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Research Article

Clinical outcomes after HBsAg clearance in chronic hepatitis B patients treated with Peg-IFN α: A study with an 11- to 173-month follow-up

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ABSTRACT

To investigate the risk and influencing factors of long-term liver adverse events in chronic hepatitis B patients achieving hepatitis B surface antigen (HBsAg) clearance after pegylated interferon α (Peg-IFN α) treatment, a retrospective analysis was conducted on 456 patients at Beijing Ditan Hospital from 2008 to 2023 who achieved HBsAg clearance and discontinued Peg-IFN α treatment. The baseline was defined as the time of HBsAg clearance and treatment cessation. The endpoint was the first occurrence of liver adverse events (hepatocellular carcinoma or ascites) or last follow-up. Subsequently, we evaluated the incidence and risk factors of liver adverse events, along with changes in liver fibrosis, cirrhosis, and liver function indicators. During a median follow-up of 70 months, the incidence of liver adverse events was 2.30%, hepatocellular carcinoma 1.76%, and ascites 0.55%. Older age and cirrhosis were significant risk factors (HR 1.075 and 41.393, both $P < 0.01$). The APRI score significantly improved at follow-up compared to baseline (0.53 vs. 0.25, $P < 0.001$), and cirrhosis prevalence decreased from 5.70% to 0.88% ($P < 0.001$). In conclusion, patients who achieved HBsAg clearance and discontinued Peg-IFN α treatment have a low risk of liver adverse events, while advanced age and cirrhosis remain major risk factors.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a major global health issue, with the potential to progress to cirrhosis, decompensated liver cirrhosis, and significantly increase the risk of hepatocellular carcinoma (HCC). To minimize the occurrence of adverse liver clinical events, the treatment goal has shifted from achieving negativity for hepatitis B virus deoxyribonucleic acid (HBV DNA) and hepatitis B e antigen (HBeAg) to achieving negativity for hepatitis B surface antigen (HBsAg) (Terrault et al., 2018; EASL, 2017).

The therapeutic drugs against chronic HBV infection encompass pegylated interferon α (Peg-IFN α) and nucleoside analogues (NAs) (Lok, 2024). NAs can effectively inhibit HBV DNA replication, but their impact on achieving HBsAg clearance is limited. Patients who take NAs orally for a long time have an HBsAg seroconversion rate of 0–4% (Marcellin et al., 2019; van Bömmel et al., 2023). Compared with NAs, the HBsAg seroconversion rate of patients treated with Peg-IFN α is notably higher, although it remains relatively low at 7.8%–10% in the overall population (Bourlière et al., 2017; Farag et al., 2024).

Due to the low HBsAg seroconversion rate, there are relatively few clinical studies on this special population compared to HBsAg-positive patients. Clinical observation showed that patients who achieve HBsAg serological clearance through Peg-IFN α treatment exhibit improved clinical outcomes (Coffin et al., 2022; Li et al., 2022,b).

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Previous reports have shown that HBsAg-negative patients have a significantly lower risk of developing HCC and cirrhosis compared to HBsAg-positive patients [adjusted hazard ratio (aHR) = 0.206, $P < 0.01$, and aHR = 0.361, respectively, $P < 0.01$] (Okada et al., 2024). However, little is known about the long-term risk of liver adverse events and associated influencing factors following HBsAg clearance and IFN-a treatment discontinuation.

Therefore, in this real-world observational cohort regression analysis study, we analyzed the clinical outcomes of patients with HBsAg clearance and discontinued Peg-IFN α treatment through long-term follow-up. Liver function indicators at treatment discontinuation (baseline) and the follow-up endpoint, including alanine aminotransferase (AST), aspartate aminotransferase (AST), albumin (ALB), hemoglobin (HGB), platelet (PLT), were collected and compared. Factors associated with the occurrence of liver adverse events were analyzed. Our findings may have clinical significance for guiding the management of this special population.

RESULTS

Patient demographics and baseline data

A total of 645 patients who achieved HBsAg loss and discontinued Peg-IFN α treatment were initially enrolled. Among them, 189 patients who were followed up for less than 48 weeks after Peg-IFN α treatment discontinuation and did not meet the inclusion criteria were excluded (Fig. 1A). Finally, 456 patients were included for analysis and study, with a median follow-up duration of 70 (11–173) months (Fig. 1B). The median age of at the time of HBsAg clearance and IFN-α treatment discontinuation was 39.00 (33.00, 45.00) years. In this study, liver-related clinical adverse events were defined as HCC and ascites. Patients with liver adverse events (Group A) were significantly older than those without liver adverse events (Group B) [48.00 (43.75, 57.50) vs. 39.00 (33.00, 45.00) years, $Z = -3.050$, $P = 0.002$].

Liver fibrosis and cirrhosis were evaluated using aspartate aminotransferase to platelet ratio index (APRI) (Amernia et al., 2021). At baseline, the proportion of patients with APRI ≥ 2 was significantly higher in group A (40%) than in group B (4.93%) ($P = 0.001$). There were no significant differences in other liver function indicators, including ALT, AST, ALB, HGB, and PLT, between group A and group B ($P > 0.05$). The overall percentage of patients achieving HBsAg loss was 81.80%. Although the rate in group A (90.00%) was higher than group B (81.61%), this difference was not significant ($\chi^2 = 0.070$, $P = 0.791$). The demographic and baseline data of patients are shown in Table 1.

