CXCL9 and CXCL10 Chemokines as Predictors of Liver Fibrosis in a Cohort of Primarily African-American Injection Drug Users With Chronic Hepatitis C

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CXCL9 (monokine induced by IFN γ [Mig]) and CXCL10 (interferon [IFN] γ−inducible protein 10 [IP-10]) have been associated with hepatic fibrosis in predominantly white hepatitis C virus (HCV)–infected patients. We investigated their potential as noninvasive markers of hepatic fibrosis and fibrosis progression in African-American patients. Peripheral chemokine levels were measured in 115 HCV-infected patients within 4 months of liver biopsy; patients underwent a second biopsy after 3–5 years. CXCL10 levels appeared to be higher in patients with advanced fibrosis on the contemporaneous biopsy and were significantly higher in patients with advanced fibrosis compared with those with minimal fibrosis on the later biopsy ($P = .0045$). Therefore, CXCL10 has potential as a marker of fibrosis progression in African-American HCV-infected patients.

Although infection with hepatitis C virus (HCV) is the most common cause of chronic liver disease in the United States, only a minority of infected individuals develop progressive disease that ultimately results in cirrhosis or hepatocellular carcinoma (HCC). Some patients progress very slowly, and even after several decades of HCV infection have minimal hepatic fibrosis [1–3]. Thus, identification of patients with accelerated fibrogenesis is of tremendous importance because early treatment can prevent development of cirrhosis and HCC [4]. Because current HCV treatment, which consists of pegylated interferon (PEG-IFN) and ribavirin (RBV), is difficult to tolerate, is effective in only 50% of patients, and is costly, it is not pursued in all patients. Consequently, some guidelines do not recommend treatment for those patients with mild disease [5].

CXCR3-associated chemokines, CXCL10 (interferon [IFN] γ−inducible protein 10 [IP-10]), CXCL9 (monokine induced by IFN γ [Mig]), and CXCL11 (IFN-inducible T cell α chemoattractant [I-TAC]), are key players in chronic HCV infection as they direct lymphocyte recruitment to the liver [6]. Both intrahepatic and peripheral levels of these chemokines are elevated in HCV-infected patients with high levels of liver inflammation and fibrosis [7, 8]. We have recently shown that the combination of peripheral CXCL10 and CXCL9 measurements has very good utility to discriminate HCV-infected patients with advanced liver fibrosis from those with mild fibrosis [8]. Moreover, chemokines had improved discriminatory ability compared with the aspartate aminotransferase (AST) to platelet ratio (APRI), another noninvasive index of hepatic fibrosis. In comparison with the performance characteristics reported in literature for FibroSure (LabCorp), 1 of only 2 commercially available noninvasive fibrosis indices in the United States, the combination of CXCL10 and CXCL9 had equivalent, if not improved, ability to identify patients with advanced fibrosis. However, that work was performed in a primarily white population, and the potential of these chemokines as noninvasive markers of fibrosis in individuals of other ethnicities is at present unknown. In addition, their potential to identify HCV-infected patients most likely to have progressive liver disease has not been investigated.

In this study, we analyzed CXCL10 and CXCL9 peripheral expression levels in a cohort of 115 HCV-infected injection drug users (IDUs), 93.9% of whom were African-American. All serum samples used for chemokine measurements were obtained within 4 months of liver biopsy, enabling analysis of the association between chemokine expression and fibrosis stage. Because all patients also underwent a second biopsy 3–5 years after the initial one, we explored potential associations between chemokine expression and fibrosis progression.

METHODS

Patient Characteristics

Patients included in this study were described previously [9]. Between 1996 and 1998, 210 patients were randomly selected

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out of 1625 participants from an HCV-infected IDU cohort to participate in a cross-sectional study designed to determine the severity and correlates of their liver disease [10]. All participants provided informed consent and were offered enrollment in the biopsy substudy. Repeat liver biopsies were offered between 2001 and 2003 and were performed on 121 of 182 eligible patients [9]. Of 121 patients who had 2 biopsies, 5 had inadequate samples for evaluation. An additional 3 patients who developed end-stage liver disease before the second biopsy was performed were also included in the study. Of 119 patients available for the study of fibrosis progression, chemokines were measured in 115 patients. All samples used for chemokine measurements were obtained within 4 months of the first liver biopsy (median 44 days).

Histopathologic evaluation of all liver biopsies was performed by a single, experienced hepatopathologist using the 7-point Ishak modified hepatic activity index (MHAI) and Ishak fibrosis index [11].

Chemokine and Other Measurements

We measured serum chemokine concentrations using commercially available enzyme-linked immunosorbent assay (ELISA) kits (BD OptEIA, BD Biosciences). Serum samples were kept frozen at −70° C and were not previously thawed.

