line therapy for CHB includes nucleos(t)ide analogs (NAs) and interferon-α (IFN-α), of which NAs are the most widely used [5]. NA therapy can strongly inhibit reverse transcriptase of HBV and reduce serum HBV DNA levels in CHB patients [6]. However, NAs cannot eliminate covalently closed circular DNA (cccDNA) of HBV [7] because cccDNA is integrated into the genome of the host hepatocytes [8]. As a result, long-term (even lifelong) NA treatment is needed, which causes some concerns, such as large economic burden, low adherence, high adverse reactions, and drug resistance [9].

Several guidelines for the prevention and treatment of CHB by different societies, including the Asian Pacific Association for the Study of the Liver (APASL) [10], European Association for the Study of the Liver (EASL) [11], American Liver Disease, The American Association for the Study of Liver Diseases (AASLD) [12], the WHO [13], and Chinese Medical Association [14], recommend the cessation of NA therapy. However, a significant proportion of patients encounter relapse within 1 year after the cessation of NA therapy [15, 16]. Relapse may cause adverse events, such as disease progression, cirrhosis, liver cancer, and liver failure. Therefore, the feasibility and safety of NA cessation still need to be further evaluated. This study aimed to report the 96-week clinical outcomes and the predictors of relapse after cessation of NAs in CHB.

Patients and methods

Study design and subjects

This was a prospective trial. The CHB patients treated in the Department of Infectious Diseases of the Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) between December 2013 and December 2018 were enrolled. All patients were previously treated with NAs before discontinuation, including entecavir (ETV), telbivudine (LDT), lamivudine (LAM), adefovir dipivoxil (ADV), or a combination of LAM and ADV. This study was approved by the institutional review board of the Third Affiliated Hospital of Sun Yat-sen University (NO. [2015]2–81) on 25 March 2015 and all patients signed informed consent forms. This trial was registered on the Clinical Trials.gov Protocol Registration and Results System (registration code: NCT02883647). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. We have previously reported the 48-week outcome of this trial [17]. Among the 74 patients included for analysis in this study, 51 patients had been included in our previous report of the 48-week outcome of this trial [17].

Enrollment criteria were as follows: (i) CHB patients (sustained positive hepatitis B surface antigen [HBsAg] or HBV DNA for >6 months [2]) receiving NA treatment; (ii) aged 18–65 years; (iii) both the duration of NA treatment and HBV DNA-negative duration exceeded 2 years (HBV DNA test was performed at least three times, at least once every 6 months); (iv) for patients who were hepatitis B e antigen (HBeAg)-positive before NA treatment, the duration of HBeAg seroconversion and consolidation exceeded 1 year; and (v) before discontinuation, the Roche Cobas TaqMan HBV test showed 'not detected' or '<20 IU/mL'. Exclusion criteria were as follows: (i) combined with cirrhosis, hepatocellular carcinoma (HCC) or other malignant tumors; (ii) combined with hepatitis caused by other factors; (iii) pregnant or lactating women; (iv) combined with human immunodeficiency virus infection or other congenital immunodeficiency diseases; (v) with diabetes or other autoimmune diseases; or (vi) with severe organ dysfunction or complications (such as infection, ascites, bleeding, hepatic encephalopathy, hepatorenal syndrome).

Follow-up

The patients were followed up monthly for the first 3 months after cessation of NA treatment and every 3 months thereafter. At each follow-up, all symptoms were recorded, such as appetite, fatigue, and jaundice; the incidences of cirrhosis and HCC were calculated. All patients underwent blood routine tests (white blood cells, red blood cells, hemoglobin, platelets), blood biochemical tests (aspartate aminotransferase, alanine aminotransferase, total bilirubin, urea nitrogen, creatinine), virological tests (HBsAg, HBeAg, hepatitis B e antibody [HBeAb], HBV DNA levels), and liver ultrasound examination.

Definition and management of relapse

According to the APASL guidelines for the management of HBV infection [4], virological relapse was defined as HBV DNA level >2,000 IU/mL and clinical relapse was defined as HBV DNA level >2,000 IU/mL and alanine transaminase (ALT) exceeding two times the upper limit of normal (ULN). After NA discontinuation, if there was no relapse, the patient continued to be followed up.

Patients with virological relapse were treated as follows: if the ALT level was no more than two times the ULN, patients continued to be followed up; if patients had clinical relapse and the ALT level was between two times the ULN and five times the ULN, patients without symptoms continued to be followed up and patients with symptoms received NA treatment again (retreatment); if the patient had clinical relapse and ALT more than five times the ULN, NA retreatment was given. The NA retreatment for clinically relapsed patients included ETV, TDF, and the original NAs. After retreatment was started, patients were followed up monthly for the first 3 months and every 3 months thereafter. The relapse-management procedure is shown in Figure 2.

For patients who were HBsAg-positive after cessation, there were four types of clinical outcomes: Type A was defined as patients without virological relapse, the ALT level was normal during follow-up, and the HBV DNA level did not exceed

