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ORIGINAL ARTICLE

Pegylated-interferon consolidation treatment versus nucleos(t)ide analogue consolidation treatment in non-cirrhotic hepatitis B patients with hepatitis B e antigen seroconversion: an open-label pilot trial

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Abstract

Background The safety of nucleos(t)ide analogue (NA) treatment cessation remains one of the most controversial topics in the management of chronic hepatitis B (CHB) patients. This study investigated the efficiency of 48-week pegylated-interferon (peg-IFN) alfa-2a consolidation therapy on viral relapse after discontinued NA treatment in CHB patients who achieved hepatitis B e antigen (HBeAg) seroconversion for > 1 year.

Methods NA-treated HBeAg-positive patients who achieved the standard of discontinued NA treatment (i.e. time of HBeAg seroconversion > 1 year) were randomly assigned to receive peg-IFN consolidation ($n = 24$) treatment or continue original NA therapy ($n = 24$) for 48 weeks. The treatments were then discontinued, and the patients were observed up to 144 weeks. The primary endpoint was the proportion of patients with viral relapse at week 144 among those who received at least one dose of study drug or had at least one study visit [modified intention-to-treat population (mITT)].

Results Of the 24 patients who received peg-IFN treatment, 6 (25%) experienced viral relapse and 8 (36.3%) showed HBsAg loss during 96 weeks of treatment-free follow-up. Of the patients who underwent NA consolidation treatment, only 1 (4.3%) of 23 patients showed HBsAg loss and 14 (58.3%) of 24 patients experienced viral relapse during follow-up. HBsAg level decline < 0.25 $\log_{10}$ IU/mL at week 96 was significantly associated with viral relapse.

Conclusion A 48-week peg-IFN alfa-2a consolidation therapy increased the rate of HBsAg loss and sustained viral replication suppression in HBeAg-positive patients who achieved HBeAg seroconversion for > 1 year after NA treatment discontinuation.

Keywords Hepatitis B virus · Pegylated-interferon alfa-2a · Hepatitis B surface antigen · Antivirus

Introduction

The aim of antiviral treatment for chronic hepatitis B virus (HBV) infection is to provide sustained suppression of viral replication, thus lessening the risk of fibrosis progression and hepatocellular carcinoma [1]. The ideal treatment endpoint of patients with chronic hepatitis B (CHB) is functional cure, defined as hepatitis B surface antigen (HBsAg) clearance with or without the formation of antibodies against HBsAg (anti-HBs), which is associated with improved outcomes [2, 3]. Unfortunately, only a limited number of patients achieve this ideal endpoint [4]. Therefore, for patients with hepatitis B e antigen (HBeAg) positive, HBeAg seroconversion may be an accepted therapy endpoint, which is indicative of partial recovery of host-specific immune control of HBV infection [5]. According to the European Association for the Study of the Liver (EASL) and American Association for the Study of Liver Diseases (AASLD) guidelines, HBeAg-positive CHB patients who show HBeAg serum conversion for > 1 year during nucleos(t)ide analogue (NA) treatment can discontinue NA treatment after 1 year of consolidation treatment [6, 7]. However, recent studies reported that 50–70% of patients had viral relapse after antiviral treatment discontinuation [8, 9]. Therefore, life-long therapy is

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usually required for these patients. As there is no realistic option to eradicate HBV in a chronic carrier within the next few years, limitation of long-term duration of NA therapy is one of the current major aims [10]. The concept of life-long therapy is not a viable option for many patients because of the economic burden and safety concerns of drug side effects, especially in women of childbearing age [11].

Therefore, studies assessing a treatment strategy are focused on increasing the rates of HBsAg loss and decreasing viral relapse after antiviral treatment discontinuation. Previous attempts to improve response rates by combining pegylated-interferon (peg-IFN) and NA have yielded varying results [12–14]. The most promising data published to date are from Ning et al. [13], who showed that when HBeAg-positive patients who pre-received entecavir treatment were switched to 1-year peg-IFN treatment, 14.9% experienced HBsAg loss. The new switch study had a similar result [15]. These studies give a reasonable indication that switching to peg-IFN consolidation treatment might decrease the rate of viral relapse for HBeAg-positive CHB patients with HBeAg seroconversion.

Randomised controlled studies have not yet been conducted on the efficacy of peg-IFN consolidation treatment in CHB patients after discontinuation of NA treatment. Therefore, we aimed to compare peg-IFN consolidation therapy versus NA consolidation treatment in HBeAg seroconversion CHB patients and to investigate the rate of viral relapse and HBsAg loss and markers of viral relapse after discontinued NA treatment.

