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Original Article

Soluble CD163 and CD163 Expression on Monocytes Associated with Chronic Hepatitis B Inflammation and HBsAg Loss

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Abstract

Background and Aims: Monocyte/macrophage-associated CD163 is an indicator of the severity of liver inflammation and cirrhosis, but the difference of soluble CD163 (sCD163) levels in chronic hepatitis B (CHB) patients and hepatitis B surface antigen (HBsAg)-loss patients is unclear. Herein, we aimed to compare the sCD163 levels in CHB patients and HBsAg-loss patients with or without antiviral treatment. ***Methods:*** sCD163 and CD163 expression on monocytes were compared among four groups, healthy subjects, treatment-naïve CHB patients, spontaneous HBsAg-loss patients, and treatment-related HBsAg-loss patients. The correlation between sCD163 levels and clinical parameters in CHB patients was analyzed. A group of 80 patients with hepatitis B virus (HBV) infection and liver biopsy were recruited. ***Results:*** sCD163 levels were higher in the CHB group than in the other three groups. sCD163 levels were higher in treatment-related HBsAg-loss patients than in spontaneous HBsAg-loss patients. sCD163 levels were negatively correlated with hepatitis B e-antigen (HBeAg) and HBsAg levels in HBeAg-positive patients. Liver biopsy results further demonstrated that sCD163 levels were elevated in CHB patients with substantial inflammation (A≥2) or fibrosis (F≥2). The sCD163 model was more sensitive in predicting inflammation than other noninvasive models. Its levels were higher in patients with normal alanine aminotransferase levels and significant inflammation (A≥2) than in patients with no or mild inflammation. ***Conclusions:*** sCD163 and CD163 expression on monocytes were associated with CHB inflammation and HBsAg loss, and may be used as markers to predict HBV-specific immune activation.

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Introduction

Hepatitis B virus (HBV) infection is a major health concern worldwide. Twenty million patients have been estimated to suffer from chronic HBV infection in China and are at a high risk of cirrhosis and hepatocellular carcinoma. Treatment with nucleos(t)ide analogues and pegylated interferon are effective in preventing HBV replication, but virus eradication has not yet been achieved.[1]

Innate and adaptive immunity play important roles in HBV infection.[2] Antigen-presenting cells (APCs) bridge innate and adaptive immunity. Peripheral monocytes/macrophages and Kupffer cells in the liver are important APCs in the innate immune system that clear viral infection by activating HBV-specific $CD4^+$ or $CD8^+$ T cells.[3,4] Monocytes/macrophages can be divided into several subsets according to the expression of cell surface antigens CD14 and CD16, including classical, intermediate, and nonclassical monocyte subsets.[5] Monocytes/macrophages express the surface molecule CD163, which is a high-affinity scavenger receptor of the hemoglobin-haptoglobin complex,[6] and in the absence of haptoglobin, with lower affinity, for hemoglobin alone.[7] It is also a marker of cells of the monocyte/macrophage lineage.[8] Activated monocytes/macrophages shed the hemoglobin-haptoglobin scavenger receptor CD163 into circulation as soluble CD163 (sCD163). Thus, sCD163 is a marker of monocytes/macrophages activation and is nega-

Keywords: CD163; Hepatitis B virus; HBsAg loss; Monocytes; Immune activation.

Abbreviations: CHB, chronic hepatitis B; HBeAb, hepatitis B e antibody; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; sCD163, soluble CD163; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; PLT, platelet count; PBMC, Peripheral blood mononuclear cells; RPR, red cell distribution width to platelet ratio; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis-4; CI, confidence intervals; ROC, receiver operating characteristic; ULN, upper limit of normal.

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tively correlated with the expression of CD163 on monocytes/macrophages.[9,10]

Recently, it has been found that CD163 and sCD163 have diagnostic and predictive value in many infectious diseases.[11–15] sCD163 has been reported as an indicator of liver inflammation and fibrosis in patients chronically infected with HBV.[16] In addition, a study showed that sCD163 was independently associated with fibrosis in patients with chronic viral hepatitis B and C.[11] Although CD163 and sCD163 are associated with inflammation and liver fibrosis during HBV infection, their levels in patients with chronic hepatitis B (CHB) or hepatitis B surface antigen (HBsAg) loss remain unclear. Comparison of CD163 levels between CHB and HBsAg-loss patients may be helpful in elucidating the dynamic change of CD163 in chronic HBV infection and provide a new marker for evaluating the specific immune status of patients with chronic HBV infection.

Methods

Selection of patients

A total of 170 adults were enrolled in the study between 2014 and 2016, including 24 healthy subjects (HS), 97 treatment-naïve CHB patients (CHB), 18 patients with spontaneous HBsAg loss (SL), and 31 patients with treatment-related HBsAg loss (TL). An additional 80 liver biopsy patients with chronic HBV infection were enrolled. Inclusion criteria for CHB patients were clinical, biochemical, and virological evidence of HBsAg positivity for at least 6 months, elevated alanine transaminase (ALT) and HBV DNA, not currently receiving antiviral therapy, or discontinuation of antiviral therapy more than 6 months previously and followed by a virological relapse. Virological response was defined as an undetectable HBV DNA level (<500 IU/mL) after 48 weeks of nucleos(t)ide analog therapy. Serological response was defined as an HBsAg decline >1 $\log_{10}$ IU/mL or an HBeAg decline >1 $\log_{10}$ IU/mL after 48 weeks of nucleos(t)ide analog therapy. All subjects were recruited at Ruijin Hospital (Shanghai, China) and were negative for antibodies against hepatitis A, C, and delta viruses and human immunodeficiency virus. Patients with liver cirrhosis and/or systematic diseases were excluded from the study. The study was approved by the Ethics Committee of Ruijin Hospital and was conducted following the ethical guidelines of the Declaration of Helsinki.

Biochemical and serologic assays

HBsAg, hepatitis B e-antigen (HBeAg), anti-HBs, anti-HBe, and hepatitis B core antibody (anti-HBc) were determined with a chemiluminescent microparticle immunoassay (Abbott Architect; Abbott Laboratories, North Chicago, IL, USA). Quantitative HBsAg (Abbott Diagnostics Division, Ireland) was assayed with the Abbott Architect system. Serum HBV DNA was quantified with Cobas AmpliPrep/Cobas TaqMan (Roche Diagnostics, Basel, Switzerland). Biochemical assays, such as ALT, aspartate transaminase (AST), platelet count (PLT), total bilirubin (TBIL), and direct bilirubin (DBIL) were also performed at the time of sampling.

