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Original Article

Peripheral Blood CD4⁺/CD8⁺ T Cell Ratio Predicts HBsAg Clearance in Inactive HBsAg Carriers Treated with Peginterferon Alpha

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Abstract

Background and Aims: T lymphocytes play a pivotal role in resolving hepatitis B virus infection. This study aimed to investigate the dynamics of peripheral blood T lymphocyte subsets during peginterferon alpha (peg-IFN-α) therapy and their association with hepatitis B surface antigen (HBsAg) clearance in inactive HBsAg carriers (IHCs). ***Methods:*** This prospective observational study enrolled 197 IHCs treated with peg-IFNα-2a/2b for 48 weeks and followed for 24 weeks (treatment group), and 221 IHCs who were regularly monitored for 72 weeks without treatment (IHC control group). Peripheral blood T lymphocyte subsets were evaluated using flow cytometry at baseline, and at 12, 24, 48, and 72 weeks in both groups. At 72 weeks, IHCs in the treatment group were categorized into an HBsAg clearance group and an HBsAg persistence group. Differences in T lymphocyte subsets among these groups were compared, and correlations between T lymphocyte subsets and HBsAg clearance were analyzed. ***Results:*** At 72 weeks, intention-to-treat analysis showed significantly higher HBsAg clearance (46.7%) and seroconversion rates (34.5%) in the treatment group compared to the IHC control group (HBsAg clearance rate of 1.4%, seroconversion rate of 0.9%; both $p < 0.001$). The median absolute counts of CD3⁺, CD4⁺, and CD8⁺ cells significantly decreased at 12, 24, and 48 weeks in both the HBsAg clearance and persistence groups, returning to baseline at 72 weeks (all $p < 0.001$). IHCs with HBsAg clearance had higher median percentages of CD3⁺ CD8⁺ cells and lower median percentages of CD3⁺ CD4⁺ cells and CD4⁺/CD8⁺ ratios at 12, 24, and 48 weeks compared to the HBsAg persistence and IHC control groups (all $p < 0.001$). Baseline HBsAg levels (below 2.0 $\log_{10}$ IU/mL) and hepatitis B virus DNA levels (below 20 IU/mL), alanine aminotransferase elevation at 12 weeks (greater than 2×upper limit of normal), and CD4⁺/CD8⁺ ratios (less than 1.5 at 12 weeks and below 1.4 at 24 weeks) were predictive of HBsAg clearance. ***Conclusions:*** Peripheral blood CD4⁺/CD8⁺ ratios at 12 and 24 weeks may serve as predictive markers for HBsAg clearance in IHCs treated with peg-IFN-α.

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Introduction

Inactive hepatitis B surface antigen (HBsAg) carriers (IHCs) represent a distinct subset of patients with chronic hepatitis B virus (HBV) infection, characterized by persistent HBsAg positivity for over six months, negative HBeAg, HBV DNA levels below 2,000 IU/mL, and normal alanine aminotransferase (ALT) levels.[1] While current guidelines from the European Association for the Study of the Liver, and the Chinese Society of Hepatology and Chinese Medical Association recommend a "watch and wait" approach for IHCs due to their low risk of disease progression, these patients remain at risk for spontaneous HBV reactivation, progressive liver function deterioration, cirrhosis, and the development of malignant liver tumors.[2,3] Given the long-term risks associated with HBV, IHCs could benefit greatly from a functional cure, which is defined by stable and undetectable levels of HBsAg and HBV DNA after a defined period of treatment.[4]

The resolution of HBV infection requires an intricate and coordinated interplay between innate and adaptive immune responses, particularly involving a robust response from HBV-specific T lymphocytes.[5–7] Both classical CD4⁺ and CD8⁺ T cells, the two primary subsets of T lymphocytes, are crucial for HBV clearance.[8] However, patients with chronic HBV infection often exhibit impaired immune responses, characterized by an imbalance in T lymphocyte subsets, a reduced number of T lymphocytes, and functional defects in HBV-specific T lymphocytes.[9] To achieve a functional cure, the immune system must be restored to effectively eradicate infected hepatocytes and prevent new infections.

Peginterferon alpha (peg-IFN-α), a cytokine with both immunomodulatory and antiviral properties, has been used as a

Keywords: Inactive HBsAg carriers; Peginterferon alpha; T lymphocyte subsets; HBsAg clearance; CD4⁺/CD8⁺ ratio; Predictive markers.

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primary therapeutic option for chronic hepatitis B (CHB).[3,10] Compared with nucleos(t)ide analogues (NAs), peg-IFN-α achieves higher HBsAg clearance rates in CHB patients. In contrast, even after more than ten years of NAs therapy, HBsAg clearance rates in CHB patients have been reported to be as low as 1–5%.[11] Due to the low functional cure rates of NAs, the application of peg-IFN-α for achieving a functional cure in IHCs has garnered increased attention from researchers in recent years. Previous studies have shown that peg-IFN-α-based regimens can significantly increase HBsAg clearance rates to approximately 44.7% - 47.9%.[12,13] Although the functional cure rate can be significantly increased by peg-IFN-α, the improved HBsAg clearance rate indicates that approximately half of IHCs respond poorly to peg-IFN-α. Furthermore, peg-IFN-α is associated with high costs and a range of adverse events (AEs). Therefore, there is a significant clinical need for reliable biomarkers with high predictive accuracy and convenient operability to predict the response to peg-IFN-α in IHCs. Some studies have explored the relationship between T lymphocyte subsets and the clinical response to peg-IFNα-2a therapy in HBeAg-positive CHB.[14] However, the specific relationship between T lymphocyte subsets and HBsAg clearance in IHCs treated with peg-IFN-α remains underexplored.

This study aimed to examine the dynamic changes in peripheral blood T lymphocyte subsets in IHCs undergoing peg-IFN-α treatment and to assess their correlation with HBsAg clearance. The findings of this investigation may help improve functional cure rates using peg-IFN-α antiviral therapy and contribute to the development of personalized treatment strategies for IHCs.

Methods

Patients and healthy controls (HCs)

This prospective, non-randomized, observational study was conducted at our institution from November 2015 to June 2021 and was approved by the Biomedical Ethics Committee of Xi'an Jiaotong University. Informed consent was obtained from all participants prior to enrollment. IHCs aged 18 to 65 years were included if they had a quantitative HBsAg ≤ 1,500 IU/mL, a liver stiffness measurement (LSM) value <12.4 kPa, no prior anti-HBV treatment history, and no evidence of cirrhosis. Exclusion criteria included co-infections with hepatitis A, C, D, or E; hepatocellular carcinoma; liver diseases caused by alcohol, drugs, or autoimmunity; and any contraindications for peg-IFN-α therapy.

HCs were recruited from individuals visiting the Physical Examination Center of our hospital during the same period. HCs were negative for HBsAg, had HBsAb levels >200 mIU/mL, and were free of viral infectious diseases or autoimmune disorders. HCs were matched with IHCs at a ratio of approximately 1:10 based on age (±5 years) and gender.

Study design

IHC participants were informed of the potential benefits, risks, and treatment outcomes associated with peg-IFN-α therapy and were given the option to participate in either the treatment group or the IHC control group. The treatment group received a 48-week course of peg-IFN α-2a (Pegasys; Roche, Shanghai) or peg-IFNα-2b (PegBeron; Xiamen Tebao Biological Engineering Co., Ltd., Xiamen) at a dosage of 180 μg/week subcutaneously, based on their preferences, followed by 24 weeks of follow-up. The IHC control group underwent regular follow-ups for 72 weeks. Peripheral blood T lymphocyte subsets were monitored throughout the study period.

At week 72, the treatment group was further divided into two subgroups based on treatment outcomes: the HBsAg clearance group (serum HBsAg levels <0.05 IU/mL, with or without HBsAb levels ≥10 mIU/mL) and the HBsAg persistence group (HBsAg levels ≥0.05 IU/mL). HBsAg clearance and seroconversion rates (seroconversion defined as HBsAg levels <0.05 IU/mL and HBsAb levels ≥10 mIU/mL) were calculated and compared between the treatment and IHC control groups. The predictive value of T lymphocyte subsets for peg-IFN-α treatment outcomes was evaluated by comparing the dynamic changes in T lymphocyte subsets among the HBsAg clearance group, the HBsAg persistence group, and the IHC control group.

