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HBV DNA and HBsAg Levels at 24 Weeks Off-Treatment Predict Clinical Relapse and HBsAg Loss in HBeAg-Negative Patients Who Discontinued Antiviral Therapy

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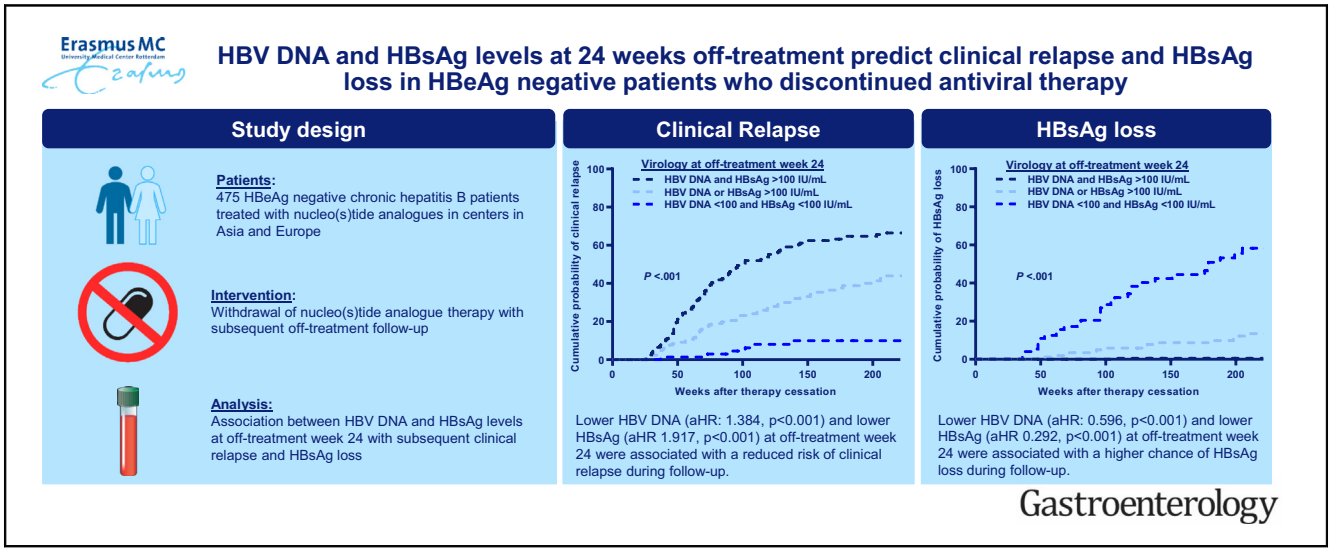

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BACKGROUND & AIMS: Patients who discontinue nucleo(s)tide analogue therapy are at risk of viral rebound and severe hepatitis flares, necessitating intensive off-treatment follow-up. **METHODS:** We studied the association between hepatitis B surface antigen (HBsAg) and hepatitis B virus (HBV) DNA levels at off-treatment follow-up week 24 (FU W24), with subsequent clinical relapse, and HBsAg loss in a multicenter cohort of hepatitis B e antigen (HBeAg)–negative patients with chronic hepatitis B who discontinued nucleo(s)tide analogue therapy. **RESULTS:** We studied 475 patients, 82% Asian, and 55% treated with entecavir. Patients with higher HBV DNA levels at FU W24 had a higher risk of clinical relapse (hazard ratio [HR], 1.576; $P < .001$) and a lower chance of HBsAg loss (HR, 0.454; $P < .001$). Similarly, patients with higher HBsAg levels at FU

W24 had a higher risk of clinical relapse (HR, 1.579; $P < .001$) and a lower chance of HBsAg loss (HR, 0.263; $P < .001$). A combination of both HBsAg <100 IU/mL and HBV DNA <100 IU/mL at FU W24 identified patients with excellent outcomes (9.9% clinical relapse and 58% HBsAg loss at 216 weeks of follow-up). Conversely, relapse rates were high and HBsAg loss rates negligible among patients with both HBsAg >100 IU/mL and HBV DNA >100 IU/mL ($P < .001$). **CONCLUSIONS:** Among HBeAg-negative patients with chronic hepatitis B who discontinued antiviral therapy and who did not experience clinical relapse before FU W24, serum levels of HBV DNA and HBsAg at FU W24 can be used to predict subsequent clinical relapse and HBsAg clearance. A combination of HBsAg <100 IU/mL with HBV DNA <100 IU/mL identifies patients with a low risk of relapse and excellent chances of HBsAg loss and could potentially be used as an early surrogate end point for studies aiming at finite therapy in HBV.

Keywords: HBsAg; HBV DNA; Clinical Relapse; HBsAg Loss.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Patients who discontinue nucleo(s)tide analogue therapy are at risk of viral rebound and severe hepatitis flares, necessitating intensive off-treatment follow-up. Early off-treatment predictors of longer-term outcomes are required to guide patient management.

NEW FINDINGS

Higher hepatitis B virus DNA and hepatitis B surface antigen levels at 24 weeks after therapy withdrawal are associated with an increased risk of subsequent clinical relapse and a lower chance of hepatitis B surface antigen loss during long-term follow-up. Patients with both hepatitis B virus DNA <100 IU/mL and hepatitis B surface antigen <100 IU/mL at off-treatment week 24 have a very limited risk of subsequent clinical relapse and a very high chance of hepatitis B surface antigen loss.

LIMITATIONS

The current study included only patients with negative hepatitis B e antigen at the start of antiviral therapy, and the findings may therefore not be applicable to patients with positive baseline hepatitis B e antigen. Most of the cohort comprised Asian patients, and further validation of the findings is therefore required in other populations.

CLINICAL RESEARCH RELEVANCE

Hepatitis B virus DNA and hepatitis B surface antigen levels at off-treatment week 24 can be used to guide patient management. Retreatment should be considered in patients with high hepatitis B virus DNA and hepatitis B surface antigen levels, whereas careful follow-up may be considered for patients with both low hepatitis B virus DNA and hepatitis B surface antigen levels.

BASIC RESEARCH RELEVANCE

The combination of low HBV DNA and hepatitis B surface antigen levels at off-treatment week 24 could be used as a surrogate for long-term favorable outcomes and could be used as a response parameter in translational studies.

Discontinuation of nucleo(s)tide analogue (NUC) therapy in patients positive for hepatitis B surface antigen (HBsAg) has recently been shown to be feasible and to result in sustained remission and even HBsAg loss in a subset of patients.[1,2] Caucasian ethnicity and low end-of-treatment viral antigen levels have been identified as important predictors of favorable outcomes after therapy cessation.[2,3] Hepatitis B virus (HBV) genotype C may also be associated with a higher chance of HBsAg clearance after treatment cessation among Asian patients, whereas no definitive data are available on the influence of HBV genotype on outcomes in non-Asians.[3]

Unfortunately, even patients with multiple favorable prognostic indicators remain at risk of relapse and hepatitis flares after therapy withdrawal. Previous studies have shown that whereas reappearance of HBV DNA is observed in most patients after therapy cessation, the timing and severity of viral rebound may vary significantly, necessitating prolonged and intensive follow-up in all patients who discontinue therapy.[4] Recent studies indicate that off-treatment viral antigen kinetics may correlate poorly with HBV DNA kinetics and may therefore offer important additional information over HBV DNA monitoring alone.[5,6] We hypothesized, based on experienced gained with finite interferon–based therapies, where remission at 6 months after therapy withdrawal is associated with excellent long-term outcomes[7,8] and the observation that the highest risk of virologic relapse occurs during the first months after NUC withdrawal,[2,9] that serum levels of HBV DNA and HBsAg at 6 months after therapy withdrawal could be used to predict subsequent relapse risk and HBsAg loss.

Patients and Methods

Patients

The current study used data from a pooled data set comprising patients with chronic hepatitis B who discontinued NUC therapy as part of studies or clinical practice in centers in Europe and Asia (the CREATE cohort).[2,10] Patients were eligible for the current analysis if they had been treated with only NUCs (no history of interferon add-on was allowed) and if they were (1) hepatitis B e antigen; (HBeAg) negative at the time of therapy initiation, (2) had undetectable HBV DNA at the time of therapy cessation, (3) had available information on HBV DNA and HBsAg levels at follow-up week 24 (FU W24), and (4) were still HBsAg

HEPATOBILIARY

* Authors share co-first authorship; § Authors share co-senior authorship.

Abbreviations used in this paper: aHR, adjusted hazard ratio; CI, confidence interval; FU W24, follow-up week 24; HBcrAg, hepatitis B core related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HR, hazard ratio; NUC, nucleo(s)tide analogue.

