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HEPATITIS B (J LIM, SECTION EDITOR)

Evidence-Based Approach to Stopping Oral Antiviral Therapy in Chronic HBV

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Abstract

Purpose of Review To review the evidence for stopping antiviral therapy with nucleos(t)ide analogues (NA) in patients with chronic hepatitis B.

Recent Findings HBsAg loss is usually stable even without anti-HBs seroconversion after stopping NA therapy. About 50% of HBeAg-positive patients who have achieved anti-HBe seroconversion and have stopped NA therapy remain in virological remission. Consolidation therapy increases the response rate. In HBeAg-negative hepatitis, stable virological remission is documented in 30% after NA discontinuation. Interestingly, some studies document unexpectedly high long-term HBsAg loss rates after stopping therapy.

Summary Evidence supports NA discontinuation, especially after HBsAg seroclearance. In HBeAg-positive patients, NA can be stopped 12 months after anti-HBe seroconversion but severe flares have to be considered. NA discontinuation is also possible in selected HBeAg-negative patients if close monitoring can be guaranteed. The high rate of HBsAg loss needs further evaluation.

Keywords Treatment discontinuation · Nucleos(t)ide analogues · HBeAg seroconversion · HBeAg negative · HBsAg loss

Introduction

The major international guidelines from AASLD, APASL, and EASL give similar recommendations for starting antiviral therapy with nucleos(t)ide analogues (NA) in chronic hepatitis B virus (HBV) infection [1–3] (Table 1). These recommendations, as well as the benefits of antiviral therapy, are backed up by an increasing amount of evidence. Antiviral therapy with NA reduces the incidence of hepatocellular carcinoma (HCC) and progression of liver cirrhosis and improves survival [1–3]. On the contrary, research focusing on stopping NA therapy is limited. Based on the available data, three possible time points have been suggested to stop antiviral therapy: (a) after HBsAg loss, (b) after anti-HBe seroconversion, and (c) in HBeAg-negative patients after a varying duration of consolidation therapy. While guidelines are in accordance on stopping antiviral therapy in patients after HBsAg loss, increasing differences exist for HBsAg-positive patients, especially in HBeAg-negative patients (Table 1) [1–3]. In recent years, more knowledge on the outcome after HBsAg loss as well as experience after stopping NA therapy in HBsAg-positive patients has accumulated. In addition, several studies have examined viral and host responses to predict the outcome after treatment is stopped. We here review the most recent publications on stopping NA therapy in all three settings, as well as currently available biomarkers, and controversial topics to provide an evidence-based approach to discontinue antiviral therapy.

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Table 1 Recommendations of the AASLD, EASL, and APASL guidelines to stop therapy with nucleos(t)ide analogues (NA) in patients with chronic hepatitis B

