Risk factors for relapse after discontinuation of tenofovir or entecavir in hepatitis B e Antigen-Negative Patients

Original Article

Running title: Risk of relapse after treatment discontinuation

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ABSTRACT

Background & Aims: This study aimed to define the relapse frequency and risk determinants in chronic hepatitis B (CHB) patients who discontinued nucleos(t)ide analog (NA) treatment, were HBeAg-negative, and had a virological and biochemical response.

Materials and Methods: This retrospective cohort study reviewed patients with HBeAg-negative CHB who received antiviral therapy for at least 65 months between January 1, 2013, and December 31, 2020, discontinued treatment, and had a biochemical and virological response. Patients were evaluated at 6, 12, and 24 months after treatment discontinuation.

Results: 67 patients with CHB who received NA therapy for at least 65 months but discontinued treatment and had negative HBV DNA and normal ALT values were evaluated. After NA cessation, relapse was observed in 38 patients (56.7%). The relapse rate was 71.0% in patients treated with tenofovir disoproxil fumarate (TDF) as the last NA type and 37.9% in patients treated with entecavir (ETV) (p=0.017). The cutoff value for the best estimate of age for relapse was 42 years. The relapse rate was 69.2% in patients aged ≥42 years and 39.2% in patients aged <42 years (p=0.007). The relapse rate was 51.3% in patients with a pre-treatment fibrosis score of 2, 56.0% in those with a fibrosis score of 3, and 100% in those with a fibrosis score of 4 (p=0.089).

Conclusion: Among HBeAg-negative CHB patients who achieved a virological and biochemical response to long-term antiviral therapy, those aged 42 years and older, those with high fibrosis scores before treatment, and those who used TDF before treatment cessation should be closely followed for relapse, especially in the first 12 months after stopping NA treatment.

Keywords: Hepatitis B, Discontinued treatment, Relapse

INTRODUCTION

The World Health Organization estimated that as of 2019, 296 million people worldwide were living with chronic hepatitis B (CHB) infection, and only 6.6 million of these patients were on treatment.1 The main purpose of CHB infection treatment is to prevent potential negative consequences of the disease such as cirrhosis, hepatic decompensation, hepatocellular carcinoma (HCC) and death.2 In current guidelines, first-line therapy for CHB is nucleos(t)ide analogues (NA) tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), and entecavir (ETV), with their high barrier to resistance and appropriate safety profiles, as well as leading to long-term undetectable HBV DNA levels in compliant patients.2, 3 CCC (covalently closed circular) DNA, defined as the stable form of HBV-DNA, is not eradicated with these treatment regimens. Therefore, long-term NA therapy, which suppresses HBV and provides regression of histological activity and fibrosis, is currently the best therapeutic strategy to prevent adverse outcomes such as HCC and cirrhosis.2, 4, 5 However, this disease, which requires long-term treatment, raises concerns regarding drug compliance, side effects, and costs.5 For this reason, the concept of NA discontinuation emerges as a strategy to consider when the treatment with the recommended rules does not provide more benefit.6

The ideal endpoint in the treatment of non-cirrhotic CHB is confirmed sustained hepatitis B surface antigen (HBsAg) seroconversion with or without anti-HBs seroconversion. 2, 3, 7 Although this is associated with favourable clinical outcomes, it is observed in long-term follow-up and the annual seroclearance rate in patients treated with NA is only 0.33% 7. Therefore, other endpoints such as HBV suppression (virological remission), normalization of alanine aminotransferase (ALT) level (biochemical remission) and hepatitis B e antigen (HBeAg) seroconversion have been defined in the evaluation of efficacy. 2 Achievement of these endpoints has also been shown to lead to histologic improvement and/or regression of liver fibrosis. 4, 6

In the European Association for the Study of the Liver (EASL) 2017 guideline, it was suggested that NA can be discontinued with some conditions if close monitoring can be guaranteed, except for HBsAg seroconversion.2 In HBe Ag positive and non-cirrhotic patients, it is recommended that NA can be discontinued with at least 12 months of consolidation treatment after stable HBeAg seroconversion and undetectable HBV DNA.2 In non-cirrhotic HBeAg-negative CHB patients, it is stated that NA therapy can be discontinued after at least 2 years of treatment and after ≥3 years of virological suppression.2, 6 In the American Association for the Study of Liver Diseases (AASLD) 2018 guideline, it is stated that it can be considered only in non-cirrhotic patients who develop HBsAg loss, although there is not sufficient and definitive evidence for discontinuation of treatment. In addition, it is recommended that people who discontinue NA treatment should be closely monitored every 3 months for at least 1 year in terms of virologic relapse, ALT exacerbations and clinical decompensation.3