Improvement of liver function and related indicators during the follow-up period

Liver function indicators were collected at medication discontinuation (baseline) and follow-up endpoint, including ALT, AST, ALB, HGB, and PLT (Table 2). At the follow-up endpoint, ALT and AST levels were significantly lower than baseline [20.50 (13.53, 30.80) vs. 31.55 (17.73, 45.40) U/L, Z = −10.481, $P < 0.001$; 19.90 (16.60, 24.78) vs. 27.75 (22.00, 37.68) U/L, Z = −13.566, $P < 0.001$]. The levels of HGB, PLT, and ALB at the follow-up endpoint were significantly higher than baseline [153.00 (139.25, 163.00) vs. 140.00 (125.00, 151.00) g/L, Z = −14.861, $P < 0.001$; 204 × 10^9/L (170.00, 239.00) vs. 138.00 × 10^9/L (109.00, 178.75), Z = −16.431, $P < 0.001$; 47.20 (45.20, 49.20) vs. 46.50 (44.50, 48.40), Z = −4.821, $P < 0.001$, respectively].

The rates of ALT abnormality [59.87% vs. 31.14 %, $\chi^2 = 80.095$, $P < 0.001$], AST abnormality [23.25% vs. 4.61 %, $\chi^2 = 68.505$, $P < 0.001$], HGB abnormality [12.28 % vs. 3.95%, $\chi^2 = 25.352$, $P < 0.001$], and PLT abnormality [33.11% vs. 6.58%, $\chi^2 = 108.271$, $P < 0.001$] at the follow-up endpoint were significantly lower than at baseline. However, there was no significant difference in the abnormality rate of ALB between baseline and endpoint [16 (3.51%) vs. 15 (3.30%), $\chi^2 = -0.186$, $P = 0.853$] (Fig. 2).

Improvement of liver fibrosis indicators during follow-up period

The baseline and follow-up endpoint APRI scores of patients were 0.53 (0.37, 0.86) and 0.25 (0.19, 0.35), respectively, with a significant improvement was observed during the follow-up period ($Z = -15.765$, $P < 0.001$). The proportion of patients diagnosed with cirrhosis (APRI ≥ 2) at the end of follow-up (0.88%) was significantly lower than the percentage of patients diagnosed with cirrhosis at the time of discontinuation (5.70%) ($P < 0.001$) (Fig. 2). At baseline, there were 26 patients had an APRI score ≥ 2, while only 4 patients had APRI ≥ 2 at the end of follow-up. There were 430 patients with baseline APRI < 2, and none of them progressed to cirrhosis after discontinuing HBsAg therapy.

Incidence and influencing factors of clinical adverse events

During the follow-up period, 10 patients experienced liver adverse events (Group A), and 446 patients did not (Group B). With the extension of follow-up time after Peg-IFN α treatment discontinuation, the incidence of liver adverse events gradually increased. During the 70 (11–173) month follow-up period, the overall incidence of liver adverse events was 2.30%, with HCC and cirrhosis ascites rates of 1.76% and 0.55%, respectively. The cumulative survival analysis curve of adverse events is shown in Fig. 3.

Univariate Cox regression analysis identified baseline age and liver cirrhosis (APRI ≥ 2) as influencing factors for liver adverse events. Multivariate Cox regression analysis was performed on baseline age and APRI ≥ 2. The results showed that cirrhosis (HR = 41.393, 95%CI 4.773–358.958, $P < 0.001$) and age (HR = 1.075, 95%CI 1.028–1.125, $P = 0.002$) were significant risk factors for adverse events (Table 3).

Receiver operating characteristic (ROC) curve analysis was performed on age (Fig. 4), with a cut-off value of 42.5 years (sensitivity 0.90, specificity 0.67, Youden index 0.557, AUC 0.782). Kaplan Meier survival curve analysis with Log-Rank tests were performed to assess HCC and ascites incidence in patients with cirrhosis and aged ≥ 42.5 years old. There was a significant difference in the incidence of HCC among age groups (HR = 125.05, $P = 0.009$), but no significant difference was observed in the cirrhosis group (HR = 5.332, $P = 0.104$). By contrast, significant differences in clinical adverse events and ascites were found between age groups (HR = 17.037, $P < 0.001$; HR = 10.064, $P = 0.012$) and in cirrhosis groups (HR = 7.970, $P < 0.001$; HR = 10.058, $P < 0.001$) (Fig. 5).

DISCUSSION

CHB patients with HBsAg clearance have good clinical outcomes, which primarily depends on the host immune response (Li et al., 2018, 2021; Anderson et al., 2021). HBsAg clearance is an surrogate marker for enhanced host immune control over hepatitis B virus covalently closed circular DNA (HBV ccDNA) (Inoue et al., 2023). However, HCC can still occur in this population (Okada et al., 2024). Therefore, further exploration of HCC risk and associated factors for liver adverse clinical events (e.g. ascites) in patients with HBsAg clearance is warranted.

