



Prediction of Sustained Response After Nucleo(s)tide Analogue Cessation Using HBsAg and HBcrAg Levels: A Multicenter Study (CREATE)

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BACKGROUND & AIMS: Predictors of successful nucleo(s)tide analogue (NA) therapy withdrawal remain elusive. We studied the relationship between end-of-treatment levels of hepatitis B core-related antigen (HBcrAg) and hepatitis B surface antigen (HBsAg) and outcome after therapy cessation.

METHODS: Patients who discontinued NA therapy in centers in Asia and Europe were enrolled. HBcrAg and HBsAg were measured at treatment cessation, and associations with off-treatment outcomes were explored. The SCALE-B (Surface antigen, Core-related antigen, Age, ALT, and tenofovir for HBV) score was calculated as previously reported. End points included sustained virologic response (VR; hepatitis B virus DNA level <2000 IU/mL), HBsAg loss, and alanine aminotransferase (ALT) flares (>3× upper limit of normal). Re-treated patients were considered nonresponders.

RESULTS: We analyzed 572 patients, 457 (80%) were Asian and 95 (17%) were hepatitis B e antigen positive at the start of NA therapy. The median treatment duration was 295 weeks. VR was observed in 267 (47%), HBsAg loss was observed in 24 (4.2%), and ALT flare was observed in 92 (16%). VR (67% vs 42%) and HBsAg loss (15% vs 1.5%) was observed more frequently in non-Asian patients when compared to Asian patients ($P < .001$). Lower HBcrAg levels were associated with higher rates of VR (odds ratio [OR], 0.701; $P < .001$) and HBsAg loss (OR, 0.476; $P < .001$), and lower rates of ALT flares (OR, 1.288; $P = .005$). Similar results were observed with HBsAg (VR: OR, 0.812; $P = .011$; HBsAg loss: OR, 0.380; $P < .001$; and ALT flare: OR, 1.833; $P < .001$). Lower SCALE-B scores were associated with higher rates of VR, HBsAg loss, and lower rates of ALT flares in both Asian and non-Asian patients ($P < .001$).

CONCLUSIONS: In this multicenter study, off-treatment outcomes after NA cessation varied with ethnicity. Lower levels of HBcrAg and HBsAg were associated with favorable outcomes. A risk score comprising both factors can be used for risk stratification.

Abbreviations used in this paper: ALT, alanine aminotransferase; AUROC, area under the receiver-operating characteristic curve; CHB, chronic hepatitis B; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NA, nucleo(s)tide analogue; OR, odds ratio; TDF, tenofovir disoproxil fumarate; VR, virologic response.

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Long-term antiviral therapy with nucleo(s)tide analogues (NA) results in near-universal hepatitis B virus (HBV) DNA suppression, improvement of liver histology, and reduces the risk of hepatocellular carcinoma.¹⁻³ However, loss of hepatitis B surface antigen (HBsAg) infrequently is achieved, necessitating long-term therapy. Various studies have explored the rates of durable disease remission after NA cessation in HBsAg-positive patients, with sustained virologic response (often defined as HBV DNA level <2000 IU/mL) observed in 30% to 50% of patients.^{4,5} Interestingly, some studies have shown increased rates of HBsAg clearance after NA cessation, although these benefits seem to be observed more frequently in Caucasian cohorts when compared with studies enrolling predominantly Asian patients.⁶⁻⁹ Cessation of treatment also has been associated with significant alanine aminotransferase (ALT) flares, which may be associated with both HBsAg decline but also may signify acute liver injury, which even may result in liver failure in patients with advanced liver disease.^{9,10} Recently, small studies have shown that higher serum levels of viral antigens, such as HBsAg and hepatitis B core-related antigen (HBcrAg), are associated with increased risks of post-treatment viral relapse, occurrence of flares, and re-treatment.¹¹⁻¹³ A combination of serum HBsAg and HBcrAg levels, together with age and ALT levels at discontinuation (the Surface antigen, Core-related antigen, Age, ALT, and tenofovir for HBV or SCALE-B score), also was shown to predict relapse after therapy discontinuation in 135 Asian patients.¹³ A subsequent smaller study enrolling Caucasian patients showed an association between SCALE-B score and HBsAg loss, but not with relapse.¹¹ As a result, there are currently no widely accepted prediction rules that could be used to guide patient management. The aim of the current study therefore was to study the relationship between HBcrAg and HBsAg at treatment cessation with off-treatment sustained response, and to assess predictive performance of the SCALE-B score in a large, multi-ethnic, multicenter cohort of chronic hepatitis B (CHB) patients.

Patients and Methods

Patients

Data were extracted from the CREATE (HBcrAg to estimate the probability of sustained response to antiviral therapy in chronic hepatitis B) database, which comprises data from patients who discontinued NA therapy as part of previously published studies originally conducted in Asia and Europe, combined with data from patients who discontinued therapy as part of clinical practice in Barcelona (Spain) and Pisa (Italy).^{7,9,12-18}

Inclusion Criteria of the Patients Included in This Analysis

Patients were enrolled from the CREATE database if they had been treated with only NA (no history of pegylated interferon add-on was allowed) and if they were (1) hepatitis B e antigen (HBeAg) negative at the time of treatment cessation, (2) had undetectable HBV DNA at NA cessation, (3) had at least 24 weeks of off-treatment follow-up evaluation (or were re-treated before this time), and (4) had available data on serum HBsAg and HBcrAg levels at the time of treatment cessation.

