Research Article
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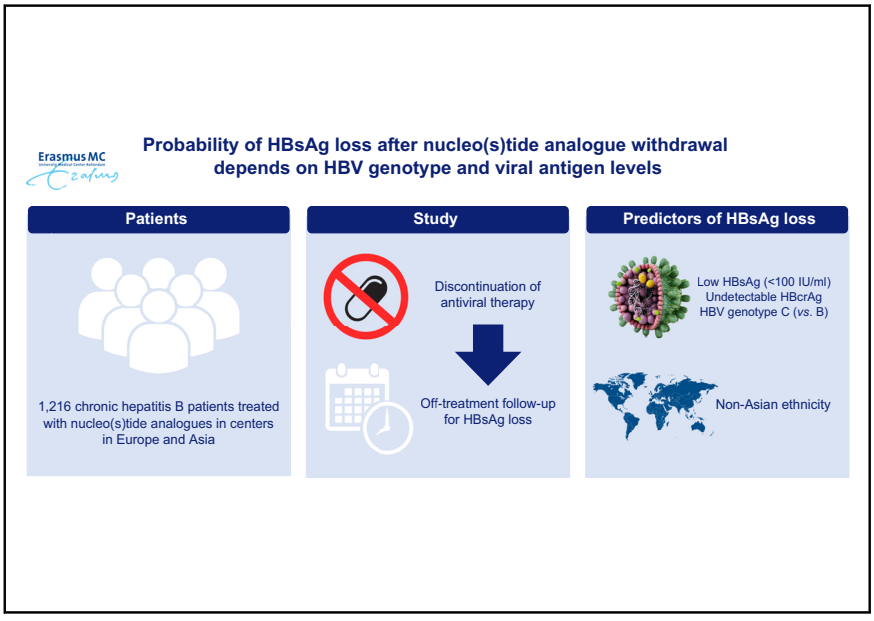
Probability of HBsAg loss after nucleo(s)tide analogue withdrawal depends on HBV genotype and viral antigen levels

Graphical abstract



Highlights

- A minority of patients with chronic hepatitis B may achieve HBsAg clearance after withdrawal of antiviral therapy.
- In this multicenter study comprising 1,216 patients, non-Asian ethnicity was associated with the highest chance of HBsAg loss.
- Among Asian patients, genotype C was associated with a higher chance of HBsAg loss.
- Low HBsAg levels (<100 IU/ml) and undetectable HBcrAg levels were associated with a higher chance of HBsAg loss.

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Lay summary

A subset of patients may achieve clearance of hepatitis B surface antigen (HBsAg) – so-called functional cure – after withdrawal of nucleo(s)tide analogue therapy. In this multicentre study of 1,216 patients who discontinued antiviral therapy, we identified non-Asian ethnicity, HBV genotype C, and low hepatitis B surface antigen and hepatitis B core-related antigen levels as factors associated with an increased chance of HBsAg loss.

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Probability of HBsAg loss after nucleo(s)tide analogue withdrawal depends on HBV genotype and viral antigen levels

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Background & Aims: Nucleo(s)tide analogue (NUC) withdrawal may result in HBsAg clearance in a subset of patients. However, predictors of HBsAg loss after NUC withdrawal remain ill-defined.

Methods: We studied predictors of HBsAg loss in a global cohort of HBeAg-negative patients with undetectable HBV DNA who discontinued long-term NUC therapy. Patients requiring retreatment after treatment cessation were considered non-responders.

Results: We enrolled 1,216 patients (991 with genotype data); 98 (8.1%) achieved HBsAg loss. The probability of HBsAg loss was higher in non-Asian patients (adjusted hazard ratio [aHR] 8.26, p <0.001), and in patients with lower HBsAg (aHR 0.243, p <0.001) and HBV core-related antigen (HBcrAg) (aHR 0.718, p = 0.001) levels. Combining HBsAg (<10, 10-100 or >100 IU/ml) and HBcrAg (<2log *vs.* ≥2 log) levels improved prediction of HBsAg loss, with extremely low rates observed in patients with HBsAg >100 IU/ml with detectable HBcrAg. HBsAg loss rates also varied with HBV genotype; the highest rates were observed for genotypes A and D, and none of the patients with HBV genotype E experienced HBsAg loss (p <0.001 for the overall comparison across genotypes; p <0.001 for genotypes A/D *vs.* genotypes B/C). HBV genotype C was independently associated with a higher probability of HBsAg loss when compared to genotype B among Asian patients (aHR 2.494; 95% CI 1.490–4.174, p = 0.001).

Conclusions: The probability of HBsAg loss after NUC cessation varies according to patient ethnicity, HBV genotype and end-of-treatment viral antigen levels. Patients with low HBsAg (<100 IU/ml) and/or undetectable HBcrAg levels, particularly if non-Asian or infected with HBV genotype C, appear to be the best candidates for treatment withdrawal.

Lay summary: A subset of patients may achieve clearance of hepatitis B surface antigen (HBsAg) – so-called functional cure – after withdrawal of nucleo(s)tide analogue therapy. In this multicentre study of 1,216 patients who discontinued antiviral therapy, we identified non-Asian ethnicity, HBV genotype C, and low hepatitis B surface antigen and hepatitis B core-related antigen levels as factors associated with an increased chance of HBsAg loss.

