Original Article
Specific chemokines CCL2 and CCL17 are correlated with hepatic necro-inflammation in chronic hepatitis B patients with normal/mildly elevated alanine aminotransaminase levels

Lin Wang*, Yaoxin Fan*, Xiaoguang Dou

*Department of Infectious Diseases, Shengjing Hospital of China Medical University, No.39, Huaxiang Road, Shenyang, Liaoning Province, China. *Equal contributors.*

Abstract: Background: A subpopulation of patients with chronic hepatitis B virus (HBV) infection coupled with moderate/severe liver necro-inflammation were analyzed. The results show normal/mildly elevated alanine transaminase (ALT) levels. Objective: The goal of this study was to investigate the role of serum chemokine profiles as a possible biomarker of hepatic necro-inflammation in patients with HBV infection and normal/mildly elevated ALT levels. Method: A total of 122 previously untreated patients with chronic HBV infection and normal/mildly elevated ALT levels underwent liver biopsy and histological grading of liver necro-inflammation (G0-G4). Levels of 13 serum chemokines was measured by flow cytometry. Expression of selected increased chemokines was further verified by enzyme-linked immunosorbent assay (ELISA). Results: Expression of (C-C motif) ligand 2 (CCL2), CCL11, CCL17, and chemokine (C-X-C motif) ligand 1 (CXCL1), CXCL5, and CXCL11 were upregulated in the patients with moderate or severe liver necro-inflammation. Serum levels of CCL2 and CCL17 were significantly elevated in patients with chronic HBV infection and significant liver necro-inflammation, compared to those with mild liver necro-inflammation. CCL2 and CCL17 were the risk factors for significant liver necro-inflammation (P=0.012 and P=0.014, respectively). The area under the receiver operating characteristics curve (AUROC) for predicting significant necro-inflammation of combined CCL2 and CCL17 were 0.83, with 78.57% and 71.43% sensitivity and specificity, respectively. Conclusion: High levels of CCL2 and CCL17 are correlated with hepatic necro-inflammation, and these chemokines may be useful as serum biomarkers for liver necro-inflammation in patients with chronic HBV infection and normal/mildly elevated ALT.

Keywords: Hepatitis B virus, liver necro-inflammation, monocyte chemoattractant protein-1 (CCL2/MCP-1), thymus- and activation-regulated chemokine (CCL17/TARC)

Introduction

Hepatitis B virus (HBV) infection is associated with a high rate of morbidity and mortality and is a worldwide public health problem [1]. Liver inflammation and necrosis are the important components of the pathogenesis in chronic liver disease, which may lead to progressive liver fibrosis, cirrhosis, and hepatocellular carcinoma [2, 3]. Moreover, early and accurate detect liver necro-inflammation should be prior to liver fibrosis [4].

Currently, liver biopsy remains the gold standard for determining the severity of the liver necro-inflammation, but clinical application has

been greatly limited because it is invasive, and has an impact on specimen and procedural complications [5]. Alanine transaminase (ALT) is an enzyme that is released from the damaged hepatocytes and can be used as an indicator of the severity of liver injury, especially for those patients with acute or active hepatitis or chronic HBV patient with flare. A minor elevation in ALT levels could be observed and is always considered to be clinically insignificant [6]. However, in clinical practice, a subpopulation of Chronic HBV infection frequently exhibits persistent and normal serum ALT levels [7, 8], this possess a great challenge to the physicians since this could mask the severity of liver injury, in turn, affect the decision when a mandatory

Table 1. Baseline characteristics of patients with chronic hepatitis B virus (HBV) infection and normal/mildly elevated serum alanine transaminase (ALT) levels