At present, the main drugs used to treat chronic HBV infection are Peg-IFN α and NAs, which can inhibit virus replication, reverse liver inflammation and fibrosis, and reduce the risk of cirrhosis and HCC, with particularly pronounced benefits for patients achieving HBsAg serological clearance (Terrault et al., 2018; EASL, 2017; Lok, 2024). However, HBsAg clearance remains infrequent across treatment modalities, including NAs monotherapy, NAs withdrawal therapy, and Peg-IFN α and NAs combination/sequential/intermittent treatment modes (Lok, 2024; Marcellin et al., 2019; Li et al., 2022,b; Pan et al., 2021). The clearance of HBsAg in NAs monotherapy is extremely rare. Patients who have received tenofovir disoproxil fumarate (TDF) treatment for up to

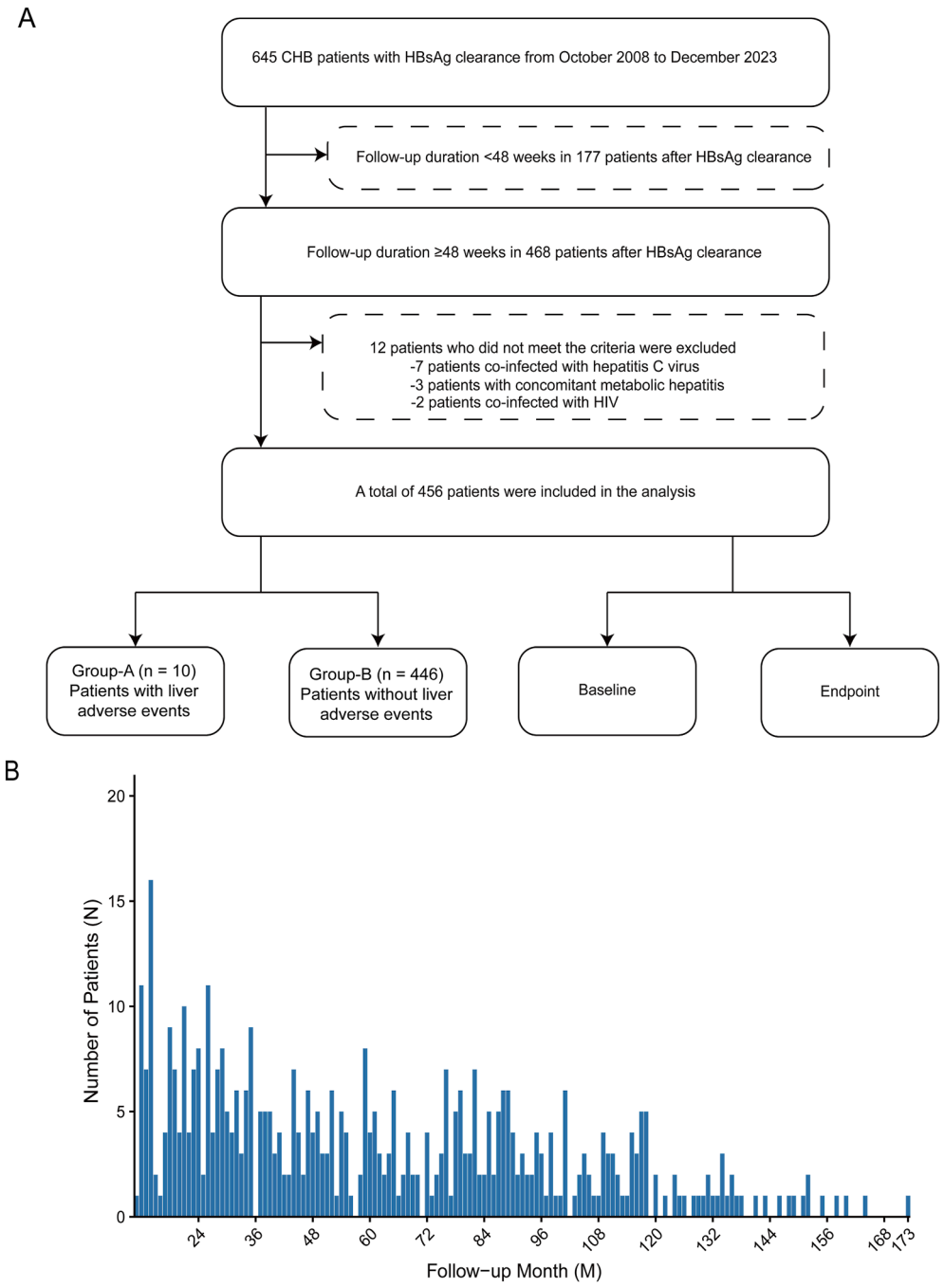

Fig. 1. Flow diagram of patient enrollment and the distribution of enrolled patients in this study. **A** Flow diagram of patients enrolled in this study. **B** Distribution of enrolled patients by month. A total of 456 patients were included in the analysis and study, with a median follow-up duration of 70 (11–173) months.

Table 1
Demographic and baseline characteristics of patients.

