Cessation of nucleos(t)ide analogues therapy in chronic hepatitis B: a systematic review and meta-analysis

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Abstract

Backgroud: To address the possibility of safe cessation of nucleos(t)ide analogues (NAs) therapy in chronic hepatitis B (CHB) and to identify factors associated with off-NAs virological relapse (VR). Methods: A published work search was performed to identify all published studies including patients who ceased NAs and were followed for ≥12 months. A meta-analysis was performed. Results: Twenty-six studies involving 2573 patients who discontinued NAs were included. The pooled rate of off-NAs VR was 0.63, being lower in initially hepatitis B e antigen (HBeAg)-positive than HBeAg-negative patients (0.57 versus 0.62, P = 0.732). The pooled rates of VR were 0.47, 0.55, 0.61, 0.51, 0.73 and 0.64 at 6, 12, 24, 36, 48 and 60 months after NAs cessation, respectively, being relatively lower in initially HBeAg-positive (0.31, 0.45, 0.55, 0.44, 0.48, 0.52) than HBeAg-negative patients (0.50, 0.55, 0.69, 0.57, 0.53, 0.68) (P = 0.405). The pooled rate of biochemical relapse was 0.44, being lower in initially HBeAg-positive than HBeAg-negative patients (0.43 versus 0.48, P = 0.554). The pooled rates of hepatitis B surface antigen (HBsAg) loss and seroconversion was 0.09 and 0.06, respectively. The pooled rates of VR at 12 months after NAs cessation were significantly different between duration of on-NAs virological response ≤24 months and >24 months in all patients (0.55 versus 0.41, P = 0.011), initially HBeAg-positive patients (0.59 versus 0.41, P = 0.031), and initially HBeAg-negative patients (0.53 versus 0.37, P = 0.025). Conclusions: Safe cessation of NAs therapy seems to be feasible in a substantial proportion of CHB patients. On-NAs virological response >24 months reduces the risk of off-NAs VR. Stopping NAs treatment may have an impact on long-term HBsAg decline and HBsAg clearance.

Background

Chronic hepatitis B virus (HBV) infection is the leading cause of liver cirrhosis and hepatocellular carcinoma (HCC) across the world, especially in Asia [1]. Compared with pegylated interferon-alfa therapy, treatment with nucleos(t)ide analogues (NAs) appears to be attractive due to their excellent tolerance, safety and efficacy. Effective suppression of HBV DNA using long-term NAs therapy has been proven to delay disease progression in patients with chronic hepatitis B (CHB) [2]. Wherease, HBV is a DNA virus that infects hepatocytes and establishes a covalently closed circular DNA (cccDNA) that acts as a template for HBV replication and antigenic production [3]. NAs therapy suppresses viral replication, but does not successfully eradicate cccDNA or the integrated HBV genome, thus leading to the need of long-term treatment [4,5]. The truth is that life-long NAs therapy represents a financial burden for patients and national health systems. Potential drug toxicity is also important consideration [6]. For these reasons, several studies have become interested in investigating the need for continuation as well as the safety of treatment withdrawal.

NAs cessation is not widely practiced and remains a controversial but highly relevant subject [7]. One reason is the lack of high-quality evidence for treatment withdrawal. Most studies are retrospective with infrequent and irregular off-treatment follow-up and variable criteria of virological relapse (VR) and retreatment [8]. In addition, some studies described very high rates of VR and advised against NAs discontinuation [9,10], whereas other studies demonstrated sustained virological response and HBV surface antigen (HBsAg) loss in some patients, suggesting that NAs discontinuation is possible [11,12].

Therefore, the aim of this study was to assess the existing data on NAs cessation in hepatitis B envelope antigen (HBeAg)-positive or HBeAg-negative CHB patients and to identify risk factors associated with virological relapse after NAs discontinuation.

Methods

Published work search

We performed a published work search for studies published from 2015 to July 2019 using the PubMed, Embase and Cochrane databases. Additional publications were sought using the reference lists of the identified papers. Published reviews on the topic were also evaluated. The following search terms were used: (“hepatitis B” OR “HBV” OR “HBeAg”) AND (“lamivudine” OR “adefovir” OR “telbivudine” OR “entecavir” OR “tenofovir” OR “NA” OR “Nucleostide Analogue” OR “Nuc” OR “treatment” OR “therapy” OR “antiviral”) AND (“cessation” OR “end” OR “stop” OR “withdrawal” OR “off-treatment” OR “discontinuation”).

Study selection

The included studies met the following criteria: (i) randomized trials or observational studies (case-control or cohort); (ii) adult CHB patients including compensated cirrhosis who ceased NAs therapy were involved; (iii) the duration of therapy was more than 12 months and that of off-NAs follow-up was at least 12 months; (iv) data on the number of patients with off-NAs VR was supplied; and (v) definition of off-NAs VR was presented.

The exclusion criteria were: (i) studies published not as original articles (including letters, abstracts, reviews and editorials); (ii) studies published not in English; and (iii) studies involving patients with coinfection (HBV and hepatitis D or C or human immunodeficiency virus), liver or renal or bone marrow transplantation, or HCC.

Data extraction

We extracted the cumulative probabilities of VR, biological relapse (BR) and HBsAg loss/seroconversion at 6, 12, 24, 36, 48 and 60 months after NAs cessation from the original papers. Data were extracted using software "Engauge Digitizer 4.0" [13] from Kaplan-Meier curves if they were not shown in the articles directly.

