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

Yandi Xie

Peking University People's Hospital <https://orcid.org/0000-0001-5002-341X>

Rui Jin

Peking University People's Hospital

Guangjun Song

Peking University People's Hospital

Hui Ma

Peking University People's Hospital

Bo Feng (✉ fengbo@pkuph.edu.cn)

Peking University People's Hospital, Peking University Hepatology Institute <https://orcid.org/0000-0001-5084-6715>

Research article

Keywords: cessation, nucleos(t)ide analogues, chronic hepatitis B, meta-analysis

Posted Date: July 29th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-16051/v3>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Journal of Hepatology on August 1st, 2020. See the published version at [https://doi.org/10.1016/S0168-8278\(20\)31675-5](https://doi.org/10.1016/S0168-8278(20)31675-5).

Abstract

Background

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

Cessation of NAs therapy seems to be safe in a small subset of CHB patients. On-NAs virological response >24 months reduces the risk of off-NAs VR.

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].

So far, there have been two meta-analysis about NAs cessation. One meta-analysis [13] only included patients with hepatitis B envelope antigen (HBeAg) negative chronic hepatitis B. The other meta-analysis [14] included studies published from 2002 to December 2014. In recent years, there have been more studies with higher quality researching discontinuation of oral antivirals in CHB, and more patients with CHB taking first-line antiviral drugs than before. Therefore, we conducted literature retrieval and analysis from 2015 to now to explore the current status of NAs cessation in both of HBeAg-positive and HBeAg-negative CHB patients. The aim of this study was to assess the existing data on NAs cessation in 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 "Nucleoside 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” [15] from Kaplan-Meier curves if they were not shown in the articles directly.

Two independent reviewers (YDX and RJ) evaluated the quality of the included studies, according to the risk of bias assessment by a tool [16]. 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 [17]. 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) [18].

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) [19-44]. 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 [24,27], including 140 off-NAs patients), which was diagnosed by histology findings and/or imaging studies.

Sixteen studies included Asian patients [19,22-28,30,33,35,38-41,44], six studies involved Caucasian patients [20,21,29,32,36,42], and four studies included mixed patients population [31,34,37,43]. Lamivudine

was used in one [32], entecavir in three [23,24,28], tenofovir in two [20,34], several NAs in fifteen [19,21,22,26,30,31, 33,35-41,44], and NAs was not reported in the remaining five studies [25,27,29,42,43] (Table 1).

Only HBeAg-positive patients were involved in three studies [25,26,43], only HBeAg-negative patients in ten [20,29,31,32,34-38,42], and both HBeAg-positive and HBeAg-negative patients in thirteen [19,21,23,24,26-28,30,33,39-41,44]. 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) levels, 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.

The definition of VR after NAs cessation was based on HBV DNA levels >20 IU/mL in one [22], >120 IU/mL in one [21], >2000 IU/mL (or 10,000 copies/mL) in 19 [19,20,22-26,28-31,33-35,38-40,43,44], and >20,000 IU/mL (or 100,000 copies/mL) in one study [27]. One study provided two definitions of VR which were based on HBV DNA levels >2000 IU/mL and >20,000 IU/mL [32]. Two studies provided three definitions of VR which were based on HBV DNA levels >200 IU/mL, >2000 IU/mL and >20,000 IU/mL [36,37]. While it was not specified in one study [42]. As to the three studies [32,36,37] provided more than one definition of VR, when calculating the overall pooled rates of VR, we adopted the definition of VR based on HBV DNA level >2000 IU/mL.

BR after NAs cessation was defined as ALT above the upper limit of normal (ULN) in three [24,31,41], >1.2×ULN in one [20], and >2×ULN in fifteen studies [19,22,25-29,32-35,38,39,43,44]. One study specified two definitions of BR which were based on ALT >ULN and > 2×ULN [36]. One study specified five definitions of BR which were based on ALT >ULN, > 2×ULN, > 3×ULN, > 5×ULN and > 10×ULN [37]. While it was not specified in five studies [21,22,30,40,42]. As to the two studies [36,37] provided more than one definition of BR, when calculating the overall pooled rates of BR, we adopted the definition of BR based on ALT level >2×ULN.

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 (17

studies included), 12 (21 studies included), 24 (17 studies included), 36 (15 studies included), 48 (12 studies included) and 60 (10 studies included) 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 NAs 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 definitions.

