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Dynamics of HBV biomarkers during nucleos(t)ide analog treatment: A 14-year study

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Abstract

Background: Circulating HBsAg, HBV RNA, and hepatitis B core-related antigen (HBcrAg) are potential biomarkers for the response to nucleos(t)ide analog (NA) treatment discontinuation in patients with chronic hepatitis B (CHB). We retrospectively investigated the long-term kinetics of HBsAg, HBV RNA, and HBcrAg in HBeAg-negative patients treated with NA for up to 14 years in a prospective cohort study.

Methods: Ninety-six patients (mean age 65 y, 77% male, 52% with cirrhosis, all HBV genotype D) who were undergoing first (n = 33, group A) or second-line (n = 63, group B) treatment with tenofovir disoproxil fumarate were included. HBV biomarkers collected during tenofovir disoproxil fumarate treatment were measured in 384 serum samples stored at −20 °C. The combined biomarker endpoints associated with functional cure following NA discontinuation included HBsAg < 1000 IU/mL, HBV RNA < 54 copies/mL, and HBcrAg < 2 log U/mL.

Results: Before NA treatment, HBV RNA and HBcrAg were detectable in 85% (mean 3.9 ± 2.3 [range, 0–9.2] $\log_{10}$ copies/mL) and 80% (mean 4.3 ± 1.9 [2–8.9] $\log_{10}$ U/mL), respectively, of the patients in group A. In groups A and B, the percentages of patients with detectable HBV RNA levels decreased to 53% and 34%, respectively, during years 8–10 of NA treatment, and to 29% in group B during years 11–14 to 29%. HBcrAg could be quantified in 2% of patients in group B NA treatment years 8–10. Combined biomarker endpoints were met at baseline and at years 1–4, 5–7, 8–10, and 11–14 of treatment by 3.3%, 12% and 14%, 13% and 38%, 26% and 29%, and 41% of patients, respectively.

Abbreviations: cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CpAM, core protein allosteric modulator; EASL, European Association for the Study of the Liver; HBcrAg, hepatitis B core-related antigen; LOD, lower limit of detection; LOESS, Locally Estimated Scatterplot Smoothing; LOQ, lower limit of quantification; NA, nucleos(t)ide analog; TDF, tenofovir disoproxil fumarate.

Conclusions: HBV biomarker endpoints are associated with functional cure after the discontinuation of NA increase during long-term NA treatment.

Keywords: functional cure, HBcrAg, HBV biomarkers, stop NUC, treatment discontinuation

INTRODUCTION

Chronic hepatitis B (CHB) infection affects 292 million people globally and causes significant morbidity and mortality in 15%–40% of infected individuals.[1,2]

Treatment of CHB infection is mostly based on highly potent nucleos(t)ide analogs (NAs), which aim to prevent complications associated with progressive inflammation and fibrosis, such as liver failure, decompensated liver cirrhosis, and HCC.[3,4]

While many patients with HBeAg-positive CHB can achieve stable endpoints that are associated with long-term immune control, such as HBeAg seroconversion or, in a minority of patients (< 1%), HBsAg loss, which is considered a functional cure, patients with HBeAg-negative CHB often need life-long NA treatment.[5,6] However, recent studies suggest that discontinuing NA after more than 2–3 years of NA treatment and persistently undetectable serum HBV DNA may result in higher percentages of functional cure in HBeAg-negative patients,[7,8] leading to this method's inclusion in international treatment guidelines, although its implementation is not well characterized.[3,9]

However, there is strong evidence that patients with low HBsAg levels at the time of NA treatment discontinuation have the highest percentages of functional cures during follow-up.[7,10–13] Additionally, low levels of the experimental HBV biomarkers HBV RNA and hepatitis B core-related antigen (HBcrAg) have recently been shown to be associated with favorable outcomes and the potential of HBsAg loss after NA treatment discontinuation.[13–20] The serum levels of both HBV RNA and HBcrAg reflect the intrahepatic transcriptional activity of the HBV covalently closed circular DNA (cccDNA).[21,22] However, the duration of NA treatment necessary to achieve HBV biomarker profiles associated with increased chances of functional cure after NA discontinuation remains unclear. We measured and compared both markers in a prospectively established real-world cohort of HBeAg-negative patients.

METHODS

Patient population

All patients were treated in the outpatient clinic of the University of Milan in Italy and were retrospectively included in this study. Patients with HBeAg-negative CHB at the start of treatment who met the treatment indications according to EASL (European Association for the Study of the Liver) guidelines; were over 18 years of age; did not have HCC; underwent treatment with tenofovir disoproxil fumarate (TDF) as a first-line or second-line therapy for at least 8 years; had available serum samples stored at −20 °C obtained at baseline and during years 1–4, 5–7, and 8–10 of TDF treatment; and had documentation of any previous NA treatments were included in the study.[3] The decision to treat with TDF was made by the attending physician. All patients provided written informed consent to participate in the study. Our study was conducted in accordance with the principles of the Declaration of Helsinki and Istanbul.

Laboratory measurements

All HBV markers were measured in 4 samples for each patient (n = 96), resulting in a biomarker measurement in a total of 384 samples.

Quantification of HBV DNA and HBsAg

HBV DNA was quantified via real-time PCR (Roche cobas, lower limit of quantification [LOQ], 10 IU/mL and lower limit of detection [LOD], 4.3 IU/mL). Results below the LOQ and above the LOD were designated < LOQ, but not provided as exact values as they are not reliably quantifiable. Results below the LOD were considered undetectable. HBsAg was quantified via ELISA (Abbott Architect, LOQ, 0.05 IU/mL) according to the manufacturer's instructions.

Quantification of HBV RNA

Full-length HBV RNA was quantified after reverse transcription using specific real-time PCR in a one-step procedure, as previously described.[23] HBV RNA results were categorized on the basis of the different definitions of detectability. Thus, the LOQ was determined to be 54 copies/mL by a serum sample used as a standard. Positive PCR signals below 54 copies/mL were not reliably quantifiable and were designated as < LOQ, whereas PCR results without HBV RNA signal detection were designated as undetectable.

