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Recurrence risk factors for chronic hepatitis B virus-infected patients who achieve functional cure with pegylated interferon-α-2b-based therapy: a multicenter pilot study

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Abstract

Background Hepatitis B surface antigen (HBsAg) clearance is an achievable treatment endpoint for chronic hepatitis B virus (HBV)-infected patients. Pegylated interferon-α (PEG-IFN-α) induces higher rate of HBsAg clearance than nucleos(t)ide analogues. However, the influencing factors associated with HBsAg recurrence have not been fully elucidated. The aim of this study was to evaluate the risk factors for recurrence in chronic HBV-infected patients who achieved functional cure with PEG-IFN-α-2b-based treatment.

Methods A multicenter retrospective study was conducted. All patients received PEG-IFN-α-2b-based therapy, achieved HBV DNA negativity and HBsAg clearance, and were followed-up for at least 48 weeks after discontinuation of medications. The demographic data, as well as virological, serological, and biochemical indicators, were collected at baseline, therapy cessation, and during followed-up. Logistic regression analysis was subsequently performed.

Results A total of 101 chronic HBV-infected patients who achieved HBsAg loss with PEG-IFN-α-2b-based therapy were enrolled. The median treatment time was 24.00 (14.50, 37.50) weeks, and the median consolidation time was 11.00 (0.00, 24.00) weeks. HBsAg recurrence was found in 16 patients after a median 70.00 (48.00, 96.00) week follow-up, with a cumulative recurrence rate of 15.84%. A higher platelet count was associated with a slightly increased HBsAg recurrence risk at therapy cessation, whereas a shorter consolidation time was associated with an elevated HBsAg recurrence risk during followed-up. The appearance of anti-HBs presented a robustly reduced HBsAg recurrence risk at both therapy cessation and followed-up. No HBV DNA positivity or occurrence of end-stage liver disease was observed during treatment or followed-up.

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Conclusion The cumulative HBsAg recurrence rate was 15.84% after discontinuation of medications in chronic HBV-infected patients who achieved functional cure with PEG-IFN-α-2b-based therapy. The presence of anti-HBs reduced the HBsAg recurrence risk.

Clinical trial registration This trial is a part of ZhuFeng Project (ClinicalTrials.gov, identifier NCT04035837) and a part of E-Cure Study (ClinicalTrials.gov, identifier NCT05182463).

Keywords Chronic hepatitis B, Pegylated interferon-α, Functional cure, Recurrence

Background

Hepatitis B virus (HBV) chronically infects approximately 257 million people worldwide, leading to 880,000 deaths annually due to HBV-induced end-stage liver diseases, such as decompensated liver cirrhosis, liver failure, and hepatocellular carcinoma [1, 2]. However, only 10.5% (~27 million) of chronic HBV-infected patients are aware of their infection, and 16.7% (~4.5 million) of whom are receiving treatment [1]. Thus, persistent inhibition of viral replication by antiviral agents to achieve the World Health Organization goals for elimination of HBV infection worldwide by 2030, which is defined as a 90% reduction in incidence and 65% reduction in mortality, is important [1, 2].

Currently, there are two main first-line therapeutic recommendations for chronic hepatitis B (CHB) patients: oral nucleos(t)ide analogue (NAs) therapy [including entecavir (ETV), tenofovir (TDF), tenofovir alafenamide (TAF), and tenofovir amibufenamide (TMF)] and subcutaneous injection of pegylated interferon-α (PEG-IFN-α) [3–6]. First-line NA treatments are well tolerated and safe. Long-term NA therapies can achieve undetectable circulating HBV DNA in more than 95% of patients, with an extremely low incidence of virological breakthrough due to the high barrier to resistance [7–11]. However, the cumulative rate of hepatitis B surface antigen (HBsAg) loss induced by NAs is low, which is even similar to that of untreated chronic HBV-infected patients [12]. PEG-IFN-α has both antiviral and immunoregulatory properties, resulting in a relatively high rate of functional cure [13, 14], which is defined as sustained HBsAg loss (≥24 weeks post therapy) with or without the appearance of hepatitis B surface antibody (anti-HBs), undetectable hepatitis B e antigen (HBeAg), and undetectable serum HBV DNA after a finite course of therapy [15, 16]. Functional cure is regarded as an ideal and achievable endpoint for antiviral therapy in terms of drug withdrawal safety and improved prognosis [17]. Importantly, PEG-IFN-α therapy could achieve high functional cure rate in inactive HBV carriers (IHC) [18–22]. With the popularization of functional cure concept for CHB, more IHC patients have a strong desire for treatment due to the higher risk of IHC for development of hepatocellular carcimoma than normal population [23].

However, the major challenges from functional cure to sterilizing cure are the presence of covalently closed circular DNA (cccDNA) and the integration of HBV DNA into host DNA [15]. Although cccDNA levels and HBsAg-positive hepatocytes were significantly lower in liver tissue obtained from functionally cured CHB patients than in those obtained from uncured and treatment-naïve patients, HBsAg-positive hepatocytes were still present in 25.5% of patients with functional cure, whereas intrahepatic HBV RNA remained present in 72.2% of patients [24]. Intergrated HBV DNA and cccDNA maintain transcriptional activity in intrahepatic HBsAg-positive patients with functional cure following PEG-IFN-α-2b-based therapy, potentially leading to virological and HBsAg relapse [24]. Furthermore, risk factors corresponding to virological and serological recurrence in chronic HBV-infected patients with functional cure have still not been fully elucidated.

Therefore, we conducted a real-world retrospective observational study to investigate HBsAg recurrence in functionally cured chronic HBV-infected patients induced by PEG-IFN-α-2b-based treatment and assess risk factors associated with HBsAg recurrence.