Values	All patients (n = 456)	Group-B (n = 446)	Group-A (n = 10)	Z/χ^2	*P*
Male (%)	312 (68.42%)	306 (68.61 %)	6 (60.00 %)	0.055	0.814
Age (years)	39.00 (33.00, 45.00)	39.00 (33.00, 45.00)	48.00 (43.75, 57.50)	−3.050	0.002
HBsAb+ (%)	373 (81.80%)	364 (81.61%)	9 (90.00%)	0.070	0.791
Abnormal ALT (%)	273 (59.87%)	267 (59.87%)	6 (60.00%)	< 0.001	> 0.999
Abnormal AST (%)	106 (23.25%)	102 (22.87%)	4 (40.00%)	0.792	0.374
Abnormal ALB (%)	16 (3.51 %)	15 (3.36%)	1 (10.00%)	Fisher	0.303
Abnormal HGB (%)	56 (12.28 %)	56 (12.56%)	0 (0.00%)	0.503	0.478
Abnormal PLT (%)	151 (33.11%)	145 (32.51%)	6 (60.00%)	2.211	0.137
APRI ≥ 2 (%)	26 (5.70%)	22 (4.93%)	4 (40.00%)	Fisher	0.001

Notes: Group-A: adverse event occurrence group; Group-B: no adverse event occurrence group. Statistical significance was defined as $P < 0.05$. HBsAb+, hepatitis B surface antibody (HBsAb)-positive; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; HGB, hemoglobin; PLT, platelet. APRI, AST to platelet ratio index, APRI = 100 × [[AST (U/L)/ULN of AST]/platelet count (10^9/L)], APRI ≥ 2 was defined as indicative of cirrhosis.

Table 2
Baseline and endpoint data.

Values	Baseline	Endpoint	Z/χ^2	P
APRI (scores)	0.53 (0.37, 0.86)	0.25 (0.19, 0.35)	−15.765	< 0.001
APRI ≥ 2 (%)	26 (5.70%)	4 (0.88%)	Fisher	< 0.001
ALT (U/L)	31.55 (17.73, 45.40)	20.50 (13.53, 30.80)	−10.481	< 0.001
AST (U/L)	27.75 (22.00, 37.68)	19.90 (16.60, 24.78)	−13.566	< 0.001
ALB (g/L)	46.50 (44.50, 48.40)	47.20 (45.20, 49.20)	−4.821	< 0.001
HGB (g/L)	140.00 (125.00, 151.00)	153.00 (139.25, 163.00)	−14.861	< 0.001
PLT (10^9/L)	138.00 (109.00, 178.75)	204 (170.00, 239.00)	−16.431	< 0.001

Notes: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; HGB, hemoglobin; PLT, platelet. APRI, AST to platelet ratio index, APRI = 100 × [[AST (U/L)/ULN of AST]/platelet count (10^9/L)], APRI ≥ 2 was defined as indicative of cirrhosis.

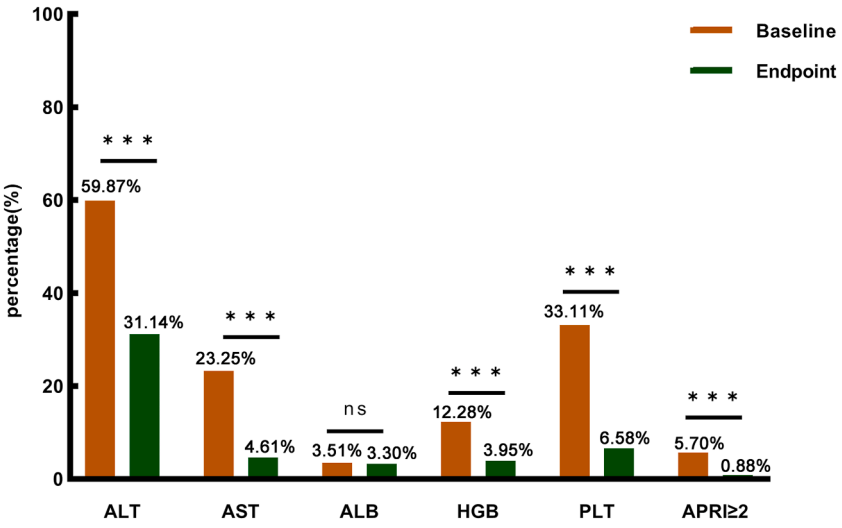

Fig. 2. The rates of patients with abnormal liver function at baseline and endpoint. Statistical significance was defined as $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$; ns indicates $P \geq 0.05$ (not statistically significant). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; HGB, hemoglobin; PLT, platelet; APRI, AST to platelet ratio index, APRI = 100 × [[AST (U/L)/ULN of AST]/platelet count (10^9/L)], APRI ≥ 2 was defined as indicative of cirrhosis.

