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Soluble inflammatory markers as predictors of liver histological changes in patients with chronic hepatitis C virus infection

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Abstract Host immune response seems to be mainly responsible for the progression of liver disease among patients with hepatitis C virus (HCV) infection. Immune activation involves the release of cytokines and their receptors that can be measured in plasma samples. The study aimed to evaluate the association between plasma levels of chemokines and soluble tumor necrosis factor receptors (sTNFR) and liver histological changes among patients with chronic HCV infection. Seventy-one treatment-naive patients were included. Plasma levels of CCL2, CCL3, CCL11, CCL24, CXCL9, CXCL10, sTNFR1, and sTNFR2 were measured and liver histological findings were reviewed. Plasma levels of CXCL9, sTNFR1, and sTNFR2 were significantly associated with liver fibrosis, with higher median levels found among patients with moderate/ severe fibrosis (F≥2) if compared to those with no or mild fibrosis (p=0.014; p=0.012; p=0.009, respectively). Plasma sTNFR2 levels were significantly associated with necroinflammatory activity, with higher median levels among patients with moderate/severe activity (A≥2) if compared to those with no or mild activity (2.34 ng/mL vs. 1.99 ng/mL; p= 0.019). In conclusion, plasma levels of CXCL9, sTNFR1, and sTNFR2 were independently associated with liver histological changes, suggesting a role of TNF activation and Th1-type cell-mediated immune response in the pathogenesis of HCV infection.

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Introduction

Hepatitis C virus (HCV) is estimated to infect around 170 million people worldwide and is currently one of the main causes of chronic liver failure [1]. The disease progression of HCV-infected patients is quite variable and only about 15% of those chronically infected will eventually progress to cirrhosis after a median of 20–30 years after infection [2]. Several factors such as age at infection, gender, alcohol use, insulin resistance, and co-infection with human immunodeficiency virus (HIV) have been described as influencing disease progression [3–6]. The immune response pattern is also expected to influence the natural course of HCV infection, as liver damage is largely immune-mediated and liver inflammation is a pre-requisite for fibrogenesis [7–10].

In HCV infection, tumor necrosis factor alpha (TNF-α) seems to play an important role in liver inflammation [11–13] and TNF-α level has been shown to be associated with liver fibrosis [14–16]. TNF-α is a cytokine produced primarily by activated monocytes and Kupffer cells in response to

immune recognition of viral antigens and seems to have both direct and indirect activity against HCV. While being a key element in host defense, excessive production of TNF-α may lead to detrimental local and systemic effects.

TNF-α action seems to depend on its binding to two specific cellular membrane receptors (TNFR), TNF-R55 and TNF-R75 [17, 18], that are present in most human cells. Extracellular domains of these receptors can be cleaved into soluble molecules (sTNFR1 and sTNFR2) that retain their ability to bind circulating TNF and are important in regulating its activity. Soluble TNF receptors are released by activated neutrophils, mononuclear blood cells, and fibroblasts [19, 20] in response to mediators such as interferon and TNF-α itself [20–23]. sTNFR1 and sTNFR2 are distinct molecules, encoded in different chromosomes and probably regulated by different mediators [24]. The latter is more abundant, suggesting an important role for this TNF inhibitor in regulating TNF bioactivity in vivo. Circulating sTNFR levels seem to reflect activation of the TNF system [25] and could be used as surrogate markers of activation of the TNF system.

A subgroup of small cytokines entitled chemokines is also involved in HCV pathogenesis. Chemokines are involved with leukocyte trafficking through a process called haptotaxis, where leukocytes move towards higher concentrations of chemokines. This process is important for the regulation of leukocyte migration into sites of infection or into lymph nodes [26–32]. Chemokines are also involved in leukocyte activation, lymphocyte differentiation, regulation of Th1/Th2 balance, angiogenesis, and fibrogenesis [31].