Patients and methods

Study design

In this single-centre pilot study, patients were randomly assigned 1:1 to the continued 48-week original NA treatment (group A) or switched to 48-week peg-IFN treatment (group B). Patients in group B received peg-IFN alfa-2a (Pegasys; F Hoffmann-La Roche, Basel, Switzerland) 180 µg/week subcutaneously. After 48 weeks, treatment was discontinued and all patients were followed up at 144 weeks (Fig. 1). For all patients, clinical visits occurred every 2 weeks in the first 3 months, then every 4 weeks until week 144. Patients with at least one of the following four criteria restarted original NA therapy after stopping treatment: confirmed (i.e. two consecutive laboratory results) increase of direct bilirubin by > 1.5 mg/dL from baseline and alanine aminotransferase (ALT) level greater than upper limit of normal (ALT > ULN); confirmed (i.e. two consecutive laboratory results) increase in prothrombin time (PT) by ≥ 2.0 s prolonged from baseline, with adequate vitamin K therapy and ALT > ULN; confirmed (i.e. two consecutive laboratory results) ALT of > 5× ULN, with or without associated symptoms; and ALT > 2× ULN and viral relapse (HBV DNA > 2000 IU/mL) persisting for ≥ 60 days (8 weeks).

If ALT or HBV DNA remained elevated but the patients did not fulfil one of the above criteria, the investigator decided whether to restart the original NA therapy, continue with the scheduled visit or discontinue the patient from the study.

Patients

All patients who fulfilled the following inclusion criteria were enrolled: age ≥ 18 years, HBsAg positivity, HBeAg positivity before NA therapy, time of HBeAg seroconversion during NA treatment > 1 year and HBV DNA < 20 IU/mL. Patients with cirrhosis, history of decompensated liver disease (defined as direct bilirubin > 1.5× ULN, PT > 1.5× ULN, platelet count < 50,000/mm^3 and serum albumin < 3.0 g/dL), concomitant treatment with immunosuppressive agents and history of clinical hepatic decompensation, hepatocellular carcinoma or hepatitis C or D virus co-infection were excluded.

Objectives

The primary objective was to evaluate the proportion of patients with HBV relapse at week 144 in HBeAg-positive CHB patients who had seroconversion during NA therapy and switched to 1-year peg-IFN consolidation treatment compared with patients showing HBeAg seroconversion who underwent 1-year NA consolidation therapy. The secondary objectives were to explore possible rate of HBsAg loss following stop therapy and to evaluate patient's safety through laboratory tests and adverse events reporting.

Procedures

Randomization was performed using a coin flip method. Routine examinations and laboratory tests were conducted with 2-week intervals for the first 3 months and 4-week intervals thereafter. Viral load was assessed before selecting or approaching eligible patients, if available from the medical chart. Furthermore, HBsAg and viral load were assessed at the time of screening and at day 1 of consolidation treatment, which was used as baseline HBsAg and HBV DNA level. Plasma HBV DNA level was determined using COBAS TaqMan assay (F Hoffmann-La Roche, Basel, Switzerland). Serum HBsAg level was quantified using HBsAg Architect assay (Abbott Diagnostics, Abbott Park, IL, USA). Qualitative detection of serum HBsAg, antibody to HBsAg (anti-HBs), HBeAg and antibody to HBeAg (anti-HBe) was done using an enzyme