End Points

Virologic response (VR) was defined as HBV DNA level less than 2000 IU/mL and was assessed at follow-up weeks 24 and 48. Re-treated patients were considered nonresponders at subsequent time points. Re-treatment criteria varied across the cohorts and were based on HBV DNA levels, ALT levels, and/or the decision of the treating physician. Patients who became HBsAg negative at any time during follow-up evaluation after treatment cessation were considered to have achieved HBsAg loss. For the analyses we used VR at week 48 as the primary end point, with a last-observation-carried-forward approach applied to patients lost to follow-up evaluation between weeks 24 and 48. Flares were defined as an ALT level greater than 3 times the upper limit of normal within the follow-up period.

Laboratory Testing

Serum HBcrAg and HBsAg levels were quantified at the time of therapy cessation. 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, using a lower limit of detection of 2 log U/mL. The assay's lower limit of quantification is 3 log U/mL. Serum HBsAg was quantified using various standardized methods. HBV DNA was measured using local polymerase chain reaction methods.

Statistical Analysis

The proportion of patients with VR, HBsAg loss, and ALT flares were compared across ethnicities using Pearson chi-square statistics and multivariate logistic regression. The relationship between HBsAg and HBcrAg levels at treatment cessation with VR, HBsAg loss, and ALT flares was analyzed using Pearson chi-square

statistics after categorization and using logistic regression, both univariate and adjusted for other potential predictors. For the evaluation of the SCALE-B model in our data set we calculated the original SCALE-B score as $35 \times \text{HBsAg (log IU/mL)} + 20 \times \text{HBcrAg (log U/mL)} + 2 \times \text{age (years)} + \text{ALT (U/L)} + 40$ for tenofovir use, with HBcrAg levels of 2 log U/mL or less set as 1.¹³ We applied previously reported cut-off values, which were shown to predict low (<260) and high risk (>320) of relapse.¹³ To assess the added value of other variables, we performed multivariable logistic regression using the variables of the SCALE-B (re-fitted SCALE-B), and compared this with a model that additionally included ethnicity, and with a model comprising additional predictor variables (ethnicity, HBeAg status, sex, and ALT levels). Discriminative performance was assessed using the area under the receiver operating characteristic curve (AUROC) and AUROCs were compared using the DeLong test. Analyses were performed using SPSS version 24.0 (SPSS, Inc, Chicago, IL) and R version 3.6.3 (2020-02-29) (R Core Team 2020, Vienna, Austria) and the package pROC (version 1.16.1, University of Geneva, Switzerland). All statistical tests were 2-sided and were evaluated at the 0.05 level of significance.

Results

Patient Characteristics

We analyzed a total of 572 patients. The majority of patients were of Asian or Caucasian ethnicity. Patients were treated predominantly with ETV (52%) or TDF (26%), and 95 (17%) were HBeAg positive at the start of treatment (Table 1). The median duration of antiviral therapy before discontinuation was 295 weeks (first quartile–third quartile, 208–434 wk). Asian patients more frequently were HBeAg positive at NA initiation (19% vs 7.0%; $P = .002$), and more frequently were treated with ETV (59% vs 22%; $P < .001$).

Off-Treatment Outcomes and Re-treatment

Overall, 246 (43%) patients were re-treated after therapy discontinuation, with the primary indications being persistent HBV DNA elevation (67%) and ALT increases or flares (29%). An ALT flare greater than 3 times the upper limit of normal was observed in 92 (16%) individuals. Two of these patients, both without a history of advanced fibrosis, developed jaundice without a concomitant increase in the international normalized ratio or encephalopathy, and both resolved without sequelae after re-treatment. VR was observed in 291 of 572 (51%) patients at week 24 and in 206 of 504 (41%) patients with available data at week 48. VR at last follow-up evaluation was documented in 267 (46.7%) patients.

What You Need to Know

Background

A limited number of patients may experience durable remission after discontinuation of nucleos(t)ide analogue therapy, but predictors of successful therapy withdrawal remain elusive.

Findings

Non-Asian ethnicity and lower end-of-treatment levels of hepatitis B surface antigen and hepatitis B core-related antigen were associated with favorable off-treatment outcomes. A risk score comprising both viral antigens (SCALE-B) predicted outcomes in both Asian and non-Asian patients.

Implications for patient care

Low end-of-treatment viral antigen levels may be used to select patients for therapy withdrawal.

HBsAg loss was observed in 24 patients (4.2%) during off-treatment follow-up evaluation. VR rates were higher in non-Asians (77 of 115; 67%) compared with Asians (190 of 457; 42%; $P < .001$), as were HBsAg loss rates (14.8 vs 1.5%; $P < .001$), with an adjusted odds ratio (OR) of 5.282 for VR (95% CI, 2.718–10.266; $P < .001$) (Table 2) in multivariable logistic regression. HBeAg positivity at the start of NA treatment was not associated with favorable virologic outcomes (VR: 49.5% vs 46.1%; $P = .550$; HBsAg loss: 3.2% vs 4.4%; $P = .581$), or with occurrence of flares (18.9% vs 15.5%; $P = .405$).