As Th1 response is particularly involved in hepatocellular damage of patients with chronic hepatitis C (CHC), there is special interest in the chemokines responsible for the recruitment of Th1 cells into the liver [33, 34]. The most important of these chemokines are CCL2 (Monocyte Chemotactic Protein-1, MCP-1), CCL3 (Macrophagic Inflammatory Protein 1 alpha, MIP-1 alpha), CCL4 (Macrophagic Inflammatory Protein 1 beta, MIP-1 beta), CCL5 (Regulated upon Activation, Normal T Cell Expressed and Secreted, RANTES), CXCL9 (Monokine Induced by IFN-gamma, MIG), and CXCL10 (Interferon-gamma Inducible Protein, IP-10). The above-mentioned CCL chemokines bind to CCR5 (C-C chemokine receptor type 5), while those CXCL chemokines bind to CXCR3 (CXC chemokine receptor type 3) [35]. In the liver, chemokines are mainly produced by activated monocytes, Kupffer cells, endothelial cells, and hepatocytes [36, 37].

We sought to investigate the association between liver histological changes and several peripheral inflammatory markers such as soluble TNF receptors (sTNFR1 and sTNFR2) and plasma chemokines (CCL2, CCL3, CCL11, CCL24, CXCL9, CXCL10) among patients with CHC infection.

Methods

Patients

Between June 2005 and December 2007, consecutive patients with CHC infection seen at Orestes Diniz Center, a public university-based referral service for chronic hepatitis patients in Belo Horizonte, Brazil, were recruited for the study. All included patients were adults, had a positive anti-HCV antibody test (ELISA-3, Ortho Diagnostic Systems), and HCV RNA detectable by polymerase chain reaction (PCR; AMPLICOR®, Roche Molecular Systems) for more than six months. All patients had available liver biopsy samples with a length ≥1 cm containing at least five portal tracts, were negative for auto-antibodies (anti-nuclear, anti-mitochondria, anti-smooth muscle antibodies), and had negative results for *Schistosoma mansoni* ova on three stool samples. Patients were excluded if they had previously used interferon with or without ribavirin or had any of the following: coinfection with hepatitis B virus or HIV, chronic use of steroids or immunosuppressant drug, or renal failure. Sociodemographic, clinical, and laboratory data were obtained through chart review and patient interview. Self-reported current and past alcohol consumption were assessed by an overall quantity–frequency measure [38].

Histological assessment

Ultrasound-guided liver biopsy was performed using a Tru-Cut needle and tissue was formalin-fixed and paraffin-embedded for histological examination. Liver biopsy sections were stained with hematoxylin–eosin and either Sirius red or Masson's trichromic stain. All samples were evaluated in a blinded fashion by an independent, experienced liver pathologist using the METAVIR scoring system [39], which gives two separate scores, one for necroinflammatory activity (A) and another for the stage of fibrosis (F). The scores are defined as follows: A0, no histologic necroinflammatory activity; A1, minimal activity; A2, moderate activity; A3, severe activity; F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Histologic steatosis was graded using Brunt's scoring system [40, 41].

Chemokines and sTNFR quantification

Plasma samples were taken from patients within a year from the time of biopsy and were frozen at −70°C until measurements were performed. For chemokine analysis, plasma samples were thawed and excess proteins, mainly albumin, were removed by acid/salt precipitation, as routinely performed in our laboratory [42]. Briefly, an equal volume of plasma and 1.2% trifluoroacetic acid/1.35 M NaCl were mixed

Table 1 Comparison of median and interquartile range (IQR) of soluble inflammatory markers (pg/mL) among chronic hepatitis C (CHC) patients with absent/mild (METAVIR A ≤1) and those with moderate/severe (METAVIR A≥2) hepatic necroinflammatory activity

Inflammatory marker	Absent/mild (n=45), median (IQR)	Moderate/severe (n=26), median (IQR)	p-value
CCL2	96.1 (0–128.0)	103.2 (57.3–160.0)	0.498
CCL3	98.5 (35.0–126.9)	108.0 (89.1–141.1)	0.194
CCL11	200.4 (174.4–259.1)	216.6 (161.4–239.5)	0.834
CCL24	690.4 (389.9–1068.2)	752.7 (574.9–1068.2)	0.463
CXCL9	349.4 (0–884.0)	626.5 (285.7–1056.7)	0.085
CXCL10	139.0 (94.2–286.1)	178.4 (75.5–289.9)	0.784
sTNFR1	677.8 (443.8–920.9)	737.8 (584.7–1040.2)	0.178
sTNFR2	1,992.8 (1,705.4–2,390.9)	2,336.9 (1,910.0–2,723.2)	0.019

and left at room temperature for 10 min. Samples were then centrifuged for 5 min at 3,000g and the supernatants adjusted for salt content (0.14 M sodium chloride and 0.01 M sodium phosphate) and pH (7.4) for the determination of chemokine and soluble TNF receptors levels. For sTNFR measurement, samples were only diluted in phosphate-buffered saline (PBS) and the removal of excess proteins was not necessary.

