The Reduction in CD8+PD1+ T Cells in Liver Histological Tissue is Related to Pegylated IFN-α Therapy Outcomes in Chronic Hepatitis B Patients

Ruyu Liu (liuruyu28@163.com)
Capital Medical University https://orcid.org/0000-0001-7561-9439
Yanhui Chen
Capital Medical University
Jiang Guo
Capital Medical University
Minghui Li
Capital Medical University
Yao Lu
Capital Medical University
Lu Zhang
Capital Medical University
Ge Shen
Capital Medical University
Shuling Wu
Capital Medical University
Min Chang
Capital Medical University
Leiping Hu
Capital Medical University
Hongxiao Hao
Capital Medical University
Henghui Zhang
Capital Medical University
Yao Xie
Capital Medical University

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Abstract

Background and Aim Antiviral therapy for patients with immune-active chronic hepatitis B (CHB) should be adopted to decrease the risk of liver-related complications. While antiviral therapy outcomes of PEG-IFN-α vary in different CHB patients. We aimed to identify the factors influencing antiviral therapy outcomes in CHB patients who received PEG-IFN-α therapy.

Methods Thirty-two CHB patients who received PEG-IFN-α therapy were enrolled in this study. All of the patients underwent two liver biopsies at baseline and 6 months later. CD8 + T cells, CD4 + T cells, CD68 + mononuclear cells, and PD-1 levels in 64 liver biopsy specimens were tested via immunofluorescence.

Results CD8 + T cells in 32 CHB patients' liver tissue significantly decreased after 6 months of PEG-IFN-α therapy ($p<0.01$). CD8 + PD1 + T cells significantly decreased after 6 months of PEG-IFN-α treatment in the FIER (fibrosis and inflammation response with HBeAg seroconversion) group ($p<0.05$), while CD8 + PD1 - T cells had no significant difference. However, in the FIENR (no fibrosis and inflammation response and HBeAg seroconversion) group, CD8 + PD1 - T cells significantly decreased after 6 months of PEG-IFN-α treatment ($p<0.05$), while CD8 + PD1 + T cells had no significant difference. CD68 + mononuclear cells at baseline were higher in the FIRENR (fibrosis and inflammation response without HBeAg seroconversion) group than the FIENR (no fibrosis and inflammation response and HBeAg seroconversion) group.

Conclusions The reduction in CD8 + PD1 + T cells in liver tissue was critical for patients who responded to PEG-IFN-α therapy. High levels of CD68 + mononuclear cells at baseline might be associated with fibrosis and inflammation response to PEG-IFN-α therapy.

Introduction

It estimated that the global prevalence of hepatitis B s-antigen (HBsAg) was 3.9%, corresponding to 291,992,000 infections.[1] Reports showed that approximately 786,000 people will die annually of chronic HBV infection-related diseases, including liver cirrhosis or hepatocellular carcinoma (HCC).[2] To decrease the risk of liver-related complications, guidelines recommend antiviral therapy for patients with immune-active chronic hepatitis B (CHB).[3, 4] Peginterferon along with tenofovir alafenamide (TAF), tenofovir disoproxil fumarate (TDF), and entecavir (ETV) are the preferred antivirals.[3, 4] Pegylated interferon α–2a (PEG-IFN-α) therapy represents a promising therapeutic alternative to the prolonged use of nucleos(t)ide analogs (NA) in chronic hepatitis B (CHB) infection.[5, 6] As a first-line drug for the treatment of chronic hepatitis B infection, PEG-IFN-α mediates the antiviral, antiproliferative, and immunomodulatory effects.[7] However, its efficacy is limited in approximately one-third of treated patients.[3, 8]

IFN-α has been used for over 20 years to treat patients with chronic HBV infection, but it is not well understood why some patients respond to treatment and others do not.[9] IFN-α is a pleiotropic cytokine that has both direct antiviral and immunomodulatory properties. Regarding the former, IFN-α induces the

expression of hundreds of interferon-stimulated genes (ISGs), many with antiviral effector functions.[9] The immunomodulatory properties of IFN-α include the activation of NK cells, dendritic cells, and B cells, as well as both the direct and indirect activation of T cell function.[9–11] IFN-α has been shown to play an important role in the differentiation of both $CD4^+$ and $CD8^+$ T cells.[10] Studies showed that PEG-IFN-α therapy led to a striking reduction in $CD8^+$ T cells.[12, 13] However, PEG-IFN-α has also been shown to increase $CD8^+$ T cells but causes no change in $CD4^+$ T lymphocytes in CHB patients' liver tissue.[14] These contradictory results between respondents and non-respondents need to be further studied.