Variables	Total (n=122)	Group A (n=52)	Group B (n=70)
Age (years)	37 (28, 43)	37.5 (28.25, 43)	37 (28, 43)
Gender			
Male	60 (49.18%)	22 (42.3%)	38 (54.29%)
Female	62 (50.82%)	30 (57.7%)	32 (45.71%)
ALT (U/L)	45.5 (29, 57.25)	47.5 (33, 59.75)	40.5 (28, 56)
AST (U/L)	32 (26, 40.25)	36 (26.25, 43.75)	30.5 (24.75, 39)
ALP (U/L)	81 (60.1, 94.53)	80.5 (59.75, 94.6)	81 (60.4, 94.6)
GGT (U/L)	23 (16, 35)	22.5 (16, 36.75)	24 (16.75, 33.25)
TB (μ/L)	12.05 (9.88, 15.1)	11.85 (9.23, 14.9)	12.1 (10.38, 15.4)
HBVDNA (log10 IU/mL)	8.09 (6.15, 8.23)	8.23 (6.74, 8.23)	7.41 (4.72, 8.23)
HBsAg (log10 IU/mL)	4.07 (3.56, 4.64)	4.03 (3.59, 4.61)	4.11 (3.46, 4.7)
Liver inflammation			
Mild necro-inflammation (G0-1)	68 (55.74%)	26 (50%)	42 (60%)
Significant necro-inflammation (G2-4)	54 (44.26%)	26 (50%)	28 (40%)

Data are expressed as median and interquartile (IQR) or numbers and percentages. Abbreviations: HBV, hepatitis B virus; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; TB, total bilirubin; HBsAg, hepatitis B surface antigen.

Table 2. Serum chemokine expression in the significant and mild liver necro-inflammation groups with chronic HBV infection and normal/mildly elevated serum ALT

Variables	G<2 (n=26)	G≥2 (n=26)	P value
CXCL8	10.67 (9.77, 30.19)	10.85 (9.77, 32.38)	0.801[a]
CXCL10	178.55 (110.08, 278.48)	217 (149.73, 278.69)	0.159[a]
CCL11	52.30±40.38	73.09±31.62	0.044[b]
CCL17	23.07 (17.82, 46.24)	111.02 (49.22, 197.81)	0.000[a]
CCL2	137.31 (93.57, 170.79)	267.23 (211.44, 371.7)	0.000[a]
CCL3	5.7 (5.01, 16.38)	7.01 (5.04, 14.72)	0.691[a]
CXCL5	142.12 (70.9, 190.35)	369.19 (199.51, 730.96)	0.000[a]
CXCL1	54.24 (31.96, 83.28)	102.06 (71.58, 149.72)	0.001[a]
CXCL11	12.59 (7.91, 20.88)	22.1 (12.65, 33.57)	0.038[a]
CCL4	10.67 (9.77, 30.19)	10.85 (9.77, 32.38)	0.801[a]

[a]Mann-Whitney U tests. [b]T-test.

may be directly related the injury [10, 11].

For example, C-C motif ligand 2 (CCL2) has been proven to be an important indicator in acute and chronic liver hepatic injury [12, 13]. Furthermore, progression of chronic liver necro-inflammation due to HBV infection is associated with differential levels of chemokines [14]. Thus, these chemokines provide a good opportunity to allow us to determine if some of them are directly related to the degree of liver injury by simply quantifying them and measuring them quantitatively. For example, CCL17 has been regarded as a promising inflammatory role of disease progression and outcome in hepatocellular carcinoma [15]. Therefore, by correlating expression of chemokines with the pathological findings via biopsy, the relationship or association of the expression patterns of some chemokines with histological status could be established and could be used as a guidance for clinical practice. Therefore, a noninvasive and chemokine-related approach could be of potential no-
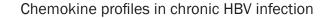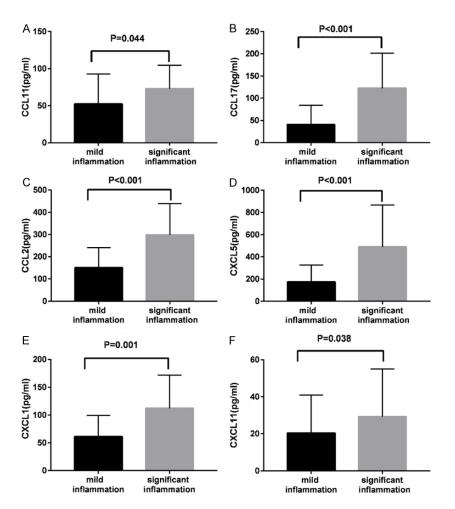
antiviral therapy should be initiated. Therefore, the levels of ALT may not always be a reliable indicator in such a scenario. Therefore, more precise and noninvasive serum markers are great in need.

