Received: 14 September 2017 | First decision: 5 October 2017 | Accepted: 21 January 2018

DOI: 10.1111/apt.14560

WILEY AP&T Alimentary Pharmacology & Therapeutics

Stopping nucleos(t)ide analogue treatment in Caucasian hepatitis B patients after HBeAg seroconversion is associated with high relapse rates and fatal outcomes

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Funding information
Foundation Against Cancer Belgium, Grant/Award Number: 2014-087

Summary

Background: Stopping nucleos(t)ide analogues (NA) after hepatitis B e antigen (HBeAg) seroconversion is associated with high relapse rates in Asian patients, but data in Caucasian cohorts are scarce. Clinical course, outcomes and immunological aspects of chronic hepatitis B infections differ substantially between distinct ethnicities.

Aim: The aim of this study was to determine relapse rates, factors predicting relapse and clinical outcomes after nucleos(t)ide analogue cessation in a large, predominantly Caucasian cohort of chronic hepatitis B patients with nucleos(t)ide analogue-induced HBeAg seroconversion.

Methods: This is a nationwide observational cohort study including HBeAg positive, mono-infected chronic hepatitis B patients with nucleos(t)ide analogue-induced HBeAg seroconversion from 18 centres in Belgium.

Results: A total of 98 patients with nucleo(s)tide analogue-induced HBeAg seroconversion were included in the study. Of the 62 patients who stopped treatment after a median consolidation treatment of 8 months, 30 relapsed. Higher gamma-glutamyl transferase levels at both treatment initiation (HR 1.004; $P = 0.001$ per unit increment) and HBeAg seroconversion (HR 1.006; $P = 0.013$ per unit increment) were associated with an increased risk of clinically significant relapse in a multivariate Cox regression model. Treatment cessation led to liver-related death in 2 patients, of whom one showed a severe flare. Of the patients who continued treatment after HBeAg seroconversion, none relapsed or developed severe hepatic outcomes.

Conclusion: Treatment withdrawal in Caucasian chronic hepatitis B patients after nucleos(t)ide analogue-induced HBeAg seroconversion results in viral relapses in more than half of patients with potential fatal outcomes. These real-world data further lend support to preferentially continue NA treatment after HBeAg seroconversion until HBsAg loss.

The Handling Editor for this article was Professor Grace Wong, and it was accepted for publication after full peer-review.
The author's complete affiliation are listed in Appendix 1.

1 | BACKGROUND

Chronic hepatitis B virus (HBV) infections are a major global health problem, affecting 250 million people worldwide.[1,2] Clinical presentation remains often subclinical for decades, but serious liver-related complications and liver-related death develop in one-fourth of all untreated patients.[1,3] Standard oral treatment consists of nucleos(t)ide analogues (NA) and suppresses, but does not eradicate the virus.

Hepatitis B e antigen (HBeAg) seroconversion is an important landmark during the natural disease course of a chronic HBV infection and is used to monitor NA treatment efficiency. It is associated with an improved immune control of the HBV and marks the transition from a HBeAg-positive hepatitis phase to a HBeAg-negative chronic infection state with limited risk of further disease progression.[4-6]

It remains controversial whether NA treatment can be stopped after NA-induced HBeAg seroconversion, as relapse rates of up to 50% have been observed.[7-9] This is reflected by contrasting recommendations of the 3 major international scientific hepatological associations: Asian guidelines recommend to stop treatment after HBeAg seroconversion, whereas American and European guidelines favour treatment continuation, but allow discontinuation in selected patients with close subsequent monitoring.[5,10,11] Several factors such as longer consolidation therapy and lower hepatitis B surface antigen (HBsAg) and HBV DNA levels at treatment cessation have previously been linked to a decreased chance of relapse. A recent meta-analysis could, however, not substantiate these associations.[12] In addition, there is currently little evidence of the effect of treatment cessation after HBeAg seroconversion on liver-related outcomes such as hepatocellular carcinoma, liver decompensation and cirrhosis.[11,13,14]

So far published studies assessing the clinical consequences of NA treatment stop after HBeAg seroconversion mainly focus on Asian patients.[12] However, it is well appreciated that the host's ethnicity influences HBV's natural history and immune responses.[4,15] Asian patients show, for example, lower spontaneous or treatment-induced HBsAg seroclearance rates, and have higher HBV relapse rates in HBeAg-negative patients after treatment cessation.[16] Clinical hepatocellular carcinoma risk scores developed in Asian cohorts seem to be unreliable in Caucasian and especially African patients, the latter developing hepatocellular carcinoma at much younger ages.[17,18] Firm associations of immune-related gene polymorphisms with HBV persistence, seroconversion, seroclearance and disease progression vary between ethnicities. For example, the HLA-DR polymorphism rs 9277535 (550 A/G) is strongly associated with chronic hepatitis B and its outcomes in Asian, but not in African-American or Caucasian patients.[19,20] In addition, Asian and Caucasian patients have different HBV-related T-cell epitopes.[21] It therefore remains to be determined whether HBV relapse rates, factors predicting relapse and clinical outcomes after NA cessation are similar in Caucasian and Asian patients. These data are, however, difficult to obtain, given the practice of NA continuation after HBeAg seroconversion in most European countries and the USA.[5,11]

In contrast to most national guidelines on HBV in Europe, the Belgian HBV reimbursement criteria impose to stop NA treatment after 6 months consolidation therapy following HBeAg seroconversion. This makes Belgium a unique country to investigate the outcome after NA cessation following HBeAg seroconversion in Caucasian patients.[22] We investigated relapse rates, factors predicting clinically significant relapse and clinical outcomes after NA treatment stop in a nationwide multicentre study.