Plasma concentrations of chemokines and soluble TNF receptors were measured using sandwich ELISA kits for CCL2, CCL3, CCL11, CCL24, CXCL9, CXCL10, sTNFR1, and sTNFR2 (DuoSet; R & D Systems, Minneapolis, MN, USA), according to the recommended procedure by the manufacturer; briefly, captured IgG antibodies were diluted in PBS, added to each well, and left overnight at 4°C. The plate was washed four times in PBS with 0.05% Tween 20 (Sigma-Aldrich Co., St. Louis, MO, USA) and blocked with 1% bovine serum albumin. It was then incubated for 1 h at room temperature before being washed four times with PBS and 0.05% Tween 20. The samples and standards were added and the plate incubated overnight at 4°C. After washing the plate as indicated above, biotinylated antibodies diluted in PBS were added. The plate was incubated for 2 h at room temperature and washed again; streptavidin (Duo-Set; R & D Systems) was added and the plate was incubated for 30 min. Finally, color reagent o-phenylenediamine (Sigma-Aldrich Co., St. Louis, MO, USA) was added to each well and the reaction allowed to develop in the dark for 15 min. The reaction was stopped with the addition of 1 M H_2SO_4 to each well. The absorbance was measured on a plate reader at a 492-nm wavelength (Emax; Molecular Devices, Minneapolis, MN, USA). The detection limit was 10 pg/ml for chemokines and 5 pg/ml for sTNFR. All samples were assayed in duplicate on the same plate.

Statistical analysis

Non-parametric analysis was performed using the Mann–Whitney test and the Kruskal–Wallis test. The Chi-square test was used for the comparison of categorical data. Correlation analyses between cytokine plasma levels and clinical parameters were performed using Spearman's correlation coefficient. Stepwise multivariate logistic regression analysis was performed when the outcome-dichotomized variable was either severity of liver necroinflammatory activity or severity of liver fibrosis. Statistical analysis was performed using the SPSS software package (version 12.0; SPSS Inc., Chicago, IL, USA). All reported p-values are two-sided and statistical significance was set at $p<0.05$.

Ethics approval

The study was approved by ethics committee at the Federal University of Minas Gerais and it has been performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Results

Seventy-one consecutive CHC patients were included. The mean age was 43.8 years, 37 (50.7%) were male, and 34
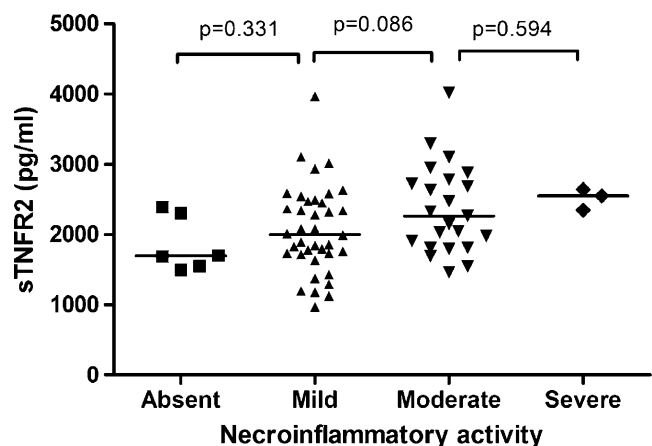

Fig. 1 Plasma levels of sTNFR2 among patients with chronic hepatitis C (CHC) infection stratified by severity of necroinflammatory activity; p-values presented as calculated with the Mann–Whitney U-test

Table 2 Multivariate logistic regression to identify factors predictive of moderate/severe necroinflammatory activity (METAVIR A≥2) in patients with CHC (n=73)

Parameter	Odds ratio (95% CI)	p-value
sTNFR2 (ng/mL)	2.85 (1.09–7.47)	0.03
Liver steatosis	3.22 (1.08–9.63)	0.04
Type II diabetes	11.43 (1.08–121.60)	0.04

Hosmer–Lemeshow: 0.73; Nagelkerke R^2: 0.28

(46.6%) patients reported current or past habit of ingesting more than 40 g of alcohol per day. The most prevalent risk factors for HCV infection were blood transfusion before 1992 (30.1%) and current or past illicit drug use (20.5%). HCV genotype 1 was found in 54 (73.9%) patients and 39 (53.4%) had alanine aminotransferase (ALT) elevation (above 1.5 times the upper normal limit). Moderate/severe hepatic necroinflammatory activity (METAVIR A≥2) was found in 26 (35.6%) patients, moderate/severe liver fibrosis (METAVIR F≥2) in 35 (47.9%), and cirrhosis in 10 (13.7%).