Keywords: HBsAg; HBcrAg; HBsAg loss; HBV genotype.
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Introduction

Currently recommended nucleo(s)tide analogues (NUCs) effectively achieve HBV DNA suppression in almost all patients with HBV infection. Therapy-maintained HBV DNA suppression is associated with significant decrease of liver-related complications but does not completely eliminate the risk of hepatocellular carcinoma. Thus, focus has recently shifted towards achieving functional cure, defined as sustained loss of HBsAg

from serum. Unfortunately, rates of HBsAg loss are low even after long-term NUC therapy.[1] Since early experiences with addition of pegylated-interferon (PEG-IFN) have been disappointing,[2] alternative strategies involving treatment cessation are being explored. Recent studies have shown that a proportion of HBeAg-negative patients may experience HBsAg loss after NUC withdrawal.[3] However, rates of HBsAg loss differ widely across studies.[4] Although some of this heterogeneity may be attributed to differences in retreatment criteria and duration of post-treatment follow-up, studies have consistently shown lower HBsAg loss rates in Asian cohorts when compared to cohorts enrolling predominantly non-Asian patients.[3,5–7] So far there has been no clear mechanistic explanation to account for the observed differences across patients from different ethnic backgrounds. One hypothesis is that this relates to HBV genotype, since previous studies have shown that HBV genotypes that predominate in Asia are associated with lower rates of HBsAg loss in patients treated with PEG-IFN[8] and NUCs.[9] In addition to ethnicity, low end-of-treatment viral antigen levels were also shown to be associated with favourable outcomes after treatment withdrawal, although the limited number of patients with HBsAg loss in previous studies limited statistical power for stratified analyses focussed on this outcome.[7] If and how the complex interplay between ethnicity and other potential predictors of response influence previously reported findings is therefore unclear.

The aim of the current study was therefore to assess predictors of HBsAg loss after NUC withdrawal in a multi-ethnic cohort of patients with chronic hepatitis B (CHB).

Patients and methods

Patients

The current study is a pooled analysis of previously published cohorts of patients with CHB who discontinued NUC therapy. We used data from patients enrolled in the CREATE study (n = 572), which comprises data from patients who stopped NUC therapy as part of studies or clinical practice in centres in Europa and Asia.[7] These data were pooled with a cohort of Taiwanese patients who discontinued NUC therapy (n = 644).[10]

Inclusion criteria of patients included in this analysis

Patients were selected from the combined database if they had been treated with only NUCs (no history of PEG-IFN add-on was allowed) and if they were i) HBeAg negative at the time of treatment cessation, ii) had undetectable HBV DNA at NUC cessation, iii) had available data on both HBsAg and HBV core-related antigen (HBcrAg) levels at treatment cessation.

Laboratory testing

HBcrAg was quantified with the Lumipulse G HBcrAg assay (Fujirebio Europe, Ghent, Belgium) on a LUMIPULSE G1200 analyser (Fujirebio Inc., Tokyo, Japan). HBcrAg levels were determined following the manufacturer's instructions. The assay's lower limit of quantification is 3 log U/ml and the lower limit of detection is 2 log U/ml. HBsAg was quantified at end of treatment using various standardised methods. HBsAg status after treatment withdrawal was assessed using local quantitative or qualitative methods applied at various intervals during off-treatment follow-up. HBV DNA was measured using local PCR methods. HBV genotyping was performed using various methods including line-probe assays, restriction fragment length polymorphism and/or sequencing. Patients enrolled from Greece were considered to have genotype D infection if no genotype data was available, as this is the predominant genotype in >95% of patients in this region.[11]

Endpoints and statistical analysis

The primary efficacy outcome was HBsAg loss, which was defined as undetectable HBsAg at any time during off-treatment follow-up. Retreated patients were not censored at the time of retreatment but considered persistently HBsAg positive. Cumulative probabilities were estimated using the Kaplan-Meier estimator, overall and across ethnicities (Asian *vs.* non-Asian), HBsAg levels (<10, 10-100 and >100 IU/ml), HBcrAg levels (<2 log, 2-3 log, >3 log), and HBV genotype, and compared using log-rank tests. The additive value of HBcrAg levels to HBsAg levels was also assessed by combining HBcrAg (detectable *vs.* undetectable) with HBsAg cut-offs (<10, 10-100 and >100 IU/ml[7]). These analyses were performed in the overall population and after stratification by ethnicity and genotype.

Multivariable analyses were adjusted for previously reported predictors of HBsAg loss, including antiviral agent, patient ethnicity, age, sex, pretreatment HBeAg status, and viral antigen and alanine aminotransferase (ALT) levels at the end of treatment, where applicable. Analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). All statistical tests were 2-sided and were evaluated at the 0.05 level of significance.

Table 1. Cohort characteristics.

Characteristics	N = 1,216
Demographics	
Age (years), median (IQR)	50 (41–58)
Male	880 (72.4%)
Duration of therapy (weeks, IQR)	167 (156–294)
HBeAg positive pretreatment	291 (23.9%)
Treatment	
TDF	372 (30.6%)
ETV	717 (59.0%)
other	127 (10.4%)
Race	
Caucasian	91 (7.5%)
Asian	1,101 (91%)
Other	24 (2.0%)
HBV genotype	
A	19 (1.6%)
B	497 (40.9%)
C	368 (30.3%)
D	81 (6.7%)
E	16 (1.3%)
Other	10 (0.8%)
Unknown	225 (18.5%)
HBcrAg (log U/ml) at EOT	
<2 log	272 (22.4%)
2–3 log	234 (19.2%)
>3 log	710 (58.4%)
HBsAg (log IU/ml) at EOT	
<10	64 (5.3%)
10–100	192 (15.8%)
>100	960 (78.9%)
ALT (U/L) at EOT	22 (16–31)

ALT, alanine aminotransferase; EOT, end of treatment; ETV, entecavir; TDF, tenofovir disoproxil fumarate.

Results

Cohort characteristics

We enrolled 1,216 patients, the majority of whom were Asian (91%), treated with ETV (59%) and infected with genotypes B or C (Table 1). After treatment discontinuation, 541 (44.5%) patients re-initiated therapy after a median of 34 (IQR 18–78) weeks of off-treatment follow-up. In the overall cohort, 98 (8.1%) patients cleared HBsAg, after a median follow-up of 102.5 (IQR 48–244) weeks. No patient cleared HBsAg after restarting therapy. The overall cumulative probability of HBsAg loss was 1.4% at 48 weeks, 4.1% at 96 weeks, and 5.9% at 144 weeks after treatment cessation.