Relationship Between Hepatitis B Core-Related Antigen and Hepatitis B Surface Antigen Levels at Treatment Cessation and Off-Treatment Outcomes

Lower HBcrAg levels were associated significantly with higher rates of VR (OR, 0.701; 95% CI, 0.614–0.800; $P < .001$) and HBsAg loss (OR, 0.476; 95% CI, 0.333–0.681; $P < .001$). Patients with an undetectable HBcrAg level (<2 log U/mL) had the highest probability of VR and HBsAg loss (Figure 1). ALT flares were observed more frequently in patients with higher HBcrAg levels (OR, 1.288; 95% CI, 1.081–1.535; $P = .005$) (Figure 1).

Lower HBsAg levels also were associated significantly with higher rates of VR (OR, 0.812; 95% CI, 0.691–0.953; $P = .011$) and HBsAg loss (OR, 0.380; 95% CI, 0.272–0.530; $P < .001$). Patients with HBsAg levels less than 50 IU/mL had the highest probability of VR and HBsAg clearance (Figure 1). ALT flares were observed more frequently in patients with higher HBsAg levels at discontinuation (OR, 1.833; 95% CI, 1.404–2.392; $P < .001$) (Figure 1).

Findings generally were consistent across ethnicities and pretreatment HBeAg status, although absolute event rates varied (Figure 2 and Supplementary Table 1).

Factors Associated With Virologic Response in Multivariable Analysis

In multivariable analysis, lower serum levels of HBsAg and HBcrAg, younger patient age, non-TDF use, and lower ALT levels (ie, the factors that comprise the SCALE-B score) were associated with VR. In addition, non-Asian ethnicity was associated strongly with higher rates of VR (adjusted OR, 5.282; $P < .001$). Patient sex and duration of therapy were not associated with VR (Table 2).

Addition of Ethnicity Improves Discriminative Performance of the Surface antigen, Core-related antigen, Age, ALT, and tenofovir for HBV (SCALE-B) score

The original SCALE-B model had an AUROC of 0.626 (95% CI, 0.580–0.672) in this data set. Addition of ethnicity to the re-fitted SCALE-B model improved the AUROC to 0.693 (95% CI, 0.650–0.735; $P = .051$). The performance of a full model comprising the factors shown in Table 2 did not significantly improve discrimination (AUROC for full model, 0.699; $P = .409$ for comparison with SCALE-B plus ethnicity). We therefore opted to proceed with the SCALE-B model, stratified by ethnicity.

Relationship Between the Surface antigen, Core-related antigen, Age, ALT, and tenofovir for HBV (SCALE-B) Score and Off-Treatment Outcomes

The median SCALE-B score was 293 (range, 114–475). Lower SCALE-B scores were associated significantly with higher rates of VR (OR, 0.990; 95% CI, 0.986–0.994; $P < .001$) and HBsAg loss (OR, 0.978; 95% CI, 0.970–0.987; $P < .001$), and with a lower risk of ALT flares (OR, 1.022; 95% CI, 1.016–1.028; $P < .001$). Patients in the lowest SCALE-B quartile (<260) had a probability of VR and HBsAg loss of 62% and 11%, compared with 35% and 1% in those with the highest SCALE-B score (>320 ; $P < .001$) (Figure 3A). Patients with the highest scores also were most likely to experience ALT flares (31% vs 3%; $P < .001$) (Figure 3A and Supplementary Table 2). The relationship between lower SCALE-B scores and favorable outcomes was consistent across ethnicities, although absolute rates of VR and HBsAg loss were higher in non-Asian patients irrespective of SCALE-B score (Figure 3B). The relationship between SCALE-B score and off-treatment outcomes was consistent irrespective of HBeAg status at baseline (Supplementary Table 1).

Table 1. Cohort Characteristics

Characteristics	Cohort (n = 572)
Demographics	
Age, median, y	52 (first–third quarter, 43–59)
Male	390 (68%)
Duration of therapy, median, y	295 (first–third quarter, 208–434)
HBeAg positive at baseline	95 (17%)
Treatment	
ETV	295 (52%)
TDF	150 (26%)
Other	127 (22%)
Race	
Caucasian	91 (16%)
Asian	457 (80%)
Other	24 (4.2%)
HBcrAg, log U/mL	
<2	127 (22%)
2–3	134 (23%)
>3	311 (54%)
HBsAg, IU/mL	
<50	81 (14%)
50–100	44 (7.7%)
100–1000	187 (33%)
>1000	260 (46%)
Outcome	
VR 24 (n = 572)	291 (51%)
VR 48 (n = 504)	206 (41%)
VR EOF (n = 572)	267 (47%)
HBsAg loss	24 (4.2%)
ALT flare	92 (16%)

NOTE. An ALT flare was an ALT level more than 3 times the upper limit of normal.