Quantification of HBcrAg

HBcrAg was quantified using the Lumipulse G HBcrAg assay (Fujirebio Europe) according to the manufacturer's instructions. The HBcrAg results were reported as quantities if they were above the LOQ of 1000 U/mL (3 $\log_{10}$ U/mL). HBcrAg signals between the LOQ and LOD of 2 $\log_{10}$ U/mL were reported as $<$LOQ. HBcrAg results below the LOD of 2 $\log_{10}$ U/mL (target not detected) were described as undetectable.

Definition of biomarker endpoints

The levels of single HBV markers associated with an increased likelihood of developing a functional cure following the cessation of NA treatment were based on recently published categories: HBsAg $<$1000 IU/mL,[5] HBcrAg $<$2 log/U/mL,[5,6] and HBV RNA below the detection limit.[7,8,18] A combined biomarker endpoint was defined as the achievement of all 3 biomarkers meeting predefined levels at the same time point in a patient during the course of NA treatment.

Statistical analysis

The dynamics of single HBV biomarkers were analyzed according to the duration of TDF treatment and total NA treatment. Descriptive statistics were calculated using means, SDs, and ranges. For group comparisons of values, the nonparametric Mann–Whitney test was used for age and weight. *t* tests were used when a normal distribution could be assumed, and the Fisher exact test was used for categorical variables. The mean smoothing method of locally weighted smoothing (LOESS—Locally Estimated Scatterplot Smoothing) was applied. All figures were created using the ggplot2 package library of R statistics software.

RESULTS

Patient population and samples

Ninety-six patients were included in the analysis at the time of TDF treatment initiation (Table 1). Thirty-three patients started NA treatment with TDF as first-line treatment (group A) and were observed for a mean duration of 88 $\pm$ 11 months (range, 66–120 mo). Sixty-three patients started TDF as second-line treatment (group B) and were observed for a mean duration of 90 $\pm$ 11 months (range, 43–96 mo) (Figure 1). All patients in group B received lamivudine prior to TDF treatment for a mean duration of 46 $\pm$ 21 months (range, 1–101 mo). In group B, patients were older (mean age 60 vs. 68 y, $p = 0.0001$), and more patients had liver cirrhosis than did those in group A (27% vs. 65%, $p = 0.0004$) (Table 1). No patients developed HCC or hepatic decompensation during the observation period. A total of 384 serum samples were collected at baseline and at years 1–4, 5–7, and 8–10 of TDF treatment and stored at −20 °C.

Response to TDF treatment

At the start of NA treatment, HBV DNA was above the limit of detection in 84% of patients in group A with a mean of 5 $\pm$ 1.9 $\log_{10}$ IU/mL (range, 1.7–9 $\log_{10}$ IU/mL) (Table 2). During TDF treatment, HBV DNA levels decreased below the LOQ in all patients in both groups (Figure 2A). The percentage of patients with undetectable HBV DNA increased from 55%–67% in years 1–4 to 75%–77% in years 5–7, 86%–87%% in years 8–10, and 92% in years 11–14 in group B (Figure 3A). The mean HBsAg level was 4 $\pm$ 0.6 $\log_{10}$ IU/mL (range, 2.3–4.4 $\log_{10}$ IU/mL) at baseline in group A and 3.1 $\pm$ 1.4 $\log_{10}$ IU/mL (range, 2.1–4.1 $\log_{10}$ IU/mL) in group B (p = n.s.) and decreased mildly in both groups during the observation period (Figures 2B and 3B). None of the patients experienced HBsAg loss during the observational period.

HBV RNA levels during NA treatment

At baseline, the mean HBV RNA level was 3.9 $\pm$ 2.3 $\log_{10}$ copies/mL (range, 0–9.2 $\log_{10}$ copies/mL) in treatment-naïve patients (group A) and 1.1 $\pm$ 1.0 $\log_{10}$ copies/mL (range, 0–3 $\log_{10}$ copies/mL) ($p = 2.6\times10^{-8}$) in treatment-experienced patients (group B) (Figure 2C). During NA treatment, the mean HBV RNA levels decreased in both groups (Figure 2C and Table 2). Thus, at the start of TDF treatment, 83% of patients in group A presented with HBV RNA above the lower LOQ, which decreased to 24% of patients after 8–10 years of TDF treatment. In group B, the percentage of patients with HBV RNA levels above the lower LOQ decreased from 36% at NA treatment initiation to 21% at years 8–10 of TDF treatment, and further to 2% at years 11–14 of TDF treatment (Figure 3C). HBV RNA signals below the lower LOQ remained detectable at variable percentages in 3%–39% of patients over the duration of TDF treatment in both groups. No differences in HBV RNA levels were found between patients with and without cirrhosis at any time point (p = n.s.).

HBcrAg levels during NA treatment

At baseline of NA treatment, the mean HBcrAg level was 4.5 $\pm$ 1.9 $\log_{10}$ U/mL (range, 3–8.9 $\log_{10}$ U/mL) in group A. At month 12 of TDF treatment, the mean HBcrAg levels were similar between the groups

TABLE 1 Patient characteristics (n = 96) at the start of TDF treatment

Group	A (n = 33)	B (n = 63)	*p*
Sex, m/f (%)	21/12 (63/37)	53/10 (84/16)	2×10^{-2}
Age (y)[a]	60 ± 9 (40–73)	68 ± 7.8 (44–84)	10^{-4}
Weight (kg)[a]	71 ± 13 (46–90)	73 ± 13 (45–128)	0.4
Cirrhosis (%)	9 (27)	41 (65)	4×10^{-4}
ALT (IU/mL)[a]	24 ± 12 (7–78)	24.5 ± 2.1 (23–26)	0.12
HBV genotype D (%)	33 (100)	63 (100)	1
HBV DNA (log_{10} IU/mL)[a]	5 ± 1.9 (1.7–9)	2.6 ± 1.2 (1.8–3.4)	0.15
Patients with detectable HBV DNA (%)			
HBsAg (log_{10} IU/mL)[a]	4 ± 0.6 (2.3–4.4)	3.1 ± 1.4 (2.1–4.1)	0.13
HBcrAg (log_{10} U/mL)[a]	4.3 ± 1.9 (2–8.9)	3.1 ± 0.5 (2–4.4)	5.2×10^{-12}
HBcrAg > LOQ (%)	20 (67)	1 (4)	
HBcrAg LOD–LOQ (%)	4 (13)	1 (4)	
HBcrAg < LOD (%)	6 (20)	26 (93)	
HBV RNA (log_{10} copies/mL)[a]	3.9 ± 2.3 (0–9.2)	1.1 ± 1.0 (0–3)	2.6×10^{-8}
HBV RNA > LOQ (%)	25 (83)	10 (36)	
HBV RNA LOD–LOQ (%)	1 (3)	11 (39)	
HBV RNA < LOD (%)	4 (13)	7 (25)	

[a]Mean ± SD (range).
Abbreviations: HBcrAg, hepatitis B core-related antigen; LOD, lower limit of detection; LOQ, lower limit of quantification; TDF, tenofovir disoproxil fumarate.