Higher rates of HBsAg loss in non-Asian patients

The probability of HBsAg loss was significantly higher in non-Asian patients (22.9% at 144 weeks) compared to Asian patients (4.7% at 144 weeks; p <0.001 by log-rank, Fig. 1). The increased probability of HBsAg loss in non-Asians was confirmed in multivariable analysis (adjusted hazard ratio (aHR) 8.289, p <0.001; Table 2).

Lower HBsAg and HBcrAg levels are associated with higher HBsAg loss rates

Higher HBsAg levels at the end of treatment were associated with a lower probability of HBsAg clearance, with an unadjusted HR of 0.274 (95% CI 0.230–0.328, p <0.001). The highest probability of HBsAg loss was observed in patients with HBsAg <10 IU/ml at the end of treatment (Fig. 2A). Lower HBsAg levels were associated with higher rates of HBsAg loss among both Asians and non-Asians (Fig. S1).

Higher HBcrAg levels at end of treatment were associated with lower probability of HBsAg clearance, unadjusted HR 0.647 (95% CI 0.561–0.747, p <0.001). The highest cumulative probability of HBsAg loss was observed in patients with undetectable (<2 log) HBcrAg (14.6% at 144 weeks *vs.* 3.5% in patients ≥2 log; Fig. 2B). Undetectable HBcrAg levels were associated with higher rates of HBsAg loss among both Asians and non-Asians (Fig. S2).

In multivariable analysis, HBsAg (aHR 0.243, p <0.001) and HBcrAg (aHR 0.718, p = 0.001) levels were independently associated with HBsAg loss (Table 2).

Table 2. Factors associated with HBsAg loss in the overall population.

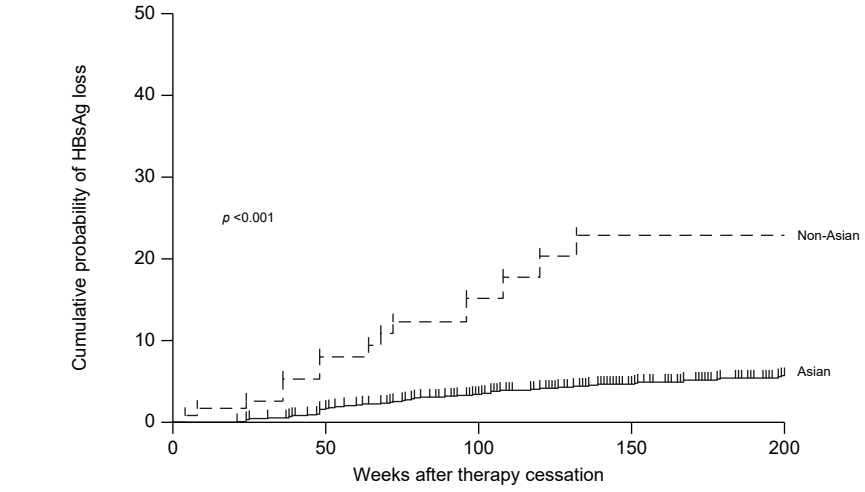
Variable	HR (95% CI)	p value
HBsAg (log IU/ml)	0.243 (0.201–0.293)	<0.001
HBcrAg (log U/ml)	0.718 (0.593–0.869)	0.001
ALT (U/L)	0.986 (0.973–1.000)	0.050
Age (years)	0.964 (0.945–0.982)	<0.001
TDF use	0.970 (0.600–1.568)	0.902
Sex	1.483 (0.900–2.445)	0.122
Non-Asian ethnicity	8.289 (4.298–15.988)	<0.001
HBeAg positive at baseline	1.342 (0.671–2.683)	0.405

Factors associated with HBsAg loss after nucleo(s)tide analogue withdrawal in multivariable Cox regression analysis.
ALT, alanine aminotransferase; HR, hazard ratio; HBcrAg, HBV core-related antigen; TDF, tenofovir disoproxil fumarate.