Study assessments

Study assessments included clinical laboratory tests and AEs monitoring. The details of these methods were described in previous publications from the same research group and are not repeated here.[12,15,16]

T lymphocyte subsets analysis via flow cytometry

Absolute counts of peripheral blood $CD3^+$, $CD4^+$, and $CD8^+$ T cells, percentages of $CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ T cells, and the $CD4^+/CD8^+$ ratio were measured at baseline (week 0), 12, 24, 48, and 72 weeks. These analyses were conducted using a Cytomics FC 500 flow cytometer (Beckman Coulter Inc., Brea, CA, USA) and a CD45/CD4/CD8/CD3 detection kit (Beckman Coulter, Inc., Brea, CA, USA) with monoclonal antibodies labeled with different fluorochromes: CD45-FITC, CD4-RD1, CD8-ECD, and CD3-PC5. All procedures strictly followed the manufacturer's instructions. Approximately 3 mL of venous EDTA-anticoagulated whole blood was collected from both IHCs and HCs. One hundred μL of EDTA-anticoagulated whole blood was pipetted into a 12×75 mm tube and mixed with 10 μL of four-color monoclonal antibodies. The mixture was vortexed and incubated for 16 m at room temperature in the dark. Following incubation, 500 μL of red blood cell lysis buffer (BD Biosciences) was added, and the sample was incubated for another 12 m in the dark at room temperature. The sample was then treated with 2 mL of saline solution, mixed thoroughly, and centrifuged at 1,500 rpm for 5 m. After discarding the supernatant, the remaining pellet was resuspended in 500 μL of saline solution for analysis.

T lymphocyte subsets were analyzed using the Cytomics FC 500 flow cytometer. Lymphocytes were initially identified based on the characteristics of their low forward scatter and side scatter. Leukocytes were selected by gating on CD45-positive cells, and T lymphocytes were further isolated by gating on CD3-positive cells. $CD4^+$ T cells ($CD3^+$ $CD4^+$) and $CD8^+$ T cells ($CD3^+$ $CD8^+$) were differentiated within the CD3+ T cell population. The $CD4^+/CD8^+$ ratio was calculated based on the percentages of $CD4^+$ and $CD8^+$ T cells. Data were automatically processed using FlowJo 7.6.1 software.

Statistical analysis

Although per-protocol analysis is commonly used in prospective observational studies, intention-to-treat (ITT) analysis was employed as the primary analytical method in this study, given the significant impact of peg-IFN-α-related AEs on patient dropout rates. ITT analysis provides a more comprehensive evaluation of clinical outcomes by including all participants, reflecting the real-world effectiveness of peg-IFN-α. Missing data were imputed using the expectation-maximization algorithm.

Quantitative results were presented as means ± standard deviation or as medians with interquartile ranges, depending on the results of the normality test (Kolmogorov-Smirnov). Comparisons between the two groups were conducted using Student's t-test or the Mann-Whitney U test, with *p*-values adjusted for multiple comparisons using the Bonferroni correction. Count data were presented as frequencies (%) and analyzed using the χ2 test or Fisher's exact test. Repeated-measures analysis of variance or generalized estimating equations were applied to within-and between-group comparisons of repeated measurements, as appropriate based on the normality test results. The cumulative HBsAg clearance and seroconversion rates over 72 weeks, as well as group comparisons, were performed using the same methods described in our previous publications.[12,15,16] Additionally, univariate and multivariate logistic regression analyses were conducted to evaluate the predictive value of T lymphocyte subsets and other clinical indicators for HBsAg clearance at 72 weeks. Variables with a univariate *p*-value < 0.05 were included in the multivariate logistic regression model and adjusted for age and gender. Receiver operating characteristic (ROC) curves and the area under the ROC curves (AUCs) were employed to assess the performance of key predictors and identify optimal cut-off values. Statistical analyses were conducted using SPSS 25.0 software (IBM Corp., USA), with statistical significance indicated by a *p*-value below 0.05 or a corrected *p*-value.

Results

Patient characteristics

Figure 1 displays a flowchart outlining the patient participation process in the study. After screening 1,494 patients, 418 IHCs were initially recruited. Among the 197 IHCs in the treatment group, 182 patients reached the study endpoint, with 8 patients (4.1%) terminating treatment due to AEs associated with peg-IFN-α and 7 patients (3.6%) lost to follow-up due to inadequate adherence. Of the 221 IHCs in the IHC control group, 14 (6.3%) were lost to follow-up, and an additional 5 participants (2.3%) withdrew for the following reasons: NA treatments for HBV reactivation (n = 3), progression to cirrhosis (n = 1), and progression to hepatocellular carcinoma (n = 1). The study was completed with 202 (91.4%) participants in the IHC control group. Additionally, 40 HBV-uninfected healthy individuals were matched as HCs. The baseline characteristics of the treatment and IHC control group were balanced (Supplementary Table 1).

ITT analysis at week 72 revealed that 92 participants in the treatment group achieved HBsAg clearance. Table 1 provides the baseline and week 72 characteristics of the HBsAg clearance group, HBsAg persistence group, IHC control group, and healthy control group. The HBsAg clearance group exhibited a higher proportion of individuals with baseline HBsAg levels <100 IU/mL, baseline HBV DNA <20 IU/mL, and baseline LSM levels <9.0 kPa compared to both the HBsAg persistence group and the IHC control group (all *p*-values < 0.01).

HBsAg clearance and seroconversion

Per-protocol analysis (Fig. 2A and C) revealed significantly higher HBsAg clearance rates in the treatment group compared to the IHC control group at both 48 and 72 weeks (45.6% vs. 1.0% at 48 weeks, 50.5% vs. 1.5% at 72 weeks; $p < 0.001$ at both time points). Similarly, the treatment group exhibited significantly higher HBsAg seroconversion rates (31.3% vs. 0.5% at 48 weeks, 37.4% vs. 1.0% at 72 weeks; all *p*-values < 0.001).

ITT analysis (Fig. 2B and D) further confirmed the superiority of the treatment group in achieving both HBsAg clearance and HBsAg seroconversion rates compared to the IHC control group. At 48 weeks, HBsAg clearance rates were significantly higher in the treatment group (42.1% vs. 0.9%, $p < 0.001$), and this advantage persisted at 72 weeks (46.7% vs. 1.4%, $p < 0.001$). Similarly, HBsAg seroconversion rates were markedly higher in the treatment group at both 48 weeks (28.9% vs. 0.5%, $p < 0.001$) and 72 weeks (34.5% vs. 0.9%, $p < 0.001$).

Peripheral blood T lymphocyte subsets of IHCs and HCs

Prior to treatment, peripheral blood T lymphocyte subsets of IHCs were analyzed using flow cytometry (Fig. 3A–D) to assess the impact of chronic HBV infection on immune status. Figure 3E–K and Supplementary Table 2 present the comparisons of baseline T lymphocyte subsets between IHCs and HCs. No significant differences were observed in the absolute counts of $CD3^+$, $CD4^+$, and $CD8^+$ T cells, the percentages of $CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ T cells, or the $CD4^+/CD8^+$ ratio (all *p*-values > 0.05).

Dynamic changes of T lymphocyte subsets in IHCs

We then analyzed the impact of peg-IFN-α treatment on peripheral blood T lymphocyte subsets in IHCs. Longitudinal observations in the IHC control group showed no statistically significant changes in the median absolute counts of $CD3^+$, $CD4^+$, and $CD8^+$ T cells, the median percentages of $CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ T cells, or the median $CD4^+/CD8^+$ ratio compared to baseline levels (all *p*-values > 0.05) (Fig. 4 and Table 2).