ALT, alanine aminotransferase; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; TDF, tenofovir disoproxil fumarate; VR, virologic response (HBV DNA, <2000 IU/mL); VR EOF, virologic response at last follow-up evaluation (ie, week 48, or week 24 if no data were available at week 48).

Discussion

In this multicenter study of CHB patients who discontinued NA therapy, VR and HBsAg loss rates varied strongly with ethnicity; non-Asian patients were more likely to achieve favorable outcomes. Irrespective of ethnicity, lower serum levels of HBcrAg and HBsAg were associated with higher rates of VR and HBsAg loss and lower rates of ALT flares. A previously reported risk score comprising both viral antigens, SCALE-B, was associated with the probability of VR HBsAg loss and ALT flares in both Asian and non-Asian patients, and thus could be applied to identify a subset of patients most likely to achieve favorable outcomes.

Because currently available NA yield only limited HBsAg loss rates, most patients require long-term therapy. Previous studies have indicated that treatment withdrawal may be successful in a subset of patients, resulting in a state of post-treatment sustained response

Table 2. Factors Associated With VR EOF in Multivariable Logistic Regression

Variable	OR (95% CI)	P
HBsAg, log IU/mL	0.754 (0.624–0.911)	.003
HBcrAg, log U/mL	0.726 (0.615–0.857)	<.001
ALT, U/L	0.980 (0.962–0.999)	.038
Age, y	0.961 (0.944–0.978)	<.001
TDF use	0.354 (0.199–0.628)	<.001
Sex	1.111 (0.747–1.653)	.603
Non-Asian ethnicity	5.282 (2.718–10.266)	<.001
Duration of therapy, wk	1.000 (0.999–1.001)	.547

ALT, alanine aminotransferase; CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; OR, odds ratio; TDF, tenofovir disoproxil fumarate; VR EOF, virologic response at last follow-up evaluation in multivariable logistic regression (ie, week 48, or week 24 if no data were available at week 48).

or immune control.⁵ Response rates after therapy discontinuation vary widely across studies, with up to 25% cumulative rates of HBsAg loss reported in 1 study.⁷ However, both VR and HBsAg loss rates were significantly lower in a recent study enrolling predominantly Asian patients.⁸ Because previous studies enrolled patients from distinct geographic areas, it has remained unclear whether these differences are the result of ethnicity or other potential confounders. The current study, which used data from expert centers from Europe and Asia, showed that non-Asian ethnicity is associated independently with higher rates of both VR and HBsAg loss. This finding is of major importance for the interpretation of response rates of future studies aimed at off-treatment sustained response.

Pending the approval of novel antiviral agents, most CHB patients with a treatment indication receive indefinite NA therapy. Because long-term therapy is associated with a limited decrease in renal function and possibly bone mineral loss (particularly in patients treated with TDF or ADV),¹⁹ strategies aimed at achieving higher rates of off-treatment sustained responses or HBsAg loss are of major clinical interest.²⁰ Because strategies involving pegylated interferon add-on or switch yield limited response rates,^{15,21} current studies focus on identifying patients most likely to achieve favorable outcomes after NA withdrawal. Because HBV DNA undetectability is a prerequisite for NA discontinuation, other factors are required to quantify response probabilities. Previous smaller studies have suggested that lower serum HBsAg and HBcrAg levels are associated with lower relapse rates after therapy discontinuation.^{11,13} In the current study, HBcrAg levels were associated strongly with both VR and HBsAg loss, and also with the risk of withdrawal flares. The current study thus confirms previous studies in a very large international data set, but also underscores that the absolute probability of response varies strongly with ethnicity. For example, response rates among patients with undetectable HBcrAg were 51% for Asian patients, compared with 79% for non-Asians. Similarly, response rates were highest in patients with low HBsAg levels at discontinuation, with 100% VR and 71% HBsAg loss observed in non-Asians with an HBsAg level less than 50 IU/mL, compared with 61% and 10% in Asians. Importantly, the risk of treatment withdrawal flares increased with higher HBcrAg and/or HBsAg levels, and these associations were consistent across ethnicities. Taken together, these findings support the association of low viral antigen levels with favorable outcomes after NA withdrawal, and patients with very