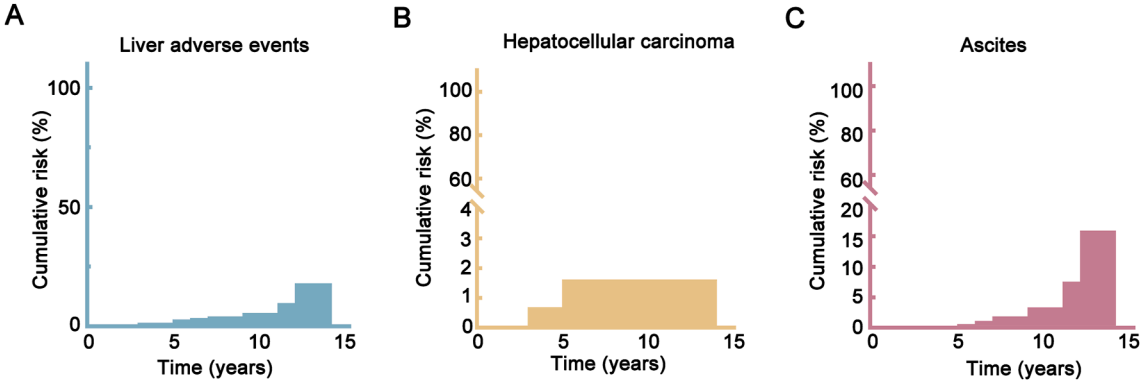

Fig. 3. Cumulative survival analysis curve for adverse events. Liver adverse events, including hepatocellular carcinoma and ascites, were analyzed. The cumulative incidences of overall liver adverse events (**A**), hepatocellular carcinoma (**B**), and ascites (**C**) after HBsAg clearance and IFN-α treatment discontinuation are presented in the figure.

10 years have an overall HBsAg seroconversion rate of 4.0% (Marcellin et al., 2019). Therefore, it is generally believed that NAs require lifelong medication. Notably, NAs withdrawal therapy may trigger beneficial immune responses leading to the clearance of HBsAg, particularly in patients with HBsAg <1000 IU/mL at the end of treatment (Johannessen et al., 2024; Hall et al., 2022). Emerging evidence even suggests that NAs withdrawal therapy may be superior to NAs sustained therapy in terms of HBsAg clearance (Brakenhoff et al., 2024; Hirode et al., 2024). In contrast, treatment regimen based on Peg-IFN α have demonstrated superior efficacy, with cumulative HBsAg seroconversion rates of 14% and 32% at 5 and 10 years, respectively (Choi et al., 2021), achieving a higher HBsAg seroconversion rate (Farag et al., 2024).

In this study, APRI score was employed as a tool to assess liver fibrosis severity, with APRI score ≥ 2 as the defining criterion to identify patients with cirrhosis. Results showed that the APRI score at follow-up endpoint were significantly lower than baseline values, and the proportion of patients diagnosed with cirrhosis also significantly decreased. For the 26 patients with baseline concomitant cirrhosis, only 4 patients remained in a state of cirrhosis at the follow-up endpoint. For patients without baseline cirrhosis, no patients progressed to cirrhosis at the end

Table 3
Cox regression analysis of hepatic adverse events.

Values	B	HR	95%CI	*P*-value	B	HR	95%CI	P-value
	Univariate analysis				Multivariate analysis			
Age (years)	0.068	1.071	1.025–1.118	0.002	0.072	1.075	1.028–1.125	0.002
Male (%)	−0.732	0.481	0.135–1.713	0.259				
HBsAb+ (%)	0.508	1.662	0.210–13.714	0.630				
APRI ≥2 (%)	2.076	7.970	2.185–29.072	0.002	3.723	41.393	4.773–358.958	< 0.001
Abnormal ALT (%)	−0.007	0.993	0.280–3.527	0.991				
Abnormal AST (%)	0.953	2.594	0.731–9.207	0.140				
Abnormal ALB (%)	0.776	2.172	0.271–17.439	0.466				
Abnormal HGB (%)	−3.166	0.042	0.000–403.780	0.498				
Abnormal PLT (%)	0.894	2.444	0.687–8.693	0.167				

Notes: Statistical significance was defined as $P < 0.05$. HBsAb+, hepatitis B surface antibody (HBsAb)-positive; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; HGB, hemoglobin; PLT, platelet. APRI, AST to platelet ratio index, APRI = 100 × [[AST (U/L)/ULN of AST]/platelet count (10^9/L)], APRI ≥ 2 was defined as indicative of cirrhosis.

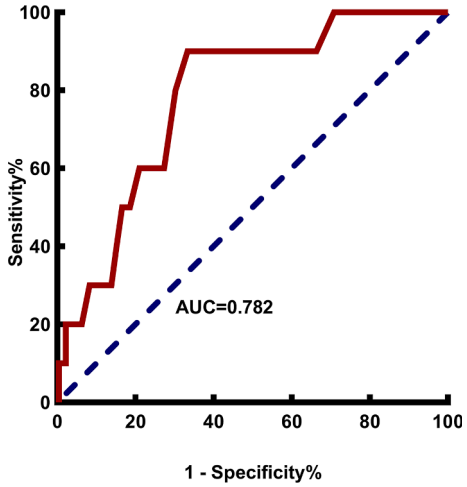

Fig. 4. Receiver operating characteristic curve for HCC. The figure demonstrated the receiver operating characteristic (ROC) age curve for the prediction of hepatocellular carcinoma (HCC). Age, AUC = 0.782, Youden index = 0.557, sensitivity = 0.90, specificity = 0.67.

of follow-up. A study conducted in Taiwan reported that HBsAg-negative patients without other viruses co-infection did not develop cirrhosis at the end of follow-up, which is consistent with our findings (Chen et al., 2002). The results of this study suggest that chronic HBV-infected patients who achieve HBsAg clearcance after interferon treatment exhibit gradual regression of liver fibrosis, with most cirrhosis patients experiencing reversal and non-cirrhotic patients rarely developing liver fibrosis.