At baseline, the median absolute counts of $CD3^+$, $CD4^+$, and $CD8^+$ cells were comparable among the HBsAg clearance group, HBsAg persistence group, and IHC control group (all *p*-values > 0.05). During treatment, the median counts of these T lymphocyte subsets in both the HBsAg clearance and HBsAg persistence groups were significantly lower than those in the control group at 12, 24, and 48 weeks (all *p*-values < 0.001). At week 72 (24 weeks after treatment), these counts returned to baseline levels, showing no significant differences from the IHC control group (all *p*-values > 0.05) (Fig. 4A–C and Table 2). No significant differences were observed between the HBsAg clearance and HBsAg persistence groups throughout the study (all *p*-values > 0.01; corrected *p*-value = 0.01). Furthermore, fluctuations in peripheral white blood cells (WBC) and lymphocytes (Fig. 4H–I) mirrored the patterns observed in the T lymphocyte subset counts (Fig. 4A–C).

Throughout the treatment, no significant differences in the median percentage of $CD3^+$ cells were observed among the three groups (all *p*-values > 0.05). However, the HBsAg clearance group exhibited a significantly lower median percentage of $CD3^+$ $CD4^+$ cells and $CD4^+/CD8^+$ ratio, and a markedly higher median percentage of $CD3^+$ $CD8^+$ cells at 12, 24, and 48 weeks compared to both the IHC control (all *p*-values < 0.01) and HBsAg persistence groups (all *p*-values < 0.05) (Fig. 4D–G and Table 2). In contrast, no significant differences were observed between the IHC control and HBsAg persistence groups (all *p*-values > 0.05).

Predictive values of T lymphocyte subsets for peg-IFN-α-induced HBsAg clearance

We assessed the predictive values of T lymphocyte subsets at early stages (baseline, week 12, and week 24) for peg-IFN-

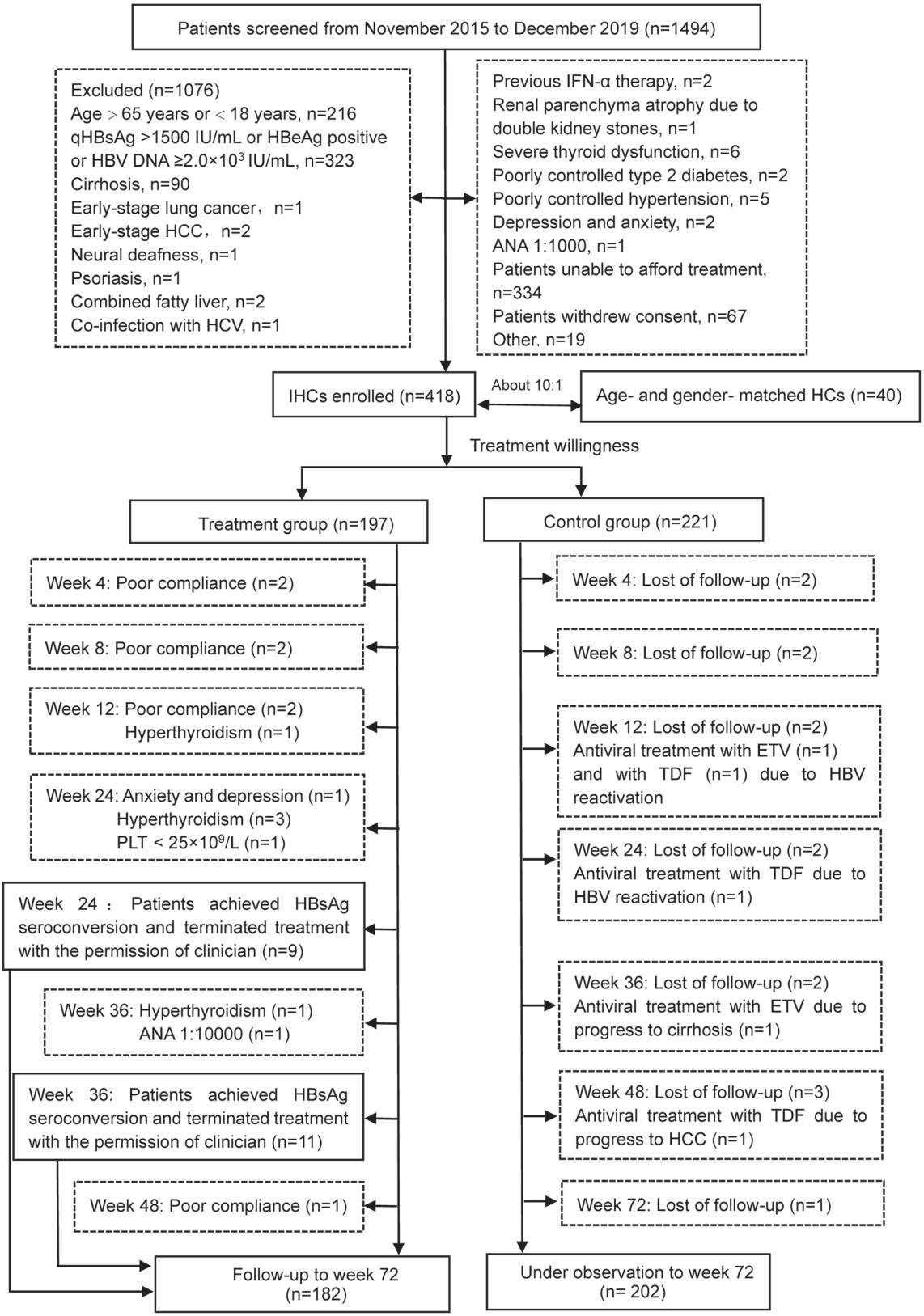

Fig. 1. Participant enrollment process. HCC, hepatocellular carcinoma; ANA, antinuclear antibody; IHCs, inactive hepatitis B surface antigen carriers; HCs, healthy controls; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; TDF, tenofovir disoproxil fumarate.

Table 1. Participants' demographic information and clinical characteristics at baseline and week 72

Characteristics	HBsAg clearance (n = 92)	HBsAg persistence (n = 105)	IHC control group (n = 221)	Healthy control group (n = 40)	*p*-value
Male (%)	56 (60.9)	78 (74.3)	142 (64.3)	26 (65.0)	0.204
Age, years, mean ± SD	36.8 ± 9.5	39.2 ± 10.7	37.0 ± 10.7	37.0 ± 9.8	0.282
BMI at baseline, kg/cm^2, mean ± SD	22.3 ± 1.2	22.5 ± 1.0	22.9 ± 1.0	23.1 ± 0.9	0.089
Mode of transmission					
Vertical (%)[†]	46 (50.0)	61 (58.1)	133 (60.2)	–	0.252
Others (%)[‡]	46 (50.0)	44 (41.9)	88 (39.8)	–	
HBsAg at baseline					
<100 IU/mL (%)	59 (64.1)	20 (19.0)	72 (32.6)	–	<0.001
100–500 IU/mL (%)	20 (21.7)	25 (23.8)	65 (29.4)	–	
500–1,500 IU/mL (%)	13 (14.1)	60 (57.1)	84 (38.0)	–	
HBV DNA at baseline					
<20 IU/mL (%)	53 (57.6)	13 (12.4)	82 (37.1)	–	<0.001
20-2,000 IU/mL (%)	39 (42.4)	92 (87.6)	139 (62.9)	–	
Genotype					0.084
A	2 (2.2)	4 (3.8)	10 (4.5)	–	
B	12 (13.0)	5 (4.8)	11 (5.0)	–	
C	1 (1.1)	20 (19.0)	28 (12.7)	–	
D	0 (0)	4 (3.8)	8 (3.6)	–	
Undetectable	77 (83.7)	72 (68.6)	164 (74.2)	–	
Transaminase at baseline, IU/L, median (Q1, Q3)					
ALT	22.0 (15.0, 31.0)	27.0 (17.0, 34.0)	24.0 (17.0, 33.0)	27.5 (18.3, 36.0)	0.141
AST	23.0 (18.0, 29.0)	25.0 (21.0, 31.0)	26.0 (21.0, 32.0)	25.5 (20.3, 33.8)	0.177
LSM at baseline, kPa					
<9.0 kPa, n (%)	83 (90.2)	75 (71.4)	174 (78.7)	–	0.005
9.0–12.0 kPa, n (%)	9 (9.8)	30 (28.6)	47 (21.3)	–	
Treatment					
Peg-IFNα-2a (%)	21 (22.8)	29 (27.6)	–	–	0.441
Peg-IFNα-2b (%)	71 (77.2)	76 (72.4)	–	–	
WBC at baseline, ×10^9/L, mean ± SD	5.6 ± 1.3	5.8 ± 1.3	6.0 ± 1.2	6.1 ± 1.1	0.222
T lymphocyte counts at baseline, ×10^9/L, mean ± SD	1.8 ± 0.6	1.8 ± 0.8	1.8 ± 0.6	1.8 ± 0.4	0.933
BMI at week 72, kg/cm^2, mean ± SD	21.8 ± 1.3	22.1 ± 1.1	22.8 ± 1.1	–	0.123