The published work search was conducted by two independent reviewers (YDX and RJ). Two lists of selected studies were compared for concordance and discrepancies, and if necessary, arbitrated by a third reviewer (GJS). Each study in the selected list was assessed by two independent reviewers (GJS, HM) to determine whether it fulfilled all the inclusion criteria. Data extraction from the selected studies was performed by two independent reviewers (YDX, RJ) based on a predefined form. Data discrepancies and potential queries were arbitrated by a third reviewer (BF).

Two independent reviewers (YDX and RJ) evaluated the quality of the included studies, according to the risk of bias assessment by a tool [14]. Studies with at least a serious risk of bias were considered to be of low quality. Studies with low or moderate risk of bias were considered to be of high or acceptable quality.

Statistical analysis

The inverse variance method, analyzing each time point separately (univariate method), was applied to calculate pooled estimates and corresponding 95% confidence intervals (CIs). In that case, for each time point, we only included studies which contributed data to that time point.

Univariate meta-regression models were used to assess whether study-level factors influenced the rate of VR at 12 months after NAs cessation, which represented the minimum off-NAs follow-up. To analyze HBeAg-positive and HBeAg-negative patients separately, subgroup analyses were carried out. The multivariate Wald test was conducted to compare the results of the two subgroups. To pool the overall rate of VR, BR, and HBsAg loss/seroconversion, logistic random effects regression models were conducted.

Heterogeneity was assessed using the Cochran Q-test ($P < 0.10$ indicated heterogeneity) and I^2 statistics [15]. Sensitivity analysis was performed using sequential omission of individual studies as a possible major source of heterogeneity. To assess publication bias, we visually inspected Begg's funnel plots and used Egger's regression asymmetry test ($P < 0.05$ indicated significant bias) [16].

Meta-analysis were performed using the commands metan, mvmeta, and metareg, meanwhile publication bias was assessed using the commands metan, metafunnel and metareg in STATA ver. 13.0 (Stata Corp, College Station, TX).

Results

Studies characteristics

Our initial search identified 349 potentially relevant publications, 36 of which were retrieved for detailed review. Twenty six studies met our inclusion criteria (Supporting Fig. S1) [17-42]. The main characteristics of the included studies are presented in Tables 1 and 2. The sample size of these studies ranged 6–1075 participants, and there were a total of 3451 CHB patients. Among these patients, 2573 (74.6%) ceased NAs. Of the 2573 patients who ceased NAs, 1899 (73.8%) were male. At baseline, 337 (13.9%) of the 2433 patients with cirrhosis (information of cirrhosis was not reported in two studies [22,25], including 140 off-NAs patients), which was diagnosed by histology findings and/or imaging studies.

There were only one randomized study [32] and the other 25 cohort studies including five retrospective [22-24,29,41], three retrospective-prospective [17,30,33] and 17 prospective [18-21,25-28,31,34-40,42]. According to the risk of bias assessments, four studies had high quality [17,20,21,30], nine had acceptable quality [23,26,27,31,32,34,37,38,42], and 13 had low quality [18,19,22,24,25,28,29,33,35,36, 39-41] (Supporting Table S1).

Sixteen studies included Asian patients [17,20-26,28,31,33,36-39,42], six studies involved Caucasian patients [18,19,27,30,34,40], and four studies included mixed patients population [29,32,35,41]. Lamivudine were used in one [30], entecavir in three [21,22,26], tenofovir in two

[18,32], several NAs in fifteen [17,19,20,24,28,29, 31,33-39,42], and NAs was not reported in the remaining five studies [23,25,27,40,41] (Table 1).

Only HBeAg-positive patients were involved in three studies [23,24,41], only HBeAg-negative patients in ten [18,27,29,30,32-36,40], and both HBeAg-positive and HBeAg-negative patients in thirteen [17,19,21,22,24-26,28,31,37-39,42]. In total, 747 (29.0%) HBeAg-positive and 1826 (71.0%) HBeAg-negative patients were included (Table 1).

Great heterogeneity was found among the 26 studies in respect of age, serum alanine aminotransferase (ALT), HBV DNA levels, HBsAg levels, duration of virological response before NAs cessation, duration of HBeAg seroconversion before NAs cessation, treatment duration and duration of follow up after NAs cessation (Table 2). The shortest duration of follow up after NAs cessation was 12 months, and the longest was 62 months.

VR after NAs cessation

VR was observed in 1747 of 2573 patients (random effects pooled estimate = 0.63, 95% CI 0.56-0.70; *P* for heterogeneity < 0.001) despite of the duration of follow up after NAs cessation (Table 3). The overall pooled rate of VR in initially HBeAg-positive patients (254/493; random effects = 0.57, 95% CI 0.38-0.75; *P* for heterogeneity < 0.001) was lower than initially HBeAg-negative patients (1019/1452; random effects = 0.62, 95% CI 0.54-0.70; *P* for heterogeneity < 0.001). However, there was no statistic difference between initially HBeAg-positive patients and HBeAg-negative patients (*P* = 0.732). The forest plots of the probabilities of VR in all, initially HBeAg-positive, and HBeAg-negative patients are presented in Fig. 1A-C.

In view of the duration of follow up after NAs cessation, the pooled rates (95% CI) of VR were 0.47 (0.38-0.57), 0.55 (0.46-0.64), 0.61 (0.51-0.70), 0.51 (0.32-0.70), 0.73 (0.62-0.84) and 0.64 (0.42-0.87) at 6, 12, 24, 36, 48 and 60 months after NAs cessation, respectively (Fig. 2A). The 6-month, 12-month, 24-month, 36-month, 48-month and 60-month pooled rates (95% CI) of VR were lower, but not significantly, in initially HBeAg-positive patients (0.31 [0.23-0.39], 0.45 [0.26-0.65], 0.55 [0.39-0.71], 0.44 [0.28-0.59], 0.48 [0.30-0.66], 0.52 [0.42-0.62]) than HBeAg-negative patients (0.50 [0.39-0.61], 0.55 [0.45-0.65], 0.69 [0.43-0.95], 0.57 [0.1-1.23], 0.53 [0.41-0.69], 0.68 [0.51-0.76]) (*P* = 0.405) (Fig. 2B-C).