Following HBsAg clearance and IFN-α treatment discontinuation, cirrhosis reversal was observed in 22 patients, which may be associated with extracellular matrix (ECM) degradation, functional hepatocyte regeneration, and restoration of normal liver architecture (Bedossa et al., 2015). ECM components, such as elastin, hyaluronic acid, and proteoglycans, possess the capacity to activate hepatic stellate cells (HSCs). Upon metalloproteinase-mediated degradation of these components, activated HSCs are eliminated through either phenotypic reversion to quiescence or programmed cell death——a process precisely regulated by signaling pathways such as TGF-β/Smad (Arthur et al., 1995; Iredale et al., 1998; Hernandez-Gea et al., 2011; Baek et al., 2024). Hepatocyte regeneration represents the core event of reversal. In viral hepatitis-induced cirrhosis, sustained virological suppression constitutes a fundamental the prerequisite for halting hepatic inflammatory responses and initiating regeneration (Sun et al., 2024). Architectural restitution of the liver parenchyma necessitates reversion from pathological nodular formations to physiologically normal lobular configurations. This reparative process is critically dependent upon reestablishing physiological perfusion gradients along the sinusoidal-central venous axis (Sun et al., 2024). In addition, we observed significant improvement in liver inflammation and synthesis function indicators, including ALT, AST, HGB, PLT, and ALB, at the follow-up endpoint compared to baseline levels. These results indicate that HBsAg-negative patients still show significant improvements in liver function status, liver fibrosis severity, and cirrhosis status after discontinuation of medication.

In this study, patients with HBsAg clearance and IFN-a treatment discontinuation were followed up for a median of 70 months (range: 11–173 months). Our showed that the cumulative incidence rates of clinical adverse events, including hepatocellular carcinoma (HCC) and ascites, were 2.30%, 1.76%, and 0.55%, respectively. Compared with untreated CHB patients or patients receiving NAs monotherapy in other studies, the risk of HCC occurrence is significantly reduced (Kumada et al., 2013). In Su et al.'s study, the cumulative incidence of HCC in untreated CHB patients and NAs monotherapy patients over four years was 17.5% and 9.4%, respectively (Su et al., 2016). In Kumada et al.'s study, the cumulative incidence rate over 10 years can reach as high as 40.0% (Kumada et al., 2013). Peg-IFN α has direct antiviral and immunomodulatory effects and is superior to NAs in inducing sustained immune control and clearance (Cao et al., 2022). Compared to NAs treatment, Peg-IFN α therapy has demonstrated superior efficacy in preventing liver adverse clinical outcomes and HCC occurrence in CHB patients, regardless of HBsAg clearance status (Lee et al., 2021). HBsAg clearance is an ideal treatment endpoint for CHB patients, with increasing evidence indicating that patients who achieve HBsAg clearance exhibit a further reduction in the risk of HCC and liver decompensation events (EASL, 2017; Anderson et al., 2021).

In the non-Peg-IFNα-treated population, a previous long-term follow-up study (223 months) of 432 inactive HBsAg carriers demonstrated spontaneous HBsAg loss in 49 patients (9.5%), with an HCC incidence of 10.2% during a mean 19.6-month post-clearance follow-up period (Ahn et al., 2005). Yip et al.'s analysis of 20,263 CHB patients receiving ETV/TDF monotherapy showed HBsAg loss in 376 cases (2.1%), with a 0.5% HCC incidence over a median 4.8-year follow-up (Yip et al., 2019). Within the Peg-IFNα-based therapeutic cohort of our investigation, patients achieving HBsAg loss exhibited a 1.76% HCC incidence during a 70-month (range: 11–173 months) post-treatment follow-up. Although the Peg-IFNα group showed numerically higher rates than the NA monotherapy group, it should be noted that significant differences in follow-up durations exist (19.6 months for spontaneous clearance vs. 4.8 years for NAs vs. 5.8 years for Peg-IFNα), and the cumulative HCC risk increases with extended follow-up, potentially influencing outcome interpretation. To comprehensively evaluate therapeutic differences, we