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positive and did not meet criteria for clinical relapse before or at FU W24 (Supplementary Figure 1).

Laboratory Testing

HBV DNA was measured using local laboratory methods. HBsAg was measured using various standardized methods. Hepatitis B core-related antigen (HBcrAg) was quantified with the Lumipulse G HBcrAg assay (Fujirebio Europe) on a Lumipulse G1200 analyser (Fujirebio Inc). The assay's lower limit of quantification is 3 log U/mL. HBV genotyping was performed using various methods, including line-probe assays, restriction fragment length polymorphism, or sequencing. Other biochemical tests were performed using local laboratory facilities.

End Points and Statistical Analysis

The association between HBV DNA and HBsAg levels at FU W24 was assessed using Pearson's correlation. Next, we explored the association between HBV DNA levels (continuous and categorized as <100, 100–1000, 1,000–10,000, and >10,000 IU/mL), and HBsAg levels (continuous and categorized as <100, 100–1000 and >1,000 IU/mL), with clinical relapse and HBsAg loss during subsequent follow-up. Analyses were performed in the overall population, and after stratification, by end-of-treatment HBsAg (<100 and >100 IU/mL) and HBcrAg (<3 log and >3 log IU/mL) levels. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason. Patients achieving HBsAg loss were considered to remain free from clinical relapse. HBsAg loss was defined as undetectable HBsAg at any time during off-treatment follow-up. Re-treated patients were considered persistently HBsAg positive. Associations were explored using the Kaplan-Meier methods and Cox's regression. Analyses were performed using SPSS 28 software (IBM). All statistical tests were 2-sided and were evaluated at the 0.05 level of significance.

Results

Cohort Characteristics

The CREATE database comprised data of 1241 patients who discontinued antiviral therapy; of these, 300 patients were excluded because of a positive HBeAg at baseline, 289 were excluded for missing data at FU W24, 126 were excluded because of clinical relapse or HBsAg loss before FU W24, and 51 patients did not have sufficient follow-up beyond W24 (Supplementary Figure 1). Characteristics of the patients included in this analysis and of those who were excluded for clinical relapse in the first 24 weeks after therapy cessation are shown in Supplementary Tables 1 and 2. This left 475 patients for further analysis, most of whom were previously treated with entecavir (54.9%) and of Asian ethnicity (82%). HBV DNA levels at FU W24 were <100 IU/mL in 177 (37%) and >10,000 IU/mL in 82 (17%). HBsAg levels at FU W24 were <100 IU/mL in 139 (29%) and >1000 in 121 (26%). Additional cohort characteristics are summarized in Table 1 and Supplementary Table 3. At FU W24 we observed a limited correlation between HBV DNA and HBsAg levels (Pearson's $r = 0.270$, $P < .001$) (Figure 1). In the overall study population, the cumulative probability of clinical relapse and HBsAg loss at 216 weeks of follow-up were 48.9% and 13.6%. Among the 193 patients who experienced clinical relapse during FU >W24, 3 (1.9%) experienced decompensation of liver disease, 1 of whom died.

Table 1. Characteristics of the Patients Included in This Cohort

Characteristics	Values (N = 475)
Demographics	
Age, *y*	52 (45–59)
Male sex	358 (75)
Asian ethnicity	391 (82)
Cirrhosis	18 (3.8)
Duration of treatment, *wk*	157 (156–211)
Treatment	
Entecavir	261 (54.9)
Tenofovir disoproxil fumarate	169 (35.6)
Other/combination	45 (9.5)
Characteristics at end of treatment	
Alanine aminotransferase, *U/L*	22 (17–32)
HBV DNA undetectable	475 (100)
HBsAg, *log IU/mL*	2.67 (2.1–3.1)
HBsAg <100 IU/mL	112 (23.6)
HBcrAg, *log U/mL*[a]	3.0 (3.0–3.5)
HBcrAg <3 log U/mL	286 (60.2)
Characteristics at FU W24	
Alanine aminotransferase, *U/L*	27 (19–38)
HBV DNA, *log IU/mL*	2.6 (1.3–3.6)
HBV DNA <100 IU/mL	177 (37.3%)
HBsAg, *log IU/mL*	2.6 (1.9–3.0)
HBsAg <100 IU/mL	139 (29.3%)

NOTE. Data are presented as n (%) or median (interquartile range).
[a]The lower limit of quantification for HBcrAg is 3 log U/mL, the lower limit of detection is 2 log U/mL.

HBV DNA Levels at Follow-up Week 24 Predict Clinical Relapse and HBsAg Loss

Patients with higher HBV DNA levels at FU W24 had a higher risk of clinical relapse (hazard ratio [HR], 1.576; 95% confidence interval [CI], 1.423–1.747; $P < .001$) and a lower chance of HBsAg loss (HR, 0.454; 95% CI, 0.360–0.573; $P < .001$). The cumulative probability of clinical relapse at 216 weeks was 27.8% among patients with HBV DNA <100 IU/mL vs 83% among patients with HBV DNA >10,000 IU/mL ($P < .001$) (Figure 2*A*). Conversely, patients with HBV DNA levels <100 IU/mL had the highest chance of subsequent HBsAg loss; the cumulative probability of HBsAg loss at 216 weeks was 31.3% vs 2.8% among patients with HBV DNA >10,000 IU/mL at off-treatment W24 ($P < .001$) (Figure 2*B*). Findings were consistent among patients with low and high viral antigen levels at the end of treatment (Supplementary Figures 2 and 3).

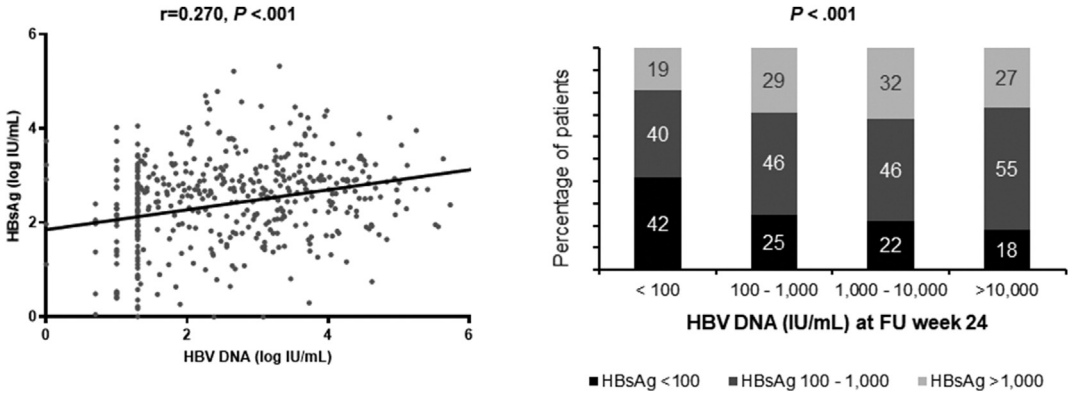


Figure 1. Association between HBV DNA and HBsAg levels at FU W24. There was a modest correlation between HBV DNA and HBsAg levels in this cohort.

HBsAg Levels at Follow-up Week 24 Predict Subsequent Clinical Relapse and HBsAg Loss

Patients with higher HBsAg levels at FU W24 had a higher risk of clinical relapse (HR, 1.579; 95% CI, 1.364–1.828; $P < .001$) and a lower chance of HBsAg loss (HR, 0.263; 95% CI, 0.212–0.328; $P < .001$). The cumulative probability of clinical relapse at 216 weeks was 24.9% among patients with HBsAg levels <100 IU/mL vs 60.3% among patients with HBsAg >1000 IU/mL ($P < .001$) (Figure 3*A*). Conversely, patients with HBsAg levels <100 IU/mL had the highest chance of subsequent HBsAg loss. The cumulative probability of HBsAg loss at 216 weeks was 40.2% vs 1.4% among patients with HBsAg >1000 IU/mL ($P < .001$) (Figure 3*B*). Findings were consistent among patients with low and high HBcrAg levels at the end of treatment and among patients with HBsAg levels >100 IU/mL at the end of treatment (Supplementary Figures 4 and 5).

Interestingly, outcomes were similar for patients who had an HBsAg level >100 IU/mL at the end of treatment but who experienced a decline to <100 at FU W24 compared with patients who had HBsAg levels <100 IU/mL at both end of treatment and FU W24 ($P > .424$) (Supplementary Figure 6).