Isolation of peripheral blood monocytes

Peripheral blood mononuclear cells (PBMCs) were isolated using Lymphoprep (Axis Shield, Dundee, Scotland) within 4 h after blood collection, cryopreserved in fetal bovine serum (Gibco, USA) containing 10% dimethylsulfoxide (Sigma-Aldrich, St. Louis, MO, USA), and stored in liquid nitrogen.

Determination of sCD163 and CD163 expression on monocytes

sCD163 was determined with an enzyme-linked immunosorbent assay (ELISA). sCD163 in plasma was measured with a DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. CD163 expression on monocytes was assayed by flow cytometry. To correlate the expression of CD163 with that of other monocyte cell surface markers, PBMCs were assayed by flow cytometry after surface antibody staining of CD14 (anti-human, FITC, Mouse IgG2a, κ, Biolegend), CD16 (anti-human, PE, Mouse IgG1, κ, Biolegend), CD163 (anti-human CD163, APC, Mouse IgG1, κ, Biolegend), incubated at 4°C for 30 min, washed once with phosphate buffered saline, fixed with 100 μL 4% paraformaldehyde. A FACS Calibur flow cytometer (BD, San Diego, USA) was used with FlowJo 9.6 software (Tree Star). The mean fluorescence intensity (MFI) of $CD163^+$ cells were detected.

Model calculations

The METAVIR score is used to evaluate the severity of inflammation and fibrosis. The grade indicates the amount of inflammation in the liver and the stage represents the amount of scarring or fibrosis. The gamma-glutamyl transpeptidase to platelet ratio (GPR), red cell distribution width-to-platelet ratio (RPR), fibrosis index based on four factors (FIB-4), and aspartate aminotransferase-to-platelet ratio index (APRI) were calculated as: GPR = (GGT/ULN of GGT) × 100/PLT; RPR=RDW (%)/PLT; FIB-4= (age × AST) / (PLT × $ALT^{1/2}$), and APRI = (AST/ULN of AST) ×100/PLT where ULN is the upper limit of normal. The AAG and AAGP diagnostic algorithm for the evaluation of significant liver inflammation were calculated as previously described.[17]

Statistical analysis

One-way analysis of variance with the Bonferroni correction was used for the multiple-group comparisons. Student's *t*-test was used to compare between-group differences in normally distributed variables. Kruskal-Wallis and Mann-Whitney tests were used for non-normally distributed data. The correlation coefficient (r) was calculated with the nonparametric Spearman correlation. Statistical significance was set at $p<0.05$. Numerical data were reported as mean ± standard error of the mean.

Results

Demographic, serological, biochemical characteristics and sCD163 levels of the subjects

Baseline demographic, serological, and biochemical characteristics for the subjects are shown in Supplementary Table 1. The four groups were healthy subjects, treatment-naïve patients with CHB, patients with spontaneous HBsAg loss, and patients with treatment-related HBsAg loss. CHB patients had the highest ALT, AST, TBIL, and DBIL levels ($p<0.001$) and the lowest PLT levels ($p<0.001$); 67% were genotype B and 23% were genotype C. The proportion of HBeAg-positive pa-

tients was 67%. The mean baseline HBsAg and HBV DNA levels were 4.25±0.25 $\log_{10}$ IU/mL and 7.87±0.59 $\log_{10}$ copies/mL, respectively (Supplementary Table 1). The ELISA results of plasma sCD163 in the four groups are shown in Figure 1. Plasma sCD163 levels in CHB patients (1,099.02±72.64 ng/mL) were significantly higher than those in the other three groups (p<0.001). In addition, the plasma sCD163 levels in patients with treatment-related HBsAg loss (558.6±58.68 ng/mL) were significantly higher than those in patients with spontaneous HBsAg loss (255±29.21 ng/mL; p=0.003). Taken together, the results indicate that sCD163 may be a sensitive serum marker for CHB and may be used as an index for evaluating HBV infection prognosis.

Correlation of plasma sCD163 level and clinical parameters of CHB patients

The correlations between plasma sCD163 levels and clinical parameters of CHB patients are shown in Figure 2A–E. Plasma sCD163 levels were positively correlated with ALT (r=0.69, p<0.001), AST (r=0.77, p<0.001), DBIL (r=0.55, p<0.001), and TBIL (r=0.25, p=0.016) and negatively correlated with PLT (r=−0.31, p=0.004). The correlations indicate that sCD163 may be associated with liver inflammation. In HBeAg-positive patients, sCD163 levels were negatively correlated with HBeAg (r=−0.38, p=0.0019; Fig. 2F), HBsAg (r=−0.45, p<0.001; Fig. 2G), and HBV DNA (r=−0.08, p=0.52; Fig. 2H). In HBeAg-positive CHB patients, the HBsAg and HBV DNA levels decreased with the progression of immune activation. The negative correlation of sCD163 levels with HBsAg and HBV DNA reflects its cor-

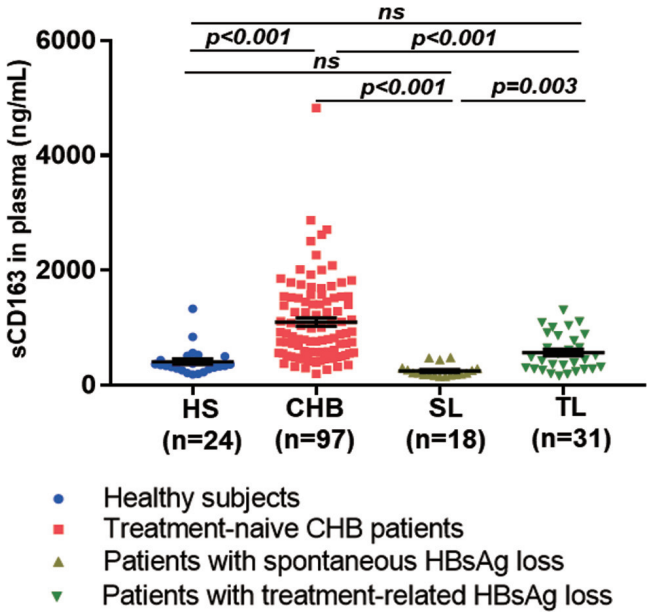

Fig. 1. Comparison of sCD163 levels in study subjects. Comparison of sCD163 levels in 24 healthy subjects (HS), 97 treatment-naïve patients with chronic hepatitis B (CHB), 18 with spontaneous hepatitis B surface antigen (HBsAg) loss (SL), and 31 with treatment-related HBsAg loss (TL). Plasma sCD163 levels were measured by enzyme-linked immunosorbent assays. *P*-values from Kruskal-Wallis tests adjusted for multiple comparisons. Significant p≤0.05 in *italic*. Data are means±SEM.