2 | METHODS

2.1 | Study design

We retrospectively included chronic HBeAg-positive HBV-infected patients with NA-induced HBeAg seroconversion between 1998 and 2016 from 18 centres in Belgium. Patients were followed at the outpatient hepatology clinics every 3-6 months according to the international treatment guidelines applicable at that time. Exclusion criteria were concomitant infection with the hepatitis C virus (HCV), hepatitis delta virus (HDV) and/or human immunodeficiency virus (HIV) and the long-term use (>2 weeks) of immunosuppressive therapy up to 6 months before NA therapy was initiated. The study was approved by the ethical committees of the Antwerp University Hospital and participating centres (no. EC15/44/464) in November 2015.

2.2 | Data collection

HBeAg-positive patients were included consecutively from the availability of electronic medical records onwards. All patient charts were individually analysed (n = 2090) for NA-use (n = 533) and subsequent NA-induced HBeAg seroconversion (n = 115). HBeAg seroconversion and HBsAg loss/seroconversion were defined as the loss of HBeAg or HBsAg and the appearance of anti-HBeAg and anti-HBsAg antibodies on two occasions, respectively, at least 1 month apart. Consolidation therapy was defined as the time between HBeAg seroconversion and treatment stop. Belgian NA reimbursement criteria impose to stop antiviral therapy 6 months after HBeAg seroconversion. However, some patients stopped treatment before the end of a 6-month consolidation therapy, either at their own initiative or at the discretion of the treating physician. Other patients continued treatment beyond 6 months after HBeAg seroconversion, either because the treating physician asked for an exception to the prevailing reimbursement criteria or because the patient was included in a NA clinical trial or medical need programme. For cirrhotic patients, antiviral treatment with lamivudine is reimbursed since 2010 irrespective of alanine aminotransferase (ALT) levels, HBeAg seroconversion and HBV viral load. This implicates that treatment continuation in cirrhotic patients after HBeAg seroconversion was not reimbursed between 2002 and 2010.

Data on demographics (date of birth, gender, ethnicity), biochemistry (ALT, aspartate aminotransferase [AST], gamma-glutamyl transferase, alkaline phosphatases [ALP]), virology (HBeAg and HBsAg

status, HBV viral load, HCV, HDV and HIV status), previous treatment type (interferon/lamivudine/adefovir/entecavir/tenofovir) and duration, fibrosis stage and liver disease severity were collected systematically in one case report form per patient.

Fibrosis was assessed histologically using the Metavir score on pre-treatment liver biopsies in 92.5% of the patients. The Belgian reimbursement criteria for HBV treatment require a liver biopsy prior to treatment start, except for patients with haemophilia or concomitant anticoagulant use. Laboratory tests were performed by the local or tertiary referral centres of the NA stop study group. Viral load, serological assays and biochemical parameters were measured using standardised automated methods. The upper limit of normal (ULN) for ALT was defined as 40 IU/mL. The limit of quantification for HBV DNA varied between 20 and 357 IU/mL depending on the time of determination (2009 and later vs 1998). Positive HBV DNA values but below the limit of quantification were given the arbitrary value of the lowest limit of quantification. Results indicating HBV DNA levels below the limit of detection ("Negative") were coded as 0.

2.3 | Clinical outcomes

Virologic relapse was defined as a single elevation of HBV DNA >2000 IU/mL. Combined relapse included ALT levels >2 × ULN (biochemical relapse) and virologic relapse. Relapse was considered persistent if detected consecutively on two or more occasions at least 3 months apart. Relapse was considered clinically significant if persistent or if immediate retreatment was deemed necessary by the treating physician. Sustained response was defined as persistent HBV DNA <2000 IU/mL after treatment stop. HBeAg reversion was defined as the reappearance of HBeAg. Both HBsAg loss and HBeAg reversion had to be confirmed on two consecutive samples at least 1 month apart. Hepatic decompensation was defined as the presence of variceal bleeding, ascites and/or encephalopathy. The diagnosis of hepatocellular carcinoma and cirrhosis was made according to international guidelines.[23]