There is a strong interaction or interplay between host defense and HBV infection [9], which lead to persistent liver inflammation and fibrosis [3]. As a result, a large number of chemokines are released into the hepatic tissues or into circulating bloodstream, and some which

Figure 1. Serum chemokine expression and liver necro-inflammation in patients with chronic HBV infection and normal/mildly elevated serum alanine transaminase levels. The levels of serum chemokines CCL11, CCL17, CCL2, CXCL5, CXCL1, and CXCL11 were increased in the group with chronic HBV infection and significant liver necro-inflammation compared to the group with chronic HBV infection and mild liver necro-inflammation.

lines of the 1975 Declaration of Helsinki and was approved by the Medical Ethics Committee of the hospital. Informed consent was sought from all patients who underwent a liver biopsy as part of this study.

The inclusion criteria in this study were as follows: (1) positive for hepatitis B surface antigen (HBsAg) for ≥6 months; (2) no previous antiviral therapy; (3) a serum alanine transaminase (ALT) level that was either normal or less than twice the upper limit of normal (ULN) (<80 U/L); and (4) underwent a liver biopsy. Patients with other concurrent liver diseases, other cause of hepatitis, ALT ≥80 U/L, and prior antiviral therapy were excluded from the study.

velty specifically for those with normal and mild ALT levels.

The aim of this study was to investigate the role of serum chemokines as a possible biomarker of hepatic necro-inflammation in chronic HBV patients with normal/mildly elevated ALT levels.

Patients and methods

Study participants

This study included 122 patients with chronic HBV infection who were hospitalized from March 2014 to October 2016 at the Department of Infectious Diseases, Shengjing Hospital. The study protocol conforms to the ethical guide-

Histopathology and grading of liver necro-inflammation

By following the liver biopsy procedural protocol strictly, liver tissues were obtained by an experienced physician, The specimens were fixed with formalin, processed for routine histopathology, and reviewed by two experienced pathologists who were unaware of the patient's clinical information.

The Scheuer histological scoring (grading) system (G0-G4) was used to classify the grade of the chronic hepatic inflammation for each liver specimen [16]. The grades are scored as follows: G0 (no or minimal portal inflammation, no lobular inflammation), G1 (portal inflammation, lobular inflammation but no necrosis), G2 (mild piecemeal portal necrosis, focal lobular necrosis or acidophil bodies), G3 (moderate piecemeal portal necrosis, severe local lobular cell damage), and G4 (severe piecemeal portal
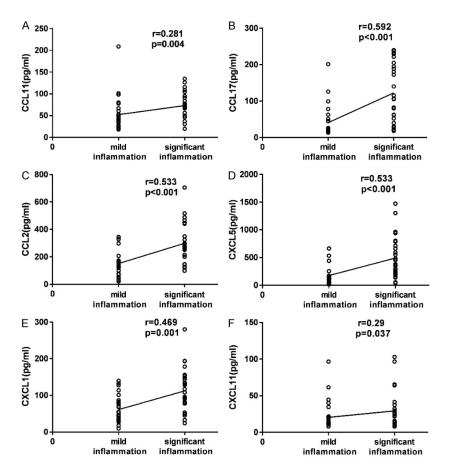
Figure 2. Correlation between the serum chemokine levels and liver necro-inflammation in CHB patients with normal/mildly elevated serum alanine transaminase levels. The chemokines CCL11, CCL17, CCL2, CXCL5, CXCL1, and CXCL11 in patients with chronic HBV infection and normal/mildly elevated alanine transaminase levels were significantly correlated with liver necro-inflammation.

necrosis, damage includes bridging necrosis). A grade ≥2 indicates significant liver necro-inflammation.

Laboratory analysis

Utilizing a fully automated biochemical analyzer (AU5800 Analyzer, Beckman Coulter Inc., Suzhou, China), the following hepatic enzymes or biochemical substrates were assessed: ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), and total bilirubin (TB). The limit of normal serum ALT level was 0-40 U/L.

HBV serological markers including HBsAg, hepatitis B envelope antigen (HBeAg), antibodies against HBsAg (anti-HBs), HBeAg (anti-HBe), and HBcAb (anti-HBc) were measured using a fully automated chemiluminescence immunoassay method (I2000SR, Abbott Laboratories, Ab-

bott Park, IL, USA). A quantitative assay of the serum HBV-DNA level was carried out using polymerase chain reaction (PCR) with a COBAS Taq Man System (Roche Diagnostics, Mannheim, Germany). The lowest limit of detection was 20 IU/mL.