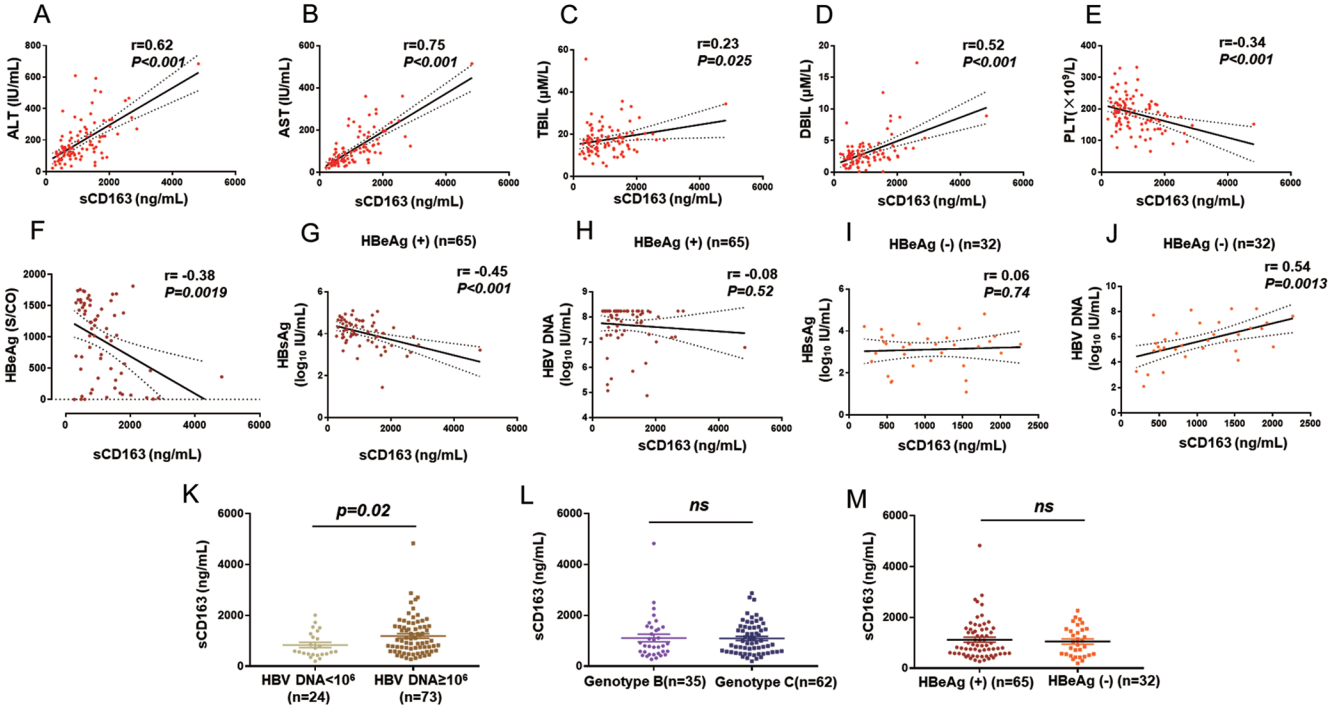

Fig. 2. Correlation of plasma sCD163 level and biochemical and virological assays in treatment-naïve CHB patients. (A) Plasma sCD163 and ALT. (B) Plasma sCD163 and AST. (C) Plasma sCD163 and total bilirubin. (D) Plasma sCD163 and direct bilirubin. (E) plasma sCD163 levels and PLT. (F) Plasma sCD163 and hepatitis B e-antigen (HBeAg, $\log_{10}$) in CHB patients. (G). sCD163 and HBsAg ($\log_{10}$) in HBeAg(+) CHB patients. (H) sCD163 and hepatitis B virus (HBV) DNA ($\log_{10}$) in HBeAg(+) CHB patients. (I) sCD163 levels and HBsAg ($\log_{10}$) in HBeAg(−) CHB patients. (J) sCD163 levels and HBV DNA ($\log_{10}$) in HBeAg(−) CHB patients. (K) sCD163 level in HBV DNA <10^6 IU/mL and HBV DNA≥10^6 IU/mL. (L) sCD163 level in HBV genotypes B and C. (M) sCD163 in HBeAg(+) and HBeAg(−) patients. Comparison (Mann-Whitney test and Spearman's rank correlation. Data are means±SEM. p-values ≤0.05 are in *italic*. CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; sCD163, soluble CD163; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; PLT, platelet count.