(continued)

Table 1. *(continued)*

Characteristics	HBsAg clearance (n = 92)	HBsAg persistence (n = 105)	IHC control group (n = 221)	Healthy control group (n = 40)	*p*-value
HBsAg at week 72					
<0.05 IU/mL (%)	92 (100)	0 (0)	3 (1.4)	–	<0.001
0.05–10 IU/mL (%)	0 (0)	24 (22.9)	36 (16.3)	–	
10–100 IU/mL (%)	0 (0)	32 (30.5)	53 (24.0)	–	
100–500 IU/mL (%)	0 (0)	34 (32.4)	63 (28.5)	–	
500–1,000 IU/mL (%)	0 (0)	11 (10.5)	50 (22.6)	–	
1,000–1,500 IU/mL (%)	0 (0)	3 (10.5)	16 (7.2)	–	
>1,500 IU/mL	0 (0)	1 (1.0)	0 (0)	–	
HBsAb at week 72					
<10 mIU/mL (%)	24 (26.1)	100 (95.2)	215 (97.3)	–	<0.001
10–100 mIU/mL (%)	42 (45.7)	5 (4.8)	6 (2.7)	–	
100–500 mIU/mL (%)	22 (23.9)	0 (0)	0 (0)	–	
>500 mIU/mL (%)	4 (4.3)	0 (0)	0 (0)	–	
HBV DNA at week 72					
<20 IU/mL (%)	92 (100.0)	86 (81.9)	87 (39.4)	–	<0.001
20- 2,000 IU/mL (%)	0 (0)	19 (18.1)	134 (60.6)	–	
Transaminase at week 72, IU/L, median (Q1, Q3)					
ALT	28.1 (20.3, 34.0)	28.0 (20.0, 35.0)	27.0 (20.3, 34.0)	–	0.372
AST	27.0 (22.0, 33.0)	29.0 (23.0, 34.5)	2.05 (22.0, 31.0)	–	0.269
LSM at week 72, kPa					
<9.0 kPa, n (%)	85 (92.4)	81 (77.1)	171 (77.4)	–	0.005
9.0–12.0 kPa, n (%)	7 (7.6)	24 (22.9)	50 (22.6)	–	
WBC at week 72, $\times 10^9$/L, mean ± SD	5.6 ± 1.4	6.0 ± 1.3	6.3 ± 1.3	–	0.435
T lymphocyte counts at baseline, $\times 10^9$/L, mean ± SD	1.8 ± 0.7	1.9 ± 0.7	1.8 ± 0.7	–	0.717

[†]Participants whose mothers had a clear history of chronic HBV infection. [‡]Participants whose mothers had no clear history of chronic HBV infection or whose history of chronic HBV infection was unclear. SD, standard deviation; BMI, body mass index; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HBsAb, hepatitis B surface antibody; IHC, inactive hepatitis B surface antigen carriers; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LSM, liver stiffness measurement; IU, international unit; mL, milliliter.

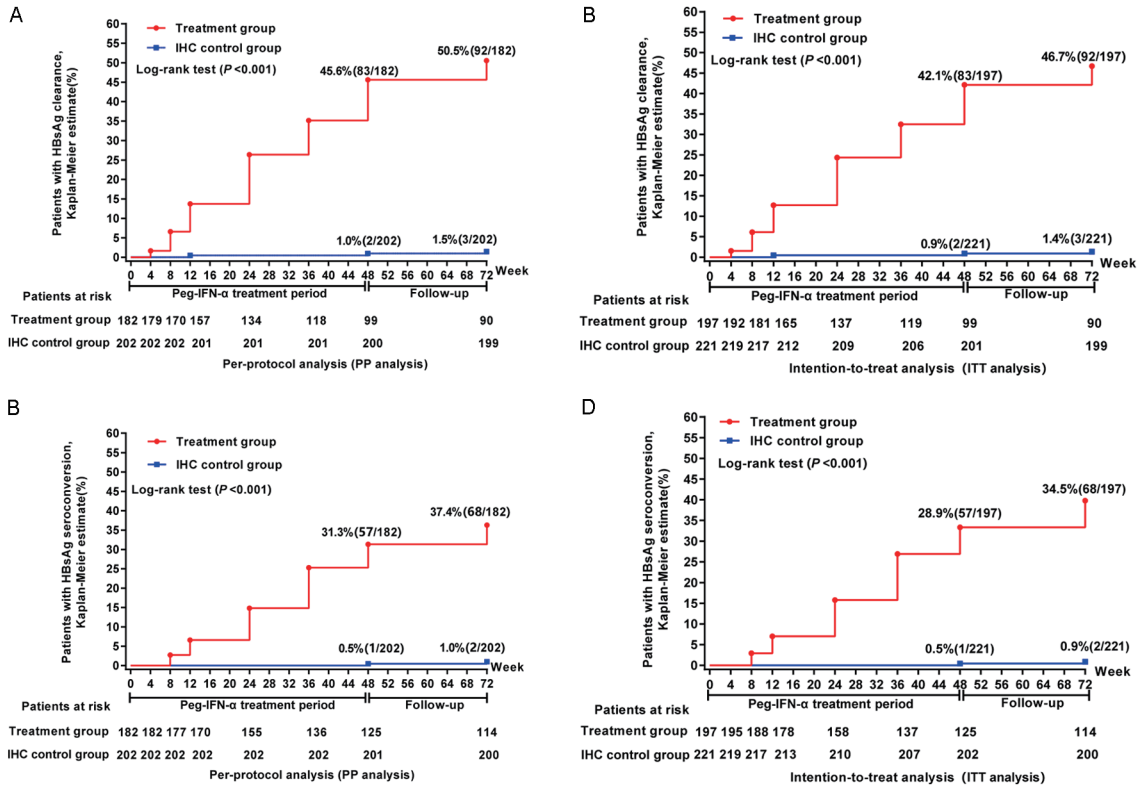

Fig. 2. HBsAg clearance and HBsAg seroconversion rates. (A) HBsAg clearance rate was analyzed by per-protocol (PP) analysis. (B) HBsAg clearance rate was analyzed by intention-to-treat (ITT) analysis. (C) HBsAg seroconversion rate was analyzed by PP analysis. (D) HBsAg seroconversion rate was analyzed by ITT analysis. HBsAg, hepatitis B surface antigen; Peg-IFN-α, peginterferon alpha.

α-induced HBsAg clearance. Univariate analysis showed a greater likelihood of HBsAg clearance at week 72 in individuals with lower percentages of $CD3^+CD4^+$ T cells at week 12 [OR (95% CI) 0.950 (0.912–0.989), $p = 0.013$] and week 24 [OR (95% CI) 0.910 (0.873–0.949), $p < 0.001$], lower $CD4^+/CD8^+$ ratios at week 12 [OR (95% CI) 0.246 (0.128–0.473), $p < 0.001$] and week 24 [OR (95% CI) 0.095 (0.044–0.203), $p < 0.001$], and higher percentages of $CD3^+CD8^+$ T cells at week 12 [OR (95% CI) 1.100 (1.049–1.154), $p < 0.001$] and week 24 [OR (95% CI) 1.158 (1.101–1.216), $p < 0.001$]. Additionally, several conventional indicators were associated with HBsAg clearance at week 72, including male sex [OR (95% CI) 0.538 (0.294–0.987), $p = 0.048$], baseline HBV DNA <20 IU/mL [OR (95% CI) 9.617 (4.715–19.618), $p < 0.001$], lower baseline LSM values [OR (95% CI) 0.886 (0.789–0.994), $p = 0.039$], baseline HBsAg levels (log_{10} IU/mL) [OR (95% CI) 0.357 (0.249–0.511), $p < 0.001$], and week 12 ALT ≥ 2×upper limit of normal (ULN) [OR (95% CI) 3.132 (1.741–5.635), $p < 0.001$].