In all patients, the pooled probabilities of VR after NAs cessation were getting lower and lower in studies defining VR by HBV DNA <200, < 2000 and < 20,000 IU/mL (VR [95% CI] at 12 months after NA cessation: 0.80 [0.72-0.87] versus 0.57 [0.47-0.67] versus 0.39 [0.29-0.48]); Odds ratio [95% CI]: 0.89 [0.03-4.8] and 0.25 [0.12-3.25], *P* = 0.006) (Table 4). Likewise, the pooled rates of off-NAs VR were significantly different according to the VR definition in initially HBeAg-negative patients (*P* < 0.001). There were too few studies of initially HBeAg-positive patients to calculate the pooled probabilities of VR according to the different VR definition.

BR after NAs cessation

BR was reported in 966 of 2030 patients (random effects pooled estimate = 0.44, 95% CI 0.36-0.51; *P* for heterogeneity < 0.001) despite of the duration of follow up after NAs cessation (Table 3). The overall pooled rate of BR in initially HBeAg-positive patients (153/407; random effects = 0.43, 95% CI 0.24-0.62; *P* for heterogeneity < 0.001) was lower than initially HBeAg-negative patients (625/1214; random effects = 0.48, 95% CI 0.39-0.57; *P* for heterogeneity < 0.001). However, there was no statistic difference between HBeAg-positive and HBeAg-negative patients (*P* = 0.554). The forest plots of the probabilities of BR in all, initially HBeAg-positive, and HBeAg-negative patients are presented in Supporting Fig. S2A-C.

In view of the duration of follow up after NAs cessation, the pooled rates (95% CI) of BR were 0.29 (0.15-0.42), 0.34 (0.27-0.41), 0.45 (0.31-0.59), 0.46 (0.35-0.56), 0.48 (0.36-0.60) and 0.49 (0.37-0.62) at 6, 12, 24, 36, 48 and 60 months after NAs cessation, respectively (Supporting Fig. S3A). The 6-month, 12-month, 24-month, 36-month, 48-month and 60-month pooled rates (95% CI) of BR were lower, but not significantly, in initially HBeAg-positive patients (0.15 [0.07-0.22], 0.36 [0.22-0.50], 0.39 [0.32-0.45], 0.44 [0.32-0.56], 0.47 [0.24-0.70], 0.40 [0.19-0.62]) than HBeAg-negative patients (0.26 [0.07-0.45], 0.39 [0.20-0.59], 0.54 [0.25-0.83], 0.52 [0.11-0.94], 0.58 [0.27-0.88], 0.49 [0.25-0.73]) (*P* = 0.201) (Supporting Fig. S3B-C).

Retreatment after NAs cessation

In ten studies suppling such data [17,22,24,29,31-33,35,37,40], retreatment was started in 447 (84%) of 534 patients with BR or 447 (52%) of 859 patients with VR.

Durability of HBeAg seroconversion after NAs cessation

As only three studies provided data about the durability of HBeAg seroconversion after NAs cessation [18,19,41], we did not calculate the pooled estimate.

HBsAg loss or seroconversion after NAs cessation

After NAs cessation, HBsAg loss was reported in 144 of 1721 patients (random effects pooled estimate = 0.09, 95% CI 0.06-0.12; *P* for heterogeneity < 0.001). There was no significant difference between initially HBeAg-positive patients (14/209; random effects = 0.06, 95% CI 0.02-0.10; *P* for heterogeneity = 0.222) and HBeAg-negative patients (99/1048; random effects = 0.14, 95% CI 0.08-0.20; *P* for heterogeneity < 0.001) (*P* = 0.131) (Table 3).

At the same time, HBsAg seroconversion was reported in 65 of 1036 patients (random effects pooled estimate = 0.06, 95% CI 0.05-0.11; *P* for heterogeneity = 0.017) (Table 3).

Factors associated with VR after NAs cessation

Results of subgroup meta-analysis found that the duration of on-therapy virological response had a strong effect in the rates of VR after NAs cessation in all, initially HBeAg-positive patients or HBeAg-negative patients (Table 4). Especially, the pooled rates (95% CI) of VR at 12 months after NAs cessation were significant different between duration of on-NAs virological response ≤ 24 months and > 24 months in all patients (0.55 [0.39-0.72] versus 0.41 [0.13-0.66], OR [95% CI] = 0.89 [0.63-3.56], *P* = 0.011), initially HBeAg-positive patients (0.53 [0.32-0.73] versus 0.37 [0.16-0.68], OR [95% CI] = 0.67 [0.01-3.17], *P* = 0.031), and initially HBeAg-negative patients (0.59 [0.21-0.84] versus 0.41 [0.21-0.53], OR [95% CI] = 0.56 [0.14-4.26], *P* = 0.025).

Results of subgroup meta-analysis found that the duration of treatment did not affect the probability of VR after NAs cessation in all or in initially HBeAg-negative patients. There was too few data from studies of initially HBeAg-positive patients to do the subgroup meta-analysis (Table 4).

