Durability of response after hepatitis B surface antigen seroclearance during nucleos(t)ide analogue treatment in a multiethnic cohort of chronic hepatitis B patients : results after treatment cessation

Durability of Response after HBsAg Seroclearance during Nucleos(t)ide Analogue Treatment in a Multi-Ethnic Cohort of Chronic Hepatitis B Patients: Results after Treatment Cessation

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ABBREVIATIONS

HBV, hepatitis B virus; CHB, chronic hepatitis B; NA, nucleos(t)ide analogue; HCC, hepatocellular carcinoma; ULN, upper limit of normal.

DISCLOSURES

T Vanwolleghem has received grants from Roche, Gilead, BMS and Janssen, and speakers honoraria from Gilead, BMS and Abbvie.

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AUTHOR CONTRIBUTIONS

H. Chi: study coordination, acquisition of data, analysis and interpretation of data, drafting of the article, and finalizing the article.

J. Peng, J. Cao, S. Van Hees, T. Vanwolleghem, Q. Xun, L. Chen, D. Wong, J.J. Feld, R.J. de Knegt: acquisition of data, critical revision of draft of article, and approval of the final version of the article.

B.E. Hansen: study concept and design, study coordination, analysis and interpretation of data, drafting of the article, and finalizing the article.

H.L.A. Janssen: study concept and design, study coordination, analysis and interpretation of data, drafting of the article, and finalizing the article.

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ABSTRACT

In 70 chronic hepatitis B patients with HBsAg seroclearance during nucleos(t)ide analogue therapy, response was sustained in all 54 patients who discontinued treatment. Clinically significant relapses as indicated by high HBV DNA and ALT levels were not observed. Anti-HBs positivity may not be required to ensure sustained off-treatment response.

INTRODUCTION

Currently, nucleos(t)ide analogues (NA) play a major role in the treatment of chronic hepatitis B (CHB) patients. NAs effectively inhibit hepatitis B virus (HBV) replication, a key driver of disease progression.[1] However, immune control over the virus and serological responses, such as HBsAg seroclearance, are rarely achieved due to the limited effect of NA on the covalently closed circular DNA (cccDNA).[2] The risk of relapse is high when NAs are stopped before HBsAg seroclearance.[3, 4] Therefore, in clinical practice NAs are generally prescribed as a long-term or even indefinite treatment.

HBsAg seroclearance is associated with a reduced risk of disease progression and is often considered as a functional cure of CHB.[5-7] HBsAg seroclearance may therefore be the ideal endpoint for successful NA discontinuation. This is also suggested by the latest CHB management guidelines, in which continued treatment until HBsAg seroclearance is increasingly recommended.[8, 9] Yet, there are very few clinical studies that provide evidence regarding the durability of response after NA-induced HBsAg seroclearance due to the rarity of this endpoint. A recent study reported good sustained off-treatment response and event-free survival after NA stop, but only Asian patients were investigated.[10] In addition, the effect of anti-HBs positivity at time of NA discontinuation or consolidation therapy beyond HBsAg seroclearance on sustained off-treatment response remains unknown.

The aim of this real-life cohort study of Caucasian and Asian CHB patients was to investigate the sustainability of response after HBsAg seroclearance during NA therapy and subsequent treatment cessation. In addition, we investigated the effect of anti-HBs positivity and consolidation therapy beyond HBsAg seroclearance on sustained response.

METHODS

Patients.

This was an international multicenter observational study of CHB patients who received NA treatment for at least one year with on-treatment HBsAg seroclearance between 1997 and 2015. Exclusion criteria were: interferon therapy during NA treatment; co-infection with human immunodeficiency virus, hepatitis C virus, or hepatitis D virus; concomitant other liver disease; liver transplantation or liver cancer.

Approval was obtained from ethics committees of all centers. The study was performed in compliance with the Declaration of Helsinki.

Methods.

Data was systematically collected according to a pre-defined protocol. Patients were evaluated at least every 3-6 months or more often if indicated. Cirrhosis was diagnosed based on histology or ultrasonographic signs of cirrhosis (nodules within liver parenchyma, spleen size >12 cm, or portal vein >16mm).[11]

Biochemical and serological tests were performed with local well-validated standardized automated techniques and commercially available immunoassays. ALT levels were standardized according to gender and the local upper limit of normal (ULN). Sensitive real-time polymerase chain assays were used to measure HBV DNA levels. Undetectable HBV DNA was defined as HBV DNA <20 IU/mL. Serum anti-HBs level >10 IU/mL was considered as positive.

Study endpoints included anti-HBs seroconversion, detectable HBV DNA (>20 IU/mL), HBsAg seroreversion, virological relapse (HBV DNA >2,000 IU/mL), and ALT elevation >2x ULN.

