# CD14+ monocytes and CD163+ macrophages correlate with the severity of liver fibrosis in patients with chronic hepatitis C

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**Abstract.** Hepatic fibrosis is a crucial pathological process involved in the development of chronic hepatitis C (CHC) and may progress to liver cirrhosis and hepatocellular carcinoma. Activated peripheral blood monocytes and intrahepatic macrophages further promote hepatic fibrogenesis by releasing proinflammatory and profibrogenic cytokines. The present study aimed to investigate the role of peripheral $CD14^+$ monocytes and intrahepatic $CD163^+$ macrophages in hepatitis C virus (HCV)-associated liver fibrosis and clarify whether serum soluble CD163 (sCD163) may serve as a fibrosis marker in patients with CHC. A total of 87 patients with CHC and 20 healthy controls were recruited. Serum sCD163 levels were measured by ELISA. Frequencies of peripheral $CD14^+$ monocytes and inflammatory cytokines expressed by $CD14^+$ monocytes were analyzed by flow cytometry. The degree of fibrosis in human liver biopsies was graded using the Metavir scoring system and patients were stratified into two groups based on those results ($F<2$ vs. $F\geq2$). Hepatic expression of CD163 was examined by immunohistochemical staining. The diagnostic values of sCD163, aspartate aminotransferase to platelet ratio index (APRI), fibrosis 4 score (FIB-4) and the aspartate aminotransferase to alanine aminotransferase ratio (AAR) in significant fibrosis ($F\geq2$) were evaluated and compared using receiver operating characteristic (ROC) curves. The results indicated that the serum sCD163 levels and the frequency of $CD14^+$ monocytes were significantly higher in the patients than that in the controls and positively correlated with liver fibrosis. The level of serum sCD163 was consistent with hepatic CD163 expression in the liver sections from patients. The frequencies of interleukin (IL)-8- and tumor necrosis factor-α-expressing monocytes were increased and that of IL-10-expressing monocytes was decreased in the patients. The area under the ROC curve (AUROC) for sCD163, APRI, FIB-4 and AAR was 0.876, 0.785, 0.825 and 0.488, respectively, and the AUROC for sCD163 was significantly higher than those for APRI and AAR. In conclusion, sCD163 may serve as a novel marker for assessing the degree of liver fibrosis in HCV-infected patients.

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*Key words:* hepatitis C virus, liver fibrosis, CD14, CD163, soluble CD163

## Introduction

Hepatitis C virus (HCV) infection is highly prevalent worldwide and has caused an extensive medical burden (1). Hepatic fibrosis is a crucial pathological process associated with chronic viral hepatitis, which facilitates the progressions towards severe hepatic outcomes, including liver cirrhosis, liver failure and hepatocellular carcinoma. With the development of therapeutic interventions, most patients are able to achieve sustained virological clearance and improved fibrosis, while the above-mentioned progressive features may not be completely reversed. Hence, it is critical to evaluate and monitor the stage of liver fibrosis prior to and after antiviral treatment (2).

Cells of the innate immune system regulate the fibrotic process in chronic liver diseases (3). Macrophages and their progenitor cells, monocytes, are key factors in the immune system. Liver fibrosis is characterized by extracellular matrix accumulation and hepatic stellate cell activation. Macrophages, which release pro-inflammatory and pro-fibrogenic cytokines, including tumor necrosis factor α (TNF-α) and transforming growth factor β1 (TGF-β1) may promote liver fibrosis by degrading matrix collagen and regulating hepatic stellate cells (4-7). Macrophages may differentiate into 'classically activated' M1 and 'alternatively activated' M2 macrophages (7). The function of M2 macrophages is to inhibit the inflammatory reaction and participate in tissue repair, anti-inflammatory cytokine production and extracellular matrix synthesis and stabilization (8,9). CD163 is predominantly expressed on M2 macrophages, particularly on Kupffer cells, the resident macrophages of the liver, which represent the largest

population of macrophages in the mammalian body. Soluble CD163 (sCD163) is a scavenger receptor, which is released from M2 macrophages upon activation. The activation of macrophages, mainly Kupffer cells, may be reflected by the levels of sCD163 in the blood circulation (10). Cytokines produced by $CD14^+$ cells, including interleukin-6 (IL-6), IL-8, TNF-α and IL-10, contribute to the pathogenesis of HCV-induced liver disease.