Patients with moderate/severe hepatic necroinflammatory activity had significantly higher levels of sTNFR2 (p= 0.019) if compared to those with mild or absent activity (Table 1). Although not reaching statistical significance, a dose–response pattern between plasma levels of sTNFR2 and necroinflammatory activity was observed (Fig. 1). The median levels of other plasma inflammatory mediators did not significantly differ between those with mild/absent necroinflammatory activity and those with moderate/severe activity (Table 1). Patients with moderate/severe necroinflammatory activity were older at the time of liver biopsy (48.7 ± 11.6 years vs. 41.7±9.2 years; p=0.006) and had a greater proportion of ALT elevation (odds ratio [OR] 3.08; 95% confidence interval [CI] 1.11–8.55), type 2 diabetes mellitus (OR 10.47; 95% CI 1.15–95.41), and any degree of liver steatosis (OR 3.54; 95% CI 1.30–9.74) when compared to those with no or mild activity.

To determine whether sTNFR2 levels were an independent predictor of necroinflammatory activity, stepwise multivariate

logistic regression analyses were performed using plasma sTNFR2 levels, ALT elevation, type II diabetes mellitus, and the presence of liver steatosis as potential explanatory variables. As seen in Table 2, when the outcome variable was dichotomized as either having mild/absent or moderate/severe necroinflammatory activity, sTNFR2 levels, the presence of liver steatosis, and type 2 diabetes were independent significant explanatory variables.

Patients with moderate/severe hepatic fibrosis showed significantly higher levels of sTNFR1 (p=0.012), sTNFR2 (p=0.009), and CXCL9 (p=0.014) if compared to those with absent/mild fibrosis (Table 3). Although non-significant, there was also a dose–response pattern between liver staging and both sTNFR1 and sTNFR2 that was not observed with CXCL9 levels (Fig. 2). Receiver operating characteristic (ROC) curves showed that the accuracy of fibrosis prediction using sTNFR1, sTNFR2, or CXCL9 was low, with values below 70% (data not shown).

Those with more advanced liver fibrosis were also older (40.9±8.6 years vs. 47.7±11.4; p=0.006), had a greater proportion of any degree of liver steatosis (OR 3.47; 95% CI 1.29–9.33), and greater necroinflammatory hepatic activity (OR 21.1; 95% CI 5.3–83.2). Gender and body weight were not associated with necroinflammatory activity or liver fibrosis.

To determine whether the levels of CXCL9, sTNFR1, and sTNFR2 were independent predictors of liver fibrosis, stepwise multivariate logistic regression analyses were performed. Due to the high correlation between the soluble inflammatory markers (Table 4), an individual model was built for each one where, besides the plasma levels of each marker (i.e., CXCL9, sTNFR1, and sTNFR2), age at the time of the liver biopsy, presence of steatosis, history of alcohol ingestion greater than 40 g/day, and severity of necroinflammatory activity were included as potential explanatory variables. As seen in Table 5, when the outcome variable was dichotomized as either having mild/ absent or moderate/severe fibrosis, sTNFR1 and CXCL9 levels were independent significant explanatory variables. The association between sTNFR2 and liver fibrosis became