Further multivariate logistic regression analysis identified lower $CD4^+/CD8^+$ ratios at week 12 [OR (95% CI) 0.202 (0.046–0.484), $p = 0.019$] and week 24 [OR (95% CI) 0.125 (0.007–0.372), $p = 0.003$], baseline HBV DNA < 20 IU/mL [OR (95% CI) 5.521 (3.765–9.402), $p < 0.001$], lower baseline HBsAg levels [OR (95% CI) 0.267 (0.156–0.459), $p < 0.001$], and week 12 ALT ≥ 2×ULN [OR (95% CI) 2.304 (1.728–10.722), $p = 0.002$] as independent predictors of HBsAg clearance at week 72 (Table 3).

The AUCs for $CD4^+/CD8^+$ ratios, which reflect the test's diagnostic accuracy in distinguishing between HBsAg clearance and persistence, increased from 0.796 at week 12 to 0.863 at week 24. The optimal cut-off values were 1.5 at week 12 (sensitivity 77.6%; specificity 71.4%) and 1.4 at week 24 (sensitivity 86.3%; specificity 82.4%). Among IHCs, those with a $CD4^+/CD8^+$ ratio <1.5 at week 12 showed an HBsAg clearance rate of 76.2%, while those with a $CD4^+/CD8^+$ ratio <1.4 at week 24 exhibited a clearance rate of 82.5%. The AUC for baseline HBsAg levels was 0.774, with an optimal cut-off value of 2.0 log_{10} IU/mL (100 IU/mL) (sensitivity 64.1%; specificity 81.0%). HBsAg clearance rates were 74.7% in patients with baseline HBsAg <2.0 log_{10} IU/mL, 80.3% in patients with baseline HBV DNA <20 IU/mL, and 59.4% in patients with week 12 ALT ≥ 2×ULN (Table 4 and Supplementary Fig. 1).

Combined analysis revealed that IHCs with baseline HBsAg <2.0 log_{10} IU/mL, HBV DNA <20 IU/mL, ALT ≥ 2×ULN at week 12, $CD4^+/CD8^+$ ratio <1.5 at week 12, and $CD4^+/CD8^+$ ratio <1.4 at week 24 achieved a 100% HBsAg clearance rate. In contrast, IHCs with baseline HBsAg ≥ 2.0 log_{10} IU/mL, HBV DNA levels between 20 and 2,000 IU/mL, ALT <2×ULN at week 12, and $CD4^+/CD8^+$ ratios ≥1.5 at week 12 and $CD4^+/CD8^+$ ratio ≥1.4 at week 24 did not achieve any HBsAg clearance (Supplementary Fig. 1).

Safety

The AEs observed in this study are detailed in Supplementary Table 3. Overall, peg-IFN-α was well tolerated by the IHCs. Eight patients discontinued peg-IFN-α treatment due to severe therapy-related AEs. Other reported AEs were consistent with those commonly observed with peg-IFN-α therapy. Notably, we identified several underreported AEs of peg-IFN-α, including fundus hemorrhage and nail lesions.

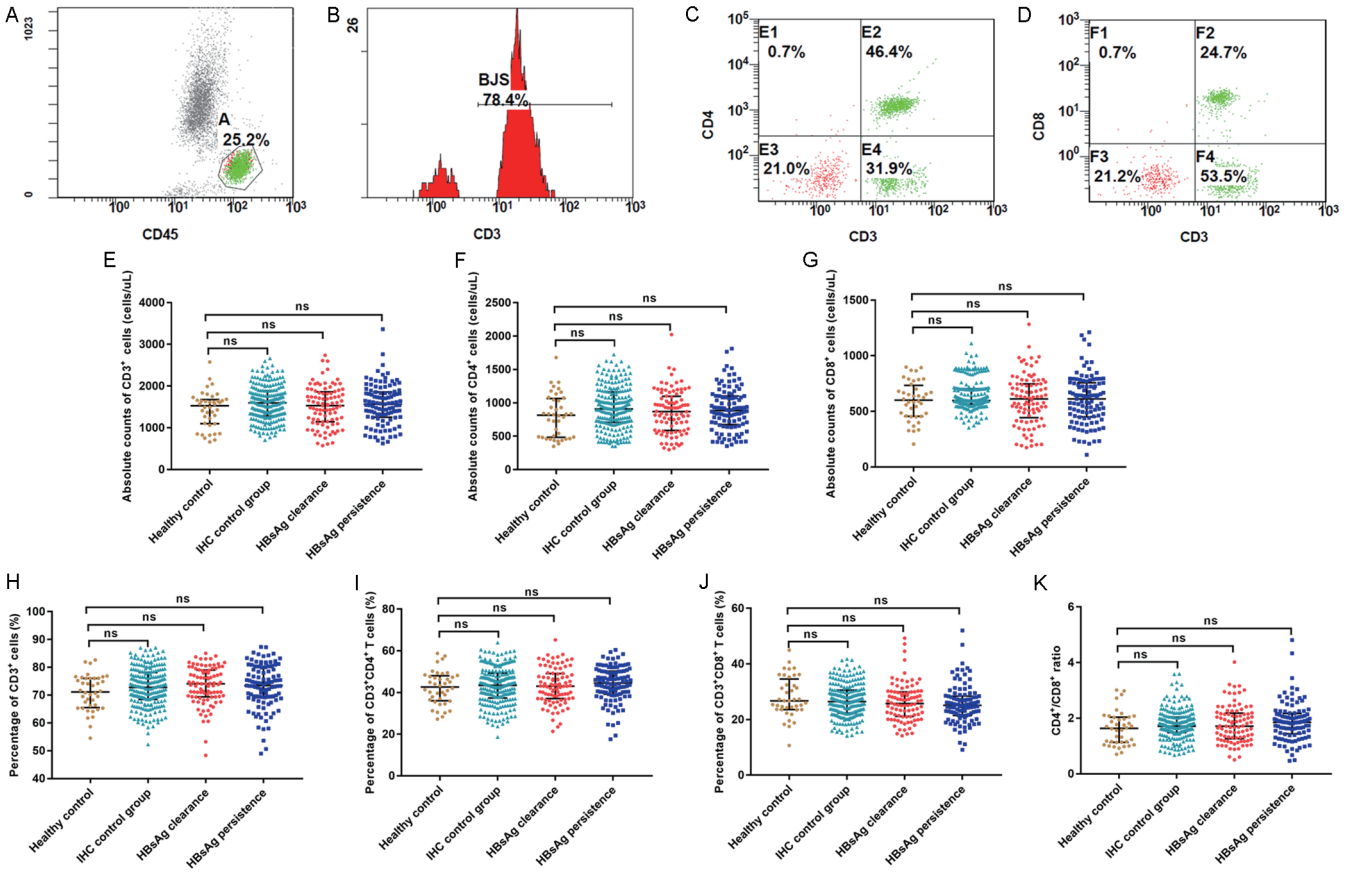

Fig. 3. Flow cytometric analysis of peripheral blood T lymphocyte subsets in IHCs and HCs at baseline. (A) Frequency of peripheral blood white blood cells (A). (B) Frequency of peripheral blood CD3⁺ cells. (C) Frequency of peripheral blood CD3⁺ CD4⁺ T cells (E2). (D) Frequency of peripheral blood CD3⁺ CD8⁺ T cells (F2). (E–G) Median absolute counts of CD3⁺, CD4⁺, and CD8⁺ T cells in HCs and IHCs at baseline. (H–J) Median percentages of CD3⁺, CD3⁺ CD4⁺, and CD3⁺ CD8⁺ T cells in HCs and IHCs at baseline. (K) Median CD4⁺/CD8⁺ ratios in HCs and IHCs at baseline. IHCs, inactive hepatitis B surface antigen carriers; HCs, healthy controls; HBsAg, hepatitis B surface antigen.