Methods

Ethics

The study protocol was approved by the Institutional Review Board of Tangdu Hospital (202301-06), Yuncheng Central Hospital (YXLL2024072), and Air Force Hospital of Southern Theatre Command (2024-01). The Ethics Committees waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this was a retrospective study, and only characteristics and laboratory indicators were collected. The data were collected in December 2024. We used an anonymized database for all analyses, and all potentially identifying variables were removed. The study involving human participants was conducted in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This trial is a part of ZhuFeng Project (The Clinical Cure Project of Chronic Hepatitis B in China; ClinicalTrials.gov, identifier: NCT04035837) and a part of E-Cure Study (Real

World Study of Peginterferon α-2b Treatment for Inactive Chronic Hepatitis B Patients; ClinicalTrials.gov, identifier NCT05182463).

Study design

A retrospective observational cohort study was performed. Patients were enrolled from August 2020 to August 2023 at Tangdu Hospital, Yuncheng Central Hospital, and Air Force Hospital of Southern Theatre Command. Inclusive criteria for CHB patients: ① Age 18 ~ 60 years; ② The diagnoses met the standard of the Chinese Guidelines for the Prevention and Treatment of Chronic Hepatitis B [3]; ③ More than 1 year history receiving NAs therapy. Inclusive criteria for IHC: ① Age 18 ~ 60 years; ② Positive for HBsAg for more than 6 months; ③ Negative for HBeAg and positive for anti-HBe; ④ Serum HBV DNA is less than 2000 IU/ml; ⑤ Normal liver function; ⑥ No antiviral agents were used before enrollment. The exclusion criteria included co-infection with other hepatitis viruses or human immunodeficiency virus, indication of decompensated liver cirrhosis, affliction with end-stage liver diseases, affliction by alcoholism or drug addiction, affliction by autoimmune diseases, and affliction by solid cancers or leukemia. The enrolled patients received PEG-IFN-α-2b (Y shape, 40 kDa; 180 μg, subcutaneous injection weekly; Xiamen Amoytop Biotech Co., Ltd., Xiamen, Fujian Province, China) therapy. IHC patients received PEG-IFN-α-2b monotherapy, whereas CHB patients received PEG-IFN-α-2b "add-on" strategy, which is defined as the addition of PEG-IFN-α to on-going NAs [25–27]. The dosage of PEG-IFN-α-2b was adjusted to 135 μg weekly if the neutrophil count was $< 0.75 \times 10^9$/L or the platelet count was $< 50 \times 10^9$/L, whereas PEG-IFN-α-2b was discontinued if the neutrophil count was $< 0.50 \times 10^9$/L or the platelet count was $< 25 \times 10^9$/L or if serious adverse events occurred according to the manufacturer's instruction [27]. "Treatment time" was defined as the duration from the initiation of PEG-IFN-α-2b therapy to HBsAg loss, whereas "consolidation time" was defined as the duration from confirmed HBsAg loss to cessation of PEG-IFN-α-2b treatment [28]. All medications, including PEG-IFN-α-2b and NAs, were discontinued after the completion of consolidation treatment. All patients underwent at least 48 weeks of followed-up after the discontinuation of medications. "Sustained responder" was defined as having continuously undetectable HBV DNA and HBsAg with normal liver function during the followed-up period. "Recurrence" was defined as the reappearance of HBsAg and/or HBV DNA at least twice over an interval of 4 weeks during the followed-up period. Clinical data were extracted from the medical records (demographic information and laboratory indicators) at three time points, including baseline (initiation of PEG-IFN-α-2b therapy), therapy cessation (end of consolidation time), and followed-up (at least 48 weeks after discontinuation of medications).

Evaluation of virological, serological, and biochemical indices

Serum HBV DNA was quantified *via* real-time fluorescence quantitative polymerase chain reaction using a commercial HBV DNA detection kit (Xiamen Amplly, Xiamen, Fujian Province, China) with a detection limit of 50 IU/ml. HBsAg, anti-HBs, HBeAg, hepatitis B e antibody (anti-HBe), and hepatitis B core antibody (anti-HBc) was quantified using the ARCHITECH HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc reagent kits (Abbott GmbH & Co., KG., Wiesbaden, Germany). The detection limit for HBsAg was 0.05 IU/ml. HBsAg loss was defined as an HBsAg level less than 0.05 IU/ml. Positive for anti-HBs was defined as an anti-HBs level higher than 10 mIU/ml. When anti-HBs level was lower than 10 mIU/ml (negative for anti-HBs), the anti-HBs value was defined as "0 mIU/ml" regardless of the detection value. Other laboratory parameters, including white blood cells (WBC), neutrophils, red blood cells (RBC), hemoglobin (HGB), platelets, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (T-BIL), total protein, albumin, and globulin, were also collected.

Statistical analyses

Statistical analyses were performed using SPSS Version 25.0 (IBM SPSS Software, Chicago, IL, USA) and SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA). For categorical variables, the data are presented as *n* (%). The Chi-squared test or Fisher's exact test was used for comparisons. Continuous variables were first analyzed for a normal distribution using the Shapiro-Wilk test. For continuous variables following a normal distribution, the data are presented as the means ± standard deviations. Student's *t* test was used for comparisons between two groups. For continuous variables following a skewed distribution, the data are presented as the median (interquartile range) [M (Q_1, Q_3)]. The Mann-Whitney *U* test was used for comparisons between two groups. The factors influencing recurrence were examined using univariate and multivariate stepwise logistic regression models, with the results expressed as odds ratios (OR) with 95% confidence intervals (CI). *P* values less than 0.05 were considered as statistically significant.