However, to date, the immunopathogenic role of peripheral blood monocytes and intrahepatic macrophages in HCV-associated fibrosis have remained to be fully elucidated. The present study aimed to investigate the effect of monocytes/macrophages and its associated cytokines in the fibrosis of chronic hepatitis C (CHC) by detecting peripheral $CD14^+$ monocyte frequencies and intrahepatic $CD163^+$ macrophage levels in HCV-associated liver fibrosis.

Various studies have demonstrated the important role of liver macrophages (Kupffer cells) during liver fibrosis (11,12), therefore, macrophage-specific markers may be useful tools to monitor liver fibrotic processes. Previous data have indicated that in patients with liver diseases, sCD163 may be used to monitor Kupffer cell activation (13). Thus, in the present study, the diagnostic relevance of sCD163 was assessed by comparing it to other well-known biomarkers of liver fibrosis.

## Materials and methods

*Subjects.* A total of 87 patients with CHC were recruited at The Third Hospital of Hebei Medical University (Shijiazhuang, China) between January 2013 and October 2013. HCV infection was diagnosed based on positivity for IgG antibodies to HCV in the serum, the presence of plasma HCV RNA and a liver biopsy with histology consistent with chronic HCV. Participants with the following conditions were excluded: i) Decompensated cirrhosis; ii) co-infection with human immunodeficiency virus (HIV); iii) co-infection with hepatitis A (HAV), B (HBV)or D virus; and iv) other chronic liver diseases. Furthermore, 20 age- and sex-matched healthy subjects with no presence of HAV, HBV, HCV, HIV or other causes of chronic liver disease were used as controls.

Liver biopsies were performed on all 87 HCV patients. In addition, 20 normal liver tissues as controls were collected from donor livers for transplantation. H&E and Masson trichrome staining were used for observation of hepatic inflammation and fibrosis in the liver sections. The grade of hepatic fibrosis was determined using the Metavir scoring system (12). A Metavir stage of F2, F3 or F4 was defined as indicating significant fibrosis. Patients were classified into two groups according to the F-score ($F \geq 2$ and $F<2$, respectively). The demographic and clinical data of the cohort are provided in Table I.

In the present study, the values of three biomarkers for liver fibrosis, namely the aspartate aminotransferase (AST) to platelet ratio index (APRI), AST to alanine aminotransferase (ALT) ratio (AAR) and fibrosis 4 score (FIB-4), were also calculated based on the following formulas: APRI=100x[AST(U/l)/upper limit of normal range]/platelets($10^9$/l), FIB-4=age(years) x AST(U/l)/[platelets($10^9$/l) x ALT(U/l)$^{1/2}$], where the age of the patient is the age at the time of liver biopsy, and AAR=AST/ALT (14-16).

*Blood sample collection.* Blood was obtained from each patient at the time-point of enrollment in this study. Samples were aliquoted and stored at -80˚C for further use.

*Biochemical assays.* The liver and kidney functions were analyzed by a Mindray BS-800M automatic chemical analyzer at the Central Laboratory of the 3rd Hospital of Hebei Medical University (Shijiazhuang, China).

*HCV antibody tests and quantitative detection of HCV RNA.* The serum antibodies to HCV were detected by ELISA with a commercial detection kit (Livzon Diagnostics Inc.). The plasma HCV RNA load was measured by using qualitative reverse transcription PCR (RT-PCR) assay (Cobas Taqman HCV Test; Roche Diagnostics) and the lower limit of quantification was 15 IU/ml.

*Immunohistochemistry detection of CD163 in liver tissues.* Paraffin-embedded liver sections (5 $\mu$m) were incubated with anti-CD163 (specific for M2 macrophages) (1:100 dilution; cat. no. MCA1853; AbD Serotec) and EnVision System HRP-conjugated secondary antibody (cat. no. K4001; Dako; Agilent Technologies, Inc.). Freshly prepared 3,3'-diaminobenzidine solution was used as the substrate, followed by counterstaining with hematoxylin according to previously described protocols (17).

*Measurement of the sCD163 concentration.* Serum sCD163 levels were detected with an ELISA kit (cat. no. DC1630; R&D Systems) according to the manufacturer's protocol.