Discussion

In recent years, research has increasingly focused on achieving a functional cure for IHCs. In this study, we prospectively observed HBsAg clearance in 197 IHCs receiving peg-IFN-α monotherapy and compared it with 221 IHCs who received no treatment. The findings demonstrated a significantly higher HBsAg clearance rate (up to 46.7%) with a favorable safety profile, corroborating our previous research.[13] By expanding the sample size, this study further validates these observations. The results align with recent studies on the use of peg-IFN-α for treating IHCs, suggesting its potential as a regimen for achieving a functional cure in the IHC population.[13,17,18]

T lymphocytes play a crucial role in the antiviral response. Dysfunctions in these cells, particularly HBV-specific T lymphocytes, are believed to contribute significantly to the development of persistent HBV infection.[9,19] Reports indicate that patients with CHB often exhibit abnormalities in T lymphocyte subsets, typically characterized by a reduction in CD4⁺ T lymphocytes, an elevation in CD8⁺ T lymphocytes, and an imbalance in Th1/Th2 lymphocytes.[9] However, our observations did not reveal significant differences in T lymphocyte subsets between IHCs and healthy individuals. Although this result was somewhat unexpected, it suggests that IHCs may exhibit a T lymphocyte immune profile similar to that of healthy individuals. This intriguing finding may be explained by the reduced levels of HBsAg and HBV DNA in IHCs, which likely exert a mild suppressive impact on host immunity.[20,21] Prior research indicates that prolonged exposure of T cells to elevated levels of HBV-related antigens, particularly HBsAg, disrupts T-cell functionality and impairs their response to interferon.[22,23] Therefore, the reduced levels of HBsAg and HBV DNA in IHCs may protect T cells from antigenic stimulation, which is crucial for restoring functional T-cell responses.[24,25] This phenomenon may help explain why IHCs exhibit a superior response to peg-IFN-α therapy compared to CHB patients, who have higher levels of HBsAg and HBV DNA.[12,26]

To investigate the impact of peg-IFN-α on the adaptive immune response in IHCs, we conducted a longitudinal analysis of peripheral blood T lymphocyte subsets in IHCs receiving peg-IFN-α monotherapy and compared them with untreated IHCs. IHCs receiving peg-IFN-α therapy exhibited a significant decrease in the absolute counts of CD3⁺, CD4⁺, and CD8⁺ T cells at 12, 24, and 48 weeks of treatment. This reduction was observed regardless of treatment response and returned to baseline levels 24 weeks after treatment. Consistent with previous findings,[27] this phenomenon is likely due to the transient myelosuppressive AEs associated with peg-IFN-α, which leads to a significant decrease in both peripheral blood WBC and T lymphocyte counts.[3] Furthermore, the parallel changes in the absolute counts of CD3⁺, CD4⁺, and CD8⁺ T cells with peripheral blood WBC and T lympho-

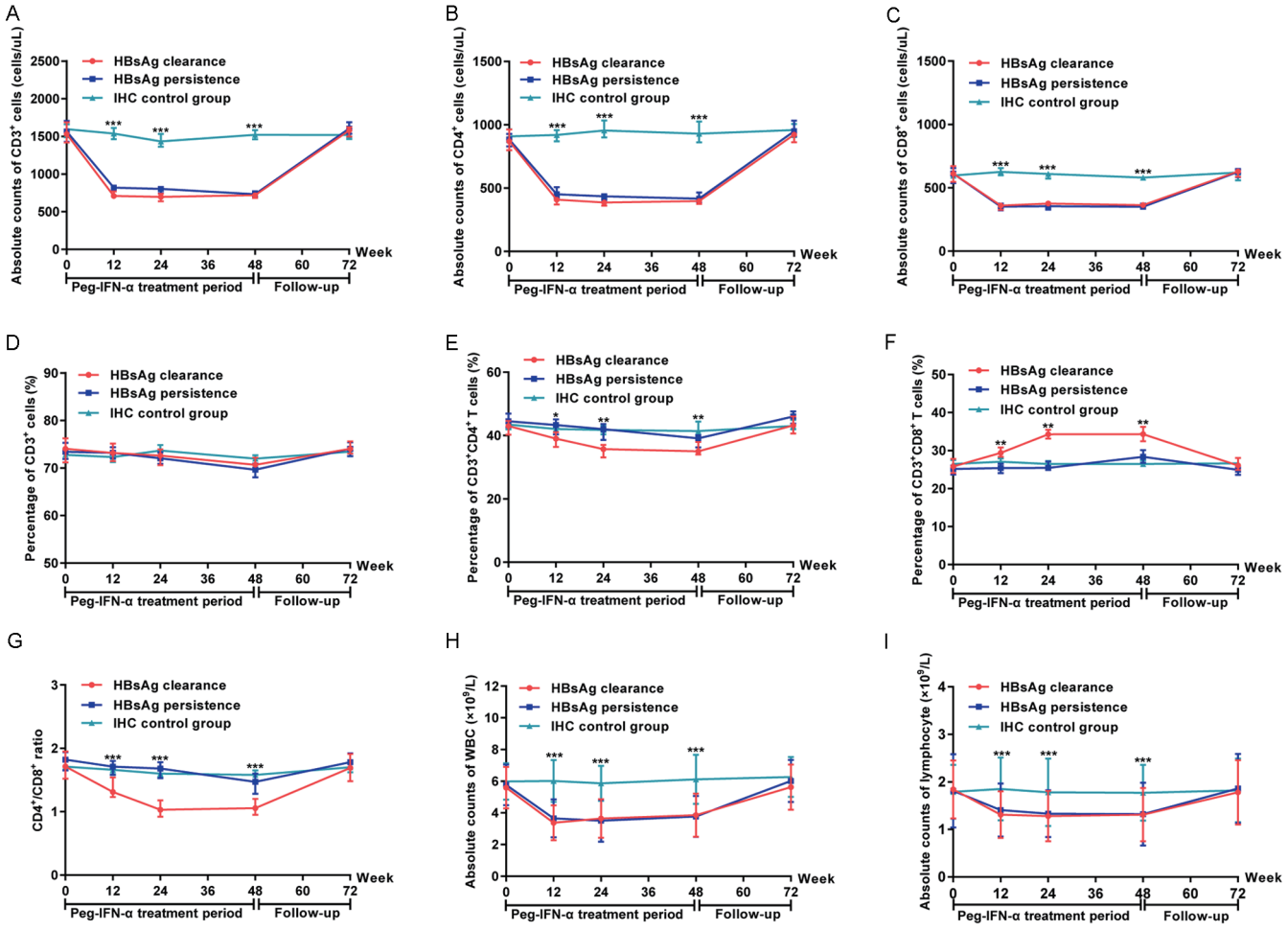

Fig. 4. Dynamics of peripheral blood T lymphocyte subsets, WBC, and T lymphocyte counts. (A-C) Dynamics of the median absolute counts of $CD3^+$, $CD4^+$, and $CD8^+$ T cells. (D) Dynamics of the median percentage of $CD3^+$ cells. (E–G) Dynamics of the median percentage of $CD3^+$ $CD4^+$ cells, $CD3^+$ $CD8^+$ cells and $CD4^+/CD8^+$ ratio. Data shown are median values and error bars represent 95% confidence interval. (H–I) Dynamics of the mean absolute counts of WBC and T lymphocytes. Data shown are mean ± standard deviation. WBC, white blood cell; HBsAg, hepatitis B surface antigen; Peg-IFN-α, peginterferon alpha. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

cytes further support the hypothesis that these effects are primarily due to myelosuppression.

To clarify the immunological mechanisms involved in HBsAg clearance, we conducted a comparative analysis of T lymphocyte subsets between two subgroups within the treatment group: individuals who achieved HBsAg clearance and those with HBsAg persistence. Notably, the HBsAg clearance group exhibited significantly higher median percentages of $CD3^+CD8^+$ cells, lower median percentages of $CD3^+CD4^+$ cells, and lower $CD4^+/CD8^+$ ratios at 12, 24, and 48 weeks. These findings suggest that peg-IFN-α enhances antiviral immunity by promoting the differentiation and expansion of cytotoxic T lymphocytes, particularly $CD8^+$ T cells, which are essential for viral clearance.[28] This observation is supported by a recent study that demonstrated an increase in effector $CD8^+$ T cell percentages via single-cell RNA sequencing.[29] The higher percentages of $CD8^+$ cells and lower percentages of $CD4^+$ T cells in the clearance group may indicate a shift toward a more robust cytotoxic-dominant immune response, facilitating the elimination of infected hepatocytes.

