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Outcome after discontinuation of nucleot(s)ide analogues in chronic hepatitis B: relapse rate and associated factors

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Abstract

The introduction of nucleot(s)ide analogues (NAs) for oral antiviral therapy has dramatically improved the clinical outcome of patients with chronic hepatitis B. NAs appear to be safe and induce potent suppression of viral replication. However, they are associated with a low rate of HBsAg seroclearance, the gold standard of successful treatment, and also with a relatively high rate of virological relapse after discontinuation. As a result, long-term treatment is needed. The optimal duration of NA treatment currently remains unclear, nevertheless in some patients NA treatment can be stopped with a relatively low probability of relapse. Whether NAs are able to induce a sustained off-treatment response is an important area for research. This article reviews the relapse rate after cessation of treatment with NAs in chronic hepatitis B patients with the goal of identifying possible predictive factors of relapse.

Keywords Chronic hepatitis B, stopping therapy, nucleot(s)ides analogues

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Introduction

Hepatitis B virus (HBV) infection is a globally important public health problem. The spectrum of disease and the natural history of chronic HBV infection range from an inactive carrier state, with a low risk of advanced liver disease, to progressive chronic hepatitis B (CHB), which may lead to the development of cirrhosis or hepatocellular carcinoma (HCC) [1-4]. Patients with CHB can be hepatitis B e antigen (HBeAg) positive or negative [2]. In particular HBeAg-negative individuals can have either inactive or active chronic hepatitis, associated with mutations in the pre-core region of the HBV genome. Serum hepatitis B surface antigen (HBsAg) correlates with the presence of covalently closed circular DNA (cccDNA) and is a key marker of infection. Clearance of HBsAg, with seroconversion to HBs antibodies (anti-HBs) is the closest correlate of cure and the ultimate goal of CHB therapy [2,5].

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In recent years, the introduction of NAs for oral antiviral therapy has dramatically improved the clinical outcome in patients with CHB due to their ability to profoundly inhibit viral replication [2]. However, the NAs tend not to eradicate HBV as they do not impact HBV cccDNA, which acts as an ongoing source of viral persistence. The ideal goal of HBsAg loss is rarely met by NAs and virological relapse is therefore common after discontinuation of treatment. Thus, although current international guidelines for the management of CHB provide information regarding when to start antiviral therapy, there is no clear consensus on when to stop treatment, especially for those who respond to therapy [2,5]. Whether NAs are able to induce a sustained off-treatment response is now an important area for research.

Lessons from spontaneous clearance and interferon (IFN) therapy

Our understanding of features associated with eradication of HBV comes from studies of both spontaneous and treatment-induced clearance [6-8]. In particular, it is known that spontaneous clearance of HBV is associated with an intact immune system and a strong immune response. For instance, infection as an infant when the immune system is immature is associated with high rates of chronicity, whereas acute infection as an adult carries a much lower risk of chronic infection and a high rate of spontaneous clearance [9-12]. Similarly, conversion from a high to low replicative carrier (accompanied by seroconversion from HBeAg-positive, anti-HBe-negative to HBeAg-negative, anti-HBe-positive) is associated with

enhanced immune reactivity and a "flare" in hepatitis [13,14]. Conversely, immunosuppression in individuals with low HBV DNA levels is associated with rebound high HBV DNA levels, as immune control is lost [15-17].

Goals of antiviral therapy with NAs and stopping rules

The goal of therapy for CHB is to improve quality of life and survival by preventing disease progression. This is best achieved by long-term HBV suppression, associated with a reduction in histological activity, a lower risk of cirrhosis, and reversal of fibrosis. However, although it may be lower, a significant risk of HCC remains after viral suppression especially in patients with cirrhosis [18-21]. Currently available NAs are lamivudine (LAM), adefovir (ADV), telbivudine (LdT), entecavir (ETV), and tenofovir (TDF). ETV and TDF are first-line treatments because of their potent antiviral activity and high barrier to resistance [2].

