Short-term Peginterferon-Induced High Functional Cure Rate in Inactive Chronic Hepatitis B Virus Carriers With Low Surface Antigen Levels

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Background. None of the current guidelines recommend antiviral therapy for inactive hepatitis B virus (HBV) carriers (IHCs).

Methods. In this real-world, multicenter, nonrandomized study, 32 participants meeting the inclusion criteria were enrolled 1:1 for treatment with peginterferon α-2b or monitoring without treatment based on participant preference. The expected treatment duration was 48 weeks. The primary end point was hepatitis B surface antigen (HBsAg) loss. The HBV vaccine could be injected after HBsAg loss.

Results. All patients had HBsAg levels of <20 IU/mL. The mean baseline HBsAg levels were 6.6 IU/mL and 5.8 IU/mL in the treated and untreated groups, respectively. Fifteen (93.8%) participants achieved HBsAg loss, 5 obtained HBsAg seroconversion after undergoing a mean of 19.7 weeks of therapy in the treated group, and no one in the follow-up group achieved HBsAg loss during a mean follow-up time of 12.6 months ($P < .0001$). Generally, the therapy was well tolerated. Nine of 11 individuals who exhibited HBsAg loss benefited from receiving the HBV vaccine.

Conclusions. This study provides justification for further studies of short-course peginterferon α-2b for the functional cure of IHCs with low HBsAg levels. Additionally, HBV vaccine injection is beneficial after interferon-induced HBsAg loss.

Keywords. functional cure; hepatitis B surface antigen loss; hepatitis B vaccine; inactive hepatitis B virus carrier; peginterferon α-2b.

China has the largest burden of individuals with chronic hepatitis B virus (HBV) infection, which includes ~93 million people [1]. The natural history of chronic HBV infection is usually classified into 4 or 5 phases according to the latest guidelines (guidance) [2–4]. Currently, at least 20 million Chinese individuals with chronic HBV infection are in immune-active phases 2 and 4 [1], which means that antiviral treatment is needed [2, 3]. Phase 3 inactive HBV carriers (IHCs), that is, those with “hepatitis B e antigen (HBeAg)-negative chronic hepatitis B infection,” are characterized by normal alanine aminotransferase (ALT) and undetectable or low (<2000 IU/mL) serum HBV DNA levels [2, 3, 5]. Commonly, IHCs have a relatively low risk of progression to cirrhosis or hepatocellular carcinoma (HCC) [2], and some individuals progress to phase 4 HBeAg-negative chronic hepatitis B [6]. Additionally, current therapy with nucleos(t)ide analogs cannot lead to hepatitis B virus surface antigen (HBsAg) loss, and treatment with peginterferon α is costly and has side effects and a relatively low HBsAg loss rate. Therefore, the current guidelines do not recommend antiviral therapy for IHCs [2, 4, 5].

Recently, a functional cure, that is, sustained HBsAg loss with undetectable HBV DNA, was proposed as the end point of antiviral treatment and aimed at clinical trials [3, 7, 8]. It is believed that functional cure is associated with improved long-term outcomes [9]. However, few studies have investigated or even considered functional cure in IHCs for the abovementioned reasons. Notably, discrimination against individuals with chronic HBV infection has continued to be severe in China [10, 11], and many IHCs strongly desire to clear their HBsAg, even if they do not meet the indications for antiviral therapy. Herein, we report the safety and efficacy of treatment of IHCs with extremely low HBsAg levels with Chinese-made peginterferon α-2b (PegBeron, Y shape, 40 kD, Xiamen Amoytop Biotech, Xiamen City, Fujian Province, China) in a real-world setting, as well as the follow-up outcome in untreated IHCs with conditions comparable to those of the treated IHCs. This study may...
provide some inspiration for forthcoming clinical decisions and contribute to the future differential management of IHCs.

**METHODS**

**Inclusion and Exclusion Criteria**

In this current multicenter, nonrandomized, real-world study, all enrolled participants met the diagnostic criteria of an IHC according to the latest guidelines from June to December 2018 [2, 4, 5], that is, an individual with noncirrhotic, non-HCC, HBeAg-negative chronic HBV infection with a normal ALT level and an HBV DNA level of <2000 IU/mL. The detailed inclusion and exclusion criteria in the current study were as follows: (1) age 20–50 years; (2) a history of chronic HBV infection; (3) serum HBsAg level <20 IU/mL; (4) serum HBeAg negative with or without antibody to HBeAg (anti-HBe); (5) HBV DNA level undetectable or <200 IU/mL; (6) normal ALT level <40 U/mL; (7) no cirrhosis, including compensated and decompensated cirrhosis; (8) no HCC or extrahepatic manifestations; (9) no prior interferon treatment and no treatment with nucleos(t)id analogs for 5 years before enrollment; and (10) negative test results for hepatitis A, C, D, and E viruses, Epstein-Barr virus, cytomegalovirus, and HIV, as well as thyroid disorders and antinuclear, antismooth muscle, and antimitochondrial autoantibodies.