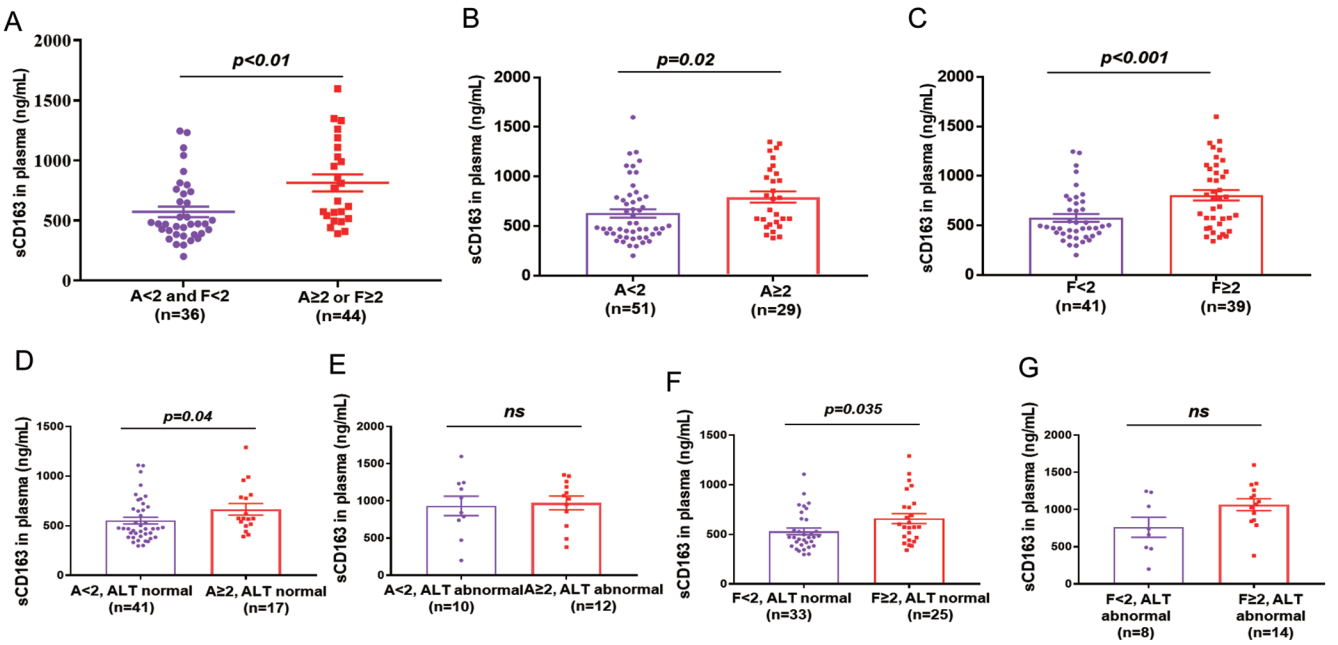

Fig. 3. Correlation of plasma sCD163 level and liver inflammation and fibrosis in liver biopsy patients with chronic HBV infection. (A) Patients with no or mild inflammation and fibrosis (A<2 and F<2) and patients with moderate or severe inflammation or fibrosis (A≥2 or F≥2). B. Patients with substantial inflammation (A≥2) and patients with no or mild inflammation (A<2) (C) Patients with substantial fibrosis (F≥2) and patients with no or portal fibrosis without septa (F<2). (D) Patients with no or mild inflammation (A<2) and patients with substantial inflammation (A≥2) and normal ALT levels. (E) Patients with no or mild inflammation (A<2) and patients with substantial inflammation (A≥2) and abnormal ALT levels (F) Patients with no significant fibrosis (F<2) and patients with substantial fibrosis (F≥2) and normal ALT levels. (G) Patients with no significant fibrosis (F<2) and patients with substantial fibrosis (F≥2) and abnormal ALT levels. Data are means±SEM, Mann-Whitney test, *p*-values <0.05 are in *italic*. HBV, hepatitis B virus; sCD163, soluble CD163; ALT, alanine aminotransferase.

relation with immune activation. In HBeAg-negative CHB patients, sCD163 levels were significantly positively correlated with HBV DNA (r=0.54, p=0.0013; Fig. 2J). Consistent with this, in HBeAg-negative CHB patients, the levels of HBV DNA fluctuated periodically or increased with the progression of immune activation, and the positive correlation between sCD163 and HBV DNA reflects the correlation between sCD163 and immune activation. In addition, we compared the sCD163 levels in patients with HBV DNA $<10^6$ IU/mL and those with HBV DNA$\geq 10^6$ IU/mL. We found that the sCD163 levels in patients with HBV DNA$\geq 10^6$ IU/mL were higher than those in patients with HBV DNA $<10^6$ IU/mL (p=0.02; Fig. 2K). However, in CHB patients, there was no significant difference in the levels of sCD163 in HBeAg-positive and HBeAg-negative patients (Fig. 2L), nor between genotype B and genotype C patients (Fig. 2M).

Expression of membrane-bound CD163 on monocytes

We assayed the presence of $CD163^+$ monocytes in the 101 subjects in the four study groups who had PBMCs sufficient for multiparameter flow cytometry. The monocyte population was divided into classical ($CD14^+CD16^-$; Fig. 3A), intermediate ($CD14^+CD16^+$; Fig. 3B), and nonclassical ($CD14^-CD16^+$; Fig. 3C) monocyte subsets. No statistically significant differences were observed in the percentages of classical (p=0.410), intermediate (p=0.617), and nonclassical (p=0.192) monocytes in the four groups (Table 1). The frequency of CD163 expression (Table 1) reveal no significant differences on classical (p=0.568), intermediate (p=0.555), and nonclassical (p=0.264) monocytes. However, the MFI of CD163 expression was significantly different on the classical (p=0.010) and intermediate (p=0.042) monocytes but not on nonclassical monocytes (p=0.098, Table 1). Interestingly, as shown in Figure 4D, in classical monocytes, the MFI of CD163 expression in healthy subjects was significantly higher than that in patients with CHB (232.2±29.61 vs. 138.9±7.4, p<0.01), spontaneous HBsAg loss (232.2±29.61 vs 146.9±19.45, p<0.01), and treatment-related HBsAg loss (232.2±29.61 vs. 142.1±11.34, p<0.01). In intermediate monocytes, the MFI of CD163 expression in healthy subjects was higher than that in patients with CHB (187.9±20.21 vs. 162.3±10.20, p<0.01) and in those with spontaneous HBsAg loss (187.9±20.21 vs. 121.7±13.12, p<0.01) but not in those with treatment-related HBsAg loss (187.9±20.21 vs. 168.9±21.37, p=0.625).