In recent years, studies evaluating the potential benefits, safety and efficacy of discontinuation of long-term NA treatment have been emerged. 6 Although there is ongoing debate on this issue, in real-life practice, discontinuation of long-term NA treatment should be practiced when better or similar outcomes can be achieved than continuing treatment. 8-10 For this, it is important to know the predictors of relapse or sustained remission in patients who terminate long-term NA treatment in order to determine which patients should not stop treatment. Therefore, our study aimed to define the frequency of relapse and risk predictors in HBeAg-negative, virologically and biochemically responded chronic hepatitis B patients who discontinued NA treatment.

MATERIALS AND METHODS

This retrospective cohort study was conducted in a tertiary center in Southern Anatolia, where the prevalence of HBV is higher than in the rest of the country. Between 1 January 2013 and 31 December 2020, all HBeAg-negative CHB patients were evaluated. A total of 67 patients

with available data who had received antiviral treatment for at least 65 months, discontinued treatment, had biochemical and virological response when discontinued were identified. The biochemical response was defined as ALT within normal limits (<40U/L), and the virological response was defined as undetectable HBV DNA.11

Age, gender, place of residence, date of the first diagnosis, presence of concomitant Anti HCV, Anti Delta, steatohepatitis, liver histology (fibrosis and HAI), Baseline HBV DNA, ALT level, date of initiation of treatment, antiviral treatment given, treatment change, if any, total treatment time and negative viral load durations of the patients included in the study were recorded. Demographic and clinical data were obtained from patients' electronic medical records.

Patients who discontinued antiviral therapy were followed monthly for the first 6 months and then every 3 months. Patients with clinical relapse were re-treated according to the severity of the relapse. ALT, HBV DNA, total bilirubin, and INR levels at 6, 12, and 24 months after discontinuation of treatment, development of any relapse or flare, and any re-initiation of therapy were recorded. In our study, relapse was defined as an increase in HBV DNA above 2000 IU/ml; ALT elevation was defined as higher than 1-2 times normal; flare was defined as HBV-DNA>2.000 IU/ML and ALT> ULN X 10.

All collected blood samples were tested on the same regularly calibrated analyser. Biochemical tests were performed at the hospital's central laboratories using routine automated techniques. Serum hepatitis markers including HBsAg, HBsAg antibody, HBeAg, HBeAg antibody, anti-hepatitis D virus and anti-hepatitis C virus were tested using the Enzyme Immunoassay kit (Abbott Diagnostics, North Chicago, IL). Serum HBV DNA was assayed using the Roche Cobas Amplicor TaqMan HBV Monitor assay (detection limit, 20 IU/mL; Roche Diagnostics, Pleasanton, CA). Patients whose file records could not be reached were excluded from the study.

Statistical Analysis

All tests were analyzed using the statistical package SPSS, version 21.0 (SPSS Inc, an IBM Company). Continuous variables were presented as median (interquartile range [IQR]). Categorical variables were summarized as frequency and percentage. Chi-square or Fisher's exact test was used to test the relationship between two categorical variables. Non-parametric comparisons between two groups were made using the Mann-Whitney test. The Receiver-operating characteristic (ROC) curve was used to evaluate the predictive power of age for relapse, and the Youden Index (J = Sensitivity + Specificity – 1) was used to determine the cut-off point. Cumulative relapse rate estimates were obtained using the Kaplan-Meier method. The Log-Rank method was used to compare the Kaplan-Meier curves. Cox proportional hazard regression models were used to estimate the risk of relapse for some variables. To identify independent prognostic factors, a multivariate Cox regression model was created with significant associations in univariate analyses and age, pre-treatment fibrosis score, and last NA type before treatment discontinuation. Factors with significance or trends ($P<0.10$) in univariate analyses were used in this model. Hazard ratios (HR) and their 95% confidence intervals (CI) along with corresponding P values are presented. A P value of <0.05 was considered to be statistically significant.