*Flow cytometric analysis of $CD14^+$ monocytes and inflammatory cytokines expressing $CD14^+$ monocytes.* All the antibodies were purchased from BD Biosciences. For marker staining with FITC-conjugated anti-human CD14 (cat. no. 347493) and phycoerythrin (PE)-conjugated anti-human IL-2 (cat. no. 340450), interferon-gamma (IFN-γ; cat. no. 554701), IL-6 (cat. no. 340527), TNF-α (cat. no. 340517), IL-8 (cat. no. 554720), IL-4 (cat. no. 559333) and IL-10 (cat. no. 559330; all 1:100 dilution), the methods were according to previously described protocols (18,19).

*Statistics analysis.* Values are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was used for multiple comparisons and Student's t-test to study differences of normally distributed variables between the groups. Following ANOVA, the Student-Newman-Keuls post-hoc test was applied. The association between sCD163 and CD163 in liver tissues was analyzed by simple linear regression. Spearman's rank correlation test was used to study associations between sCD163, CD163, CD14 and histological scores. $P<0.05$ was considered to indicate statistical significance.

The diagnostic values of four markers (sCD163, APRI, FIB-4 and AAR) were assessed by calculating the area under the receiver operating characteristic (ROC) curves (AUROC) as the best cut-off values. The diagnostic performance was evaluated by determining the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). All data were analyzed using SPSS version 17.0 for Windows software (SPSS, Inc.).

Table I. Clinical characteristics of the patients and controls enrolled in the present study.

| Item | Healthy controls (n=20) | Patients with CHC (n=87) | P-value |
|---|---|---|---|
| Sex (male/female) | 9/11 | 38/49 | 1.000 |
| Age (years) | 44.9±11.7 | 46.6±13.8 | 0.627 |
| Body mass index (kg/m$^2$) | 23.4±3.3 | 22.3±2.5 | 0.588 |
| ALT (0-40 IU/l) | 28.66±1.9 | 83.2±9.4 | 0.001 |
| AST (0-40 IU/l) | 23.1±6.3 | 67.1±7.9 | 0.006 |
| HCV RNA (IU/ml) | | $9.1 \times 10^5$ (128-$2.5 \times 10^7$) | |
| Possible route of contamination | | | |
| Transfusion | | 62 (71.3) | |
| Previous surgery | | 6 (6.9) | |
| Stomatologic treatments | | 3 (3.4) | |
| Others or unknown | | 16 (18.4) | |
| HCV genotype, 1b/2a | | 81/6 | |
| Fibrosis score | | | |
| F<2 | | 43 | |
| F≥2 | | 44 | |

Values are expressed as the mean ± standard deviation, median (range) or n (%). ALT, alanine transaminase; AST, aspartate transaminase; HCV, hepatitis C virus; CHC, chronic HCV infection.

## Results

*Hepatic macrophages are markedly increased in HCV infection patients with fibrosis.* The hepatic distribution of CD163$^+$ cells of patients with CHC and healthy controls was examined. As presented in Fig. 1, patients with F≥2 had a higher CD163$^+$ cell density in the liver than patients with F<2. As CD163 was widely expressed on Kupffer cells in the lobular area, CD163$^+$ cells in the portal area were taken as the macrophages for quantitative analysis. It was revealed that the number of CD163$^+$ cells in the portal area was markedly higher in CHC patients with high fibrosis scores (Fig. 1). Box plots of the CD163 count in relation to the fibrosis stage are presented in Fig. 2 ($r^2$=0.942, P<0.001). There were no differences among the groups regarding the ratios of CD163 to CD68 (CD163/CD68 data not shown).

*Serum sCD163 levels are significantly higher and gradually increased with the progression of hepatic fibrosis in CHC patients.* The serum sCD163 levels of CHC patients and in healthy subjects are presented in Fig. 1D. The mean serum sCD163 levels in patients with CHC were markedly higher than those in the control subjects (88.3±11.2 $\mu$g/l vs. 49.5±7.6 $\mu$g/l, P<0.001). Serum sCD163 levels were markedly elevated in patients with F≥2 as compared with those in patients with F<2 (102.3±9.98vs. 76.0±12.2 $\mu$g/l, P<0.001). There was a positive correlation between sCD163 and fibrosis ($r^2$=0.899, P<0.001; Fig. 2). Furthermore, serum sCD163 and the number of CD163$^+$ cells in the portal area increased in parallel in association with the histological fibrosis stage in HCV patients. There was a correlation between sCD163 and hepatic CD163$^+$ cells in CHC patients ($r^2$=0.701, P<0.001; Fig. 2).