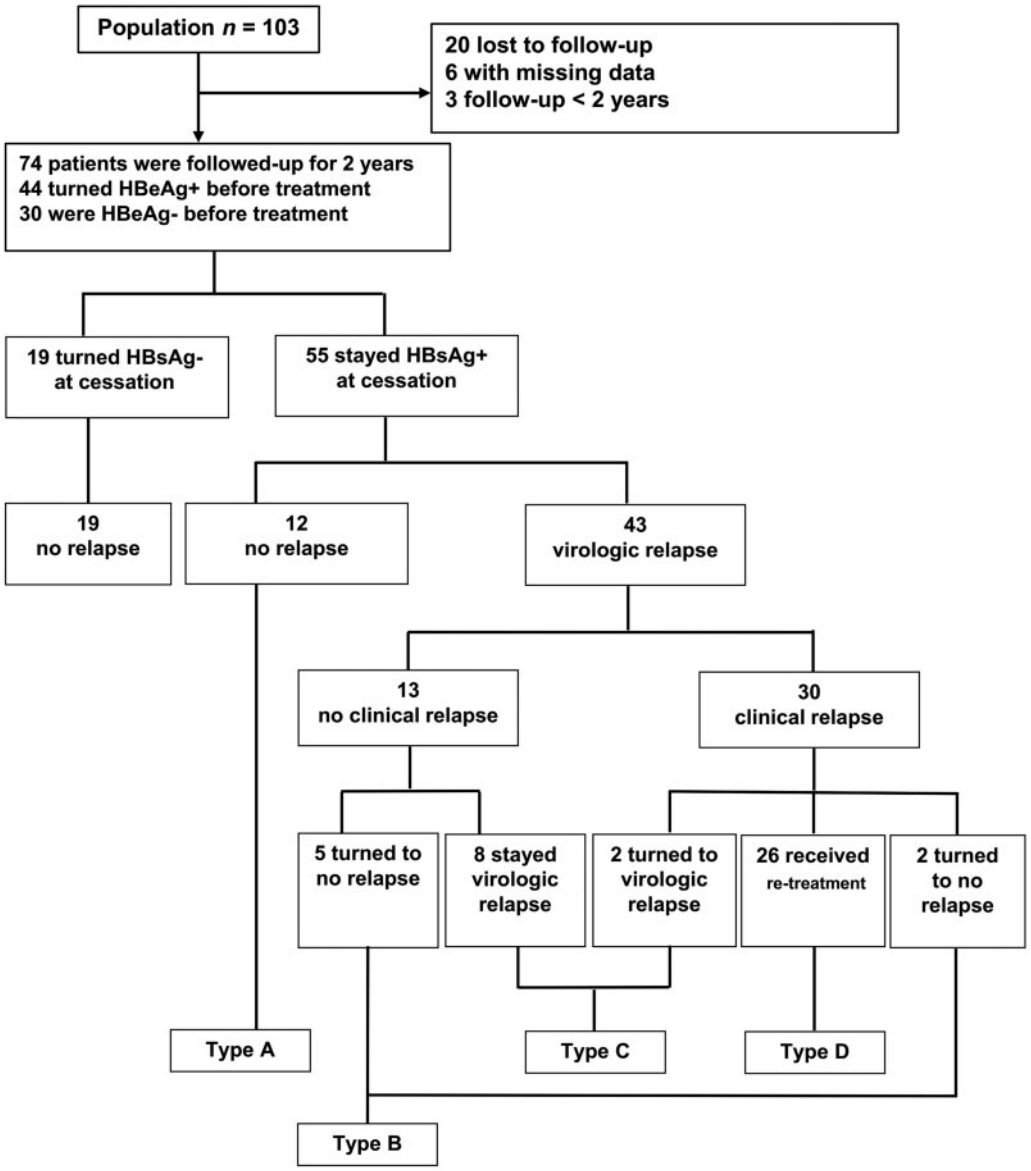

Figure 1. The CONSORT diagram of patient enrollment and clinical outcome

2,000 IU/mL; Type B was defined as patients with virological relapse that turned into no virological relapse without antiviral treatment and the HBV DNA level did not exceed 2,000 IU/mL at the end of the 96-week follow-up; Type C was defined as patients who had maintained virological recurrence, the ALT level was less than two times the ULN, and the HBV DNA level was >2000 IU/mL; Type D was defined as those who received antiviral therapy again (retreatment), the ALT level became normal and negative conversion of HBV DNA was achieved at 48 weeks after retreatment.

Statistical analysis

Continuous data are presented as mean ± standard deviation, whereas categorical data are presented as count and percentage (%). Student's independent *t*-test and one-way Analysis of Variance (ANOVA) were used to test the difference of means between two groups or among multiple groups (more than two). If the data normality assumption was not met, non-parametric tests were used to compare means between groups, including the Mann–Whitney *U* test and the Kruskal–Wallis test. Categorical data were tested with Chi-square test or Fisher's exact test (if there was any expected value <5).

The outcome variables included the occurrences of virological or clinical relapse at baseline (month 0) and 1, 2, 3, 6, 12, 15, 18, 21, and 24 months after cessation. Associations between independent variables and outcome variables were analysed using univariate/multivariate generalized estimating equation (GEE), binary logistic-regression models. An independent working correlation matrix was adopted for the repeated measurement data. The statistical significance level for all the tests was set at a *P*-value of <0.05. All statistical analyses were performed using IBM SPSS Version 20 (SPSS Statistics V20, IBM Corporation, Somers, New York).