	AASLD (2)	EASL (1)	APASL (3)
Patients who achieved HBsAg loss (no cirrhosis)	Time point: Discontinuation of treatment after HBsAg loss in HBeAg-positive patients who seroconvert to anti-HBe. Discontinuation of treatment in HBeAg-negative patients may be considered; however, there is currently insufficient data to definitely guide treatment decision in these patients. Monitoring: Every 3 months for at least 1 year (recurrent viremia, seroconversion, ALT flares, and clinical decompensation)	Time point: Discontinuation of treatment after HBsAg loss in HBeAg-positive patients who seroconvert to anti-HBe and HBeAg-negative patients. Monitoring: Close monitoring (ALT and HBV DNA determination) at least in the first year after discontinuation must be warranted (no accurate time points are given)	Time point: Discontinuation of treatment after HBsAg loss in HBeAg-positive patients who seroconvert to anti-HBe. In HBeAg-negative patients, it is recommended to either wait for anti-HBs seroconversion or do at least 12 months of a post-HBsAg-clearance consolidation therapy. Monitoring: Monthly for the first 3 months, and from there on, every 3–6 months for relapse
HBeAg-positive patients after anti-HBe seroconversion (no cirrhosis)	Time point: Discontinuation of treatment after 12 months of consolidation therapy (persistent normal ALT levels and undetectable HBV DNA) after seroconversion. Monitoring: Every 3 months for at least 1 year (recurrent viremia, seroconversion, ALT flares, and clinical decompensation)	Time point: Discontinuation of treatment after 12 months of consolidation therapy (persistent normal ALT levels and undetectable HBV DNA) after seroconversion. Monitoring: Close monitoring (ALT and HBV DNA determination) at least in the first year after discontinuation must be warranted (no accurate time points are given)	Time point: Discontinuation of treatment after 12 months of consolidation therapy, but preferably 3 years (persistent normal ALT levels and undetectable HBV DNA) after seroconversion. Monitoring: Monthly for the first 3 months, and from there on, every 3–6 months for relapse
HBeAg-positive patients after anti-HBe seroconversion (with cirrhosis)	Time point: Indefinite treatment unless there is a strong competing rationale for stopping treatment. Treatment cessation may be considered if patients lose HBsAg. Monitoring: Closely (e.g., every month the first 6 months, then every 3 months (recurrent viremia, seroreversion, ALT flares, and clinical decompensation)	Time point: Indefinite treatment	Time point: Discontinuation of treatment may be considered. Monitoring: Careful off-therapy monitoring plan (no accurate time points are given) must be warranted
HBeAg-negative hepatitis (no cirrhosis)	Time point: Indefinite treatment unless there is a compelling rationale for treatment discontinuation. Monitoring: Every 3 months for at least 1 year (recurrent viremia, ALT flares, and clinical decompensation)	Time point: Discontinuation of treatment after 3 years of virological suppression under NA treatment. Monitoring: Close monitoring (ALT and HBV DNA determination) at least in the first year after discontinuation must be warranted (no accurate time points are given)	Time point: Discontinuation of treatment after HBsAg loss following either anti-HBs seroconversion or at least 12 months of a post-HBsAg-clearance consolidation therapy, or after treatment for at least 2 years with undetectable HBV DNA documented on three separate occasions, 6 months apart. Monitoring: Monthly for the first 3 months, and from there on, every 3–6 months for relapse
HBeAg-negative hepatitis (with cirrhosis)	Time point: Indefinite treatment	Time point: Indefinite treatment	Time point: Discontinuation of treatment may be considered. Monitoring: Careful off-therapy monitoring plan (no accurate time points are given) must be warranted

Discontinuation of Nucleos(t)ide Analogues after HBsAg Loss

Loss of HBsAg is referred to as "functional cure," providing the best long-term outcome currently achievable [1–3]. Loss of HBsAg is an important step which is associated with lower mortality. A large outcome study of 2946 HBsAg-positive patients demonstrated that patients with successive loss of HBsAg had a lower risk for HCC development in comparison with patients with "only" suppressed HBV DNA on follow-up, with a lifetime risk of 4% and 6.6%, respectively [4•]. Remaining risk factors for HCC development in HBsAg-negative patients were the presence of cirrhosis, male gender, and age > 50 years [5]. No differences for mortality, HCC development, and anti-HBs (after 6 years) could be observed between patients with spontaneous HBsAg loss or HBsAg

loss during NA therapy, with 0% vs. 1%, 2% vs. 1.1%, and 62.3% vs. 61.5%, respectively [6].

Many studies have shown that the annual rate of HBsAg loss is very rare, a finding that was very recently confirmed in a meta-analysis demonstrating an annual rate of HBsAg seroclearance of 1.02% including patients with and without antiviral therapy [7••]. A higher probability for HBsAg seroclearance was found for HBeAg-negative patients in comparison with HBeAg-positive patients (1.33% vs. 0.40%) and lower HBV DNA (6.61 log10 IU/mL vs. 7.71 log10 IU/mL) and HBsAg levels (2.74 log10 IU/mL vs. 3.90 log10 IU/mL) [7••]. Importantly, HBsAg seroclearance was not associated with HBV genotype or treatment history [7••].

Once HBsAg is lost, HBsAg seroclearance is stable and the risk of HBsAg seroreversion is rather low [8, 9]. In two studies on this topic, one including 54, the other one including 110 CHB individuals, seroreversion occurred in 4% and 7% of the patients, respectively [8, 9]. Notably, HBsAg seroreversion was only transient for the majority of patients and most showed an immediate seroclearance after seroreversion. The patients with persistent HBsAg after initial seroclearance and seroreversion had very low levels of HBsAg and HBV DNA suggesting immune control [8, 9]. However, many patients with HBsAg seroclearance do not develop anti-HBs, even several years of follow-up after losing HBsAg [6].