(Figure 2D, and Table 2). In group A, at baseline, HBcrAg was above the LOQ in 67% of patients (Figure 3D). During TDF treatment, the percentage of patients with HBcrAg levels above the LOQ decreased from years 1–4, 5–7, and 8–10 to 21%, 6%, and 0%, respectively, in group A, and to 4%, 3%, and 2%, and at years 11–14 to 0%, respectively, in group B. No differences in HBcrAg levels were found in patients with or without cirrhosis (p = n.s.). HBcrAg signals in the non-linearity range remained detectable in variable percentages of patients.

ALT levels during NA treatment

At the beginning of TDF treatment, the mean ALT levels were significantly greater in group A (Table 2). After 12 months of TDF treatment, the ALT levels were similar between groups A and B (Figure 2E).

Combined biomarker endpoints

A combined biomarker endpoint for increased potential of HBsAg loss after NA treatment discontinuation was met by 3% of patients in group A at the initiation of NA treatment (Figure 4A). At years 1–4 of NA treatment, a combined biomarker endpoint was achieved by 12% and 14% of patients in groups A and B, respectively. At years 5–7 and 8–10 of NA treatment, the percentage of patients who achieved combined biomarker endpoints increased to 13% and 29% in group A and to 38% and 26% in group B, and there was a further increase from years 11–14 of NA treatment to 41% in group B. Most patients in group A presented at the start of NA treatment unfavorable single biomarker profiles for increased likelihood of HBsAg loss after NA treatment discontinuation, including HBsAg, HBV RNA, and HBcrAg levels (Figure 4B). However, starting at years 1–4 of NA treatment, the majority of patients reached

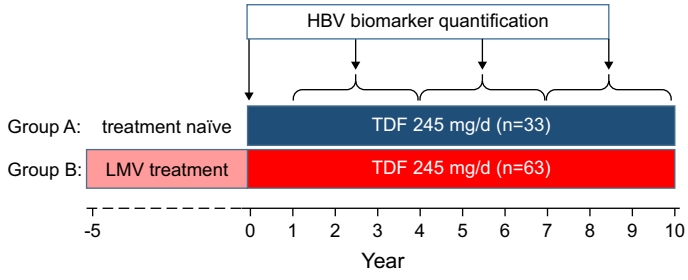

FIGURE 1 Study design and time points of HBV biomarker measurements. Before treatment with TDF, patients were either naïve to NA treatment (group A) or treated with LMV (group B). Abbreviations: NA, nucleos(t)ide analog; LMV, lamivudine; TDF, tenofovir disoproxil fumarate.

TABLE 2 HBV biomarker quantification before and during first-line (group A) or second-line (group B) treatment with TDF

	Baseline		Years 1–4		Years 5–7		Years 8–10	
Group	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
HBV DNA (log_{10} IU/mL)[a]	5.0 (2.0)	2.6 (1.1)	0.4 (0.5)	0.3 (0.5)	0.2 (0.4)	0.1 (0.3)	1.0 (1.8)	0.1 (0.3)
HBsAg (log_{10} IU/mL)[a]	4.0 (0.6)	3.1 (1.4)	3.4 (0.5)	2.9 (0.5)	3.1 (0.6)	2.5 (0.6)	2.9 (0.7)	2.3 (0.7)
HBV RNA (log_{10} U/mL)[a]	4.2 (1.9)	1.9 (0.3)	2.4 (1.0)	1.9 (0.4)	2.2 (0.6)	1.9 (0.3)	2.0 (0.8)	1.8 (0)
HBcrAg (U/mL)[a]	4.5 (1.9)	3.1 (0.3)	3.2 (1.0)	3.0 (0.4)	3.1 (0.6)	3.0 (0.3)	3.0 (0.4)	3.0 (0)

[a]Mean ± SD.
Abbreviations: HBcrAg, hepatitis B core-related antigen; TDF, tenofovir disoproxil fumarate.