Results

Baseline characteristics of the enrolled patients

A total of 101 patients with chronic HBV infection were enrolled in this study. The baseline characteristics of the enrolled subjects are shown in Table 1. The average age of the enrolled patients was approximately 40 years, and 57 participants (56.44%) were male, with

Table 1 Baseline characteristics of enrolled patients

Characteristic	Total	CHB	IHC	*P* value (IHC vs. CHB)
Patients enrolled, *n*	101	70	31	
Male gender, *n* (%)	57 (56.44%)	37 (52.86%)	20 (64.51%)	0.278
Age (years)	39.93 ± 9.18	40.34 ± 10.05	39.00 ± 6.84	0.500
HBV DNA undetectable (< 50 IU/ml), *n* (%)	89 (89.11%)	52 (74.29%)	27 (87.10%)	0.150
HBV DNA detectable (> 50 IU/ml), *n* (%)	22 (20.79%)	18 (15.71%)	4 (12.90%)	
HBsAg (IU/ml)	47.13 (4.88, 218.9)	57.04 (8.92, 256.7)	18.15 (2.29, 147.7)	0.074
HBsAg < 10 IU/ml, *n* (%)	33 (32.67%)	19 (27.14%)	14 (45.16%)	
HBsAg 10 ~ 100 IU/ml, *n* (%)	32 (31.68%)	24 (34.29%)	8 (25.81%)	
HBsAg 100 ~ 1000IU/ml, *n* (%)	29 (28.72%)	22 (31.43%)	7 (22.58%)	
HBsAg > 1000 IU/ml, *n* (%)	7 (6.93%)	5 (7.14%)	2 (6.45%)	
HBeAg positive, *n* (%)	5 (4.95%)	5 (7.14%)	0 (0.00%)	0.127
PEG-IFN-α-2b monotherapy, *n* (%)	31 (30.69%)	0	31	
PEG-IFN-α-2b add-on NAs therapy, *n* (%)	70 (69.31%)	70	0	
ETV, *n* (%)	42 (60.00%)	42	0	
TDF, *n* (%)	17 (24.29%)	17	0	
TAF, *n* (%)	6 (8.57%)	6	0	
TMF, *n* (%)	5 (7.14%)	5	0	
Blood routine test				
White blood cells ($\times10^9$/L)	5.29 ± 1.39	5.03 ± 1.15	5.88 ± 1.71	0.004
Neutrophils ($\times10^9$/L)	3.14 ± 1.01	2.99 ± 0.81	3.49 ± 1.30	0.020
Platelets ($\times10^9$/L)	205.3 ± 57.60	201.0 ± 60.14	214.9 ± 51.00	0.263
Red blood cells ($\times10^{12}$/L)	4.91 ± 0.55	4.87 ± 0.59	5.01 ± 0.44	0.222
Hemoglobin (g/L)	148.7 ± 19.45	147.8 ± 19.28	150.7 ± 19.99	0.484
Liver function				
ALT (U/L)	23.00 (16.00, 30.75)	23.00 (16.00, 31.00)	21.50 (14.75, 28.25)	0.625
AST (U/L)	21.50 (18.00, 26.00)	22.00 (18.75, 26.00)	20.00 (18.00, 23.75)	0.159
T-BIL (μmol/L)	17.75 (10.60, 21.30)	17.00 (11.10, 22.23)	11.55 (9.35, 17.03)	0.036
Total protein (g/L)	73.46 ± 8.15	73.00 ± 9.10	74.60 ± 4.99	0.384
Albumin (g/L)	48.83 ± 3.38	48.94 ± 3.30	48.55 ± 3.60	0.613
Globulin (g/L)	25.41 ± 3.26	25.02 ± 3.43	26.27 ± 2.61	0.064

IHC: inactive hepatitis B virus carrier; CHB: chronic hepatitis B; HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; ETV: entecavir; TDF: tenofovir disoproxil fumarate; TAF: tenofovir alafenamide; TMF: tenofovir amibufenamide; ALT: alanine aminotransferase; AST: aspartate aminotransferase; T-BIL: total bilirubin

a male-to-female ratio of 1.30:1. Eighty-nine patients (88.11%) had undetectable serum HBV DNA at baseline, and the median HBsAg level was 47.13 (4.88, 218.9) IU/ml before PEG-IFN-α-2b treatment. Most of the participants (93.07%) had HBsAg levels less than 1000 IU/ml, and 65 patients (64.35%) had HBsAg levels less than 100 IU/ml. Five patients were positive for HBeAg. Thirty-one IHC patients who were treatment-naïve received PEG-IFN-α-2b monotherapy. Seventy CHB patients who had on-going NA treatment (including 42 with ETV, 17 with TDF, 6 with TAF, and 5 with TMF) received PEG-IFN-α-2b add-on therapy. The mean or median levels corresponding to the clinical indices, including blood routine tests and liver function, were within the normal ranges (Table 1). There were no remarkable differences in gender ratio, average age, and HBV DNA status between CHB patients and IHC (Table 1). Although CHB patients had slightly higher baseline HBsAg level than IHC, this difference failed to achieve statistical significance ($P = 0.074$, Table 1). CHB patients had lower baseline WBC and neutrophils counts and higher T-BIL level compared with IHC ($P < 0.05$, Table 1).

Sixteen patients (eight CHB and eight IHC) experienced HBsAg recurrence during the followed-up period, with a cumulative recurrence rate of 15.84%. The HBsAg recurrence rate was comparable between CHB patients (8/70, 11.43%) and IHC (8/23, 25.81%, $P = 0.068$). There were no significant differences in either demographic information (gender ratio and average age) or baseline laboratory indicators (virological, serological, biochemical, or blood routine indices) between the sustained responder group and the recurrence group in all enrolled patients (Table 2), in CHB patients (Table S1), or in IHC (Table S2) ($P > 0.05$).