A Combination of HBsAg <100 IU/mL With HBV DNA <100 IU/mL at Follow-up Week 24 Identifies Patients With Excellent Long-term Outcomes

Among the 475 patients in this cohort, HBsAg and HBV DNA levels at FU W24 were both >100 IU/mL in 233 (49%), 168 (35%) had HBsAg >100 IU/mL with HBV DNA <100 IU/mL or HBsAg <100 IU/mL with HBV DNA >100 IU/mL, and 74 (16%) had both HBsAg and HBV DNA levels <100 IU/mL. A combination of both HBsAg <100 IU/mL and HBV DNA <100 IU/mL at FU W24 identified patients with the best outcomes: at 216 weeks of follow-up, the cumulative risk of clinical relapse was only 9.9%, and the cumulative incidence of HBsAg loss was 58.3% in this subset of the cohort ($P < .001$) (Figure 4). Conversely, patients with both HBV DNA >100 IU/mL and HBsAg >100 IU/mL had a very high risk of clinical relapse (66.5% at 216 weeks) and virtually no chance of HBsAg loss (<1% at 216 weeks). Importantly, among the patients who had an end-of-treatment HBsAg level <100 IU/mL, only 57 (50.9%) had both HBsAg <100 IU/mL and HBV DNA <100 IU/mL at off-treatment W24.

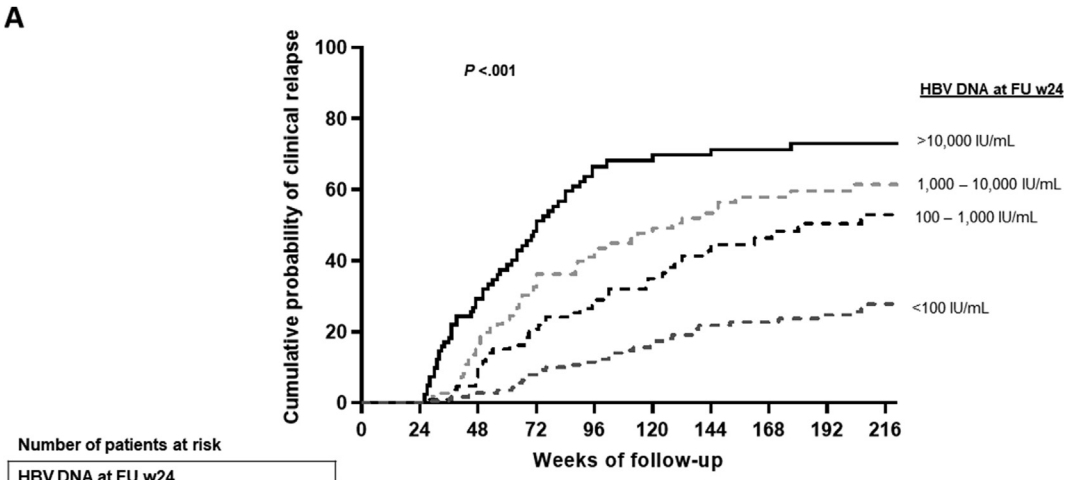
HBV DNA and HBsAg Levels at Follow-up Week 24 Predict Subsequent Relapse and HBsAg Loss: Multivariable Analysis

In multivariable Cox's regression analysis, adjusting for other established predictors of outcomes after therapy cessation, higher HBV DNA (adjusted HR [aHR], 1.384; $P < .001$) and HBsAg (aHR, 1.917; $P < .001$) levels at FU W24 were independently associated with an increased risk of clinical relapse (Table 2). Similarly, lower HBV DNA (aHR, 0.596; $P < .001$) and HBsAg levels (aHR, 0.292; $P < .001$) were independently associated with an increased chance of HBsAg loss (Table 3). In similar models, the combined presence of both HBV DNA <100 IU/mL with HBsAg <100 IU/mL was associated with a very low risk of clinical relapse (aHR, 0.158; $P < .001$) (Supplementary Table 4) and a very high chance of HBsAg loss (aHR, 13.874; $P < .001$) (Supplementary Table 5).

Discussion

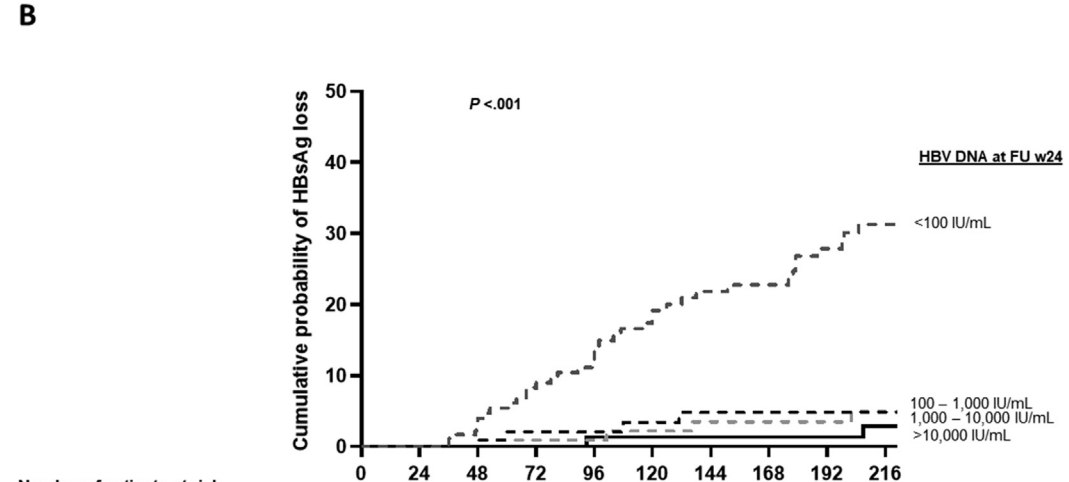
Most patients who discontinue NUC therapy will experience virologic relapse during follow-up and will not achieve HBsAg clearance. In this multicenter study, we show that serum levels of HBV DNA and HBsAg at FU W24 can be used to stratify future relapse risk and to predict which patients are (un)likely to achieve HBsAg clearance. These findings can be used to guide patient management and suggest that FU W24 HBV DNA and HBsAg levels could be used as surrogate end points for trials aiming for finite treatment.

Withdrawal of NUCs has gained major clinical interest because a substantial proportion of patients may achieve sustained disease remission and even HBsAg loss during



Number of patients at risk

HBV DNA at FU w24										
>10,000 IU/mL		82				20				15
1,000 – 10,000 IU/mL		110				37				21
100 – 1,000 IU/mL		106				43				18
< 100 IU/mL		177				97				69



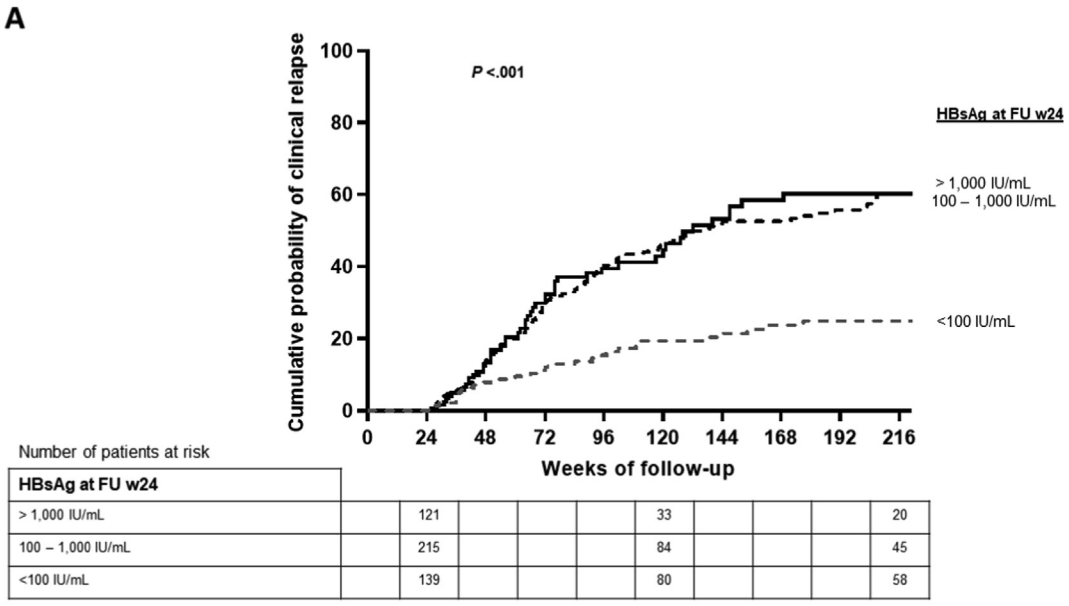
Number of patients at risk

HBV DNA at FU w24										
< 100 IU/mL		177				95				59
100 – 1,000 IU/mL		106				71				50
1,000 – 10,000 IU/mL		110				76				67
>10,000 IU/mL		82				71				65