Data analysis.

In order to assess endpoints after treatment discontinuation, off-treatment follow-up time was calculated from NA stop (time=0) until an endpoint or last follow-up visit. For anti-HBs seroconversion, the time of HBsAg seroclearance was considered as time=0. Kaplan-Meier analysis and the log-rank test were used to analyze time-to-event endpoints.

Statistical tests were two-sided with a *p*-value <0.05 considered as statistically significant. All tests were performed with SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Study population.

In a cohort of 5872 CHB patients treated with NA therapy, a total of 70 patients were identified who cleared serum HBsAg. Of these 70 patients, 46% were Caucasians and 44% were Asians (Supplementary Table 1). At start of treatment, 31 patients (44%) were HBeAg-positive and 39 (56%) were HBeAg-negative. The median time from NA initiation until HBsAg seroclearance was shorter in pretreatment HBeAg-positive patients compared to pretreatment HBeAg-negative patients (3.6 vs. 5.1 years, p=0.011). After HBsAg seroclearance, anti-HBs seroconversion developed in 51 of the 70 patients resulting in cumulative rates of 63%, 82%, and 91% at year 1, 3, and 5, respectively (Supplementary Figure 1).

Durability of response after HBsAg seroclearance.

Of the 70 patients, 16 were still on NA at last follow-up visit. Overall, the 70 patients contributed to 760 person-months between HBsAg seroclearance and the last on-treatment visit. During these 760 months, no HBV DNA elevations or HBsAg reversions were observed.

NA was discontinued in 54 (77%) patients with all patients being negative for HBV DNA, HBeAg and HBsAg at treatment discontinuation (Supplementary Table 1). Cirrhosis (compensated) was present in 8 of the 54 patients (15%). Treatment was continued for a median of 7 months (IQR 6-14) beyond HBsAg seroclearance. At least two consecutive confirmations of HBsAg negativity were done before treatment discontinuation in 52 of the 54 patients (96%). At NA cessation, 30 (56%) patients were anti-HBs positive with a median duration of anti-HBs positivity of 5 months (IQR 0-10).

During a median off-treatment follow-up of 1.6 years (IQR 0.5-2.7), 5 of the 54 patients developed detectable HBV DNA (range 23-1320 IU/mL) while remaining HBsAg negative (Table

1). Of these 5 patients, one had cirrhosis and was retreated immediately. In addition, 2 of the 54 patients developed HBsAg reversion while maintaining undetectable HBV DNA (Table 1). Of these two patients, one was HBsAg positive until the end of follow-up, while the other became HBsAg negative again. The cumulative rates of the composite development of detectable HBV DNA or HBsAg reversion were 10% and 16% at year 1 and 5, respectively (Supplementary Figure 2). None of the patients developed a full virological relapse as indicated by combined HBsAg reversion and high HBV DNA levels. No patients developed ALT levels >2x ULN. None of the patients developed hepatic decompensation or hepatocellular carcinoma (HCC) and no patients died.

DISCUSSION

In this multi-ethnic cohort of Caucasian and Asian CHB patients with HBsAg seroclearance during NA therapy, we demonstrated that the response was durable in most patients regardless of anti-HBs positivity, even after subsequent NA discontinuation. None of the patients developed a clinically significant relapse with high HBV DNA and ALT levels. A minority of patients developed detectable HBV DNA or HBsAg reversion, which were mostly mild and transient, and never in combination with each other.

Studies are scarce regarding the durability of response after HBsAg seroclearance during NA therapy and were mostly performed in Asian patients. Our study included a significant amount of Caucasian patients (46%) demonstrating good durability of response after HBsAg seroclearance and subsequent NA stop. Detectable HBV DNA and HBsAg reversion were observed, but these episodes were transient and did not lead to any ALT flares. These episodes did not result in true disease reactivation such as frequently seen in HBsAg positive patients who have discontinued NA treatment.[3, 4] In line with our results, a study of Korean patients showed that none of their patients developed HBV DNA levels >2,000 IU/mL when NA was stopped after HBsAg seroclearance.[10]

Although HBsAg seroclearance appears to be a safe endpoint for NA therapy discontinuation according to the guidelines,[8, 9] there is limited evidence regarding the importance of anti-HBs positivity or consolidation therapy beyond HBsAg seroclearance. Important to note is that in our study, 44% of the patients were anti-HBs negative when NA was stopped. The absence of relapses in this group after NA cessation suggests that HBsAg negativity by itself indeed likely reflects disease remission and/or immune control and may be sufficient to stop NA.[5, 12] Likewise, low level of HBsAg at end-of-treatment has been demonstrated as a strong,

independent predictor of sustained response in HBsAg positive patients who discontinued NA therapy.[13]