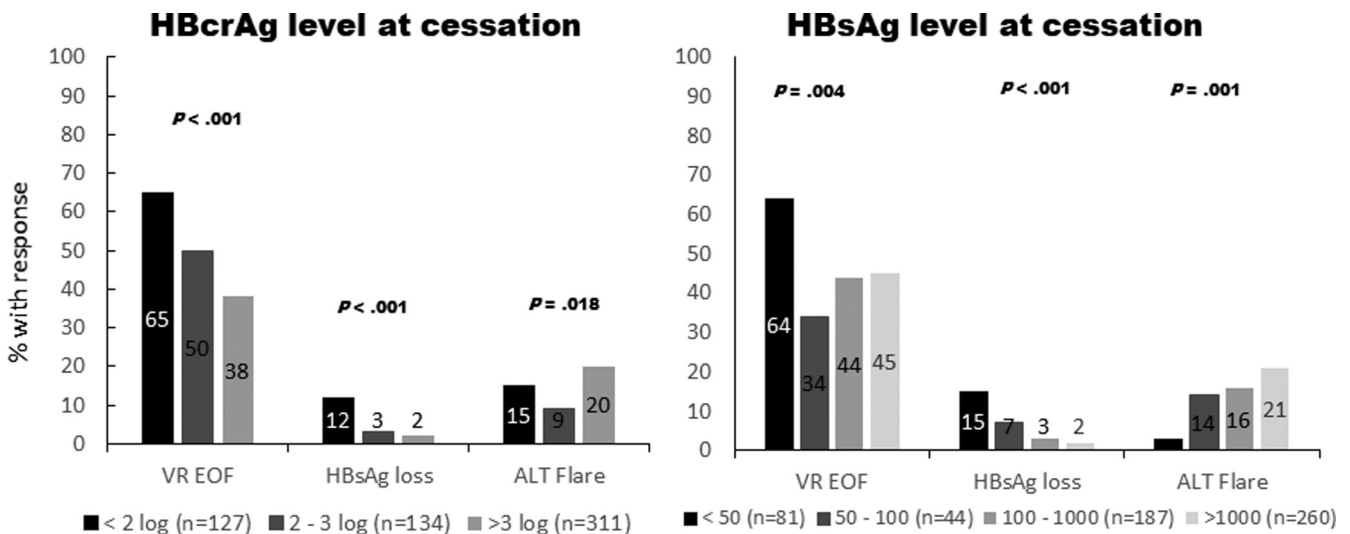


Figure 1. Outcome after therapy withdrawal according to hepatitis B core-related antigen (HBcrAg) and hepatitis B surface antigen (HBsAg) levels at treatment cessation. A flare consisted of an ALT level at least 3 times the upper limit of normal. VR EOF was defined as a virologic response (HBV DNA level, <2000 IU/mL) at the last follow-up evaluation (ie, week 48, or week 24 if no data available at week 48).