Combining HBsAg and HBcrAg to predict HBsAg loss

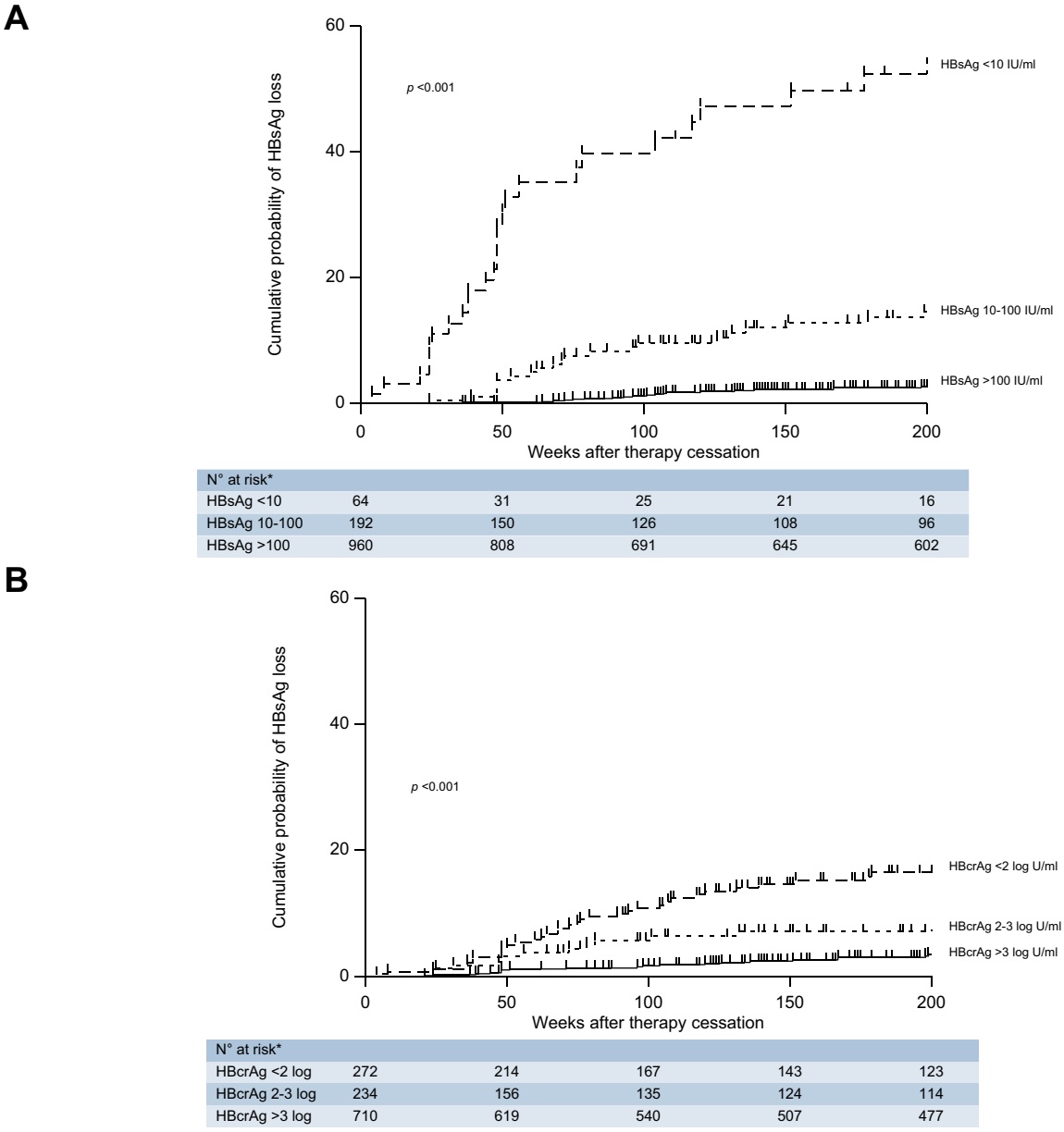
Since HBsAg levels and HBcrAg were independently associated with HBsAg loss we assessed the additive value of HBcrAg assessment (undetectable [<2 log] *vs.* detectable [≥2 log]) to previously reported HBsAg cut-offs (<10 IU/ml, 10-100 IU/ml and >100 IU/ml).

Among patients with HBsAg <10 IU/ml there was no additive value of HBcrAg (HR 1.080, p = 0.833). However, HBcrAg undetectability was associated with a significantly higher probability of HBsAg loss among patients with HBsAg levels of 10–100 IU/ml (HR 3.397, p = 0.001) and patients with HBsAg levels of >100 IU/ml (HR 3.702, p <0.001). Patients with both HBsAg >100 IU/ml and detectable HBcrAg had a very low chance of HBsAg loss (Fig. 3A). Based on Fig. 3A, we collapsed HBsAg and HBcrAg combinations into 4 categories: very high probability of HBsAg loss (HBsAg <10 IU/ml, irrespective of HBcrAg; 5% of cohort), high



N° at risk*					
Non-Asian	115	64	33	30	30
Asian	1,101	925	809	744	684

Fig. 1. HBsAg loss according to ethnicity. Cumulative probability of HBsAg loss after treatment cessation in Asian and non-Asian patients. *Retreated patients were considered persistently HBsAg positive. Comparison performed with the log-rank test.



N° at risk*					
HBsAg <10	64	31	25	21	16
HBsAg 10-100	192	150	126	108	96
HBsAg >100	960	808	691	645	602

N° at risk*					
HBcrAg <2 log	272	214	167	143	123
HBcrAg 2-3 log	234	156	135	124	114
HBcrAg >3 log	710	619	540	507	477

Fig. 2. HBsAg loss according to HBsAg and HBcrAg levels. Cumulative probability of HBsAg loss after treatment cessation according to (A) HBsAg and (B) HBcrAg levels at treatment cessation. HBsAg levels shown in IU/ml, HBcrAg levels shown in log U/ml. *Retreated patients were considered persistently HBsAg positive. HBcrAg, HBV core-related antigen. Comparisons performed with the log-rank test.

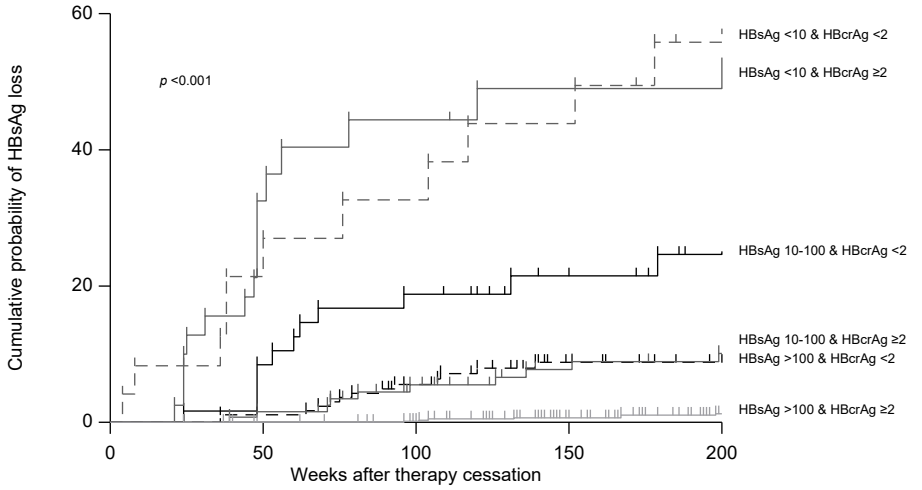
probability of HBsAg loss (HBsAg 10-100 IU/ml with undetectable HBcrAg; 5% of cohort), limited probability (HBsAg >100 IU/ml with undetectable HBcrAg OR HBsAg 10-100 IU/ml with detectable HBcrAg; 27% of cohort) and low probability of HBsAg loss (HBsAg >100 IU/ml and detectable HBcrAg; 63% of cohort) as shown in Fig. 3B.