Correlation between plasma sCD163 levels and liver inflammation and fibrosis in chronic HBV-infected patients with liver biopsy

The clinical parameters, including baseline demographic, serological, and biochemical characteristics on the 80 liver biopsy patients with HBV infection are shown in Supplementary Table 2. The correlation between sCD163 levels and HBV was assessed with the meta-analysis of histological data in viral hepatitis (METAVIR) scores in each group. We compared the sCD163 levels in those with no or mild inflammation and fibrosis (A<2 and F<2) and those moderate-to-severe inflammation or fibrosis (A≥2 or F≥2). As shown in Figure 3A, sCD163 levels were higher in moderate or severe inflammation or fibrosis (A≥2 or F≥2) than in no or mild inflammation and fibrosis (A<2 and F<2; p<0.01). In addition, our results showed that sCD163 levels were significantly associated with moderate and severe inflammatory activity in liver biopsy (A<2 vs. A≥2; p=0.02; Fig. 3B). sCD163 levels were also significantly higher in moderate and severe liver fibrosis than no or mild fibrosis (F<2

Table 1. Expression of membrane-bound CD163 on monocytes

	Healthy subjects (N=9)	Treatment-naïve CHB patients (N=53)	Patients with spontaneous HBsAg loss (N=13)	Patients with treatment-related HBsAg loss (N=26)	P-value
$CD14^+CD16^-$ (%)	3.2±0.92	4.3±0.43	3.9±1.09	4.8±0.72	0.410
$CD14^+CD16^-CD163^+$ (%)	61.5±7.93	57.2±2.68	62.3±4.45	62.3±3.12	0.568
$CD14^+CD16^-CD163^+$MFI	232.2±29.61	138.9±7.40	146.9±19.45	142.1±11.34	0.010
$CD14^+CD16^+$ (%)	0.9±0.19	1.1±0.12	0.9±0.19	1.2±0.18	0.617
$CD14^+CD16^+CD163^+$ (%)	71.8±4.34	64.2±2.70	63.6±3.16	66.5±3.55	0.555
$CD14^+CD16^+CD163^+$MFI	187.9±20.21	162.3±10.20	121.7±13.12	168.9±21.37	0.042
$CD14^-CD16^+$ (%)	10.7±2.12	13.5±0.83	11.6±1.51	10.9±1.11	0.192
$CD14^-CD16^+CD163^+$ (%)	2.5±0.9	1.2±0.23	0.9±0.29	1.4±0.51	0.264
$CD14^-CD16^+CD163^+$MFI	74.5±16.40	87.6±5.22	73.2±12.07	74.4±8.45	0.098

The monocyte expression is expressed as a percentage. Data are mean±standard error of the mean. HBsAg, hepatitis B surface antigen.

vs. F≥2; $p<0.001$; Fig. 3C). We also observed that despite significant liver inflammation (A≥2), several CHB patients had normal ALT levels, which indicates that ALT had a false-negative rate in predicting significant liver inflammation. As shown in Figure 3D, the levels of sCD163 were compared between patients with HBV infection (A<2) and patients with CHB (A≥2) and normal ALT levels. The results show that although ALT levels were normal, sCD163 levels were significantly higher ($p=0.04$) in patients with moderate or severe inflammation (A≥2) than in those with no or mild inflammation (A<2). However, sCD163 levels were not significantly different in those no or mild inflammation (A<2) and moderate or severe inflammation (A≥2) under abnormal ALT levels (Fig. 3E). These results suggest that sCD163

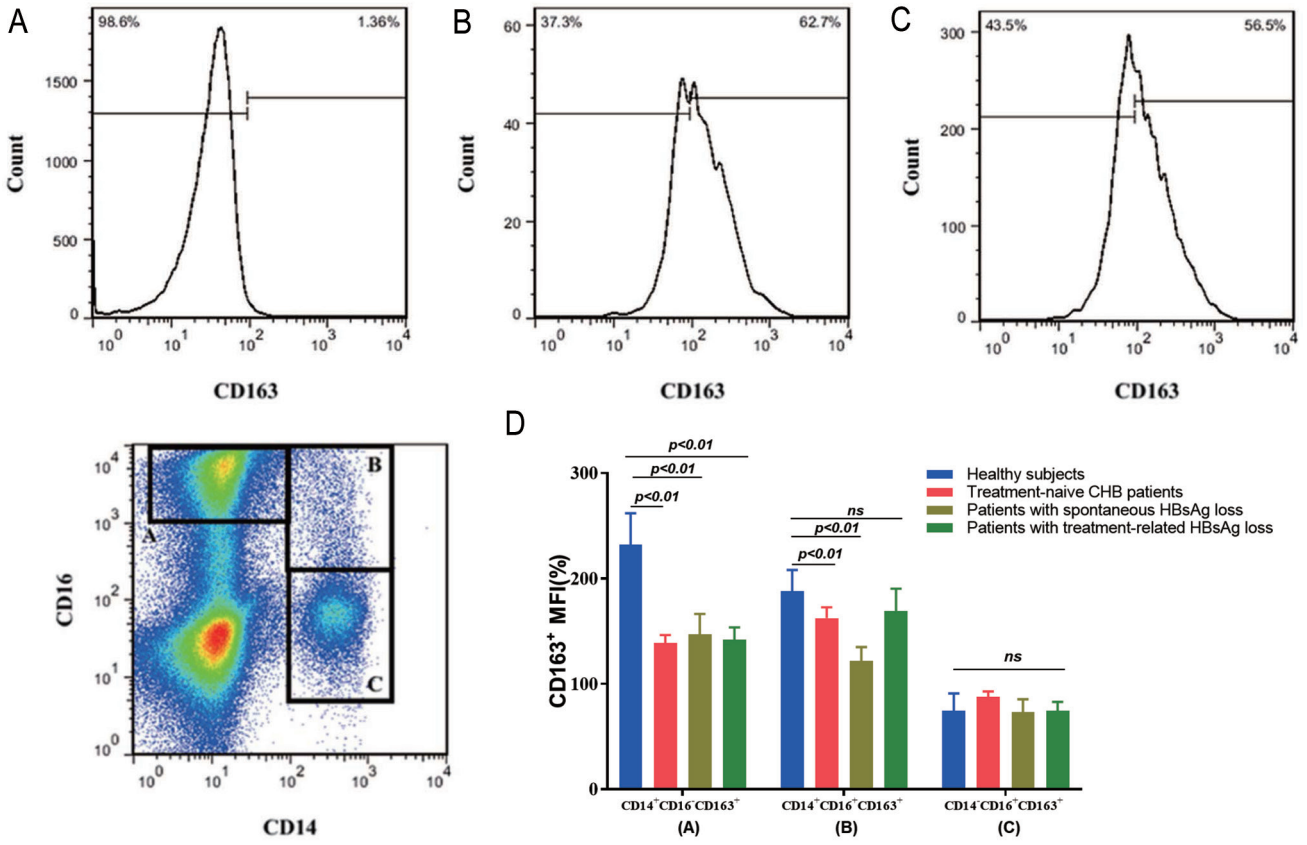

Fig. 4. Mean fluorescence intensity (MFI) of CD163 expression on monocytes subsets. Gating of monocyte/macrophage subsets by CD14 and CD16 expression. (A) $CD14^+CD16^-$. (B) $CD14^+CD16^+$. (C) $CD14^-CD16^+$. (D) Comparison of the MFI of CD163 expression on classical ($CD14^+CD16^-CD163^+$) and intermediate ($CD14^+CD16^+CD163^+$) monocyte subsets in healthy subjects, treatment-naïve CHB patients, patients with spontaneous HBsAg loss, and patients with treatment-related HBsAg loss. Data are means±SEM. *p*-values <0.05 are in *italic*. CHB, chronic hepatitis B; HBsAg, hepatitis B surface antigen; sCD163, soluble CD163.