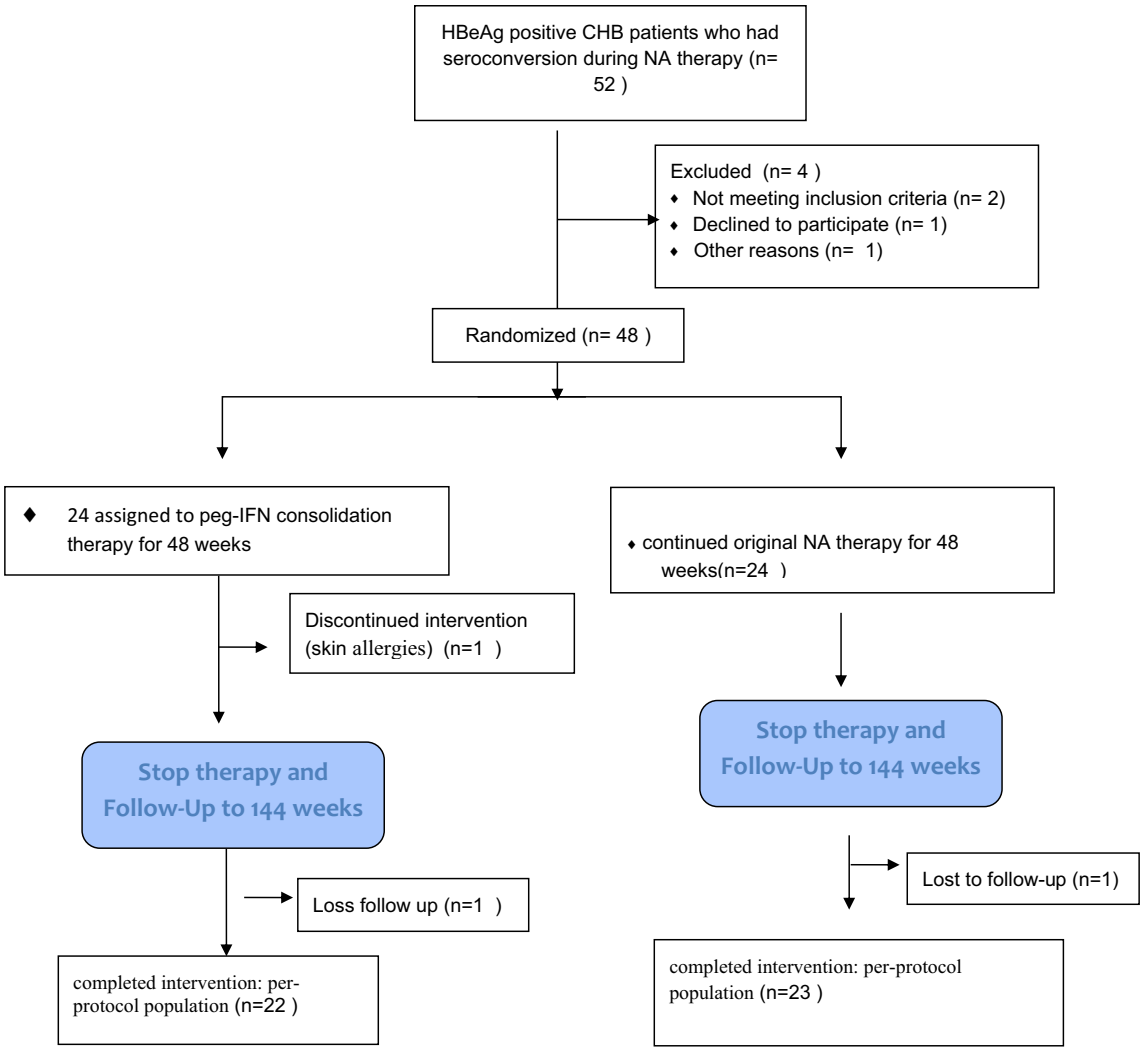


Fig. 1 Patient flow diagram up to 144 weeks

immunoassay (AxSYM; Abbott Laboratories). ALT levels were expressed as absolute values (U/L).

Study endpoints

The primary efficacy endpoint was the proportion of patients with HBV relapse at week 144 in both study groups (defined as two consecutive HBV DNA > 2000 IU/mL or HBV DNA < 2000 IU/mL or > 20 IU/mL with ALT > ULN). The secondary endpoints were the proportion of patients with HBsAg loss at week 144 and HBsAg seroconversion (defined as anti-HBs > 10 IU/L) in both groups at weeks 96 and 144. HBsAg loss was defined as undetectable serum HBsAg by AxSYM (< 0.05 IU/mL).

Statistical analysis

Sample size calculation was based on the primary endpoint (viral relapse at week 144). Assumed viral relapse rates were 10% for patients who switched to peg-IFN treatment versus 50% for patients who continued with the NA treatment [16, 17]. A group sample of 22 patients in the control group was determined to have power to detect a difference with either of the treatment arms at the α level of 0.05 (two-sided Fisher's exact test), with a power of 81%. Assuming a 10% dropout rate, 24 patients or more were needed in each group. Overall, 48 patients were enrolled in this study. Patients who entered 96 weeks of untreated follow-up were included in the modified intention-to-treat (mITT) population. In this analysis, an mITT model was applied, including all patients

who received at least one dose of study drugs (treatment groups). Patients who prematurely discontinued treatment were scored as having a viral relapse. Patients with missing data were also considered to have viral relapse. Logarithmic transformation was performed in case of skewed data. Data are presented as mean ± standard deviation.

In the primary analysis, differences in the proportion of patients with viral relapse in each group were compared with Pearson's χ^2 test. In the secondary analysis of HBsAg loss or decrease, only patients who completed 48 weeks of treatment and 96 weeks of treatment-free follow-up were included (per-protocol model). Baseline and on-treatment variables were compared between the study groups using Student's *t* test, Mann-Whitney *U* test, Kruskal–Wallis *H* test, Pearson's χ^2 test or Fisher's exact test. The difference in viral relapse rate between groups A and B was examined using log-rank test. Associations between variables as potential predictors of viral relapse were examined using Cox regression analysis. Data were analysed with SPSS Statistics version 23 (IBM, Armonk, NY, USA). All *p* values are two sided, and $p < 0.05$ was considered statistically significant.