Correspondingly, all major guideline value HBsAg loss as the optimal treatment endpoint, feasible for the cessation of therapy [1–3] (Table 1). It should be noted that the effect of consolidation therapy after HBsAg seroclearance is largely unknown. Given the excellent tolerability of current NA treatment, treatment prolongation until anti-HBs seroconversion in patients with cirrhosis seems reasonable. After stopping NA treatment, the AASLD guidelines recommend monitoring every 3 months for the first year [2]. After the first year, monitoring may be extended to 6- to 12-month intervals, as relapse rate decreases and such a close follow-up is not feasible for all patients [2, 10•]. It should be noted that all patients who lost HBsAg with cirrhosis, advanced age (> 50 years), and male gender remain at an increased HCC risk, and HCC surveillance should be continued.

Importantly, even after HBsAg loss, cessation of antiviral therapy is not recommended for people with decompensated cirrhosis, as well as patients currently under immunosuppressive treatment or receiving chemotherapy, unless stable anti-HBs levels are present [1, 2]. Of note, one has to be reminded that "functional cure" cannot be equalized with "virological cure" as there might be still cccDNA persisting in the liver, which could possibly lead to virological reactivation [11].

Discontinuation of Nucleos(t)ide Analogues after Anti-HBe Seroconversion

Whereas HBsAg loss is titled "functional cure," anti-HBe seroconversion may reflect immune control often accompanied by a low replicative status of the virus [1–3]. In 90% of HBsAg-positive patients, HBeAg loss occurs before the age of 40 years [3]. However, patients may be incorrectly classified as HBeAg-negative carriers or HBeAg-negative infection due to variants with mutations in the precore and basal core promoter region, leading to an escape from the anti-HBe antibody response and HBeAg-negative hepatitis [12]. Thus, patients should be further monitored after anti-HBe seroconversion.

The most comprehensive data on therapy termination in initial HBeAg-positive patients after anti-HBe seroconversion were collected in a systematic review and meta-analysis with a total of 1217 HBeAg-positive patients [10•]. HBeAg seroconversion is stable with pooled rates of 95.4%, 91.9 %, and 88.0% at 6 months, 12 months, and 24 months, respectively [10•]. Virological remission rates were lower with 62.5%, 53.4%, and 51.5% of the patients at 12, 24, and 36 months after cessation of therapy [10•]. However, the included studies were heterogeneous concerning the definition of virological remission and duration of anti-HBe seroconversion before NA treatment was ceased [10•].

An increased likelihood for a permanent remission in younger patients and patients with lower HBsAg was shown, but overall, the data are limited. Continued antiviral therapy was associated with a higher rate of virological and biochemical remission; HBeAg reversion does not occur if antiviral therapy is continued. It should be noted that data on long-term mortality, progress of liver disease, and risk of hepatocellular carcinoma or hepatic decompensation compared with continued therapy are not available.

To increase the frequency of virological remission, consolidation therapy after anti-HBe seroconversion has been advocated. Several studies showed a benefit of a consolidation therapy ≥ 12 months compared with a consolidation therapy < 12 months [13–15]. One study investigated an even longer consolidation therapy and found a consolidation therapy of 3 years was linked to a higher likelihood of HBsAg loss on further follow-up as well as lower risk for relapse in comparison with 1-year consolidation therapy [13]. In contrast, the systematic analysis could not provide strong evidence that a consolidation therapy < 12 months after anti-HBe seroconversion is inferior, but the strong heterogeneity of the studies should be considered [10•]. Nevertheless, in accordance with international guidelines and practice in studies conducted to date, consolidation therapy of at least 12 months after anti-HBe seroconversion should be administered before therapy is discontinued (Table 1).

No influence of the genotype on the probability of a permanent remission could be demonstrated; however, the data are based mostly on HBV genotype B and C. A lower HBsAg level (< 2.5 log IU/ml) is associated with permanent remission [10•].

Given the significant relapse rates, mostly within the first year, monitoring at least every 3 months for the first year is recommended and currently suggested by the AASLD [2]. APASL even recommends monthly visits for the first 3 months and every 3 months afterward (Table 1) [3]. The optimal post-treatment control intervals are not well defined and should be tighter in advanced fibrosis [2, 3]. Attention should be paid to clinical signs of hepatic decompensation. Fulminant reactivations have been described in HBeAg-positive patients after stopping NA therapy [16•].

In all guidelines, stopping antiviral therapy (before HBsAg loss) is only recommended in patients without liver cirrhosis [1–3]. As the risk for virological relapse is high and prediction of sustained virological remission after stopping NA in patients with anti-HBe seroconversion is poor, continuing NA therapy until HBsAg loss is a viable strategy and a recommended alternative in the AASLD guideline [2].

In the case of confirmed clinical relapse, patients should be treated according to the guideline recommendations [1–3].