Collection of blood samples

Blood was collected from each fasting patient on the morning before the liver biopsy was performed. Serum samples were stored at -80°C for analysis.

Flow cytometry analysis of serum chemokines

A total of 13 serum chemokines in 52 of the enrolled patients (group A) were detected by flow cytometry using a BioLegend LEGENDplex™ Multi-Analytic Flow Assay Kit (BioLegendInc, San Diego, CA, USA) following the manufacturer's instructions. The following 13 chemokines were measured: CCL2, CCL3, CCL4, CCL5, CCL-11, CCL17, CCL20, and C-X-C motif ligand 1 (CXCL1), CXCL5, CXCL8, CXCL9, CXCL10, and CXCL11.

Enzyme-linked immunosorbent assay analysis of serum CCL2 and CCL17

Serum CCL2 and CCL17 levels in 70 of the enrolled patients (group B) were detected by ELISA using a BioLegend LEGEND MAX™ ELISA Kit with pre-coated plates (BioLegend Inc, San Diego, CA, USA) following the manufacturer's instructions.

Statistical methods

The data were analyzed using SPSS version 22.0 (SPSS Inc, Chicago, IL, USA) and expressed

Table 3. Independent risk factors for significant liver necro-inflammation in chronic HBV infected patients with normal/mildly elevated serum ALT

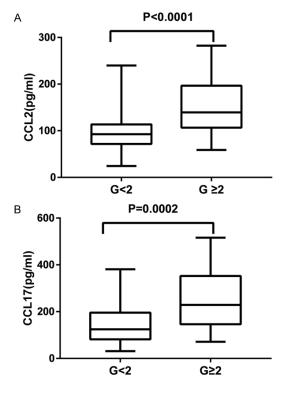| Chemokine	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P	OR (95% CI)	P
CXCL8	1.008 (0.989-1.028)	0.418		
CXCL10	1.003 (0.998-1.008)	0.227		
CCL11	1.018 (1.000-1.036)	0.054		
CCL17	1.021 (1.008-1.035)	0.001	1.015 (1.003-1.027)	0.014
CCL2	1.013 (1.005-1.020)	0.001	1.010 (1.002-1.018)	0.012
CCL3	1.007(0.967-1.050)	0.733		
CXCL5	1.005 (1.002-1.009)	0.003	1.000 (0.995-1.004)	0.811
CXCL1	1.023 (1.008-1.038)	0.003	1.007 (0.990-1.026)	0.413
CXCL11	1.018 (0.991-1.054)	0.190		
CCL4	1.008 (0.989-1.028)	0.418		

Figure 3. Serum expression of CCL2 and CCL17 in CHB patients with liver necro-inflammation and normal/mildly elevated serum alanine transaminase levels. The levels of serum chemokines CCL17, and CCL2, were increased in the group with chronic HBV infection and significant liver necro-inflammation (G≥2, n=28), compared to the group with chronic HBV infection and mild liver necro-inflammation (G<2, n=42).

as mean ± standard deviation (SD), or median and interquartile (IQR) or numbers and percentages. Comparisons between groups (significant [G≥2] vs. mild [G<2] necro-inflammation) were carried out using T-test or Mann-Whitney U tests. Pearson or Spearman's rank correlation tests were performed to assessthe associations between serum chemokine levels and liver necro-inflammation grades. A logistic regression analysis was performed to identify possible predictors of significant liver necro-inflammation. Area under the receiver operating characteristics curve (AUROC) was calculated to assess the discriminatory ability for significant necro-inflammation. Sensitivities, specificities, positive predictive value (PPV), and negative predictive value (NPV) were used to assess the accuracy of diagnosis. All the tests were two-sided, and p<0.05 was considered statistically significant.

Results

Patient characteristics

A total of 122 patients with chronic HBV infection and ALT levels <2 ULN were included in this study. The mean age of the patients was 37 (28, 43) years, and 60 (49.18%) were male patients. The mean serum ALT level for all patients was 45.5 (29, 57.25), and significant liver necro-inflammation (G2-G4) was identified in 54/122 patients (44.26%) via liver biopsy. The baseline clinical characteristics and histopathological findings for all patients are summarized in **Table 1**.