2.4 | Statistical analysis

The date of treatment cessation was defined as baseline. Predictive factors for clinically significant relapse were calculated using a Cox proportional hazard regression model. Follow-up time was calculated as time from baseline to the first date of relapse detection or loss-to-follow-up (LTFU) or HBsAg loss. Cumulative rates of clinically significant relapse were plotted on a Kaplan-Meier curve. A Receiver Operating Characteristics (ROC) curve was applied to assess the discriminatory power of predictive factors for clinically significant relapse. Continuous data were presented as Mean ± *SD* or median (interquartile range [IQR]). A Mann-Whitney *U* test, student *t* test or one-way ANOVA was used to compare continuous variables, whereas a chi-squared test was used to compare categorical variables between two groups. Data were analysed in SPSS version 23.0 (SPSS, Chicago, IL, USA) All statistical tests were two-sided and $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

Of 2090 identified HBeAg-positive patients, 533 fulfilled the NA treatment reimbursement criteria and subsequently started NA treatment during the inclusion period (1998-2016). 356/533 had a HBV mono-infection and were not taking immunosuppressive drugs (see Figure 1). Confirmed NA-induced HBeAg seroconversion was observed in 115/356 (32%) patients after a median treatment duration of 17.7 months (IQR 9.7-32.4). Nine patients showed simultaneous HBsAg loss. Eight patients were LTFU immediately after treatment stop and were therefore excluded (Figure 1). The analysed cohort thus consisted of 98 predominantly male (75%) patients of mostly Caucasian (68%) origin with a median follow-up duration of 5.8 years (IQR 3.1-9.9) after treatment start. Treatment was stopped in 62/98 patients after a median consolidation therapy of 8.0 (IQR 4.6-14.9) months and was continued in 36/98 patients upon the discretion of the treating physician. An overview of all patient characteristics after HBeAg seroconversion is depicted in Table 1. The continuous treatment group consisted of more cirrhotic patients (39% vs 18%; $P = 0.028$) and had a shorter follow-up time (median 2.1 years vs 5.3 years; $P < 0.001$) after HBeAg seroconversion than the NA stop group. There was no difference in time to HBeAg seroconversion (median 18.8 vs 14.2 months; $P = 0.916$) between both groups.

3.2 | Relapse rates

During a median follow-up time of 3.8 years (IQR 1.6-7.0) after treatment stop, 30/62 patients (48.3%) relapsed of whom 16 showed only a virologic and 14 a combined virologic and biochemical relapse. Relapse was transient in 7 patients, persistent in 10 patients and another 13 patients were retreated after a single relapse detection (HBV DNA elevation >2000 IU/mL). The cumulative rate of clinically significant relapse (either persistent relapse or immediately retreated relapse) was 31%, 45%, 51%, 51%, 58% and 63% after 1, 2, 3, 4, 5 and 6 years, respectively, with 33, 24, 16, 14, 12 and 8 patients still in follow-up and event-free at the respective time points (Figure 2).

Twenty relapsed patients started retreatment (4 lamivudine, 12 tenofovir, 1 adefovir, 3 entecavir) with subsequent complete viral DNA suppression in all. Three patients showed persistent virologic relapse without ALT-rise and were therefore not retreated. Two more patients in the sustained response group were retreated proactively due to a HBV DNA rise <2000 IU/mL, despite having normal ALT levels and were therefore considered to be sustained responders.

Demographic and treatment-related characteristics were similar between patients irrespective of their virologic outcome after NA stop (Table 2). Similarly, there was no difference in HBV DNA levels at treatment start and HBeAg seroconversion. At treatment start and cessation, however, higher ALT levels were noted in patients going on to develop transient flares ($P = 0.010$). One patient in the

