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SHORT COMMUNICATION

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Sustained off-treatment viral control is associated with high hepatitis B surface antigen seroclearance rates in Caucasian patients with nucleos(t)ide analogue–induced HBeAg seroconversion

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1 | INTRODUCTION

The hepatitis B virus (HBV) is a non-cytopathic DNA virus that infects hepatocytes and frequently causes a lifelong infection. As such, it accounts for up to 780 000 deaths annually. Nucleos(t)ide analogues (NA) efficiently suppress viral replication and substantially improve survival of chronic HBV patients. However, loss of the hepatitis B surface antigen (HBsAg) is rare in patients under NA treatment, often requiring years to decades.[1]

Over the last years, the feasibility of stopping NA treatment prior to HBsAg loss has been extensively investigated in long-term virally suppressed hepatitis B e antigen (HBeAg)-negative hepatitis B patients.[2] Although viral relapse was found to be frequent, subsequent HBsAg loss has been reported in up to 39% of the patients.[3] This has been ascribed to beneficial alanine aminotransferase (ALT) flares with concomitantly restored HBV-specific T-cell responses.[4,5] However, in a recent, large cohort study of 691 HBeAg-negative hepatitis patients, sustained viral suppression rather than any type of relapse following treatment withdrawal was associated with HBsAg seroclearance.[6]

Abbreviations: ALT, Alanine aminotransferase; Anti-HBe, Anti-hepatitis B e antigen antibody; Anti-HBs, Anti-hepatitis B surface antigen antibody; AST, Aspartate aminotransferase; Gamma-GT, Gamma-glutamyl transferase; HBeAg, Hepatitis B e antigen; HBsAg, Hepatitis B surface antigen; HBV, Hepatitis B virus; IQR, Interquartile range; NA, Nucleos(t)ide analogues; SD, Standard deviation; ULN, Upper limit of normal.

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HBsAg loss following NA withdrawal in HBeAg-positive hepatitis patients with NA-induced HBeAg seroconversion has been less well studied. These data are relatively difficult to obtain, given the low prevalence of these patients and the common practice of continuing treatment until HBsAg loss in many countries.[7] In the current study, we examine HBsAg loss following treatment cessation in an international, multi-ethnic cohort of patients with recent NA-induced HBeAg seroconversion.

2 | METHODS

2.1 | Patients

This retrospective, observational cohort study included all consecutive NA-treated chronic hepatitis B patients who experienced treatment-induced HBeAg seroconversion and subsequently stopped treatment between December 2000 and October 2016 at 20 hospitals in Belgium, the Netherlands and Canada. Exclusion criteria were concomitant infection with the hepatitis C virus, hepatitis delta virus or human immunodeficiency virus, the use of long-term immunosuppressive medication (>2 weeks), peg-interferon add-on treatment and a lack of follow-up after treatment cessation. Outpatient visits with routine laboratory assessment were scheduled at least every 3-6 months at all participating centres.

2.2 | Data collection

Data on demographics (date of birth, ethnicity and gender), virology (HBeAg status, HBsAg status, HBV viral load and HBV genotype), biochemistry (ALT, aspartate aminotransferase (AST), gamma-glutamyl transferase (gamma-GT)), treatment (type and duration) and the presence of cirrhosis were collected systematically according to predefined criteria using the same case record form for all patients. The diagnosis of cirrhosis was made according to international guidelines, via a combination of clinical, elastographical and imaging studies, or histologically. The limit of quantification for HBV DNA varied between 12 IU/mL and 2000 IU/mL, depending on the year of determination. HBV DNA levels below the limit of detection and below the limit of quantification were assigned the arbitrary values of 0 and the lower limit of quantification, respectively. HBV genotyping was performed using the INNO-LiPA test (Fujirebio, Ghent, Belgium).