Discontinuation of Nucleos(t)ide Analogues in HBeAg-Negative Chronic Hepatitis

Two large systematic reviews and meta-analyses have investigated the discontinuation of NA therapy in HBeAg-negative patients, one including 1732 and 967 patients, respectively [10•, 17]. The included studies showed strong heterogeneity concerning the duration of therapy (6–96 months and 14–79 months), as well as follow-up (6–82 months and 12–69 months) [10•, 17]. In the review of Papatheodoridis et al., 43.7%, 31.3%, and 30.1% of the patients showed virological remission after 12, 24, and 36 months of follow-up, respectively [10•].

The high relapse rates in the first year are the reason why all three guidelines recommend close monitoring within the first year after cessation of therapy [1–3], with the AASLD not recommending NA discontinuation in HBeAg-negative patients at all. HBeAg-negative patients also profit from a prolonged time of consolidation therapy [10•, 17]. Consolidation therapy > 24 months has been linked to a 3-fold increase (95% CI 0.39–23.30) in virological remission in comparison with < 12 months of consolidation therapy [10•]. Similar findings were made by Chi et al. who found that there is a significant difference between < 1 year and > 3 years of consolidation therapy in HBeAg-negative individuals (24% vs. 57%, $p = 0.036$) [13].

Recent studies have shown that virological relapse occurs mostly within the first 3 months after discontinuation of tenofovir, whereas relapse might occur later after stopping entecavir [18•, 19, 20] (Table 2). In addition, relapse after discontinuation of tenofovir may be more severe [19]. The reason for the different timing of HBV relapse after stopping tenofovir or entecavir in the studies is not understood. A potential bias is possible as entecavir was approved earlier and treatment duration may have been longer in entecavir cohorts. However, a biological reason could also be possible. For example, one study suggested that tenofovir induced type III interferon [21]. Further studies need to explore reasons for the documented differences.

Similar to the studies after anti-HBe seroconversion, patients with other liver diseases, immunosuppression, advanced liver diseases, or severe comorbidities were excluded. It is well known that those patients have a lesser hepatic reserve and severe flares might lead to hepatic decompensation or death. Thus, NA discontinuation should not be done in those patients. Hepatic decompensation in patients with liver cirrhosis has been reported [17, 22].

Biological Effects of NA Discontinuation in HBeAg-Negative Patients

Although initial goals of treatment discontinuation have been to achieve an off-therapy virological remission, several studies of different groups have shown that treatment discontinuation may lead to increased rates of HBsAg seroclearance [23–25)], also in comparison with patients who continue NA therapy, with rates of HBsAg loss of 19–39% in the long-term follow-up.

The first study that suggested a high rate of HBsAg loss after stopping NA therapy was from Greece. Thirty-three HBeAg-negative patients treated for > 4 years with adefovir stopped treatment [26]. All patients experienced a transient HBV DNA rebound and 76% had an ALT flare. In the long-term follow-up of 5.5 years, 39% achieved HBsAg loss [26]. Another study from Greece including 57 patients treated with entecavir or tenofovir discontinued NA therapy after being 5.3 years in virological remission [26]. One year after stopping NA treatment, 16% of patients achieved HBsAg loss, which increased to 25% after 18 months [23]. In our own prospective single-center study, 3 out of 15 HBeAg-negative patients cleared HBsAg following discontinuation of therapy [25]. A randomized trial with 42 HBeAg-negative Caucasian patients (21 stopped and 21 continued NA therapy) demonstrated that 19% of the patients who stopped NA treatment achieved HBsAg loss by week 144 off-therapy [24••]. Patients in the control group did not show any HBsAg decline while the median HBsAg change was − 0.59 log IU/mL in the stop group [24••].

Table 2 Timing of HBV relapse after stopping entecavir (ETV) or tenofovir (TDF). Results of three studies with a direct comparison