Table 3 Comparison of median and IQR of soluble inflammatory markers (pg/mL) among CHC patients with absent/mild (METAVIR F ≤1) and those with moderate/severe (METAVIR F≥2) hepatic fibrosis

| Inflammatory marker	Absent/mild (n=45), median (IQR)	Moderate/severe (n=26), median (IQR)	p-value
CCL2	97.9 (0–129.8)	96.1 (59.1–144.0)	0.583
CCL3	96.1 (22.5–122.1)	108.0 (89.1–157.7)	0.183
CCL11	200.4 (164.7–249.3)	219.9 (171.2–266.5)	0.379
CCL24	665.1 (372.4–1045.5)	761.7 (557.3–1090.3)	0.490
CXCL10	132.7 (86.7–267.0)	180.3 (114.7–293.3)	0.424
CXCL9	285.7 (0–733.6)	626.5 (211.6–1273.6)	0.014
sTNFR1	636.7 (413.7–782.8)	833.1 (622.1–1044.9)	0.012
sTNFR2	1,951.7 (1,697.8–2,332.8)	2,345.1 (1,834.5–2,703.3)	0.009
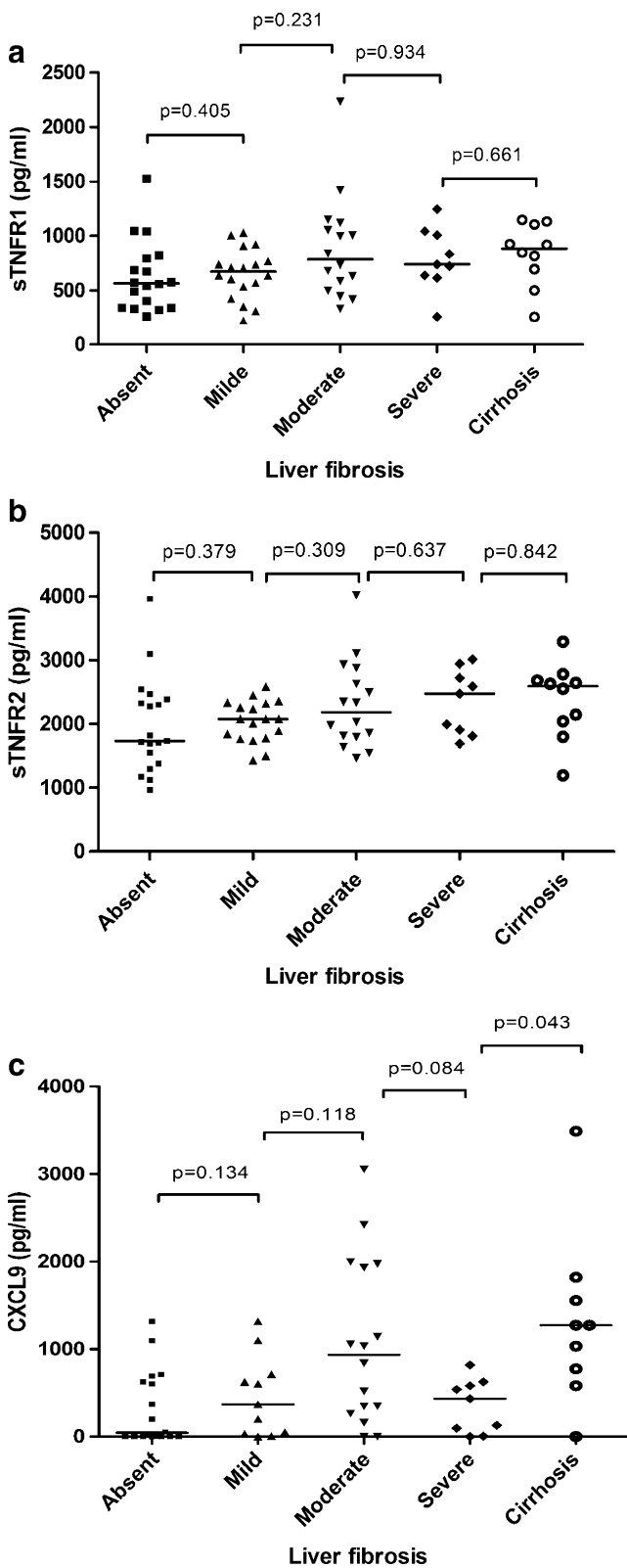
Fig. 2 Plasma levels of sTNFR1 (**a**), sTNFR2 (**b**), and CXCL9 (**c**) among CHC patients with different severities of liver fibrosis; *p*-values presented as calculated with the Mann–Whitney *U*-test

non-significant after adjustment for necroinflammatory activity, suggesting that liver inflammation could mediate the association.