Figure 2. Association between HBV DNA levels at FU W24 with subsequent outcomes. Association between (*A*) HBV DNA levels at FU W24 with clinical relapse and (*B*) HBsAg loss. The study excluded patients who experienced clinical relapse or HBsAg loss at $\leq$24 weeks of follow-up. Clinical relapse was defined as the occurrence of HBV DNA $>$2000 IU/mL with alanine aminotransferase $>$2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

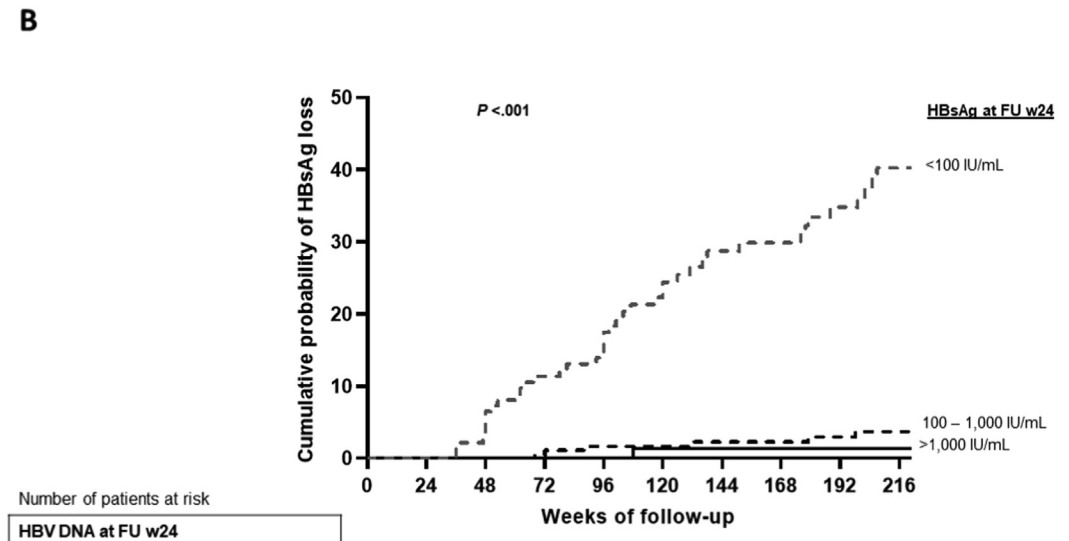
off-treatment follow-up. However, most patients will not achieve these outcomes and will remain at risk for virologic relapse and severe hepatitis flares during long-term follow-up. These findings have prompted various studies to focus on identifying predictors of successful therapy withdrawal. Potential predictors of off-treatment outcomes are patient age, ethnicity, HBV genotype, antiviral agent, and lower end-of-treatment levels of alanine aminotransferase, HBsAg, HBcrAg, and HBV RNA.[1,2,10–12] Importantly, even combinations of these factors cannot guarantee successful therapy withdrawal, and careful follow-up remains essential in all patients who discontinue therapy.

Prospective studies have shown that although relapse may occur at any time during off-treatment follow-up, the risk is highest during the first months after therapy withdrawal.[9] We therefore hypothesized that HBV DNA and HBsAg levels at 6 months after therapy withdrawal could potentially be used as predictors of longer-term outcomes.



Number of patients at risk

HBsAg at FU w24										
> 1,000 IU/mL		121				33				20
100 – 1,000 IU/mL		215				84				45
<100 IU/mL		139				80				58



Number of patients at risk

HBV DNA at FU w24										
< 100 IU/mL		139				75				42
100 – 1,000 IU/mL		215				166				131
> 1,000 IU/mL		121				72				68

Figure 3. Association between HBsAg levels at FU W24 with subsequent outcomes. Association between (*A*) HBsAg levels at FU W24 with clinical relapse and (*B*) HBsAg loss. The study excluded patients who experienced clinical relapse or HBsAg loss at ≤24 weeks of follow-up. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

This approach is analogous to that which has been applied to interferon-based therapies, where responses observed at 6 months after therapy withdrawal have been shown to be durable.[7,8]

In the current study, we were able to analyze data from 475 patients who were still off-treatment at FU W24. Interestingly, we observed a wide range of HBV DNA and HBsAg levels at off-treatment FU W24 in these patients, with only limited correlations observed between HBV DNA and HBsAg levels. This finding supports previous observations that these factors may be complimentary.[5,6]

In our cohort, lower levels of HBV DNA and HBsAg at FU W24 were associated with a lower risk of clinical relapse and a higher chance of HBsAg loss. Importantly, patients with both HBV DNA <100 IU/mL and HBsAg <100 IU/mL had a very low risk of clinical relapse during prolonged follow-up and a very high chance of HBsAg loss. However, patients with both HBsAg >100 IU/mL and HBV DNA

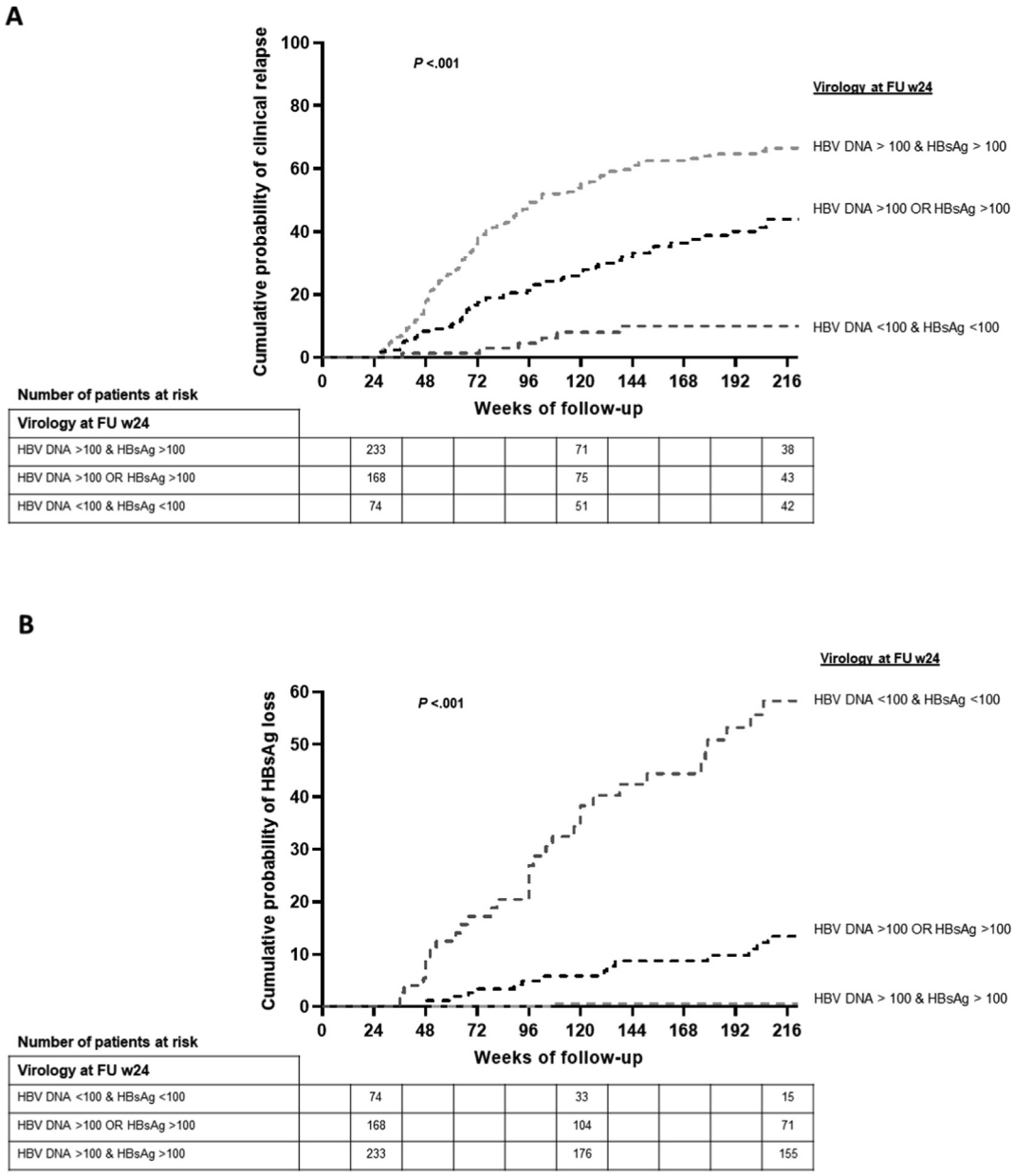


Figure 4. A combination of HBV DNA and HBsAg levels at FU W24 predicts clinical relapse and HBsAg loss. Association between (*A*) a combination of HBV DNA and HBsAg levels at FU W24 with clinical relapse and (*B*) HBsAg loss. The study excluded patients who experienced clinical relapse or HBsAg loss at $\leq$24 weeks of follow-up. Clinical relapse was defined as the occurrence of HBV DNA $>$2000 IU/mL with alanine aminotransferase $>$2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

$>$100 IU/mL had a very high risk of clinical relapse and virtually no chance of HBsAg loss. These findings were consistent across several clinically relevant subgroups, including those with high or low end-of-treatment viral antigen levels, and were also robust in multivariable analysis.