We performed FibroSure testing on serum samples stored at −70°C, as described previously [9]. We calculated APRI from blood collected within 45 days of the first biopsy [9].

Statistical Analysis

We summarized continuous variables through their medians (range) and categorical variables through frequency counts. For comparisons of continuous variables, we used analysis of variance (ANOVA) or Kruskal–Wallis nonparametric tests. We used Fisher exact test to assess associations between categorical variables. We used logistic regression to model binary data. We evaluated the diagnostic performance of CXCL10 and FibroSure based on their ability to correctly classify participants relevant to their hepatic status. We computed the area under the receiver operating curve (AUROC) for CXCL10, FibroSure, and APRI as well as performance characteristics (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV]). We tested the hypothesis that the AUROC of CXCL10 for detection of insignificant levels of fibrosis differs significantly from that of FibroSure and APRI [12]. The significance level was defined at $P = .05$ and all statistical comparisons were performed with SAS and R (R Language, http://www.r-project.org).

RESULTS

Study Participants

We measured chemokine levels in serum samples obtained from 115 HCV-infected patients within 4 months of the initial liver biopsy, which was performed between November 1996 and June 1998. Patients underwent a second biopsy between March 2001 and May 2003, as described previously [9]. The median age of study participants at the time of the initial biopsy was 41.8 years (range, 27.4–59.5 years), 84.4% were male, 93.9% were African-American, 96% were infected with HCV genotype 1, and 26% of patients were coinfected with HIV.

Chemokine Expression and Contemporaneous Fibrosis

Most participants (91%) had minimal fibrosis (Ishak modified fibrosis score 0–2) on the first liver biopsy, whereas none of the patients had cirrhosis (Ishak modified fibrosis score 5–6) (Table 1). We did not find a significant association between fibrosis stage and peripheral CXCL9-10 expression on the contemporaneous biopsy (Figure 1*A*). CXCL10 levels were decreased in patients with stage 0–1 fibrosis compared with levels in other patients, although the difference in expression was not statistically significant ($P = .071$) (Figure 1*B*).

Chemokine Expression and Future Fibrosis

Only a few patients in this cohort developed progressive hepatic fibrosis. On the second biopsy, most patients still had either mild or undetectable fibrosis (81%), and only 8 patients (7%) had developed cirrhosis (Table 1). CXCL10 expression levels were elevated in patients who developed higher stages of fibrosis on the second biopsy, although the differences were not statistically significant when we divided patients into 7 fibrosis categories ($P = .08$) (Figure 1*C*). We did not find a significant correlation between CXCL9 expression and fibrosis stage on the second biopsy. When we grouped patients in 2 categories based on their fibrosis stage (minimal fibrosis: stage ≤ 2; advanced fibrosis: stage ≥ 3), CXCL10 levels were significantly higher in patients with advanced fibrosis compared with levels in those with minimal fibrosis on the later biopsy ($P = .0045$) (Figure 1*D*). The odds ratio of having advanced fibrosis at the second biopsy per unit increase in ln(IP-10) is 2.368 (95% confidence

Table 1. Fibrosis Scores at First and Second Liver Biopsy among 115 HCV-infected Patients

		First biopsy fibrosis results					
		0	1	2	3	4	Total (%)
	0	19	8	5	0	0	32 (28)
Second	**1**	9	12	8	0	1	30 (26)
biopsy	**2**	7	8	13	3	0	31 (27)
fibrosis	**3**	2	2	4	1	0	9 (8)
results	**4**	0	3	1	0	1	5 (4)
	5	0	0	1	1	0	2 (2)
	6	0	2	1	2	1	6 (5)
Total (%)		37 (32)	35 (30)	33 (29)	7 (6)	3 (3)	115

NOTE. Fibrosis assessed using the Ishak fibrosis index.