The ideal endpoint of therapy in HBeAg-positive and HBeAg-negative patients is sustained off-therapy HBsAg loss with or without seroconversion to anti-HBs. This is associated with complete and definitive remission of the activity of CHB and an improved long-term outcome, but is rarely achieved with the currently available antiviral agents [22,23]. A more realistic and satisfactory endpoint is the induction of sustained off-therapy virological and biochemical response for both HBeAg-negative patients and HBeAg-positive patients with durable anti-HBe seroconversion for the latter group. The next most desirable endpoint is long-term virological remission (undetectable HBV DNA using a sensitive PCR assay) under long-term therapy in the HBeAg-negative and in HBeAg-positive individuals who do not achieve anti-HBe seroconversion [2].

International guidelines (European Association for the Study of the Liver [EASL], Asian-Pasific Association for Study of the Liver [APASL], American Association for the Study of the Liver Diseases [AASLD]) suggest that treatment can be stopped after HBeAg seroconversion in HBeAg-positive individuals if HBV DNA remains undetectable by a sensitive PCR assay for at least 12 months (EASL, APASL) or 6 months (AASLD). For patients with HBeAg-negative CHB, however, there is no clear optimal duration of antiviral therapy with NAs. Both the EASL and AASLD guidelines recommend long-term NAs therapy until HBsAg seroclearance. Conversely, the APASL guidelines suggest that cessation of NAs can be considered after at least 2 years of treatment if HBV DNA remains undetectable on 3 separate occasions 6 months apart [1-3].

Outcome after discontinuation of NA therapy

The definition of relapse

One of the important points to consider is the definition of hepatitis relapse, which will subsequently determine the follow up of patients after stopping NA therapy. Transient abnormalities in the alanine aminotransferase (ALT) level or the HBV DNA level may be observed in the majority of the patients who ultimately achieve inactive carrier status. Therefore, even if the ALT or HBV DNA levels show mild elevations, these individuals can still be monitored without restarting treatment. However, there are no clear criteria that identify relapse and therefore no consensus as to when to re-start treatment.

In studies with HBeAg-negative patients there is no single description of the term "relapse", with variability in the HBV DNA, ALT and timepoint criteria used to define a relapse. Initially relapse was defined as the detection of serum HBV DNA but the assays for HBV DNA quantification were not the same in all the studies and as a result detection limits were different [24-37]. Currently, using the same limits as the guidelines for the initiation of therapy, an HBV DNA level >2000 IU/mL can be considered as relapse, [24,28,30,32,33]. Furthermore, virological relapse can be distinct from biochemical relapse which is an elevation of ALT levels >x1, 1.5 or 2 the upper limit of normal (ULN) depending on study criteria. In some studies the term clinical relapse is used. This takes into account both virological and biochemical relapses [27,31,37]. In addition to this variability in the criteria defining the terms, some of the authors have used different time points during the off-treatment period for the definition of relapse [29]. Last but not least, the criteria for retreatment may differ from those for relapse in the same study.

In the majority of studies the definition of relapse after stopping therapy in HBeAg-positive patients is considered to be the re-appearance of HBe-antigen and the detection of serum HBV DNA. However some studies consider purely the level of viremia, with HBV DNA levels ranging from >20,000 IU/mL to >100 IU/mL depending on the study [38-50].

Thus, it is clear that it is difficult to draw a consensus from these studies with varying definitions. Clinically the decision to retreat or not may include an assessment of liver disease stage, with a lower retreatment threshold for individuals with severe fibrosis or cirrhosis.

Relapse rate in HBeAg-negative CHB and off-treatment durability of sustained response

There are only few reports in the literature on the risk of relapse after stopping anti-viral therapy in HBeAg-negative patients (Table 1). The overview of these studies will be presented according to the different definitions of relapse after treatment cessation.

In a study of 458 patients treated with ETV (257) or LAM (201) for 12 months therapy, virological relapse (HBV DNA >60 IU/mL) occurred in almost all patients (97% and 95%) during a 6 month follow-up period [25]. Similar relapse rates were found in a small study of 9 HBeAg-negative LAM-resistant patients treated with ADV for 12 months after HBV DNA became undetectable [35]. Additionally, in a study of 54 patients taking LAM, ETV or clevudine for more than 12 months after virological response, the relapse rate was 81.5% during a median follow-up period of 22 months [51]. On the other hand, in a population given LAM for 48 months, Fung *et al* [37] found a virological relapse rate (HBV DNA >40 IU/mL) of almost one third in 27 patients after 6 months