Serum chemokine profiles and liver necro-inflammation in patients with chronic HBV infection and serum ALT levels <2 ULN

We first screened an array of serum chemokines and their serum levels were measured in 52 of total patients (group A). According to the pathological results, the patients were divided into significant liver necro-inflammation (G≥2) and mild necro-inflammation group (G<2), respectively. In a total of 13 chemokines measured, both CCL20 [38/52 (73.08%)] and CXCL9 [43/52 (82.69%)] levels were below the limits of detection, CCL5 [30/52 (57.69%)] was upper limits of detection. In the remaining 10 detectable chemokines, there were no signifi-
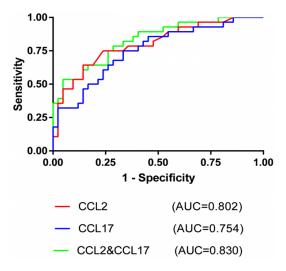
Figure 4. ROC curves for prediction of significant liver necro-inflammation in CHB patients and normal/mildly elevated serum alanine transaminase levels. ROC curves of CCL2, CCL17, CCL2, and CCL17 for the diagnosis of significant liver necro-inflammation in patients with chronic HBV infection and normal/mildly elevated alanine transaminase are shown.

cant differences in CXCL8, CXCL10, CCL3, and CCL4 levels between the patients with significant and mild necro-inflammation (P>0.05 for all). However, significant differences were detected in the serum levels of CCL11, CCL17, CCL2, CXCL5, CXCL1, and CXCL11 between the patients with significant or mild necro-inflammation (P<0.05 for all) (**Table 2**, **Figure 1**).

Correlations between serum chemokines and liver necro-inflammation

We next determined if there is a correlation between each serum chemokine level and the degree of liver necro-inflammation. CXCL11, CCL11, CXCL1, CCL17, CCL2 and CXCL5 were found to be positively correlated with liver necro-inflammation (r=0.29, r=0.281, r=0.469, r=0.592, r=0.533, r=0.533, respectively, P<0.05 for all). CCL17, CCL2, and CXCL5 exhibit a strong correlation with the degree of liver necro-inflammation (**Figure 2**).

Independent risk factors for significant liver necro-inflammation

Univariate logistic regression analyses were carried out to analyze the possible predictors of significant liver necro-inflammation. These findings showed that CXCL8, CXCL10, CCL11, CCL3, CCL4, and CXCL11 were not associated

with liver necro-inflammation (P>0.05 for all). In contrast, serum levels of CCL2, CCL17, CXCL5, and CXCL1 were significant associated with significant liver necro-inflammation (P<0.05 for all) (**Table 3**).

A multivariate logistic regression analysis showed that serum levels of CXCL5 (P=0.811) and CXCL1 (P=0.413) were not independently associated with significant necro-inflammation, while CCL2 (P=0.012) and CCL17 (P=0.014) were associated with significant liver necro-inflammation (**Table 3**).

Serum expression of CCL2 and CCL17 in CHB patients with liver necro-inflammation and serum ALT levels <2 ULN

Based on the analyzation of chemokine selection, CCL2 and CCL17 were independent factors for significant liver necro-inflammation. Furthermore, CCL2 and CCL17 were further detected in 70 chronic HBV infected patients with ALT levels <2 ULN (group B). In accordance with flow cytometry results, expression of CCL2 in patients with significant necro-inflammation was higher than patients with mild necro-inflammation (139.4 (106.55, 196.75) pg/ml vs. 92.54 (71.52, 113.57) pg/ml, p<0.001). Expression of CCL17 in patients with significant necro-inflammation was higher than patients with mild necro-inflammation (228.97 (146.3, 352.6) pg/ml vs. 124.71 (81.92, 195.64) pg/ml, p<0.001) (**Figure 3**). The serum expression of CCL2 and CCL17 was positively correlated with the degree of liver necro-inflammation (r=0.513, r=0.432, P<0.05, respectively).