In spite a total of 14 studies provided data about variable additional predictors of VR after NAs cessation [17,20-23,26,30,31,34,35,37-39,42], they often reported conflicting results. Off-NAs VR was found to be associated with higher baseline ALT and platelet in one [22], higher baseline gamma-glutamyltransferase (GGT) in one [30], high baseline HBV DNA (>10^6copies/ml) in one [22], baseline advanced fibrosis in one [34], and higher end of treatment (EOT) ALT in one study [21], but not in any other study included in this review. Male gender was associated with off-NAs VR only in one study [22]. In addition, older age was associated with off-NAs VR in seven studies (all with both HBeAg-positive and HBeAg-negative patients) [17,21,31,37-39,42], and two of these studies found that higher rates of off-NAs VR in patients >35 years old than patients ≤35 years old [31,42]. They did not found off-NAs VR was association with alcohol consumption in one [34], diabetes and hypertension in one [26], and body mass index (BMI) in two studies [27,34].

The rate of VR after NAs cessation was not related to the type of NAs in six studies using first-line (tenofovir or entecavir) and second-line NAs (adefovir, lamivudine or telbivudine) involving 683 patients (330 HBeAg-positive, 353 HBeAg-negative) [31,34,35,37,38,42]. Whereas, the rate of VR was higher in 22 tenofovir-treated than 113 entecavir-treated HBeAg-negative CHB patients in one study [39]. The rate of VR after NAs cessation was not related to HBV genotype (genotypes B, C and D) only in one study with HBeAg-negative patients reporting such data [35]. No relationship was found between the risk of off-NAs VR and EOT liver stiffness in three studies [17,30,31].

Higher EOT HBsAg levels were related to VR after NAs cessation in six studies with both HBeAg-positive and HBeAg-negative patients (269 HBeAg-positive, 327 HBeAg-negative) [17,21,26,37,39,42], but not in one study with 18 HBeAg-positive patients [20], two studies with 80 HBeAg-negative patiens [30,34], and in one study with both HBeAg-positive and HBeAg-negative patients (60 HBeAg-positive, 22 HBeAg-negative) [31]. Total serum level of EOT antibody against the HBV core protein (anti-HBc) [37] and EOT hepatitis B core-related antigen (HBcrAg) [39] were associated with risk of VR after NAs cessation in each one study.

When compared with initially HBeAg-positive and HBeAg-negative patients, only one study suggested initially HBeAg-positive patients with higher off-NAs VR [22], whereas no difference was found in other seven studies [21,23,26,31,37,39,42]. In the studies including HBeAg-positive patients at the treatment onset, the rate of VR after NAs cessation was associated with time to HBeAg seroconversion in one study with 138 patients [38], but not in another study with 58 patients [23]. Moreover, the rates of off-NAs VR were affected by consolidation duration in two studies including 196 patients [23,38], but not in another three studies including 152 patients [17,21,37].

Clinical outcomes after NAs cessation

Data of clinical outcomes after NAs cessation was provided in 11 studies including 1365 CHB patients [19,24,25,27,30-34,37,38].

During follow-up after NAs cessation, 7 of 337 patients with cirrhosis at baseline suffered from hepatic decompensation, all of whom were initially HBeAg-negative patients. They were retreated promptly but three patients died at 3, 15 and 32 months after NAs cessation, respectively.

Among 1166 patients, twenty-three patients developed HCC after NAs cessation, in whom, 19 patients were cirrhosis at baseline, and 2 patients were non-cirrhosis. Moreover, all of them were initially HBeAg-negative patients.

Discussion

We analyzed cumulative rates of VR at 6, 12, 24, 36, 48 and 60 months after NAs cessation in 26 studies, including 2573 patients who discontinued NAs. The results of all studies showed that the pooled overall rate of off-NAs VR was 0.63, being lower in initially HBeAg-positive than HBeAg-negative patients (0.57 versus 0.62, $P = 0.732$). In view of the duration of follow up after NAs cessation, the pooled rates of VR were 0.47, 0.55, 0.61, 0.51, 0.73 and 0.64 at 6, 12, 24, 36, 48 and 60 months after NAs cessation, respectively, being relatively lower in initially HBeAg-positive than HBeAg-negative patients ($P = 0.405$). Remarkable variations was found across these 26 studies, including the study design (prospective or retrospecvie), NAs stopping rule, duration of NAs treatment and off-NAs follow-up, duration of virological response before NAs cessation, definition of VR, and other clinical factors.

The pooled rate of BR after NAs cessation was 0.44, which was about 20% lower than those of off-NAs VR (0.63) in all. Although depend on the definitions of VR and BR, such differences were accordant among these included studies, suggesting that VR do not always means clinical relapse (CR) (both of VR and BR), especially with the low serum HBV DNA levels. It remains controversial when to start retreatment after NAs cessation. Generally, only VR (without BR) does not indicate retreatment. Some studies suggested that VR is an important trigger for immune responses that can mediate killing of infected hepatoctyes or noncytolytic degradation of cccDNA [43]. Whether CR may be allowed or should be retreated is the conundrum at the centre of controversy. The association between ALT levels increase and hepatic decompensation is well established [44], which suggests that all patients experiencing substantial ALT elevations should restart therapy. However, there is also evidence that ALT flare prior to HBsAg loss. It is clearly important to identify when an ALT flare is detrimental or beneficial to the patient's long-term outcome. Therefore, vigilant monitoring at least during the first 12 months after NAs cessation is mandatory for a timely retreatment.

The results of our study showed a higher incidence of HBsAg loss (random effects pooled estimate = 0.09) and HBsAg seroconversion (random effects pooled estimate = 0.06) after NAs cessation. It is unexpected that the incidence is so much higher than the on-treatment annual incidence [25,45-49]. The loss of HBsAg is regarded as the optimal treatment endpoint, termed 'functional cure', but it is only rarely achieved with current antiviral agents. Spontaneous HBsAg seroreversion with reactivation of the inflammatory liver process after HBsAg loss is rare and may occur in patients with a significant impairment of their immune function [50]. However, several studies have shown higher incidences of HBsAg loss, varing from 7% to 39%, after NAs cessation [32,34,51,52], and it is even discussed as interventional strategy to have an impact on long-term HBsAg decline and also HBsAg clearance [27,52].