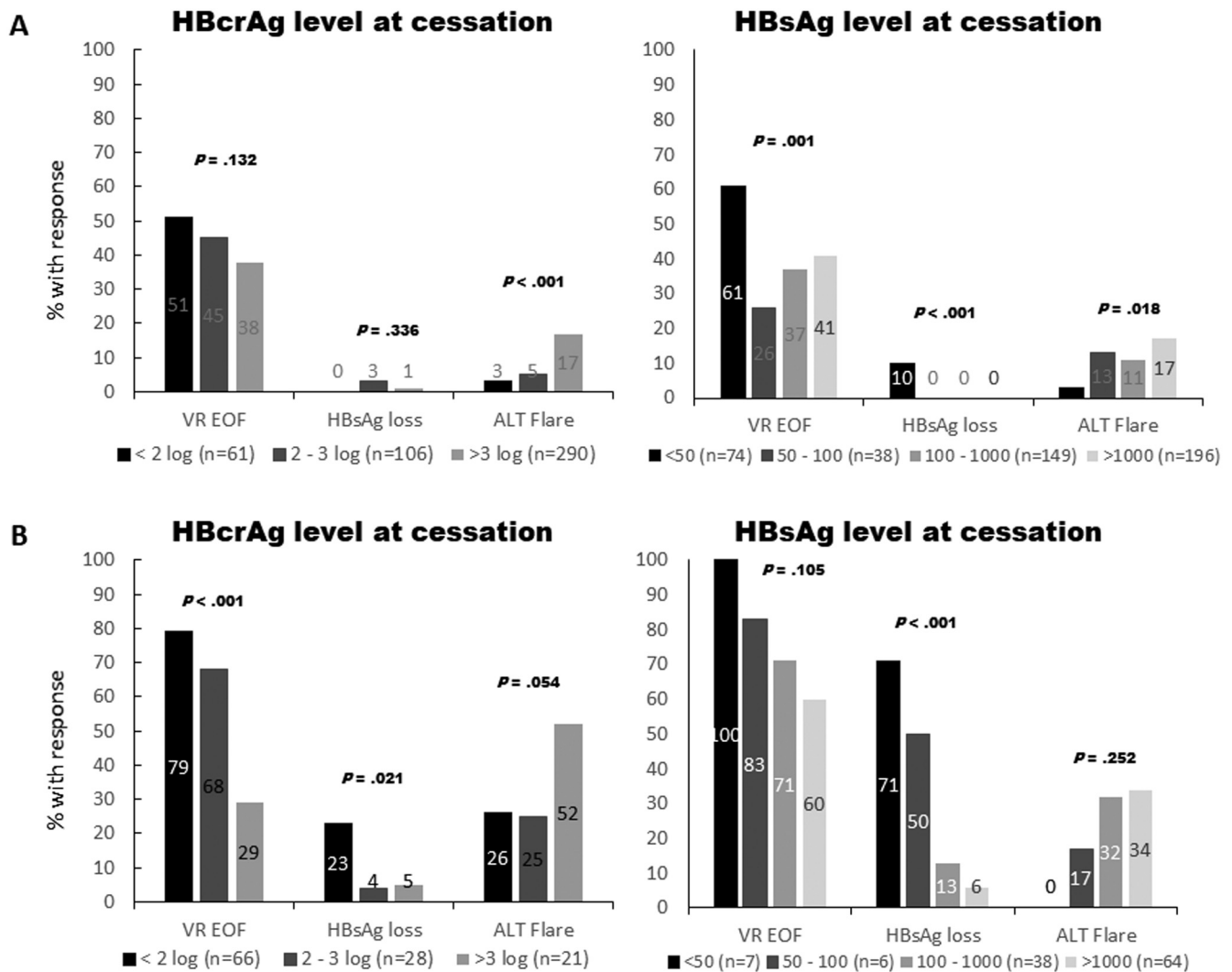


Figure 2. Outcome after therapy withdrawal according to hepatitis B core-related antigen (HBcrAg) and hepatitis B surface antigen (HBsAg) levels at treatment cessation in (A) Asian and (B) non-Asian patients. A flare consisted of an ALT level at least 3 times the upper limit of normal. VR EOF was defined as a virologic response (HBV DNA level, <2000 IU/mL) at the last follow-up evaluation (ie, week 48, or week 24 if no data available at week 48).

low antigen levels (especially those with HBsAg level <50 IU/mL) appear to be excellent candidates for treatment withdrawal. However, the absolute number of patients complying with these criteria is relatively small and alternative predictors therefore are required to increase the number of patients who might be eligible for therapy withdrawal.

In addition to ethnicity and HBcrAg and HBsAg levels, serum ALT, age, and TDF use were associated with VR. Because these factors were incorporated in the previously published SCALE-B score, we studied the performance of this score stratified by ethnicity. The discriminative performance of SCALE-B, as assessed by AUROC, was lower in our cohort (0.626) than reported in the original study (>0.87). This may be accounted for by the use of different end points used in the studies and the heterogeneity of our multicenter cohort. However, despite the relatively low AUROCs, there appears to be a certain clinical use of the SCALE-B score. In our cohort,

lower SCALE-B scores were associated with higher rates of VR, HBsAg loss, and very low (<5%) probability of withdrawal flares, irrespective of ethnicity. Importantly, patients in the highest quartile had both the lowest response rates and highest flare rates, suggesting that these patients are not good candidates for NA withdrawal.

Another interesting finding in our study is the lower VR response rates observed in patients treated with TDF. This finding is in line with previous studies,^{9,13} but clear pathophysiological explanations currently are lacking. One study suggested differential interferon- γ -associated gene expression profiles in patients treated with TDF vs ETV, which could be a contributing factor.²² Another potentially important issue could be previous NA treatment failure, which may have been over-represented in the TDF-treated subgroup in our cohort. Unfortunately, these data are not available for most patients in this cohort.