**Group Division and Treatment Regimen**

The enrolled IHCs were divided into 2 groups according to their own choice, that is, the treated group and the untreated follow-up group. Participants meeting the inclusion criteria were enrolled 1:1 for treatment with peginterferon α-2b or monitoring without treatment, based on participant preference. All IHCs in the treated group received weekly subcutaneous injections of 180 µg of Chinese-made peginterferon α-2b (PegBeeron), which is currently the principal interferon regimen in China [12, 13]. The expected treatment duration for the treated group was 48 weeks, and participants could discontinue therapy at the time of HBsAg loss (functional cure) or at any time during therapy. All IHCs in the untreated follow-up group did not receive any therapy.

**Main Assessments at Baseline, During Therapy, and on Follow-up**

Quantification of the levels of HBsAg, antibody to HBsAg (anti-HBs), anti-HBe, HBV DNA, serum biochemical markers, and routine blood parameters was performed in the treated group and the untreated follow-up group. In a real-world setting, the enrolled IHCs were monitored with a flexible interval of ~1–3 months in the treated group and ~3–6 months in the untreated follow-up group. Serum HBsAg was tested using the Abbott ARCHITECT assay (Abbott Diagnostics, Abbott Park, IL, USA) with a lower limit of quantification of 0.05 IU/mL (HBsAg <0.05 IU/mL is nonreactive, ie, HBsAg loss), and an anti-HBs level >10 mIU/mL was defined as positive. Serum HBV DNA (lower limit of quantification was 15 or 25 IU/mL depending on the institute’s settings) was measured using COBAS AmpliPrep/COBAS TaqMan tests (Roche Molecular Systems, Inc., Branchburg, NJ, USA).

Cirrhosis was diagnosed based on the results of at least 2 imaging modalities (ie, abdominal ultrasonography, FibroScan, computed tomography, or magnetic resonance imaging) plus clinical evidence of manifestations. A liver stiffness >12.5 kPa was considered to indicate cirrhosis in patients without ascites [14]. Patients were screened for HCC by ≥2 imaging modalities or by 1 diagnostic imaging modality plus determination of a serum alpha-fetoprotein level of ≥400 ng/mL [15].

**Efficacy and Safety Evaluations**

The primary efficacy and follow-up end points were HBsAg loss, which was defined as HBsAg <0.05 IU/mL. The secondary efficacy end point was undetectable HBV DNA. In addition, all adverse events and laboratory abnormalities that occurred during therapy were reported in the treated group.

**HBV Vaccine Injection After HBsAg Loss**

Individuals with HBsAg loss could be injected with 20 µg of HBV vaccine after HBsAg loss according to their own choice.

**Sample Size Estimation**

One important aim of this study was designed to demonstrate that the difference in the HBsAg loss rate between the 2 groups (treated group – untreated follow-up group) is more than the known “rate difference” (Δ), which was 0.274 (0.298–0.024) at week 48 of peginterferon therapy in a previous study [16]. In consideration of the extremely reduced HBsAg levels in IHCs that may have higher treatment-induced and spontaneous HBsAg loss rates, the expected treatment-induced HBsAg loss rate, spontaneous HBsAg loss rate, and Δ were all set as high as 2 times higher in the current study, that is, 0.596, 0.048, and 0.548, respectively. The level of 2-sided significance was set at .05, with a high power (1 – β) of 0.9. Therefore, the estimated sample size was 11 in each group, which was rounded up to 12 to allow for a high rate of withdrawal (10%). The total sample size required for the 2 groups in this study was thus calculated to be ≥24.

**Statistical Analysis**

Continuous variables are summarized as either the mean ± SD or median and range, as appropriate. The percentage of patients in each category was calculated for categorical variables. The percentages were compared between the 2 groups using the chi-square test. Mann-Whitney U tests were performed for comparisons of continuous variables between the 2 groups. The Kaplan-Meier method was used to calculate the cumulative rates of HBsAg loss, and differences were determined using the log-rank test. A 2-sided P < .05 was considered significant.
The sample size was estimated by PASS 15 for Windows (NCSS statistical software, Kaysville, UT, USA) using the Z-test with unpooleed variance for the equality of 2 proportions [17]. The analyses were performed using SPSS software 25.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Informed Consent and Ethical Evaluations
All procedures used were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2008. Informed consent for the observational process was obtained from all patients before inclusion in the study. The observational protocol was approved by the institutional review board or ethics committee before study initiation.