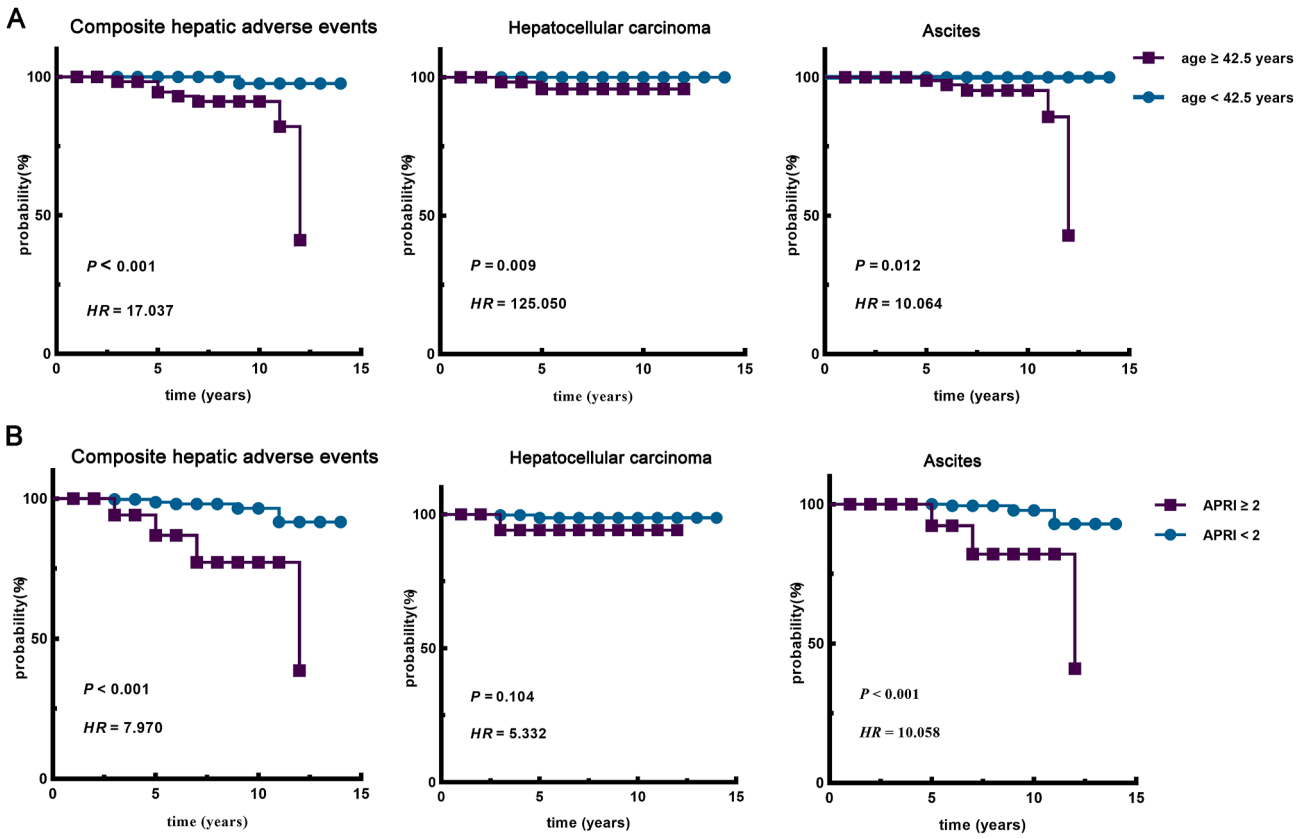

Fig. 5. Incidence of hepatic adverse events in the group with age ≥ 42.5 years (**A**) and liver cirrhosis (**B**). Statistical analysis was conducted using a two-tailed test. $P < 0.05$ is considered statistically significant. Composite hepatic adverse events, including hepatocellular carcinoma and ascites, were analyzed.

will conduct prospective multicenter studies on HBsAg loss patients in non-interferon treatment groups in future research.

HBsAg serological clearance can reduce liver decompensation events, but there is limited research on the influencing factors of liver decompensation events. Our study showed that age and cirrhosis are significant HCC-related risk factors for liver adverse clinical events after HBsAg clearance. In stratified analysis, the significant risk factors for ascites during follow-up were baseline age ≥ 42.5 years or concomitant cirrhosis, while the significant risk factor for HCC is baseline age ≥ 42.5 years. Although baseline cirrhosis was not identified as an independent risk factor for HCC development in our analysis, its presence conferred a significantly elevated HCC incidence compared to non-cirrhotic patients. Previous studies have reported that age, gender, cirrhosis, and family history of HCC at the time of HBsAg clearance and IFN-a treatment discontinuation are independent risk factors for HCC (Park et al., 2021). Therefore, when interpreting the risk factors for HCC in this study, we need to maintain a cautious attitude. This non-significant result may be attributed to the sample size limitation, HBV genotype variability, immune status heterogeneity, and the method used to evaluate cirrhosis.

This study is a retrospective analysis and did not collect potential confounding factors that may affect clinical outcomes, such as HBV genotype, family history, disease duration, and duration of interferon treatment. At the end of follow-up, only 10 patients experienced liver adverse events. The relatively small sample size may have some impact on the statistical power and analytical reliability of the findings. In addition, although we required a minimum follow-up of 48 weeks after HBsAg loss, the observation period remains insufficient to fully capture late-onset clinical outcomes, which may affect the assessment of long-term risks. Prospective studies with extended follow-up durations and collection of additional data are needed to comprehensively evaluate clinical outcomes and associated factors following HBsAg clearance.

CONCLUSIONS

This study demonstrated that chronic hepatitis B patients who achieved HBsAg clearance and discontinued IFN-α treatment had a low incidence of liver adverse events during long-term follow-up, indicating a favorable prognosis. Advanced age and baseline cirrhosis were identified as significant risk factors for liver adverse events. In addition, significant improvements in liver fibrosis and a marked reduction in the proportion of patients with cirrhosis were observed at the end of follow-up.