Research showed that the extended upregulation of PD–1 is associated with T cell exhaustion and persistent viral infection.[15] Our past study demonstrated that in PEG-IFN-α therapy respondents, PD–1 levels in $CD4^+$ and $CD8^+$ T cells decreased in the peripheral blood, indicating that PD–1 expression in $CD4^+$ and $CD8^+$ T might influence PEG-IFN-α therapy outcomes.[8] Whether this is the same as the peripheral blood in the liver tissue of CHB patients must be further studied. In liver tissue, $CD68^+$ mononuclear cells were regarded as Kupffer cells (KCs) and liver-infiltrating monocytes/macrophages.[16, 17] Studies showed that these cells could induce pro-inflammatory response inhibition of viral replication, which is important for the inhibition of viral replication.[18] KC virus interactions can also inhibit the development of effective viral immunity, facilitate viral persistence, or promote liver damage.[18] Furthermore, KCs are thought to be involved in fibrogenesis via the release of various pro-fibrinogenic factors.[18] Recent studies of experimental animal models demonstrate that these activities are only partially conducted by liver-resident macrophages, but largely depend on the recruitment of monocytes as precursors of macrophages into the inflamed and damaged liver.[19, 20] Few publications reported the changes in $CD68^+$ mononuclear cells in liver tissue during PEG-IFN-α therapy; this also should be further studied.

In this study, we assessed the changes in $CD4^+$ T, $CD8^+$ T, and $CD68^+$ mononuclear cells and PD–1 levels in liver tissue at baseline and 6 months later in 32 respondents/non-respondents CHB patients who received PEG-IFN-α therapy. We further analyzed the differences between respondents and non-respondents in order to find the baseline and on-treatment factors that influence PEG-IFN-α treatment outcomes.

Materials And Methods

Patients

A total of 32 HBeAg positive CHB patients were enrolled in this retrospective study from December 2008 to September 2011 at Beijing Ditan Hospital, Beijing, China. All of the patients were positive for HBsAg for more than 6 months and received antiviral therapy at treatment commencement according to guidelines. All of the patients received PEG-IFN-α therapy for one year according to guidelines. Liver biopsies were performed at baseline and 6 months later in all of the patients. Patients whose liver fibrosis stages and

histologic activity were determined as F0 to F4 and A0 to A3 (METAVIR scoring system) received 180 μg of PEG-IFN-α weekly. The exclusion criteria were (i) co-infection with hepatitis C, D, and human immunodeficiency virus; (ii) HCC development after PEG-IFN-α commencement during the study period; and (iii) lack of compliance with PEG-IFN-α treatment for more than 3 months. In this study, virological response was defined as a HBV-DNA level undetectable by quantitative polymerase chain reaction assay with a lower limit detection of 20 IU/mL during PEG-IFN-α therapy. HBeAg seroconversion was defined as the disappearance of hepatitis B e-antigen (HBeAg) with detectable anti-HBe. This study was approved by the Institutional Review Board of Beijing Ditan Hospital （Reference number 161508）, and informed consent was waived.

Laboratory data

The follow-up schedule for all of the patients was as follows: biochemical, serological, and virological parameters were performed at baseline and after 3, 6, 9, and 12 months of PEG-IFN-α treatment and were measured using standard laboratory procedures. HBV serology included HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc testing. Serum HBsAg testing was performed using commercial kits (Abbott Laboratories; Lake Bluff, IL, USA). Serum HBV-DNA levels were assessed via real-time polymerase chain reactions at our hospitals (COBAS TaqMan HBV Test v2.0, Roche Diagnostics, Branchburg, NJ, USA). A serum HBV-DNA level of <20 IU/mL was defined as the limit of detectability.

Liver histological assessment

Percutaneous liver biopsies were performed, and paraffin sections were made according to a previous study.[21] The biopsy samples were assessed by two independent pathologists who were blinded to the results of noninvasive fibrosis tests. Discordant cases were reviewed by a third highly experienced pathologist. The METAVIR scoring system was adopted as the pathological diagnosis standard of liver inflammation and fibrosis. Liver inflammation was divided into four grades: A0, no inflammation; A1, mild inflammation (focal, few portal areas); A2, moderate inflammation (most portal areas, and even extending to beyond the portal areas); and A3, severe inflammation (significant confluent necrosis and bridging necrosis). Liver fibrosis was divided into five stages: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, cirrhosis.[21] Inflammation response was defined as a decrease in the METAVIR inflammation score ≥2 points from baseline and no worsening of the fibrosis score. Fibrosis response was defined as a decrease in the METAVIR fibrosis score ≥1 point from baseline.