Table 1 Relapse rate in HBeAg-negative patients after treatment (NAs) cessation

Reference	HBeAg(-) patients (n)	Ethnicity	NAs	Duration of therapy (months, range or ±SD)	Genotype	Cumulative relapse rate	Definition of relapse
Shouval *et al* [25]	257 201		ETV LAM	12 12		97% in 6 months 95% in 6 months	HBV DNA >60 IU/mL
Sohn *et al* [51]	54	Korean	ETV, LAM, Clevudine	22 (12-56)		81.5% in 22 months	
Ha *et al* [36]	145		ADV	26 (24-66)	B, C	65.5% in 60 months	HBV DNA >200 IU/mL
Liang *et al* [34]	43		LAM, ADV, ETV	32.7±7.9		47% in 12 months	
Chan *et al* [26]	53		LAM	34±23		83% in 12 months	
Liu *et al* [28]	61	Asian	LAM	27 (24-66)		26.2% in 6 months 43.6% in 12 months 49.7% in 24 months 52.1% in 36 months 56.1% in 48 months 56.1% in 60 months	HBV DNA ≥2000 IU/mL
Seto *et al* [52]	184	Asian	ETV	36.7±7.7		74.2% in 6 months 91.4% in 12 months	
Jin *et al* [53]	26	Asian	LAM	35 (14-80)		18% in 36 months	
He *et al* [24]	64	Asian	LAM, ADV ETV(n=7), LDT (n=2)	40 (32.5-47.5)	B, C	29.7% in 27 months	HBV DNA >2000 IU/mL in 2 occasions 1 month apart
Chen *et al* [33]	105		LAM	22±9	B or C	43.4% in 12 months 60.1% in 36 months 68.4% in 72 months	HBV DNA >2000 IU/mL in 2 occasions 6 months apart
Hadziyannis *et al* [29]	33	Caucasian	ADV	69 (67-72)	D	9% in 6 months 21% in 12 months 33% in 24 months 42% in 36 months 42% in 48 months 45% in 60 months 45% in 72 months	HBV DNA >2000 IU/mL & ALT >x1 ULN following post-treatment month 6 sustained to the end of follow up
Jeng *et al* [30]	95	Asian	ETV	25 (14-63)	B or C	45.3% in 12 months	HBV DNA >2000 IU/mL & ALT >x2 ULN
Kim *et al* [32]	45		ETV, LAM, ADV	38.42±10.49		VR: 48.9% in 6 months CR: 35.6% in 6 months VR: 73.3% in 12 months CR: 53.3% in 12 months	
Fung *et al* [37]	27	Chinese	LAM	24	B or C	CR: 12% in 6 months VR: 30% in 6 months CR: 18% in 12 months VR: 50% in 12 months CR: 30% in 18 months VR: 50% in 18 months	Virological relapse (VR): HBV DNA >0.5 pg/mL Clinical relapse (CR): HBV DNA >0.5 pg/mL & ALT >x1.5 ULN
Chien *et al* [31]	85		LAM	6-12		61% in 12 months	HBV DNA >0.5 pg/mL & ALT >x1 ULN
Paik *et al* [27]	43	Asian	LAM	24		41.8% in 45±20 months	
Jung *et al* [35]	9 LAM-Resistant		ADV	33 (12-47)		100% 33% 22%	1) HBV DNA >70 copies/mL or 2) HBV DNA >10^5 copies/mL or 3) ALT >x1 ULN

HBV DNA 0.5 pg/mL equal to 1.4×10^5 copies/mL

NAs, nucleot(s)ide analogues; ALT, alanine aminotransferase; HBV DNA, hepatitis B virus DNA; CR, clinical relapse; VR, virological relapse; ADV, adefovir; ETV, entecavir; LAM, lamivudine; LdT, telbivudine; TDF, tenofovir

and of one half after 12 months. There are two studies [26,34] with similar population sizes (53 and 43 patients given LAM or LAM, ETV, ADV respectively) where the relapse (HBV DNA >200 IU/mL) rates were 83% and 47% after 12 months of follow up. The second study thus met the APASL criteria for stopping therapy. Another study [36] of 145 patients treated with ADV which also followed the APASL stopping guidelines showed a 65% relapse rate (HBV DNA >200 IU/mL) and noted that 12 of the 50 patients (24%) with sustained response had seroconversion to anti-HBs after 5 years of follow up.