Table 2 Comparison of baseline characteristics between sustained responder and recurrence

Characteristic	Sustained responder group	Recurrence group	*P* value
Patients enrolled, *n*	85	16	
Male gender, *n* (%)	47 (55.29%)	10 (62.50%)	0.723
Age (years)	39.95 ± 8.90	39.81 ± 10.86	0.956
HBV DNA undetectable (< 50 IU/ml), *n* (%)	66 (77.65%)	13 (81.25%)	0.749
HBV DNA detectable (> 50 IU/ml), *n* (%)	19 (22.35%)	3 (18.75%)	
HBsAg (IU/ml)	46.52 (4.88, 218.9)	57.12 (3.91, 286.2)	0.790
HBsAg < 10 IU/ml, *n* (%)	28 (32.94%)	5 (31.25%)	
HBsAg 10 ~ 100 IU/ml, *n* (%)	27 (31.77%)	5 (31.25%)	
HBsAg 100 ~ 1000IU/ml, *n* (%)	25 (29.41%)	4 (25.00%)	
HBsAg > 1000 IU/ml, *n* (%)	5 (5.88%)	2 (12.50%)	
HBeAg positive, *n* (%)	5 (5.88%)	0 (0.00%)	0.319
IHC with PEG-IFN-α-2b monotherapy, *n* (%)	23 (27.06%)	8 (50.00%)	0.421
CHB with PEG-IFN-α-2b add-on NAs therapy, *n* (%)	62 (72.94%)	8 (50.00%)	
ETV, *n* (%)	38 (61.30%)	4 (50.00%)	
TDF, *n* (%)	14 (22.58%)	3 (37.50%)	
TAF, *n* (%)	5 (8.06%)	1 (12.50%)	
TMF, *n* (%)	5 (8.06%)	0 (0.00%)	
Blood routine test			
White blood cells ($\times10^9$/L)	5.25 ± 1.37	5.50 ± 1.55	0.515
Neutrophils ($\times10^9$/L)	3.13 ± 0.96	3.18 ± 1.27	0.873
Platelets ($\times10^9$/L)	202.4 ± 54.97	220.7 ± 69.98	0.245
Red blood cells ($\times10^{12}$/L)	4.92 ± 0.57	4.87 ± 0.44	0.718
Hemoglobin (g/L)	149.4 ± 19.56	144.6 ± 18.91	0.368
Liver function			
ALT (U/L)	22.00 (15.50, 30.50)	24.00 (16.00, 32.00)	0.909
AST (U/L)	22.00 (18.50, 26.00)	20.00 (18.00, 26.00)	0.291
T-BIL (μmol/L)	16.60 (10.65, 22.25)	13.30 (10.20, 16.50)	0.172
Total protein (g/L)	73.61 ± 8.38	72.59 ± 6.78	0.667
Albumin (g/L)	49.08 ± 3.20	47.32 ± 4.08	0.071
Globulin (g/L)	25.40 ± 3.29	25.46 ± 3.20	0.943

HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; IHC: inactive hepatitis B virus carrier; CHB: chronic hepatitis B; ETV: entecavir; TDF: tenofovir disoproxil fumarate; TAF: tenofovir alafenamide; TMF: tenofovir amibufenamide; ALT: alanine aminotransferase; AST: aspartate aminotransferase; T-BIL: total bilirubin

Characteristics of chronic HBV-infected patients who achieved HBsAg loss based on PEG-IFN-α-2b treatment at therapy cessation

All enrolled patients achieved functional cure with HBV DNA negativity, HBsAg loss, anti-HBe positivity, and anti-HBc positivity. The median total course of PEG-IFN-α-2b-based therapy was 42.00 (24.00, 59.50) weeks. The median treatment time of all patients was 24.00 (14.50, 37.50) weeks. Seventy-three patients (72.28%) received consolidative treatment, and the median consolidation time of all patients was 11.00 (0.00, 24.00) weeks. There were no remarkable differences in total course PEG-IFN-α-2b injection or treatment time between the sustained responder group and the recurrence group (Table 3). Although the median consolidation time was longer in the sustained responder group (12 weeks) than in the recurrence group (6 weeks), this difference failed to achieve statistical significance ($P = 0.116$, Table 3). There were no remarkable differences in treatment or consolidation time between sustained responder group and recurrence group in either CHB (Table S3) patients or IHC (Table S4) ($P > 0.05$). Importantly, fifty-nine patients (58.42%) achieved anti-HBs positivity at therapy cessation. The percentage of anti-HBs-positive patients was robustly higher in the sustained responder group (55/85, 64.71%) than in the recurrence group (4/16, 25.00%) ($P = 0.003$, Table 3). The median anti-HBs titer was also significantly higher in the sustained responder group than in the recurrence group (28.70 mIU/ml vs. 0.00 mIU/ml, $P = 0.017$, Table 3) in all enrolled patients. Similar trends were also found in CHB patients, revealing that sustained responder group had higher percentage of anti-HBs positivity (69.35% vs. 12.50%, $P = 0.002$, Table S3) and higher anti-HBs titer (52.45 mIU/ml vs. 0.00 mIU/ml, $P = 0.006$, Table S3) than recurrence group. However, these differences failed to achieve statistical significance in IHC (Table S4). Compared with the patients in the recurrence group, those in the sustained responder

Table 3 Comparison of characteristics at therapy cessation between sustained responder and recurrence