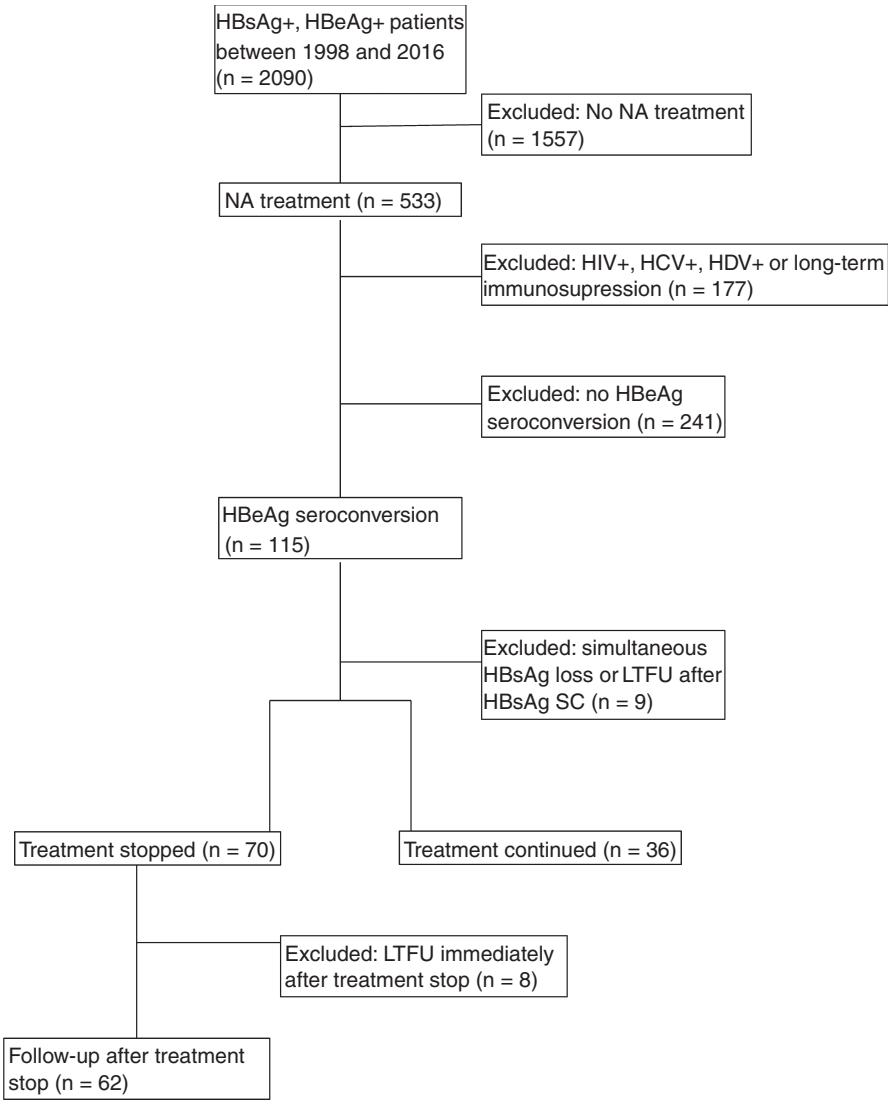

FIGURE 1 Flowchart of the patients included in the study. NA, nucleos(t)ide analogues; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; SC, seroconversion; HIV, human immunodeficiency virus; HDV, hepatitis delta virus; HCV, hepatitis C virus; LTFU, loss-to-follow-up

transient relapse group stopped therapy at his own initiative without HBV DNA suppression <2000 IU/mL, resulting in a transient peak HBV DNA flare of 4.16 log IU/mL, but with subsequent virus control <2000 IU/mL. There was no difference in peak HBV DNA (Mean 6.74 ± 2.08 log IU/mL vs 6.64 ± 1.40 log IU/mL; $P = 0.998$) and peak ALT (median 2.8 ULN vs 2.5 ULN; $P = 0.980$) after treatment stop between patients who developed a persistent relapse and those who were immediately retreated, indicating that the decision of the treating physician to immediately restart treatment was justified (Table 2). Patients with a persistent relapse showed a milder onset of relapse as compared to those in need of immediate retreatment, but the final severity of relapse was not different between the persistent relapse group and the immediate retreatment group (Table 2). As the clinical relapse severity did not differ between both groups, they were combined in further statistical modelling to predict clinically significant relapse rates.[24] A total of 6 noncirrhotic patients showed HBeAg reversion within 1 year after treatment cessation. This was accompanied by combined virologic and biochemical relapse. No relapse was detected in the group who continued treatment after HBeAg seroconversion.

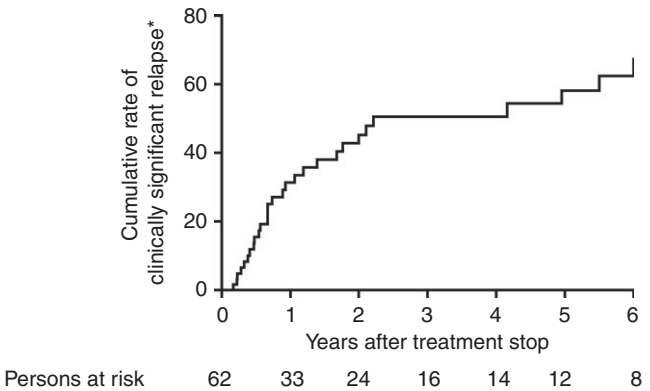

FIGURE 2 Cumulative rates of clinically significant relapse after treatment cessation. *Clinically significant = persistent or immediately retreated relapse

3.3 | Factors predicting clinically significant relapse

Factors assessed for predicting clinically significant relapse in an univariate cox regression model are depicted in Table 3. Among

TABLE 1 Patient characteristics grouped per treatment status after HBeAg seroconversion