RESULTS

Participant Characteristics
Thirty-two participants meeting the inclusion criteria were enrolled 1:1 for treatment or monitoring without treatment, based on participant preference. The baseline characteristics of the participants in the treated group and the untreated follow-up group are shown in Table 1 and Supplementary Table 1. All participants had a history of >10 years of known chronic HBV infection, and the levels of ALT, total bilirubin, and alpha-fetoprotein and the liver stiffness measurements were all in normal ranges at baseline. In addition, no patients had cirrhosis, extrahepatic manifestations, imaging findings of HCC, or a family history of HCC. The median baseline HBsAg levels (interquartile range) were 3.5 (0.1–12.7) IU/mL and 2.6 (0.1–11.9) IU/mL in the treated group and untreated follow-up group, respectively. All participants were negative for HBeAg at baseline, while 12 and 10 participants were positive for anti-HBe in the abovementioned 2 groups, respectively. Only 2 participants in the treated group and 3 participants in the untreated follow-up group had detectable HBV DNA levels. The detailed characteristics of the treated group at baseline are shown in Table 2.

HBsAg Loss in 2 Groups
Fifteen (93.8%) participants achieved HBsAg loss, and 5 (31.2%) participants achieved HBsAg seroconversion after undergoing a mean (median) of 19.7 (16) weeks of peginterferon α-2b therapy in the treated group (Table 2). Two individuals with detectable HBV DNA showed undetectable results after 5 and 8 weeks of treatment. The HBeAg and anti-HBe statuses were unchanged in all individuals at HBsAg loss or discontinuation of therapy compared with baseline in the treated group. Over a mean follow-up time of 12.6 months in the untreated follow-up group, no one achieved spontaneous HBsAg loss (Figure 1), no one showed an altered anti-HBe status, and 1 participant progressed to phase 4 HBeAg-negative chronic hepatitis B with an HBsAg level of 110.2 IU/mL, an HBV DNA level of 1890 IU/mL, and an ALT level of 86 U/L at 1-year follow-up.

Compliance, Safety, and Adverse Events
Generally, peginterferon α-2b therapy was well tolerated, and the main side effects were pyrexia, fatigue, and muscle pain (Table 3). The main laboratory abnormalities were leukopenia, thrombocytopenia, and ALT elevation. Interestingly, 12 participants had a white blood cell count decrease of >1 ×10⁹/L, a platelet count decrease of >50 ×10⁹/L, and an ALT increase of more than the upper limit of normal compared with baseline. Notably, none of the patients had an elevated total bilirubin.

Table 1. Demographic and Clinical Characteristics of Peginterferon α-2b (PegBeron)–Treated and Untreated Inactive HBV Carriers With Low HBsAg Levels at Baseline

| Parameters | Treated Group (n = 16) | Untreated Follow-up Group (n = 16) |
|------------|------------------------|-----------------------------------|
| Male, No. (%) | 12 (75)                | 12 (75)                           |
| Han Chinese, No. (%) | 16 (100)              | 16 (100)                          |
| Age, median (range), y | 34 (32–46.8)          | 36 (32–44.8)                      |
| HBsAg, median (range), IU/mL | 3.5 (0.1–12.7) | 2.6 (0.1–11.9) |
| Anti-HBe positivity, No. (%) | 12 (75)              | 10 (62.5)                         |
| HBV DNA detectable,a No. (%) | 2 (12.5)             | 3 (18.8)                          |
| ALT, median (range), U/L | 20.5 (15.5–25)       | 20.5 (17.3–25.5)                  |
| AST, median (range), U/L | 21 (17.3–23)         | 19.5 (17–26.8)                    |
| TBIL, median (range), µmol/L | 11.8 (8.3–15.7)    | 7.4 (6.4–13)                      |
| Platelet, median (range), x10¹²/L | 192 (124.5–228.8) | 170 (135.3–215.8)                |
| Splenomegaly, No. (%) | 0 (0)                 | 0 (0)                             |
| LSM, median (range), kPa | 5.8 (4.7–6.3)        | 5.6 (5.1–6.1)                     |
| AFR, median (range), ng/mL | 2.1 (1.8–2.9)       | 2.5 (1.8–3.5)                     |
| Imaging findings of HCC, No. (%) | 0 (0)                | 0 (0)                             |