HBV genotype predicts HBsAg loss after therapy withdrawal

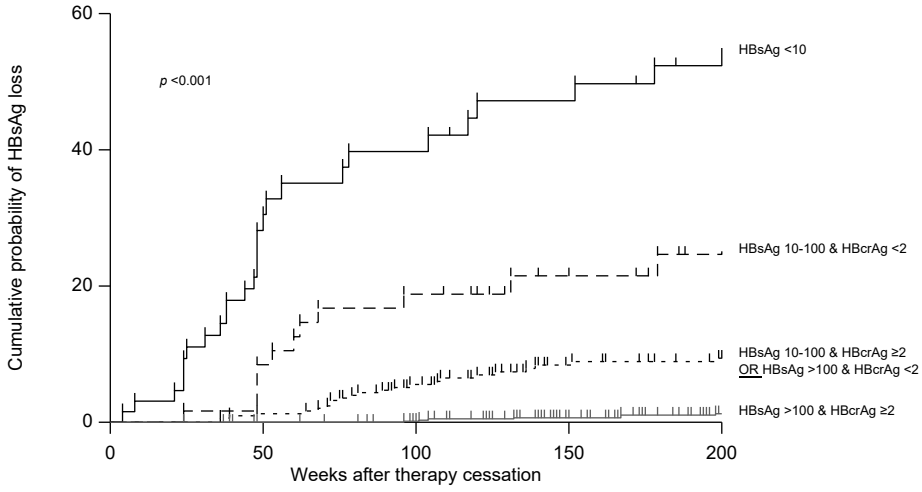
HBV genotype data were available for 991 patients, with HBV genotypes A, B, C, D, E and other/mixed observed in 1.9%, 50%, 37%, 8.2%, 1.6% and 1.0%, respectively. As expected, HBV genotype was highly collinear with ethnicity; only 1 non-Asian patient had genotype C and none had genotype B.

Serum HBcrAg and HBsAg levels at end of treatment varied with HBV genotype. Around 60% of patients with genotypes A or D had undetectable HBcrAg, compared to 26% and 10% of patients with genotype B and C, respectively (Fig. 4, p <0.001 for the overall comparison). HBsAg and HBcrAg levels were comparable in patients with genotype A when compared to patients with genotype D (p >0.538). However, patients with genotype C had both higher HBsAg and HBcrAg levels at treatment cessation when compared to patients with HBV genotype B (p = 0.001). All patients with HBV genotype E had HBsAg levels >100 IU/ml.

HBsAg loss rates also varied significantly with HBV genotype, the highest rates were observed with genotypes A and D, while none of the patients with HBV genotype E experienced HBsAg



N° at risk*					
HBsAg <10 & HBcrAg <2	24	14	12	10	5
HBsAg <10 & HBcrAg ≥2	40	17	13	11	11
HBsAg 10-100 & HBcrAg <2	59	45	36	28	20
HBsAg 10-100 & HBcrAg ≥2	133	105	90	80	76
HBsAg >100 & HBcrAg <2	189	155	119	105	98
HBsAg >100 & HBcrAg ≥2	771	653	572	540	504



N° at risk*					
HBsAg <10	64	31	25	21	16
HBsAg 10-100 & HBcrAg <2	59	45	36	28	20
HBsAg 10-100 & HBcrAg ≥2 OR HBsAg >100 & HBcrAg <2	322	260	209	185	174
HBsAg >100 & HBcrAg ≥2	771	653	572	540	504

Fig. 3. Combining HBsAg and HBcrAg levels to predict HBsAg loss. Cumulative probability of HBsAg loss according to (A) a combination of HBsAg and HBcrAg levels and (B) after collapsing groups with similar probabilities of HBsAg loss. HBsAg levels shown in IU/ml, HBcrAg levels shown in log U/ml. *Retreated patients were considered persistently HBsAg positive. HBcrAg, HBV core-related antigen. Comparisons performed with the log-rank test.

loss (p <0.001 by log-rank; Fig. 5). Multivariable analysis among patients with genotypes A, B, C or D confirmed the association between HBV genotype and HBsAg loss (Table 3).

Given the collinearity between genotype and ethnicity we also performed pairwise comparisons after stratification by ethnicity (HBV genotype A *vs.* D among non-Asians and HBV genotype B *vs*. C among Asians). Among 94 non-Asian patients with genotypes A or D, no difference in the probability of HBsAg loss between the genotypes was detected in univariate analysis (p = 0.921 by log-rank) nor in multivariable analysis (p = 0.387, in a model adjusted only for HBcrAg and HBsAg levels because of small sample size).

Among 864 Asian patients with genotypes B or C, no difference between the genotypes was found in the probability of HBsAg loss in univariate analysis (p = 0.553 by log-rank). However, after adjusting for the differences in HBsAg and HBcrAg levels at treatment cessation in multivariable Cox regression analysis, HBV genotype C was independently associated with a higher probability of HBsAg loss than genotype B (aHR 2.494; 95% CI 1.490–4.174; p = 0.001; Table S1).