Prediction of CCL2 and CCL17 for liver necro-inflammation in CHB patients with serum ALT levels <2 ULN

In order to assess if a unique serum pattern of CCL2 and CCL17 expression could provide the necessary information for distinguishing mild from significant necro-inflammation in CHB patients, a receiver operating characteristics curve (ROC) was constructed and the area under curve for CCL2 and CCL17 was determined, respectively. Use the maximum Youden index as the best cut-off point. When a cut-off value of 113.1 pg/ml of serum CCL2 was used, the AUROC was 0.802, 95% confidence interval (CI) was 0.689-0.888 with sensitivity and specificity of serum CCL2 for diagnosing significant

Table 4. Comparative diagnostic performance of CCL2 and CCL17 for significant liver necro-inflammation in chronic HBV infected patients with normal/mildly elevated serum ALT

Variable	AUROC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CCL2	0.802	75	76.19	67.74	82.05
CCL17	0.754	75	66.67	60	80
CCL2&CCL17	0.830	78.57	71.43	64.71	83.33

Abbreviations: AUROC, Area under the receiver operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value.

necro-inflammation of 75% and 76.19%, positive predictive value (PPV) and negative predictive value (NPV) of 67.74% and 82.05%, respectively. When a cut-off value of 147.88 pg/ml of serum CCL17 was used, the AUROC was were 0.754, 95% CI was 0.637-0.849, sensitivity and specificity of serum CCL17 for diagnosing significant necro-inflammation were 75% and 66.67%, PPV and NPV were 60% and 80%, respectively (**Figure 4**, **Table 4**).

Combination of CCL2 and CCL17 for predict significant liver necro-inflammation in chronic HBV infected patients and serum ALT levels <2 ULN

To improve the diagnostic performance for significant liver necro-inflammation, binary logistic regression analysis was conducted by the combination of CCL2 and CCL17. The two variables were transformed into a new but combined probability. The optimal cut-off for the combined probability was 0.308, AUROC 0.83, 95% CI (0.721-0.909), sensitivity 78.57%, specificity 71.43%, PPV 64.71% and NPV 83.33% (**Figure 4**).

Discussion

In the study, we advanced the important role of chemokines in the development of liver injury in chronic HBV infected patients with normal/mildly elevated ALT levels. Moreover, we characterized the diagnostic value of CCL2 and CCL17, and the combination of CCL2 with CCL17 could be considered as the potential noninvasive biomarkers for detecting significant liver necro-inflammation in those CHB patients with normal or mild ALT levels.

Numbers of chemokines could participate in the damage to liver tissue caused by the immune response, and the dysregulated ex-

pression of chemokines may result in excessive inflammation [17-19]. In this study, we detected the major CC and CXC chemokine levels in chronic HBV infected patients with different degrees of liver necro-inflammation and normal/mildly elevated ALT levels. The data showed that the levels of CCL11, CCL17, CCL2, CXCL5, CXCL1, and CXCL11 were upregulated in patients with chronic HBV infection and significant liver necro-inflammation compared to those with mild liver necro-inflammation, indicating that different levels of CC and CXC chemokines could be involved in the pathogenesis of liver necro-inflammation in HBV infections. The findings of this study are consistent with those of previously published studies that showed that the CC and CXC chemokine subfamilies are modulators of immune system-induced hepatocyte damage and inflammation [20, 21].

More importantly, in this study, the chemokines CCL2 and CCL17 were identified to be independent risk factors for moderate/severe liver necro-inflammation. CCL2 is also known as monocyte chemoattractant protein-1 (MCP-1) and have known to play the major role in the recruitment and activation of Kupffer cells to the injured liver and was acknowledged as a promotor to liver inflammation [14, 22]. Indeed, our result showed that serum CCL2 was elevated in patients with chronic HBV infection who had significant liver necro-inflammation, even with normal or slightly elevated ALT. This may mean that measurement of serum CCL2 is a more sensitive reflection of liver necro-inflammation, in accordance with the recent research that CCL2 levels can change prior to significant changes in the levels of serum aminotransferases in mouse models of liver injury [23]. CCL17 is also called thymus- and activation-regulated chemokine (TARC) and the function role of CCL17 in liver inflammation remains limited and controversial. It has been reported that elevated CCL17 could suppress immune response and contribute to immune tolerance in liver disease [24, 25], while another study reported that CCL17 is absent from the liver under normal conditions and upregulated in chronic liver inflammation [26]. In our study, the

results showed that the serum levels of CCL17 were positive and significantly elevated in patients with chronic HBV infection and significant liver necro-inflammation. Taken together, serum CCL2 and CCL17 expressions were positive with histological injury and could be used as biomarkers for clinical practice.