	NA stopped (n = 62)	NA continued (n = 36)	*P*-value
Male gender (n)	45 (73%)	30 (83%)	0.210
Previous interferon treatment (n)	18 (29%)	12 (33%)	0.660
Ethnicity			
Asian	11	7	0.947
African	7	5	
Caucasian	43	24	
Other	1	0	
HBeAg seroconversion			
Age[a] (y)	42.9 ± 16.9	43.2 ± 17.5	0.924
ALT[b] (ULN)	0.8 (0.6-1.0)	0.9 (0.6-1.5)	0.126
HBV DNA[a] (^{10}log)	1.57 ± 1.12	1.55 ± 1.22	0.963
Gamma-GT[b] (IU/mL)	22 (16-50)	35 (18-68)	0.721
Treatment at HBeAg seroconversion			
Lamivudine	28	9	0.569
Tenofovir	9	18	
Adefovir	7	3	
Entecavir	14	6	
Lamivudine + Adefovir	4	0	
Time to HBeAg seroconversion (mo)[b]	18.8 (10.1-28.6)	14.2 (6.1-28.3)	0.916
FU-time post-HBeAg seroconversion (mo)[b]	64.1 (28.0-100.6)	24.7 (12.2-49.0)	<0.001
FU-time post-treatment stop (mo)[b]	46.4 (19.7-85.3)	/	
Start of treatment			
ALT[b] (ULN)	2.8 (2.0-5.1)	4.0 (1.7-8.1)	0.407
HBV DNA[a] (^{10}log)	6.91 ± 1.63	6.92 ± 1.81	0.950
Presence of cirrhosis (n)	11 (18%)	14 (39%)	0.028
Gamma-GT[b] (IU/mL)	53 (23-98)	70 (48-192)	0.205
Histological fibrosis at start of treatment			
Metavir F0-F1	18	8	0.215
Metavir F2	19	7	
Metavir F3-F4	21	17	

Continuous variables were compared using a student *t* test, categorical variables using a Chi-squared test.
ULN, Upper Limit of Normal (40 IU/mL); SC, Seroconversion; NA, nucleos(t)ide Analogues: /, not applicable; FU, follow-up; ALT, alanine aminotransferase; FU, follow-up; HBV, hepatitis B virus.
[a]Mean ± *SD*.
[b]Median (IQR).

those, only gamma-GT levels at start of treatment (HR 1.004; $P = 0.001$ per unit increment) and at HBeAg seroconversion (HR 1.006; $P = 0.014$ per unit increment), but not at treatment stop (HR 1.004; $P = 0.083$ per unit increment), were predictive of clinically significant relapse (Table 3). Gamma-GT levels at HBeAg seroconversion (Table 3) and treatment start (not shown) remained significantly associated with clinically significant relapse when corrected for age in multivariate cox regression models. Of the 9 patients with a gamma-GT <14 IU/mL at HBeAg seroconversion, none relapsed within 2 years after treatment cessation. Patients with a gamma-GT above 50 IU/mL at HBeAg seroconversion (n = 13) had a 75% chance of clinically significant relapse within 2 years after treatment cessation. Relapse and subsequent retreatment was observed in 7 patients with gamma-GT >50 IU/mL at HBeAg seroconversion in our cohort. None of the patients mentioned excessive alcohol use (>10 Units/week). A ROC curve (Figure S1) showed an area under the curve (AUC) of 0.613 for gamma-GT at HBeAg seroconversion, indicating a moderate discriminatory power to predict clinically significant HBV relapse. No other factors were significantly related to the risk of clinically significant relapse, except for gamma-GT to platelet ratio (GPR), a non-invasive fibrosis marker, at treatment start (Table 3). When corrected for gamma-GT levels at treatment start in a multivariate cox regression model, only gamma-GT at treatment start ($P = 0.025$) remained predictive of clinically significant relapse. Overall, this suggests that the association of gamma-GT levels at

TABLE 2 Patient and treatment-related characteristics according to the outcome after treatment stop