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 studies included), 12 (19 studies included), 24 (15 studies included), 36 (11 studies included), 48 (9 studies included) and 60 (8 studies included) 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).

In all patients, the pooled probabilities of BR after NAs cessation were getting lower and lower in studies defining BR by ALT >1×ULN, >2×ULN and >5×ULN (BR [95% CI] at 12 months after NAs cessation: 0.48 [0.13-0.95] versus 0.35 [0.28-0.81]) versus 0.19 [0.02-0.58]); Odds ratio [95% CI]: 0.38 [0.19-1.4] and 0.34 [0.11-2.84], $P = 0.013$) (data not shown). There were too few studies of initially HBeAg-positive and HBeAg-negative patients to calculate the pooled probabilities of BR according to the different BR definitions.

Retreatment after NAs cessation

In ten studies supplying such data [19,24,26,31,33-35,37,39,42], 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 [20,21,43], 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).

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 of 14 studies provided data about predictors of VR after NAs cessation [19,22-25,28,32,33,36,37,39-41,43], they often reported conflicting results. Off-NAs VR was found to be associated with higher baseline ALT and platelet in one [24], higher baseline gamma-glutamyltransferase (GGT) in one [32], high baseline HBV DNA ($> 10^6$ copies/ml) in one [24], baseline advanced fibrosis in one [36], and higher end of treatment (EOT) ALT in one study [23], but not in any other study included in this review. Male gender was associated with off-NAs VR only in one study [24]. In addition, older age was associated with off-NAs VR in seven studies (all with both HBeAg-positive and HBeAg-negative patients) [19,23,33,39-41,43], and two of these studies found that higher rates of off-NAs VR in patients > 35 years old than patients ≤ 35 years old [33,44]. They did not found off-NAs VR was association with alcohol consumption in one [36], diabetes and hypertension in one [28], and body mass index (BMI) in two studies [29,36].

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) [33,36,37,39,40,44]. Whereas, the rate of VR was higher in 22 tenofovir-treated than 113 entecavir-treated HBeAg-negative CHB patients in one study [41]. 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 [37]. No relationship was found between the risk of off-NAs VR and EOT liver stiffness in three studies [19,32,33].

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) [19,23,28,39,41,44], but not in one study with 18 HBeAg-positive patients [22], two studies with 80 HBeAg-negative patients [32,36], and in one study with both HBeAg-positive and HBeAg-negative patients (60 HBeAg-positive, 22 HBeAg-negative) [33]. Total serum level of EOT antibody against the HBV core protein (anti-HBc) [39] and EOT hepatitis B core-related antigen (HBcrAg) [41] 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 [23,25,28,33,39,41,44]. 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 [40], but not in another study with 58 patients [25]. Moreover, the rates of off-NAs VR were affected by consolidation duration in two studies including 196 patients [25,40], but not in another three studies including 152 patients [19,23,39].

Clinical outcomes after NAs cessation

Data of clinical outcomes after NAs cessation was provided in 11 studies including 1365 CHB patients [21,26,27,29,32-36,39,40].

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.

Sensitivity and publication bias

Sensitivity analysis was performed by sequential omission of individual studies. For analyses that included more than three pooled individual studies, the significance of the VR was not significantly altered by omitting any single study (data not shown).

Among 26 studies that estimated the rates of VR, no evidence of publication bias was found in the main analysis ($p > 0.05$) (Supporting Fig. S4).

Study quality

There were only one randomized study [34] and the other 25 cohort studies including five retrospective [24-26,31,43], three retrospective-prospective [19,32,35] and 17 prospective [20-23,27-30,33,36-42,44]. According to the risk of bias assessments, four studies had high quality [19,22,23,32], nine had acceptable quality [25,28,29,32,34,36,39,40,44], and 13 had low quality [20,21,24,26,27,30,32,35,37,38, 41-43] (Supporting Table S1).

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 retrospective), 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. In the previous meta-analysis of Papatheodoridis [14] overall rate of VR was 54%. That means, more than a half of CHB patients may be virological relapse after NAs cessation and only a small subset of CHB patients can discontinue NAs safely.