levels were more accurate than ALT levels in predicting liver inflammation. Similarly, the levels of sCD163 were higher in patients with moderate or severe fibrosis (F≥2) than in those with no or mild fibrosis (F<2) under normal ALT levels (p=0.035; Fig. 3F) but were not significantly different between the two groups with abnormal ALT levels (Fig. 3G).

Predictive value of sCD163 levels in chronic HBV-infected patients with liver biopsy

It is well known that when the liver has severe inflammation, most patients with CHB have abnormal changes in liver function, including elevated ALT and AST levels, and fluctuation of HBsAg and HBV DNA. Receiver operating characteristic (ROC) curve analysis was used to evaluate the sensitivity of sCD163, ALT, AST, HBsAg, and HBV DNA in detecting significant liver inflammation (Fig. 5A). The areas under the ROC curve (AUROCs) of sCD163, ALT, AST, HBsAg, and HBV DNA for differentiating patients with A≥2 from those with A<2 were 0.68 (p=0.008), 0.68 (p=0.008), 0.68 (p=0.008), 0.55 (p=0.48), and 0.62 (p=0.06), respectively. Multiple logistic regression analysis was performed with the METAVIR inflammation score as the dependent variable and sCD163, ALT, and AST as the explanatory variables. The ROC curves are shown in Figure 5B, and the new AUROC was 0.70 (p=0.001). The results suggest that sCD163 combined with ALT and AST was a better predictor of liver inflammation. We further evaluated the performance of other noninvasive models for predicting significant liver inflammation. The AUROCs and 95% confidence intervals (CI) of the various noninvasive models are shown in Table 2. In general, the diagnostic performance of other noninvasive models was lower than that of our model. The results show that sCD163 was a better predictor of liver inflammation. In addition, the presence of significant fibrosis (F≥2) is normally used as an indicator for initiating antiviral therapy. We compared the AUROC of sCD163 with those of GPR, RPR, APRI, and FIB-4, as shown in Figure 5C. The AUROC

Table 2. Comparison of AUROC between sCD163 model and other non-invasive models for liver inflammation

Non-invasive models	AUROC	95%CI
sCD163 model	0.70	0.58–0.81
AAGP model	0.61	0.48–0.74
AAG model	0.6	0.47–0.73
RPR model	0.58	0.43–0.74

sCD163, soluble CD163; RPR, red cell distribution width to platelet ratio; ROC, Receiver operating characteristic

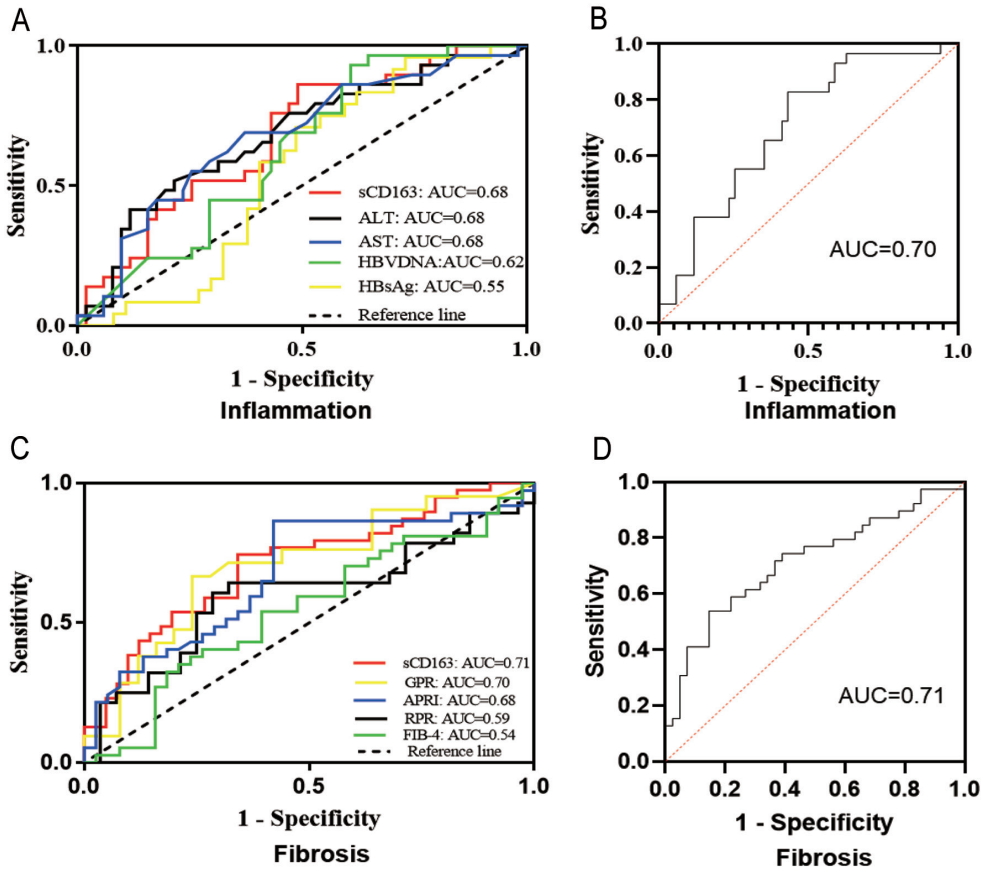

Fig. 5. Predictive value of sCD163 level in patients with chronic HBV infection and liver biopsy. (A) Receiver operating characteristic (ROC) curve analysis for identifying patients with significant inflammation (A≥2) by the sCD163, ALT, AST, HBsAg, or HBV DNA level. (B) ROC curve analysis for identifying patients with significant inflammation (A≥2) by combining the sCD163, ALT, and AST levels. (C) ROC curve analysis to identify patients with significant fibrosis (F≥2) by the sCD163, GPR, RPR, APRI, or FIB-4 level. (D) ROC curve analysis for identifying patients with significant fibrosis (F≥2) by combining the sCD163, ALT and, AST levels. HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; sCD163, soluble CD163; ALT, alanine aminotransferase; AST, Aspartate aminotransferase; RPR, red cell distribution width to platelet ratio; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis-4.