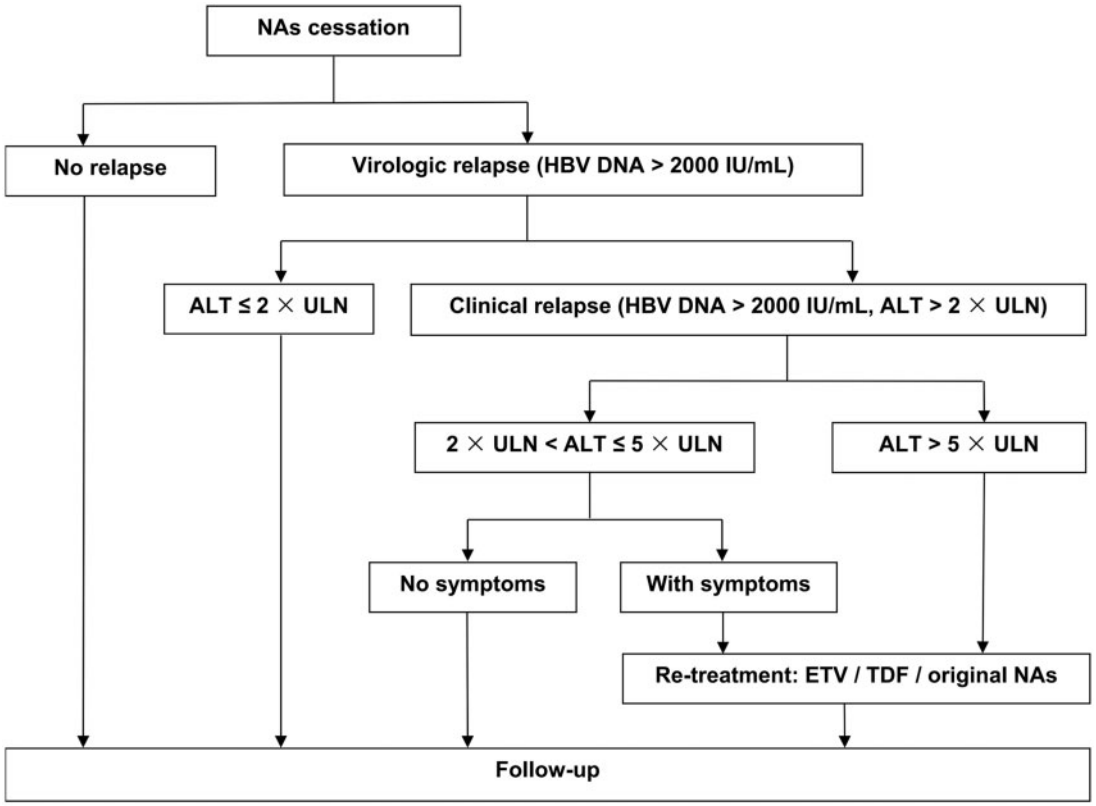

Figure 2. The flowchart of management of relapse after cessation of NA therapy. NAs, nucleos(t)ide analogs; ALT, alanine transaminase; HBV, hepatitis B virus; ULN, upper limit of normal; ETV, entecavir; LDT telbivudine; LAM, lamivudine; ADV, adefovir dipivoxil.

Results

Baseline characteristics

A total of 103 CHB patients were enrolled. There were 20 cases lost to follow-up, 6 cases with missing data, and 3 patients with a follow-up period of <2 years. Therefore, 74 eligible patients (51 males and 23 females) were finally included in this study. After treatment cessation, the mean age of the entire cohort was 36.6 ± 9.9 years and the body mass index was 21.8 ± 3.1 kg/m^2. The CONSORT diagram of patient enrollment and clinical outcome is shown in Figure 1. The demographic and clinical characteristics of the patients are shown in Table 1. The most common NA treatment before cessation was LDT (n = 28, 31.1%), followed by ETV (n = 23, 31.1%). As for the original status of HBeAg, 44 cases were positive while 30 cases were negative. After the cessation of NAs, 55 cases were positive for HBsAg while 19 cases were negative (Table 1).

Subgroup analysis stratified by HBeAg and HBsAg status

Subgroup analyses of patients' characteristics stratified by HBeAg and HBsAg status were conducted. As shown in Table 1, the only significant variable between HBeAg-negative and HBeAg-positive groups was age, where the mean age of the HBeAg-negative group was significantly higher than that of the HBeAg-positive group (41.8 ± 9.8 vs 33.0 ± 8.1 years, P < 0.001).

In the comparisons between HBsAg-negative and HBsAg-positive groups, it was found that the HBsAg-negative group had a significantly longer duration of NA treatment (5.6 ± 2.6 vs 4.3 ± 1.9 years, P = 0.044) and time of the negative conversion of HBV DNA (1.4 ± 1.4 vs 0.7 ± 1.0 years, P = 0.018), as well as lower levels of HBsAg after cessation (–0.2 ± 1.3 vs 3.0 ± 0.6 log10 (IU/mL), P < 0.001) than the HBsAg-positive group.

Subgroup analysis stratified by NA treatment before the cessation

Subgroup analysis stratified by different NA treatments showed that sex, duration of NA treatment, time of the negative conversion of HBV DNA, duration of negative HBV DNA maintenance, and the ratio of NA retreatment during follow-up were significantly different among the five subgroups (all P < 0.05; Table 2). An approximately equal sex ratio was found in the LDT group, whereas there were more males in the other four NA groups. The ADV groups had the longest duration of NA treatment and duration of negative HBV DNA maintenance, whereas the LDT group had the shortest duration. The LAM+ADV group and LAM group had the longest and shortest time of negative conversion of HBV DNA, respectively.

Clinical outcomes

Clinical outcomes of all patients are shown in Figure 2. Of the 55 patients who were HBsAg-positive after cessation, 12 became non-relapse (type A) and 43 had virological relapse. In the 43 patients with virological relapse, 7 spontaneously became non-relapse (type B), 10 maintained clinical relapse (type C), whereas the other 26 had clinical relapse and received retreatment (type D). Figure 3 demonstrates the changes in the levels of HBV DNA

Table 1. Subgroup analysis of patients' characteristics stratified by HBeAg and HBsAg status