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 depending 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 hepatocytes or noncytolytic degradation of cccDNA [45]. 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 [46], 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 [27,47-51]. 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 [52]. In the previous meta-analysis of Papatheodoridis [14] HBsAg loss was reported to occur in a

minority of cases (2% by random effects model), however, several studies have shown higher incidences of HBsAg loss, varying from 7% to 39%, after NAs cessation [34,36,53,54], and it is even discussed as interventional strategy to have an impact on long-term HBsAg decline and also HBsAg clearance [29,54].

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 [11,55,56]. 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 [57]. 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 [58,59]. 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, cessation of NAs therapy seems to be safe in a small subset 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.

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

Funding

This study was funded by the National Major Science and Technology Project of China (the 13th Five-Year Plan) (No.2017ZX10302201-004-001, No. 2017ZX10203202-003) and Beijing Municipal Science and Technology Commission of Major Projects (No. D161100002716002). Funding bodies only provided financial support, but did not have a role in study design, or collection, analysis and interpretation of data, or manuscript writing.

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.

Acknowledgements

Not applicable

Author details

¹Peking University People's Hospital, Peking University Hepatology Institute, Beijing Key Laboratory of Hepatitis C and Immunotherapy for Liver Diseases, Beijing International Cooperation Base for Science and Technology on NAFLD Diagnosis, Beijing 100044, China.

Supplementary Material

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

References

1. Cooke GS, Andrieux-Meyer I, Applegate TL, et al. Accelerating the elimination of viral hepatitis: a Lancet Gastroenterology & Hepatology Commission. *Lancet Gastroenterol Hepatol* 2019; 4(2): 135-84.
2. Indolfi G, Easterbrook P, Dusheiko G, et al. Hepatitis B virus infection in children and adolescents. *Lancet Gastroenterol Hepatol* 2019; 4(6): 466-76.
3. Seeger C, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; 64(1): 51-68.
4. Honer ZSC, Rinker F, Maasoumy B, et al. Viral and host responses after stopping long-term nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B. *J Infect Dis* 2016; 214(10): 1492-7.
5. Seeger C, Mason WS. Molecular biology of hepatitis B virus infection. *Virology* 2015; 479: 672-86.
6. Gill US, Zissimopoulos A, Al-Shamma S, et al. Assessment of bone mineral density in tenofovir-treated patients with chronic hepatitis B: can the fracture risk assessment tool identify those at greatest risk? *J Infect Dis* 2015; 211(3): 374-82.
7. Reijnders JG, Janssen HL. Relapse of chronic hepatitis B after discontinuation of nucleos(t)ide analogs: is the glass half full or half empty? *Hepatology* 2013; 58(6): 1885-7.
8. Chang ML, Liaw YF, Hadziyannis SJ. Systematic review: cessation of long-term nucleos(t)ide analogue therapy in patients with hepatitis B e antigen-negative chronic hepatitis B. *Aliment Pharmacol Ther* 2015; 42(3): 243-57.
9. Chaung KT, Ha NB, Trinh HN, et al. High frequency of recurrent viremia after hepatitis B e antigen seroconversion and consolidation therapy. *J Clin Gastroenterol* 2012; 46(10): 865-70.
10. Seto WK, Hui AJ, Wong VW, et al. Treatment cessation of entecavir in Asian patients with hepatitis B e antigen negative chronic hepatitis B: a multicentre prospective study. *Gut* 2015; 64(4): 667-72.
11. Chi H, Hansen BE, Yim C, et al. Reduced risk of relapse after long-term nucleos(t)ide analogue consolidation therapy for chronic hepatitis B. *Aliment Pharmacol Ther* 2015; 41(9): 867-76.
12. Hadziyannis SJ, Sevastianos V, Rapti I, et al. Sustained responses and loss of HBsAg in HBeAg-negative patients with chronic hepatitis B who stop long-term treatment with adefovir. *Gastroenterology*