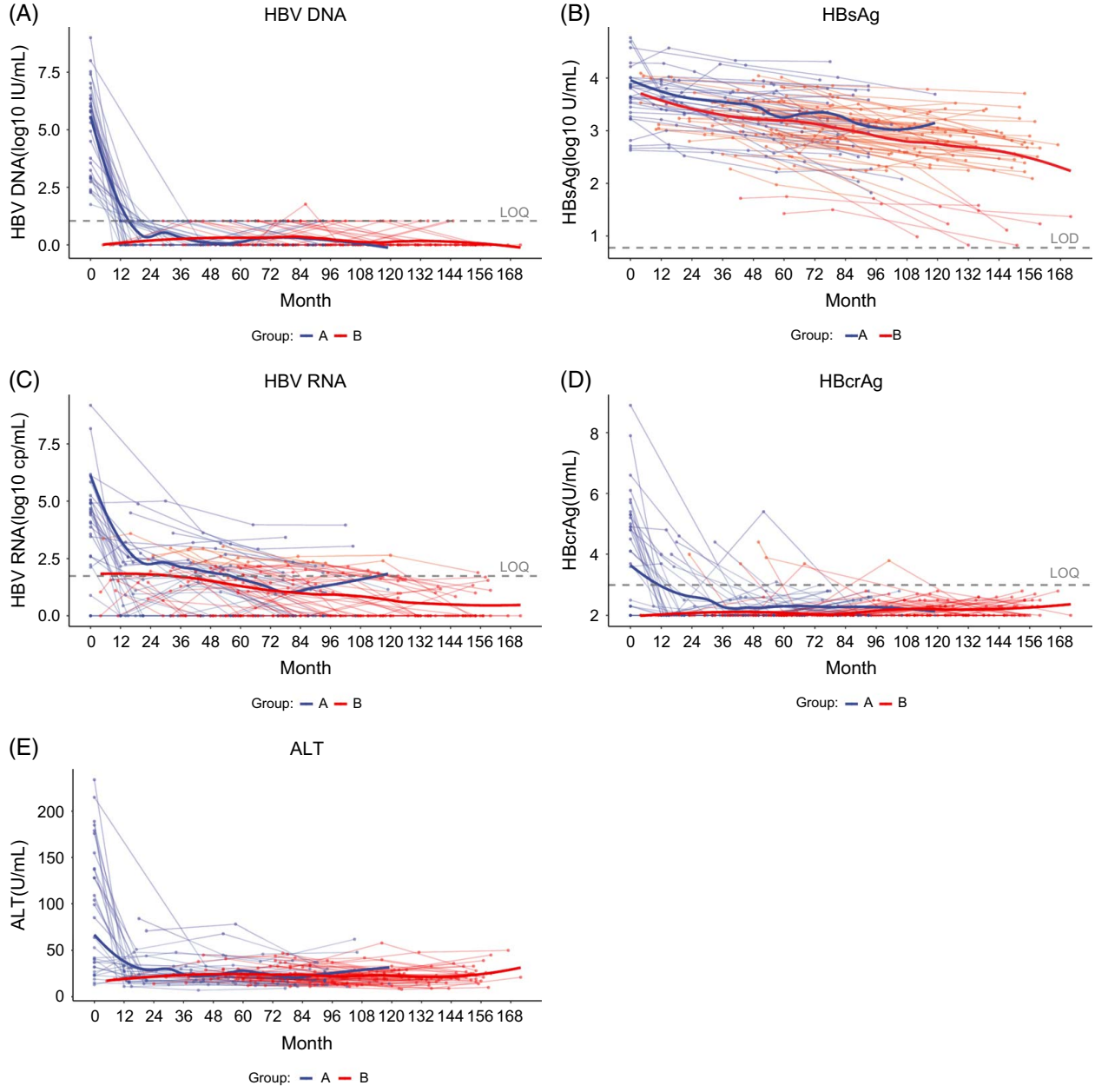

FIGURE 2 Kinetics of the circulating HBV biomarkers HBV DNA (A), HBsAg (B), HBV RNA (C), and HBcrAg (D), and the ALT levels (E) during up to 10 years of TDF treatment in HBeAg-negative patients. The values of patients receiving first-line TDF treatment (group A) are displayed in blue, and the values of patients receiving TDF as second-line treatment after lamivudine (group B) are displayed in red. Abbreviations: HBcrAg, hepatitis B core-related antigen; TDF, tenofovir disoproxil fumarate.

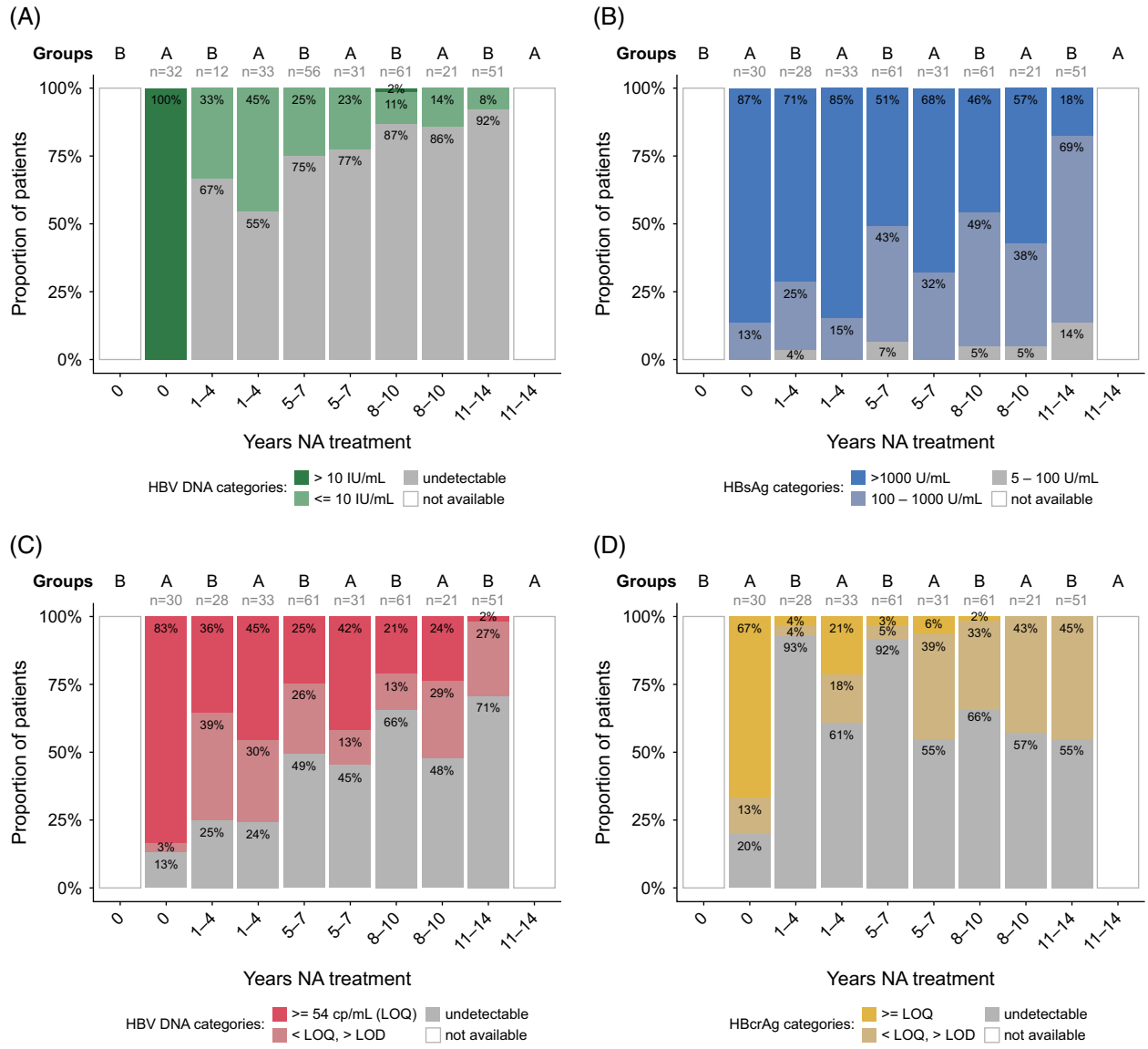

FIGURE 3 Categories of different circulating HBV biomarkers during long-term NA treatment. Biomarker categories of HBV DNA (A), HBsAg (B), HBV RNA (C), and HBcrAg (D) are shown for patients treated with TDF as first-line treatment (group A, n = 33) or second-line treatment (group B, n = 63). Abbreviations: NA, nucleos(t)ide analog; HBcrAg, hepatitis B core-related antigen; TDF, tenofovir disoproxil fumarate.