RESULTS

Patients' main characteristics

After stopping the treatment, 67 CHB patients who had received NA treatment for at least 65 months were negative for serum HBV DNA, serum HBeAg, Anti-HCV, and Delta antibody and had normal ALT values. Their median age was 44 (youngest – oldest; 29 – 69), and 36 (53.7%) were male. The main characteristics of the patients are presented in **Table 1**. Initial

NA therapy was changed in 7 (10.4%) patients. After initial treatments, two (3.0%) patients were switched from ETV, ADF, and 3TC to TDF, and one (1.5%) switched from TDF to ETV. The last type of NA used before cessation was ETV in 43.3% and TDF in 56.7% of patients. The median duration of treatment with NA was 78 months, the duration of virological remission during treatment was 69 months, and the lowest duration of virological remission was 61 months.

Virological and biochemical relapse

The frequencies of relapses at 6 and 12 months according to the HBV DNA cut-off points of 2,000-20,000 IU/ml and >20,000 IU/ml and the upper limit of the ALT value are presented in **Figure 1**. After cessation of NA, relapse was observed in 38 (56.7%) patients. After discontinuation of NA, relapse developed at 6 months in 22 (32.8%) patients and 12 months in 16 (23.9%) patients. No virological and biochemical relapses were observed in the 24th-month follow-up. Virological relapse occurred mostly at the defined cut-off point of HBV DNA 2000-20000 IU/ml. At both 6 and 12 months after treatment cessation, ALT was 1-2 times higher than normal in those with HBV DNA of 2,000-20,000 IU/ml and 10 times above normal in those with HBV DNA >20,000 IU/ml. In 3 patients who developed flare with ALT> 10x ULN and HBV DNA> 20,000 IU / ml, total bilirubin was between 2-10 mg/dl and INR was high. Re-treatment was started in all patients who developed relapse. Virological and biochemical remission was achieved at 12 months in all patients who relapsed at 6 months, and at 24 months in all patients who relapsed at 12 months.

Relapse indicators

In univariate Cox regression analyses, a higher fibrosis score before NA treatment (p=0.043) and use of TDF as the last NA treatment before discontinuation (p=0.017) were found to be significantly associated with the risk of relapse. There was a trend towards a significant

relationship with older age (p = 0.066). A multivariate Cox regression model was established with age, pre-treatment fibrosis score, and last NA type before discontinuation of treatment. In this model, there was no independent risk factor, but there was a significant trend between the probability of relapse and use of TDF as the last NA treatment before discontinuation (p=0.070) (**Table 2**).

At 24 months after discontinuing treatment, relapse was observed in 71.0% of patients with TDF as the last NA type and in 37.9% of patients with ETV (p=0.012) (**Figure 2A**). In the ROC analysis performed to evaluate the predictive power of age for relapse, the best predictive power was 0.611 (95% CI; 0.529 – 792 p=0.025), and the cut-off value was 42 years, with 71.0% sensitivity and 62.0% specificity. The relapse rate at the end of 24 months was 69.2% in patients aged ≥42 years and 39.2% in patients aged <42 years (p=0.007) (**Figure 2B**). At the end of 24 months, the relapse rate was 51.3% in patients with a pre-treatment fibrosis score of 2, 56.0% in those with a fibrosis score of 3, and 100% in those with a fibrosis score of 4 (p=0.089) (**Figure 2C**).

DISCUSSION

In the management of CHB, how long NA should be continued in patients with long-term virologic suppression has not yet been concluded due to the concern of HBV relapse.12, 13 Studies evaluating the frequency of virological relapses after NA discontinuation in CHB patients who achieved a virological response with long-term treatment have reported variable cut-off values for relapse. For example, studies in the literature that accepted the lowest value of HBV-DNA as the cut-off for virological relapse showed virological relapse over 80%.12, 14 In the first of the two previous systematic reviews, the virological remission rate at 12 months after discontinuation of therapy in HBeAg-negative patients was 43.7% at 12 months, while the virological relapse rate was <70% and the clinical relapse rate was 50% in the other one.15, 16. Similar to our study, in a prospective observational study in which virological relapse in

HBeAg-negative patients was defined as HBV DNA >2000 IU/mL, virological relapse was reported in approximately half of the patients within 24 months after discontinuation of treatment despite long-term viral suppression. 17 The majority of relapses occur within the first year after discontinuation of treatment.16, 18 This is consistent with the fact that all relapses occurred in the first 12 months during our 24-month follow-up study.