Our observations may have important clinical applications. Patients with both low HBsAg and HBV DNA levels at FU W24 could potentially enter a less restrictive follow-up protocol, given their limited risk of relapse. Furthermore, our findings suggest that patients with both high HBsAg and high HBV DNA levels may be better off restarting antiviral therapy, because they are at very high risk of clinical relapse, with virtually no chance of achieving HBsAg clearance. These patients are therefore unlikely to benefit from further off-treatment follow-up and are potentially at risk of developing severe hepatitis flares, as highlighted by a recent case report and the cases of hepatic decompensation after therapy withdrawal observed in this cohort.[13]

In addition to the potential clinical application of our findings in patients who discontinue NUC therapy, our results also suggest that low off-treatment HBsAg and HBV DNA levels could have utility as a potential surrogate end point for future studies investigating finite treatment

Table 2. Factors Associated With Clinical Relapse

Variable	aHR (95% CI)	*P*
HBV DNA (log IU/mL) FU W24	1.384 (1.238–1.548)	<.001
HBsAg (log IU/mL) FU W24	1.917 (1.568–2.342)	<.001
Alanine aminotransferase (U/L) FU W24	1.011 (1.001–1.021)	.026
Age	1.040 (1.026–1.056)	<.001
Male sex	1.271 (0.890–1.816)	.188
Asian ethnicity	5.976 (2.560–13.95)	<.001
Tenofovir therapy	1.257 (0.907–1.742)	.169

NOTE. Factors associated with clinical relapse after NUC withdrawal in multivariable Cox's regression analysis. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

approaches. At present, such studies are challenging to perform because HBsAg loss is infrequently achieved. There is therefore a major unmet need for other more easily achievable intermediate end points, a so-called partial cure, to evaluate the efficacy of novel compounds and treatment strategies. Based on our findings, we propose that a combination of low HBsAg and HBV DNA levels at 6 months after therapy withdrawal could potentially be used as such a surrogate end point, although this should still be validated for novel antiviral agents.

Although our study is relatively large and studied patients from centers in Europe and Asia, it does have several limitations. First, because we excluded patients re-treated before week 24, only a limited subset of the cohort was eligible for enrollment. Furthermore, other off-treatment factors, such as HBV RNA and HBcrAg, were not available to us, and future studies should focus on assessment of the additive value of these biomarkers.

Table 3. Factors Associated With Hepatitis B Surface Antigen Loss

Variable	aHR (95% CI)	*P*
HBV DNA (log IU/mL) FU W24	0.596 (0.466–0.761)	<.001
HBsAg (log IU/mL) FU W24	0.292 (0.232–0.369)	<.001
Alanine aminotransferase (U/L) FU W24	1.023 (1.007–1.038)	.004
Age	0.988 (0.963–1.013)	0.348
Male sex	0.725 (0.424–1.239)	0.239
Asian ethnicity	0.206 (0.096–0.440)	<0.001
Tenofovir therapy	1.303 (0.774–2.193)	0.319

NOTE. Factors associated with HBsAg loss after NUC withdrawal in multivariable Cox's regression analysis.

Additionally, we studied a relatively small number of non-Asian patients. Although findings were consistent across Asian and non-Asian patients (Supplementary Figure 7), further validation in non-Asian patients is required.

It is also important to note that the type of antiviral therapy was not associated with clinical relapse in our analyses, which appears to contradict other reports.[14,15] This is likely due to exclusion of patients with early clinical relapse, which is more likely to be observed in patients previously treated with tenofovir.[14,15]

Conclusion

Our multicenter study shows that, among patients with chronic hepatitis B who are HBeAg negative and who discontinued antiviral therapy and who did not experience clinical relapse before FU W24, serum levels of HBV DNA and HBsAg at FU W24 can be used to predict subsequent clinical relapse and HBsAg clearance. A combination of low HBsAg levels and low HBV DNA levels identifies patients with very low risk of clinical relapse and excellent chances of HBsAg loss and could potentially be used as an early surrogate end point for studies aiming at finite therapy in HBV. Patients with both high HBsAg and HBV DNA levels have a very high risk of relapse and virtually no chance of HBsAg loss and may benefit from reinitiation of antiviral therapy.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2023.09.033.