Results

A total of 52 patients with CHB were screened for participation in the study from the Liver Centre of Zhe Jiang Provincial People's Hospital from December 1, 2012, to August 1, 2014. 48 patients were randomly assigned to one of the two groups (two did not meet inclusion criteria and two withdrew before randomization). 24 patients each were randomized to group A (continued NA therapy) and group B (peg-IFN treatment). Of the 48 patients, three prematurely withdrew from the study: two in the peg-IFN and one in the NA treatment group (Fig. 1). One patient in the peg-IFN group discontinued because of IFN-related side effects (severe skin allergies) at 12 weeks and another patient was lost to follow-up after 12 weeks. One patient in group A was lost to follow-up after 6 weeks. Thus, the per-protocol population consisted of 22 patients in group B and 23 patients in group A.

The patients characteristics are shown in Table 1. The majority of the patients were male (34/48, 70.8%). The predominant NA drug was entecavir (27/48, 56.2%). Of the patients who received peg-IFN treatment, 18 (81.8%) remained off-therapy up to week 144. Six patients experienced viral relapse. Of these patients, five were previously treated with entecavir and one was treated with lamivudine plus adefovir. The total duration of NA therapy was 3.81 ± 1.83 years, and the total duration of HBeAg seroconversion was 2.48 ± 0.97 years. In contrast, of the patients who received NA consolidation treatment, 14 experienced viral relapse; of those, 8 patients were previously treated with entecavir, 4 patients were treated with lamivudine, and 2 patients were treated with lamivudine plus adefovir. The total duration of NA therapy was 4.65 ± 2.78 years, and the total duration of HBeAg seroconversion was 3.51 ± 2.43 years.

Table 1 Baseline demographics and clinical characteristics of 48 chronic hepatitis B patients with HBeAg seroconversation

Characteristic	Group A NA therapy (*n* = 24)	Group B peg-IFN (*n* = 24)	*p* value
Age at initial treatment (years)	37.8 ± 8.1	34.5 ± 7.2	0.17
Sex			
Male	18	16	0.57
Female	6	8	
Antivirus drugs at treatment discontinued			
Lamivudine	3	5	0.2
Lamivudine + adefovir	4	4	
Entecavir	15	12	
Tebivudine	2	3	
ALT started consolidation therapy (U/L)	27.3 ± 15.1	31.2 ± 13.2	0.38
HBsAg started consolidation therapy (IU/mL)	409 (10.1–8187.4)	1006.6 (17.8–7938)	0.27
HBsAg at discontinued therapy (IU/mL)	574.5 (0.03–9761)	68 (0–4714)	0.21
Duration of NA therapy (*years*)	*4.65 ± 2.78*	*3.81 ± 1.83*	*0.26*
Duration of HBeAg seroconversion (*years*)	*3.51 ± 2.43*	*2.48 ± 0.97*	*0.06*
Virological relapse (*n*, %)	15 (62.5)	6 (25)	0.03
Clinical relapse (*n*, %)	*8* (*36.3*)	3 (12.5)	0.03
Restarted on antiviral therapy (*n*, %)	*12* (*50*)	*3* (*12.5*)	0.03

Clinical relapse is defined as HBV DNA > 2000 IU/mL and ALT > 2 × ULN are given in italics