Characteristic	Sustained responder group	Recurrence group	*P* value
Patients enrolled, *n*	85	16	
Total PEG-IFN-α-2b injection (weeks)	44.00 (24.00, 59.50)	36.00 (21.00, 59.00)	0.852
Treatment time (weeks)	24.00 (13.50, 36.00)	24.50 (15.75, 43.50)	0.450
Received consolidative therapy, *n* (%)	63 (74.12%)	10 (62.50%)	0.341
Consolidation time (weeks)	12.00 (0.00, 24.00)	6.00 (0.00, 12.00)	0.116
Anti-HBs positive, *n* (%)	55 (64.71%)	4 (25.00%)	0.003
Anti-HBs titer (mIU/ml)	28.70 (0.00, 203.0)	0.00 (0.00, 68.18)	0.017
Blood routine test			
White blood cells ($\times10^9$/L)	2.84 (2.30, 3.44)	3.21 (2.78, 3.92)	0.116
Neutrophils ($\times10^9$/L)	1.49 (1.14, 1.79)	1.62 (1.14, 2.30)	0.618
Platelets ($\times10^9$/L)	114.5 ± 45.26	150.3 ± 57.87	0.007
Red blood cells ($\times10^{12}$/L)	4.32 ± 0.58	4.26 ± 0.67	0.719
Hemoglobin (g/L)	131.0 ± 18.67	129.1 ± 22.97	0.710
Liver function			
ALT (U/L)	45.00 (29.50, 69.50)	48.00 (25.50, 96.50)	0.981
AST (U/L)	45.00 (28.50, 70.00)	40.00 (32.75, 82.00)	0.996
T-BIL (μmol/L)	13.50 (10.60, 17.00)	12.30 (9.30, 16.58)	0.421
Total protein (g/L)	72.14 ± 3.89	74.11 ± 2.98	0.067
Albumin (g/L)	45.85 ± 3.25	45.51 ± 3.75	0.714
Globulin (g/L)	26.26 ± 3.72	28.58 ± 2.92	0.024

PEG-IFN-α-2b: pegylated interferon-α-2b; Anti-HBs: hepatitis B surface antibody; ALT: alanine aminotransferase; AST: aspartate aminotransferase; T-BIL: total bilirubin

group had significantly lower platelet counts ($P = 0.007$) and globulin levels ($P = 0.024$) (Table 3). Similarly, sustained responder group also had reduced platelet counts than recurrence group in CHB patients ($P = 0.007$, Table S3). However, globulin levels were comparable between sustained responder group and recurrence group in CHB patients ($P = 0.084$, Table S3) and IHC ($P = 0.081$, Table S4). There were no statistical differences in other blood routine or liver function indicators between the sustained responder group and the recurrence group at therapy cessation in all enrolled patients (Table 3), in CHB patients (Table S3), or in IHC (Table S4) ($P > 0.05$).

Characteristics of chronic HBV-infected patients who achieved HBsAg loss based on PEG-IFN-α-2b treatment during followed-up

All patients received at least 48 weeks of followed-up after the discontinuation of medications, with a median followed-up of 70.00 (48.00, 96.00) weeks. The longest followed-up time was 180 weeks. The median followed-up time for the sustained responder group was longer than that for the recurrence group (72 weeks vs. 48 weeks), but this difference still did not achieve statistical significance in all enrolled patients ($P = 0.124$, Table 4). The median followed-up time was longer in sustained responder group compared with recurrence group in CHB patients ($P = 0.011$, Table S5), but not in IHC ($P = 0.762$, Table S6). Sixteen patients who experienced recurrence were negative for serum HBV DNA, and only had HBsAg recurrence with very low median HBsAg levels [0.54 (0.16, 2.01) IU/ml]. One recurrence patient was positive for anti-HBs after discontinuation of medications, but the percentage of anti-HBs-positive patients was still significantly lower in the recurrence group (5/16, 31.25%) than in the sustained responder group (50/85, 58.82%) ($P = 0.042$, Table 4). The median anti-HBs titer was still higher in sustained responder group than in the recurrence group (31.00 mIU/ml vs. 0.00 mIU/ml, $P = 0.011$, Table 4). However, only median anti-HBs titer was higher in CHB patients with sustained responder than in recurrence (49.70 mIU/ml vs. 0.00 mIU/ml, $P = 0.041$, Table S5), but not in IHC ($P > 0.05$, Table S6). Compared with the recurrence group, the sustained responder group had lower WBC, neutrophil, platelet counts ($P < 0.05$, Table 4). The RBC count and hemoglobin level were also lower in the sustained responder group than in the recurrence group, but these differences just missed statistical significances ($P = 0.054$ and $P = 0.071$, respectively, Table 4). Similar trends were also found in IHC (Table S6), but not in CHB patients (Table S5). There were no remarkable differences in liver function indicators between the sustained responder group and the recurrence group during the followed-up period in all enrolled patients (Table 4), in CHB patients (Table S5), or in IHC (Table S6) ($P > 0.05$). No patients suffered from end-stage liver diseases (including decompensated liver cirrhosis, liver failure, and hepatocellular carcinoma) during the followed-up period.

Twelve patients who suffered with HBsAg recurrence (eight CHB patients and four IHC) restarted PEG-IFN-α-2b monotherapy, and four patients (three CHB patients and one IHC) achieved functional cure again within 24

Table 4 Comparison of characteristics at followed-up between sustained responder and recurrence

Characteristic	Sustained responder group	Recurrence group	*P* value
Patients enrolled, *n*	85	16	
Followed-up time (weeks)	72.00 (48.00, 96.00)	48.00 (48.00, 82.00)	0.124
HBsAg level (IU/ml)	< 0.05	0.54 (0.16, 2.01)	-
Anti-HBs positive, *n* (%)	50 (58.82%)	5 (31.25%)	0.042
Anti-HBs titer (mIU/ml)	31.00 (0.00, 295.3)	0.00 (0.00, 32.58)	0.011
Blood routine test			
White blood cells ($\times10^9$/L)	3.48 (2.61, 4.72)	4.63 (3.82, 5.92)	0.004
Neutrophils ($\times10^9$/L)	2.01 (1.22, 2.72)	2.45 (2.19, 3.34)	0.014
Platelets ($\times10^9$/L)	153.1 ± 62.86	195.0 ± 50.90	0.023
Red blood cells ($\times10^{12}$/L)	4.46 ± 0.71	4.87 ± 0.64	0.054
Hemoglobin (g/L)	135.8 ± 22.42	147.8 ± 20.53	0.071
Liver function			
ALT (U/L)	23.00 (16.00, 38.00)	23.00 (18.00, 30.00)	0.670
AST (U/L)	27.00 (20.50, 40.50)	20.00 (18.00, 31.00)	0.058
T-BIL (μmol/L)	14.00 (11.40, 16.30)	15.20 (11.50, 19.25)	0.513
Total protein (g/L)	73.73 ± 4.56	73.38 ± 4.75	0.713
Albumin (g/L)	46.09 ± 3.53	45.52 ± 2.32	0.575
Globulin (g/L)	27.68 ± 4.10	28.25 ± 3.97	0.649