IHC staining

The slides were deparaffinized in xylene, rehydrated, and washed in tap water before boiling in Tris-EDTA buffer (pH 9; 643901; Klinipath) for epitope retrieval/microwave treatment (MWT). Endogenous peroxidase was blocked using Antibody Diluent/Block (72424205, PerkinElmer). Protein blocking was performed using Antibody Diluent/Block (72424205, PerkinElmer) for 10 min at room temperature. Then primary Abs were incubated for 1 h at 37℃ or 12 h at 4℃. The primary Abs used were grouped into a 6-antibody panel consisting of CD4 (ZM−0418, clone UMAB64; Zsbio, dilution 1:100), CD8 (ZA−0508, clone EP334; Zsbio, dilution 1:100), CD68 (ZA−0060, clone KP1; Zsbio, dilution 1:400), PD−1 (ZM−0381, clone UMAB199; Zsbio, dilution 1:100), and IFN-r (ab218426, clone 466; Abcam, dilution 1:100). Next, incubation with Polymer HRP Rb (PV−6001, Zsbio) or Polymer HRP Ms (PV−6002, Zsbio) was performed at 37℃ for 10 min. TSA visualization was performed with an Opal seven-color IHC Kit (NEL797B001KT, PerkinElmer) containing fluorophores DAPI, Opal 570 (CD4), Opal 620 (CD8), Opal 650 (CD68), Opal 690 (PD1), and TSA Coumarin system (NEL703001KT, PerkinElmer). MWT was performed with Tris-EDTA buffer (pH 9) to remove the Ab TSA complex. TSA single stain slides were finished with MWT and counterstained with DAPI for 5 min and were enclosed in Antifade Mounting Medium (I0052; NobleRyder). The IHC staining results are shown in Fig 1.

Tissue imaging and analysis

The slides were scanned using PerkinElmer Vectra (Vectra 3.0.5, PerkinElmer). Multispectral images were unmixed using spectral libraries built from images of single stained tissues for each reagent using inForm Advanced Image Analysis software (inForm 2.3.0, PerkinElmer). A selection of 5–15 representative original multispectral images was used to calibrate the inForm software (tissue segmentation, cell segmentation, phenotyping tool, and positivity score). All of the settings applied to the calibration images were saved in an algorithm to allow batch analysis of multiple original multispectral images of the same tissue.

Statistical analyses

The results are as follows: normal distribution data as mean±SD, non-normal distribution continuous data as median (interquartile range), and categorical variables as number (percentage). The *t* test, χ^2, or Mann-Whitney test were performed between the different groups. Statistical significance was accepted for p-values of <0.05. *Represents p<0.05 and ** represents p<0.01. The analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA).

Results

Patients' baseline characteristics and improvement after 6 months of PEG-IFN-α treatment
The characteristics of the 32 patients at baseline and 6 months later are shown in Table 1. A total of 24 (75%) patients were male, and 8 were female (25%). According to the METAVIR scoring system, the

patients in each METAVIR fibrosis stage were as follows: F1 = 18 (56.3%), F2 = 9 (28.1%), F3 = 4 (12.5%), and F4 = 1 (3.1%), respectively. The patients in each METAVIR inflammation stage were as follows: A1 = 6 (18.75%), A2 = 14 (43.75%), and A3 = 12 (37.5%), respectively. After 6 months of PEG-IFN-α therapy, the WBC, PLT, ALT, AST, HBV-DNA, and HBeAg levels decreased significantly ($p<0.05$) (Table 1). The ALT, AST normal, and HBVDNA negative rates increased during PEG-IFN-α therapy (Table 2). In this study, 43.8% (14/32) of the CHB patients achieved fibrosis response and 68.8% (22/33) achieved inflammation response (Table 2). The baseline mean fibrosis and inflammation stages were 1.630.83 and 2.190.74, respectively. After 6 months of PEG-IFN-α treatment, they significantly decreased to 1.190.86 and 1.50.72, respectively ($p<0.05$) (Table 1).

$CD8^+PD1^+$ T cells were significantly decreased in the FIER (fibrosis and inflammation response with HBeAg seroconversion) group after 6 months of PEG-IFN-α treatment.
$CD8^+$ T cells in the 32 CHB patients' liver tissue at baseline and 6 months later are shown in Table 1. They significantly decreased after 6 months of PEG-IFN-α therapy ($p<0.01$) (Table 1, Fig 2A). Compared with baseline, $CD4^+$ T cells in the liver tissue of all of the patients had no significant difference after 6 months of PEG-IFN-α treatment ($p>0.05$) (Table 1, Fig 2B). We also assessed the changes in $CD68^+$ mononuclear cells at baseline and after 6 months of PEG-IFN-α treatment in all of the patients, but there was no significant difference ($p>0.05$) (Table 1). $PD1^+$ cells showed a reduction trend after 6 months of PEG-IFN-α treatment, but there was no significant difference ($p>0.05$) (Table 1).

According to the outcomes after 6 months of PEG-IFN-α treatment, the patients were divided into different groups (Table 3). Compared with baseline, $CD8^+PD1^+$ T cells significantly decreased after 6 months of PEG-IFN-α treatment in the liver tissue in the FIER group (n = 7) (Fig 3A, $p<0.05$), while $CD8^+$ T cells and $CD8^+PD1^-$ T cells had no significant difference (Fig 2C, Fig 3B, $p>0.05$). $CD4^+$ T cells and $CD68^+$ mononuclear cells had no significant difference (data not shown).