- 2012; 143(3): 629-36.
13. Chang ML, Liao YF, Hadziyannis SJ. Systematic review: cessation of long-term nucleos(t)ide analogue therapy in patients with hepatitis B e antigen negative chronic hepatitis B. *Aliment Pharmacol Ther* 2015; 42: 243-257.
 14. Papatheodoridis G, Vlachogiannakos I, Cholongitas E, et al. Discontinuation of oral antivirals in chronic hepatitis B: A systematic review. *Hepatology* 2016; 63: 1481-92.
 15. Mitchell M. Available at: <http://digitizer.sourceforge.net/>. Accessed June 20, 2019.
 16. Sterne JAC, Hernán MA, Reeves BC, et al. [ROBINS-I: a tool for assessing risk of bias in non-randomized studies of interventions](#). *BMJ* 2016; 355; i4919.
 17. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; 21(11): 1539-58.
 18. Sterne JA, Gavaghan D, Egger M. Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *J Clin Epidemiol* 2000; 53(11): 1119-29.
 19. Peng J, Cao J, Yu T, et al. Predictors of sustained virologic response after discontinuation of nucleos(t)ide analog treatment for chronic hepatitis B. *Saudi J Gastroentero* 2015; 21(4): 245.
 20. Buti M, Casillas R, Riveiro-Barciela M, et al. Tenofovir discontinuation after long-term viral suppression in HBeAg negative chronic hepatitis B. Can HBsAg levels be useful? *J Clin Virol* 2015; 68: 61-8.
 21. Fontaine H, Kahi S, Chazallon C, et al. Anti-HBV DNA vaccination does not prevent relapse after discontinuation of analogues in the treatment of chronic hepatitis B: a randomised trial-ANRS HB02 VAC-ADN. *Gut* 2015; 64(1): 139-47.
 22. Chen L, Gan QR, Zhang DQ, et al. Increased intrahepatic quasispecies heterogeneity correlates with off-treatment sustained response to nucleos(t)ide analogues in e antigen-positive chronic hepatitis B patients. *Clin Microbiol Infec* 2016; 22(2): 201-7.
 23. Hsu YC, Mo LR, Chang CY, et al. Association between serum level of hepatitis B surface antigen at end of entecavir therapy and risk of relapse in E antigen-negative patients. *Clin Gastroenterol Hepatol* 2016; 14(10): 1490-8.
 24. Park C, Kim HY, Lee SW, et al. On-treatment and off-treatment efficacy of entecavir in a real-life cohort of chronic hepatitis B patients. *Eur J Gastroen Hepat* 2016; 28(10): 1179-87.
 25. Jun BG, Lee SH, Kim HS, et al. Predictive factors for sustained remission after discontinuation of antiviral therapy in patients with HBeAg-positive chronic hepatitis B. *Korean J Gastroenterol* 2016; 67(1): 28-34.
 26. Lin C, Bair M, Chen C, et al. Off-treatment efficacy of 3-year nucleos(t)ide analogues in chronic hepatitis B patients. *The Kaohsiung Journal of Medical Sciences* 2016; 32(1): 10-5.
 27. Nagata N, Kagawa T, Hirose S, et al. Off-treatment durability of antiviral response to nucleoside analogues in patients with chronic hepatitis B. *BMC Gastroenterol* 2016; 16(1).
 28. Lee HA, Seo YS, Park SW, et al. Hepatitis B surface antigen titer is a good indicator of durable viral response after entecavir off-treatment for chronic hepatitis B. *Clinical and Molecular Hepatology* 2016; 22(3): 382-9.