It remains controversial whether it is safe for patients with cirrhosis to stop NA due to their increased risk of hepatic decompensation and HCC.[3, 4] In our study, high off-treatment HBV DNA levels or ALT flares were not observed. In contrast, in a Chinese study of 22 patients who stopped NA after HBsAg loss, one patient developed HBV DNA elevation >5 log IU/mL and ALT >500 U/L.[14] Therefore, the risk of hepatic decompensation may be present in patients with cirrhosis. Furthermore, studies have demonstrated that HCCs do occur after HBsAg seroclearance mainly in those with cirrhosis.[7, 10, 15] Considering the risk on these adverse outcomes, HCC surveillance and confirmation of sustained off-treatment response should be continued in cirrhotic patients who cleared serum HBsAg.

Our study is limited by the retrospective design. However, due to the rarity of NA-induced HBsAg seroclearance, it may be difficult to study this in a prospective manner. Long-term follow-up studies with larger number of patients are warranted to assess the incidence and risk factors of adverse clinical outcomes after NA-induced HBsAg seroclearance.

In conclusion, in this real-life, multiethnic cohort of CHB patients treated with NA, HBsAg seroclearance was a safe endpoint for treatment discontinuation with subsequent sustained off-treatment response. Clinically significant relapses as indicated by high HBV DNA and ALT levels were not observed. Anti-HBs positivity may not be required to ensure sustained off-treatment response. Future studies are warranted regarding HCC development after NA-induced HBsAg seroclearance in order to identify high risk patients.

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SUPPLEMENTARY FILE

Durability of Response after HBsAg Seroclearance during Nucleos(t)ide Analogue Treatment in a Multi-Ethnic Cohort of Chronic Hepatitis B Patients: Results after Treatment Cessation

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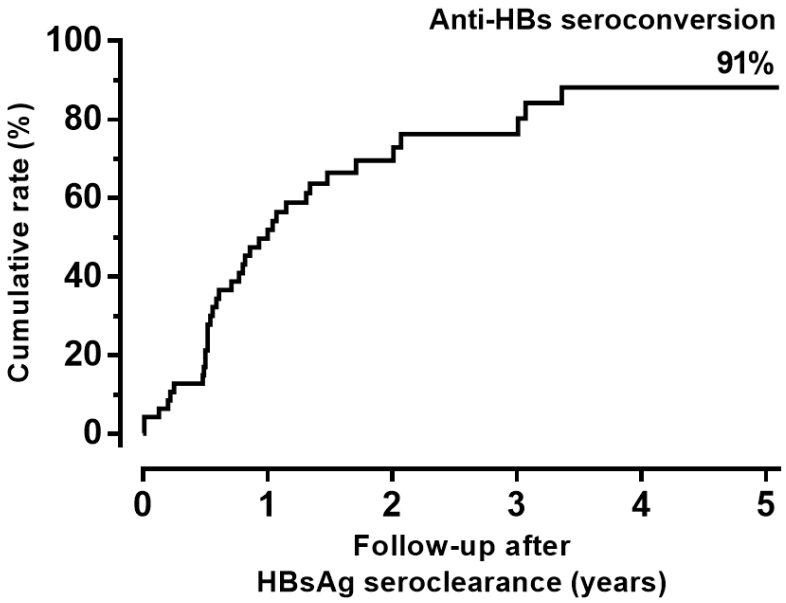
Supplementary Table 1. Characteristics of all patients with HBsAg seroclearance during nucleos(t)ide analogue treatment and patients who subsequently stopped treatment.

	Total	**Nucleos(t)ide analogue discontinuation**
	N=70	**N=54**
Characteristics	**At HBsAg loss**	**At end-of-treatment**
Age, *years**	49 ±12	48 ±12
Male gender	58 (83%)	47 (87%)
Race		
Caucasian	32 (46%)	27 (50%)
Asian	31 (44%)	23 (43%)
Other	7 (10%)	4 (7%)
Presence of cirrhosis	14 (20%)	8 (15%)
(PEG-)IFN-experienced	22 (31%)	19 (35%)
NA therapy†		
First-line	40 (57%)	29 (54%)
Second-line	30 (43%)	25 (46%)
Therapy duration, *years***	5.0 (3.3-8.2)	4.8 (3.3-7.5)
Time to HBsAg seroclearance, *years***	3.9 (2.2-7.2)	3.7 (2.0-6.8)
Duration of undetectable HBV DNA, *years***	2.5 (0.9-4.3)	3.0 (1.3-4.6)
Duration of HBsAg negativity, *years***	-	0.6 (0.5-1.2)
Anti-HBs		
Positive	18 (26%)	30 (56%)
Negative	35 (50%)	23 (42%)
Unknown	17 (24%)	1 (2%)
Duration of anti-HBs positivity, *years***	-	0.4 (0.0-0.9)
ALT, *xULN***	0.6 (0.4-0.8)	0.6 (0.4-0.8)
HBV DNA, *log IU/mL**	Undetectable	Undetectable
Platelet count, *10^9/L**	202 ±54	206 ±47
Start-of-treatment characteristics		
HBeAg-positive	31 (44%)	27 (50%)
ALT, *xULN***	3.2 (1.8-10.1)	2.8 (1.8-8.8)
HBV DNA, *log IU/mL**	6.2 ±2.2	6.2 ±1.4
Platelet count, *10^9/L**	180 ±71	184 ±71