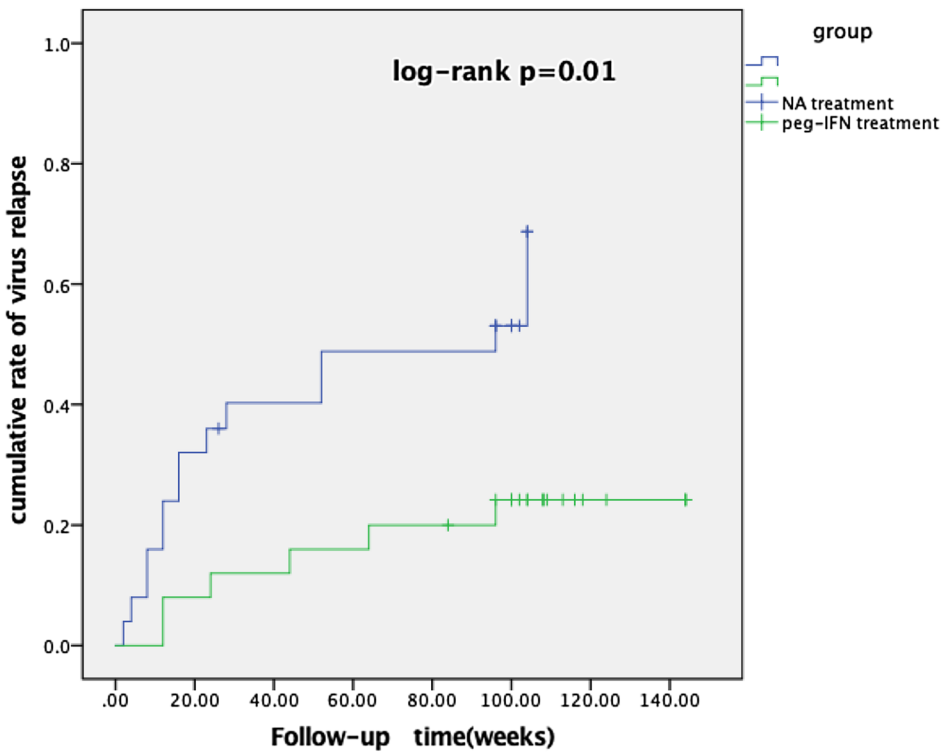
ALT alanine aminotransferase, *HBsAg* hepatitis B surface antigen, *Peg-IFN* peg-interferon-alfa-2a *NA* nucleos(t)ide analogue

Efficacy

There was a significant difference in the rate of viral relapse (25% vs. 58.3%, $p=0.01$) between the two groups. From baseline to week 144, HBV DNA became detectable in 6 (25%) of 24 patients who switched to peg-IFN therapy. Among these 24 patients, 4 (16.6%) experienced viral relapse and 3 (12.5%) had been restarted on NA treatment because of hepatitis reoccurrence (ALT > 2 × ULN). In contrast, at week 144, in the 24 patients who received NA therapy, 15 (62.5%) had detectable HBV DNA, of whom 14 (58.3%) had HBV DNA > 2000 IU/mL. 12 (50%) of 24 patients were qualified to restart NA treatment at week 8 because of hepatitis reoccurrence. Eight patients experienced clinical relapse (defined as HBV DNA > 2000 IU/mL and ALT > 2 × ULN) and four patients experienced mildly elevated ALT levels (< 2 × ULN). Of these patients, eight were previously treated with entecavir, two with lamivudine and one with lamivudine combined plus adefovir. The time of viral relapse was mostly within week 12 during the treatment-free follow-up. The cumulative rate of HBV DNA detected was significantly higher in patients who had NA consolidation therapy than those in the peg-IFN therapy group during 96 weeks follow-up (56% versus 25%, $p=0.01$) (Fig. 2).

HBsAg loss was observed in 8 (8/22, 36.3%) in group B, with 6 (6/22, 27.2%) occurring during peg-IFN treatment and 2 (9.09%) during treatment-free follow-up time. HBsAg seroconversion was observed in 6 (6/22, 27.2%) of 22 patients. In group A, only 1 (1/22, 4.5%) of 24 patients had HBsAg loss at week 92 and no patients had HBsAg seroconversion. Moreover, all patients that lost HBsAg had sustained HBsAg loss and HBsAg seroconversion during the 96-week follow-up. The mean HBsAg level had declined significantly in both study groups at weeks 96 and 144 compared with the time of discontinuation of therapy (Fig. 3a, b): mean −2.3 $\log_{10}$ IU/mL reduction for the peg-IFN treatment group ($p=0.0001$) and − 0.2 $\log_{10}$ IU/mL reduction for patients who stopped NA therapy ($p=0.001$) at week 96. The mean change in HBsAg was −2.2 $\log_{10}$ IU/mL for group B and −0.51 $\log_{10}$ IU/mL for group A (Table 2) at week 144. HBsAg declined more strongly in the peg-IFN treatment group ($p=0.005$) than that in the NA treatment group.

Significant predictors of viral relapse in univariable analysis were non-peg-IFN therapy ($p=0.04$), higher baseline HBsAg level ($p=0.01$) at follow-up and HBsAg level decline < 0.25 $\log_{10}$ IU/mL at week 96 ($p<0.001$). In the



Time(weeks)	0	24	48	96	124	144
Number at risk						
NA treatment group	24	16	14	11	1	0
Peg-IFN group	24	22	20	17	3	1

Fig. 2 Comparison of the cumulative rate of the hepatitis B virus relapse between NA therapy group and peg-INF therapy group. *peg-IFN* pegylated-interferon alfa-2a; *NA* nucleos(t)id