HBcrAg levels < 2 log U/mL and the increase in the combined biomarker endpoint during the follow-up period was triggered by decreasing HBsAg and HBV RNA levels. The achievement of the combined biomarker endpoints in group B was triggered by decreases in HBcrAg and HBV RNA levels over time in group A, as the majority of patients already had HBcrAg levels < 2 log U/mL at the start of the observation period (Figure 4C).

DISCUSSION

In the present study, we assessed the kinetics of experimental biomarkers of cccDNA activity, HBV RNA, and HBcrAg in a cohort of well-characterized HBeAg-negative patients during long-term treatment with NA. To our knowledge, this is the first study of these markers in patients treated with NA for up to 14 years. We found that the percentage of patients with quantifiable HBV RNA or HBcrAg levels decreased over time during NA treatment. Thus, HBV RNA was above the LOQ in 78% of patients at the start of NA treatment and subsequently decreased to 24% at years 8–10 of treatment (Figures 2C and 3C). Among patients who were treated for up to 14 years with NA, only 4% presented detectable HBV RNA levels (Figure 3C). In contrast, HBcrAg was above the LOQ in 50% of patients at baseline, in 2%–17% of patients at years 8–10 of treatment, and in no patients at years 11–14 of

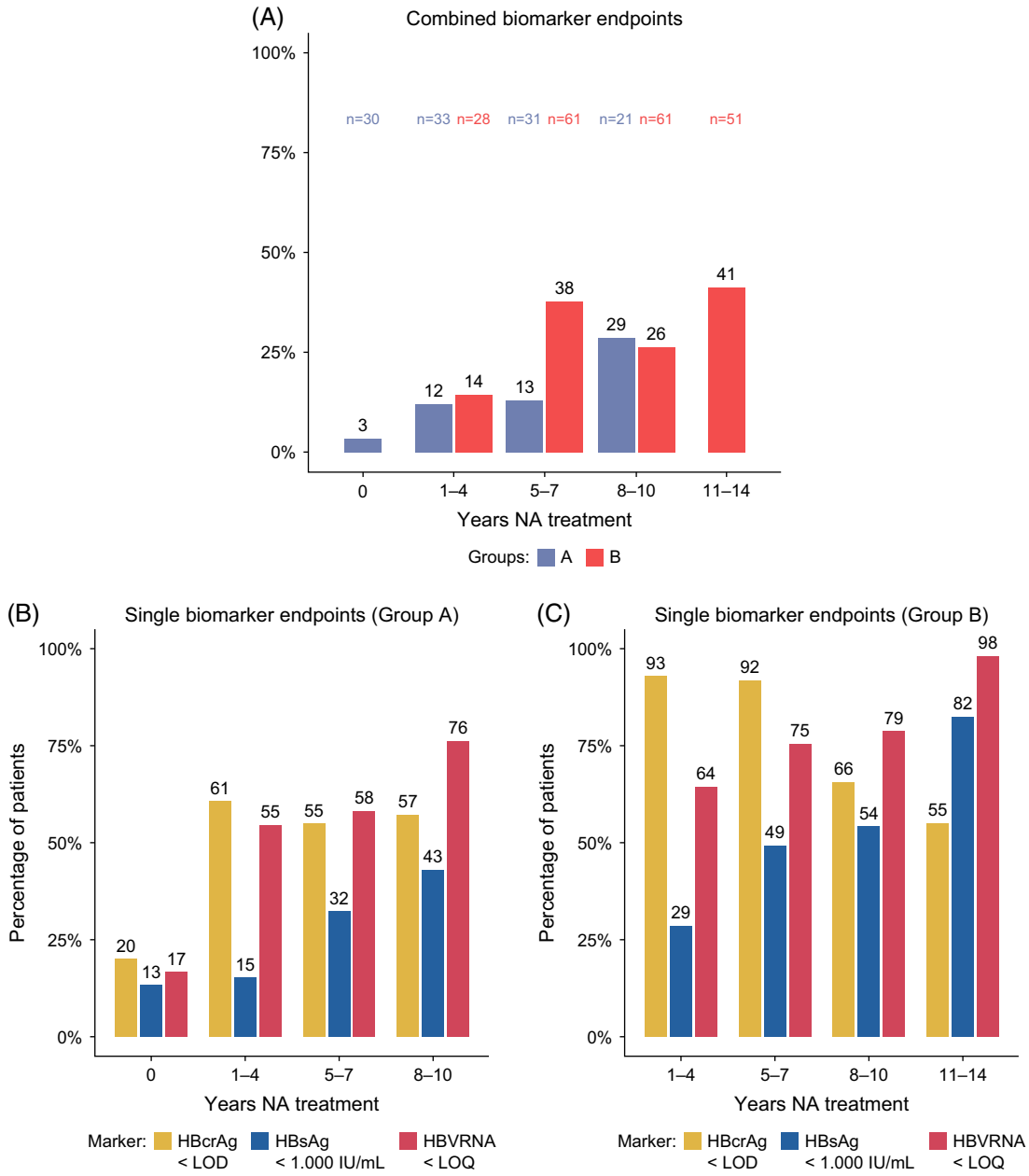

FIGURE 4 HBV biomarker endpoints are favorable for the response to treatment discontinuation during long-term NA therapy. A combination endpoint of HBsAg < 1000 IU/mL, HBcrAg < 2 log U/mL, and HBV RNA < 54 copies/mL was rare during the first 4 years, though it continuously increased during the following treatment period (A). The achievement of combined endpoints in group A (B) and group B (C) was driven mostly by the kinetics of HBsAg and HBV RNA, as HBcrAg was less than 2 log in most patients. Abbreviations: NA, nucleos(t)ide analog; HBcrAg, hepatitis B core-related antigen; LOD, lower limit of detection; LOQ, lower limit of quantification.

treatment (Figure 3D). During NA treatment, the percentage of patients whose combined HBV biomarker profile was potentially associated with the development of a functional cure after the discontinuation of NA treatment increased.