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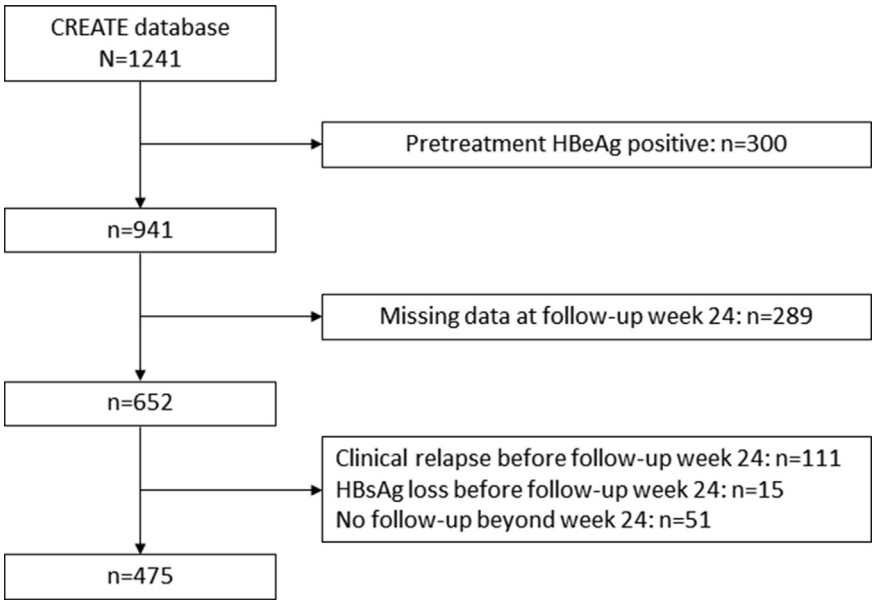
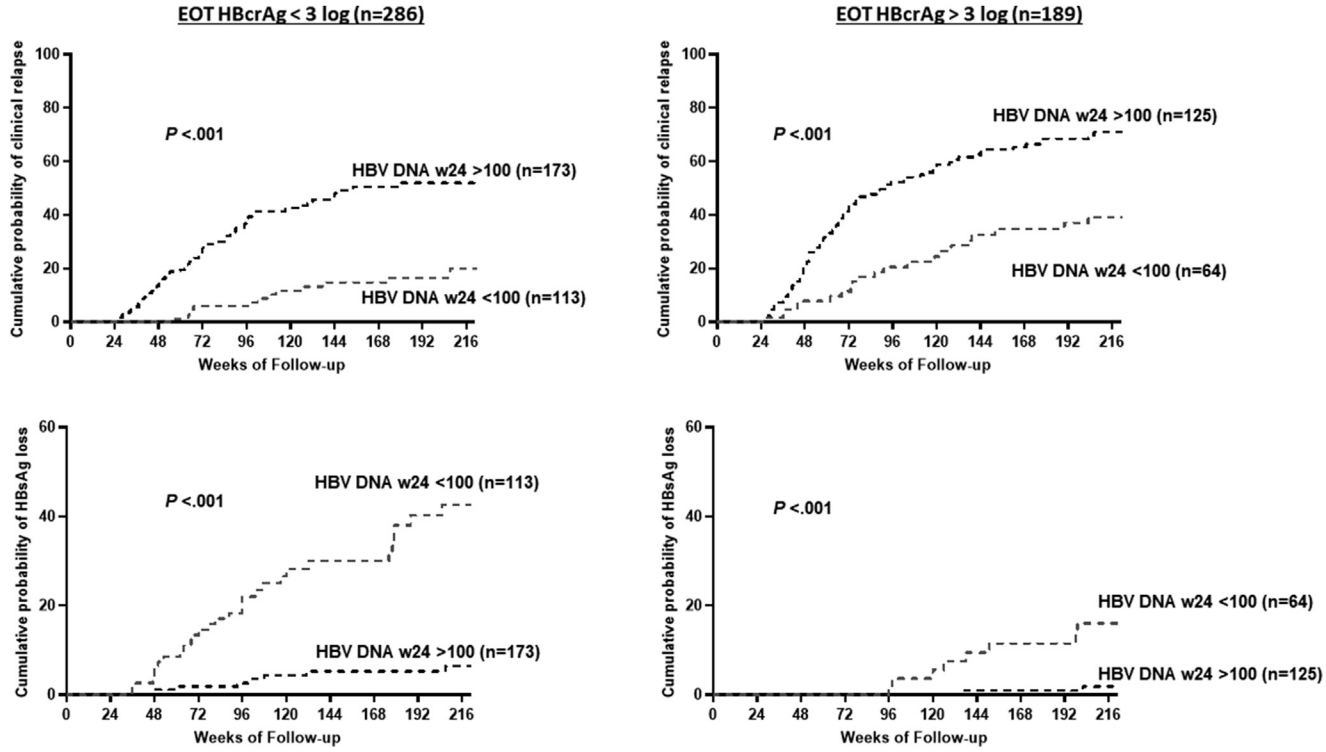
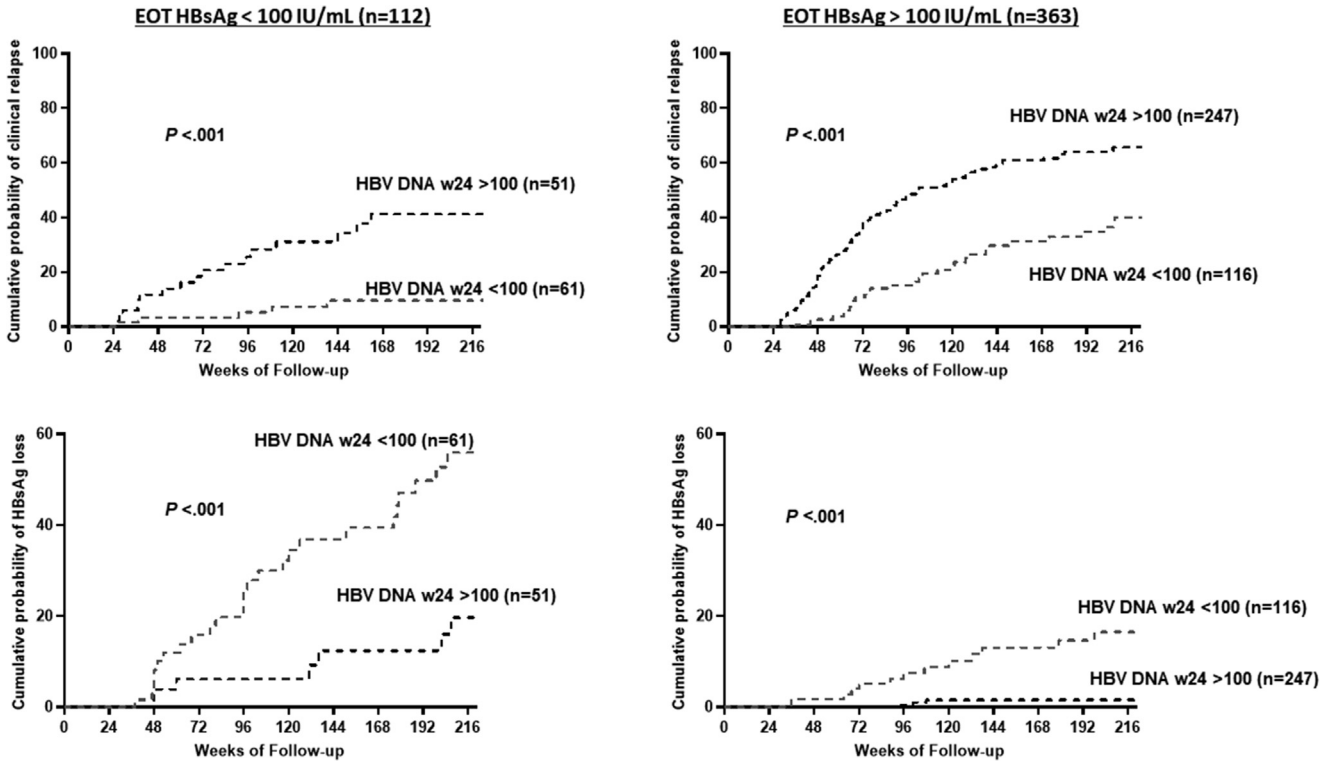
Data Availability
The data used for the current analysis were derived from previously published cohorts and clinical data sets. The data cannot be shared.



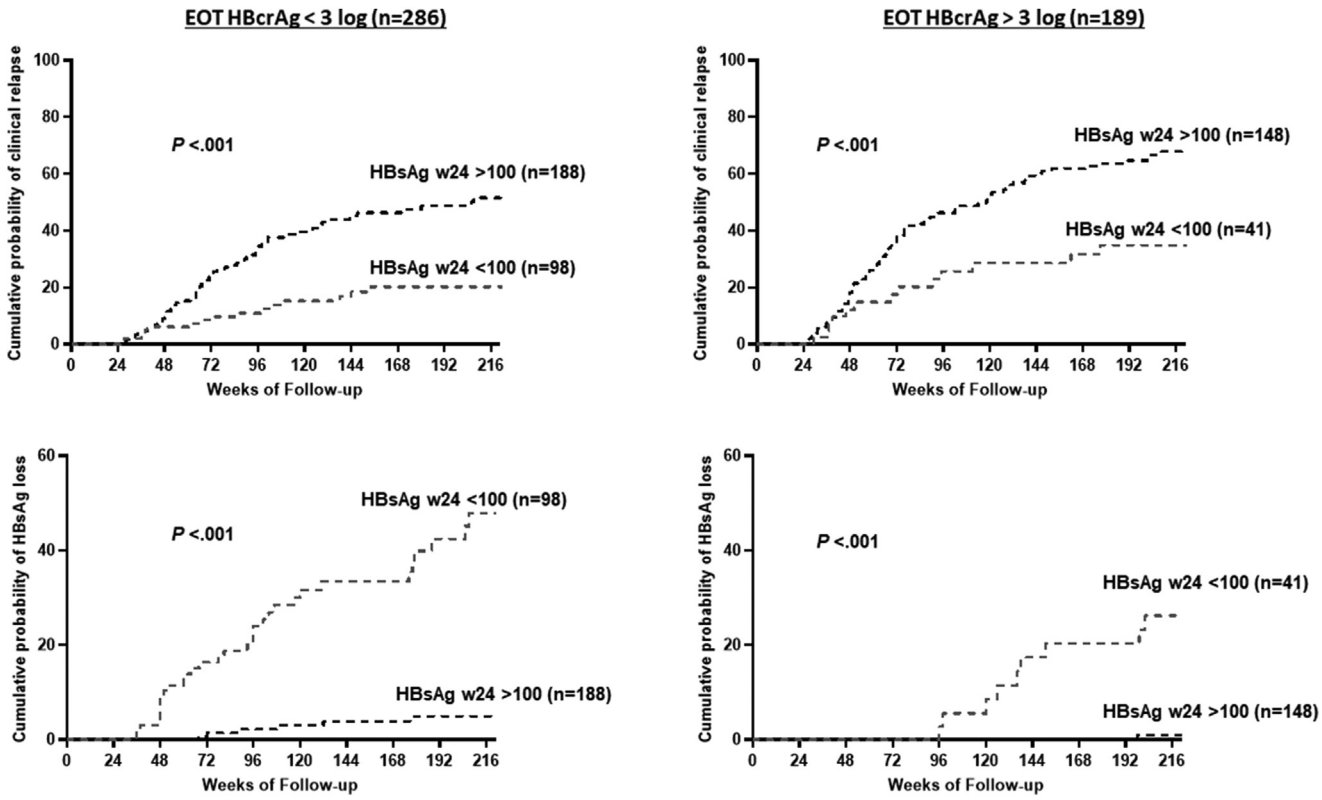
Supplementary Figure 1. Patient flow chart. Selection of patients for the current analysis. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.