Achieving a functional cure remains a critical goal for patients with chronic HBV infection. However, the clinical application of peg-IFN-α is limited by its variable efficacy, AEs, high cost, and the lack of reliable early-stage efficacy predictors. Therefore, we aimed to identify patients who are most likely to benefit from peg-IFN-α therapy in achieving a functional cure. Given the differences in the percentage of $CD3^+CD4^+$ cells, $CD3^+CD8^+$ cells, and the $CD4^+/CD8^+$ ratio between the HBsAg clearance and HBsAg persistence groups, we assessed the potential of these parameters as early indicators of HBsAg clearance. Our findings indicated a correlation between $CD4^+/CD8^+$ ratios at 12 and 24 weeks of peg-IFN-α treatment and HBsAg clearance at 72 weeks. Specifically, lower $CD4^+/CD8^+$ ratios at these time points were associated with a greater likelihood of achieving a favorable treatment outcome. To our knowledge, this study is the first to establish a link between the $CD4^+/CD8^+$ ratio and HBsAg clearance, providing insight into the immune mechanisms underlying a functional cure in IHCs treated with peg-IFN-α. Furthermore, our study showed that lower baseline HBsAg levels, baseline HBV DNA <20 IU/mL, and week 12 ALT ≥ 2×ULN were associated with a higher likelihood of HBsAg clearance, confirming the conclusions of previously published studies.[12,15,30] Additionally, univariate analysis identified a potential correlation between lower baseline LSM and HBsAg clearance, suggesting that patients with lower LSM values

Table 2. Comparisons of T lymphocyte subsets among the three groups

T lymphocyte subsets	HBsAg clearance (n = 92)	HBsAg persistence (n = 105)	IHC control group (n = 221)	p_1-value	p_2-value	p_3-value
CD3$^+$ absolute counts, cells/μL						
Baseline	1,533.0 (1,144.0, 1,861.5)	1,566.0 (1,251.5, 1,859.5)	1,596.0 (1,282.5, 1,882.0)	0.580	0.266	0.674
Week 12	709.0 (622.0, 845.5)	819.0 (696.0, 905.0)	1,539.0 (1,224.0, 1,870.0)	0.015	<0.001	<0.001
Week 24	726.5 (604.8, 790.5)	783.0 (647.5, 866.5)	1,435.0 (1,139.0, 1,538.0)	0.011	<0.001	<0.001
Week 48	720.5 (620.5, 824.5)	733.0 (617.0, 823.0)	1,522.0 (1,223.0, 1,851.5)	0.869	<0.001	<0.001
Week 72	1,581.0 (1,325.0, 1,745.5)	1,605.0 (1,404.5, 1,745.3)	1,519.0 (1,250.0, 1,788.5)	0.350	0.648	0.140
CD4$^+$ absolute counts, cells/μL						
Baseline	870.0 (587.8, 1,098.8)	884.0 (676.5, 1,104.0)	909.0 (710.0, 1,163.0)	0.576	0.186	0.533
Week 12	409.0 (332.5, 499.3)	452.0 (379.5, 526.5)	920.0 (694.0, 1,168.5)	0.034	<0.001	<0.001
Week 24	386.5 (305.0, 447.8)	436.0 (371.0, 501.5)	974.0 (695.5, 1,203.5)	0.013	<0.001	<0.001
Week 48	397.5 (345.5, 502.5)	417.0 (347.5, 507.0)	930.0 (723.0, 1,206.5)	0.873	<0.001	<0.001
Week 72	922.5 (757.8, 1,051.5)	949.0 (826.0, 1,103.5)	959.0 (735.0, 1,162.5)	0.085	0.089	0.967
CD8$^+$ absolute counts, cells/μL						
Baseline	611.5 (442.5, 747.0)	612.0 (450.5, 757.0)	597.0 (564.0, 701.0)	0.982	0.151	0.100
Week 12	359.5 (277.0, 403.8)	352.0 (288.5, 419.0)	626.0 (526.5, 721.5)	0.549	<0.001	<0.001
Week 24	376.0 (306.5, 425.8)	354.0 (278.0, 397.4)	609.0 (500.5, 688.0)	0.097	<0.001	<0.001
Week 48	363.5 (325.0, 433.0)	351.0 (284.5, 426.5)	580.0 (504.5, 633.5)	0.180	<0.001	<0.001
Week 72	629.0 (522.0, 671.0)	626.0 (531.0, 703.1)	621.0 (517.0, 747.5)	0.546	0.436	0.953
Percentage of CD3$^+$ cells, (%)						
Baseline	74.1 (69.4, 79.0)	73.4 (69.1, 80.0)	72.8 (68.5, 78.1)	0.911	0.291	0.437
Week 12	73.2 (69.0, 76.4)	73.2 (67.4, 76.4)	72.3 (67.5, 76.7)	0.673	0.221	0.569
Week 24	72.6 (68.9, 75.7)	72.1 (67.3, 75.2)	73.2 (69.7, 77.5)	0.530	0.052	0.057
Week 48	70.7 (68.5, 75.2)	69.8 (64.2, 73.1)	72.6 (68.5, 75.9)	0.010	0.459	0.051
Week 72	74.0 (70.2, 78.3)	74.1 (69.8, 78.3)	73.5 (70.2, 76.9)	0.989	0.283	0.281
Percentage of CD3$^+$ CD4$^+$ cells, (%)						
Baseline	43.0 (37.1, 49.1)	44.5 (39.2, 50.2)	43.5 (37.4, 49.3)	0.251	0.774	0.318
Week 12	39.0 (35.1, 44.4)	43.3 (37.4, 47.5)	41.1 (34.9, 47.6)	0.005	0.049	0.389
Week 24	35.7 (30.5, 40.7)	42.1 (34.3, 46.1)	41.8 (36.2, 47.1)	0.001	<0.001	0.145
Week 48	35.2 (31.7, 42.1)	38.6 (32.1, 43.1)	41.4 (34.6, 48.6)	0.009	<0.001	0.057
Week 72	43.2 (38.9, 49.2)	46.1 (40.5, 50.3)	43.0 (37.8, 49.9)	0.139	0.970	0.089
Percentage of CD3$^+$ CD8$^+$ cells, (%)						
Baseline	25.8 (21.2, 29.9)	25.1 (21.4, 28.4)	26.5 (22.3, 30.6)	0.430	0.306	0.050
Week 12	29.4 (26.0, 33.9)	25.4 (22.3, 29.1)	27.1 (22.7, 32.0)	<0.001	0.003	0.075
Week 24	34.3 (30.7, 37.6)	25.4 (23.1, 30.6)	26.5 (22.3, 30.3)	<0.001	<0.001	0.978
Week 48	34.3 (38.4, 27.6)	28.3 (25.0, 32.2)	26.5 (23.1, 30.7)	<0.001	<0.001	0.061
Week 72	26.1 (23.1, 30.8)	25.0 (21.6, 30.3)	26.6 (23.0, 30.5)	0.012	0.750	0.114
CD4$^+$/CD8$^+$ ratio						
Baseline	1.7 (1.3, 2.2)	1.8 (1.4, 2.2)	1.7 (1.5, 2.0)	0.371	0.975	0.140
Week 12	1.3 (1.1, 1.7)	1.7 (1.3, 2.0)	1.7 (1.3, 1.9)	<0.001	<0.001	0.331
Week 24	1.0 (0.8, 1.3)	1.7 (1.3, 1.9)	1.6 (1.3, 1.9)	<0.001	<0.001	0.964
Week 48	1.1 (0.9, 1.5)	1.5 (1.1, 1.6)	1.6 (1.3, 1.8)	<0.001	<0.001	0.063
Week 72	1.7 (1.3, 2.1)	1.8 (1.5, 2.2)	1.7 (1.4, 2.0)	0.201	0.655	0.514

Data are presented as medians with interquartile ranges [M (Q1, Q3)]. p_1-values represent comparisons between the HBsAg clearance group and the HBsAg persistence group (the intervention for patients in both groups was identical; comparisons between the groups at different time points were conducted using the Mann-Whitney U test with a corrected p-value of 0.01). p_2-values represent comparisons between the HBsAg clearance group and the IHC control group; p_3 values represent comparisons between the HBsAg persistence group and the IHC control group (intergroup comparisons at different time points align with the analysis of repeated measures data and were conducted using generalized estimating equations [GEE], with a p-value of 0.05). HBsAg, hepatitis B surface antigen; IHC, inactive hepatitis B surface antigen carriers.