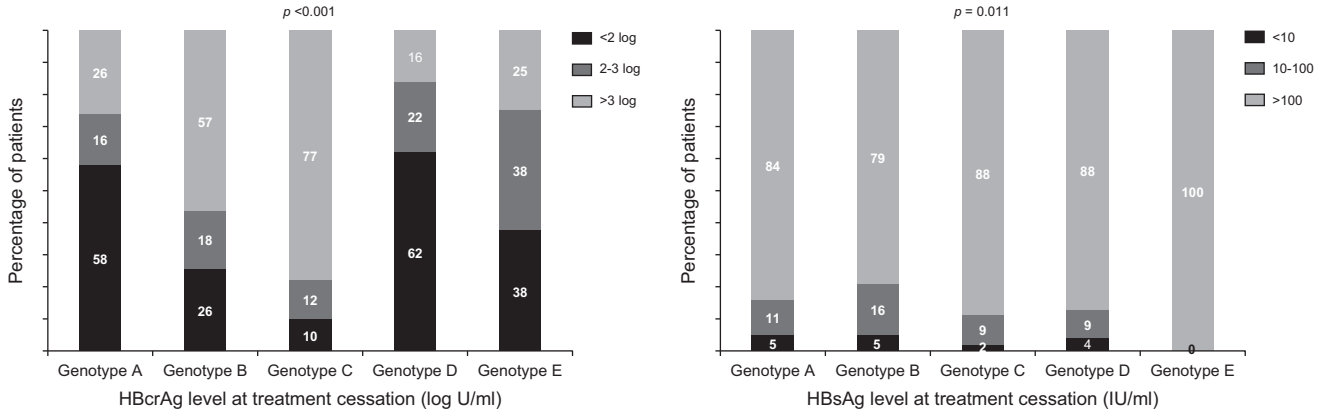


Fig. 4. HBsAg and HBcrAg levels at treatment cessation across HBV genotypes. Patients with genotypes A and D had similar HBsAg and HBcrAg distributions, whereas patients with HBV genotype C had higher HBsAg and HBcrAg levels than patients with HBV genotype B. HBsAg levels shown in IU/ml, HBcrAg levels shown in log U/ml. *Retreated patients were considered persistently HBsAg positive. HBcrAg, HBV core-related antigen. Comparisons performed with the chi-squared test.

The combination of HBcrAg and HBsAg predicts HBsAg loss across HBV genotypes

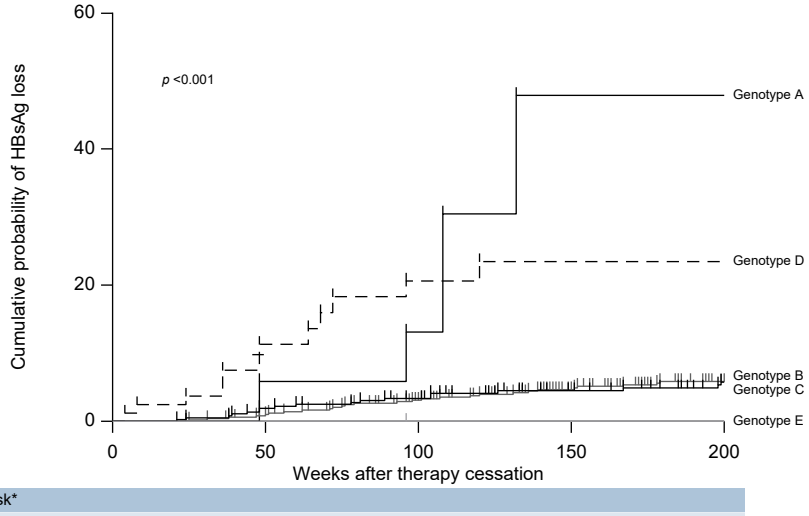
The association between HBsAg and HBcrAg levels and HBsAg loss was also assessed after stratification by HBV genotype (A+D, B, or C). Similar to the overall population, lower HBsAg and HBcrAg were associated with higher rates of HBsAg loss, although the absolute probabilities differed by genotype. Patients with HBsAg >100 IU/ml and detectable HBcrAg had a very low chance of HBsAg loss irrespective of HBV genotype (Fig. S3A-C).

Discussion

The current multi-ethnic multicentre study of patients who discontinued NUC therapy identified HBV genotype, age and end-of-treatment serum HBsAg and HBcrAg levels as independent predictors of HBsAg loss after therapy withdrawal. These findings may help in the selection and counselling of patients regarding treatment cessation.

All CHB management guidelines currently recognise the importance of HBsAg clearance in patients with CHB as HBsAg negativity is associated with a marked improvement in prognosis.[12,13] The importance of HBsAg loss is evident even among patients with suppressed HBV DNA, underscoring the superiority of this endpoint.[14] Unfortunately, rates of HBsAg loss during long-term NUC therapy are low and alternative options including trials of NUC cessation are being explored.

We have previously shown that patient ethnicity is an important predictor of off-treatment outcomes in patients who discontinue NUC therapy: both sustained virological response and HBsAg loss were less often achieved in Asian patients.[7] We



N° at risk*					
Genotype A	19	13	5	3	3
Genotype B	497	478	450	404	361
Genotype C	368	342	256	239	222
Genotype D	81	38	28	27	27
Genotype E	16	16	4	4	4

Fig. 5. HBsAg loss according to HBV genotype. Cumulative probability of HBsAg loss across the major HBV genotypes. *Retreated patients were considered persistently HBsAg positive. Comparison performed with the log-rank test.

Table 3. Association between HBV genotype and HBsAg loss.

Variable	HR (95% CI)	*p* value
HBsAg (log IU/ml)	0.213 (0.171–0.262)	<0.001
HBcrAg (log U/ml)	0.729 (0.603–0.882)	0.001
ALT (U/L)	0.988 (0.975–1.001)	0.067
Age (years)	0.963 (0.941–0.985)	0.001
TDF use	0.889 (0.551–1.867)	0.629
Sex	1.392 (0.822–2.359)	0.219
HBV genotype		<0.001
A	Ref.	
B	0.178 (0.060–0.532)	
C	0.417 (0.137–1.265)	
D	1.759 (0.514–6.014)	
HBeAg positive at baseline	0.865 (0.401–1.867)	0.712

Factors associated with HBsAg loss after nucleo(s)tide analogue withdrawal in multivariable Cox regression analysis.
ALT, alanine aminotransferase; HR, hazard ratio; HBcrAg, HBV core-related antigen; TDF, tenofovir disoproxil fumarate.