Abbreviations: AFR, alpha-fetoprotein; ALT, alanine aminotransferase; anti-HBe, antibody to hepatitis B e antigen; AST, aspartate aminotransferase; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; TBIL, total bilirubin.

aAll 5 individuals had detectable (15–199 IU/mL) HBV DNA of <200 IU/mL in current study.
level. Additionally, none of the participants had a severe adverse event or discontinued injection due to intolerable side effects. One participant discontinued treatment at week 28 in the treated group because of unsatisfactory changes in HBsAg level.

**HBV Vaccine Injection and Follow-up**

Notably, a total of 11 individuals were injected with 20 µg of HBV vaccine immediately after HBsAg loss, including 6 participants without anti-HBs and 5 participants with anti-HBs who were injected with 1 dosage (20 µg) of HBV vaccine after HBsAg loss (Table 4). Interestingly, anti-HBs appeared in 4 individuals who originally did not have anti-HBs, the anti-HBs titer increased in all 5 individuals who originally had anti-HBs, and the remaining 2 individuals who originally did not have anti-HBs maintained the status of HBsAg loss but without the appearance of anti-HBs (Table 4). In addition, anti-HBs positivity did not appear in 4 anti-HBs-negative individuals who did not receive the HBV vaccine. These findings indicate that the HBV vaccine may contribute to the appearance of anti-HBs or elevation of preexisting positive anti-HBs levels in individuals with therapy-induced HBsAg loss (Table 5). The off-therapy HBsAg loss was stable after a mean of 9.9 months of off-treatment follow-up, including 4 participants with HBsAg loss who did not receive the HBV vaccine (Table 4).
The current study has the integrated characteristics of a high functional cure rate and low HBsAg level in IHCs, a short duration regimen, and low cost when compared with a previous study [16]. To the best of our knowledge, this is the first work that shows an unexpectedly short-course (mean, 19.7 weeks; median, 16 weeks) and low-cost peginterferon α-2b regimen leading to the highest functional cure rate in chronic HBV-infected individuals.

In the current study, only inactive carriers with an HBsAg level <20 IU/mL were enrolled to pursue the best cost-effectiveness, and all treated patients received Chinese-made peginterferon α-2b (PegBeron, Y shape, 40 kD), which is different from other studies [16, 18]. Furthermore, the mean treatment course (~20 weeks) was less than half of the standard full course, which was 48 or 72 weeks in many previous studies [18, 19]. The functional cure rate for IHCs was <50% in almost all prior reports [2, 4, 5], even in IHCs treated with peginterferon for 96 weeks [16], and the current study shows a functional cure rate as high as 93.8% with ~20 weeks of therapy. Additionally, the current treatment regimen is cost-effective. One dosage of peginterferon α-2b costs 820 Chinese Yuan (~117 US dollars) in China before insurance program reimbursement, and the total cost for these patients is relatively low, that is, USD$2343 and USD$1874 for 20 dosages (mean weeks) and 16 dosages (median weeks), respectively. The total price of the presented regimen was much lower than that of Epclusa, which costs ~10 000 US dollars in China and leads to the cure of hepatitis C infection. Notably, the cure rate in the current study is similar to that of Epclusa, and the natural disease progression and long-term prognosis may also be comparable between regular chronic hepatitis C patients and IHCs with an HBsAg level of <20 IU/mL, although the (clinical) cure for these 2 types of diseases may have essential differences, and HBsAg loss is not a true cure due to the persistence of covalently closed circular DNA.

Current HBV guidelines recommend that patients in phases 2 and 4 receive a finite course of peginterferon therapy [2, 4, 5]. However, few studies have investigated or even considered the possibility and necessity of HBsAg loss in IHCs, even those with extremely low HBsAg levels, not to mention the further differential or tailored management of these IHCs. It is interesting and important to consider whether a safe therapy with a low cost, high functional cure rate, and short duration exists for IHCs with an HBsAg level of <20 IU/mL; in addition, if the patients have a strong desire to eliminate their chronic HBV infection, why not treat them? Notably, ALT elevation during treatment commonly occurred, and 13 of 16 patients had ALT ≥40 U/L at HBsAg loss or discontinuation of therapy, which indicates that peginterferon may lead to immune activation for patients with an inactive immune status. Furthermore, immune activation may have contributed to HBsAg loss in the current study.