	HBeAg (original status)			HBsAg (at cessation)			
Parameter	Negative ($n=30$)	Positive ($n=44$)	P	Negative ($n=19$)	Positive ($n=55$)	P	All ($n=74$)
Age, years	42.0 ± 9.8	33.0 ± 8.1	<0.001	36.7 ± 10.7	655	0.824	36.6 ± 9.9
Sex			0.089			0.603	
Male	24 (80.0)	27 (61.4)		14 (73.68)	37 (67.27)		51 (68.92)
Female	6 (20.0)	17 (38.6)		5 (26.32)	18 (32.73)		23 (31.08)
BMI, kg/m^2	22.5 ± 3.0	21.2 ± 3.0	0.125	21.5 ± 3.2	21.8 ± 3.1	0.619	21.8 ± 3.1
NA treatment			0.257			0.084	
ETA	11 (36. 7)	12 (27.3)		5 (26.3)	18 (32.7)		23 (31.1)
LDT	7 (23.3)	21 (47.7)		4 (21.1)	24 (43.6)		28 (37.8)
LAM	2 (6. 7)	3 (6.8)		1 (5.3)	4 (7.3)		5 (6.8)
ADV	4 (13.3)	4 (9.1)		5 (26.3)	3 (5.5)		8 (10.8)
LAM+ADV	6 (20.0)	4 (9.1)		4 (21.1)	6 (10.9)		10 (13.5)
Duration of NA treatment, years	4.7 ± 2.0	4.6 ± 2.3	0.608	5.6 ± 2.6	4.3 ± 1.9	0.044	4.6 ± 2.2
Time of the negative conversion of HBV DNA, years	0.8 ± 1.1	0.9 ± 1.2	0.313	1.4 ± 1.4	0.7 ± 1.0	0.018	0.9 ± 1.2
Duration of negative HBV DNA maintenance, years	3.9 ± 1.5	3.7 ± 2.0	0.384	4.2 ± 2.4	3.6 ± 1.5	0.603	3.8 ± 1.8
Duration of HBeAg seroconversion maintenance, years	-	3.1 ± 2.3	-	2.8 ± 2.5	3.3 ± 2.2	0.524	3.1 ± 2.3
EOT HBsAg, log10 (IU/mL)	2.1 ± 1.7	2.3 ± 1.6	0.578	−0.2 ± 1.3	3.0 ± 0.6	<0.001	2.2 ± 1.7
Reuse of NAs			0.170			<0.001	
No	15 (50.0)	29 (65.9)		19 (100.0)	25 (45.5)		44 (59.5)
ETV	10 (33.3)	5 (11.4)		0	15 (27.3)		15 (20.3)
LAM	0	1 (2.3)		0	1 (1.8)		1 (1.4)
LDT	2 (6.7)	5 (11.4)		0	7 (12.7)		7 (9.5)
TDF	3 (10.0)	4 (9.1)		0	7 (12.7)		7 (9.5)
Relapse after cessation group			0.291			<0.001	
No relapse	8 (26.7)	11 (25.0)		19 (100.0)	0		19 (25.7)
A	2 (6.7)	10 (22.7)		0	12 (21.8)		12 (16.2)
B	2 (6.7)	5 (11.4)		0	7 (12.7)		7 (9.5)
C	5 (16.7)	5 (11.4)		0	10 (18.2)		10 (13.5)
D	13 (43.3)	13 (29.6)		0	26 (47.3)		26 (35.1)
Relapse after cessation			0.062			–	
No	2 (9.1)	10 (30.3)		–	12 (21.8)		12 (21.8)
Yes	20 (90.9)	23 (69.7)		–	43 (78.2)		43 (78.2)

NA, nucleos(t)ide analogs; ETV, entecavir; LDT, telbivudine; LAM, lamivudine; ADV, adefovir dipivoxil; EOT, end of treatment.

Table 2. Subgroup analysis of patients' characteristics stratified by NA treatment

Parameter	ETA ($n = 23$)	LDT ($n = 28$)	LAM ($n = 5$)	ADV ($n = 8$)	LAM+ADV ($n = 10$)	P
Age, years	36.0 ± 9.9	34.7 ± 8.1	33.6 ± 13.0	43.9 ± 10.7	39.2 ± 10.8	0.191
Sex						0.003
Male	18 (78.3)	13 (46.4)	3 (60.0)	8 (100.0)	9 (90.0)	
Female	5 (21.7)	15 (53.6)	2 (40.0)	0 (0.0)	1 (10.0)	
BMI, kg/m^2	21.8 ± 3.2	21.9 ± 2.9	21.3 ± 4.8	21.0 ± 3.2	22.3 ± 2.6	0.829
Duration of NA treatment, years	4.5 ± 2.0	3.7 ± 1.6	5.0 ± 2.5	6.9 ± 2.1	5.5 ± 2.8	0.005
Time of the negative conversion of HBV DNA, years	0.9 ± 1.1	0.5 ± 0.6	0.4 ± 0.1	1.1 ± 0.8	1.9 ± 2.3	0.025
Duration of negative HBV DNA maintenance, years	3.7 ± 1.7	3.1 ± 1.3	4.7 ± 2.4	5.8 ± 1.8	3.7 ± 1.7	0.006
Duration of HBeAg seroconversion maintenance, years	2.9 ± 1.9	2.7 ± 1.6	2.9 ± 0.9	5.7 ± 2.7	3.8 ± 4.9	0.215
EOT HBsAg, log10(IU/mL)	2.3 ± 1.6	2.5 ± 1.6	2.5 ± 2.2	1.4 ± 1.3	1.7 ± 1.8	0.474
Reuse of NAs						0.037
No	13 (56.5)	16 (57.1)	3 (60.0)	7 (87.5)	5 (50.0)	
ETV	9 (39.1)	2 (7.1)	1 (20.0)	1 (12.5)	2 (20.0)	
LAM	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	
LDT	1 (4.4)	5 (17.9)	0 (0.0)	0 (0.0)	1 (10.0)	
TDF	0 (0.0)	5 (17.9)	0 (0.0)	0 (0.0)	2 (20.0)	
Relapse after cessation group						0.325
No relapse	5 (21.7)	4 (14.3)	1 (20.0)	5 (62.5)	4 (40.0)	
A	4 (17.4)	6 (21.4)	1 (20.0)	1 (12.5)	0 (0.0)	
B	2 (8.7)	4 (14.3)	0 (0.0)	0 (0.0)	1 (10.0)	
C	3 (13.0)	5 (17.9)	1 (20.0)	1 (12.5)	0 (0.0)	
D	9 (39.1)	9 (32.1)	2 (40.0)	1 (12.5)	5 (50.0)	
Relapse after cessation						0.505
No	4 (22.2)	6 (25.0)	1 (25.0)	1 (33.3)	0 (0.0)	
Yes	14 (77.8)	18 (75.0)	3 (75.0)	2 (66.7)	6 (100.0)	