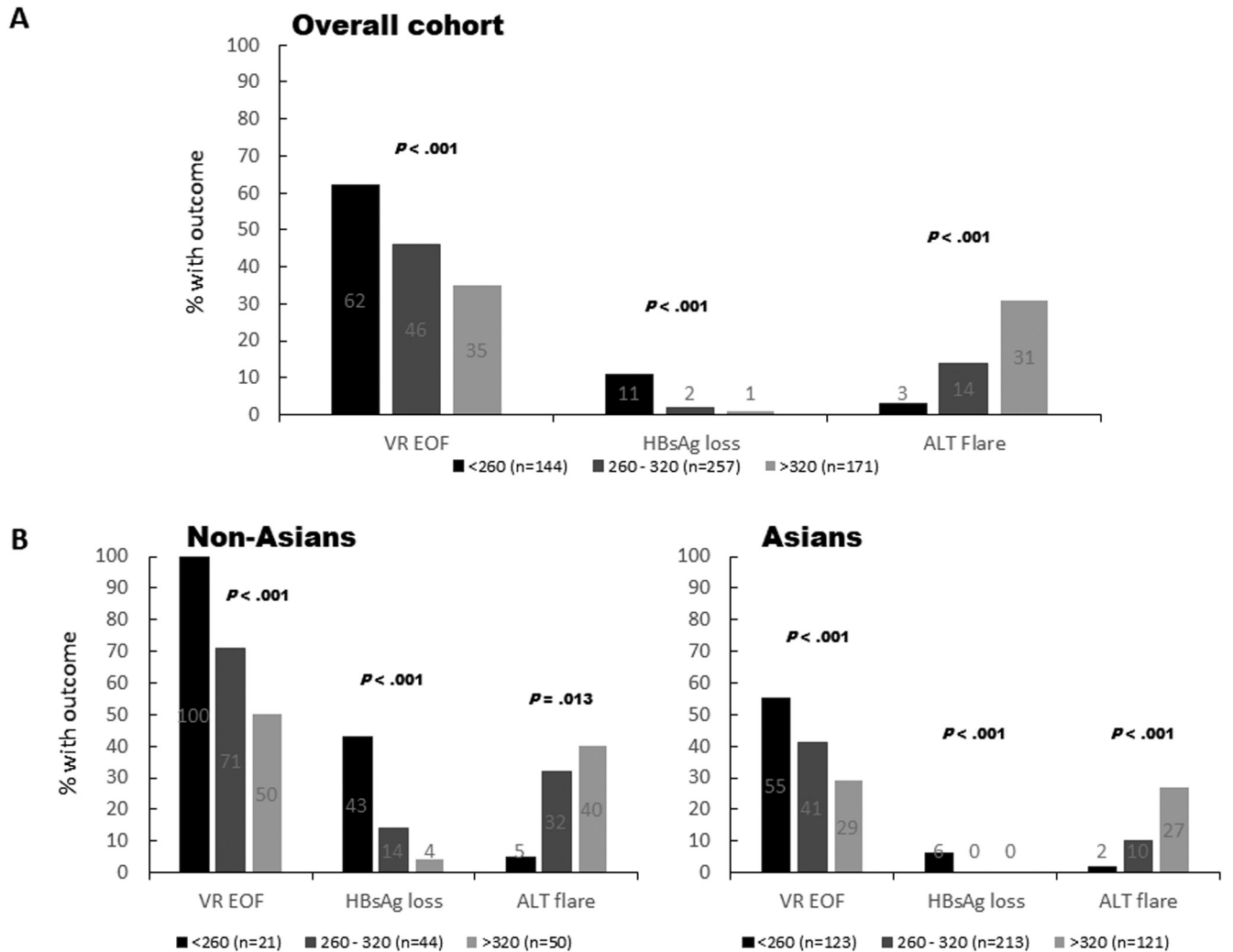


Figure 3. Outcome after therapy withdrawal according to the Surface antigen, Core-related antigen, Age, ALT, and tenofovir for HBV (SCALE-B) score at treatment cessation (A) in the overall population and (B) stratified by ethnicity. A flare consisted of an ALT level at least 3 times the upper limit of normal. VR EOF was defined as a virologic response (HBV DNA level, <2000 IU/mL) at the last follow-up evaluation (ie, week 48, or week 24 if no data available at week 48).

Although our study was large and enrolled patients from different ethnic backgrounds and used data on both HBcrAg and HBsAg, there were some caveats. First, because we enrolled patients from different cohorts, re-treatment criteria varied, and a significant proportion of patients was re-treated for HBV DNA flares without an ALT increase. Some of these patients still might have progressed to an inactive carrier state without antiviral therapy, and early re-treatment therefore may have contributed to an underestimation of sustained response rates. Despite the heterogeneity in re-treatment criteria, response rates were consistently lower in cohorts enrolling predominantly Asian patients (34%–50%) compared with cohorts with predominantly non-Asian patients (66%–84%), and findings were consistent in a sensitivity analysis excluding the predominantly Asian cohort with the lowest response rates. Furthermore, not all patients in our cohort had data available at week 48 after treatment. For these patients we used a last-observation-carried-forward approach to preserve

statistical power. We performed a sensitivity analysis for VR at week 48 (ie, excluding patients without data at this time point) and observed similar associations. This supports the robustness of our findings. However, future studies should focus on the long-term risk of relapse in relation to end-of-treatment biomarker levels because the majority of our patients did not have data beyond week 48 after therapy discontinuation. In addition, we defined off-treatment response based on HBV DNA levels and/or HBsAg loss, whereas other studies used combined end points comprising both HBV DNA and ALT. In our cohort, the vast majority of patients with a VR (82%) had normal ALT levels, and the associations of HBcrAg, HBsAg, and SCALE-B with response were similar when we applied a combined end point (HBV DNA <2000 IU/mL with normal ALT level) (Supplementary Figure 1). Furthermore, it should be appreciated that although we confirmed the predictive performance of HBcrAg, HBsAg, and the SCALE-B¹³ score in this independent data set, discriminative performance was rather limited, and

absolute response rates remain relatively low in Asians despite low HBsAg and HBcrAg levels and SCALE-B scores at the time of treatment cessation. Future studies therefore should explore the additive value of other factors, for example, HBV RNA and antibody to hepatitis B core antigen levels, to further improve prediction of favorable outcomes after NA cessation, particularly in Asian patients. Another issue that warrants further exploration is the influence of HBV genotype, for which data were not available for the majority of our cohort. Finally, the safety of NA withdrawal remains a potential concern. In our cohort, 2 patients with viral rebound after treatment discontinuation developed a flare with a concomitant increase in bilirubin—both resolved with re-treatment. Importantly, neither of these patients had a low SCALE-B score.

In conclusion, our multicenter study comprising a unique multi-ethnic cohort of CHB patients who discontinued NA therapy shows that off-treatment VR and HBsAg loss rates vary with patient ethnicity. Non-Asian patients are more likely to achieve favorable outcomes. Lower levels of HBcrAg and HBsAg are associated with favorable outcomes and these factors, either alone or combined in a risk score (SCALE-B), can be used for risk stratification. Further studies investigating other biomarkers are required to optimize selection of patients with NA discontinuation.

Supplementary Material

Note: To access the supplementary material accompanying this article, please click [here](#).