In the FIENR (no fibrosis and inflammation response and HBeAg seroconversion) group (n = 9), $CD8^+PD1^-$ T cells significantly decreased after 6 months of PEG-IFN-α treatment in the liver tissue (Fig 3D, $p<0.05$), while $CD8^+$ T cells and $CD8^+PD1^+$ T cells had no significant difference (Fig 2D, Fig 3C, $p>0.05$). $CD4^+$ T cells and CD68+ mononuclear cells had no significant difference (data not shown).

We also assessed the changes in the $CD4^+$ T, $CD8^+$ T, $CD8^+PD1^-$ T, $CD8^+PD1^+$ T cells, and $CD68^+$ mononuclear cells in the FIRENR (fibrosis and inflammation response without HBeAg seroconversion) group (n = 5); there were no significant differences between baseline and 6 months of PEG-IFN-α therapy (data not shown). In the IRFENR (inflammation response without fibrosis response without HBeAg seroconversion) group (n = 8), only the $CD8^+PD1^+$ T cells decreased significantly in the portal areas after 6 months of antiviral therapy, while there was no significant difference in the lobular areas and the total (portal and lobular) areas (data not shown). There was only 1 patient in the FRIENR (fibrosis response without inflammation response without HBeAg seroconversion) group, so it could not be analyzed.

Fibrosis and inflammation response might be associated with a high number of $CD68^+$ mononuclear cells at baseline

The $CD4^+$ T, $CD8^+$ T, $CD8^+PD1^-$ T, $CD8^+PD1^+$ T, and $CD68^+$ mononuclear cells were compared between the different groups at baseline. The $CD4^+$ T cells, $CD8^+$ T cells, $CD8^+PD1^-$ T cells, and $CD8^+PD1^+$ T cells had no significant difference (data not shown). Compared with the FIENR group, the $CD68^+$ mononuclear cells were higher in the FIRENR group (Fig 4A, $p<0.05$). This indicated that high levels of $CD68^+$ mononuclear cells at baseline might be associated with fibrosis and inflammation response. Compared with baseline, the $CD68^+$ mononuclear cells significantly increased in the FIER group after 6 months of PEG-IFN-α therapy, which also supports this conclusion (Fig 4B, $p<0.05$).

Discussion

IFN-α has been used for many years to treat patients with chronic HBV infection, but is it unclear why some patients respond to treatment and others do not. IFN-α has both direct antiviral and immunomodulatory properties.[9] The immunomodulatory properties of IFN-α include the activation of NK cells and B cells and both the direct and indirect activation of $CD8^+$ T cell function.[9] Treatment with PEG-IFN-α can led to increased IFN-γ production of NK cells.[22, 23] IFN-α has been shown to influence both $CD4^+$ and $CD8^+$ T cells,[10] but the findings are contradicted. Reports documented that responders to IFN-α exhibited significant increases in intrahepatic $CD8^+$ T cells.[14] The authors thought the intrahepatic $CD8^+$ T lymphocyte, but not the $CD4^+$ T lymphocyte or NK/NKT-cell response, is important for HBV clearance during interferon-a therapy. [14] Recent studies showed that PEG-IFN-α therapy led to a striking reduction of $CD8^+$ T cells.[12, 13] Our research found that $CD8^+$ T cells significantly decreased after 6 months of PEG-IFN-α therapy in 32 CHB patients, similar to previous reports.[12, 13] We also assessed the changes in the $CD4^+$ T cells; there were no significant differences between baseline and after 6 months of PEG-IFN-α therapy, which was also the same as in a previous report.[14]

Studies showed that the upregulation of PD−1 is associated with T cell exhaustion and persistent viral infection.[15] Our past study showed that in PEG-IFN-α therapy respondents, PD−1 levels in $CD4^+$ and $CD8^+$ T cells decreased in the peripheral blood, while in non-respondents, $CD4^+PD1^+$ T and $CD8^+PD1^+$ T cells had no significant reduction.[8] In this study, $CD8^+PD1^+$ T cells significantly decreased after 6 months of PEG-IFN-α therapy in the FIER group in the liver tissue, while $CD8^+PD1^-$ T cells had no significant difference. Furthermore, $CD8^+PD1^-$ T cells significantly decreased in the FIENR group after 6 months of PEG-IFN-α therapy while $CD8^+PD1^+$ T had no significant difference. This was similar to our peripheral blood study and indicated that the reduction in $CD8^+PD1^+$ T cells in the liver tissue was critical for patients who responded to PEG-IFN-α therapy.

Studies showed that monocytes can bind HBV or HBV proteins, leading to their activation.[18, 24] In liver tissue, $CD68^+$ mononuclear cells were regarded as KCs and liver-infiltrating monocytes/macrophages.[16, 17] Reports showed HBV particles and HBsAg induce IL−1b, IL−6, CXCL8, and TNF production by human

$CD68^+$ cell-enriched non-parenchymal cells via NF-κB activation and subsequently inhibit HBV replication in primary hepatocytes.[25] Our study showed that $CD68^+$ mononuclear cells in the FIRENR group were higher than in the FIENR group at baseline, and $CD68^+$ mononuclear cells significantly increased in the FIER group after 6 months of PEG-IFN-α therapy. This indicated that PEG-IFN-α might partly through $CD68^+$ mononuclear cells inhibit HBV replication, and high levels of $CD68^+$ mononuclear cells at baseline might be associated with fibrosis and inflammation response.