	Sustained response (n = 32)	Transient relapse (n = 7)	Persistent relapse (n = 10)	Immediate retreatment (n = 13)	*P*-value
General					
Male gender	23 (71.9%)	5 (71.4%)	7 (70.0%)	10 (76.9%)	0.984
Caucasian ethnicity	24 (75.0%)	5 (71.4%)	5 (50.0%)	9 (69.2%)	0.538
Cirrhotic patients	5 (15.6%)	2 (28.6%)	1 (10.0%)	1 (7.6%)	0.695
Treatment at HBeAg SC	14 LAM, 4 TDF, 3 ADV, 9 ETV, 2 LAM+ADV	3 LAM, 3 ADV, 1 ETV	4 LAM, 2 TDF, 2 ETV, 2 LAM+ADV	7 LAM, 3 TDF, 1 ADV, 2 ETV	0.587
Age at HBeAg SC (y)[a]	43.53 ± 17.99	42.71 ± 18.38	43.50 ± 16.76	41.15 ± 15.10	0.979
Time to HBeAg SC (mo)[b]	12.0 (8.2-21.5)	26.4 (23.5-72.9)	24.7 (18.0-58.4)	13.6 (10.6-47.6)	0.018
Consolidation therapy (mo)[b]	7.5 (4.0-15.5)	14.7 (7.6-25.2)	7.1 (4.0-14.5)	7.8 (4.3-13.8)	0.473
Follow-up time after treatment stop (mo)[b]	33.5 (15.4-82.3)	59.4 (20.2-79.1)	75.0 (21.5-96.5)	62.4 (29.6-114.9)	0.599
At treatment start					
HBV DNA (^{10}log IU/mL)[a]	7.23 ± 1.75	5.89 ± 1.37	7.14 ± 1.50	6.55 ± 1.46	0.202
ALT (ULN)[b]	2.8 (1.9-4.9)	7.8 (3.5-10.5)	2.4 (1.9-11.7)	2.5 (1.7-3.75)	0.019*
Gamma-GT (IU/mL)[b]	43 (19-73)	132 (51-190)	83 (37-169)	47 (23-198)	0.144
At HBeAg seroconversion					
HBV DNA (^{10}log IU/mL)[a]	1.41 ± 1.29	1.77 ± 1.34	1.65 ± 0.85	1.98 ± 0.79	0.753
ALT (ULN)[b]	0.8 (0.5-1.0)	0.7 (0.6-1.3)	0.8 (0.6-0.9)	0.9 (0.6-1.4)	0.885
Gamma-GT (IU/mL)[b]	19 (16-33)	50 (20-74)	28 (21-57)	27 (19-237)	0.067
AT treatment stop					
HBV DNA (^{10}log IU/mL)[b]	0.00 (0.00-2.55)	2.55 (1.99-3.22)	1.28 (0.00-1.30)	1.28 (0.83-2.65)	0.093
ALT (ULN)[b]	0.6 (0.5-0.8)	1.0 (0.6-1.1)	0.8 (0.7-1.0)	0.7 (0.6-1.0)	0.014*
Gamma-GT (IU/mL)[b]	19 (14-33)	35 (17-45)	21 (17-52)	29 (15-66)	0.214
Relapse					
HBV DNA at first relapse detection (^{10}log IU/mL)	1.42 ± 1.17	3.87 ± 0.21	4.66 ± 1.64	6.64 ± 1.40	<0.001*
HBV DNA peak (^{10}log IU/mL)[a]	1.42 ± 1.17	3.87 ± 0.21	6.74 ± 2.08	6.64 ± 1.40	<0.001*
ALT at first relapse detection (ULN)[b]	0.7 (0.5-1.0)	0.9 (0.5-2.5)	1.1 (0.9-1.5)	2.8 (1.6-11.4)	<0.001*
ALT peak (ULN)[b]	0.7 (0.5-1.0)	0.9 (0.5-2.5)	2.5 (1.1-16.6)	2.8 (1.6-11.4)	<0.001*
HBeAg reversion (n)	0	1	2	3	0.025

Characteristics were compared using a one-way ANOVA with, if *$P < 0.05$, a Tukey test to determine the origin of the significant values.
SC, seroconversion; ULN, upper limit of normal (40 IU/mL for ALT); LAM, lamivudine; TDF, tenofovir; ETV, entecavir; ADV, adefovir; ALT, alanine aminotransferase; HBV, hepatitis B virus.
[a]Mean ± *SD*.
[b]Median (IQR).

HBeAg seroconversion or treatment start with clinically significant relapse is independent of liver fibrosis.

3.4 | Clinical outcomes

Of the 14 cirrhotic patients in the continuous treatment group, 3 had an episode of ascites, respectively, 1.3, 0.5 months before and 0.4 months after HBeAg seroconversion, but all recovered. Of the patients who stopped treatment, two developed severe hepatic outcomes leading to liver-related death: one patient with histological fibrosis grade Metavir F2-F3 at start of treatment developed a severe hepatitis flare 6 months after tenofovir treatment cessation and subsequently died due to a spontaneous bacterial peritonitis and septic shock. Another patient with biopsy-proven cirrhosis at start of treatment developed a multifocal hepatocellular carcinoma 10 years after adefovir treatment cessation. Both patients did not show any concomitant risk factors for liver disease.

4 | DISCUSSION

In the present study, we investigated the long-term clinical consequences of stopping NA after HBeAg seroconversion in a chronic hepatitis B cohort consisting of 69% (43/62) Caucasian patients.

Almost half (45%) of the patients showed signs of clinically significant relapse within 2 years after treatment cessation (Figure 2). Two of the stopped patients died due to liver-related death. To the best of our knowledge, this is the largest study to date assessing the risk of HBV relapse after treatment cessation in a cohort of chronic hepatitis B patients of mixed, but predominant Caucasian ethnicity.

No differences were observed between Caucasian and other ethnicities in our cohort. Relapse rates (up to 63% 6 years after treatment stop) are similar to those previously reported in Asian cohorts, but higher than those generally reported after spontaneous HBeAg seroconversion.[9,12,24-26] In 234 Taiwanese patients, only 33.2% relapses were observed after spontaneous HBeAg seroconversion during a median follow-up of 8.6 years.[27] These results were confirmed in a Caucasian study including 61 patients with spontaneous HBeAg seroconversion, of whom only 21 (34%) showed signs of relapse after a median follow-up of 22.8 years.[28] In the present study, we further substantiate that NA-induced HBeAg seroconversion is less durable than spontaneous HBeAg seroconversion.