MATERIALS AND METHODS

Research subjects

Patients with chronic hepatitis B (CHB) who achieved HBsAg loss through Peg-IFN α treatment in Beijing Ditan Hospital affiliated with Capital Medical University from October 2008 to December 2023 and underwent long-term follow-up observation were included.

Inclusion criteria: 1) HBsAg clearance (HBsAg ≤ 0.05 IU/mL), along with HBV DNA and HBeAg negativity, was achieved through Peg-IFN α treatment; 2) Follow-up time after HBsAg loss ≥ 48 weeks; 3) Age ranged from 18 to 65 years old.

Exclusion criteria: 1) Complicated by other liver diseases, such as viral hepatitis A, C, D, and E, alcoholic hepatitis, autoimmune hepatitis, metabolic hepatitis, and non-alcoholic fatty liver disease; 2) Complicated by other viral infections that cause liver function damage, such as

EBV, CMV, and HIV; 3) At the time of enrollment, various malignant tumors were combined.

Data collection

During the patient's HBsAg loss and discontinuation of medication, liver function, kidney function, HBV virology, and serological indicators were tested every 3–6 months. Liver imaging (color Doppler flow imaging, computed tomography, or magnetic resonance imaging) was performed. Treatment information of CHB patients was collected via the Hospital Information System (HIS) and Laboratory Information System (LIS), including baseline data at the time of discontinuation of drug treatment (HBsAg loss and demographic data) and clinical endpoint data (the occurrence and timing of the first-onset liver adverse events, such as HCC and ascites). During follow-up period, liver function indicators [alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), hemoglobin (HGB), platelet (PLT)], HBV serum: [HBsAg, Hepatitis B surface antibody (HBsAb), HBeAg, HBV DNA], and liver imaging examination results were collected.

Clinical indicator detection method

All clinical biochemical and virological-related indicators were tested at the Clinical Laboratory Center of Beijing Ditan Hospital. The detection of HBsAg and HBsAb is carried out using Abbott Micro-Particle Chemiluminescence (Abbott i2000 Automatic Luminescence Immunoassay Analyzer). The upper limit of detection is HBsAg $>$ 250 IU/mL and HBsAb $>$1000 mIU/mL. HBsAb $\geq$ 10 mIU/mL is defined as HBsAb positive. HBV DNA was detected using fluorescence quantitative polymerase chain reaction technology (Roche, Switzerland, Light Cycler 480 PCR system), with a detection limit of 20 IU/mL. Liver function testing is conducted using Hitachi's 7180 fully automatic biochemical analyzer.

Evaluation indicators

Main evaluation indicators: incidence and influencing factors of liver adverse events (including HCC and ascites) during the follow-up period; Secondary evaluation indicators: Improvement in liver fibrosis, cirrhosis, and liver function indicators (Chinese Society of Hepatology, Chinese Medical Association and Chinese Society of Infectious Diseases, Chinese Medical Association, 2022).

Data analysis

All data were analyzed using SPSS 19.0. Count data are represented by frequency, with independent samples using Chi-square test and paired samples using McNemar test. The Shapiro-Wilk test was conducted to assess normality of the measurement data. Measurement data that conform to a normal distribution were described as mean $\pm$ standard deviation, while those that don't conform to a normal distribution were described as median (quartiles). Two sample *t*-test is used for independent samples with normal distribution, and Mann-Whitney *U* test is used for independent samples whose data do not conform a normal distribution. For paired samples, the paired samples *t*-test is appropriate when the differences between paired observations follow a normal distribution, while the Wilcoxon signed-rank test is applied when such differences do not meet the normality assumption.

Estimating the incidence of liver adverse events through Kaplan Meier and exploring the influencing factors of liver adverse events through COX regression analysis. All analyses were conducted in GraphPad Prism9.5 software using a two-tailed test, with $P < 0.05$ indicating statistically significant differences.

DATA AVAILABILITY

All the data generated during the current study are included in the manuscript.

ETHICS STATEMENT

The trial was approved by the Ethics Committee of Beijing Ditan Hospital, affiliated to Capital Medical University (Ethics Approval No. Beijing Dilun Research Zi [2023] No. (009)-01) and registered at the clinicaltrials.gov (ID: NCT04301908). This is a retrospective, observational study, and it does not affect the patients' treatment plan, progression or prognosis, and it meets the criteria for a waiver of informed consent.

AUTHOR CONTRIBUTIONS

Wen Deng: Data curation, Formal analysis, Writing – original draft, Hongxiao Hao: Investigation, Methodology, Ziyu Zhang: Visualization, Investigation; Xinxin Li: Investigation, Data curation; Weihua Cao: Investigation; Yaqin Zhang: Investigation; Shiyu Wang: Investigation; Zixuan Gao: Investigation; Linmei Yao: Investigation; Shuojie Wang: Investigation; Xin Wei: Investigation; Wei Yi: Conceptualization, Resources; Linqing Zhao: Conceptualization, Methodology; Yao Xie: Conceptualization, Writing – review & editing; Minghui Li: Resources, Writing – review & editing, Funding acquisition.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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