*Frequencies of CD14$^+$ monocytes andCD14$^+$ monocytes expressing IL-2, IFN-γ, IL-6, TNF-α, IL-8, IL-4 and IL-10.* CD14$^+$ monocyte frequencies were higher in CHC patients than those in healthy controls. Of note, CD14$^+$ monocyte frequencies were increased in patients with F≥2 as compared with those in patients with F<2 (72±7% vs. 78±5%, P=0.04; Fig. 1). The correlation between the frequencies of CD14$^+$ monocytes and fibrosis was then analyzed in these patients (Fig. 2). There was a significant positive correlation between CD14$^+$ monocyte frequencies and the fibrosis stage ($r^2$=0.604, P<0.001), but no correlation was observed with serum HCV RNA (data not shown).

Although CD14 is a marker of anti-inflammatory monocytes, it also transduces signals upon binding its ligands that leads to the release of anti-inflammatory mediator IL-10 and pro-inflammatory cytokines, including IL-6, TNF-α and IL-8. To further investigate the changes of CD14$^+$ monocytes expressing associated cytokines during the progression of HCV-associated liver fibrosis, the levels of IL-2-, IFN-γ-, IL-6-, TNF-α-, IL-8-, IL-4- and IL-10-expressing CD14$^+$ monocytes were determined. Compared with the controls, the IL-2-, IL-4-, IFN-γ-, IL-6-, IL-8- and TNF-α-expressing CD14$^+$ monocytes were increased in patients with CHC, but IL-10-expressing CD14$^+$ monocytes were decreased in the F<2 group (P<0.05; Fig. 3). With the progression of fibrosis, IL-8-expressing CD14$^+$ monocytes were significantly upregulated as compared with those in the F<2 group (IL-8, P<0.05; TNF-α, P>0.05; Fig. 3), while the IL-2 and IFN-γ-expressing CD14$^+$ monocytes were significantly downregulated (IL-2 and IFN-γ, P<0.05; IL-4, IL-6 and IL-10; P>0.05; Fig. 3).

*Predictive value of sCD163 as a non-invasive biomarker of fibrosis in patients with CHC.* As sCD163 was higher in