29. Höner Zu Siederdisen C, Rinker F, Maasoumy B, et al. Viral and host responses after stopping long-term nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B. *J Infect Dis* 2016; 214(10): 1492-7.
30. Höner Zu Siederdisen C, Hui AJ, Sukeepaisarnjaroen W, et al. Contrasting timing of virological relapse after discontinuation of tenofovir or entecavir in hepatitis B e antigen-negative patients. *J Infect Dis* 2018; 218(9): 1480-4.
31. Muche M, Meyer U, Siegmund B, et al. Sustained off-treatment response after discontinuation of long-term nucleos(t)ide analogue treatment in HBeAg-seronegative hepatitis B: a case series. *Top Antivi Med* 2017; 25(3): 114-7.
32. Karakaya F, Ozer S, Kalkan C, et al. Discontinuation of lamivudine treatment in HBeAg-negative chronic hepatitis B: a pilot study with long-term follow-up. *Antivir Ther* 2017; 22(7): 559-70.
33. Cao J, Chi H, Yu T, et al. Off-Treatment hepatitis B virus (HBV) DNA levels and the prediction of relapse after discontinuation of nucleos(t)ide analogue therapy in patients with chronic hepatitis B: a prospective stop study. *J Infect Dis* 2017; 215(4): 581-9.
34. Berg T, Simon K, Mauss S, et al. Long-term response after stopping tenofovir disoproxil fumarate in non-cirrhotic HBeAg-negative patients-FINITE study. *J Hepatol* 2017; 67(5): 918-24.
35. Jeng WJ, Chen YC, Chien RN, et al. Incidence and predictors of hepatitis B surface antigen seroclearance after cessation of nucleos(t)ide analogue therapy in hepatitis B e antigen-negative chronic hepatitis B. *Hepatology* 2018; 68(2): 425-34.
36. Papatheodoridis GV, Rigopoulou EI, Papatheodoridi M, et al. DARING-B: discontinuation of effective entecavir or tenofovir disoproxil fumarate long-term therapy before HBsAg loss in non-cirrhotic HBeAg-negative chronic hepatitis B. *Antivir Ther* 2018; 23(8): 677-85.
37. Papatheodoridis GV, Manolakopoulos S, Su T, et al. Significance of definitions of relapse after discontinuation of oral antivirals in HBeAg-negative chronic hepatitis B. *Hepatology* 2018; 68(2): 415-24.
38. Rivino L, Le Bert N, Gill US, et al. Hepatitis B virus-specific T cells associate with viral control upon nucleos(t)ide-analogue therapy discontinuation. *J Clin Invest* 2018; 128(2): 668-81.
39. Chi H, Li Z, Hansen BE, et al. Serum level of antibodies against hepatitis B core protein is associated with clinical relapse after discontinuation of nucleos(t)ide analogue therapy. *Clin Gastroenterol Hepatol* 2019; 17(1): 182-91.
40. Liu F, Liu ZR, Li T, et al. Varying 10-year off-treatment responses to nucleos(t)ide analogues in patients with chronic hepatitis B according to their pretreatment hepatitis B e antigen status. *J Digest Dis* 2018; 19(9): 561-71.
41. Hsu Y, Nguyen MH, Mo L, et al. Combining hepatitis B core-related and surface antigens at end of nucleos(t)ide analogue treatment to predict off-therapy relapse risk. *Aliment Pharm Ther* 2019; 49(1): 107-15.
42. Kranidioti H, Manolakopoulos S, Kontos G, et al. Immunological biomarkers as indicators for outcome after discontinuation of nucleos(t)ide analogue therapy in patients with HBeAg-negative chronic hepatitis B. *J Viral Hepatitis* 2019; 26(6): 697-709.