Because of the stability of cccDNA in the hepatic nuclei and the difficulty of eradicating it by NAs, a high rate of VR was observed in patients after NAs cessation. Identifying the useful factors to predict VR after NAs cessation remained to be a challenge for CHB patients management. In these included 26 studies, some factors were associated with VR after treatment cessation, including age, gender, baseline ALT, baseline HBV DNA, EOT HBsAg levels, treatment duration and consolidated therapy duration. However, there are some discrepancies among these studies, as to these parameters are not practical for determining when to stop antiviral therapy. Our results of subgroup meta-analysis found that the duration of on-therapy virological response had a strong effect in the rates of VR after NAs cessation in all, initially HBeAg-positive and HBeAg-negative patients. On-NAs virological response >24 months reduces the risk of off-NAs VR. Many other studies suggested that long-term consolidation after on-NAs virological response was associated with a reduced rate of VR. The consolidation time varied between 15 months to 3 years [11,53,54]. On the basis of these results, the current consolidation duration recommended by international guidelines may not suffice for a virological non-relapse.

Liver cirrhosis and HCC development may be a concern after NAs cessation. However, in our study, only 11 included studies provided information about clinical outcomes in patients after NAs cessation, leading to too few data to analyse. Our results showed that the HCC incidence rate was 2.0% (23/1166), which was not higher than that during treatment of the patients with or without cirrhosis [55]. Too few data to analyse the factors related to progression to liver cirrhosis and HCC in CHB patients after NAs cessation. Long-term follow-up studies are needed to determine the risk of liver cirrhosis and HCC development after NAs cessation.

There is a hierarchy in the methods used for the calculation of cumulative probabilities of VR and BR [56,57]. Direct methods make no assumptions and are preferable, followed by the various indirect methods based on reported statistics. The curve methods are likely to be the least reliable. Also, it is not clear how different schemes for dividing up the Kaplan-Meier curves may impact the resulting statistics. In spite of this, in our meta-analysis, the selected studies had different duration of follow up, and the VR and BR distributions were evaluated at different time points. We had to extract cumulative survival rate data from Kaplan-Meier curves in some studies.

There are some limitations in this study. First, it is interesting and significant that a higher incidence of HBsAg loss after NAs cessation was reported. However, the few included studies with small sample sizes and short follow-up duration may limit the applicability of the results. More studies with large sample sizes and longer follow-up duration are in need to address the incidence and factors of HBsAg loss after NAs cessation and to research whether NAs cessation has impact on long-term HBsAg decline and also HBsAg clearance. Second, As some retrospective studies were included, selection or measurement bias could have interfered with the results. Third, there were potential bias in a meta-analysis such as incomplete retrieval of identified research, reporting bias or performance bias.

In summary, safe cessation of NAs therapy seems to be feasible in a substantial proportion of CHB patients. NAs may be discontinued only in patients who can be followed closely with ALT and HBV DNA determinations at least during the first year after NAs cessation. On-NAs virological response >24 months reduces the risk of off-NAs VR. Long-term consolidation after on-NAs virological response would further reduce rates of virological relapse after NAs cessation. A higher incidence of HBsAg loss and HBsAg seroconversion after NAs cessation was found in our study. Stopping NAs treatment before HBsAg loss is feasible in some CHB patients and it may be a interventional strategy to have an impact on long-term HBsAg decline and HBsAg clearance.

Abbreviations

ALT: Alanine aminotransferase; anti-HBc: Antibody against the hepatitis B virus core protein; BMI: Body mass index; BR: Biological relapse; cccDNA: Covalently closed circular DNA; CHB: Chronic hepatitis B; CIs: Confidence intervals; CR: Clinical relapse; EOT: End of treatment; GGT: Gamma-glutamyltransferase; HBcrAg: Hepatitis B core-related antigen; HBeAg: Hepatitis B envelope antigen; HBsAg: HBV surface antigen; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; NAs: Nucleos(t)ide analogues; VR: Virological relapse

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

YDX and BF conceived and designed the study. YDX, RJ, GJS and HM acquired the data. YDX and RJ analysed and interpreted the data. YDX drafted the initial manuscript. YDX, RJ, GJS, HM and BF critically reviewed the manuscript for intellectual content. All authors approved the final version of the report.

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Supplementary Material

Additional supporting information may be found online in the Supporting Information section.