In the literature, one of the determinants of relapse after discontinuation of treatment in CHB patients with long-term virological suppression is the last type of NA used.13 In our study, the median of NA treatment was 78 months, and the frequency of virological and biochemical relapses 24 months after cessation of treatment was lower in those who received ETV as the last treatment compared to those who received TDF. In two previous studies by Jeng W-J et al., the frequency of clinical relapse within 1 year after discontinuation of ETV treatment was 45.3%, while it was higher (52%) in TDF, as in our study.18, 19 It has been shown that the timing of clinical and virological relapses after TDF cessation in HBeAg-negative patients is much earlier than after ETV cessation, as in our study. 13, 20 In addition, in one of these studies, multivariate analyses identified being in the TDF group as an independent factor for relapse. 13 In our study, although no statistically significant independent risk factor was found in the multivariate analysis, receiving TDF treatment increased the risk of recurrence by 1.97 times, which tended to be statistically significant. The mechanism underlying the different relapse frequencies between ETV and TDF discontinuation has not been clearly explained. The different relapse behavior of NAs has been attributed to the different immunomodulatory abilities of these therapies 13, 20, 21

Several studies on HBeAg-negative CHB patients after discontinuation of NAs have shown that age is the only predictive factor for relapse, with lower relapse rates found in younger patients. 9, 21 In our study, although age was not an independent indicator for relapse, it was found that older age had a significant relationship trend. In a study conducted in Asia-Pacific, where CHB

infection mostly develops through perinatal transmission, relapse developed in more than three quarters of patients aged 25 years and over after NA discontinuation, and 25 years of age was considered a cutoff value for continued response in HBeAg-negative CHB patients.22 A longer period of HBV infection in the elderly was thought to affect persistence by complicating viral clearance. In addition, it was noted that younger patients may have a stronger immune system, which may have an impact on the eventual eradication of the virus. 9, 21 In our study, this cut-off value was determined as 42 years, and the rate of relapse was found to be higher at the end of 24 months in those aged ≥42 years. The higher age limit determined in our study compared to previous studies may be related to intrafamilial transmission as well as vertical transmission of CHB infection in the region where our study was conducted.23

One of the main reasons for the renewed interest in discontinuing antiviral therapy is the potential for HBsAg loss. An observational study found a very high HBsAg seroclearance rate in HBeAg-negative patients during a 5-year follow-up after ceasing adefovir therapy, though this could not be demonstrated in subsequent studies.9 However, it remains controversial whether the rate of HBsAg loss is higher after discontinuation of therapy than with continuous therapy.6 After a mean follow-up of 3 years, a randomized trial reported a significantly greater loss of HBsAg and reduction in quantitative HBsAg in patients who discontinued treatment compared to those who continued treatment.10 On the other hand, another study showed that the rate of HBsAg loss was approximately 1% in those who stopped or continued treatment, and there was no difference between the groups. However, it is noteworthy that the median post-treatment follow-up period (72 months) in this study was shorter. 24 Follow-up time may explain the different results in these studies. In our study, HBsAg loss was not observed in any patient during the 24-month follow-up period. Although evaluation with longer follow-up times is needed, this finding may also be related to the limited benefit of discontinuing NA therapy.

In patients with cirrhosis, treatment discontinuation is not recommended under any circumstances before HBsAg loss due to the risk of liver decompensation.2, 3 Besides, in a study conducted among HBeAg-negative patients with compensated cirrhosis who were treated with ETV for at least 12 months and achieved HBV DNA suppression, there was no significant difference between those who continued and discontinued treatment in terms of liver-related complications or death after a median 59-month follow-up.25 Based on these studies originating from the Asia Pacific, the APASL (The Asian Pacific Association for the Study of the Liver) guideline stated that possible discontinuation of NA therapy before HBsAg seroclearance may be considered in patients with compensated cirrhosis, provided a careful non-treatment monitoring plan.11 Another study with an 18-month follow-up period reported that among HBeAg-negative CHB patients who received treatment for 4 years or more, the rate of not re-initiating treatment was higher in patients with pre-treatment fibrosis score ≤ 3 compared to those with a fibrosis score of 4 (cirrhosis). In addition, an independent relationship was demonstrated between the severity of fibrosis score and the need for re-treatment.26 In our study, all patients with a baseline fibrosis score of 4 developed relapses at 12 months, and treatment was re-initiated. None of these 5 patients died or developed liver decompensation.