Study	Frequency of virological relapse after stopping therapy (HBV DNA > 2,000 IU/ml)	Cohort
[20]	*n* = 100 HBeAg negative (ETV *n* = 66; TDF *n* = 34) 3 months: ETV 6.1%; TDF 52.9% 6 months: ETV 33.3%; TDF 58.8% 12 months: ETV 53.0%; TDF 65.2% 24 months: ETV 65.2%; TDF 72.1%	Prospectively followed cohort, > 12 months HBV-DNA < LLQ, median treatment duration 37 months, stopping according to APASL guidelines
[19]	*n* = 507 (ETV *n* = 342; TDF *n* = 165) HBeAg positive (ETV *n* = 108; TDF *n* = 46): 3 months: ETV 5.6%; TDF 28.3% 6 months: ETV 13%; TDF 56.5% 12 months: ETV 31.6%; TDF 66.9% 24 months: ETV 41.3%; TDF 72.4% HBeAg negative (ETV *n* = 234; TDF *n* = 119): 3 months: ETV 5.6%; TDF 20.2% 6 months: ETV 29.1%; TDF 45.4% 12 months: ETV 50%; TDF 63.2% 24 months: ETV 62.7%; TDF 70.3%	Retrospective-prospective study. All patients had post-treatment follow-up for at least 6 months. VR was defined as an HBV DNA level of > 2,000 IU/mL in two consecutive measurements
[18•]	*n* = 220 (ETV *n* = 154; TDF *n* = 66) 12 weeks: ETV 4.5%; TDF 71.2% 24 weeks: ETV 48.7%; TDF 77.2%	Multicenter study (HBeAg negative at NA introduction 189 (85.9%)). Patients had to be HBV DNA negative 18 months prior to the discontinuation of antiviral therapy

A large prospective study from Taiwan documented an HBsAg loss rate of 13% in 691 patients after 6-year follow-up [27•]. Their data showed that HBeAg-negative patients with clinical relapse who remained untreated had a 7.34 times higher incidence of HBsAg loss than those who were retreated [27•]. The group of Chen et al. observed an 18-fold higher frequency of HBsAg loss, if clinical relapse was left untreated [28•]. In our own small trial, the peak of HBV DNA or HBcrAg was associated with the decline of HBsAg suggesting that virological relapse might induce host immune responses after stopping therapy in selected patients [25]. Liaw et al. suggest two types of patients with hepatic flare, one that shows a strong immune response in which HBsAg levels rise before the ALT peak, and decrease along with ALT normalization, and the second that shows an insufficient, ineffective immune response depicted by elevated HBsAg levels even after ALT normalization, requiring immediate retreatment [29, 30]. We could show that cytokines such as CXCL-10, IL-12, TNF but also IL-10 were induced after HBV DNA rebound [25]. In addition, T cell responsiveness and NK cell functions are altered during HBV DNA rebound after the cessation of NA therapy [31, 32, 33•].

Based on these data, immediate retreatment may be counterproductive if the flare would be important to achieve HBsAg loss. In clinical practice, monitoring patients every 4 weeks and maybe even closer (e.g., weekly) in the case of relapse seems reasonable. However, starting NA therapy too late in the case of high HBV DNA may also be counterproductive. We would suggest restarting NA treatment if HBV DNA is > 20.000 IU/ml and ALT > 2 ULN confirmed on at least 2 occasions. In addition, strong ALT flares > 10 ULN should also warrant retreatment based on clinical judgment. HBV DNA level > 2.000 IU/ml with elevated ALT levels in the long-term follow-up should also lead to retreatment according to the treatment indication of international guidelines.

However, there are conflicting data about stopping NA treatment. In other studies with Asian patients, the largest HBsAg declines have been observed in patients without signs of virological or clinical relapse [27•, 28•]. Also, in the DARING trial, HBsAg loss was not associated with ALT flares [23]. Importantly, some patients in the DARING trial achieved HBsAg loss very early after stopping NA therapy [23]. It may be important to consider HBsAg levels before stopping NA therapy (see Fig. 1). Patients that show an HBsAg decline during NA therapy may not require a strong flare and immune responses have already been restored during NA therapy.

Even without HBsAg decline (before or after stopping NA therapy), patients may remain in virological remission or show just very mild and transient flares after stopping therapy. Possible reasons for this are that cccDNA is inactive or already cleared and the source of HBsAg may be from integrated HBV DNA [34, 35•] or HBV DNA can be controlled by existing immune responses. The group of Bertoletti has shown that the absence of hepatic flares following discontinuation of NA therapy was associated with a certain HBV-specific T cell subset that may control HBV after stopping therapy [36].