2.3 | Definitions

HBeAg and HBsAg loss/seroconversion was defined as the loss of HBeAg and HBsAg respectively and the appearance of antibodies against HBeAg (anti-HBe) and HBsAg (anti-HBs) respectively on two occasions at least 1 month apart. HBeAg reversion was defined as the reappearance of HBeAg after confirmed HBeAg loss/seroconversion. Sustained viral control was defined as continuous HBV DNA suppression <2000 IU/mL. Virologic relapse was defined as a single HBV DNA elevation >2000 IU/mL after treatment cessation and clinical relapse as virologic relapse with ALT rise >2× upper limit of normal (ULN). The ULN for ALT was set at 40 IU/mL.

2.4 | Statistical analysis

Continuous data are shown as mean ± standard deviation (SD) or median (interquartile range (IQR)). Variables were compared between groups using a chi-square test for categorical variables and a Mann-Whitney *U* test, Student's *t* test or one-way analysis of variance (ANOVA) for continuous variables. Consolidation treatment duration was calculated as time from HBeAg seroconversion until treatment cessation. A clock-reset approach was applied to investigate HBsAg loss rates according to the virologic outcome and retreatment status after HBeAg seroconversion. Patients were considered as sustained viral controllers (n = 98) until relapse occurred (n = 62) with (n = 31) or without (n = 31) need for subsequent retreatment. Data were analysed with SPSS version 24.0 (IBM, Armonk, New York, USA). All statistical tests were two-sided, and *P*-values < 0.05 were considered statistically significant.

3 | RESULTS

A total of 98 predominantly male (74.4%) patients of mixed ethnicity (43.9% Asians, 49.0% Caucasians) who stopped treatment after NA-induced HBeAg seroconversion were included. Median consolidation treatment duration was 11.4 (6.1-18.0) months and median follow-up time after treatment cessation 42.8 (22.7-83.2) months. An overview of the patient characteristics is depicted in Table S1.

Viral relapse (HBV DNA > 2000 IU/mL) was noted in 62 patients at a median of 6.1 (3.5-16.7) months after treatment cessation with concomitant ALT rise >2 × ULN in 30/62 and HBeAg reversion in 12/62. Retreatment was started in 31/62 relapsed patients, of whom 16 started retreatment immediately and 15 at a median of 3.1 (2.4-15.2) months after relapse detection. HBsAg loss was observed in 14 patients off-treatment at a median of 9.7 (5.3-20.7) months after treatment cessation. Retreatment led to HBsAg loss in another 2 patients respectively 5.1 years and 7.4 years after retreatment start with subsequent long-term viral suppression. Both patients showed an at onset severe clinical relapse (HBV DNA 8.25 log IU/mL, ALT 4.35 ULN; and HBV DNA 7.89 log IU/mL, ALT 10.63 ULN) for which immediate retreatment was deemed necessary.

Remarkably, all patients with off-treatment HBsAg loss showed persistently low ALT (<1.5 × ULN) and HBV DNA (<2000 IU/mL) levels after HBeAg seroconversion (Figure 1). Using a clock-reset approach, annual HBsAg loss rates were examined according to the virologic outcome and retreatment status (Table S2). The annual HBsAg seroclearance rate was 8.4% in patients with off-treatment persistent viral control, compared to (a) 0.0% if relapse occurred but no retreatment was initiated (*P* = 0.009) and (b) 1.5% if relapse occurred with subsequent retreatment (*P* = 0.008). Indeed, no HBsAg loss was observed in patients (n = 31) that showed a mild relapse