Discontinuation of NA treatment has become an accepted treatment to achieve a functional cure in HBeAg-negative patients. The benefits may outweigh the risks of severe relapses of HBV replication, especially in patients with lower HBsAg levels and undetectable HBV replication at the time of NA treatment discontinuation.[7,8,24] An HBsAg threshold of 100 IU/mL for Asian and 1000 IU/mL for Caucasian patients was recently defined to identify patients with high chances of response.[17] In our study, we found that an increasing percentage of patients achieved HBsAg levels < 1000 IU/mL during up to 14 years of NA treatment, suggesting that with prolonged duration of NA treatment, more patients become more likely to develop a functional cure after NA treatment withdrawal (Figure 3C). However, the strength of the association between HBsAg and HBV replication is debatable as

HBsAg is formed not only from cccDNA but also from the integrated HBV genome, which is why HBV RNA and HBcrAg markers are currently being studied for their potential to enhance the prediction of functional cure after discontinuation of NA treatment.[25–30] Previous studies have shown that HBcrAg levels are associated with the response to NA treatment as well as to viral and ALT flares after discontinuing NA treatment.[31,32] Moreover, when HBcrAg levels are combined with HBsAg levels in serum samples from HBeAg-negative patients who discontinue NA treatment, there is a stronger correlation with HBsAg loss than with HBsAg levels alone.[7,11] The combination of serum HBsAg and HBcrAg is recommended as a predictor for viral relapse after discontinuation of NA therapy in the Japanese CHB guidelines for the management of HBV infection (Drafting Committee for Hepatitis Management Guidelines and the Japan Society of Hepatology, 2014). The detection of HBcrAg signals in the range of 2–3 log U/mL, which is unspecific according to the manufacturer, was included in the analysis.[11] These nonlinear and detected, but not quantifiable, signals may indicate the presence of the marker in spite of uncertainty and lack of reproducibility in this range. It needs to be researched whether such signals may contribute to better identification of patients who achieved HBsAg loss or other clinical endpoints. In our cohort, a large proportion of patients had HBcrAg values after up to 14 years of NA treatment (Figure 3C). Although different quantification methods were used, HBV RNA PCR yielded positive results in many patients whose HBV RNA levels were above the limit of detection (Figure 3B). We believe that these nonlinear test results should be investigated for their origin and for their value as response markers in larger studies.

HBV RNA is detectable in serum as encapsulated virion-containing pgRNA, and it may predominantly consist of pgRNA, spliced pgRNA variants, and HBx species[28,33] Serum levels of HBV RNA in combination with HBcrAg are associated with favorable outcomes and a functional cure after the discontinuation of NA treatment.[8,34,35] Interestingly, HBV RNA levels seem to be less associated with the achievement of a functional cure when used alone than when combined with the HBcrAg levels.[36] However, a comprehensive study of the significance of HBV RNA levels, alone or in combination with other serum markers, on the probability of HBsAg loss after NA termination has not yet been conducted.

In our study, we analyzed single HBV biomarker kinetics as well as the achievement of described single biomarker thresholds that have previously been associated with the achievement of functional cure following NA treatment discontinuation. To account for the superiority of a combination of different markers over a single marker, we defined the combined biomarker endpoint. Our combined biomarker endpoint was met by only a minority of patients at the initiation and during the first 1–4 years of NA treatment (Figure 4A). The increase in the number of patients who achieved this combined endpoint was driven mainly by the continuous decrease in HBV RNA and HBsAg levels (Figures 4B, C). This difference compared with that of HBcrAg is likely due to the comparatively small dynamic range of the HBcrAg assay, which has a lower detection limit of 3 log. According to previous studies, we included the nonlinear and unspecific range of HBcrAg test results between 2 and 3 log U/mL in our analysis.[7,8] However, this use of the test results for HBcrAg, as it was in our and other studies in clinical practice, requires a more profound understanding of the assay and the nature of its targets. During the later time points in our observations, an increasing number of patients achieved a combined biomarker profile. Although the association of the duration of NA treatment with the response to NA treatment cessation is not consistent across different reports, observations in line with our findings have been reported previously.[7,8] Accordingly, the assumption that a longer treatment duration results in greater HBsAg loss after NA discontinuation due to a greater proportion of patients achieving combined biomarker endpoints requires validation in future studies. However, our results indicate that a minimum duration of 4–5 years of treatment may be necessary to achieve favorable conditions to respond to this therapeutic approach in a significant number of HBeAg-negative patients. However, a limited percentage of patients reached a combined biomarker endpoint even after 11–14 years of NA treatment (Figure 4A). These findings suggest that not all patients can achieve this presumably favorable profile of HBV replication markers with the help of long-term treatment and that only a certain proportion of patients will be eligible for therapeutic discontinuation of NA therapy.

In addition to being potential markers for NA treatment discontinuation, HBV RNA and HBcrAg have been previously studied in different clinical scenarios. HBV RNA and HBcrAg reflect disease stages and the efficacy of NA-based or interferon-based treatments in HBeAg-positive patients with CHB.[37,38] Due to the lack of serological endpoints for HBeAg-negative patients, the associations of HBcrAg and HBV RNA with the response to NA treatment in these patients have not yet been assessed. However, serum HBV RNA has been evaluated for its association with the efficacy of core protein allosteric modulators (CpAM).[39] In our study, HBV RNA levels were detectable in 85% of patients at the initiation of NA treatment and in 45%–56% of patients at year 10 of NA treatment (Figure 3C). Based on the decreasing percentage of patients with detectable HBV RNA in our study, it is unclear whether HBV RNA can be used as a response marker in HBeAg-negative patients during long-term NA treatment.

However, it is possible that the detectability of HBV RNA represents a valuable predictor for novel treatments. We believe that developing and evaluating more sensitive assays for HBV RNA quantification is essential to better understand its value as a response marker for patients undergoing long-term NA treatment.