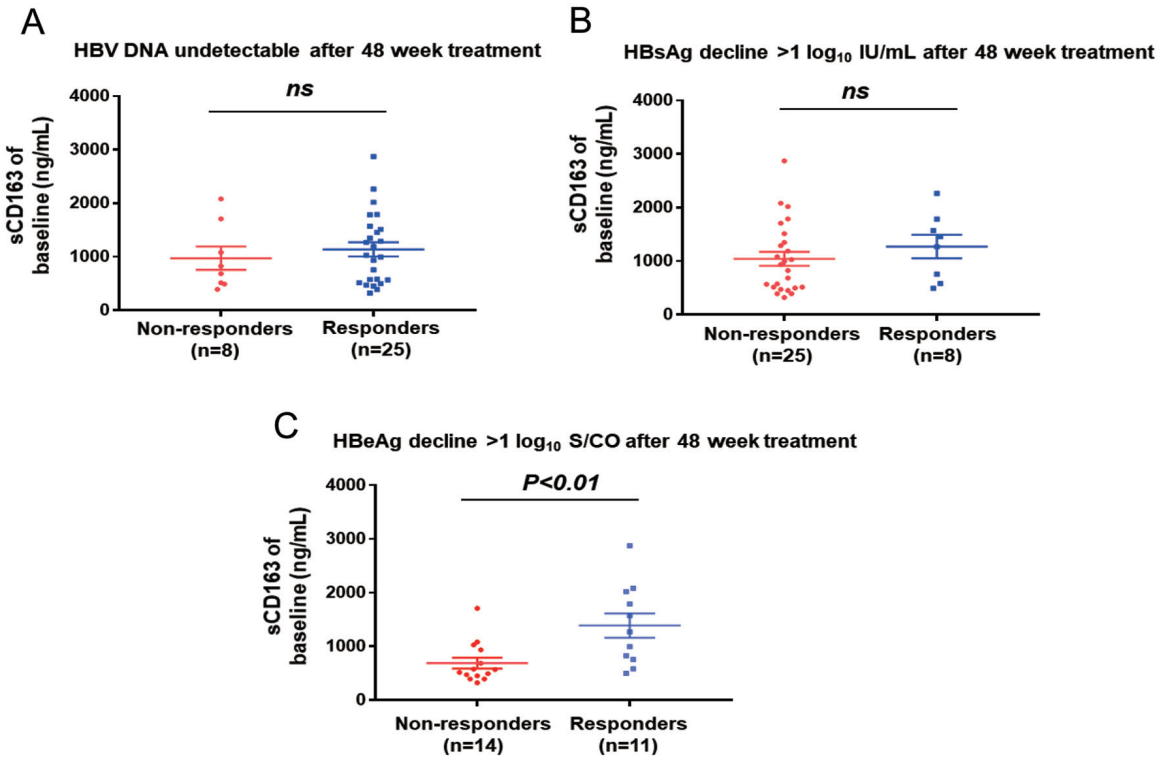

Fig. 6. sCD163 level for prediction of antiviral treatment response after 48 weeks of nucleos(t)ide analog treatment. (A) Comparison of baseline sCD163 levels in antiviral treatment responders and nonresponders based on no detectable HBV DNA undetectable. (B) Comparison of baseline sCD163 levels in antiviral treatment responders and nonresponders based on an HBsAg decline >1 $\log_{10}$ IU/mL (C) Comparison of baseline sCD163 levels in antiviral treatment responders and nonresponders based on an HBeAg decline >1 $\log_{10}$ signal/cutoff ratio. Mann-Whitney test; *p*-values adjusted for multiple comparisons. *p*-values ≤0.05 are in *italic*. Data are means±SEM. HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; sCD163, soluble CD163.

of sCD163 was 0.71 (95% CI: 0.597–0.824, p=0.0012), those of GPR, RPR, APRI, and FIB-4 were 0.70, 0.59, 0.68, and 0.54, respectively. The levels of sCD163 were significantly higher than those of GPR, RPR, APRI, and FIB-4 in the prediction of significant fibrosis. We performed multiple logistic regression analysis with the METAVIR fibrosis score as the dependent variable and sCD163, ALT, and AST as the explanatory variables. The ROC curves are shown in Figure 5D, and the new AUROC was 0.71 (p=0.001). The results suggest that sCD163 is a better predictor of liver fibrosis than GPR, RPR, APRI, or FIB-4.

Prediction of antiviral treatment response using sCD163

Some studies have reported that antiviral treatment significantly reduced the levels of sCD163.[18] Our study further investigated whether sCD163 levels could predict antiviral treatment response. As shown in Figure 6A, defining virological response with the undetectable HBV DNA levels after 48 weeks of nucleos(t)ide analog treatment, there was no difference in the baseline levels of sCD163 at baseline between virological response (n=25) and nonresponse (n=8). Similarly, in Figure 6B, defining serological response a decrease of ≥1 $\log_{10}$ IU/mL in HBsAg after 48 weeks of nucleos(t)ide analog treatment, there were no difference in the baseline levels of sCD163 in the serological responders (n=8) and nonresponders (n=25). However, because there were only 33 patients in the antiviral treatment groups, and the numbers of responders and nonresponders were limited, which might have caused statistical bias. The correlation between the sCD163 levels and the efficacy of antiviral therapy is worthy of further investigation in larger groups of patients. Also, the addition of another serological response defined as a decrease of ≥1 $\log_{10}$ in HBeAg signal/cutoff ratio after 48 weeks of nucleos(t)ide analog treatment shown in Figure 6C. We found that the baseline level of sCD163 was significantly higher in the serological responders(n=11) than the nonresponders (n=14; p<0.01), which indicates that the correlation between sCD163 levels and antiviral response needs further investigation.