HBsAg: hepatitis B surface antigen; Anti-HBs: hepatitis B surface antibody; ALT: alanine aminotransferase; AST: aspartate aminotransferase; T-BIL: total bilirubin

weeks. Four IHC did not want to restart PEG-IFN-α-2b therapy due to the side effects and were only followed-up every 12 to 24 weeks.

Recurrence risk factors for chronic HBV-infected patients who achieve functional cure with PEG-IFN-α-2b-based therapy

We first included all enrolled patients to analyze the risk factors for recurrence at therapy cessation. In all enrolled subjects, univariate analyses revealed that gender, age, baseline HBsAg level, treatment time, consolidation time, PEG-IFN-α-2b-based treatment regimens (monotherapy or add-on NA therapy), WBC, neutrophil, RBC, HGB, aminotransferases, T-BIL, total protein, or albumin levels were not significantly associated with HBsAg recurrence at therapy cessation ($P > 0.05$, Table 5). Importantly, the presence of anti-HBs at therapy cessation robustly reduced the risk of recurrence in both the univariate analysis (OR: 0.18; 95% CI: 0.05–0.61; $P = 0.006$; Table 5) and multivariate-adjusted regression analysis (OR: 0.20; 95% CI: 0.05–0.75; $P = 0.017$; Table 5). Similar findings were also presented in CHB patients receiving PEG-IFN-α-2b add-on NAs therapy, showing that anti-HBs positive at therapy cessation strongly down-regulated the recurrence risk ($P < 0.05$, Table S7). In contrast, the platelet count slightly increased the risk of recurrence according to both the univariate analysis and multivariate-adjusted regression analysis in all enrolled patients (Table 5), in CHB patients (Table S7), and in IHC (Table S8) ($P < 0.05$).

We then analyzed the risk factors for HBsAg recurrence during the followed-up period after discontinuation of medications. The results of univariate analysis revealed that gender, age, baseline HBsAg level, treatment time, PEG-IFN-α-2b-based treatment regimens, and liver function indicators were not significantly associated with HBsAg recurrence during the followed-up period ($P > 0.05$, Table 6). The WBC, neutrophil, and RBC counts remarkably increased the HBsAg recurrence risk according to the univariate analysis ($P < 0.05$, Table 6). A longer consolidation time was associated with a reduced recurrence risk according to multivariate-adjusted regression analysis (OR: 0.92; 95% CI: 0.86–0.99; $P = 0.017$; Table 6), but this difference just missed the statistical significance according to the univariate model ($P = 0.097$, Table 6). Similar to the analysis at therapy cessation, HBsAg recurrence was strongly lower in anti-HBs-positive patients in both the univariate analysis and multifactorial analysis during the followed-up period in all enrolled patients (Table 6), in CHB patients (Table S9), and in IHC (Table S10) ($P < 0.05$).

Discussion

In the present study, we analyzed the clinical characteristics of chronic HBV-infected patients with HBsAg loss in response to PEG-IFN-α-2b-based therapy. All patients received at least 48 weeks of followed-up after discontinuation of antiviral medications. Thus, all patients could be identified as functional cure, which was defined as those who were negative for HBsAg, HBeAg and HBV DNA for at least 24 weeks after treatment [15, 16]. A total of 84.16% (85/101) of patients achieved sustained response with continuous HBsAg seroclearance during a median of 72 weeks of followed-up, whereas 15.84% (16/101) of patients experienced HBsAg recurrence but

Table 5 Logistic regression analysis of factors influencing recurrence at therapy cessation

Characteristics	Univariate model		Multivariate model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Male gender	1.35 (0.45–4.04)	0.595		
Age	0.99 (0.94–1.06)	0.955		
Baseline HBsAg level	0.99 (0.99–1.02)	0.966		
Treatment time	1.01 (0.99–1.03)	0.340		
Consolidation time	0.96 (0.91–1.01)	0.097		
Anti-HBs positive	0.18 (0.05–0.61)	0.006	0.20 (0.05–0.75)	0.017
IHC with PEG-IFN-α-2b monotherapy	1.00			
CHB with PEG-IFN-α-2b add-on ETV therapy	0.30 (0.08–1.12)	0.073		
CHB with PEG-IFN-α-2b add-on TDF therapy	0.62 (0.14–2.72)	0.522		
CHB with PEG-IFN-α-2b add-on TAF therapy	0.58 (0.06–5.69)	0.636		
CHB with PEG-IFN-α-2b add-on TMF therapy	< 0.01	0.999		
Blood routine test				
White blood cells	1.07 (0.74–1.54)	0.720		
Neutrophils	0.95 (0.58–1.54)	0.824		
Platelets	1.01 (1.00-1.02)	0.012	1.01 (1.00-1.02)	0.049
Red blood cells	0.85 (0.34–2.09)	0.716		
Hemoglobin	0.99 (0.97–1.02)	0.707		
Liver function				
ALT	1.00 (0.10–1.01)	0.365		
AST	1.00 (0.99–1.01)	0.859		
T-BIL	0.99 (0.92–1.08)	0.923		
Total protein	1.15 (0.99–1.13)	0.071		
Albumin	0.97 (0.82–1.11)	0.711		
Globulin	1.18 (1.01–1.37)	0.033	1.20 (1.02–1.42)	0.031