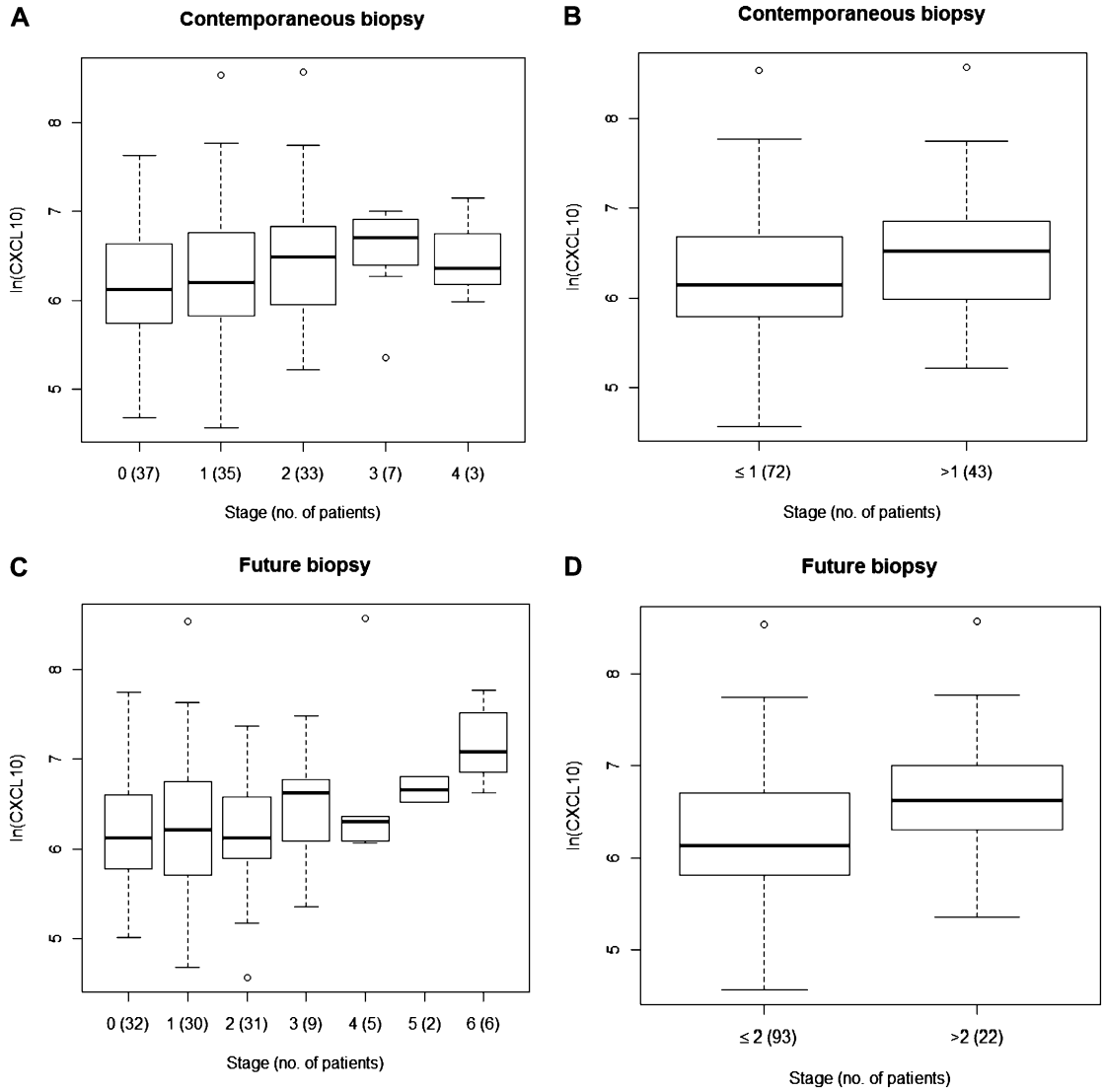

Figure 1. Box-and-whisker plots illustrating differences in the serum CXCL10 (interferon [IFN] γ−inducible protein 10 [IP-10]) levels of patients according to differences in fibrosis stage. *A* and *B*, association between serum CXCL10 levels and fibrosis on the contemporaneous liver biopsy (serum samples were obtained within 4 months of the biopsy). *C* and *D*, association between serum CXCL10 levels and fibrosis on the later biopsy (performed 3–5 years after the initial biopsy).

interval [CI], 1.15–4.877), adjusting for stage of fibrosis at the first biopsy.

When we evaluated the abilities of CXCL10, FibroSure, and APRI to discriminate between patients with advanced versus minimal fibrosis on the second biopsy, the AUROC for CXCL10 was 0.7, for FibroSure, 0.746, and for APRI 0.694 (Table 2). When we compared the AUROCs of the 3 tests, we did not find them to differ significantly. After computing tests' sensitivities, specificities, PPVs and NPVs for the selected cut-off values, CXCL10 was found to have better ability to predict minimal fibrosis (stage ≤ 2) than advanced fibrosis (stage ≥ 3) on the later biopsy (Table 2). Among the 29 patients with ln(CXCL10) $\leq$ 5.86, only 1 patient had fibrosis stage ≥ 3 on the second biopsy.