Some studies have used levels of HBV DNA >2000 IU/mL to define relapse. One recent prospective study of 184 Asian patients taking ETV for at least 24 months and using the APASL criteria to guide treatment cessation had a cumulative rate of virological relapse 74.2% and 91.4% at 6 and 12 months respectively [52]. In two other studies, patients treated with LAM had relapse rates of 26%, 43%, 56-60% and 68% at 6, 12, 60 and 72 months respectively [28,33]. In one study of 45 patients of long-term (more than 3 years) LAM, ETV or ADV therapy the relapse rate was 49% and 73% at 6 and 12 months [32]. Conversely, a very low 3-year cumulative virological relapse rate (18%) was found in a retrospective study of 26 patients with undetectable DNA at the time of cessation of LAM therapy [53].

If clinical relapse is considered as a combination of virological and biochemical relapse the results are slightly different. Detectable HBV DNA and ALT >x1 or 1.5 ULN gave relapse rates of 12-35%, 18-61% and 42% for patients at 6, 12 and 48 months respectively after cessation of long-term LAM therapy [27,31,37]. HBV DNA >2000 IU/mL and ALT >x2 ULN is a reasonable definition of clinical relapse used to guide initiation of treatment. In two studies [30,32] almost the half the patients taking ETV or ETV/LAM/ADV relapsed during the 12 months off-treatment period, including one study in which the APASL stopping rules were used.

A prospective cohort study [29] of 33 patients who had been treated with ADV for 4-5 years and monitored for 5.5 years after cessation of treatment showed an overall relapse rate of 45% during the follow-up period. This study defined relapse as an HBV DNA >2000 IU/mL and ALT >x1 ULN, from 6 months post-treatment until the end of follow up. Interestingly, in the same study in the 18 of 33 patients who achieved sustained response, 13 (72%) showed HBsAg clearance and 9 of these (69%) developed anti-HBs.

In HBeAg- negative patients most of the virological relapse occurs within the first 3-9 months after treatment discontinuation [24,25,28,37] and the majority of patients who eventually relapse have done so by the end of second year of follow up [27]. Thus, 24 months off-treatment is a reasonable indicator of sustained response. Jeng *et al* [30] reported that relapses after cessation of ETV therapy occurred later (74% after 6 months) than those after cessation of LAM or LdT (≤3 months). In the study of Hadziyannis *et al* [29] the HBV DNA of all patients became detectable in the first month after treatment cessation and in the majority of patients (88%) post-treatment HBV DNA levels peaked either in the first or second post-treatment month. Usually the biochemical relapse occurred 1-2 months [29] or longer [37] after the virological relapse.

Relapse rate in HBeAg-positive CHB and off-treatment durability of sustained response

Several reports have evaluated the durability of HBeAg seroconversion with LAM therapy, resulting in a wide range of durable HBeAg seroconversion rates (Table 2). Dienstag *et al* [40] published encouraging results of sustained response in Western countries and the relapse rate after HBeAg seroconversion was only 27% with a median period of 3-year follow up. This study had a potential selection bias because the time of entry into this trial was 4.3 months (median) after stopping therapy, meaning that patients who relapsed in the first 4 months were excluded and 5 of the 40 patients were entered with undetectable HBsAg. However, similar results were also demonstrated in two Korean and one Chinese population [41, 45]. Ryu *et al* [41] reported a cumulative relapse rate in 61 patients of 15% at 6 months and 31% at 2 years after stopping LAM therapy. Lee *et al* [45] also reported a 1-year relapse rate of 16% and 5-year of 30% in 178 patients with LAM-induced complete response. These results were supported by two more recent studies. Firstly, a prospective of 82 patients demonstrated a cumulating relapse rate of 23.4% at 6 months and 29.4% at 4 years after discontinuation of LAM [47], and, secondly, a retrospective had a 3-year cumulative relapse rate of 34.4% in 42 patients [53].