Discussion

We showed that the plasma levels of sTNFR1, sTNFR2, and CXCL9 were independently associated to histological changes in the liver biopsy of patients with CHC. It is the first time that the association between plasma CXCL9 levels and liver fibrosis has been shown in patients with CHC infection and that the association between sTNFR1 and sTNFR2 and histological changes is found to be independent of other factors. Plasma levels of both sTNFR1 and sTNFR2 were significantly associated with liver fibrosis, whereas plasma levels of sTNFR2 were also associated with necroinflammatory activity. These soluble receptors result from the cleavage of TNF receptor of inflammatory cell membranes such as activated neutrophils and monocytes [43] and contribute to TNF system homeostasis.

Earlier studies have shown an association between serum sTNFR1 and sTNFR2 levels and aminotransferases levels and different severity of liver necroinflammatory activity and liver fibrosis [16, 44–46]. Kallinowski et al. [46] and Itoh et al. [44] have found an association between sTNFR1 and sTNFR2 levels and necroinflammatory activity in univariate analysis and Zylberberg et al. [16] showed that serum sTNFR2 levels were also significantly higher among patients with more advanced fibrosis. Kakumu et al. [45] compared patients with hepatic C virus infection at different stages and showed that the serum levels of sTNFR2 gradually increased with disease progression (i.e., normal aminotransferases < CHC < cirrhosis < hepatocarcinoma).

In human experimental studies with endotoxemia, elevation of serum TNF levels after endotoxin administration correlated with elevation of sTNFR levels [25, 47], suggesting the feasibility of using these soluble receptors as surrogate markers of TNF system activation [48]. In patients with CHC, correlation between TNF serum levels and its soluble receptors, sTNFR1 and sTNFR2, were shown by Nelson et al. [49]. Zylberberg et al. [16], however, were not able to demonstrate the association between serum TNF and sTNFR levels, suggesting that other inflammatory mediators besides TNF could be involved in sTNFR release.

A greater activation of the TNF system could be one explanation for the association found between elevated sTNFR2 levels and necroinflammatory activity. TNF-α is an apoptosis inductor in infected hepatocytes [50] and is also involved in damage caused by cytotoxic T lymphocytes to surrounding non-infected hepatocytes [51]. Greater activation of the TNF system would result in more severe damage to the liver parenchyma. Zylberberg et al. [16]

Table 4 Correlation between plasma levels of soluble inflammatory markers in patients with CHC ($n=71$)

	Correlation coefficient (p-value*)		
	CXCL9	sTNFR1	sTNFR2
CXCL9	1.0	0.54 (<0.001)	0.37 (0.002)
sTNFR1	0.54 (<0.001)	1.0	0.79 (<0.001)
sTNFR2	0.37 (0.002)	0.79 (<0.001)	1.0

*Spearman's rho

showed that serum TNF levels in CHC patients with more severe necroinflammatory activity were significantly more elevated than those with mild or moderate activity.

The lack of a significant association between plasma levels of sTNFR1 and necroinflammatory activity found in our study was also found by Zylberberg et al. [16]. In healthy volunteers, serum sTNFR2 levels are more abundant and show greater affinity to TNF if compared to sTNFR1 [48]. In patients with CHC, intrahepatic expression of TNFR2 is much more intense than that of TNFR1 [46] and TNFR2 is released at a much greater concentration in response to inflammatory cytokines [16].

Regarding the chemokines studied, only CXCL9 plasma levels were significantly associated with liver histological changes. The results of our study show that patients with more advanced liver fibrosis had more elevated plasma CXCL9 levels. Although we cannot state that plasma CXCL9 levels represent intrahepatic production, several studies showed that the intrahepatic expression of CXCL9 is increased among patients with CHC [52–54] and a recent study has shown that intrahepatic mRNA expression of CXCL9 increases with fibrosis severity both in patients with CHC and those with nonalcoholic steatohepatitis [55].

Peripheral levels of CXCL9 among CHC patients were evaluated in two other studies that showed an elevation of serum levels of this chemokine when compared to healthy volunteers [56, 57]. Apolinario et al. [57] evaluated 63 patients with CHC and found elevated serum CXCL9 levels compared to the control group. Butera et al. [56] assessed the plasma levels of CXCL9 in 82 patients and also found an elevation when compared to a control group. However, none of these studies evaluated the association between CXCL9 and liver histology. We have shown, for the first time, an association between CXCL9 peripheral levels and liver histological changes.