Table 3. Baseline and on-treatment factors associated with HBsAg clearance

Predictors	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p*	OR	95% CI	*p*
Male	0.538	(0.294–0.987)	0.048	0.605	(0.997–1.808)	0.651
Baseline BMI, kg/cm^2	0.833	(0.640–1.084)	0.175	–	–	–
Smoking	1.111	(0.491–2.514)	0.800	–	–	–
Drinking	1.262	(0.531 –2.995)	0.598	–	–	–
Mode of HBV transmission	1.386	(0.789–2.435)	0.255	–	–	–
Types of Peg-IFN-α	1.290	(0.675–2.467)	0.441	–	–	–
Baseline LSM values, kPa	0.886	(0.789–0.994)	0.039	0.720	(0.426–1.216)	0.219
Age, years	0.977	(0.950–1.005)	0.101	0.943	(0.898–1.990)	0.618
Baseline HBsAg, $\log_{10}$ IU/mL	0.357	(0.249–0.511)	<0.001	0.267	(0.156–0.459)	<0.001
Baseline HBV DNA <20 IU/mL	9.617	(4.715–19.618)	<0.001	5.521	(3.765–9.402)	<0.001
Baseline ALT, IU/L	0.979	(0.953–1.006)	0.132	–	–	–
Week 12 ALT ≥ 2×ULN	3.132	(1.741–5.635)	<0.001	2.304	(1.728–10.722)	0.002
Week 24 ALT ≥ 2×ULN	1.563	(0.751–3.253)	0.231	–	–	–
Baseline $CD3^+$ T cells, %	1.006	(0.968–1.045)	0.768	–	–	–
Baseline $CD3^+$ $CD4^+$ T cells, %	0.987	(0.955–1.020)	0.421	–	–	–
Baseline $CD3^+$ $CD8^+$ T cells, %	1.014	(0.972–1.056)	0.524	–	–	–
Baseline $CD4^+/CD8^+$ ratio	0.833	(0.548–1.267)	0.393	–	–	–
Week 12 $CD3^+$ T cells, %	1.027	(0.982–1.073)	0.249	–	–	–
Week 12 $CD3^+$ $CD4^+$ T cells, %	0.950	(0.912–0.989)	0.013	0.978	(0.827–1.156)	0.794
Week 12 $CD3^+$ $CD8^+$ T cells, %	1.100	(1.049–1.154)	<0.001	1.105	(0.918–1.329)	0.291
Week 12 $CD4^+/CD8^+$ ratio	0.246	(0.128–0.473)	<0.001	0.202	(0.046–0.484)	0.019
Week 24 $CD3^+$ T cells, %	1.032	(0.989–1.076)	0.144	–	–	–
Week 24 $CD3^+$ $CD4^+$ T cells, %	0.910	(0.873–0.949)	<0.001	1.032	(0.859–1.241)	0.733
Week 24 $CD3^+$ $CD8^+$ T cells, %	1.158	(1.101–1.216)	<0.001	1.077	(0.832–1.395)	0.572
Week 24 $CD4^+/CD8^+$ ratio	0.095	(0.044–0.203)	<0.001	0.125	(0.007–0.372)	0.003

ULN, upper limit of normal; OR, odds ratio; CI, confidence interval; IU, international unit; mL, milliliter.

are more likely to achieve HBsAg clearance. However, after adjusting for potential confounders in the multivariate analysis, this correlation was no longer statistically significant. Larger sample sizes in future research would help clarify the role of LSM in predicting HBsAg clearance.

We further identified optimal cutoff values for predicting HBsAg clearance using ROC analysis. Specifically, we found that a baseline HBsAg <2.0 $\log_{10}$ IU/mL (100 IU/mL), a $CD4^+/CD8^+$ ratio <1.5 at week 12, and a $CD4^+/CD8^+$ ratio <1.4 at week 24 were associated with higher HBsAg clearance rates. Furthermore, the prediction of HBsAg clearance in IHCs was optimized through a combination of these factors: baseline HBsAg <2.0 $\log_{10}$ IU/mL, baseline HBV DNA <20 IU/mL, an ALT level ≥ 2×ULN at week 12, a $CD4^+/CD8^+$ ratio <1.5 at week 12, and a $CD4^+/CD8^+$ ratio <1.4 at week 24.

Several limitations in our study should be acknowledged. Firstly, the lack of randomization is a limitation. Randomization was not feasible because some participants expressed a strong preference for peg-IFN-α treatment in pursuit of a functional cure, while others explicitly declined it. Secondly, 75.6% (149/197) of the patients in the treatment group had undetectable or very low HBV DNA levels prior to enrollment, which made it impossible to assess the impact of HBV genotypes on the response to peg-IFN-α in this study. Thirdly, further investigation is needed to clarify the correlation between peg-IFN-α-mediated HBsAg clearance and T lymphocyte

Table 4. ROC curves for favorable predictors of HBsAg clearance

Predictors	Area	SD	95% CI	Cut-off value	Sensitivity & Specificity	HBsAg clearance rate
Baseline HBsAg, $\log_{10}$ IU/mL	0.774	0.034	(0.708–0.840)	2.0	64.1%, 81.0%	74.7% (59/79)
Week12 $CD4^+/CD8^+$ ratio	0.796	0.038	(0.604–0.852)	1.5	77.6%, 71.4%	76.2 % (64/84)
Week24 $CD4^+/CD8^+$ ratio	0.863	0.034	(0.713–0.945)	1.4	86.3%, 82.4%	82.5% (85/103)

ROC, receiver operating characteristic; SD, standard deviation; HBsAg, hepatitis B surface antigen; IU, international unit; mL, milliliter.

phenotype/function, such as T cell-related cytokine profiles, secretion capacity of perforin and granzyme, T cell receptor repertoire, and markers of T cell exhaustion (e.g., PD-1, Tim-3, CTLA-4). Our capability to perform an in-depth analysis of T lymphocyte phenotype/function, especially HBV-specific T lymphocytes, was limited by experimental conditions and funding. In future research, we plan to use single-cell sequencing technology to analyze the comprehensive transcriptomic profiles of all immune cells in IHCs. This approach will enhance our understanding of the disease's pathophysiology and the mechanisms underlying HBsAg clearance induced by peg-IFN-α.

Conclusions

This clinical prospective observational study demonstrated that peg-IFN-α can significantly enhance the rates of HBsAg clearance in IHCs. Additionally, the peripheral blood $CD4^+$/$CD8^+$ ratios at 12 and 24 weeks could potentially serve as predictive markers for HBsAg clearance in IHCs undergoing peg-IFN-α treatment.

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None to declare.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study design (SD, FW, XZ, XJ), data analysis (FW, CL), blood specimen collection (CL, LH, YW, RL, PK, YL, ML), flow cytometry analysis (ML), manuscript drafting (FW, CL, SD), research concept and overall supervision (SD). All authors contributed to data collection and interpretation. All authors have approved the final version and publication of the manuscript.

Ethical statement

The study complies with Good Clinical Practice and the Declaration of Helsinki and was approved by the Biomedical Ethics Committee of Xi'an Jiaotong University (No. 2015-2045). All patients provided informed consent prior to screening, in accordance with relevant regulatory and local ethical guidelines.

Data sharing statement

Data supporting the findings of this study are available from the corresponding authors upon reasonable request.

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