In the study, 54/122 (44.26%) chronic HBV infected patients had significant liver necro-inflammation with normal/mildly elevated ALT. Due to the limitation of ALT, no accurate noninvasive marker could be used to the evaluation of liver necro-inflammation in chronic HBV infected patients with normal/mildly elevated ALT levels. As described above, we have detected CCL2 and CCL17 was up-regulated and confirmed them as risk factors in significant liver necro-inflammation. We further analyzed the diagnosis ability of CCL2 and CCL17. The AUC curve of CCL2 and CCL17 for predicting significant liver necro-inflammation was 0.802 and 0.754. Across the further analyze, the diagnostic efficacy of combined CCL2 and CCL17 was superior than single CCL2 or CCL17. The AUC curve for predicting significant necro-inflammation was increased from 0.754 to 0.83, 74.3% (52/70) in patients that were correctly evaluated.

This initial study has several limitations. It was conducted at a single center and involved a small study sample, with a small number of subjects being enrolled in each of the two liver necro-inflammation groups. We suggest that large-scale, controlled, multicenter studies should be carried out in the future. In this study, chemokines were detected from serum samples, and the expression of chemokines in the liver tissues was unknown. Therefore, in future studies, serum chemokine levels and liver chemokine levels should be studied and compared. In addition, diagnosis accuracy needs to be improved, and additional serum markers like soluble CD163[27] might need further comparison and combination.

In conclusion, the findings of this study show that serum levels of CCL2 and CCL17 could distinguish mild and significant liver necro-inflammation in patients with chronic HBV infection and normal/mildly elevated serum ALT levels, and that the combination of CCL2 and CCL17

may improve the diagnosis accuracy for liver necro-inflammation.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaoguang Dou, Department of Infectious Diseases, Shengjing Hospital of China Medical University, No.39, Huaxiang Road, Shenyang, Liaoning Province, China. Tel: +86-18940251121; E-mail: guang40@163.com

References

[1] Schweitzer A, Horn J, Mikolajczyk RT, Krause G and Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet 2015; 386: 1546-1555.

[2] Ding YF, Wu ZH, Shu L and Peng YR. Hepatic inflammation-fibrosis-cancer axis in the rat hepatocellular carcinoma induced by diethylnitrosamine. J Cancer Res Clin Oncol 2017; 143: 821-834.

[3] Koyama Y and Brenner DA. Liver inflammation and fibrosis. J Clin Invest 2017; 127: 55-64.

[4] Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM and Murad MH. AASLD guidelines for treatment of chronic hepatitis B. Hepatology 2016; 63: 261-283.

[5] Tublin ME, Blair R, Martin J, Malik S, Ruppert K and Demetris A. Prospective study of the impact of liver biopsy core size on specimen adequacy and procedural complications. AJR Am J Roentgenol 2017; 210: 183-188.

[6] Kim WR, Flamm SL, Di Bisceglie AM and Bodenheimer HC. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 2008; 47: 1363-1370.

[7] Gui HL, Wang H, Yang YH, Wu YW, Zhou HJ, Guo SM, Lin LY, Wang L, Cai W, Chen R, Guo Q, Zhou

XQ, Bao SS and Xie Q. Significant histopathology in Chinese chronic hepatitis B patients with persistently high-normal alanine aminotransferase. J Viral Hepat 2010; 17 Suppl 1: 44-50.

[8] Zheng X, Wang J and Yang D. Antiviral therapy for chronic hepatitis B in China. Med Microbiol Immunol 2015; 204: 115-120.

[9] Bertoletti A and Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Gut 2012; 61: 1754-1764.

[10] Heymann F and Tacke F. Immunology in the liver-from homeostasis to disease. Nat Rev Gastroenterol Hepatol 2016; 13: 88-110.