Several factors predictive for relapse after treatment cessation have previously been suggested, but none of these factors proved to be a reliable predictor in a recent meta-analysis, probably due to the heterogeneity in relapse definitions used.[12,16,29-31] In our study, we aimed to identify factors predictive of clinically significant relapse, defined as either persistent relapse or severe relapse with immediate retreatment. The peak HBV DNA and ALT after treatment stop were not different between both groups. In addition, peak HBV DNA levels in the immediately retreated patients were higher than the previously established cut-off of 5-log IU/mL HBV DNA to predict the development of persistent relapse and clinical flare after NA stop.[24] From a clinical point of view, identifying patients who will develop a persistent relapse and those who will develop an immediate severe relapse is highly relevant, as opposed to those developing no or only transient relapses. We therefore combined the first two groups in statistical modelling to predict clinically significant relapse, which is in line with the methodological approach of a previously published retrospective NA stop cohort study.[16]

Interestingly, in our cohort gamma-GT levels at HBeAg seroconversion proved to be predictive of clinically significant relapse after treatment cessation. Gamma-GT levels <14 IU/mL at treatment cessation were 100% predictive of sustained remission after treatment cessation and levels >50 IU/mL were associated with a 75% chance of clinically significant relapse after treatment stop. None of the patients with gamma-GT levels >50 IU/mL reported excessive alcohol use (>10 Units/week). However, the discriminatory value of Gamma-GT is moderate (ROC curve: AUC = 0.613), indicating that more factors are needed to reliably predict clinically significant relapse after treatment cessation.

The pathogenetic basis for the association between Gamma-GT levels and relapse is currently unclear. However, the biological relevance of gamma-GT as a biomarker in overall disease outcome and in particular in viral hepatitis has become increasingly substantiated in recent years. Elevated gamma-GT levels are associated with an increased overall mortality.[32-36] They are associated with treatment-induced HBeAg seroconversion in hepatitis B patients and with sustained viral response after interferon treatment in hepatitis C patients.[35,37,38] In addition, gamma-GT levels have been suggested to predict significant fibrosis in hepatitis B patients.[39] We carefully assessed the predictive value of FIB-4, APRI, GPR and histological fibrosis stage. None of them were predictive of persistent relapse, suggesting that the underlying pathobiological mechanism between the association of gamma-GT and clinically significant relapse is not related to hepatic fibrosis. More detailed studies are clearly indicated to unravel this mechanism as well as to investigate the value of Gamma-GT as a prognostic factor for treatment stop.

In our cohort, we observed a fatal flare in a patient with baseline histologically proven Metavir F2-F3 fibrosis. It is difficult to ascertain whether there was an initial sampling error and baseline cirrhosis or a true rapid disease progression after treatment cessation in this patient. Given a biopsy length of 11 mm with 7 portal fields, an underlying cirrhosis seems possible.[40] In addition, one patient developed a multifocal hepatocellular carcinoma 10 years after treatment cessation. Whether the observed tumour development was the result of treatment cessation is hard to prove. However, previously published studies show that continuing NA treatment is associated with an up to threefold lower risk of hepatocellular carcinoma.[41-43] International guidelines suggest not to stop NA therapy in cirrhotic patients due to the risk for hepatic decompensation.[44] Lim et al previously reported two cases of fatal reactivation when lamivudine therapy was stopped in start-of-treatment HBeAg negative, cirrhotic patients.[14] Another Asian study reported one fatal hepatic decompensation after lamivudine withdrawal following HBeAg seroconversion in a patient who was cirrhotic at start of therapy.[13] The combination of fatal outcomes and high relapse rates in our national cohort study urges for a re-evaluation of Belgian national treatment reimbursement policies. Currently, there are no uniform, validated markers to guide safe NA stop after HBeAg seroconversion. Based on our findings, it would seem prudent to only stop NA treatment in patients without fibrosis and—pending further studies—those with the lowest gamma-GT levels at HBeAg seroconversion. In most HBeAg seroconverted chronic hepatitis B patients, however, NA treatment should be continued until HBsAg loss.

This study has some limitations. First, while our study design is intrinsically retrospective, the inclusion of hard biological or clinical endpoints will have partly overcome this selection bias. Nevertheless, there were 11% LTFU patients after treatment stop, which may have impacted our study results. Second, we were not able to evaluate the predictive role of HBsAg levels and HBV genotype as these parameters were not routinely measured in clinical practice. However, several studies suggest that HBV genotype would not influence relapse rates after nucleos(t)ide analogue stop.[12,45,46] Third—although not substantiated by the virologic and biochemical relapse profiles—we cannot firmly exclude that any of the immediately retreated patients would be transient.