Our study has several limitations. First, although we included a large, homogenous, and well-characterized patient cohort, our findings are restricted to Caucasian patients with the HBV genotype D. This may be important, as ethnicity may be another factor to consider regarding viral biomarker kinetics in addition to the HBV genotype. Second, HBcrAg and HBV RNA are experimental markers that have not been validated for use in clinical settings. Additionally, assays for HBV RNA and HBcrAg are not standardized; the HBV RNA assay used in this study is an in-house assay, and the results may differ from those of other assays.

In conclusion, our long-term observations of HBeAg-negative CHB patients receiving NA treatment revealed that HBsAg, HBV RNA, and HBcrAg levels steadily decreased, which may increase the likelihood of HBsAg loss in these patients after the cessation of NA treatment. However, these endpoints were only achieved by approximately half of the patients despite the long duration of treatment. A favorable time point to consider stopping NA treatment might thus be achievable for some patients after an individualized duration of therapy. However, even with prolonged treatment, many patients may not attain a full functional cure. The actual significance of these biomarker profiles for the management of HBeAg-negative patients with CHB must be assessed in future prospective studies.

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CONFLICTS OF INTEREST

Florian van Bömmel has served as a speaker for and provided consulting services to Gilead, Roche, Janssen, Ipsen, ADVANZ, Norgine, MSD, Esai, and AstraZeneca, and has served as an advisory board member of Janssen, Eisai, AstraZeneca, and Roche. He has received travel support from AstraZeneca, ADVANZ, and Gilead Sciences. He has received research funding from Gilead Sciences, Roche, VIR, Janssen, Ipsen, and Fujirebio. Pietro Lampertico: Advisory Board/Speaker Bureau for BMS, Roche, Gilead Sciences, GSK, Abbvie, MSD, Arrowhead, Alnylam, Janssen, Spring Bank, MYR, Eiger, Antios, Aligos, and Vir. Thomas Berg received grants from Abbvie, BMS, Gilead, MSD/Merck, Humedics, Intercept, Merz, Norgine, Novartis, Orphalan, and Sequana Medical and provided consulting services to Abbvie, Alexion, Bayer, Gilead, GSK, Eisai, Enyo Pharma, HepaRegeniX GmbH, Humedics, Intercept, Ipsen, Janssen, MSD/Merck, Novartis, Orphalan, Roche, Sequana Medical, SIRTEX, SOBI, and Shionogi. Thomas Berg has served as a speaker for Abbvie, Alexion, Bayer, Gilead, Eisai, Falk Foundation, Intercept, Ipsen, Janssen, MedUpdate GmbH, MSD/Merck, Novartis, Orphalan, Sequana Medica, SIRTEX, and SOBI and serves as an advisory board member for Gilead, Assembly, and GSK. All other authors disclose any conflicts of interest related to the present work.

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REFERENCES

1. Lok AS. Chronic hepatitis B. N Engl J Med. 2002;346:1682–3.
2. Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: A modelling study. Lancet Gastroenterol Hepatol. 2018;3:383–403.
3. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67:370–98.
4. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67:1560–99.
5. Jeng WJ, Lok AS. Should treatment indications for chronic hepatitis B be expanded? Clin Gastroenterol Hepatol. 2021;19:2006–14.
6. Yip TC, Wong GL, Wong VW, Tse YK, Lui GC, Lam KL, et al. Durability of hepatitis B surface antigen seroclearance in untreated and nucleos(t)ide analogue-treated patients. J Hepatol. 2018;68:63–72.
7. Wong GLH, Gane E, Lok ASF. How to achieve functional cure of HBV: Stopping NUCs, adding interferon or new drug development? J Hepatol. 2022;76:1249–62.
8. van Bömmel F, Stein K, Heyne R, Petersen J, Buggisch P, Berg C, et al. A multicenter randomized-controlled trial of nucleos(t)ide analogue cessation in HBeAg-negative chronic hepatitis B. J Hepatol. 2023;78:926–36.
9. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: A 2015 update. Hepatol Int. 2016;10:1–98.