[11] Yang Q, Shi Y, Yang Y, Lou G and Chen Z. The Sterile Inflammation in the exacerbation of HBV-associated liver injury. Mediators Inflamm 2015; 2015: 1-13.

[12] Mandrekar P, Ambade A, Lim A, Szabo G and Catalano D. An essential role for monocyte chemoattractant protein-1 in alcoholic liver injury: regulation of proinflammatory cytokines and hepatic steatosis in mice. Hepatology 2011; 54: 2185-2197.

[13] Baeck C, Wehr A, Karlmark KR, Heymann F, Vucur M, Gassler N, Huss S, Klussmann S, Eulberg D, Luedde T, Trautwein C and Tacke F. Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. Gut 2012; 61: 416-426.

[14] Marra F and Tacke F. Roles for chemokines in liver disease. Gastroenterology 2014; 147: 577-594, e571.

[15] Zhou SL, Zhou ZJ, Hu ZQ, Huang XW, Wang Z, Chen EB, Fan J, Cao Y, Dai Z and Zhou J. Tumor-associated neutrophils recruit macrophages and T-regulatory cells to promote progression of hepatocellular carcinoma and resistance to sorafenib. Gastroenterology 2016; 150: 1646-1658, e1617.

[16] Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991; 13: 372-374.

[17] Antoniades CG, Quaglia A, Taams LS, Mitry RR, Hussain M, Abeles R, Possamai LA, Bruce M, McPhail M, Starling C, Wagner B, Barnardo A, Pomplun S, Auzinger G, Bernal W, Heaton N, Vergani D, Thursz MR and Wendon J. Source and characterization of hepatic macrophages in acetaminophen-induced acute liver failure in humans. Hepatology 2012; 56: 735-746.

[18] Harvey CE. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates with histological severity and lobular inflammation. J Leukoc Biol 2003; 74: 360-369.

[19] Nishitsuji H, Funami K, Shimizu Y, Ujino S, Sugiyama K, Seya T, Takaku H and Shimotohno K. Hepatitis C virus infection induces inflammatory cytokines and chemokines mediated by the cross talk between hepatocytes and stellate cells. J Virol 2013; 87: 8169-8178.

[20] Karlmark KR, Wasmuth HE, Trautwein C and Tacke F. Chemokine-directed immune cell infiltration in acute and chronic liver disease. Expert Rev Gastroenterol Hepatol 2008; 2: 233-242.

[21] Wasmuth H, Tacke F and Trautwein C. Chemokines in liver inflammation and fibrosis. Semin Liver Dis 2010; 30: 215-225.

[22] Tacke F. Targeting hepatic macrophages to treat liver diseases. J Hepatol 2017; 66: 1300-1312.

[23] Cai SY and Boyer JL. The role of inflammation in the mechanisms of bile acid-induced liver damage. Dig Dis 2017; 35: 232-234.

[24] Meng X, Gao W, Tang Y, Shen Z and Wang Z. Alterations of serum IP-10 and TARC in patients with early acute rejection after liver transplantation. Cell Physiol Biochem 2017; 41: 1063-1071.

[25] Pere H, Tanchot C, Bayry J, Terme M, Taieb J, Badoual C, Adotevi O, Merillon N, Marcheteau E, Quillien Vr, Banissi C, Carpentier A, Sandoval F, Nizard M, Quintin-Colonna F, Kroemer G, Fridman WH, Zitvogel L, Oudard Sp and Tartour E. Comprehensive analysis of current approaches to inhibit regulatory T cells in cancer. OncoImmunology 2014; 1: 326-333.

[26] Oo YH, Weston CJ, Lalor PF, Curbishley SM, Withers DR, Reynolds GM, Shetty S, Harki J, Shaw JC, Eksteen B, Hubscher SG, Walker LSK and Adams DH. Distinct roles for CCR4 and CXCR3 in the recruitment and positioning of regulatory T cells in the inflamed human liver. J Immunol 2010; 184: 2886-2898.

[27] Dultz G, Gerber L, Farnik H, Berger A, Vermehren J, Pleli T, Zeuzem S, Piiper A, Kronenberger B and Waidmann O. Soluble CD163 is an indicator of liver inflammation and fibrosis in patients chronically infected with the hepatitis B virus. J Viral Hepat 2015; 22: 427-432.