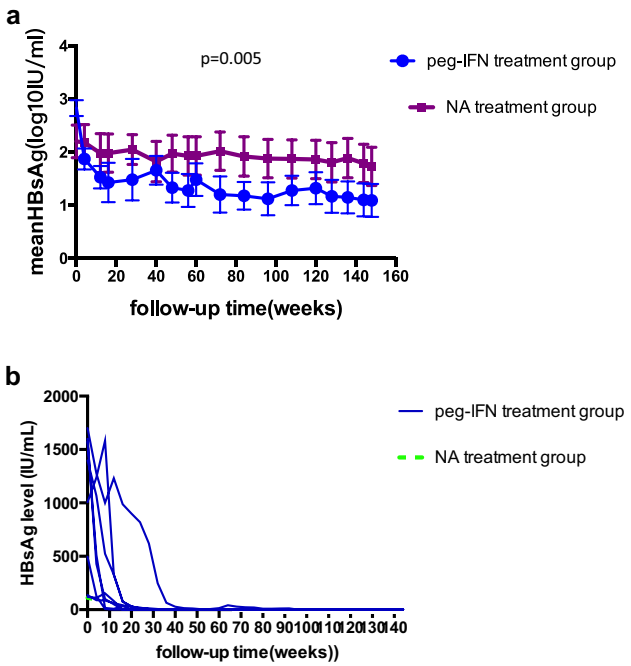


Fig. 3 a Changes in HBsAg level from baseline according to HBsAg level at the time of stopping therapy. *HBsAg* hepatitis B surface antigen. b Dynamic change of serum HBsAg levels in patients with HBsAg loss

multivariate analysis, only HBsAg level decline < 0.25 $\log_{10}$ IU/mL at week 96 was significantly associated with viral relapse. No significant associations were found among age, sex, duration of antiviral treatment, duration of HBeAg seroconversion and baseline HBsAg levels with viral relapse (Table 3). According to multivariable analysis, no significant associations were found among age, sex, duration of antiviral treatment, baseline HBsAg level, HBsAg decline > 1 $\log_{10}$ IU/mL at week 48 and HBsAg decline > 1 $\log_{10}$ IU/mL at week 96 with HBsAg loss.

ALT level elevation (ALT > 2× ULN) was observed in two patients in the peg-IFN group: one at week 17 and the other at week 120. ALT levels > 2× ULN were also observed in five patients in the group who discontinued NA treatment. All patients restarted the original NA therapy.

All patients in the peg-IFN therapy group maintained HBeAg seroconversion from the end of treatment to the end of untreated follow-up. Meanwhile, 2 (0.9%) of 22 patients in the NA therapy group lost HBeAg but maintained HBeAg loss.

Safety

The most frequent adverse events (> 30%) were fatigue, headache, fever and myalgia, which were attributed to peg-IFN dosing (Table 4). One serious adverse event was

Table 2 HBsAg responses

	Group A NA therapy	Group B peg-IFN therapy	*p* value
Number of participants	23	22	
Mean HBsAg at baseline (IU/mL)	1753 ± 482.7	1523 ± 385.3	0.67
HBsAg at week 48	835 (12–9761)	38.5 (0–4614.3)	0.01
HBsAg at week 96	270.1 (0–12,016)	11.7 (0–8671)	0.02
HBsAg at week 144	108 (0–16,290)	2.2 (0–8897)	0.009
Mean HBsAg decline at week 48 ($\log_{10}$ IU/mL)	0.6 ± 0.23	2.6 ± 0.35	0.001
Mean HBsAg decline at week 96 ($\log_{10}$ IU/mL)	0.2 ± 0.1	2.3 ± 0.37	0.001
Mean HBsAg decline at week 144 ($\log_{10}$ IU/mL)	0.5 ± 0.35	2.2 ± 0.38	0.01
HBsAg loss at week 48	0	6	0.09
HBsAg loss at week 96	1	7	0.04
HBsAg loss at week 144	1	8	0.01
HBsAg seroconversation at week 144	0	6	0.09
Week 48 HBsAg < 10 IU/mL	3	6	0.26
Week 48 HBsAg > 0.25 $\log_{10}$ IU/mL decline	2	21	0.001
Week 48 HBsAg > 1 $\log_{10}$ IU/mL decline	1	21	0.001
Week 96 HBsAg < 10 IU/mL	6	14	0.03
Week 96 HBsAg > 0.25 $\log_{10}$ IU/mL decline	3	20	0.001
Week 96HBsAg > 1 $\log_{10}$ IU/mL decline	2	20	0.001
Week 144 HBsAg < 10 IU/mL	8	17	0.04
Week 144 HBsAg > 0.5 log 10 IU/mL decline	4	17	0.001
Week 144 HBsAg > 1 log IU/mL decline	5	15	0.02