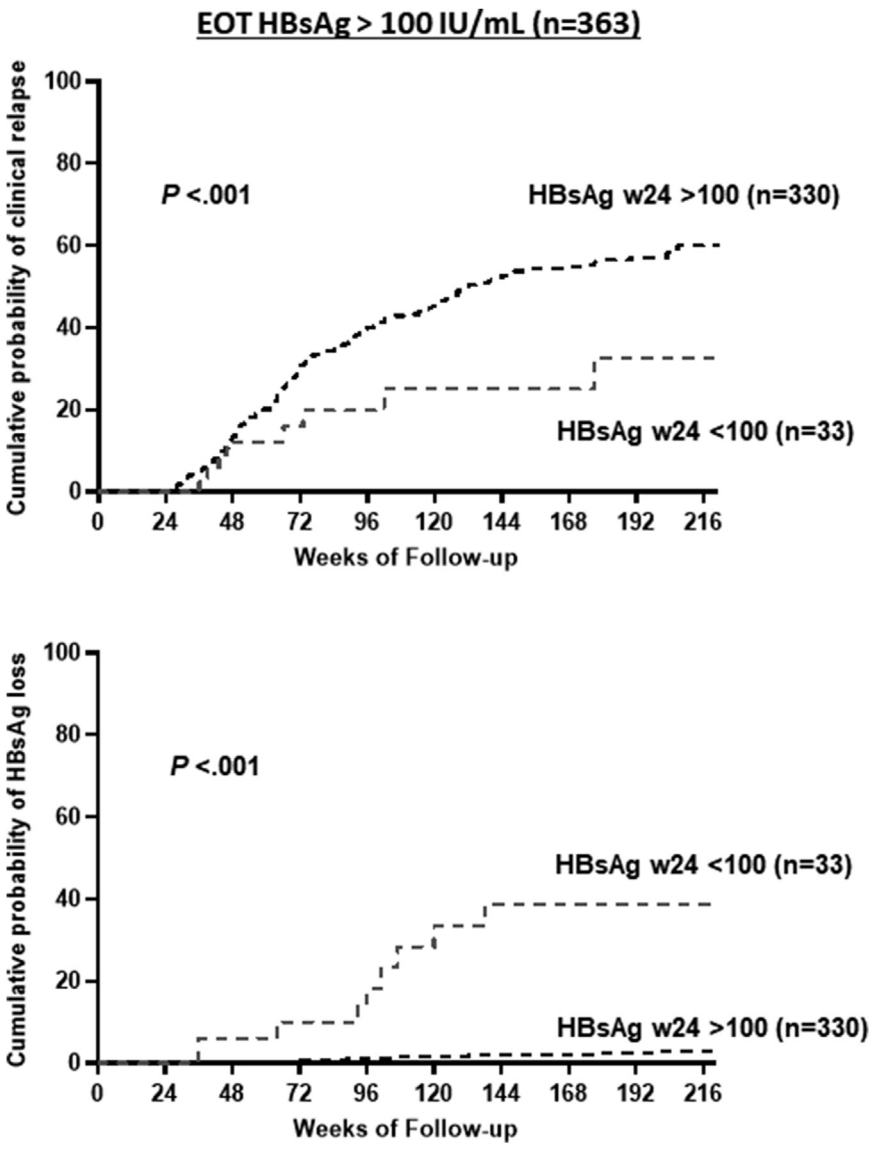
Supplementary Figure 2. Association between HBsAg levels at FU W24 with clinical relapse and HBsAg loss for patients with low and high HBcrAg levels at end of treatment (EOT). Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal, or reinitiation of antiviral therapy for any reason.



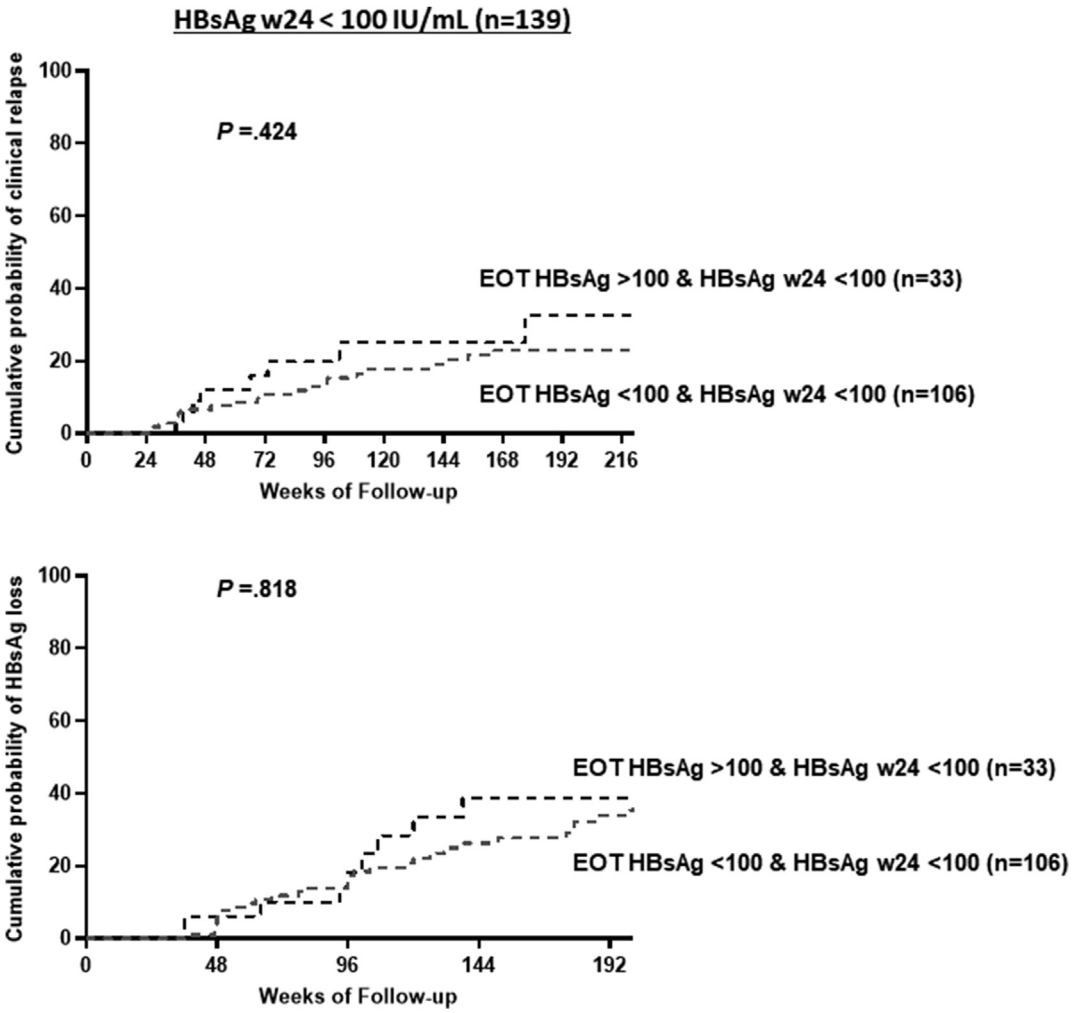
Supplementary Figure 3. Association between HBV DNA levels at FU W24 with clinical relapse and HBsAg loss for patients with low and high HBsAg levels at end of treatment (EOT). Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.