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Tables

Table 1. Characteristics of studies including CHB patients who ceased NAs

Study	All patients(n)	Patients off-NAs(n)			End of NAs treatment						Before NAs treatment	
		Total	HBeAg positive	HBeAg negative	Age(years)	Males (n)	Race	Cirrhosis	HBsAg (log_{10}IU/mL)	Type of NAs	ALT(U/L)	HBV DNA(log_{10} IU/mL)
Peng J,2015[17]	65	65	44	21	33.8†/39.1‡	56	Asian	0	2.2†/2.9‡	LAM 1,LdT 13,ADV 21,ADV+LAM 5,ETV 25	222†/263‡	6.6†/6.3$
Buti M,2015[18]	8	8	0	8	63	7	Caucasian	1	3	TDF	130.5	6.1
Fontaine H,2015[19]	70	36	5	31	46	31	Caucasian	0	3.3	LAM 2,ADV 5,ADV+LAM 7,ETV 4,LAM+TDF 1,FTC+TDF 30	NR	6.1
Chen L,2015[20]	18	18	18	0	28†/28‡	10	Asian	0	2.9†/3.6‡	ADV 4,LAM 4,LdT 5	142†/191‡	6.9†/7.1$
Hsu YC,2016[21]	161	161	37	124	48.1	122	Asian	0	2.9	ETV	164	6.6
Park CH,2016[22]	223	103	23	80	NR	0	Asian	NR	NR	ETV	NR	NR
Jun BG,2016[23]	58	58	58	0	41.1†/39.5‡	40	Asian	0	NR	NR	242†/150‡	6.0†/6.6‡
Lin CC,2016[24]	266	80	33	47	43.5†/48.6‡	65	Asian	7§/4¶	NR	LAM 14,LdT 19,ETV 47	246†/231‡	7.2†/7.2‡
Nagata N,2016[25]	94	37	14	23	NR	0	Asian	NR	NR	NR	NR	NR
Lee HA,2016[26]	44	44	25	19	44.6§/44.7¶	28	Asian	17	3	ETV	202§/338¶	7.2§/6.2¶
Siederdissen CH,2016[27]	15	15	0	15	49.1	12	Caucasian	0	3.1	NR	149.7	NR
Siederdissen CH,2016[28]	224	220	31	189	52	155	Asian	0	2.9	ETV 154,TDF 66	NR	NR
Muche M,2017[29]	6	6	0	6	55.5	4	Mixed	0	2.8	LAM 5,ETV 1	NR	NR
Karakaya F,2017[30]	39	23	0	23	51	16	Caucasian	0	3.5	LAM	73	6.3
Cao JW,2017[31]	82	82	60	22	49.4	73	Asian	0	2.8	LAM+ADV 50,ETV+TDF 32	252§/196¶	6.6§/6.2¶
Berg T,2017[32]	42	21	0	21	44.5	18	Mixed	0	4.3	TDF	NR	2.1
Jeng WJ,2018[33]	1075	691	0	691	55.2	594	Asian	308	2.6	ADV 10,LAM 10,LdT 40,ETV 537,TDF 104	147	6.2
Dalekos GN,2018[34]	60	57	0	57	60	37	Caucasian	0	2.8	ETV 13,TDF 11	90	5.6
Papatheodoridis GV,2018[35]	130	130	0	130	57	90	Mixed	0	NR	ETV 54,TDF 63,others 13	105	NR
Rivino L,2018[36]	54	48	0	48	48.3†/51.8‡	34	Asian	0	3.1†/3.2‡	LAM 21,ETV or TDF 29	94.5/NR	6.6/NR
Chi H,2018[37]	100	100	71	29	33§/41¶	86	Asian	0	2.8	first-line 43,second-line 57	252§/200¶	6.1§/5.4¶
Liu F,2018[38]	223	223	138	85	25.8§/33.2¶	164	Asian	0	NR	ADV 31,LAM 108,LdT 19	201§/167¶	6.9§/6.3¶
Hsu YC,2018[39]	135	135	31	104	49.5	109	Asian	0	2.8	ETV 113, TDF 22	134	5.7
Kranidioti H,2019[40]	70	23	0	23	57	0	Caucasian	0	3.4	NR	23.2	NR
Hees SV,2019[41]	98	98	98	0	NR	73	Mixed	0	NR	NR	NR	NR
Xie LQ,2019[42]	91	91	61	30	35.7	75	Asian	0	2.9	first-line 39	212	6.2

Note: †/‡, nonrelapers/relapers; §/¶, HBeAg positive/HBeAg negative;
Abbreviations:ADV,adefovir dipivoxil;ALT,alanine aminotransferase;ETV,entecavir;FTC,Emtricitabine;LAM,lamivudine;LdT,telbivudine;NAs,nucleos(t)ide analogues;NR,not reported;TDF,tenofovir disoproxil fumarate.

Table 2. Treatment characteristics of studies including CHB patients Who ceased NAs