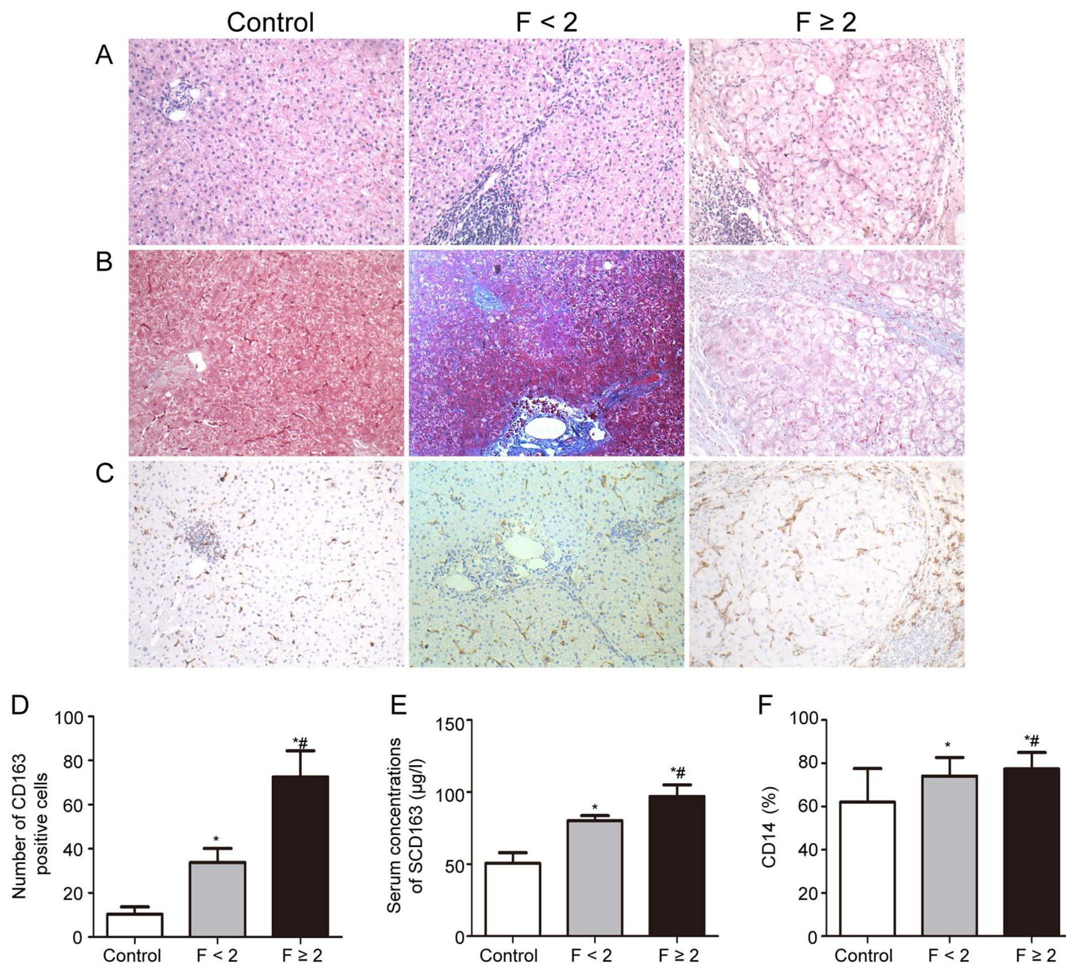


Figure 1. Expression of CD163in liver sections, serum concentration of sCD163 and proportion of CD14 in controls and patients with CHC with different severities of fibrosis (F<2 or F≥2). (A-C) Representative histology images of liver sections with (A) H&E, (B) Masson-trichrome and (C) CD163 immunohistochemical staining (original magnification, x200). In CHC liver tissues, enhanced expression of CD163 was localized in the fibrous septum. (D) Semiquantitative analysis of the immunohistochemistry results suggested that the number of CD163-expressing cells was higher in samples with F≥2 than that in F<2. (E) The serum concentration of sCD163 was measured by ELISA. Compared with controls and patients with F<2, patients with F≥2 exhibited a significantly higher concentration of sCD163. (F) Flow cytometric analysis of the proportion of CD14 in the blood in patients with CHC and controls. The proportion of CD14 was enhanced in patients with CHC and was significantly higher in F≥2 than in F<2. Data were presented as the mean ± standard deviation. *P<0.05, compared with control; #P<0.05, compared with F<2. sCD163, soluble CD163; CHC, chronic hepatitis C.

patients with considerable hepatic fibrosis, a ROC curve analysis for sCD163 with the cut-off Metavir score F≥2 was performed to distinguish patients with F≥2 from those with F<2 (Fig. 4). The AUROC for sCD163 to differentiate patients with F≥2 from those with F<2 was 0.876 (95% confidence interval: 0.795-0.958, P<0.001) with an optimal cut-off value of 73.985 $\mu$g/l. Serum sCD163 levels of ≥116.54 $\mu$g/l had a >90% specificity to identify subjects with F≥2, with an optimal cut-off value of 73.985 $\mu$g/l. Regarding the discrimination of subjects with significant fibrosis, the AUROCs for APRI, FIB-4 and AAR were 0.785, 0.825 and 0.488, respectively (Fig. 4). The optimal cut-off values of APRI, FIB-4 and AAR were 1.549, 0.74 and 0.583, respectively. In the comparison of the AUROCs, sCD163 exhibited a significantly higher AUROC as compared with APRI and AAR (P=0.028, P<0.001, respectively), while no differences were observed for sCD163 vs. FIB-4 (P=0.48). When compared to liver biopsy, AAR values >1.2 had a PPV of 77% for the diagnosis of significant fibrosis, while AAR<0.5 was able to exclude significant fibrosis with an NPV of 77%. Similar results were obtained by applying the APRI, FIB-4 and sCD163 original cutoffs. FIB-4>3.25 had a PPV of 92%, while FIB-4<1.45 was able to exclude significant fibrosis with an NPV of 81%.

## Discussion

Kupffer cells are involved in liver cirrhosis development. Studies have indicated that macrophage subsets have bidirectional roles in the progression and reversal of liver fibrosis (8,20). Macrophages not only initiate and accentuate inflammatory responses after tissue injury, but also participate in the resolution of inflammation and injury. In certain relevant studies, liver tissues from only a small number of cases or no liver tissues were included, or studies were limited to females only (21-23). The exact role of hepatic macrophages in CHC remains elusive. CD163, a member of the scavenger receptor cysteine-rich family, is involved in anti-inflammatory functions and is predominantly expressed on M2 macrophages (24,25). The present results suggested that CD163 expression was significantly increased in liver tissues of CHC