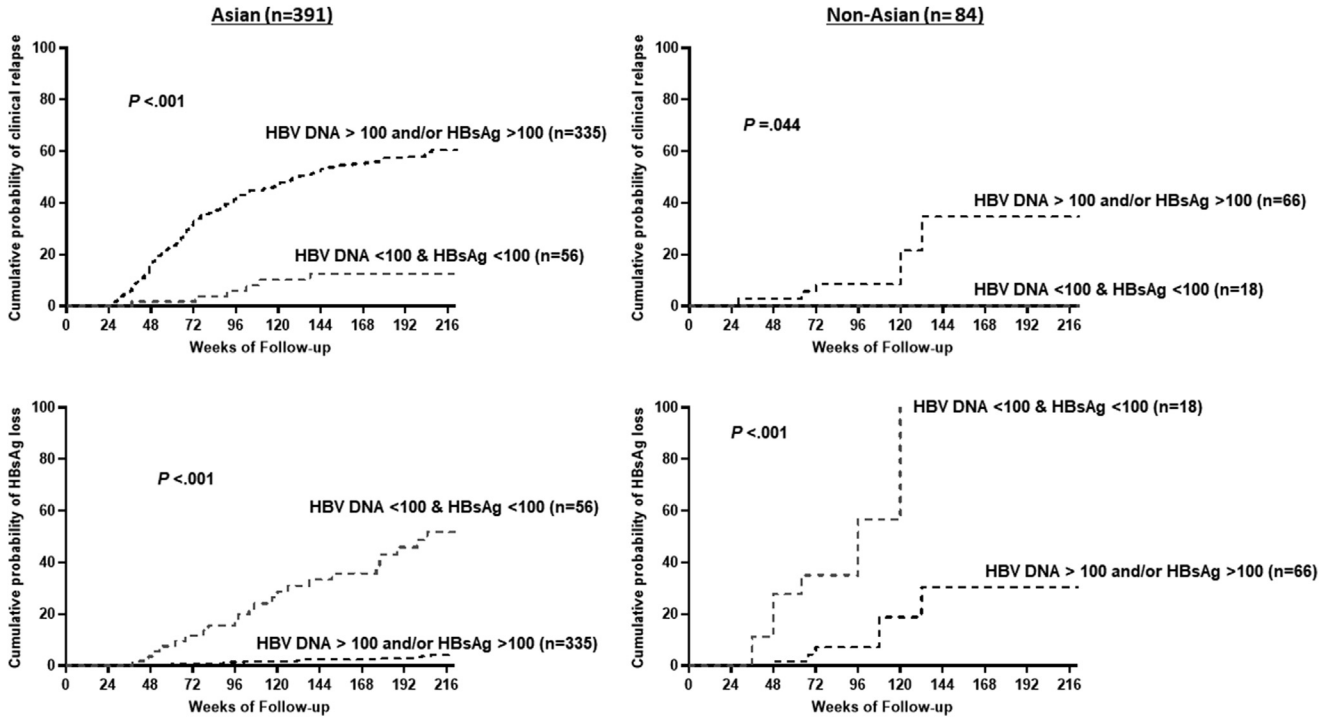
Supplementary Figure 4. Association between HBsAg levels at FU W24 with clinical relapse and HBsAg loss for patients with low and high HBcrAg levels at end of treatment (EOT). Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.



Supplementary Figure 5. Association between HBsAg levels at FU W24 with clinical relapse and HBsAg loss in the subset of patients with high HBsAg levels at end of treatment (EOT). Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.



Supplementary Figure 6. Cumulative incidence of clinical relapse and HBsAg loss for patients with an HBsAg level <100 IU/mL at both end of treatment (EOT) and FU W24 and for patients with an HBsAg level >100 IU/mL at end of treatment who achieved a decline to <100 IU/mL by FU W24. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.



Supplementary Figure 7. Cumulative incidence of clinical relapse and HBsAg loss for patients with both HBV DNA <100 IU/mL and HBsAg <100 IU/mL at FU W24 compared with patients with HBV DNA >100 IU/mL or HBsAg >100 IU/mL, or both, stratified by ethnicity. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

Supplementary Table 1. Comparison of the Characteristics of Patients Included in the Study vs Those Who Were Excluded for Clinical Relapse Within 24 Weeks After Therapy Cessation

Characteristics	Included in study (n = 475)	Clinical relapse <24 weeks (n = 111)	*P*
Demographics			
Age, y	52 (45–59)	53 (44–60)	.053
Male sex	358 (75)	87 (78.4)	.504
Asian ethnicity	391 (82)	97 (87.4)	.197
Cirrhosis	18 (3.8)	2 (1.8)	.289
Duration of treatment, *wk*	157 (156–211)	157 (156–200)	.083
Alanine aminotransferase, *U/L*	22 (17–32)	27 (19–40)	<.001
Treatment			.003
Entecavir	261 (54.9)	48 (43.2)	
Tenofovir disoproxil fumarate	169 (35.6)	58 (52.3)	
Other/combination	45 (9.5)	5 (4.5)	
Virology at end of treatment			
HBsAg <100 IU/mL	112 (23.6)	17 (15.3)	.059
HBcrAg <3 log U/mL	286 (60.2)	59 (53.2)	.174

NOTE. Data are presented as n (%) or median (interquartile range). Clinical relapse was defined as the occurrence of either HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

Supplementary Table 2. Comparison of the Characteristics of Patients With Hepatitis B Virus DNA <100 IU/mL and Hepatitis B Surface Antigen <100 IU/mL at Follow-up Week 24 vs Those Who Experienced Clinical Relapse Within 24 Weeks After Therapy Cessation

Characteristics	HBV DNA <100 and HBsAg <100 IU/mL at FU W24 (n = 74)	Clinical relapse <24 weeks (n = 111)	*P*
Demographics			
Age, *y*	52 (45–58)	53 (44–60)	.182
Male sex	55 (74.3)	87 (78.4)	.522
Asian ethnicity	56 (75.7)	97 (87.4)	.039
Cirrhosis	9 (12.5)	2 (1.8)	.003
Duration of treatment, *wk*	157 (156–283)	157 (156–200)	.718
Alanine aminotransferase, *U/L*	24 (19–39)	27 (19–40)	.112
Treatment			.862
Entecavir	35 (47.3)	48 (43.2)	
Tenofovir disoproxil fumarate	36 (48.6)	58 (52.3)	
Other/combination	3 (4.1)	5 (4.5)	
Virology at end of treatment			
HBsAg <100 IU/mL	57 (77.0)	17 (15.3)	<.001
HBcrAg <3 log U/mL	51 (68.9)	59 (53.2)	.032

NOTE. Data are presented as n (%) or median (interquartile range). Clinical relapse was defined as the occurrence of either HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or re-initiation of antiviral therapy for any reason.

Supplementary Table 3. Comparison of the Characteristics of Patients With Hepatitis B Virus DNA <100 IU/mL and Hepatitis B Surface Antigen <100 IU/mL at Follow-Up Week 24 vs Those Who Had Hepatitis B Virus DNA or Hepatitis B Surface Antigen Levels >100 IU/mL, or Both

Characteristics	HBV DNA <100 IU/mL and HBsAg <100 IU/mL at FU W24 (n = 74)	HBV DNA >100 IU/mL or HBsAg >100 IU/mL at FU W24, or both (n = 401)	*P*
Demographics			
Age, *y*	52 (45–58)	52 (45–59)	.973
Male sex	55 (74.3)	303 (75.6)	.821
Asian ethnicity	56 (75.7)	335 (83.5)	.103
Cirrhosis	9 (12.5)	9 (2.2)	<.001
Duration of treatment, *wk*	157 (156–283)	157 (156–208)	.933
Alanine aminotransferase, *U/L*	24 (19–39)	22 (17–31)	.460
Treatment			.020
Entecavir	35 (47.3)	226 (56.4)	
Tenofovir disoproxil fumarate	36 (48.6)	133 (33.2)	
Other/combination	3 (4.1)	42 (10.5)	
Virology at end of treatment			
HBsAg <100 IU/mL	57 (77.0)	55 (13.7)	<.001
HBcrAg undetectable	51 (68.9)	235 (58.6)	.096

NOTE. Data are presented as n (%) or median (interquartile range).

Supplementary Table 4. Factors Associated With Clinical Relapse

Variable	aHR (95% CI)	*P*
HBsAg <100 and HBV DNA <100 IU/mL at FU W24	0.158 (0.078–0.323)	<.001
Alanine aminotransferase (U/L) at FU W24	1.010 (1.001–1.020)	.033
Age	1.022 (1.008–1.036)	.002
Male sex	1.152 (0.808–1.642)	.433
Asian ethnicity	4.880 (2.138–11.137)	<.001
Tenofovir therapy	1.024 (0.746–1.406)	.884

NOTE. Factors associated with clinical relapse after NUC withdrawal in multivariable Cox's regression analysis. Clinical relapse was defined as the occurrence of either HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

Supplementary Table 5. Factors Associated With Hepatitis B Surface Antigen Loss

Variable	aHR (95% CI)	*P*
HBsAg <100 and HBV DNA <100 IU/mL at FU W24	13.874 (8.405–22.902)	<.001
Alanine aminotransferase (U/L) at FU W24	1.013 (0.998–1.029)	.088
Age	1.011 (0.988–1.035)	.343
Male sex	0.719 (0.427–1.210)	.214
Asian ethnicity	0.229 (0.116–0.455)	<.001
Tenofovir therapy	0.942 (0.566–1.568)	.818

NOTE. Factors associated with HBsAg loss after NUC withdrawal in multivariable Cox's regression analysis.