Study	Treatment duration (months)	Duration of virological response before NAs cessaion (months)	Duration of HBeAg seroconversion before NAs cessation (months)*	Time to first undetectable HBV DNA during treatment (months)	Time to HBeAg seroconversion (months)	Follow-up after NAs cessation (months)	Lower limit of detection of HBV DNA	Definition of off-NAs VR	Definition of off-NAs BR
Peng J,2015[17]	56.1	33.3	NR	36.7	NR	12	<20 IU/mL	HBV DNA>2000 IU/mL	ALT>2ULN
Buti M,2015[18]	72	NR	-	NR	-	18	<20 IU/mL	HBV DNA>2000 IU/mL	ALT>1.2ULN
Fontaine H,2015[19]	48	NR	NR	NR	NR	12	<12 IU/mL	HBV DNA>120 IU/mL	NR
Chen L,2015[20]	37.5†/48‡	35.5†/46.8‡	NR	1.5†/2‡	NR	12	<20 IU/mL	HBV DNA >20 IU/mL	NR
Hsu YC,2016[21]	36.6	NR	20.6	NR	NR	17	NR	HBV DNA>2000 IU/mL	ALT>2ULN
Park CH,2016[22]	NR	18.3	NR	NR	NR	24	NR	HBV DNA>10000 copies/ml	ALT>ULN
Jun BG,2016[23]	41.8†/42.2‡	21†/13‡	NR	4†/9‡	12†/23‡	20.7†/20.2‡	<20 IU/mL	HBV DNA>2000 IU/mL	ALT>2ULN
Lin CC,2016[24]	36	NR	NR	NR	NR	12	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Nagata N,2016[25]	40	NR	NR	NR	NR	60	NR	HBV DNA>100000 copies/mL	ALT>2ULN
Lee HA,2016[26]	37.0§/31.6¶	31.0§/27.1¶	23.4	5.5	57.9	20.8	NR	HBV DNA >2000 IU/mL	ALT>2ULN
Siederdissen CH,2016[27]	NR	NR	-	NR	-	12	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Siederdissen CH,2016[28]	NR	NR	NR	NR	NR	6	<20 IU/mL	HBV DNA >2000 IU/mL	NR
Muche M,2017[29]	138	84	-	NR	-	40	NR	HBV DNA >2000 IU/mL	ALT>ULN
Karakaya F,2017[30]	63	36	-	NR	-	62	NR	HBV DNA >2000IU/mL*	ALT>2ULN
Cao JW,2017[31]	48§/46.8¶	25.2§/34.8¶	NR	NR	NR	22.8	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Berg T,2017[32]	NR	NR	-	NR	-	36	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Jeng WJ,2018[33]	39	27.8	-	27	-	38.8	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Dalekos GN,2018[34]	≥48	63.6	-	NR	-	18	NR	HBV DNA>200 IU/mL#	ALT>ULN$
Papatheodoridis GV,2018[35]	60	43	-	NR	-	15	<45 IU/mL	HBV DNA >200 IU/mL#	ALT>ULN%
Rivino L,2018[36]	18	NR	-	NR	-	12	NR	HBV DNA>2000 IU/mL	ALT>2ULN
Chi H,2018[37]	46.8§/57.6¶	26.4§/34.8¶	NR	NR	NR	30	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Liu F,2018[38]	27.5§/32.0¶	18§/30¶	NR	3§/2¶	8	60	<1000 copies/mL	HBV DNA >10000 copies/mL	NR
Hsu YC,2018[39]	36.7	25.2	NR	NR	NR	25.9	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>ULN
Kranidioti H,2019[40]	96	≥48	-	NR	-	54	NR	NR	NR
Hees SV,2019[41]	NR	11.4	NR	NR	NR	42.8	varied between 12 and 2000 IU/mL@	HBV DNA >2000 IU/mL	ALT>2ULN
Xie LQ,2019[42]	56.7	28	NR	NR	NR	60	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN

Note. †/‡, nonrelapers/relapers; §/¶, HBeAg positive/HBeAg negative; *Only for studies with HBeAg-positive patients; @depending on the year of determination; * also provided data of HBV DNA >20000 IU/mL; #also provided data of HBV DNA>2000, >20000 IU/mL; $ also provided data of ALT >2ULN; % also provided data of ALT>2ULN,>3ULN,>5ULN, >10ULN.
Abbreviations: BR, biological relapse; NAs, nucleos(t)ide analogues; NR, not reported; VR, virological relapse

Table 3. Data on off-NAs VR, BR, retreament and HBsAg loss/seroconversion in studies including CHB patients who ceased NAs

	VR			BR			Retreatment	HBsAg loss	HBsAg seroconversion
Study	All patients (n/N)	HBeAg-positive patients(n/N)	HBeAg-negative patients(n/N)	All patients (n/N)	HBeAg-positive patients(n/N)	HBeAg-negative patients(n/N)	All patients (n/N)	All patients (n/N)	All patients (n/N)
Peng J,2015[17]	28/65	18/44	10/21	18/65	11/44	7/21	NR	2/65	NR
Buti M,2015[18]	2/8	-	2/8	2/8	-	2/8	2/8	1/8	NR
Fontaine H,2015[19]	25/36	5/5	20/31	NR	NR	NR	NR	2/36	NR
Chen L,2015[20]	8/18	8/18	-	NR	NR	-	NR	NR	NR
Hsu YC,2016[21]	115/161	30/37	95/124	68/161	30/37	38/124	NR	8/161	NR
Park CH,2016[22]	59/103	21/23	38/80	44/103	NR	NR	NR	NR	NR
Jun BG,2016[23]	31/58	31/58	-	10/58	10/58	-	17/58	NR	NR
Lin CC,2016[24]	29/80	12/33	17/47	27/80	NR	NR	NR	NR	NR
Nagata N,2016[25]	15/37	4/14	11/23	15/37	4/14	11/23	8/37	NR	NR
Lee HA,2016[26]	32/44	20/25	12/19	24/44	18/25	6/19	NR	NR	NR
Siederdissen CH,2016[27]	13/15	-	13/15	12/15	-	12/15	NR	3/15	2/15
Siederdissen CH,2016[28]	126/220	NR	NR	NR	NR	NR	NR	NR	NR
Muche M,2017[29]	1/6	-	1/6	1/6	-	1/6	NR	3/6	2/6
Karakaya F,2017[30]	12/23	-	12/23	NR	-	NR	8/23	2/23	2/23
Cao JW,2017[31]	58/82	NR	NR	28/82	19/60	9/22	NR	5/82	NR
Berg T,2017[32]	4/21	-	4/21	0/21	-	0/21	8/21	13/21	4/21
Jeng WJ,2018[33]	547/691	-	547/691	419/691	-	419/691	269/691	42/691	29/691
Dalekos GN,2018[34]	41/57	-	41/57	41/57	-	41/57	16/57	12/57	9/57
Papatheodoridis GV,2018[35]	108/130	-	108/130	55/130	-	55/130	NR	NR	NR
Rivino L,2018[36]	31/48	-	31/48	17/48	-	17/48	26/48	NR	NR
Chi H,2018[37]	76/100	NR	NR	38/100	31/71	7/29	NR	6/100	NR
Liu F,2018[38]	87/223	43/138	44/85	NR	NR	NR	62/223	23/223	17/223
Hsu YC,2018[39]	123/135	NR	NR	76/135	NR	NR	NR	8/135	NR
Kranidioti H,2019[40]	13/23	-	13/23	NR	-	NR	NR	NR	NR
Hees SV,2019[41]	62/98	62/98	-	30/98	30/98	-	31/98	14/98	NR
Xie LQ,2019[42]	74/91	NR	NR	41/91	NR	NR	NR	NR	NR

Abbreviations: BR, biological relapse; NAs, nucleos(t)ide analogues; NR, not reported; VR, virological relapse.