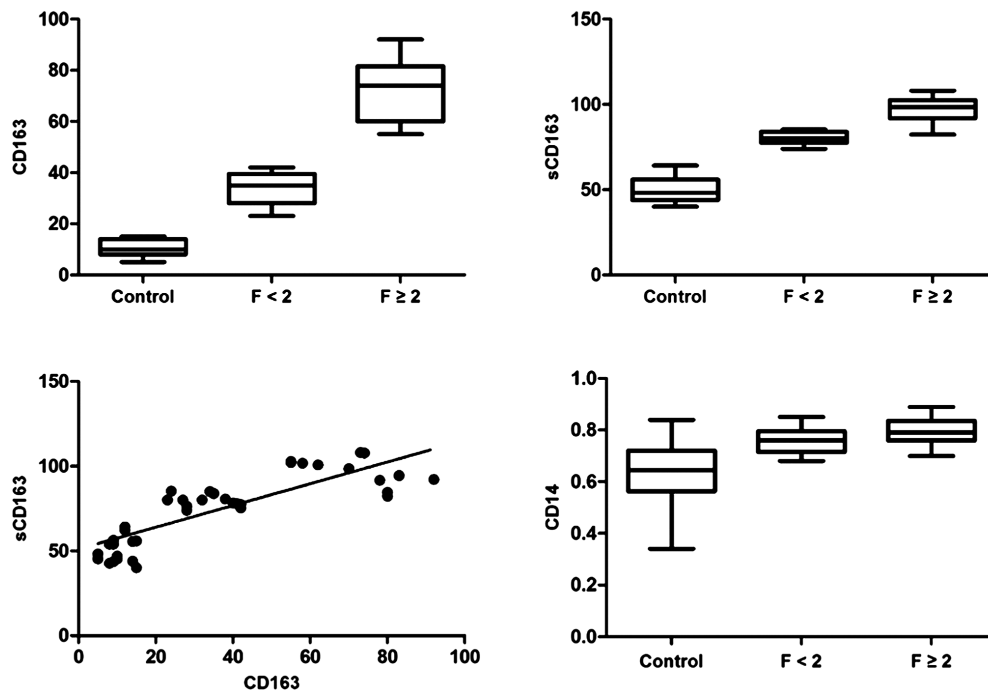


Figure 2. CD163, sCD163 and CD14 are correlated with fibrosis. A steady stepwise increase in median velocity was observed with increasing severity of hepatic fibrosis in patients with chronic hepatitis C (all P<0.01). There was a positive correlation between the serum concentration of sCD163 and liver fibrosis. sCD163, soluble CD163.

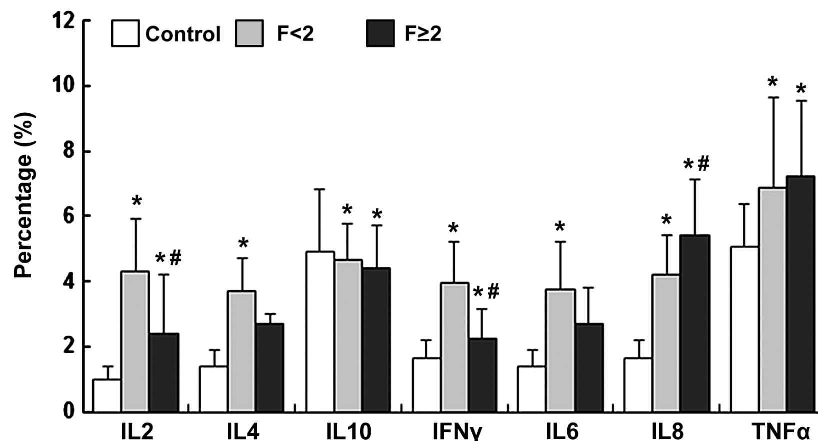


Figure 3. Proportions of IL-2-, IL-4-, IL-10-, IFN-γ-, IL-6-, IL-8- and TNF-α-expressing $CD14^+$ monocytes were measured by flow cytometry and data were presented as the mean ± standard deviation. *P<0.05, compared with control; #P<0.05, compared with F<2. IL, interleukin; IFN, interferon; TNF, tumor necrosis factor.

patients, which correlated with the degree of hepatic fibrosis. Therefore, M2 macrophages are considered pro-fibrotic under certain conditions.