NA, nucleos(t)ide analogs; ETV, entecavir; LDT, telbivudine; LAM, lamivudine; ADV, adefovir dipivoxil; EOT, end of treatment.

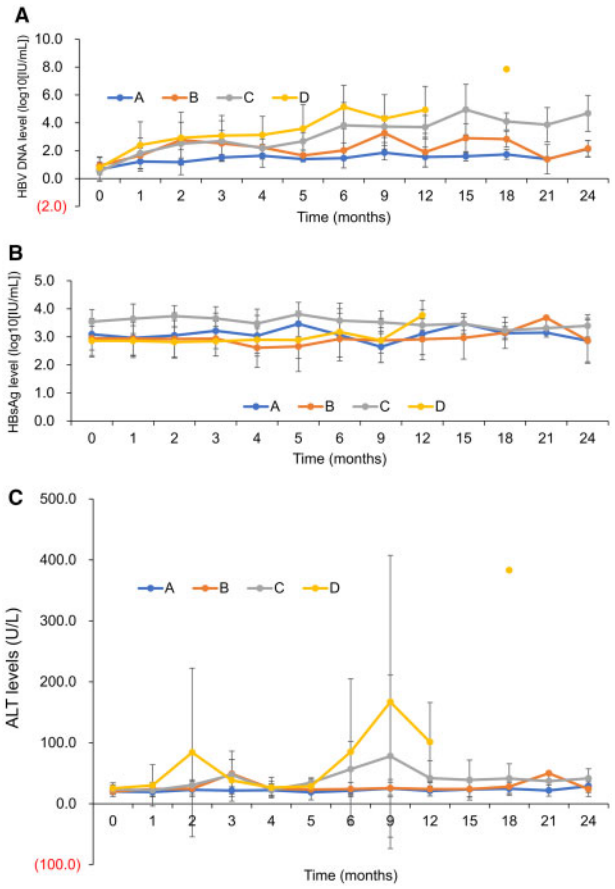

Figure 3. Time-dependent changes in the levels of HBV DNA (A), HBsAg (B), and ALT (C) of four types of clinical outcomes. ALT, alanine transaminase; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

(Figure 3A), HBsAg (Figure 3B), and ALT (Figure 3C) among these four types of clinical outcomes.

Comparison of virological and clinical-relapse cumulative rates stratified by HBeAg and HBsAg status

The 2-year cumulative rates of virological relapse of HBeAg-negative, HBeAg-positive, HBsAg-negative, HBsAg-positive, and all patients were 70.0%, 50.0%, 0%, 76.4%, and 58.1%, respectively (Figure 4A; log-rank test, $P < 0.001$). The 2-year cumulative clinical-relapse rates of HBeAg-negative, HBeAg-positive, HBsAg-negative, HBsAg-positive, and all patients were 26.7%, 22.7%, 0%, 32.7%, and 24.3%, respectively (Figure 4B; log-rank test, $P = 0.097$).

Comparison of virological and clinical-relapse cumulative rates stratified by NA treatment before the cessation

The 2-year cumulative rates of virological relapse of patients with ETA, LDT, LAM, ADV, and LAM+ADV before cessation were 60.9%, 60.7%, 60.0%, 25.0%, and 70.0%, respectively (Figure 5A; log-rank test, $P = 0.260$). The 2-year cumulative rates of clinical relapse of patients with ETA, LDT, LAM, ADV, and LAM+ADV before cessation were 17.4%, 25.0%, 20.0%, 12.5%, and 50.0%, respectively (Figure 5B; log-rank test, $P = 0.193$).