Table 4. Factors associated with the rates of VR at 12 months after NAs cessation

	Probability of VR,%(95% CI)	Odds ratio(95% CI)	*P*-value
All patients			
VR defined by HBV DNA			0.006
<200 IU/mL	0.80（0.72-0.87）	1	
<2000 IU/mL	0.57（0.47-0.67）	0.89（0.03-4.8）	
<20000 IU/mL	0.39（0.29-0.48）	0.25（0.12-3.25）	
Duration of on-NAs virological response			0.011
≤24 months	0.55（0.39-0.72）	1	
>24 months	0.41（0.13-0.66）	0.89（0.63-3.56）	
Treatment duration			0.537
≤48 months	0.60（0.51-0.69）	1	
>48 months	0.54（0.41-0.66）	0.38（0.06-1.20）	
HBeAg-negative patients			
VR defined by HBV DNA			<0.001
<200 IU/mL	0.83（0.77-0.88）	1	
<2000 IU/mL	0.68（0.60-0.76）	0.42(0.13-3.81)	
<20000 IU/mL	0.44（0.36-0.51）	0.29(0.02-2.93)	
Duration of on-NAs virological response			0.025
≤24 months	0.59（0.21-0.84）	1	
>24 months	0.41（0.21-0.53）	0.67（0.01-3.17）	
Treatment Duration			0.235
≤48 months	0.57（0.44-0.69）	1	
>48 months	0.50（0.39-0.62）	0.49（0.22-4.30）	
HBeAg-positive patients			
Duration of on-NAs virological response			0.031
≤24 months	0.53（0.32-0.73）	1	
>24 months	0.37（0.16-0.68）	0.56（0.14-4.26）	

Figures

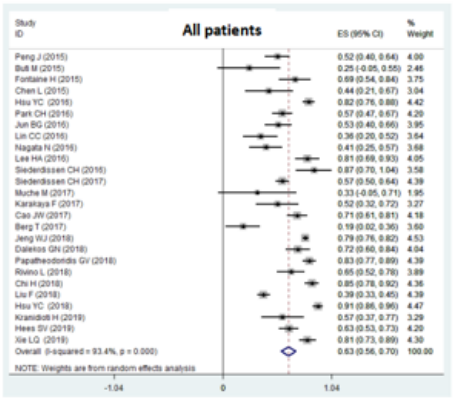

Figure 1

Forest plots of overall probabilities of virological relapse (VR) after NAs cessation. A: All patients, B: HBeAg-positive patients, C: HBeAg-negative patients. ES means effect size, which refers to estimated probability.

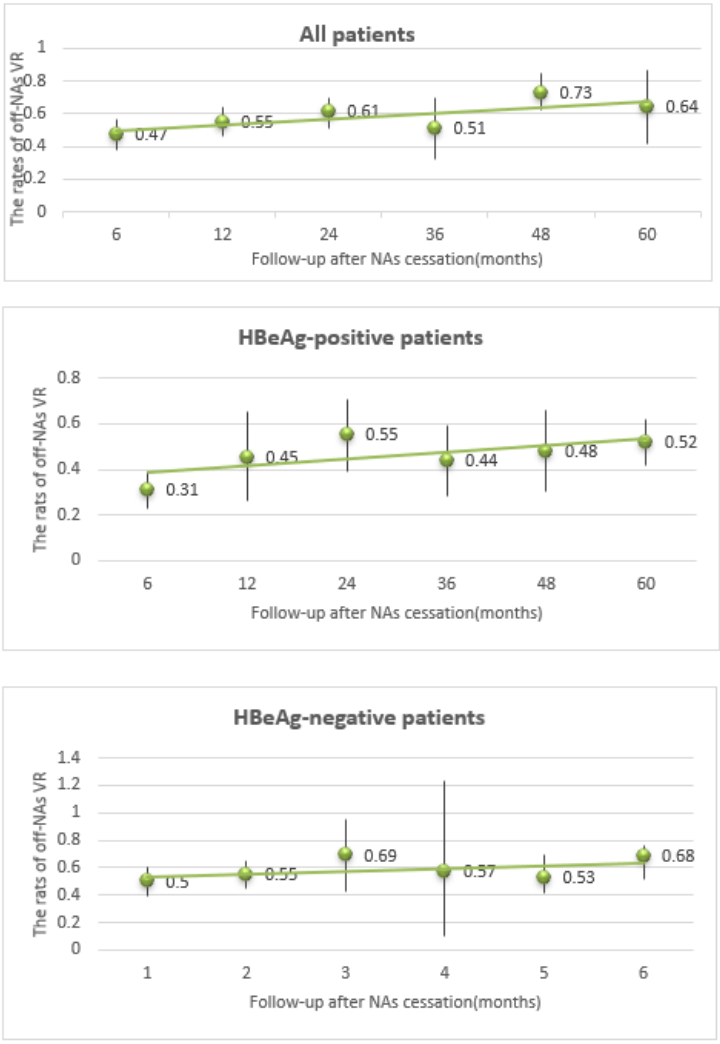

Figure 2

The rates of off-NAs virological relapse (VR) during follow-up after NAs cessation. A: All patients, B: HBeAg-positive patients, C: HBeAg-negative patients. Bars are 95% CI. Green lines are trend lines.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Revisedsupplementarymaterial.docx
- PRISMA2009checklist.doc