TABLE 3 Univariate and multivariate Cox regression model to clinically significant relapse after treatment cessation

Univariate model to predict clinically significant relapse			Adjusted, multivariate model	
	P	HR (95% CI)	*P*	Adjusted HR (95% CI)
General				
Ethnicity (Caucasian vs non-Caucasian)	0.804	1.112 (0.388-2.083)		
Male gender	0.956	0.974 (0.381-2.492)		
Previous interferon treatment	0.096	0.400 (0.136-1.178)		
First vs second generation NA	0.375	0.680 (0.290-1.596)		
Total treatment time (mo)	0.082	1.000 (1.000-1.001)		
At treatment start				
ALT (per unit)	0.519	0.999 (0.997-1.001)		
AST (per unit)	0.784	1.000 (0.998-1.002)		
Gamma-GT (per unit)	0.001	1.004 (1.002-1.007)		
ALP (per unit)	0.077	1.003 (1.000-1.006)		
Total bilirubin	0.708	0.875 (0.436-1.757)		
HBV DNA (^{10}log)	0.432	1.112 (0.853-1.450)		
Platelets	0.855	0.999 (0.993-1.006)		
Cirrhosis (yes or no)	0.242	0.417 (0.096-1.804)		
Age	0.568	1.008 (0.982-1.034)		
APRI	0.593	0.949 (0.784-1.149)		
FIB-4	0.712	0.968 (0.813-1.152)		
GPR	0.031	1.721 (1.051-2.818)		
At HBeAg seroconversion				
ALT (per unit)	0.474	0.994 (0.976-1.011)		
AST (per unit)	0.907	0.998 (0.972-1.026)		
Gamma-GT (per unit)	0.014	1.006 (1.001-1.010)	0.013	1.006 (1.001-1.010)
ALP (per unit)	0.552	1.002 (0.995-1.009)		
Total bilirubin	0.730	1.029 (0.438-2.421)		
HBV DNA (^{10}log)	0.733	1.070 (0.725-1.580)		
Platelets	0.875	1.001 (0.994-1.007)		
APRI	0.652	0.795 (0.294-2.154)		
FIB-4	0.698	0.929 (0.648-1.331)		
GPR	0.185	1.542 (0.813-2.924)		
Consolidation therapy duration (per month)	0.319	0.980 (0.941-1.020)	0.588	0.989 (0.949-1.030)
Age	0.392	1.011 (0.986-1.037)	0.457	0.987 (0.952-1.022)
At treatment stop				
ALT (per unit)	0.897	1.001 (0.980-1.023)		
AST (per unit)	0.473	1.015 (0.975-1.056)		
Gamma-GT (per unit)	0.083	1.004 (0.999-1.009)		
ALP (per unit)	0.447	1.003 (0.995-1.011)		
Total bilirubin	0.702	0.774 (0.207-2.886)		
HBV DNA (^{10}log)	0.467	0.884 (0.636-1.231)		
Undetectable HBV DNA (yes/no)	0.447	0.680 (0.252-1.836)		
Platelets	0.908	1.000 (0.995-1.006)		
APRI	0.750	0.784 (0.176-3.500)		
FIB-4	0.801	0.948 (0.627-1.433)		
GPR	0.247	1.543 (0.740-3.220)		
Age	0.412	1.011 (0.985-1.037)		

HR, hazard ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Gamma-GT, gamma-glutamyl transferase; ALP, alkaline phosphatases; APRI, AST to platelet ratio index; FIB-4, fibrosis-4 score; GPR, gamma-GT to platelet ratio.

In conclusion, stopping treatment after HBeAg seroconversion in a large, predominant Caucasian population with long-term follow-up led to high relapse rates with potential fatal outcome. Gamma-GT levels at treatment start and at HBeAg seroconversion were predictive for clinically significant relapse.

ACKNOWLEDGEMENT

Declaration of personal interests: TV is recipient of a 2014 mandate from the Belgian Foundation Against Cancer (mandate number: 2014-087) and received research funding from Gilead Sciences, Bristol-Myers-Squib and Roche Diagnostics.

Declaration of funding interests: This study was supported by the Foundation Against Cancer Belgium (mandate number: 2014-087). The organisation had no role in the design of the study, the acquisition of the data, the data analysis nor in the writing, revision or the final approval of the manuscript.

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Author contributions: T. Vanwolleghem and S. Van Hees designed the study, analysed the data and wrote the manuscript. S. Van Hees collected the data. All authors acquired the data, critically revised the manuscript and approved the final version of the manuscript, including the authorship list.

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SUPPORTING INFORMATION

Additional Supporting Information will be found online in the supporting information tab for this article.

How to cite this article: Van Hees S, Bourgeois S, Van Vlierberghe H, et al. Stopping nucleos(t)ide analogue treatment in Caucasian hepatitis B patients after HBeAg seroconversion is associated with high relapse rates and fatal outcomes. *Aliment Pharmacol Ther*. 2018;47:1170–1180. https://doi.org/10.1111/apt.14560

APPENDIX 1

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