This study also has limitations. First, the $CD8^+PD1^+$ T cell reduction in liver tissue during PEG-IFN-α treatment was critical to the respondents, but the mechanisms need to be investigated further. Second, the study sample size was insufficient and should be enlarged to more precisely evaluate the efficacy of PEG-IFN-α treatment. Third, we also assessed the changes in NK cells at baseline and after 6 months of PEG-IFN-α therapy, but failed to obtain immunofluorescence staining results. This requires further study.

In conclusion, Our results indicated that $CD8^+PD1^+$ T cell reduction in the liver tissue was critical for patients who responded to PEG-IFN-α therapy and high $CD68^+$ mononuclear cells in the liver tissue at baseline might be associated with fibrosis and inflammation response. Monitoring the changes of $CD8^+PD1^+$ T cells in liver tissue during antiviral therapy might be useful for predicting the outcomes of PEG-IFN-α therapy.

Declarations

Conflict of interest The authors declare no competing financial interests.

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Role of the sponsor The funding organizations are public institutions and had no role in the design and conduct of the study, collection, management, and analysis of the data; or preparation, review, and approval of the manuscript.

Ethical approval and informed consent All patients signed the informed consent before liver biopsy, and all clinical procedures were in accordance with the Declaration of Helsinki in 1983. The study protocol was permitted by the Institutional Review Board of Beijing Ditan Hospital.

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Tables

Table 1. Characteristics of baseline and PEG-IFN-α treated CHB patients

haracteristics	Baseline	6 months of PEG-IFN-α therapy
ge (year, M±SD)	29.27±7.51	29.74±7.38
ender (male/female)	24/8	24/8
WBC count (10^9/L)	5.69±1.48	3.64±1.01**
BC count (10^{12}/L)	4.87±0.64	4.61±0.64
latelet count (10^9/L)	190.74±60.21	122.69±44.02**
LT, U/L (median)	98.95 (74.8-189.48)	66.2 (44.9-118)**
ST, U/L (median)	57.8 (44.25-83.95)	43.9 (35.2-58)*
BIL, (umol/L, M±SD)	18.7±7.61	15.04±9.39
BIL, (umol/L, M±SD)	5.62±3.7	5.32±3.34
LB, (g/L, M±SD)	45.71±4.9	45.66±4.7
BeAg (median)	864.31 (362.12-1236.09)	395.27 (0.56-996.45)*
BeAg positive/negative	32/0	22/10
BVDNA (log10) (median)	7.21 (6.7-7.73)	6.29 (3.51-7.33)*
ibrosis stage (METAVIR)	1.63±0.83	1.19±0.86*
nflammation stage METAVIR)	2.19±0.74	1.5±0.72*
D4$^+$ T cells (%) (median)	16.5 (10.16-23.94)	12.55 (7.6-19)
D8$^+$ T cells (%) (median)	2.18 (1.23-3.98)	0.62 (0.27-2.46)**
D68$^+$ cells (%) (median)	1.39 (0.61-3.63)	1.16 (0.16-4.36)
D1$^+$ cells (%) (median)	0.5 (0.10-0.90)	0.29 (0.08-0.64)

Represents $p<0.05$, **represents $p<0.01$.

Table 2. Changes in ALT, AST, HBeAg, and HBVDNA after 6 months of therapy

Characteristics	Antiviral therapy	
	Baseline	6 months
ALT normal/total [n (%)]	3/32 (9.4%)	12/32 (37.5%)
AST normal/total [n (%)]	5/32 (15.6%)	10/32 (31.3%)
HBVDNA negative [n (%)]	0/32 (0%)	6/32 (18.8%)
HBeAg seroconversion [n (%)]	0/32 (0%)	10/32 (31.3%)*
Fibrosis improvement [n (%)]	0/32 (0%)	14/32 (43.8%)
Inflammation improvement [n (%)]	0/32 (0%)	22/32 (68.8%)

*Represents $p<0.05$.

Table 3. Different groups according to the outcome of 6 months of PEG-IFN-α treatment

Groups	Number of patients
FR group (n)	14
IR group (n)	22
ER group (n)	10
FIER group (n)	7
FIRENR group (n)	5
IRFENR group (n)	8
FRIENR group (n)	1
FIENR group (n)	9

FR=fibrosis response. IR=inflammation response. ER=HBeAg seroconversion.

FIER=fibrosis and inflammation response with HBeAg seroconversion.

FIRENR=fibrosis and inflammation response without HBeAg seroconversion.

IRFENR=inflammation response without fibrosis response without HBeAg seroconversion.