†First-line: entecavir, tenofovir. Second-line: lamivudine, adefovir, telbuvidine.
*Mean (standard deviation).
**Median (interquartile range).

Supplementary Figure 1. Cumulative rate of anti-HBs seroconversion after HBsAg seroclearance in all patients (n=70). The time of HBsAg seroclearance was considered as time=0.



Supplementary Figure 2. Cumulative rate of the composite development of detectable HBV DNA or HBsAg reversion after HBsAg seroclearance and subsequent nucleos(t)ide analogue discontinuation (n=54). The time of treatment discontinuation was considered as time=0.

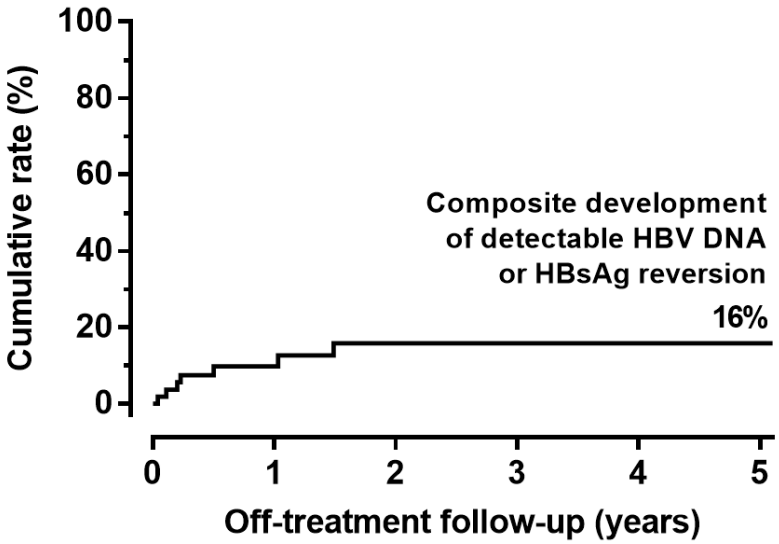


Table 1. Summary of the 7 patients with detectable HBV DNA or HBsAg reversion after nucleos(t)ide analogue therapy discontinuation.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
End-of-treatment characteristics							
Therapy	Entecavir	Entecavir	Tenofovir	Tenofovir	Tenofovir	Adefovir	Tenofovir
Age and sex	64y male	48y female	24y female	63y male	51y male	37y male	63y female
Race	Caucasian	Caucasian	Other	Caucasian	Caucasian	Asian	Other
Cirrhosis	Yes	No	No	No	No	No	No
Therapy duration, *months*	108	19	43	154	66	84	18
Duration of HBsAg negativity, *months*	63	6	5	15	14	4	7
Anti-HBs status	Negative	Positive	Positive	Negative	Negative	Negative	Positive
Duration of anti-HBs positivity, *months*	-	6	5	-	-	-	0
Start-of-treatment characteristics							
HBeAg-status	Positive	Positive	Positive	Negative	Positive	Negative	Positive
HBV DNA, *log IU/mL*	8.5	9.3	6.9	6.6	9.7	4.8	9.1
ALT, *xULN*	11.7	17.4	3.2	2.2	2.2	1.7	17.7
Endpoint characteristics							
Time to endpoint, *months*	18	0.5	5	15	2	6	3
Detectable HBV DNA	31 IU/mL	23 IU/mL	UD	1320 IU/mL	54 IU/mL	UD	223 IU/mL
HBsAg reversion	No	No	Yes	No	No	Yes, 0.08 IU/mL	No
ALT >2x ULN	No	No	No	No	No	No	No
Endpoint outcomes							
Retreatment	Yes	No	No	Yes	No	No	No
Undetectable HBV DNA	Yes	Yes	Yes	Yes	Yes	-	Yes
HBsAg seroclearance	-	-	No	-	-	Yes, 3m later	-

UD, undetectable.