hypothesized that HBV genotype might, at least in part, account for these differences. HBV genotype is an important predictor of HBsAg loss in patients treated with PEG-IFN.[15] Natural history studies have also revealed higher rates of spontaneous HBsAg loss in patients with genotype C than in patients with genotype B,[16] and HBsAg loss in HBeAg-negative patients treated with long-term tenofovir disoproxil fumarate (TDF) was mostly observed in patients with HBV genotypes A or D.[9] In our cohort, patients with genotypes A and D had the highest rates of HBsAg loss, with no differences between the 2 genotypes after adjusting for other predictors. HBsAg loss rates were lower in patients with genotype C than in those with genotype A and D, but still significantly higher than those observed among patients with HBV genotype B. It is important to note that the differences between HBV genotypes B and C were only evident after adjustment for other relatively unfavourable characteristics (*i.e.* higher HBsAg and HBcrAg levels) that were present in the genotype C patients. These findings are important for the interpretation of future studies on the influence of HBV genotype and underscore the complex interplay between HBV genotypes and other factors in determining the probability of response to treatment strategies. An important aspect to consider is the collinearity between ethnicity and HBV genotype. The prevalence of the different HBV genotypes varies widely across the globe, with HBV genotypes B and C being endemic in South East Asia, and genotype D being most prevalent in patients from countries bordering the Mediterranean basin.[17] This distinct geographical distribution is also reflected in the current cohort: only 1 non-Asian patient had HBV genotype C and no non-Asian patient had genotype B. Furthermore, 79 of 81 genotype D patients was non-Asian. Whether the higher rates of HBsAg loss observed in genotype A/D *vs.* genotype B/C reflect HBV genotype or are (partly) accounted for by ethnicity is therefore unclear. To specifically study the influence of HBV genotype we therefore performed analyses after stratification by ethnicity. Within the non-Asian population, we did not detect significant differences between genotypes A and D. However, among the Asian subset genotype C was independently associated with a higher chance of HBsAg loss than genotype B (aHR 2.494, $p = 0.001$). The association between HBV genotype and the chance of HBsAg clearance after NUC withdrawal corroborates evidence from natural history studies.[16] However, mechanistic explanations are currently lacking. Previous studies have shown differences in the prevalence of viral mutants and histological disease severity across HBV genotypes B and C,[17,18] and it is likely that some of these factors contribute to the associations reported here. Differences in the mode of transmission could also potentially influence duration of infection and viral antigen levels, although the effect is probably limited as most of the patients enrolled in this cohort are likely to have been infected at a very young age. Unfortunately, the current dataset does not allow for further investigations into these matters.

Our observations do provide important information on the clinical utility of HBV genotype assessment in patients being assessed for NUC withdrawal. Based on our data, it appears that assessment of HBV genotype in a patient that is most likely to be infected with genotypes A or D (*i.e.* a non-Asian patient) will probably not change management, whereas in regions where genotypes B and C are predominant assessment of HBV genotype may yield additional prognostic information. However, genotyping-based strategies are hampered by the need for a significant viral load, which is typically absent in patients who are eligible for treatment discontinuation. Genotyping must therefore be performed on stored historical samples, limiting usefulness in clinical practice. Nevertheless, our findings are of importance when interpreting HBsAg loss data from different centres across the globe, as background prevalence of the different genotypes may significantly influence outcomes and comparability across cohorts.

Fortunately, other more readily available factors may be used for prediction of HBsAg loss. In our cohort age was a significant, albeit non-modifiable, predictor of HBsAg loss. Furthermore, we identified low end-of-treatment levels of HBsAg and HBcrAg as independent predictors of HBsAg clearance. Very low HBsAg levels (<10 IU/ml) were associated with very high rates of HBsAg clearance and these patients therefore appear to be excellent candidates for treatment withdrawal. Among patients with slightly higher HBsAg levels (10–100 IU/ml), HBsAg loss rates were still high, but prediction could be further optimized by considering HBcrAg levels; detectable HBcrAg levels were associated with a significantly reduced probability of HBsAg clearance. Among patients with HBsAg >100 IU/ml and detectable HBcrAg the probability of HBsAg loss was negligible. Importantly, these findings were consistent across ethnicities and HBV genotypes. Future studies applying novel HBcrAg testing platforms allowing for more sensitive assessment of HBcrAg are expected to further optimize HBcrAg-based prediction of response by allowing for use of continuous rather than categorised measurements at the lower end of the measurement range.[19]

Our findings regarding the association between low viral antigen levels with favourable outcomes after treatment cessation are in line with other studies from our group[7] and others,[20] but adds significantly to the existing body of evidence by focussing specifically on HBsAg loss, something that was not possible in other cohorts since this endpoint is so infrequent. Hsu *et al.* previously derived the SCALE-B score (HBsAg and HBcrAg together with age, ALT and TDF use) for prediction of clinical relapse after NUC cessation.[20] We and others have subsequently shown that lower SCALE-B scores are associated with higher rates of HBsAg loss.[7] However, It is important to note that this model was not calibrated to predict HBsAg loss, and several components of this score, notably ALT and TDF use, were not associated with HBsAg clearance. Use of the full score is therefore unnecessary and adequate risk stratification can be

performed using only a simple combination of HBsAg and HBcrAg levels. Furthermore, in the original SCALE-B study, HBsAg clearance was only observed in patients with scores <260. Of the 98 patients who cleared HBsAg in the current cohort, 37 had a SCALE-B score >260, and therefore 38% of patients with HBsAg clearance would not have been selected for treatment withdrawal based on their SCALE-B score. Furthermore, the HBsAg and HBcrAg cut-offs identified in the current study were able to predict HBsAg loss even among patients with a SCALE-B score <260 (Fig. S4).