On the other hand, some studies have reported significantly higher relapse rates after cessation of therapy. A study of 34 patients [38] showed a cumulative relapse rate of 49.2% after 2 years of discontinuation. This study includes an unusually high 37% initial HBeAg seroconversion rate compared to the 16-18% in the majority of phase III studies [54-56] and the fact that 49% of the patients were previously IFN non-responders. Similarly, Chien *et al* [31] also reported that 48% of the patients relapsed after stopping LAM for 12 months. In parallel, with these results, several studies [42-44,49] from different groups showed that off-treatment durability of LAM-induced HBeAg seroconversion was not sustained in a large proportion of patients, with a cumulative relapse rate of more than 50% after 1 year. Conversely, in a series of recent studies [46,48,49,51] with various NAs the off-treatment response was not durable despite consolidation therapy after the HBeAg seroconversion. A study [48] of 39 patients treated with various NAs reported that almost all patients (90%) who stopped NA therapy after achieving HBeAg seroconversion, virological and clinical response experienced recurrent viremia (HBV DNA >100 IU/mL) despite consolidation therapy prior to discontinuation of NAs. This compares with none of 49 patients who continued treatment.

The reasons for these differences in rate are not always clear. However, there is variation amongst these studies in terms of: study design (prospective or retrospective), the definition of relapse, re-appearance of HBeAg or detectable HBV DNA (with different HBV DNA assays) or both; in the duration of therapy; in ethnicity; and in HBV genotype. Usually there is a combination of differences between each study, such that broad generalizations may be difficult to make.

In HBeAg-positive patients the mean time of relapse was at 12 months after treatment discontinuation and the

Table 2 Relapse rate in HBeAg-positive patients after treatment (NAs) cessation

Reference	HBeAg(+) patients (n)	Ethnicity	NAs	Duration of therapy (months, range or ±SD)	Genotype	Cumulative relapse rate	Definition of relapse
Dienstag *et al* [40]	39	International (Caucasian 78%)	LAM	36.6 (4.8-45.6)		23%	Reappearance of HBeAg
Van Nunen *et al* [42]	59	Caucasian 33% Asian 66%	LAM			54% in 36 months	
Chien *et al* [31]	82	Korean	LAM	16 (3-55)	B or C	48% in 12 months	Reappearance of HBeAg or / and HBV DNA >0.5 pg/mL
Lee *et al* [45]	178	Korean	LAM	26 (12-77)	C	15.9% in 12 months 30.2% in 60 months	
Ryu *et al* [41]	61	Korean	LAM	35±10		15% in 6months 21% in 12 months 31% in 24 months	HBV DNA >1 pg/mL
Byun *et al* [43]	132	Korean	LAM	14±7		58% in 6 months 66% in12 months	HBV DNA >1 pg/mL
Song *et al* [38]	34	Korean	LAM	9 (6-15)		37.5% in 12 months 49.2% in 24 months	HBV DNA >1.6 pg/Ll
Yoon *et al* [44]	95	Korean	LAM	25.8±13.9	C	52% in 12 months 55.7% in 24 months	HBV DNA >2.5 pg/Ll
Reijnders *et al* [46]	42	Caucasian 58% Asian 41% Other	LAM, ADV, ETV	26 (16-43)	A, B, C or D	69% in 60 months	HBV HBVDNA >2000 IU/mL
Fung *et al* [50]	22	Chinese	LAM	23 (5-91)		64% in 20 months	HBV DNA >60 copies/mL
Sohn *et al* [51]	41	Korean	ETV, LAM, Clevudine	22 (12-56)		85%	HBV DNA >60 IU/mL
Chaung *et al* [48]	39	Asian American	LAM , ADV , ETV, TDF (3 patients)	Consolidation therapy 12 (1-55)		90% in 8 months	HBV DNA >100 IU/mL
Pan *et al* [49]	136	Chinese	LAM, ETV, ADV, LdT	Group A without consolidation therapy Group B with consolidation therapy	C	Group A: 52.5% in 6 months 82.5% in 48 months Group B: 29.2% in 6 months 41.7% in 48 months	HBV DNA >200 IU/mL
Wang *et al* [47]	82	Chinese	LAM	24 (12-54)		23.4% in 12 months 25% in 24 months 29.4% in 48 months	HBV DNA >2000 IU/mL
Jin *et al* [53]	42	Korean	LAM	35.6 (13-98)		34.4 in 36 months	

HBV DNA 0.5 pg/mL equal to 1.4×10^5 copies/mL

NAs, nucleot(s)ide analogues; ALT, alanine aminotransferase; HBV DNA, hepatitis B virus DNA; CR, clinical relapse; VR, virological relapse; ADV, adefovir; ETV, entecavir; LAM, lamivudine; LdT, telbivudine; TDF, tenofovir

majority of the patients had relapsed by the end of the second year [41,45,46].