Just recently, the largest prospective stop NA study with 45 patients in the stop arm and 21 patients in the continue arm was published [16•]. In contrast to

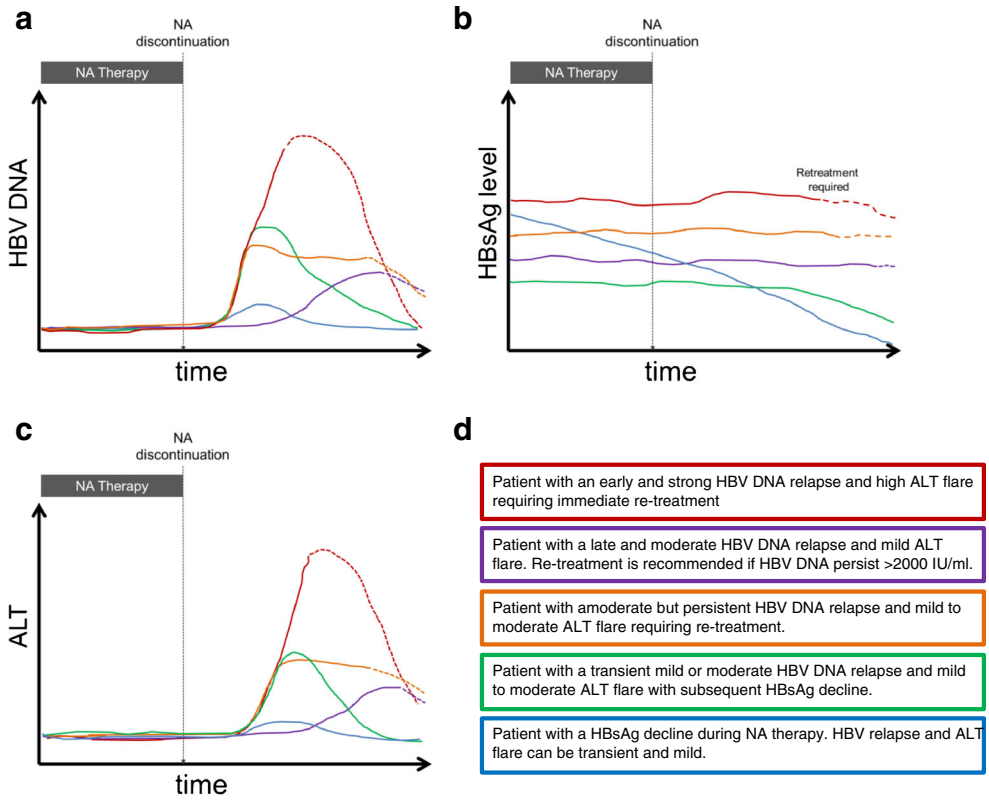

Fig. 1 Five different patterns of response after cessation of NA therapy. **a** HBV DNA relapse can occur at different times. **b** ALT flares usually occur after HBV DNA relapse. HBsAg level (**c**) pattern can differ. The different colors represent different cases (**d**)

previous trials, the Toronto trial documented no change in HBsAg levels after stopping therapy, and HBsAg decline was not associated with peak ALT or HBV DNA values [16•]. The different outcome of this study was not explained by a different consolidation therapy [16•]. Of note, 18/45 patients in the stop group were initially HBeAg positive which were 3 times more likely to require retreatment compared with HBeAg-negative patients [16•]. Only one out of 27 (4%) HBeAg-negative patients lost HBsAg after cessation of therapy. Importantly, the majority of patients in the Toronto trial were Asians [16•]. Similar findings in Asian patients were also recently published, suggesting that HBsAg loss may occur preferentially in Caucasians [37].

Presently, the reasons for the high variability in the outcome after stopping NA therapy in HBeAg-negative patients are largely unknown. Potential factors that influence the response after NA withdrawal might be virologic factors such as genotype, viral quasispecies, duration of viral infection, and host factors such as genetics or immune responses as discussed above or even environmental factors that may influence B cell responses [38]. Further research is necessary to understand the different outcomes after stopping NA therapy.

A topic of debate is whether NA discontinuation influences mortality or the risk for HCC development in comparison with patients who continue NA. So far, no increased risk could be documented in patients who discontinue NA [28•, 39]. Thus, further studies should be without a serious safety concern.

In summary and based on the available studies, 4 potential outcomes have been defined after stopping NA therapy in HBeAg-negative patients: (a) HBsAg loss (about 20% after 3 years in well-selected Caucasian patients), (b) HBeAg-negative infection ("healthy carrier") off-therapy (20–30%), (c) indeterminate state without immediate retreatment criteria (about 10–20%), and (d) HBeAg-negative hepatitis requiring retreatment (about 40%) [40].