Activation of Kupffer cells, the resident macrophages in the liver, is an important component of inflammation, cell death and fibrosis development (20). sCD163 is a surrogate parameter for macrophage activation, which may be a useful tool to assess the prognosis and complications of liver cirrhosis. In the present cohort of CHC patients, the serum sCD163 levels were higher in patients with significant fibrosis as compared to subjects with no or mild fibrosis. Furthermore, a strong correlation between sCD163 levels and the severity of liver fibrosis has been observed in the present study, which is in line with a recent publication confirming sCD163 as a fibrosis predictor (26). In addition, the present study indicated a positive correlation between the serum levels of sCD163 and hepatic CD163 expression. Therefore, to a certain extent, sCD163 levels reflect the changes of CD163 in liver tissue. These results all support the notion that hepatic macrophage activation is linked to fibrosis in CHC patients. Hiraoka *et al* (27) reported elevated levels of plasma sCD163 in patients with acute and chronic viral hepatitis. They also demonstrated that the cells expressing CD163 in the liver were Kupffer cells. Another study indicated increased hepatic expression of CD163 mRNA in patients with CHC (25). In patients with cirrhosis, sCD163 levels are associated with portal hypertension (12,28) and a recent study, sCD163 was demonstrated to be an independent predictor of variceal bleed

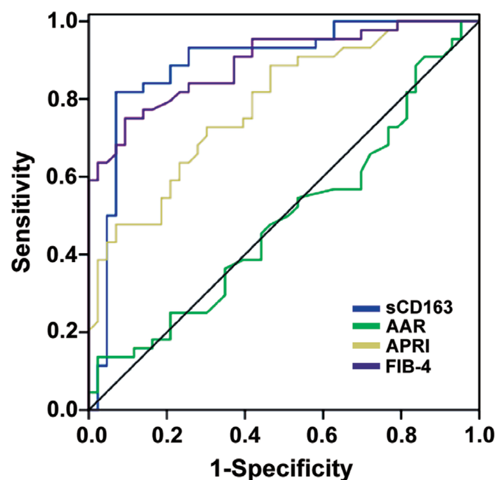


Figure 4. ROC curves for AAR, APRI, FIB-4 and sCD163 in significant fibrosis (F≥2). Comparison of areas under the ROCs indicated superior diagnostic accuracy of sCD163 and FIB-4 when compared to AAR and APRI. ROC, receiver operating characteristic; sCD163, soluble CD163. APRI, aspartate aminotransferase to platelet ratio; AAR, aspartate aminotransferase to alanine aminotransferase ratio; FIB-4, fibrosis 4 score.

and mortality in cirrhotic patients (10). These studies support the present results of elevated CD163 and sCD163 in patients with CHC. The present study suggested that the elevation of sCD163 and CD163 are associated with the progression of liver fibrosis, the most severe outcome of chronic viral hepatitis. Thus, the present study provided strong evidence for macrophage activation in patients with CHC.

Macrophages have a critical role in innate and adaptive immune responses (22). Monocytes are precursors of tissue macrophages and may exhibit special functions in the progression of HCV infection. The presence of elevated levels of $CD14^+$ monocytes has been demonstrated in various pathological conditions, including infection, inflammatory syndrome, sepsis and cancer. Most monocytes express cell surface CD14. In the present study, the levels of $CD14^+$monocytes increased in CHC patients, which was associated with the severity of fibrosis. There were significant positive correlations between CD163, CD14 and fibrosis, which suggested the involvement of $CD14^+$ monocytes and $CD163^+$ macrophages in CHC-associated liver fibrosis.

$CD14^+$ monocytes represent 90% of circulating monocytes, which produce cytokines including IL-6, IL-8, TNF-α and IFN-γ. In the present study, changes in the levels of $CD14^+$ monocytes expressing IL-6, IL-8 and TNF-α obtained from CHC patients were observed. The results confirmed that the frequencies of IL-6- and TNF-α-expressing $CD14^+$ monocytes were significantly increased in CHC patients compared with those in the normal control group, and higher levels of IL-8- and TNF-α-expressing $CD14^+$ monocytes in patients with fibrosis were determined. IL-6-, IL-8- and TNF-α-expressing $CD14^+$ monocytes are composed mainly of mononuclear macrophages induced by external stimuli (such as viral infection or endotoxin), whose levels may reflect the activation of monocytes and macrophages and have an important role in the pathogenesis of HCV infection. This suggests that IL-8- and TNF-α-expressing $CD14^+$ monocyte levels have a certain utility in the evaluation of HCV fibrosis.