CXCL9 is produced by different cell types, including sinusoidal endothelial cells, hepatocytes, and hepatic stellate cells, mainly through IFN-γ stimulus [26, 53]. CXCL9 has an important role in liver inflammation by promoting chemotaxis of CXCR3+ activated T lymphocytes with Th1 phenotype [35, 58–62]. Shields et al. [53] and Zeremski et al. [52] showed through immunohistochemistry that lymphocytes infiltrating portal space and liver lobule expressed CXCR3 and that ligands of this receptor such as CXCL9 are present in greater amounts in the sinusoidal endothelium and hepatocytes of patients with chronic hepatitis when compared to controls.

Immunohistochemistry analysis conducted by Apolinario et al. [54] showed greater CXCL9 expression and stronger presence of lymphocytes expressing CXCR3 and CCR5 in hepatic acines with greater leukocyte infiltration. Although they did not investigate the association between CXCL9 expression and liver fibrosis that was shown in the present study, it is known that the severity of liver necroinflammatory activity is a predictor of liver progression in CHC patients [6, 63, 64]. An independent association between necroinflammatory activity and liver fibrosis was also found in our study.

Table 5 Multivariate logistic regression to identify factors predictive of moderate/severe liver fibrosis in patients with CHC ($n=71$)

Parameter	Odds ratio (95% CI)	p-value
a. Logistic regression model including the inflammatory marker sTNFR1		
sTNFR1 (ng/mL)	19.85 (1.57–251.22)	0.021
Necroinflammatory activity (A≥2)	40.96 (7.09–236.56)	<0.001
Alcohol ingestion (≥40 g/d)	5.95 (1.24–28.61)	0.026
Hosmer–Lemeshow: 0.62; Nagelkerke R^2: 0.59		
b. Model including the inflammatory marker sTNFR2		
Necroinflammatory activity (A≥2)	25.31 (6.03–103.14)	<0.001
Alcohol ingestion (≥40 g/d)	3.68 (0.93–14.54)	0.063
Hosmer–Lemeshow: 0.85 ; Nagelkerke R^2: 0.47		
c. Model including the inflammatory marker CXCL9		
CXCL9 (ng/mL)	1.24 (1.03–1.51)	0.03
Necroinflammatory activity (A≥2)	1.08 (1.02–1.15)	<0.01
Hosmer–Lemeshow: 0.78; Nagelkerke R^2: 0.54		

Another explanation for the association between CXCL9 and liver fibrosis would be its role as a ligand for CXCR3 in liver stellate cells. These cells have an important role in liver fibrogenesis [8, 65] and express membrane CXCR3. However, it has been recently shown that the binding of CXCL9 to hepatic stellate cell receptors strongly suppressed collagen protein expression, producing an antifibrotic effect in vitro [55]. Therefore, the positive association between liver fibrosis and CXCL9 found in our study and also by Wasmuth et al. [55] would mean that, in vivo, the profibrotic effect associated with the inflammatory infiltrate induced by this chemokine would overcome its antifibrotic activity in patients with CHC.

CXCL10, a soluble inflammatory marker that has been extensively studied in the past several years, binds to the same receptor as CXCL9 and exerts similar functions. Two studies found that peripheral CXCL10 levels were associated, in a dose–response manner, to inflammation and fibrosis [57, 66]. In the present study, however, CXCL10 were not significantly associated to liver histological changes. Similarly, no significant association was found between liver histological changes and plasma levels of chemokines from the CC subfamily assessed here (CCL2, CCL3, CCL11, and CCL24).

Due to the cross-sectional design of the study, we cannot assume a causal relationship between the plasma levels of inflammatory markers and liver histological changes. Prospective studies to address this issue are difficult to implement due to the protracted course of hepatitis C infection that would require a long follow-up period.

In summary, we have shown that plasma levels of sTNFR1, sTNFR2, and CXCL9 are associated with histological changes in the liver of CHC patients. Due to their low accuracy, the use of soluble inflammatory markers as sole predictors of liver histological changes in chronic HCV infection is not supported by our findings. Nonetheless, these findings could add to the understanding of the role of soluble inflammatory markers in HCV pathogenesis aiding in the identification of new therapeutic targets. As chemokines and TNF seem to be important to HCV pathogenesis, the inhibition of their action with monoclonal antibodies or receptors antagonists might interfere with the disease progression.

Conflict of interest The authors declare that they have no conflict of interest.

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