OR: odds ratio; 95% CI: 95% confidence interval; HBsAg: hepatitis B surface antigen; anti-HBs: hepatitis B surface antibody; IHC: inactive hepatitis B virus carrier; CHB: chronic hepatitis B; ETV: entecavir; TDF: tenofovir disoproxil fumarate; TAF: tenofovir alafenamide; TMF: tenofovir amibufenamide; ALT: alanine aminotransferase; AST: aspartate aminotransferase; T-BIL: total bilirubin

not HBV DNA positivity during 48 ~ 148 weeks of followed-up. The HBsAg recurrence rate was comparable between CHB patients and IHC. No patients experienced severe adverse events or progressed to end-stage liver disease during treatment or followed-up. Importantly, the presence of anti-HBs strongly reduced the HBsAg recurrence risk, which was pivotal for the cessation of antiviral therapy and might be a beneficial treatment endpoint for functionally cured HBV-infected patients.

Five HBeAg-positive patients achieved both HBsAg and HBeAg seroconversion at therapy cessation and maintained a sustained response during followed-up. This finding was consistent with the findings that HBeAg positivity at cessation of treatment was a predictor of HBsAg recurrence after HBsAg loss in HBeAg-positive CHB patients [29], and was partly due to the immunomodulatory effects of HBeAg during HBV infection. HBeAg could not only promote inflammatory cytokines production to induce the expression of macrophage miR-155 [30], but also activate liver sinusoidal endothelial cells to enhance intrahepatic $CD8^+$ T cell immunity and HBV clearance [31]. Our previous study also revealed that HBeAg stimulation in vitro elevated Toll-like receptor 2/4 expression on monocytes, leading to the monocytes activation [32]. The immune activation property of HBeAg might contribute to sustained HBsAg clearance. The cumulative HBsAg recurrence rate was 15.84%, and no occurrence of viremia was observed, which was similar to previous reports of approximately 10 ~ 15% HBsAg seroreversion in functionally cured CHB patients [28, 29, 33, 34]. Two major factors were associated with HBsAg seroreversion in functionally cured patients. On the one hand, functional cure is not equivalent to sterilizing cure, which is defined as the elimination of cccDNA and integrated HBV DNA [15]. cccDNA was the transcriptional template for both pregenomic RNA (the template for reverse transcription into HBV DNA) and messenger RNAs (the template for translation into viral proteins). A high level of HBV DNA integration, which was randomly distributed among chromosomes, was detected in all immune stages of chronic HBV infection [35]. NA therapy resulted in a minimal reduction in the cccDNA concentration even after many years of treatment [36]. CHB patients with functional cure had robust reductions in both intrahepatic cccDNA and HBV integration, but 72.92% (35/48) of the functionally cured CHB patients were still positive for intrahepatic cccDNA and integrated HBV DNA could also be detected [37].

Table 6 Logistic regression analysis of factors influencing recurrence at followed-up

Characteristics	Univariate model		Multivariate model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Male gender	1.35 (0.45–4.04)	0.595		
Age	0.99 (0.94–1.06)	0.955		
Baseline HBsAg level	0.99 (0.99–1.02)	0.966		
Treatment time	1.01 (0.99–1.03)	0.340		
Consolidation time	0.96 (0.91–1.01)	0.097	0.92 (0.86–0.99)	0.017
Anti-HBs positive	0.32 (0.10-1.00)	0.049	0.13 (0.03–0.52)	0.004
IHC with PEG-IFN-α-2b monotherapy	1.00			
CHB with PEG-IFN-α-2b add-on ETV therapy	0.30 (0.08–1.12)	0.073		
CHB with PEG-IFN-α-2b add-on TDF therapy	0.62 (0.14–2.72)	0.522		
CHB with PEG-IFN-α-2b add-on TAF therapy	0.58 (0.06–5.69)	0.636		
CHB with PEG-IFN-α-2b add-on TMF therapy	< 0.01	0.999		
Blood routine test				
White blood cells	1.52 (1.05–2.20)	0.029		
Neutrophils	2.12 (1.15–3.90)	0.016		
Platelets	1.01 (0.99–1.02)	0.183		
Red blood cells	3.13 (1.09–9.01)	0.034		
Hemoglobin	1.01 (0.98–1.03)	0.639		
Liver function				
ALT	0.99 (0.96–1.02)	0.413		
AST	0.96 (0.90–1.02)	0.146		
T-BIL	1.06 (0.99–1.15)	0.106		
Total protein	0.93 (0.84–1.03)	0.147		
Albumin	0.90 (0.78–1.05)	0.183		
Globulin	0.94 (0.84–1.05)	0.270		

OR: odds ratio; 95% CI: 95% confidence interval; HBsAg: hepatitis B surface antigen; anti-HBs: hepatitis B surface antibody; IHC: inactive hepatitis B virus carrier; CHB: chronic hepatitis B; ETV: entecavir; TDF: tenofovir disoproxil fumarate; TAF: tenofovir alafenamide; TMF: tenofovir amibufenamide; ALT: alanine aminotransferase; AST: aspartate aminotransferase; T-BIL: total bilirubin