43. Van Hees S, Chi H, Hansen B, et al. Sustained off-treatment viral control is associated with high hepatitis B surface antigen seroclearance rates in Caucasian patients with nucleos(t)ide analogue-induced HBeAg seroconversion. *J Viral Hepatitis* 2019; 26(6): 766-9.
44. Xie L, Liao G, Chen H, et al. Elevated expression of serum soluble ST2 in clinical relapse after stopping long-term Nucleos(t)ide analogue therapy for chronic hepatitis B. *BMC Infect Dis* 2019; 19(1).
45. Xia Y, Stadler D, Lucifora J, et al. Interferon-gamma and tumor necrosis factor-alpha produced by T cells reduce the HBV persistence form, cccDNA, without cytolysis. *Gastroenterology* 2016; 150(1): 194-205.
46. Chang ML, Liaw YF. Hepatitis B flares in chronic hepatitis B: pathogenesis, natural course, and management. *J Hepatol* 2014; 61(6): 1407-17.
47. Chang TT, Lai CL, Kew YS, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2010; 51(2): 422-30.
48. Gish RG, Chang TT, Lai CL, et al. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naive HBeAg-positive patients with chronic hepatitis B. *J Viral Hepatol* 2010; 17(1): 16-22.
49. Zoutendijk R, Reijnders JG, Brown A, et al. Entecavir treatment for chronic hepatitis B: adaptation is not needed for the majority of naive patients with a partial virological response. *Hepatology* 2011; 54(2): 443-51.
50. Hosaka T, Suzuki F, Kobayashi M, et al. Clearance of hepatitis B surface antigen during long-term nucleot(s)ide analog treatment in chronic hepatitis B: results from a nine-year longitudinal study. *J Gastroenterol* 2013; 48(8): 930-41.
51. Kobayashi M, Hosaka T, Suzuki F, et al. Seroclearance rate of hepatitis B surface antigen in 2,112 patients with chronic hepatitis in Japan during long-term follow-up. *J Gastroenterol* 2014; 49(3): 538-46.
52. Seto WK, Cheung KS, Wong DK, et al. Hepatitis B surface antigen seroclearance during nucleoside analogue therapy: surface antigen kinetics, outcomes, and durability. *J Gastroenterol* 2016; 51(5): 487-95.
53. Buti M, Wong DK, Gane E, et al. Safety and efficacy of stopping tenofovir disoproxil fumarate in patients with chronic hepatitis B following at least 8 years of therapy: a prespecified follow-up analysis of two randomised trials. *Lancet Gastroenterol Hepatol* 2019; 4(4): 296-304.
54. Hadziyannis SJ, Sevastianos V, Rapti I, et al. Sustained responses and loss of HBsAg in HBeAg-negative patients with chronic hepatitis B who stop long-term treatment with adefovir. *Gastroenterology* 2012; 143(3): 629-36.
55. Sohn HR, Min BY, Song JC, et al. Off-treatment virologic relapse and outcomes of re-treatment in chronic hepatitis B patients who achieved complete viral suppression with oral nucleos(t)ide analogs. *BMC Infect Dis* 2014; 14: 439.
56. Song MJ, Song DS, Kim HY, et al. Durability of viral response after off-treatment in HBeAg positive chronic hepatitis B. *World J Gastroenterol* 2012; 18(34): 6277-83.
57. Raffetti E, Fattovich G, Donato F. Incidence of hepatocellular carcinoma in untreated subjects with chronic hepatitis B: a systematic review and meta-analysis. *Liver Int* 2016; 36(9): 1239-51.

58. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med* 1998; 17: 2815-34.
59. Williamson PR, Smith CT, Hutton JL, et al. Aggregate data meta-analysis with time-to-event outcomes. *Stat Med* 2002; 21: 3337-51.

Tables

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

Study	All patients(n)	Patients off-NAs(n)			End of NAs treatment					Type of NAs	Before NAs treatment	
		Total	HBeAg positive	HBeAg negative	Age(years)	Males (n)	Race	Cirrhosis	HBsAg (log ₁₀ IU/mL)		ALT(U/L)	HBV DNA(log ₁₀ IU/mL)
Peng J,2015 ¹⁹	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 ²⁰	8	8	0	8	63	7	Caucasian	1	3	TDF	130.5	6.1
Fontaine H,2015 ²¹	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 ²²	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 ²³	161	161	37	124	48.1	122	Asian	0	2.9	ETV	164	6.6
Park CH,2016 ²⁴	223	103	23	80	NR	0	Asian	NR	NR	ETV	NR	NR
Jun BG,2016 ²⁵	58	58	58	0	41.1 [†] /39.5 [‡]	40	Asian	0	NR	NR	242 [†] /150 [‡]	6.0 [†] /6.6 [‡]
Lin CC,2016 ²⁶	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 ²⁷	94	37	14	23	NR	0	Asian	NR	NR	NR	NR	NR
Lee HA,2016 ²⁸	44	44	25	19	44.6 ^{\$} /44.7 [¶]	28	Asian	17	3	ETV	202 ^{\$} /338 [¶]	7.2 ^{\$} /6.2 [¶]
Siederdisen CH,2016 ²⁹	15	15	0	15	49.1	12	Caucasian	0	3.1	NR	149.7	NR
Siederdisen CH,2016 ³⁰	224	220	31	189	52	155	Asian	0	2.9	ETV 154,TDF 66	NR	NR
Muche M,2017 ³¹	6	6	0	6	55.5	4	Mixed	0	2.8	LAM 5,ETV 1	NR	NR
Karakaya F,2017 ³²	39	23	0	23	51	16	Caucasian	0	3.5	LAM	73	6.3
Cao JW,2017 ³³	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 ³⁴	42	21	0	21	44.5	18	Mixed	0	4.3	TDF	NR	2.1
Jeng WJ,2018 ³⁵	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 ³⁶	60	57	0	57	60	37	Caucasian	0	2.8	ETV 13,TDF 11	90	5.6
Papatheodoridis GV,2018 ³⁷	130	130	0	130	57	90	Mixed	0	NR	ETV 54,TDF 63,others 13	105	NR
Rivino L,2018 ³⁸	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 ³⁹	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 ⁴⁰	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 ⁴¹	135	135	31	104	49.5	109	Asian	0	2.8	ETV 113, TDF 22	134	5.7
Kranidioti H,2019 ⁴²	70	23	0	23	57	0	Caucasian	0	3.4	NR	23.2	NR
Hees SV,2019 ⁴³	98	98	98	0	NR	73	Mixed	0	NR	NR	NR	NR
Xie LQ,2019 ⁴⁴	91	91	61	30	35.7	75	Asian	0	2.9	first-line 39	212	6.2