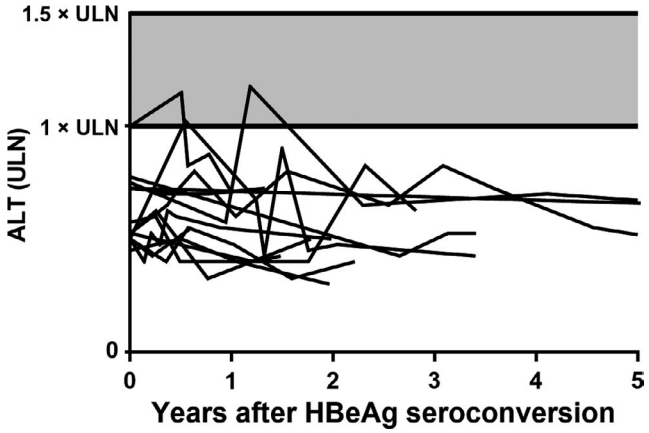

FIGURE 1 Individual alanine aminotransferase levels until HBsAg loss after HBeAg seroconversion (n = 14). ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; ULN, upper limit of normal

(peak median HBV DNA 4.02 log IU/mL; peak median ALT 0.9 ULN) but were not retreated during a median follow-up time of 1.9 (0.8-3.8) years after relapse detection. We next analysed whether baseline parameters would be associated with off-treatment HBsAg loss (Table S3). Caucasian patients had significantly higher off-treatment annual HBsAg loss rates compared to non-Caucasian patients (7.2% vs 1.0%; $P = 0.002$; Table S3), while other baseline parameters were not significantly associated with HBsAg loss. Sustained viral suppression resulted in annual HBsAg loss rates of 14.8% in Caucasian patients as opposed to 2.3% in non-Caucasian patients ($P = 0.004$; Table S4). Annual relapse rates tended to be lower in Caucasian patients (29.6% for Caucasian vs 44.2% for non-Caucasian patients; $P = 0.052$). With 48 Caucasian and 50 non-Caucasian patients, our cohort was well balanced for ethnicity.

4 | DISCUSSION

In the present study, we investigated off-treatment HBsAg loss in a multi-ethnic, international cohort of patients who stopped NA treatment following HBeAg seroconversion. We demonstrate that off-treatment HBsAg loss is exclusively seen in patients without viral relapse. In those that do relapse, HBsAg loss requires at least 5 years of viral suppression upon retreatment initiation.

These observations are in contrast with several small-scale NA stop studies in start-of-treatment HBeAg-negative patients, in which ALT flares after viral rebound have been found to facilitate HBsAg loss via a T-cell–dependent mechanism.[4,5] Similar to our results however, most off-treatment HBsAg losses occurred in patients with sustained viral control in a large cohort of 691 Taiwanese start-of-treatment HBeAg-negative patients.[6] These clinical observations raise questions on the concept of beneficial ALT flares and call for extensive translational studies in both HBeAg-positive and HBeAg-negative patients to unravel the immune responses that lead to off-treatment HBsAg loss.

Next to sustained viral control, we observed sevenfold higher annual HBsAg seroclearance rates in Caucasian patients compared to non-Caucasian patients. However, no other baseline characteristics correlated with annual HBsAg loss rates, underlining the importance of immune responses and possibly HBV genotypes in the observed HBsAg loss rates.[8]

Although this is to our knowledge the largest multi-ethnic study as of today assessing off-treatment HBsAg loss in patients with NA treatment–induced HBeAg seroconversion, the retrospective data collection might imply a variable number of laboratory values available per patient. Nevertheless, most patients were seen at the outpatient clinic every 3-6 months, which is current real-life practice in large hepatitis centres. In addition, genotype testing and quantitative hepatitis B surface antigen levels were available in only a small proportion (n = 15 and n = 14, respectively). Thus, we could not reliably evaluate the contribution of HBV genotypes or the diagnostic value of quantitative hepatitis B surface antigen levels for HBsAg loss. Second, despite pooling of all patients who stopped treatment at 20 centres across Belgium, the Netherlands and Canada, the number of patients that showed HBsAg loss after treatment cessation (n = 16) is limited and hampers multivariate statistical modelling approaches.

In conclusion, persistent viral control after treatment cessation following NA-induced HBeAg seroconversion was associated with high HBsAg loss rates in Caucasian patients. Our data raise questions on the concept of beneficial ALT flares and call for extensive translational studies into the immune mechanisms of off-treatment HBsAg loss.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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