Risks of relapse

Cessation of antiviral treatment with NAs may be associated with a flare in viremia, hepatitis and potential hepatic decompensation. These occur in approximately 10% of patients [25]. However, in general withdrawal of anti-viral therapy has proved relatively safe after virological and biochemical response in HBeAg-negative patients. Jeng *et al* reported successful retreatment with ETV of a hepatic decompensation episode following withdrawal of treatment [30]. Supporting these data, He *et al* [24] reported that a small proportion of relapsers (10%) had levels of ALT and HBV DNA higher than baseline levels, but none had fulminant hepatitis.

Importantly, the therapeutic response to retreatment is good, especially with the newer antiviral agents, which

have high efficacy and high genetic barriers to resistance. The therapeutic response was similar between retreatment and first-course therapy. In one study, reported in abstract form [57], the patients achieved rapid virological suppression (95%) and ALT normalization (86%) after salvage therapy with ETV. Likewise, salvage therapy either with LAM or ADV had a 100% response [27,29].

Thus, flares after NA withdrawal are generally self-limiting and severe episodes can be managed with retreatment [58]. However, in cirrhotic patients they may be life threatening [59]. This has led to the suggestion that withdrawal of NA in individuals with cirrhosis should not be undertaken lightly [60] and accompanied by stringent monitoring with a view to prompt retreatment. On the other hand, episodes of severe HBV reactivation and flares after treatment cessation may lead to viral clearance with HBsAg loss while under anti-viral treatment the majority of the patients will need decades for HBsAg seroclearance [9,28,61].

Predictors of relapse after discontinuation of NA therapy

Researchers have studied host and viral factors that predict relapse or sustained response after treatment cessation. Patient characteristics such as age and gender, or baseline clinical characteristics as stage of liver fibrosis, levels of ALT, HBV DNA and HBsAg, and the kinetics of these during the treatment or the off-treatment period have been analyzed. Additionally, treatment-related factors, including the duration of consolidation therapy, type of NA and virus-related factors such as genotype and mutations have also been considered.

HBeAg-negative patients

Three studies have demonstrated that patients with sustained response are younger than relapsing patients and in one of them an age of 20 years served as a cut-off [28,31,36]. Disease stage may have some effect, but at present this is not clear. In the largest study addressing this issue, 39/95 patients (41%) were cirrhotic and the relapse rate in this group was similar to the overall population [30]. Paik *et al* [27] found a weak association with cirrhosis and relapse, as did Kim *et al* [32].

Higher levels of ALT at baseline and at the end of treatment predicted sustained response in two studies [29,31]. HBV DNA levels are also a key factor. Lower levels of HBV DNA pretreatment were a predictive factor for sustained response in two studies [29,30] of patients treated with ETV and ADV, but there was no correlation in the study of Chan *et al* [26] with 53 patients on LAM. In addition delayed suppression of HBV DNA levels to undetectable during treatment has been observed in relapsers in one study [34]. Low levels of serum HBsAg at the end of treatment, or decreasing levels during therapy or after its cessation are positive predictive factors for sustained response [26,29,33]. According to Chan *et al* [26] all 5 patients with HBsAg ≤2 log IU/mL and reduction >1 log at the end of treatment achieved a sustained response at month 12 and all 40 patients with HBsAg >2 log IU/mL and reduction by ≤1 log did not have sustained response. Moreover, in another study HBsAg cut-off values of 120 and 200 IU/mL predicted a 79% HBsAg loss and 93% post-treatment sustained response respectively [33]. In this study, a reduction in HBsAg (0.22 logIU/mL) at month 6 after stopping treatment from the end was an independent predictor for HBsAg loss. However, in contrast to the previous studies Seto *et al* showed no influence of HBsAg kinetics and relapse rate [52]. There was no association between relapse and serum HBsAg level at the initiation or the end of treatment. Also, the rate of HBsAg reduction after treatment cessation had no significant association with virological relapse, with no patients achieving HBsAg seroclearance and 10 patients with a significant HBsAg reduction after stopping therapy having a virological relapse (HBV DNA >2000 IU/mL) after 6 months off-treatment.