All of the patients included in our study were HBeAg-negative and had positive Anti-HBe titres. The absence of HBeAg-positive patients in the study group is a limitation of our study. In addition, HBsAg levels and other promising predictors such as anti-HBc, HBV RNA and HBcrAg were not evaluated in this study. Finally, cessation of NA treatment was assumed to be a trigger for re-induction of the T cell response against hepatocytes, and no immunological evaluation was performed to define this interaction.

In conclusion, among HBeAg-negative CHB patients who achieved a virological and biochemical response with long-term antiviral therapy, those aged 42 years and older, those with high fibrosis scores before treatment, and those who used TDF before treatment cessation

should be followed closely for relapse, especially in the first 12 months after stopping NA treatment.

Ethics Committee Approval: Ethics: Ethics committee approval was obtained from xxxxx University Faculty of Medicine, Non-Interventional Clinical Studies Ethics Committee (number 77 on 21.01.2022).

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Table 1. Main characteristics of CHB patients who discontinued effective and long-term NA therapy

Initiation of nucleos(t)ide analogue therapy	
Age (years), median (IQR)	44 (38 – 51)
Gender (male), n (%)	36 (53.7)
ALT† (U/L), n (%)	
Normal	15 (22.4)
>1–2 x ULN	19 (28.4)
>2 x ULN	33 (49.3)
HBV-DNA (IU/ml), n (%)	
>2 million	10 (14.9)
2 thousand – 20 thousand	16 (23.9)
20 thousand – 2 million	41 (61.2)
Presence of steatohepatitis, n (%)	14 (20.9)
Fibrosis score, median (IQR)	2 (2 – 3)
Histological activity index, median (IQR)	7 (7 – 8)
Initial NA treatment, n (%)	
Adefovir	2 (3.0)
Lamivudine	2 (3.0)
Tenofovir disoproxil fumarate	30 (44.8)
Entecavir	33 (49.3)
Change in initial therapy, n (%)	7 (10.4)
Last NA before treatment discontinuation, n (%)	
Tenofovir disoproxil fumarate	38 (56.7)
Entecavir	29 (43.3)
NA‡ treatment duration (months), median (IQR)	78 (73 – 81)
Virological remission duration during NA treatment (months), median (IQR)	69 (64 – 73)

†ALT: Alanine Aminotransferase

‡NA: Nucleos(t)ide Analogue

IQR: Interquartile range

Table 2. Regression analysis of cumulative relapse with cox proportional hazards

	Cumulative relapse (n=36)			
	Univariate analysis HR (95% CI)	*P	Multivariate analysis HR (95% CI)	P*
Initiation of nucleos(t)ide analogue therapy				
Age (Per year increase)	1.032 (0.998 – 1.067)	**0.066**	1.027 (0.992 – 1.064)	0.128
Gender, male vs female	1.314 (0.685 – 2.521)	0.412		
Steatohepatitis, yes vs no	1.641 (0.794 – 3.392)	0.181		
Fibrosis score (Per score increase)	1.737 (1.019 – 2.961	**0.043**	1.498 (0.869 – 2.582)	0.146
Histological activity index (Per index increase)	0.850 (0.682 – 1.058)	0.145		
ALT†>1-2 X ULN vs Normal ALT	0.806 (0.349 – 1.864)	0.615		
ALT†≥ 2 X ULN vs Normal ALT	0.672 (0.309 – 1.461)	0.316		
HBV DNA 20 thousand-2 million vs 2 thousand – 20 thousand	1.091 (0.489 – 2.435)	0.831		
HBV DNA >2 million vs 2 thousand – 20 thousand	1.055 (0.361 – 3.083)	0.922		
Last type of NA‡ before treatment discontinuation, TDF vs ETV§	2.358 (1.163 – 4.779)	**0.017**	1.973 (0.947 – 4.111)	0.070
NA‡ treatment duration (months)	0.965 (0.922 – 1.009)	0.121		
Virological remission duration during NA treatment (months)	1.000 (0.952 – 1.051)	0.992		

†ALT: Alanine Aminotransferase ‡NA: Nucleos(t)ide Analogue, §TDF: Tenofovir Disoproxil Fumarate, ETV: Entecavir

CI: Confidence intervalİ

*P<0.05

Figure Legend:

Fig.1. Frequencies of virological relapses according to HBV DNA cut-off points at 6 and 12 months after discontinuation of NA treatments

Fig.2. Relapse rates after discontinuation of the nucleos(t)ide analogue in chronic hepatitis B patients; (A) last NA treatment type, (B) age and (C) pre-treatment fibrosis score