FRIENR=fibrosis response without inflammation response without HBeAg seroconversion.

FIENR=no fibrosis and inflammation response and HBeAg seroconversion.

Figures

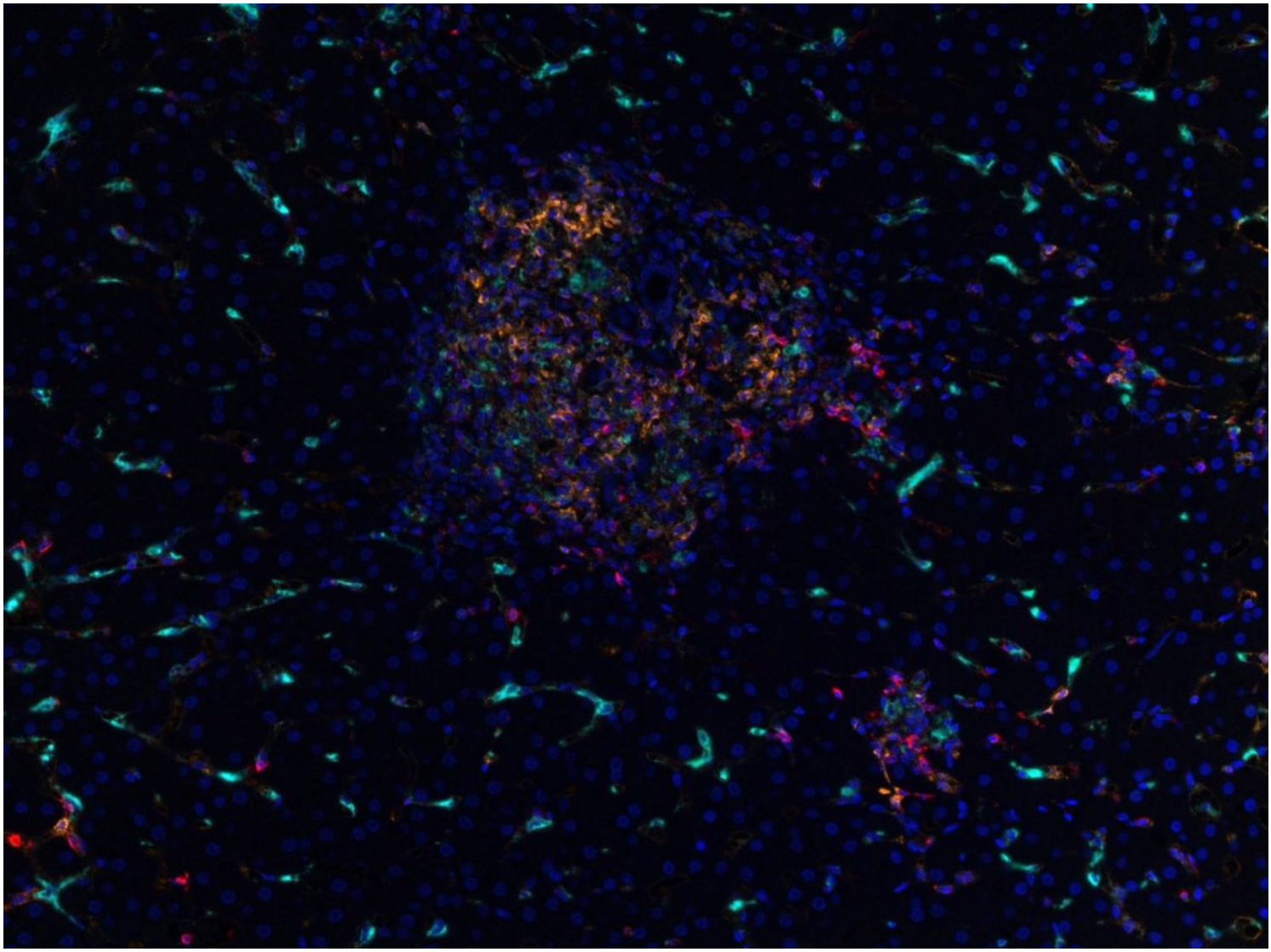

Figure 1

IHC staining. Orange are CD4+ T cells. Red are CD8+ T cells. Cyan are CD68+ mononuclear cells. Green are PD1 positive cells. Blue are cell nuclei.