Predictors for Relapse and HBsAg Loss (HBeAg-Negative Patients)

With the recent experience for stopping NA therapy, the major questions have become in whom and when to stop NA therapy to achieve best possible outcomes. Although there is an increasing amount of studies among the topic of discontinuation of NA treatment in CHB patients, a reliable biomarker to predict the patients' outcome after cessation of therapy is missing yet.

As discussed above, prolonged consolidation therapy may be a good option to improve virological remission after stopping NA therapy. However, the optimal duration is not known, although all available data hints that consolidation therapy > 1

year in HBeAg-positive patients and > 2 years (ideally 3 years) in HBeAg-negative patients is beneficial.

Viral Markers

One recently published systematic review showed that HBsAg levels at baseline (prior to NA start) and at end of therapy, age, as well as consolidation therapy duration were associated with relapse in HBeAg-negative CHB patients [41]. After HBeAg seroconversion, lower age, lower baseline ALT values, and lower HBsAg levels were linked to a lower relapse rate [42, 43]. Similar findings were made by two other studies showing that lower HBsAg levels are not only associated with less virological and clinical relapse but also with HBsAg loss [27•, 44]. Also, the meta-analysis by Chang et al. showed that low HBsAg (< 200 IU/ml) was associated with a higher rate of permanent remission [17]. However, the frequency of patients with HBsAg levels < 2 $\log_{10}$ IU/ml is low, limiting the usefulness in clinical practice.

Recently, two new biomarkers have emerged: HBcrAg and HBV RNA. In contrast to HBsAg which can be produced from either integrated HBV DNA or intrahepatic cccDNA, HBV RNA can only be produced by intrahepatic cccDNA [45]. The second marker is HBcrAg which consists of hepatitis B envelope antigen (HBeAg), hepatitis B core antigen (HBcAg), and a truncated 22 kDa precore protein (p22Cr) [46]. In one recent study, Chen et al. compared the correlation of HBsAg, HBcrAg, and HBV RNA with cccDNA in untreated CHB patients. HBcrAg had the highest correlation irrespective of HBeAg status, making it an interesting marker for prediction of relapse after discontinuation of NA treatment [46].

HBV RNA was described as a predictor for anti-HBe seroconversion during treatment with NA [47]. Preliminary data show the utility of HBV RNA to assess the risk of HBV DNA reactivation after HBsAg loss [48]. In 19 patients, 2 patients developed HBV DNA reactivation (but not HBsAg reactivation). Both patients had detectable HBV RNA at the time point of NA cessation. Thus, measurement of HBV RNA as a predictor of durable remission after HBsAg loss may become relevant, if these findings can be confirmed. Detection of HBcrAg and HBV RNA also predicted severe ALT flares in HBeAg-negative patients after NA discontinuation, which only occurred in patients with detectable levels [48]. Similar results were observed in a previous study, which showed that HBcrAg > 3.7 log IU/ml was predictive for virological relapse after NA cessation [49].

However, it should be noted that specificity, sensitivity, false positivity, and false negativity for certain cutoffs are not established.

Host Markers

Several clinical parameters, such as lower ALT, younger age (< 40 years), female sex, and absence of liver cirrhosis, have been associated with a higher probability of virological remission [10•]. However, virological control is mediated by the immune response against the virus, sparking interest in specific markers of the immune system to predict the host response.

One recently published study showed that Plasma CXCL-13 could work as a potential biomarker in a mixed HBeAg-negative, HBeAg-positive cohort to detect patients that will achieve HBsAg loss [50]. Kranidioti et al. showed that HBeAg-negative patients in stable virological remission 3 years after treatment cessation can be characterized by a distinct immune profile of IFNγ, IL-8, FASLG, and CCL4 [51].

Studies have demonstrated exhaustion of immune responses during chronic infection, but also restoration of cellular immune response during NA therapy [31, 32]. This may explain the effect of consolidation therapy. Importantly, T cells of patients that cleared HBsAg showed a less exhausted phenotype and increased functionality in our own study [33•]. Additionally, NK cell functionality increased, particularly in patients with ALT flares and subsequent HBsAg loss [52]. However, signs of T cell exhaustion, as well as increase of immunosuppressive cytokines, such as IL-10, have also been observed after treatment cessation. The latter can lead to a suppression of virus-specific T cells in the liver, suggesting potential counter regulatory pathways in case of persisting viral replication [25, 33•, 53, 54]. Therefore, the optimal time point to restart treatment needs careful consideration as discussed above. Interestingly, the studies from Asia reporting the highest frequency of patients with HBsAg loss in patients without virological relapse may be explained by a distinct PD-1+ HBV-specific T cell subset [36], suggesting different patterns of immune responses before and after stopping NA therapy.