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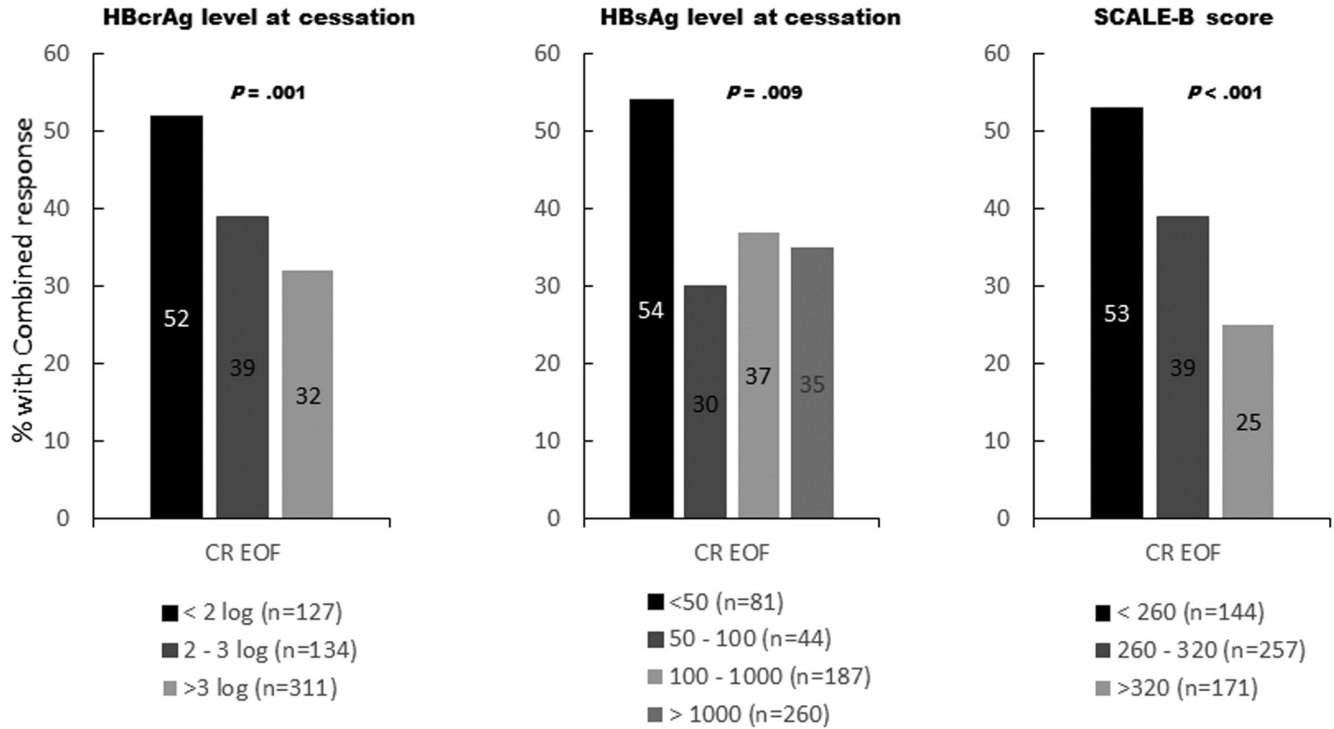
Conflicts of interest

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Supplementary Figure 1. Relationship between HBcrAg and HBsAg levels, and the Surface antigen, Core-related antigen, Age, ALT, and tenofovir for HBV (SCALE-B) score, with combined response at end of follow-up evaluation. CR EOF, combined response of HBV DNA level <2000 IU/mL with normal alanine aminotransferase level at end of follow-up evaluation; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen.

Supplementary Table 1. Off-Treatment Outcomes Across Surface antigen, Core-related antigen, Age, ALT, and tenofovir for HBV (SCALE-B) Scores Stratified by HBeAg Status at Baseline

	<60	260-320	>320	<i>P</i>
VR				
HBeAg-	58%	47%	34%	<.001
HBeAg+	93%	43%	39%	.001
HBsAg loss				
HBeAg-	10	3	1	.001
HBeAg+	20%	0%	0%	<.001
ALT flare				
HBeAg-	3.1%	14%	29%	<.001
HBeAg+	0%	11%	39%	.001

NOTE. An ALT flare consisted of an ALT level more than 3 times the upper limit of normal.
 HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; VR, virologic response (HBV DNA level, <2000 IU/mL).

Supplementary Table 2. Diagnostic Accuracy of the Surface antigen, Core-related antigen, Age, ALT, and tenofovir for HBV (SCALE-B) score in the Overall Population and Across Ethnicities

	SCALE-B < 260			
	Sensitivity	Specificity	NPV	PPV
Overall				
VR	33%	82%	58%	62%
HBsAg loss	67%	77%	98%	11%
ALT flare	4%	71%	79%	3%
Non-Asian				
VR	27%	100%	40%	100%
HBsAg loss	53%	88%	92%	43%
ALT flare	3%	75%	64%	5%
Asian				
VR	36%	79%	64%	55%
HBsAg loss	100%	74%	100%	6%
ALT flare	5%	70%	84%	2%

NOTE. An ALT flare consisted of an ALT level more than 3 times the upper limit of normal.
 ALT, alanine aminotransferase; NPV, negative predictive value; PPV, positive predictive value; VR, virologic response (HBV DNA level, <2000 IU/mL).