Peg-IFN,peg-interferon-alfa-2a; HBsAg, hepatitis B surface antigen; nucleos(t)ide analogue

Table 3 Cox regression analysis of the relationship between patient characteristics and virus relapse

Variable	Hazard ratio	95% CI	*p* value
Age	1.02	0.92–1.12	0.74
peg-INF therapy	1.02	0.08–1.83	0.23
Duration of HBeAg seroconversion	0.13	0.002–19.3	0.69
HBsAg levels starting follow-up	0.2	1.00–1.00	0.19
ALT level starting follow-up	1.02	0.98–1.03	0.63
HBsAg levels decline > 0.25 log 10 IU/ml at week 48	0.07	0.61–24.4	0.15
HBsAg levels decline > 0.25 log 10 IU/ml at week 96	0.16	0.03–0.90	0.04

Table 4 Overview of adverse events profile up to week 144

Adverse events	Group A ($n=23$)	Group B ($n=22$)
Pyrexia	1	2
Fatigue	1	4
Neutrophil count decrease	0	1
platelet count decrease	0	2
Rash	0	3
Headache	0	2
Thyroid abnormalities	0	1
SAE related to peg-INF[a]	0	3
SAE related to stop NA&	2	0
Discountinuations	0	1

SAE serious adverse event, *NA* nucleos(t)ide analogue, *Peg*-IFN peg-interferon-alfa-2a

[a]Including three patients with peg-IFN therapy

reported in the peg-IFN group (severe skin rash at week 12, $n=1$). The patient withdrew from the study. No unexpected adverse events were observed in group A. Two patients in the peg-IFN therapy group and five patients in the group who discontinued NA treatment experienced an increase in ALT levels classed as a severe adverse event.

Discussion

The result of this study shows that the rates of sustained viral replication suppression at 144 weeks were significantly higher in patients receiving peg-IFN consolidation treatment for 48 weeks among the HBeAg-positive CHB patients who had HBeAg seroconversion for > 1 year during NA treatment than in those receiving NA consolidation treatment after NA treatment cessation. The rationale for the study was based on the hypothesis that switching to peg-IFN treatment from NA therapy results in increased rates of HBsAg loss and anti-HBs seroconversion. Owing to the relatively higher rate of HBsAg loss in a previous new switch research [15], we explored the effects of peg-IFN consolidation therapy on partially cured patients, i.e. those with HBeAg seroconversion. We hypothesized that immunomodulation might be effective in patients who had HBeAg seroconversion because they have residual HBV-specific T-cell activity and are possibly sensitive to a boost with immunomodulatory agent [16]. Although the sample size in this study is relatively small, the results are encouraging, which indicate that 75% patients with HBeAg seroconversion can maintain viral replication suppression after discontinued NA treatment and 36.3% patients can achieve HBsAg loss with 1 year of peg-IFN consolidation treatment. Peg-IFN consolidation treatment appeared to be a useful strategy to discontinue antiviral treatment for NA-treated CHB patients who achieved HBeAg seroconversion.

The goal of researching the management of hepatitis B is to identify strategies for stopping therapy in patients who have received long-term NA therapy and showed HBeAg seroconversion and to increase rates of functional cure [1]. The study did not aim to make any recommendation regarding stopping NA therapy in CHB patients during routine clinical practice. However, discontinuation of long-term NA therapy may be attempted if close follow-up can be guaranteed in HBeAg-positive patients without advanced liver disease and with HBeAg seroconversion. As stated in the recent EASL guidelines [1], NA therapy can be discontinued in non-cirrhotic HBeAg-positive CHB patients who achieve stable HBeAg seroconversion and complete at least 12 months of therapy. However, many studies demonstrated that the proportion of viral relapse (defined as HBV DNA > 2000 IU/mL) in HBeAg-positive CHB patients who had achieved stable HBeAg seroconversion is higher, ranging from 50 to 70%, after discontinuation of NA therapy [18–20]. Our study obtained a similar result, which found that 14 (58.3%) of 22 patients experienced viral relapse after stopping and restarting NA therapy. The timeframe of detectable viral DNA was mostly within 12 weeks (11/12, 91.6%) during the treatment-free follow-up. In contrast, our results also found that 1-year consolidation therapy of peg-IFN significantly increased the rate of sustaining viral replication suppression (VS) for these patients. The results were consistent with the research of Peng et al. [17]. They demonstrated that patients on long-term NA treatment who are unlikely to meet therapeutic goals could achieve high

rates of HBsAg loss and sustain HBeAg seroconversion while stopping entecavir treatment by switching to peg-IFN alfa-2a. The exact mechanism is unclear, but it might be due to differential boosting of innate and adaptive antiviral responses during peg-IFN and NA treatments [18, 19].