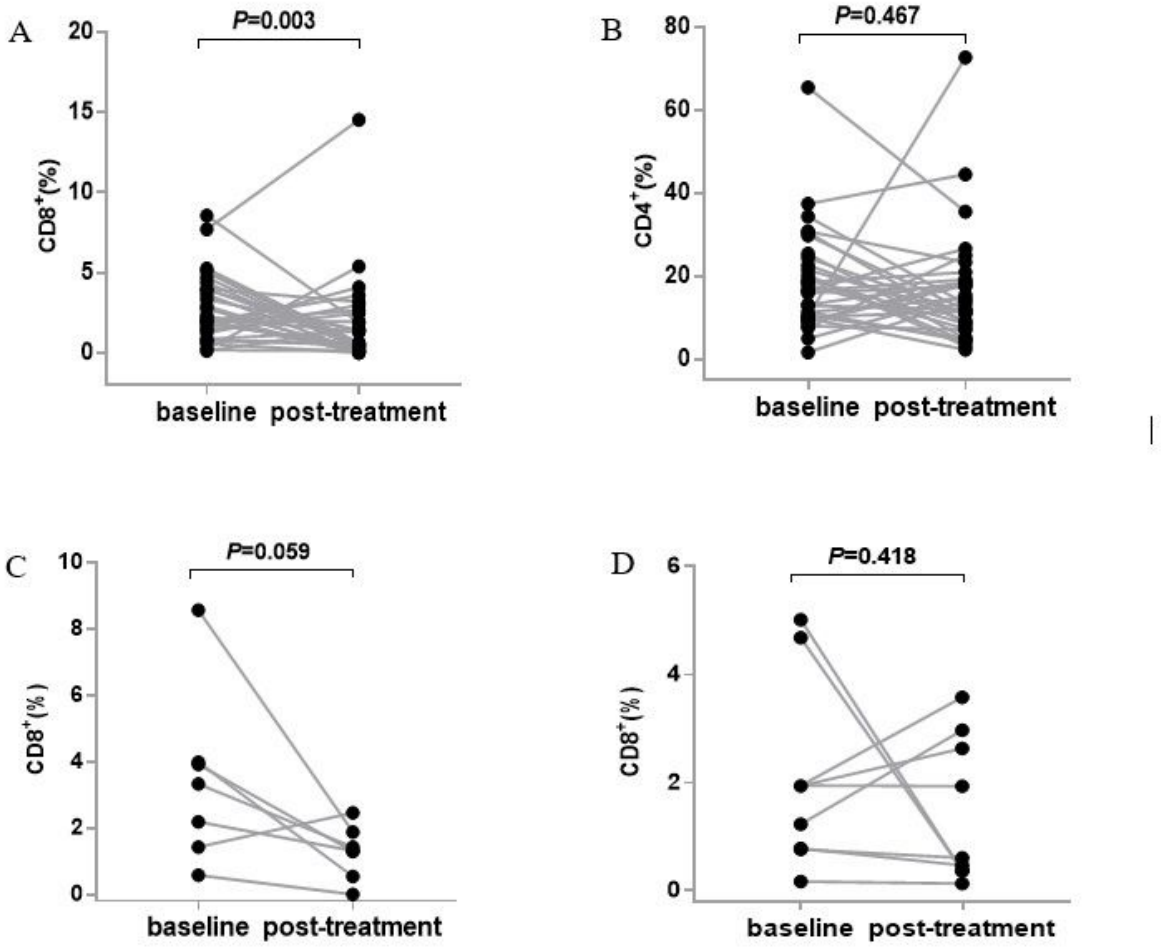

Figure 2

The changes in CD8+ T cells and CD4+ T cells at baseline and post-treatment. CD8+ T cells were significantly decreased in all of the patients (Fig 2A, $p<0.05$), while CD4+ T cells had no significant difference (Fig 2B, $p>0.05$). CD8+ T had no significant difference in the FIER and FIENR groups (Fig 2C, 2D, $p<0.05$) after 6 months of PEG-IFN-α therapy.