The type of antiviral treatment (LAM, ETV, ADV) has not significantly influenced the relapse rate *per se* in trials to date [25,32], although relapses may occur later in ETV as compared to LAM [37]. This implies that sustained response depends on both host and virological factors rather than the class of NA, despite the fact that TDF and ETV are more potent antiviral agents with high genetic barriers to resistance. Most researchers analyzed the total duration of treatment or the duration from the virological response until the end of treatment as one of the possible factors in relapse but there was no evidence of correlation even in studies with stringent criteria for stopping therapy [24,28,30,34].

Viral genotype of HBV does not appear to be a major factor. Most studies have recruited patients with genotypes B or C, with one Greek study exclusively studying genotype D individuals [24,30,33,37]. All appear to have comparable relapse rates. Furthermore, pre-core or core codon mutations did not affect the clinical relapse rates in the study of Fung *et al* [37]. Although no statistically significant difference was observed in relapse rates between the patients with or without LAM resistance mutations, this may be related to a lack of study power [25,30,37]. Thus, one study did demonstrate LAM resistance mutation as an important predictor factor of virological relapse but this study considered HBeAg-negative and -positive patients together [34].

HBeAg-positive patients

The duration of consolidation therapy is the predictive factor that most of the researchers have analyzed. There is a consensus that prolonged additional administration of NAs after HBeAg loss or seroconversion may enhance the durability of sustained response after treatment cessation and most of them consider that a 12-month period offers a more durable sustained response [31,38,41,43-47,49].

Age is also a predictive factor for sustained response after treatment cessation. Some authors have used an age of 40 years as a cut-off value and others a younger age. The combination of young age and prolonged consolidation treatment gives lower relapse rates [43-45,47].

The majority of studies have enrolled patients with genotypes B and C. In one study [31], patients with genotype C had higher relapse rates than those with B. Song *et al* [38] reported that high pre-treatment HBV DNA levels were predictive for relapse and Pan *et al* [49] reported an inverse

correlation between baseline ALT levels and relapse rate. Interestingly, host genetics may also play a role, with an effect of the IL-28B (IFNλ3/4) genotype on the response to LAM at one year [62].

Importance of HBsAg seroclearance

Serum HBsAg quantification has been an important marker of response due to its correlation with the presence of cccDNA and it is considered a surrogate marker of infected hepatocytes. It is produced by the translation of messenger RNAs generated from transcriptionally active cccDNA or integrated HBV DNA sequences in the host genome. HBsAg is present in the envelope of infectious HBV virions and in non-infectious spheres and tubules. These particles are produced in a large excess in comparison to intact virions and their production continues after replication is controlled, either spontaneously (inactive carriers) or by antiviral therapy [5].