To date, no distinct host-related marker is available to predict a certain outcome, because of the complex interplay of the immune system. However, with new therapies on the horizon, including combination therapies and immune therapies, it is more important than ever to understand the underlying immune responses.

Antiviral Drugs

A very recent controversial topic in the field of hepatitis B is the potential biological difference between tenofovir and entecavir, most notably due to the observed differences in HCC development [55••]. However, differences between tenofovir and entecavir have also been reported after NA treatment cessation with earlier virological relapse with tenofovir. Interestingly, a recent meta-analysis has revealed tenofovir to

have the most efficient antiviral activity [37]. In Table 2, we have listed three head to head studies which investigated stopping NA treatment in which patients have received one of the two antiviral drugs. In the most recent and largest of those studies, Kuo et al. compared 342 patients receiving ETV vs. 165 patients receiving TDF [19]. Interestingly, patients receiving TDF exhibited significantly higher relapse rates at all time points; however, patients in the ETV group had a significantly longer treatment duration [19]. Nevertheless, in the other head to head study of Su et al., patients had similar treatment durations and the differences in relapse rates between ETV and TDF were still present [20]. In our study, TDF patients had a median relapse time of 6 weeks, whereas ETV patients relapsed after a median of 24 weeks [18•]. Former studies investigating clinical relapse in ETV or TDF cohorts came up with similar results: In the TDF groups, clinical relapse occurred usually at a median of 12 weeks [22], whereas in the ETV group, clinical relapse was documented at a median of almost 33 weeks [56].

The fact that patients who receive TDF will relapse earlier than those who receive ETV has an important consequence for physicians, TDF-treated patients may require closer monitoring in the weeks after stopping NA, whereas ETV-treated patients may need close follow-up even 6–12 months after NA cessation due to later relapse. Currently, little is known about reasons and ongoing mechanisms that could explain those differences between the two NAs.

Conclusion

In clinical practice, treatment can be discontinued based on current evidence in 3 scenarios: (i) after HBsAg loss, (ii) after anti-HBe seroconversion, and under certain circumstances in (iii) HBeAg-negative hepatitis with undetectable HBV DNA for at least 2 years (ideally > 3 years). Importantly, treatment should not be stopped in HBsAg-positive patients with cirrhosis.

Treatment discontinuation seems safe after HBsAg loss. Although HBsAg loss is not necessarily stable, the risk for virological relapse is very low. As many patients do not develop anti-HBs within years after HBsAg loss, treatment continuation until anti-HBs seroconversion may take years with unknown benefits, but also little risks [6]. However, consolidation therapy until anti-HBs, as suggested by the AASLD guidelines, should be strongly considered in patients with advanced liver cirrhosis [2]. Of note, HCC monitoring should be continued despite HBsAg loss in patients with cirrhosis, male patients older than 50 years.

HBsAg-positive patients without pre-existing cirrhosis may discontinue NA therapy after HBeAg seroconversion and 12 months of consolidation therapy. Non-cirrhotic HBeAg-negative patients may discontinue NA therapy after 3 years of undetectable HBV DNA values and ALT values in normal range during NA therapy. In both cases, close post-NA monitoring must be granted [1–3].

Interestingly, stopping NA treatment in selected HBeAg-negative patients may increase HBsAg loss rates in the long term. So far, the mechanisms are not well understood. To date, no reliable viral or host marker exists to predict the outcome of patients after NA discontinuation. However, the present evidence supports the view that virological relapse, virological remission, and HBsAg seroclearance are immune mediated. Thus, it is likely that these events may be depicted in different levels of gene expression, cytokines, immune responses, and viral markers such as HBV-RNA, HBcrAg, and HBsAg. Continuing efforts are important to understand the virus-host interactions for better selection and better timing of stop NA strategies. This may also help to assess and guide future therapies, which are currently developed and target both the virus as well as the immune system.

Compliance with Ethical Standards

Conflict of Interest Maximilian Wübbolding declares no potential conflicts of interest. Markus Cornberg reports personal fees for lectures and advisory boards from Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag (Data Safety Board), Roche, Merck (MSD), Biogen, Falk Foundation, Boehringer Ingelheim, Siemens, and Spring Bank. Dr. Cornberg reports a grant from Roche. Christoph Höner zu Siederdissen reports travel grants from Gilead Sciences and Novartis.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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