The availability of an effective, finite therapy may motivate patients to start and adhere to therapy while providing them with the chance to achieve the closest alternative to a clinical cure of CHB. The interesting strategy of providing a finite course of therapy and increase rates of HBsAg loss is to combine immunomodulation therapy with IFN and NA therapy. There are several ways to combine therapy: initiate both agents simultaneously in treatment-naive patients [20], add peg-IFN to patients with VS after long-term NA therapy [21, 22] or switch therapy to peg-IFN in patients previously treated with oral antivirals [6]. All the studies focused on functional cure of CHB, but very few investigated a strategy to safely stop NA therapy. The identification of predictors of durable VS after NA discontinuation is of great importance. However, despite several attempts to identify predictors of durable VS at the time of NA discontinuation, no clear and reliable predictor of durable VS was found. Studies have demonstrated that HBsAg level at the end of treatment was a useful predictor to guide therapy discontinuation [23, 24]. For example, Chen et al. [25] determined that HBsAg cutoff values < 100 IU/mL could predict sustained viral replication suppression at the end of treatment [25]. Furthermore, HBsAg reduction (0.22 $\log_{10}$ IU/mL) at month 6 after stopping treatment from the end of treatment was an independent predictor of HBsAg loss after adjusting for HBsAg level at the end of treatment. In the current study, patients who achieved > 0.25 $\log_{10}$ IU/mL decrease in HBsAg levels at week 48 when therapy was stopped had durable viral replication suppression. It was an independent factor associated with VS after NA therapy discontinuation. Whether a strong HBsAg decline can lead to future HBsAg loss in peg-IFN treatment CHB patients with HBeAg seroconversion will be determined in our subsequent 5-year follow-up study.

Peg-IFN treatment is associated with severe side effects and safety issues. In our study, with the exception of one patient who withdrew because of severe skin allergies, the remaining patients tolerated peg-IFN treatment well. Judging by the high rate of adverse events and treatment discontinuation, peg-IFN treatment was well tolerated. In this study, no unexpected safety issues associated with stopping NA therapy were identified. Notably, seven patients who restarted NA therapy experienced an increase in ALT levels > 2× ULN.

This study has several potential limitations. The NA drug in our study is not consistent and the sample size for study HBsAg loss is relative small, which can be attributed to (1) a long history lamivudine treatment in China due to economic reasons and (2) few patients with HBeAg seroconversion who were willing to stop NA therapy and follow the strict protocol of initial biweekly follow-up evaluations when stopping NA therapy. Thus, additional studies are required to confirm our finding. Furthermore, the majority of patients were Asian men, which may limit the applicability of the study results to a more diverse population. Although this study provided evidence supporting the potential strategy of a finite course of peg-IFN therapy to offer higher rates of virological remission and HBsAg loss in HBeAg-positive CHB patients who discontinue NA therapy after receiving consolidation therapy for more than 1 year following HBeAg seroconversion, the actual duration of NA consolidation therapy in this study was considerably longer (3.51 ± 2.43 versus 2.48 ± 0.97 years) than currently recommended (1 year). Therefore, whether the present finding can be extrapolated to patients who minimally fulfil the duration for consolidation therapy (1 year) according to current guidelines remains unclear. Additional studies are required to confirm this possibility.

In conclusion, among HBeAg-positive Asian patients who achieved HBeAg seroconversion during long-term NA therapy, a substantial proportion of patients who switched to a finite 48-week peg-IFN-therapy showed an increased rate of sustained suppression of viral replication after NA therapy discontinuation. HBsAg loss and HBeAg seroconversion were maintained during follow-up. Therefore, switching from long-term NA therapy to finite treatment with peg-IFN-a2a appears to be a viable strategy for the discontinuation of NA therapy in HBeAg-positive CHB patients with HBeAg seroconversion > 1 year during NA therapy.

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Author contributions Hong Wang designed the research. Ying Zhou and Rong Yan collected the data and established the database. Jiong Yao presided over the enrolment and exclusion of the research subjects. Hong Wang analysed the data statistically and drafted the manuscript. Guo Qing Ru and Li Li Yu collected the pathologic data

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Compliance with ethical standards

Conflict of interest Ying Zhou, Rong Yan, Guo Qing Ru, Li Li Yu, Jiong Yao and Hong Wang declare that they have no conflict of interesting.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was reviewed and approved by the ethics committee of the hospital.

Informed consent All patients signed an informed consent form before screening in accordance with regulatory and local ethics committee requirements.

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