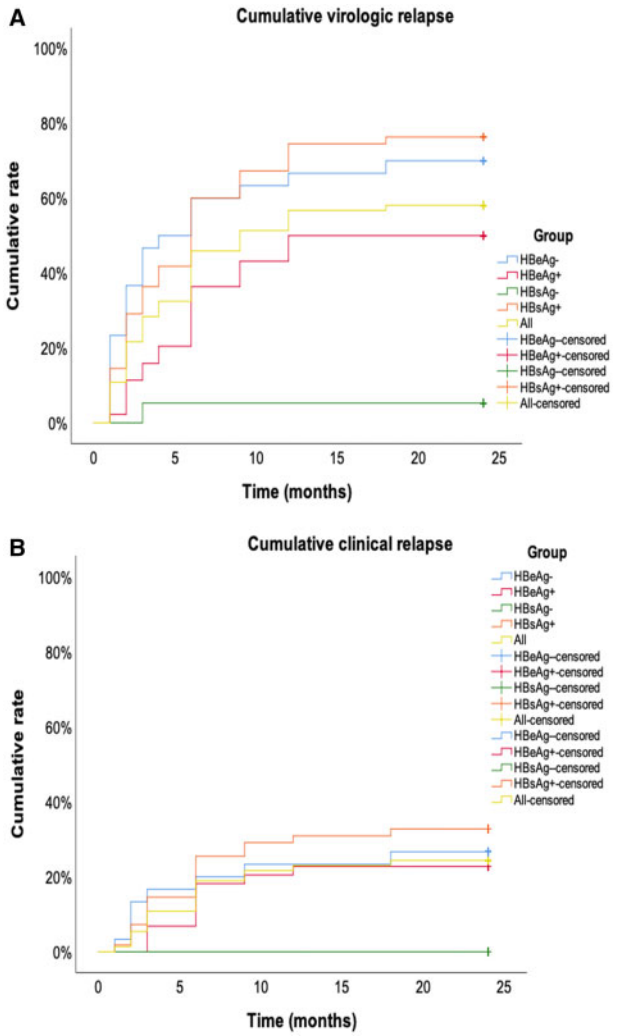

Figure 4. The cumulative relapse rates of HBeAg-negative, HBeAg-positive, HBsAg-negative, HBsAg-positive, and all patients, including virological relapse (A) and clinical relapse (B) HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B surface antigen.

Independent factors associated with virological and clinical relapse

To investigate the risk factor associated with virological and clinical relapse, logistic regression under the GEE model was performed. Independent variables that were significant in univariate results were included in the multivariate model.

As shown in Table 3, the significant factors associated with virological relapse were duration of negative HBV DNA maintenance (odds ratio [OR] = 0.71, 95% confidence interval [CI] = 0.55–0.92; $P = 0.010$), EOT (end of treatment) HBsAg (OR = 1.97, 95% CI = 1.47–2.64; $P < 0.001$), and original status of HBeAg (OR = 0.38, 95% CI = 0.18–0.77; $P = 0.008$). The longer duration of the negative HBV DNA maintenance period was associated with lower risk of virological relapse, whereas the higher EOT HBsAg level was associated with higher risk of virological relapse. A positive status of the original HBeAg was also a protective factor.

Table 4 shows the results of clinical relapse. Since the only significant variable in the univariate results was EOT HBsAg, the marginally significant variable, duration of negative HBV

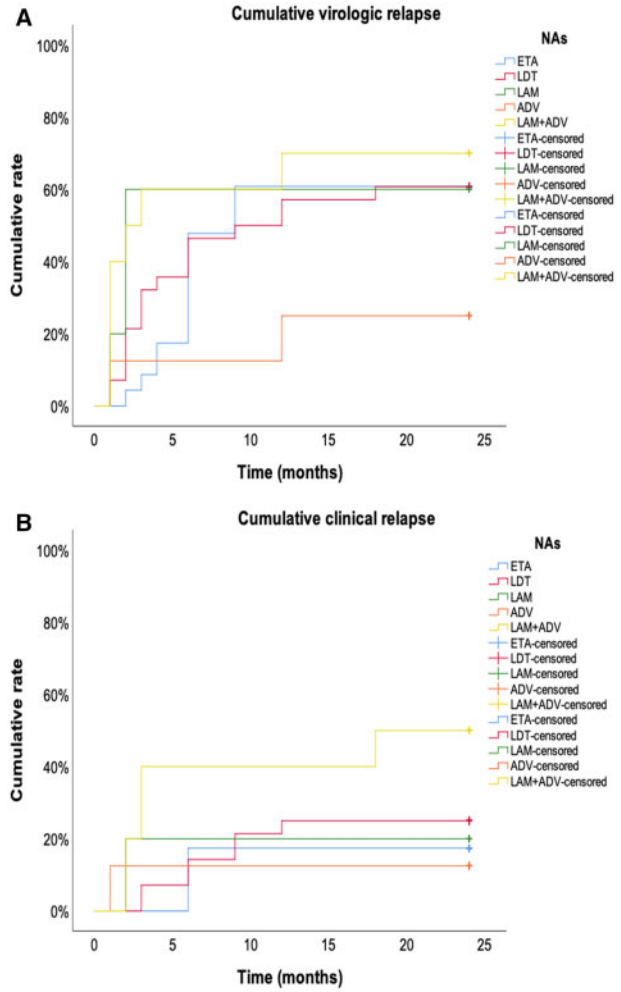

Figure 5. The cumulative relapse rates of patients using ETA, LDT, LAM, ADV, and LAM+ADV before cessation, include ng virological relapse (A) and clinical relapse (B) ETV, entecavir; LDT telbivudine; LAM, lamivudine; ADV, adefovir dipivoxil.

DNA maintenance ($P = 0.062$), was also included in the multivariate model as a controlled covariate. The only significant variable in multivariate results was EOT HBsAg (OR = 1.48, 95% CI = 1.11–1.97; $P = 0.008$). The higher EOT HBsAg level was associated with a higher risk of clinical relapse.

Discussion

Our results showed that, among the 74 enrolled CHB patients after the cessation of NAs, 19 were HBsAg-negative without relapse during the 96-week follow-up. The disappearance of HBsAg is defined as a functional cure for CHB. Of the 55 patients who were HBsAg-positive after cessation, 12 had no relapse during the 96-week follow-up (type A); 7 with virological relapses had non-virological relapse (type B); 10 maintained virological relapse (type C, 18.2%); and 26 had clinical relapse undergoing retreatment (type D). The 2-year cumulative virological and clinical-relapse rates were 58.1% and 24.3%, respectively.

In this study, 55 HBsAg-positive patients after cessation presented four different types of clinical outcomes during the 96-week follow-up. It has been found that eliminating viral antigens is important for T-cell-mediated restoration of immune function [18]. The use of NAs to reduce HBV viremia helps the restoration of HBV-specific T-cell function [19]. After cessation of NA treatment, different clinical outcomes might be attributed to the different extent of impairment of HBV-specific T-cell function. It is worth investigating the function of HBV-specific T-cells among CHB patients with different clinical outcomes after NA cessation. Our results showed that no significant difference was found in the HBsAg level among the patients with four types of clinical outcomes. These results indicated that the HBsAg level cannot be a predictive factor for relapse, which is in line with the findings reported by Seto *et al.* [20]. Among the 26 patients receiving NA retreatment (type D), 3 (11.5%) achieved HBsAg seroconversion, whereas none of the other three types of patients achieved HBsAg seroconversion during the 96-week follow-up, implying that NA retreatment at the appropriate time may have certain benefits.