Note: †/‡, nonrelapsers/relapsers; \$/¶, 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 cessation (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 ¹⁹	56.1	33.3	NR	36.7	NR	12	<20 IU/mL	HBV DNA>2000 IU/mL	ALT>2ULN
Buti M,2015 ²⁰	72	NR	-	NR	-	18	<20 IU/mL	HBV DNA>2000 IU/mL	ALT>1.2ULN
Fontaine H,2015 ²¹	48	NR	NR	NR	NR	12	<12 IU/mL	HBV DNA>120 IU/mL	NR
Chen L,2015 ²²	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 ²³	36.6	NR	20.6	NR	NR	17	NR	HBV DNA>2000 IU/mL	ALT>2ULN
Park CH,2016 ²⁴	NR	18.3	NR	NR	NR	24	NR	HBV DNA>10000 copies/ml	ALT>ULN
Jun BG,2016 ²⁵	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 ²⁶	36	NR	NR	NR	NR	12	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Nagata N,2016 ²⁷	40	NR	NR	NR	NR	60	NR	HBV DNA>100000 copies/mL	ALT>2ULN
Lee HA,2016 ²⁸	37.0 [§] /31.6 [¶]	31.0 [§] /27.1 [¶]	23.4	5.5	57.9	20.8	NR	HBV DNA >2000 IU/mL	ALT>2ULN
Siederdisen CH,2016 ²⁹	NR	NR	-	NR	-	12	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Siederdisen CH,2016 ³⁰	NR	NR	NR	NR	NR	6	<20 IU/mL	HBV DNA >2000 IU/mL	NR
Muche M,2017 ³¹	138	84	-	NR	-	40	NR	HBV DNA >2000 IU/mL	ALT>ULN
Karakaya F,2017 ³²	63	36	-	NR	-	62	NR	HBV DNA >2000IU/mL*	ALT>2ULN
Cao JW,2017 ³³	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 ³⁴	NR	NR	-	NR	-	36	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Jeng WJ,2018 ³⁵	39	27.8	-	27	-	38.8	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>2ULN
Dalekos GN,2018 ³⁶	≥48	63.6	-	NR	-	18	NR	HBV DNA>200 IU/mL*	ALT>ULN [§]
Papatheodoridis GV,2018 ³⁷	60	43	-	NR	-	15	<45 IU/mL	HBV DNA >200 IU/mL [#]	ALT>ULN [%]
Rivino L,2018 ³⁸	18	NR	-	NR	-	12	NR	HBV DNA>2000 IU/mL	ALT>2ULN
Chi H,2018 ³⁹	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 ⁴⁰	27.5 [§] /32.0 [¶]	18 [§] /30 [¶]	NR	3 [§] /2 [¶]	8	60	<1000 copies/mL	HBV DNA >10000 copies/mL	NR
Hsu YC,2018 ⁴¹	36.7	25.2	NR	NR	NR	25.9	<20 IU/mL	HBV DNA >2000 IU/mL	ALT>ULN
Kranidioti H,2019 ⁴²	96	≥48	-	NR	-	54	NR	NR	NR
Hees SV,2019 ⁴³	NR	11.4	NR	NR	NR	42.8	varied	HBV DNA	ALT>2ULN

							between 12 and 2000 IU/mL [@]	>2000 IU/mL	
Xie LQ,2019 ⁴⁴	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, retreatment and HBsAg loss/seroconversion in studies including CHB patients who ceased NAs

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

- [Revised supplementary material.docx](#)
- [PRISMA2009 checklist.doc](#)