Discussion

Our study demonstrated that monocyte/macrophage expression and sCD163 levels were closely correlated with liver inflammation and fibrosis in HBV infection. Importantly, we revealed a correlation between CD163 expression and immune activation in patients with CHB. The ultimate endpoint of chronic HBV treatment was sustained HBsAg loss with or without seroconversion to hepatitis B surface antibody (anti-HBs). Current antiviral therapies using pegylated interferon or nucleos(t)ide analogues to suppress HBV replication and improve the prognosis of CHB, but they fail to clear HBsAg. As HBsAg might contribute to the impairment of innate and adaptive immunity and the exhaustion of T cell and B cell responses, a reduction of serum HBsAg could facilitate the recovery of the host's immune system.[19] Our results showed that the levels of sCD163 were higher in patients with CHB than in HBsAg-loss patients and healthy subjects. Interestingly, we found that sCD163 levels were lower in patients with spontaneous HBsAg loss than in those

with treatment-related HBsAg loss. However, the mechanism underlying the lower levels of sCD163 in patients with spontaneous HBsAg loss needs to be further explored.

In patients with CHB, sCD163 was negatively correlated with HBsAg and HBeAg in HBeAg-positive patients, which reflects its correlation with immune activation considering that HBsAg and HBeAg levels decreased with the progression of immune activation in HBeAg-positive patients. Similarly, in HBeAg-negative patients with CHB, the levels of HBV DNA fluctuated periodically or increased with the progression of immune activation, and the positive correlation between sCD163 and HBV DNA reflected the correlation between sCD163 and immune activation during HBV infection. The results indicate that sCD163 is closely associated with immune activation during HBV infection.

Some studies have reported that sCD163 is an indicator of liver inflammation and fibrosis in patients with HBV infection.[11–13,16,20] Our study further demonstrated that sCD163 levels were positively correlated with the severity of liver inflammation and fibrosis in patients with CHB. Importantly, we found that sCD163 was higher in CHB patients with significant inflammation (A≥2) than in those with no or mild inflammation (A<2) with normal ALT levels. The results suggest that sCD163 was more accurate than ALT in identifying significant liver inflammation. Meanwhile, ROC analysis showed that sCD163 combined with ALT and AST was better predictor of liver inflammation. In addition, we compared the AUROC of sCD163 with those of GPR, RPR, APRI, and FIB-4, and found that sCD163 had the highest AUROC value, suggesting that sCD163 was a better predictor of liver fibrosis than ALT.

When inflammation and fibrosis were more severe, the levels of sCD163 were elevated, suggesting the activation of monocytes. Some studies have demonstrated that patients with CHB and no, mild, or severe hepatitis tended to have increased T cell activation linked with liver inflammation.[21] Therefore, the results indicate that as a marker of monocyte activation, CD163 is associated with the activation of HBV-specific T cells.

Because of the strong interplay between specific T cell immunity and elimination of HBV, restoration of T cell immunity against HBV is considered an important outcome in current novel therapeutic approaches. Bertoletti et al. reported that the ability of HBV-specific $CD8^+$ T cells to secrete Type 1 T helper cytokines in patients with spontaneous HBsAg loss was significantly higher than that in patients with chronic HBV infection.[22,23] HBV-specific immunity in patients with resolved HBV infection is robust and multifunctional, whereas CHB is characterized by dysfunctional innate and adaptive antiviral immunity.[24] Although regular antiviral therapy can partially restore the host-specific immune response, it cannot completely reconstruct the host's HBV-specific immune function.[25] In addition, more effective immunotherapy and representative markers are needed to reflect immune status in patients with HBV infection. In our study, the decrease in sCD163 levels in patients with spontaneous HBsAg loss compared with patients with CHB may partly reflect the restoration of the host anti-HBV-specific immune response.

In addition, classical monocytes can phagocytize foreign pathogens and present antigens, intermediate monocytes mainly have pro-inflammatory functions, and nonclassical monocytes produce anti-inflammatory cytokines.[26–28] T cells and classical monocytes are closely related, as the latter interacts with the former to induce T cell activation in target organs. Activated monocytes phagocytize other cells and are able to digest their proteins and present them to T cells, which recognize the molecular signatures of particular proteins and activate an immune response.[29] CD163, a specific monocyte marker, is closely associated with the activation of the immune response. In patients with HBV infection, antigen-presentation by classical monocytes and inflammatory factor secretion by intermediate monocytes are involved in the establishment of HBV-specific immunity. Our study showed that the MFI of CD163 expression in classical and intermediate monocytes was higher in healthy subjects than in patients with HBV infection. The results indicate monocyte activation with HBV infection and that activated monocytes shed the hemoglobin-haptoglobin scavenger receptor CD163 into circulation as sCD163. Therefore, the expression of CD163 on monocytes may be a potential marker of HBV-specific immune activation.

As previously described, monocytes are essential for the establishment of innate and adaptive immune responses. Progression status in HBV infection is strongly associated with monocyte activation, but it is necessary to elucidate how the virus directly or indirectly modifies monocyte function and how the altered function affects the HBV-specific immune response. Importantly, we need to further understand the mechanism of the immune response and the dynamic changes in CD163 expression during immunotherapy. Based on the results of our study, CD163 may be used as a marker, which is of great significance for assessing immune activation in patients with HBV infection.

In summary, this study indicated that expression of sCD163 and CD163 expression on monocytes was associated with CHB inflammation and HBsAg loss, and might be used as more sensitive markers to predict HBV-specific immune activation and worthy of further validation in larger groups of patients. The study included only CHB genotype B and genotype C patients, and it was descriptive. The underlining mechanisms need to be further investigated.

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Conflict of interest

XZ has been an editorial board member of *Journal of Clinical and Translational Hepatology* since 2013. The other authors have no conflict of interests related to this publication.

Author contributions

Conception and design of the study (XZ, QG), collection of the data (DH, BY, YC, QG), analysis of the data (PX, DH), and writing of the paper (PX, XZ).

Ethical Statement

The study was approved by the Ethics Committee of Ruijin Hospital and was conducted following the ethical guidelines of the Declaration of Helsinki.

Data sharing statement

All data are available upon request.

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