Accumulating evidence from previous studies [14, 21, 22] and systematic reviews [23, 24] has suggested that the EOT HBsAg level is a predictive factor for relapse after cessation of NA therapy. Liu *et al.* [24] conducted a systematic review (including 11 studies with 1,716 patients) to explore the optimal cut-off value of the EOT HBsAg level for the cessation of NA therapy and found that an HBsAg level of <100 IU/mL at EOT could be a marker for the cessation of NA therapy. The guidelines of several liver-disease societies also recommend this notion [10–14]. Our results showed that the 19 cases who were negative for HBsAg after the cessation of NA therapy did not have a relapse during the 96-week follow-up. Moreover, logistic-regression analysis showed that the EOT HBsAg level was an independent factor for both virological and clinical relapses. As a downstream product of HBV cccDNA, HBsAg can reflect the level of cccDNA [25]. Nevertheless, there are no current drugs to clear HBsAg. Chevaliez *et al.* [26] have modeled long-term HBV DNA and HBsAg-level kinetics to estimate the time to clear HBsAg during NA therapy and found that the predicted median time to HBsAg clearance was 52.2 years. Zoutendijk *et al.* [27] have reported that the predicted median time to HBsAg loss was 36 and 39 years for HBeAg-positive and HBeAg-negative patients, respectively. Therefore, HBsAg clearance is difficult to be achieved by NA treatment.

We have previously reported the 48-week outcome of the current trial [17]. Among the 74 patients included in this study, 51 had been included in our 48-week report [17]. Consistently with this study, our 48-week outcome showed that the EOT HBsAg level (OR = 2.21, 95% CI = 1.47–3.32; $P < 0.001$) and original status of HBeAg (OR = 0.32, 95% CI = 0.14–0.74; $P = 0.008$) were predictive factors for virological relapse [17]. Nevertheless, we also observed some inconsistent findings between these two studies. For example, age was a predictive factor for virological relapse in the 48-week outcome but not in the 96-week outcome. Given the shorter follow-up duration and smaller sample size of the 48-week study, the data from the 96-week follow-up should be more reliable.

There are still some limitations of this study. First, it should be noted that HBsAg-level quantification is not accurate in the lower range of HBsAg. In addition, the sample size of this study is relatively small and the follow-up duration is short. Nevertheless, our short-term outcomes still provide useful reference data for clinicians. Moreover, the mechanism underlying different clinical outcomes after cessation of NA treatment remains to be further elucidated.

In summary, our results suggested that four types of clinical outcomes were observed in CHB patients after NA cessation.

Table 3. Independent variables associated with virological relapse in GEE models

	Univariate		Multivariate	
Parameter	OR (95% CI)	P	OR (95% CI)	P
Age, years	1.0 (1.0–1.1)	0.096		
Sex				
Male	ref.	–		
Female	0.7 (0.3–1.6)	0.446		
BMI, kg/m^2	1.0 (0.9–1.1)	0.735		
NA treatment				
ETA	ref.	–		
LDT	1.5 (0.7–3.3)	0.344		
LAM	1.2 (0.2–6.5)	0.837		
ADV	0.3 (0.1–1.6)	0.156		
LAM+ADV	1.7 (0.6–4.8)	0.299		
Duration of NA treatment, years	0.82 (0.7–1.0)	0.084		
Time of the negative conversion of HBV DNA, years	0.9 (0.6–1.3)	0.582		
Duration of negative HBV DNA maintenance, years	0.8 (0.6–1.0)	0.018	0.7 (0.6–1.0)	0.010
Duration of HBeAg seroconversion maintenance, years	1.0 (0.8–1.3)	0.872		
EOT HBsAg, log10(IU/mL)	1.9 (1.5–2.5)	<0.001	2.0 (1.5–2.6)	<0.001
HBeAg (original status)				
Negative	ref.	–	ref.	–
Positive	0.5 (0.2–0.9)	0.031	0.4 (0.2–0.8)	0.008

NA, nucleos(t)ide analogs; ETV, entecavir; LDT, telbivudine; LAM, lamivudine; ADV, adefovir dipivoxil; EOT, end of treatment.

Table 4. Independent variables associated with clinical relapse in GEE models

	Univariate		Multivariate	
Parameter	OR (95% CI)	P	OR (95% CI)	P
Age, years	1.0 (1.0–1.1)	0.298		
Sex				
Male	ref.	–		
Female	0.7 (0.3–1.9)	0.512		
BMI, kg/cm^2	1.0 (0.9–1.1)	0.751		
NA treatment				
ETA	ref.	–		
LDT	1.5 (0.4–5.0)	0.548		
LAM	0.8 (0.1–7.5)	0.868		
ADV	0.6 (0.1–6.3)	0.701		
LAM+ADV	3.1 (0.9–11.2)	0.079		
Duration of NA treatment, years	0.9 (0.7–1.1)	0.212		
Time of the negative conversion of HBV DNA, years	1.0 (0.6–1.4)	0.795		
Duration of negative HBV DNA maintenance, years	0.8 (0.6–1.0)	0.062	0.8 (0.6–1.0)	0.088
Duration of HBeAg seroconversion maintenance, years	0.9 (0.6–1.4)	0.540		
EOT HBsAg, log10(IU/mL)	1.5 (1.1–2.0)	0.007	1.5 (1.1–2.0)	0.008
HBeAg (original status)				
Negative	ref.	–		
Positive	1.0 (0.4–2.4)	0.939		

NA, nucleos(t)ide analogs; ETV, entecavir; LDT, telbivudine; LAM, lamivudine; ADV, adefovir dipivoxil; EOT, end of treatment.