IL-10 is a multifunctional negative regulatory cytokine, mainly produced by monocytes and macrophages. IL-10 activates B cells and type 2 T-helper (Th2) cells. $CD14^+$ monocytesexpressingIL-10 regulate immune and other cells and have a pivotal role in various diseases, including autoimmune diseases, severe infections and cancer. In the present study, IL-10-expressing $CD14^+$ monocyte levels were decreased in CHC patients. IL-10 has strong immune suppressive effects and an immune regulatory function. Therefore, it was speculated whether decreased levels of IL-10 expressing $CD14^+$ monocytes are insufficient to inhibit inflammation, thus resulting in fibrosis. Thus, modulation of IL-10 expressing $CD14^+$ monocytes in the early stage of HCV may slow the progression of fibrosis. Aroucha *et al* (29) indicated a protective role of IL-10 in patients with moderate fibrosis, confirming the present hypothesis that IL-10 has a protective role in HCV infection regarding the progression of hepatic fibrosis. Another study emphasized the protective role of IL-10 used in the treatment of CHC, which decreased the severity of fibrosis in the patients enrolled (30). In another study on animal models, it was demonstrated that the absence of IL-10 was associated with liver fibrosis (31).

The present study also indicated that IL-2- and IFN-γ-expressing $CD14^+$ monocytes were significantly increased in CHC patients as compared to controls, while they declined gradually with the progression of fibrosis. IL-2 and IFN-γ expressing $CD14^+$ monocytes were predominant in CHC patients with no or mild fibrosis. IL-2 and IFN-γ are Th1 cytokines. It was therefore presumed that patients with HCV infection and fibrosis exhibited a distinct immunoregulatory cytokine pattern that was shifted towards the Th2 response.

Liver biopsy has traditionally been considered the gold standard for the evaluation of liver fibrosis. However, the liver biopsy technique is an invasive procedure with a risk of complications (32). Noninvasive biomarkers of liver fibrosis have been proposed and their clinical utilities have been evaluated (33,34). Hence, several noninvasive indexes, including the APRI, FIB-4 and AAR, have been developed, compared and validated as markers of liver fibrosis in patients with chronic liver diseases. In the present study, the serum concentration of sCD163 was consistently higher in patients with significant fibrosis as compared with that in patients with no/mild fibrosis and the AUROC (0.88) was on a par with what was obtained by combined marker algorithms, including liver biopsy, APRI, FIB-4 and AAR. The present results suggested that the sCD163 had the best-performing ROC curve in the diagnosis of moderate and severe fibrosis. FIB-4 was the second-best noninvasive biomarker of liver fibrosis after sCD163. Since blood samples are readily obtainable, it is appealing to search for serum markers that are able to replace liver biopsies or liver stiffness measurements. Soluble CD163 is readily available as a promising parameter for the noninvasive determination of HCV-associated fibrosis.

Taken together, the present results demonstrated that $CD14^+$ monocytes participate in the modulation of fibrosis in patients with CHC. Targeting inflammatory monocytes in CHC

patients may not only lead to a decrease in pro-inflammatory cytokine production but also reduce liver fibrosis.

The macrophage-associated marker sCD163 is significantly higher in CHC patients with advanced fibrosis than in those with no/mild liver fibrosis. Furthermore, serum sCD163 correlated with CD163 in liver tissue and its AUROC was higher than that for APRI, AAR, representing a promising novel fibrosis marker for the non-invasive diagnosis of fibrosis in patients with CHC.

In conclusion, serum sCD163 levels are increased in patients with CHC, reflecting hepatic macrophage activation. Increased sCD163 is positively correlated with fibrosis. It may be used to monitor the progression of liver fibrosis in the management of CHC. The levels of $CD14^+$ monocytes and $CD163^+$ macrophages may serve as markers for the disease progression in patients with CHC and pathogenic macrophage targets for specific drug development.

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## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Authors' contributions

YN designed the study; SZ, LK, YZ NF, QZ, JD, BW, RW and WR performed the experiments; SZ, WL, LK, FH and PC analyzed data; YN, SZ and RW wrote the manuscript. All authors read and approved the final version of the manuscript.

## Ethics approval and consent to participate

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008). The present study was approved by the Ethics Committee of the 3rd Hospital of Hebei Medical University (Shijiazhuang, China; Oct 13th, 2010). All participants provided written informed consent.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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