Although our study is large and enrolled patients from around the globe there are some limitations. First of all, some authors have suggested that ALT flares after treatment withdrawal may be associated with subsequent HBsAg decline and clearance and advocate prolonged watchful waiting in patients with flares. Such strategies were not applied in the cohorts included in this study and many patients with flares where therefore retreated. This might have underestimated HBsAg loss rates because retreatment was considered a non-response in our analyses. It is however important to note that we previously showed that low HBsAg and HBcrAg are associated with low rates of flares and retreatment.[7] Also, the collinearity between HBV genotype and ethnicity precluded adjustment for ethnicity in models comparing genotypes A/D *vs.* B/C. Whether the higher probabilities of HBsAg loss observed with genotypes A/D *vs.* B/C were independent of patient ethnicity is therefore unclear. However, the association between HBV genotype C and higher HBsAg loss rates was independent of ethnicity. Furthermore, not all patients had available data on HBV genotype. This was mostly accounted for by 2 sites where no genotyping was performed in any of the patients; selection for genotyping in these groups was therefore not based on viral antigen levels or off-treatment outcomes; the risk of bias therefore appears to be limited. Additionally, it is important to note that we considered retreated patients as persistently HBsAg positive. This method yields conservative estimates of HBsAg loss rates when compared to previous studies that censored retreated patients. We opted for this approach because retreatment is often initiated in patients with sustained viremia with hepatitis, which is a state that is associated with reduced rates of subsequent HBsAg loss. Censoring these patients could therefore potentially violate an important assumption of the Kaplan-Meier estimator, namely that censoring is independent of the probability of the event of interest. Finally, the current study used data from multiple centres across the globe. The participating centres enrolled patients from distinct geographical areas, with specific genotype distributions and they applied varying retreatment criteria, potentially leading to centre-related variations that are difficult to control for. We therefore performed careful stratified analyses by ethnicity (Fig. S1), HBV genotype (Fig. S2) and across cohorts. These analyses showed consistent results: lower HBsAg and HBcrAg levels were associated with higher rates of HBsAg loss in all relevant subgroups.

In conclusion, our multicentre study shows that HBsAg loss may be achieved in a significant number of patients after NUC cessation. The probability of HBsAg loss varies with ethnicity, HBV genotype, age, and viral antigen levels. Patients with low HBsAg (<100 IU/ml) and/or undetectable HBcrAg levels, particularly if non-Asian or infected with HBV genotype C, appear to be the best candidates for treatment withdrawal.

Abbreviations

ALT, alanine aminotransferase; CHB, chronic hepatitis B; HBcrAg, hepatitis B core-related antigen; NUC, nucleos(t)ide analogue; PEG-IFN, pegylated-interferon; TDF, tenofovir disopro xil fumarate.

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Conflicts of interest

MJS has received speaker's fees and research support from Roche, Gilead, BMS, Fujirebio. SMC: Nothing to disclose. JYP is an investigator in clinical trials sponsored by Abbvie, Gilead Sciences, Hanmi and Norvatis. AK: Nothing to disclose. WKS has received speaker's fees from Mylan, received speaker's fees and provided consultancy for AbbVie, received speaker's fees, provided consultancy and research funding from Gilead Sciences. YT reports lecture fees from Fujirebio, Sysmex, Gilead Sciences. Research fees from Fujifilm Corp, Janssen Pharmaceutical K.K, Gilead Sciences, Glaxosmithkline pharmaceuticals ltd, and Stanford Junior University and Scholarship Donations from Chugai Pharmaceutical Co., Ltd. IC: Nothing to disclose. MP: Nothing to disclose. FvB has received research support and provided consultancy for Roche. TB currently acts as an advisor to Abbvie, Alexion, Bayer, BMS, Gilead, Intercept, Janssen, MSD/Merck, Merz, Novartis, and Sequana Medical. He has received speaking honoraria from Abbvie, Alexion, Bayer, BMS, Eisai, Gilead, Intercept, Ipsen, Janssen, MSD/Merck, Merz, Novartis, Sirtex and Sequana Medical in the past 2 years. He has received grant support from Abbvie, BMS, Gilead, Humedics, Intercept, Janssen, MSD/Merck, Merz, Novartis, and Sequana Medical. FZ: Advisor for Aligos, Assembly, Gilead, GSK, Myr Pharma, Roche MD, Spring-Bank, Transgene; Research grants: Evotec, Roche. SHA has acted as advisors and lecturers for BMS, Gilead Sciences, MSD, AbbVie, Janssen, Assembly Biosciences, Arbutus Biopharma, GreenCross, Ildong, and he has received unrestricted grant from Gilead Sciences for the investigator-initiated trials. GND is an advisor or lecturer for Ipsen, Pfizer, Genkyotex, Novartis, received research grants from Abbvie, Gilead and has served as PI in studies for Abbvie, Novartis, Gilead, Novo Nordisk, Genkyotex, Regulus Therapeutics Inc, Tiziana Life Sciences, Bayer, Astellas. NSE: Nothing to disclose. CHS: received travel grants from Novartis and Gilead. HW: received research grants from Abbott, AbbVie, BMS, Gilead, Merck, Novartis, Roche, Roche Diagnostics, Siemens; Consultant fees from Abbott, AbbVie, BMS, Boehringer Ingelheim, Gilead, JJ/Janssen-Cilag, Merck/Schering-Plough, Novartis, Roche, Roche Diagnostics, Siemens, Transgene, ViiV; Speaker fees from Abbott, AbbVie, BMS, Boehringer Ingelheim, Gilead, JJ/Janssen- Cilag, Merck/Schering-Plough, Novartis, Roche, Roche Diagnostics, Siemens, Transgene, ViiV. MC: received personal fees from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck (MSD), Biogen, Falk Foundation, Boehringer Ingelheim, Siemens, Spring Bank as well as grants and personal fees from Roche. MFY: provided consultancy and/or received research funding from AbbVie, Arbutus Biopharma, Assembly Biosciences, Bristol-Myers Squibb, Dicerna Pharmaceuticals, GlaxoSmithKline, Gilead Sciences, Janssen, Merck Sharp and Dohme, Clear B Therapeutics, Springbank Pharmaceuticals; and received research funding from Arrowhead

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Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study design, collection of data, data analysis, writing of the manuscript and approval of final version: MJS SMC CHC NSE BM. Study design, collection of data, critical review of the manuscript and approval of final version: JYP AK WKS YT IC MP FvB TB FZ SHA GND CHS HW MC MFY KA AB MB TP GP BM. All authors approve submission of the manuscript.

Data availability statement

The data used for the current analysis were derived from previously published cohorts and clinical datasets. The data cannot be shared.

Role of the funding source

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Supplementary data

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