Table 3. Adverse Event Frequency and Severity

| Parameters                                                                 | Treated Group (n = 16) |
|---------------------------------------------------------------------------|------------------------|
| Adverse event occurring in ≥1 individual, No. (%)                        |                        |
| Pyrexia                                                                   | 13 (81.3)              |
| Fatigue                                                                   | 12 (75)                |
| Muscle pain                                                               | 9 (56.3)               |
| Liposchitria                                                              | 8 (50)                 |
| Headache                                                                  | 5 (31.3)               |
| Anorexia                                                                  | 4 (25)                 |
| Gingival bleeding                                                         | 3 (18.8)               |
| Insomnia                                                                  | 2 (12.5)               |
| Diarrhea                                                                  | 1 (6.3)                |
| Cough                                                                     | 1 (6.3)                |
| Constipation                                                              | 1 (6.3)                |
| Vomiting                                                                  | 1 (6.3)                |
| Dizziness                                                                 | 1 (6.3)                |
| Rash                                                                      | 1 (6.3)                |
| Laboratory abnormalities, No. (%)                                         |                        |
| Leukopenia (WBC decrease >1 ×10^9/L from baseline)                        | 12 (75)                |
| Thrombocytopenia (platelet decrease >50 ×10^9/L from baseline)            | 12 (75)                |
| ALT elevation (>ULN)                                                      | 12 (75)                |
| TBIL elevation (>ULN)                                                     | 0 (0)                  |
| Adverse event leading to therapy discontinuation, No. (%)                 | 0 (0)                  |
| Unsatisfactory efficacy leading to therapy discontinuation,a No. (%)      | 1 (6.3)a               |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; TBIL, total bilirubin; ULN, upper limit of normal; WBC, white blood cell.

*Participant 16 discontinued therapy at week 28 due to unsatisfactory HBsAg changes.

DISCUSSION

The current study has the integrated characteristics of a high functional cure rate and low HBsAg level in IHCs, a short duration regimen, and low cost when compared with a previous study [16]. To the best of our knowledge, this is the first work that shows an unexpectedly short-course (mean, 19.7 weeks; median, 16 weeks) and low-cost peginterferon α-2b regimen leading to the highest functional cure rate in chronic HBV-infected individuals.

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Table 4. HBV Vaccine and Follow-up of Participants With HBsAg Loss

| Participant No.: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|
| Follow-up period, mo | 6 | 8 | 11 | 7 | 7 | 15 | 10 | 12 | 18 | 6 | 9 | 12 | 8 | 9 | 10 |
| HBV vaccine injection after HBsAg loss | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes | No | No | Yes | No | Yes |
| The latest HBsAg level (<0.05), IU/mL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 |
| The latest anti-HBs level (0–10), mIU/mL | 3.3 | 136.58 | 54.68 | 110.01 | 0.77 | 43.81 | 955.39 | 213.52 | 724.15 | 702 | 3.07 | 51.6 | 5.09 | 3.88 | 60.6 |
| The latest status of HBsAg seroconversion | No | Yes | Yes | Yes | No | Yes | Yes | Yes | No | Yes | No | Yes | No | Yes | Yes |

The results show that the off-therapy HBsAg loss was stable over a mean follow-up period of 9.9 months; notably, 4 other (of a total of 9) individuals achieved HBsAg seroconversion.

Abbreviations: anti-HBs, antibody to hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.
However, the elevated ALT level returned to the normal range 1 to 3 months after stopping peginterferon therapy.

Based on the current study, we suggest that IHCs can be further divided into different stratifications by HBsAg level. Future studies should formulate an optimal HBsAg cutoff value to facilitate the differential or tailored management of IHCs. IHCs with an HBsAg level lower than a certain cutoff value can pursue a functional cure by peginterferon therapy first or preferentially. For some IHCs with an HBsAg level higher than the optimal cutoff value, nucleos(t)ide analogs may be used to arrive at this optimal cutoff value first, and peginterferon may be sequentially applied to pursue a functional cure. This preliminary management roadmap may improve the efficiency and decrease the expenditure on peginterferon therapy for some IHCs to achieve a functional cure.