A recent study also reported that HBsAg transcription shifted from cccDNA to integrated HBV DNA during treatment [38]. Thus, residual cccDNA and integrated HBV DNA contributed to HBsAg recurrence in functionally cured chronic HBV-infected patients. On the other hand, Architect HBsAg (Abbott) quantification was used in the present study and an HBsAg level less than 0.05 IU/ml was considered negative. However, a serum HBsAg < 0.05 IU/ml did not completely indicate HBsAg clearance. Anderson et al.. investigated the serum HBsAg level in 36 patients with HBsAg loss (HBsAg < 0.05 IU/ml using the conventional HBsAg assay) by using the HBsAg Next Qualitative assay (Abbott Labaratories), which has an analytical sensitivity cutoff of 0.005 IU/ml [18, 39]. A total of 30.56% (11/36) of patients experienced HBsAg seroreversion 24 weeks after discontinuation of antiviral medications, and 90.91% (10/11) of these individuals were HBsAg positive (0.005 ~ 0.05 IU/ml) at therapy cessation [18]. Furthermore, HBsAg was still detectable in 7.38% (59/800) of CHB patients with HBsAg seroclearance *via* the HBsAg Next Qualitative assay [40]. Thus, functionally cured chronic HBV-infected patients might still have residual serum HBsAg, which could not be detected by conventional HBsAg assays.

The risk factors associated with HBsAg recurrence in patients with PEG-IFN-α-2b-induced functional cure were then assessed. First, at therapy cessation, the platelet count was significantly lower than it was at baseline, whereas platelet count was lower in sustained responders in all enrolled subjects. The lower platelet count was also found in sustained responders in CHB patients, but not in IHC. During followed-up, WBC, neutrophil, platelet, and RBC counts were also remarkably lower in sustained responders in all enrolled subjects. However, the lower WBC, neutrophil, and RBC counts were only found in IHC, but not in CHB patients. Univariate models revealed that platelet count slightly increased the risk factors for HBsAg recurrence. Second, a longer consolidation time, but not treatment time, was significantly associated with a sustained functional cure. A previous study revealed that treatment time to attain HBsAg loss > 28 weeks is a risk factor for HBsAg seroreversion [33]. The median treatment time in our study was 24 weeks, with the longest treatment time being 123 weeks. However, we did not observe a statistical association between HBsAg recurrence and treatment time. Moreover, several reports have also indicated that a consolidation treatment for ≥ 12 weeks was a strong predictor for

sustained HBsAg loss [28, 29, 34]. In our study, 72.28% (73/101) of patients received consolidative treatment, and the median consolidation time of all patients was 11.00 (0.00, 24.00) weeks. Thus, we could not identify the cutoff time for consolidative treatment time for HBsAg recurrence risk. Third, the appearance and maintenance of anti-HBs was an independent predictor of sustained HBsAg seroclearance in both CHB patients and IHC, which was consistent with previous reports [28, 33, 34]. Although we did not confirm the cutoff value for anti-HBs titer during followed-up, it is still suggested that anti-HBs positivity might be important for sustained functional cure in PEG-IFN-α-2b-treated chronic HBV-infected patients.

There were several limitations in this study. First, this was a retrospective observational analysis in a real-world setting. The time points of patient enrollment were not consistent and several data (such as the time from diagnosis to treatment, the time on treatment in NAs) were not collected, leading to potential bias and missing data. A large-scale prospective cohort study with a longer observational time is needed to confirm the current findings. Second, the differences in immune status (such as cytokine profiles and cytotoxic T-cell count and functions) between patients who are susceptible to sustained response and those who have experienced recurrence should be monitored to further elucidate the potential mechanisms corresponding to HBsAg recurrence.

Conclusion

In summary, our present results provided the evidence that the cumulative HBsAg recurrence rate was 15.84% after discontinuation of medications in chronic HBV-infected patients who achieved functional cure with PEG-IFN-α-2b-based treatment. The appearance and maintenance of anti-HBs reduced the HBsAg recurrence risk. HBsAg loss with anti-HBs positivity might be more beneficial for chronic HBV-infected patients receiving PEG-IFN-α-2b-based therapy.

Abbreviations

ALT	Alanine aminotransferase
anti-HBc	Hepatitis B core antibody
anti-HBe	Hepatitis B e antibody
anti-HBs	Hepatitis B surface antibody
AST	Aspartate aminotransferase
CHB	Chronic hepatitis B
CI	Confidence interval
cccDNA	Covalently closed circular DNA
ETV	Entecavir
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HGB	Hemoglobin
IHC	Inactive HBV carrier
OR	Odds ratio
PEG-IFN-α	Pegylated interferon-α
NAs	Nucleos(t)ide analogue
RBC	Red blood cell
TAF	Tenofovir alafenamide
T-BIL	Total bilirubin
TDF	Tenofovir
TMF	Tenofovir amibufenamide
WBC	White blood cell

Supplementary Information

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Supplementary Material 1

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Author contributions

YZ, WLZ, LML, SHW, LJC, and JQL performed study conception and design. LJC, CQH, GRR, LLX, JL, YC, LJZ, CWW, HXC, ZRC, JQL, SHW, LML, WLZ, and YZ performed data collection and analysis. LLX, WLZ, and YZ performed statistical analysis. YZ and WLZ wrote the first draft of the manuscript. LJC, CQH, GRR, LLX, JL, YC, LJZ, CWW, HXC, ZRC, JQL, SHW, and LML made critically review for the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of Tangdu Hospital (202301-06), Yuncheng Central Hospital (YXLL2024072), and Air Force Hospital of Southern Theatre Command (2024-01). The Ethics Committees waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this was a retrospective study, and only characteristics and laboratory indicators were collected. We used an anonymized database for all analyses, and all potentially identifying variables were removed. The study involving human participants was conducted in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This trial is a part of ZhuFeng Project (The Clinical Cure Project of Chronic Hepatitis B in China; ClinicalTrials.gov, identifier: NCT04035837) and a part of E-Cure Study (Real World Study of Peginterferon α-2b Treatment for Inactive Chronic Hepatitis B Patients; ClinicalTrials.gov, identifier NCT05182463).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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