10. Jeng WJ, Chen YC, Chien RN, Sheen IS, Liaw YF. Incidence and predictors of hepatitis B surface antigen seroclearance after cessation of nucleos(t)ide analogue therapy in hepatitis B e antigen-negative chronic hepatitis B. Hepatology. 2018;68: 425–34.
11. Hirode G, Choi HSJ, Chen CH, Su TH, Seto WK, Van Hees S, et al.; RETRACT-B Study Group. Off-Therapy Response After Nucleos(t)ide Analogue Withdrawal in Patients With Chronic Hepatitis B: An International, Multicenter, Multiethnic Cohort (RETRACT-B Study). Gastroenterology. 2022;162: 757–71.
12. Liu J, Li T, Zhang L, Xu A. The role of hepatitis B surface antigen in nucleos(t)ide analogues cessation among Asian patients with chronic hepatitis B: A systematic review. Hepatology. 2019;70: 1045–55.
13. Sonneveld MJ, Park JY, Kaewdech A, Seto WK, Tanaka Y, Carey I, et al.; CREATE Study Group. Prediction of sustained response after nucleo(s)tide analogue cessation using HBsAg and HBcrAg levels: A multicenter study (CREATE). Clin Gastroenterol Hepatol. 2022;20:e784–93.
14. Brakenhoff SM, de Knegt RJ, van Campenhout MJH, van der Eijk AA, Brouwer WP, van Bömmel F, et al. End-of-treatment HBsAg, HBcrAg and HBV RNA predict the risk of off-treatment ALT flares in chronic hepatitis B patients. J Microbiol Immunol Infect. 2023;56:31–9.
15. Wang ML, Wang FD, Chen EQ. Letter: Serum HBV RNA and HBcrAg may help to evaluate safely stopping nucleot(s)ide analogues in patients with HBeAg-negative chronic hepatitis B and without cirrhosis. Aliment Pharmacol Ther. 2022;56: 1219–20.
16. Mak LY, Wong D, Kuchta A, Hilfiker M, Hamilton A, Chow N, et al. Hepatitis B virus pre-genomic RNA and hepatitis B core-related antigen reductions at week 4 predict favourable hepatitis B surface antigen response upon long-term nucleos(t)ide analogue in chronic hepatitis B. Clin Mol Hepatol. 2023;29: 146–62.
17. Sonneveld MJ, Chiu SM, Park JY, Brakenhoff SM, Kaewdech A, Seto WK, et al.; CREATE study group. Probability of HBsAg loss after nucleo(s)tide analogue withdrawal depends on HBV genotype and viral antigen levels. J Hepatol. 2022;76: 1042–50.
18. Fan R, Peng J, Xie Q, Tan D, Xu M, Niu J, et al.; Chronic Hepatitis B Study Consortium. Combining hepatitis B virus RNA and hepatitis B core-related antigen: Guidance for safely stopping nucleos(t)ide analogues in hepatitis B e antigen-positive patients with chronic hepatitis B. J Infect Dis. 2020; 222:611–8.
19. Xia M, Chi H, Wu Y, Hansen BE, Li Z, Liu S, et al. Serum hepatitis B virus RNA level is associated with biochemical relapse in patients with chronic hepatitis B infection who discontinue nucleos(t)ide analogue treatment. Aliment Pharmacol Ther. 2021;54:709–14.
20. Papatheodoridi M, Papachristou E, Moschidis Z, Hadziyannis E, Rigopoulou E, Zachou K, et al. Significance of serum HBV RNA in non-cirrhotic HBeAg-negative chronic hepatitis B patients who discontinue effective antiviral therapy. J Viral Hepat. 2022;29: 948–57.
21. Wang J, Yu Y, Li G, Shen C, Meng Z, Zheng J, et al. Relationship between serum HBV-RNA levels and intrahepatic viral as well as histologic activity markers in entecavir-treated patients. J Hepatol. 2017;21:S0168-8278(17)32261-4.
22. Testoni B, Lebossé F, Scholtes C, Berby F, Miaglia C, Subic M, et al. Serum hepatitis B core-related antigen (HBcrAg) correlates with covalently closed circular DNA transcriptional activity in chronic hepatitis B patients. J Hepatol. 2019;70:615–25.
23. van Bömmel F, van Bömmel A, Krauel A, Wat C, Pavlovic V, Yang L, et al. Serum HBV RNA as a predictor of peginterferon alfa-2a response in patients with HBeAg-positive chronic hepatitis B. J Infect Dis. 2018;218:1066–74.
24. Liaw YF, Chien RN. Finite nucleos(t)ide analogue therapy in hepatitis B e antigen-negative chronic hepatitis B: From an "option" to an "active recommendation". Kaohsiung J Med Sci. 2022;38:295–301.
25. Wooddell CI, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D, et al. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. Sci Transl Med. 2017;9: eaan0241.
26. Sonneveld MJ, Park JY, Kaewdech A, Seto WK, Tanaka Y, Carey I, et al. CREATE Study Group. Prediction of sustained response after nucleo(s)tide analogue cessation using HBsAg and HBcrAg levels: A multicenter study (CREATE). Clin Gastroenterol Hepatol. 2022;20:e784–93.
27. Carey I, Gersch J, Wang BO, Moigboi C, Kuhns M, Cloherty G, et al. Pregenomic HBV RNA and hepatitis B core-related antigen predict outcomes in hepatitis B e antigen-negative chronic hepatitis B patients suppressed on nucleos(t)ide analogue therapy. Hepatology. 2020;72:42–57.
28. Wang J, Shen T, Huang X, Kumar GR, Chen X, Zeng Z, et al. Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound. J Hepatol. 2016;65:700–10.
29. van Bömmel F, Berg T. Risks and benefits of discontinuation of nucleos(t)ide analogue treatment: A treatment concept for patients with HBeAg-negative chronic hepatitis B. Hepatol Commun. 2021;5:1632–48.
30. Mak LY, Wong DK, Cheung KS, Seto WK, Lai CL, Yuen MF. Review article: Hepatitis B core-related antigen (HBcrAg): An emerging marker for chronic hepatitis B virus infection. Aliment Pharmacol Ther. 2018;47:43–54.
31. Mak LY, Cloherty G, Wong DK, Gersch J, Seto WK, Fung J, et al. HBV RNA profiles in chronic hepatitis B patients under different disease phases and anti-viral therapy. Hepatology. 2021;73: 2167–79.
32. Tsuge M, Murakami E, Imamura M, Abe H, Miki D, Hiraga N, et al. Serum HBV RNA and HBeAg are useful markers for the safe discontinuation of nucleotide analogue treatments in chronic hepatitis B patients. J Gastroenterol. 2013;48: 1188–204.
33. Stadelmayer B, Diederichs A, Chapus F, Rivoire M, Neveu G, Alam A, et al. Full-length 5'RACE identifies all major HBV transcripts in HBV-infected hepatocytes and patient serum. J Hepatol. 2020;73:40–51.
34. Fan R, Peng J, Xie Q, Tan D, Xu M, Niu J, et al. Combining hepatitis B virus RNA and hepatitis B core-related antigen: Guidance for safely stopping nucleos(t)ide analogues in hepatitis B e antigen-positive patients with chronic hepatitis B. J Infect Dis. 2020;222:611–8.
35. Fan R, Zhou B, Xu M, Tan D, Niu J, Wang H, et al.; Chronic Hepatitis B Study Consortium. Association between negative results from tests for HBV DNA and RNA and durability of response after discontinuation of nucleos(t)ide analogue therapy. Clin Gastroenterol Hepatol. 2020;18:719–27.
36. Brakenhoff SM, de Man RA, Boonstra A, van Campenhout MJH, de Knegt RJ, van Bömmel F, et al. Hepatitis B virus RNA decline without concomitant viral antigen decrease is associated with a low probability of sustained response and hepatitis B surface antigen loss. Aliment Pharmacol Ther. 2021;53: 314–20.
37. van Bömmel F, Bartens A, Mysickova A, Hofmann J, Krüger DH, Berg T, et al. Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors. Hepatology. 2015;61: 66–76.

38. Farag MS, van Campenhout MJH, Pfefferkorn M, Fischer J, Deichsel D, Boonstra A, et al. Hepatitis B virus RNA as early predictor for response to PEGylated interferon alfa in HBeAg negative chronic hepatitis B. Clin Infect Dis. 2022;72:202–11.
39. Yuen MF, Kim DJ, Weilert F, Chan HLY, Lalezari JP, Hwang SG, et al. NVR3-778, a first-in-class HBV core inhibitor, alone and in combination with Peg-interferon (Pe-gIFN), in treatment-naive HBeAg-positive patients: Early reductions in HBV DNA and HBeAg. J Hepatol. 2016;64:S210–1.

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