Spontaneous and treatment-induced HBsAg loss in chronic HBV infection is associated with the resolution of residual liver injury and a decreased risk of cirrhosis and HCC over time [7, 20]. It is well known that spontaneous HBsAg loss is rare, with an approximate rate of 1%–2.4% per year [7, 16, 21]. Of individuals who participated in the current study, 16 treated participants with extremely low HBsAg levels may achieve spontaneous HBsAg loss in the future. However, it is difficult to achieve spontaneous HBsAg loss with such a short treatment course, and this point was well demonstrated by the untreated control group in our study (Figure 1). Importantly, HBsAg-positive individuals were proven to have a high risk of developing HCC in the REVEAL study in early 2002; compared with patients who were negative for both HBsAg and HBeAg, the relative risk of HCC was 9.6 and 60.2 times in patients who were positive for HBsAg alone and those who were positive for both HBsAg and HBeAg, respectively [22]. Therefore, we believe that early HBsAg loss may have early potential benefits [20, 22, 23], and treatment to achieve early HBsAg loss may also be suitable for IHCs with low levels of HBsAg. However, large-scale prospective studies are needed in the future. Hence, we will further investigate the long-term outcomes as well as the cost-effectiveness of peginterferon for IHCs with low HBsAg levels.

Table 5. Efficacy of HBV Vaccine Injection After Treatment-Induced HBsAg Loss

| Parameters | HBV Vaccine Injection | No HBV Vaccine Injection |
|------------|-----------------------|--------------------------|
| Participants, No. (%) | 11 | 4 |
| Anti-HBs appearance for anti-HBs-negative participants or titer increase for anti-HBs-positive participants, No. (%) | Yes | 9 | 0 |
| | No | 2 | 4 |

Abbreviations: anti-HBs, antibody to hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

Notably and importantly, it is largely unknown whether the HBV vaccine can contribute to the appearance of anti-HBs after HBsAg clearance, that is, HBsAg seroconversion. In the treated group in the current study, 4 other individuals achieved HBsAg seroconversion after HBV vaccine injections; 5 anti-HBs-positive participants had higher levels of anti-HBs, and 2 individuals maintained anti-HBs-negative status (Table 4). These findings indicate that the HBV vaccine may play an important role in contributing to HBsAg seroconversion or the appearance of higher anti-HBs titers after treatment-induced HBsAg loss (Table 5). However, it is important to note that the anti-HBs titers may theoretically spontaneously increase in some individuals with HBsAg loss.

There are some limitations to the current study, mainly including the small sample size, lack of long-term sustainability of off-therapy HBsAg loss, randomization of intention-to-treat participants, nonuniform approach of using peginterferon and HBV vaccine, and variable follow-up duration. However, the results of the current study already suggest a difference in the HBsAg loss rate between the 2 groups. Furthermore, we believe that enlarging the sample size will not change the HBsAg loss rates or lead to a significant difference between the treated and untreated follow-up groups because the untreated spontaneous HBsAg loss rate is well known from many previous studies, that is, only ~1%–2.4% per year [7, 16, 21]. Additionally, our study shows that off-therapy HBsAg loss was stable over a mean follow-up period of 9.9 months; in addition, prior studies have definitively demonstrated that HBsAg loss persisted over a long-term follow-up period, regardless of whether the loss was induced by nucleos(t)ide analog therapy or interferon therapy [24–26]. Third, it is important to note that the randomization of IHCs is difficult to perform because none of the guidelines recommend antiviral therapy for IHCs and not all IHCs want to be treated due to the slow progression of disease in a relatively short period. Furthermore, for IHCs who have a strong desire to clear their HBsAg, it is unacceptable for them to be randomized to the untreated follow-up group. Last, the nonuniform usage of peginterferon (response-guided) and HBV vaccine and variable follow-up duration should be controlled for or noted in future studies.

In conclusion, this real-world observational study is the first to report a functional cure rate as high as 93.8% in IHCs with HBsAg levels of <20 IU/mL obtained by a mean of 20 weeks of well-tolerated treatment with Chinese-made peginterferon α-2b (PegBeron, Y shape, 40 kD) (Supplementary Figure 1), which provides justification for further studies of short-course peginterferon α-2b for the functional cure of IHCs with low HBsAg levels. Furthermore, these findings may promote the future differential management of IHCs, and it is reasonable to presume that future antiviral indications may expand from phase 2 and 4 patients to phase 3 IHCs with preferentially low or even moderate HBsAg levels, eventually leaving patients in an immune-tolerant phase on monitoring or on an unnecessary-to-treat list. Additionally, this study shows that the HBV vaccine may contribute to the appearance or increase of anti-HBs after treatment-induced HBsAg loss. Given the limited sample
size, our HBsAg-guided approach for functional cure in IHCs should be interpreted cautiously and addressed by large-scale studies.

**Supplementary Data**

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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