The EOT HBsAg level is an independent factor associated with both virological and clinical relapse.

Author's Contributions

All authors contributed to the concept and design, patient recruitment, and follow-up of this study. L.P. and W.X. had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. W.X., Y.L., and J.L. were responsible for the acquisition, analysis, and interpretation of the data. W.X. drafted the manuscript. L.P. obtained the funding. L.P. and Z.G. took responsibility for the supervision. All authors approved the final version of the manuscript.

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Conflict of Interest

None declared.

References

1. Lavanchy D, Kane M, Global epidemiology of hepatitis B virus infection. In: YF Liaw and F Zoulim (eds). *Hepatitis B Virus in Human Diseases*. Cham: Humana Press, 2016, 187–203.
2. WHO. *Global Hepatitis Report*, 2017. https://cms.who.int/publications-detail-redirect/global-hepatitis-report-2017
3. Schweitzer A, Horn J, Mikolajczyk RT *et al.* Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet* 2015;**386**:1546–55.
4. Zhang S, Wang F, Zhang Z. Current advances in the elimination of hepatitis B in China by 2030. *Front Med* 2017;**11**:490–501.
5. Sarin SK, Kumar M, Lau GK *et al.* Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 2016;**10**:1–98.
6. Huang H, Wang J, Li W *et al.* Serum HBV DNA plus RNA shows superiority in reflecting the activity of intrahepatic cccDNA in treatment-naïve HBV-infected individuals. *J Clin Virol* 2018;**99-100**:71–8.
7. Seeger C, Mason WS. HBV replication, pathobiology and therapy: unanswered questions. *J Hepatol* 2016;**64**:S1–3.
8. Allweiss L, Dandri M. The role of cccDNA in HBV maintenance. *Viruses* 2017;**9**:156.
9. Serviddio G. *Practical Management of Chronic Viral Hepatitis*. Rijeka: InTech, 2013.
10. Liaw YF, Kao JH, Piratvisuth T *et al.* Asian-Pacific consensus statement on the management of chronic hepatitis B: A 2012 update. *Hepatol Int* 2012;**6**:531–61.
11. EASL. European Association for the Study of the Liver (EASL) 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017;**67**:370–98.
12. Terrault NA, Chang KM, Hwang JP *et al.* Update on prevention, diagnosis, and treatment and of chronic hepatitis B: AASLD 2018 hepatitis B guidance purpose and scope of the guidance HHS public access. *Hepatology* 2018;**67**:1560–99.
13. WHO. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. *https://www.who.int/hepatitis/publications/hepatitis-b-guidelines/en/*
14. Chen CH, Hung CH, Hu TH *et al.* Association between level of hepatitis B surface antigen and relapse after entecavir therapy for chronic hepatitis B virus infection. *Clin Gastroenterol Hepatol* 2015;**13**:1984–92.e1.
15. Papatheodoridis GV, Manolakopoulos S, Su TH *et al.* Significance of definitions of relapse after discontinuation of oral antivirals in HBeAg-negative chronic hepatitis B. *Hepatology* 2018;**68**:415–24.
16. Ma TL, Hu TH, Hung CH *et al.* Incidence and predictors of retreatment in chronic hepatitis B patients after discontinuation of entecavir or tenofovir treatment. *PLoS One* 2019;**14**:e0222221.
17. Xu WX, Zhang Q, Zhu X *et al.* 48-week outcome after cessation of nucleos(t)ide analogue treatment in chronic hepatitis B patient and the associated factors with relapse. *Can J Gastroenterol Hepatol* 2018;**2018**:1–11.
18. Bertoletti A, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. *Gut* 2012;**61**:1754–64.
19. Zhang E, Kosinska A, Lu M *et al.* Current status of immunomodulatory therapy in chronic hepatitis B, fifty years after discovery of the virus: search for the "magic bullet" to kill cccDNA. *Antiviral Res* 2015;**123**:193–203.
20. Seto WK, Hui AJ, Wong VWS *et al.* Treatment cessation of entecavir in Asian patients with hepatitis B e antigen negative chronic hepatitis B: a multicentre prospective study. *Gut* 2015;**64**:667–72.
21. Liu F, Wang L, Li XY *et al.* Poor durability of lamivudine effectiveness despite stringent cessation criteria: a prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients. *J Gastroenterol Hepatol* 2011;**26**:456–60.
22. Liang Y, Jiang J, Su M *et al.* Predictors of relapse in chronic hepatitis B after discontinuation of anti-viral therapy. *Aliment Pharmacol Ther* 2011;**34**:344–52.
23. Papatheodoridis G, Vlachogiannakos I, Cholongitas E *et al.* Discontinuation of oral antivirals in chronic hepatitis B: a systematic review. *Hepatology* 2016;**63**:1481–92.
24. Liu J, Li T, Zhang L *et al.* 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**:1045–55.
25. Wang DD, Lz YI, Ln WU *et al.* Relationship between maternal PBMC HBV cccDNA and HBV serological markers and its effect on HBV intrauterine transmission. *Biomed Environ Sci* 2019;**32**:315–23.
26. Chevaliez S, Hézode C, Bahrami S *et al.* Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. *J Hepatol* 2013;**58**:676–83.
27. Zoutendijk R, Hansen BE, Van Vuuren AJ *et al.* Serum HBsAg decline during long-term potent nucleos(t)ide analogue therapy for chronic hepatitis B and prediction of HBsAg loss. *J Infect Dis* 2011;**204**:415–8.