HBsAg seroclearance rarely occurs in patients with CHB. The annual incidence of spontaneous seroclearance is estimated to be approximately 0.1-0.8% in CHB patients, who acquire infection early in life [10] and the cumulative HBsAg seroclearance rate is 8% at 10 years, 25% at 20 years and 45% at 25 years of follow up [9]. It is also rarely observed in patients treated with NAs or IFN [7,8,18,63,64]. It has been reported that spontaneous or IFN-induced seroclearance is durable and is associated with improvement in liver histology, decreased risk of HCC, and prolonged survival [6-8,11]. These data have been extrapolated to patients who are treated with NAs and therefore HBsAg seroclearance has been regarded an optimal endpoint of treatment in clinical practice guidelines [2]. However, little is known about the long-term clinical outcomes and durability of NA-induced HBsAg seroclearance. Moreover, in some cases, an apparent seroclearance during LAM therapy may not indicate viral clearance but may reflect a point of mutation in the *S* gene that results in failure to detect HBsAg [65]. In a recent large study [23] investigating HBsAg seroclearance achieved after NA treatment, this correlated well with favorable clinical outcomes and was durable in most cases. More precisely, during a median follow-up period of 6 years 110 of 5409 CHB patients initially treated with LAM or ETV, achieved HBsAg seroclearance (0.33% annual seroclearance rate), corresponding to observations of naturally occurring or IFN-induced HBsAg loss. Additionally, seroconversion to anti-HBs in those losing HBsAg was found in 67.4% at 4 years. HBsAg loss was positively associated with an ALT >x5 ULN, the use of ETV, and negatively associated with HBeAg positivity, high HBV DNA and cirrhosis. Rates of HBsAg loss were lower in HBeAg-negative individuals in a multicenter study of TDF, following 2 years treatment with either TDF or ADV [66,67]. Conversely, in a smaller study of 75 patients, a similar rate of HBsAg decline was found for both as HBeAg-positive and -negative patients [66,67]. Due to its association with cccDNA, HBsAg loss may also have an impact on clinically relevant endpoints. In the large study of Kim *et al* [9], the annual risk for death or HCC in the patients with HBsAg seroclearance was 0.7% and the overall transplant-free survival was nearly 100%. Only two patients with baseline cirrhosis had clinical events (HCC or transplantation). HBsAg reversion was found in 11.7% of patients after 36 months, none required retreatment as the HBV DNA levels were <2000 IU/mL and about half experienced re-clearance. Patients with HBsAg seroclearance had shown an increased drop in HBsAg levels in the last year before seroclearance. Likewise, Heathcote *et al* demonstrated that a greater median change in HBsAg levels from baseline predict HBsAg loss [67]. Baseline HBsAg levels are less well correlated to HBsAg seroclearance [23,68]. Higher baseline ALT levels and IFN-inducible protein-10 levels were associated with stronger HBsAg decline in patients under NA treatment [66,67]. This suggests that host immune responses are important in achieving HBsAg seroclearance. However, the direct effect of NAs in this context may be very limited because NAs do not directly affect cccDNA. This may explain why the annual rate of HBsAg seroclearance during NA therapy is not substantial compared to the natural course of untreated patients and is unaffected by the type or potency of NA used.

Hence, HBsAg decline may occur only in patients who have some level of immune response to HBV. The additional HBV DNA suppression by NAs may restore exhausted T cell responses, which could lead to some additional effect compared to the untreated natural course [69]. However, this may only be transient effect and T-cell exhaustion is generally difficult to overcome if HBV persists for a long period. This may help explain why NA-induced HBsAg seroclearance remains rare especially in HBeAg-negative patients may have been infected for longer. This hypothesis is supported by the observation that HBV DNA vaccination did not alter the risk of relapse in treated patients or restore the anti-HBV immune response despite effective viral suppression by NAs [70]. Thus, the current parameters used to assess the optimal time to stop NAs therapy are inadequate. The dynamic of HBV infection and the immune status of the host at the time of treatment maybe another important variable in understanding why the studies produce conflicting results.

Concluding remarks

Generally, treatment of CHB with NAs is efficient and safe. At present, long-term therapy with NAs is required in order to generate sustained responses. The "gold-standard" of HBsAg clearance is rarely achieved but appears durable by all recognized clinical criteria. Conversely, less stringent criteria of low DNA levels may be applicable if endpoints of therapy are clinical in nature, such as halting disease progression, with an option for retreatment if DNA levels return to levels that meet retreatment criteria. Long-term treatment with third generation NAs is rarely associated with viral resistance. Last, but not least, the financial burden of long-term treatment represents another important problem, especially in developing countries where hepatitis B is endemic.

Definitely, there is a high risk of relapse after treatment discontinuation with a variable range among the studies for both HBeAg-negative and -positive patients. The complications of relapse could be eliminated by a very close, individualized follow up of the patients, but, to date, consistent stopping rules with

broad applicability remain elusive. A search of predictive factors that could provide information for selection of the correct candidates who could stop safely the NAs therapy is warranted, perhaps taking into account new biomarkers of disease. Clinical and basic research studies, especially with the more potent anti-viral agents should provide us with novel insights into the optimal management of this disease in the future.

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