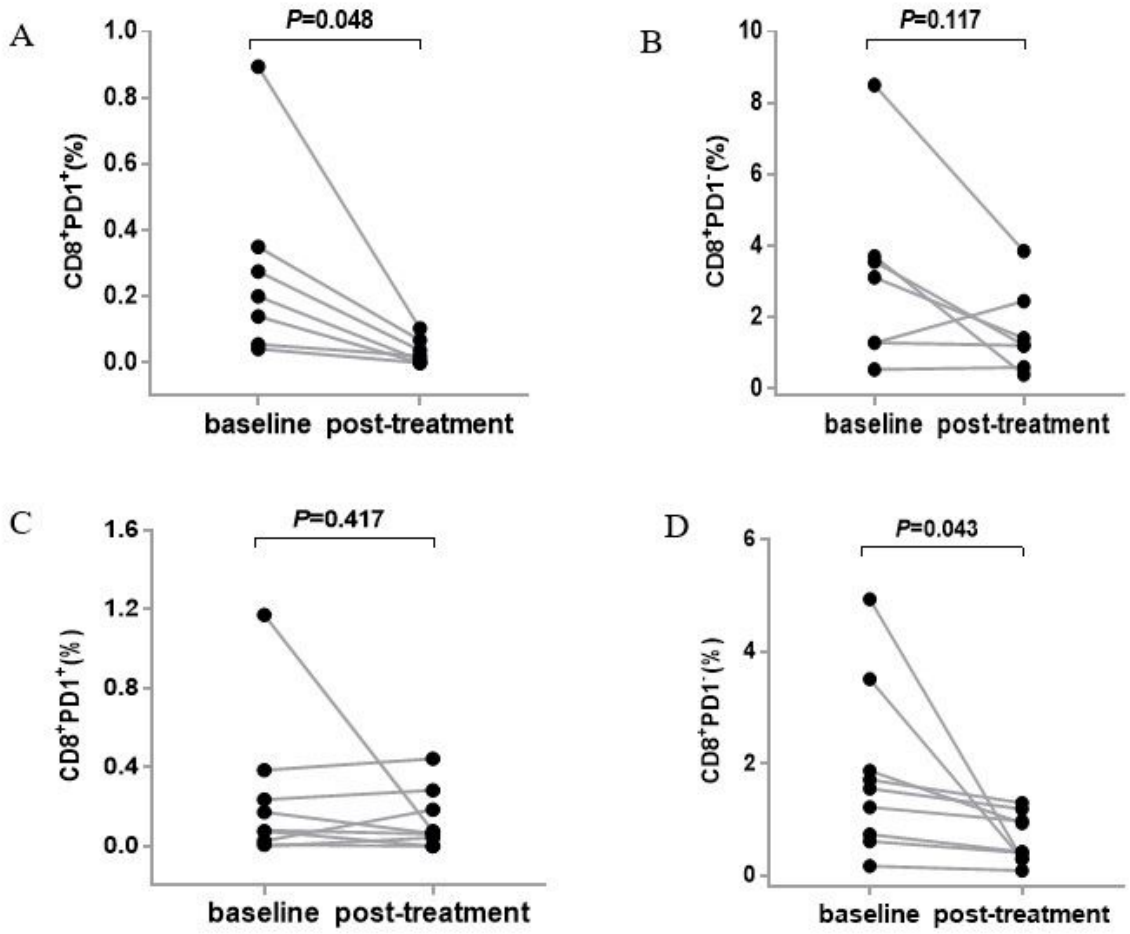

Figure 3

The changes in CD8+PD1+ T cells and CD8+PD1- T cells at baseline and post-treatment in the FIER and FIENR groups. CD8+PD1+ T cells were significantly decreased in the FIER group after 6 months of PEG-IFN-α therapy (Fig 3A, $p<0.05$), while CD8+PD1- T cells had no significant difference (Fig 3B, $p>0.05$). CD8+PD1+ T cells had no significant difference in the FIENR group (Fig 3C, $p>0.05$), while CD8+PD1- T cells were significantly decreased after 6 months of PEG-IFN-α therapy (Fig 3D, $p<0.05$).

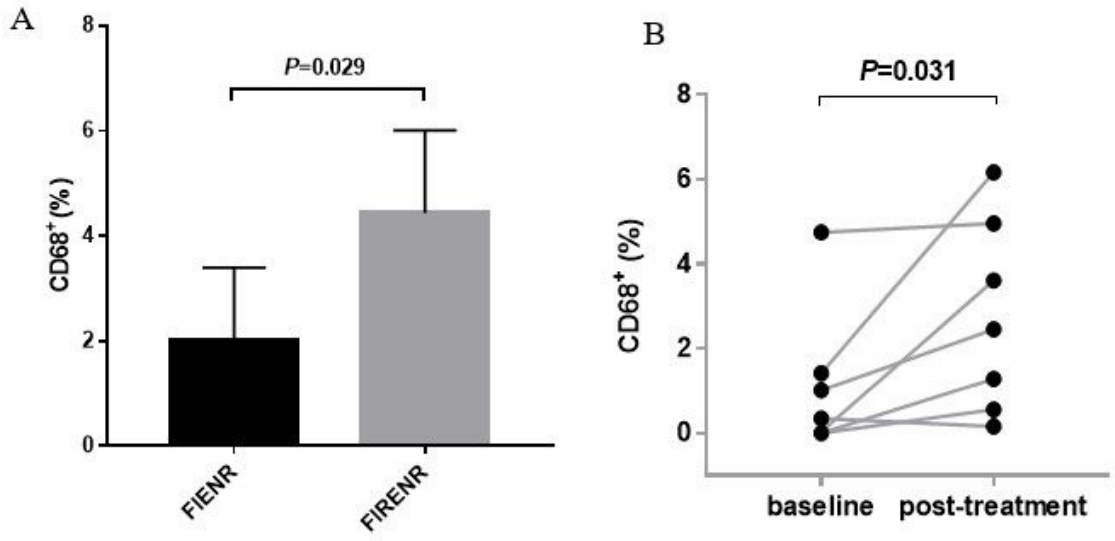

Figure 4

Baseline CD68+ mononuclear cells in different groups. CD68+ mononuclear cells were higher than in the FIENR group at baseline (Fig 4A, $p<0.05$). CD68+ mononuclear cells were significantly increased in the FIER group after 6 months of PEG-IFN-α therapy (Fig 4B, $p<0.05$).