Progression of fibrosis, defined as an increase in Ishak fibrosis score of at least 2, occurred in only 23 (20%) of study participants. On previous evaluation of patients described in this manuscript, no significant associations were found between FibroSure or APRI and fibrosis progression [9]. Although we did not find a significant association between fibrosis progression and chemokine expression as a continuous variable (OR = 1.725, 95% CI[0.92, 3.25], $P = .09$), there was a trend for an association between ln(CXCL10) $\leq$ 5.86 and fibrosis nonprogression ($P = .058$). Of 29 patients with ln(CXCL10) levels equal or $<$5.86 at the first liver biopsy, 27 (93.1%) did not have fibrosis progression in the following 3–5 years, and the remaining 2 patients progressed from stage 0 to stage 2.

Table 2. Performance of FibroSure, APRI, and CXCL10 for Detection of Liver Fibrosis on the Second Biopsy

	Sensitivity	Specificity	PPV	NPV	AUROC (SE)
Significant fibrosis (≥3)					
FibroSure > 0.48	61.9	70.3	32.5	88.9	0.746 (0.0657)
APRI >1.5	13.6	94.6	37.5	82.2	0.694 (0.0673)
ln(CXCL10) > 6.75	45.5	80.6	35.7	86.2	0.7 (0.067)
Insignificant fibrosis (≤2)					
FibroSure < 0.31	51.6	81	92.2	27.9	
APRI < 0.5	61.3	68.2	89.1	29.4	
ln(CXCL10) ≤ 5.86	30.1	95.5	96.6	24	

NOTE. Fibrosis assessed using the Ishak fibrosis index. APRI, aspartate aminotransferase to platelet ratio; AUROC, area under the receiver operating curve; CXCL10, interferon [IFN] γ–inducible protein 10 [IP-10]; NPV, negative predictive value; PPV, positive predictive value; SE, standard error.

DISCUSSION

In our previous work, we found peripheral levels of CXCR3-associated chemokines, particularly CXCL10, to be significantly associated with liver fibrosis in primarily white chronic HCV-infected patients [8]. CXCL10 in combination with CXCL9 had a strong potential to identify patients with advanced liver fibrosis. In this work, we analyzed the diagnostic abilities of CXCL9 and CXCL10 in a primarily African-American population. We found peripheral levels of CXCL10 to have potential to identify HCV-infected patients who are unlikely to have progressive liver fibrosis. CXCL9 levels were not associated with fibrosis stage on initial or later biopsy.

In contrast to our previous work [8], this study found that CXCL9-10 expression was not significantly correlated with contemporaneous liver fibrosis in this patient population. However, several major differences in the 2 cohorts could explain the discrepancy in the results between these studies. For example, most patients described in this study were African-American (93.9%), whereas white patients (85%) made up most of our previous cohort. We have found CXCL10 expression levels to be considerably higher in African-American patients from this cohort in comparison with levels in white patients from the previous study (median ln(CXCL10) 6.27 [IQR 5.86–6.73] versus 5.56 [IQR 4.93–6.04], $P < .0001$). Although the 2 studies did not use the same specimen type for chemokine measurements (plasma versus serum), significantly higher CXCL10 expression in African-Americans compared with white patients has been reported previously [13]. Therefore, the capacity of CXCL10 to differentiate among HCV-infected patients with different levels of liver fibrosis might be, at least in part, race dependent. Secondly, whereas most (91%) of the patients from this cohort had insignificant or mild liver fibrosis on the first biopsy, only 38% of the patients from the previous cohort were in that category. We have previously reported that CXCL10 has the better potential to identify patients with advanced liver fibrosis compared with those with milder stages [8]. Because none of the patients analyzed in this study had advanced fibrosis and only 9% had moderate fibrosis on the first biopsy, the discriminatory capacity of CXCL10 was consequently decreased in this cohort. Lack of advanced fibrosis in this cohort not only impacted the results reported here but also reduced the estimated performance of FibroSure and APRI for prediction of fibrosis [9]. Finally, although our prior study used plasma samples, in this work chemokine expression was measured in serum. Although we did not expect to find significant differences in chemokine levels between serum and plasma, sample type might have at least partially contributed to the discrepant results obtained in these 2 studies.

The capacity of CXCR3-associated chemokines to predict fibrosis progression or future fibrosis in chronic HCV-infected patients has not been previously assessed. We have shown here that CXCL10 has reasonable ability to identify patients who are unlikely to develop advanced fibrosis in a 3–5 year interval. Although the calculated sensitivity of CXCL10 was suboptimal, patients with low chemokine levels were extremely unlikely to develop advanced fibrosis during the next several years. Therefore, observation of these individuals could be recommended instead of pursuing treatment using pegylated interferon and ribavirin combination therapy. Additionally, considering racial differences in CXCL10 expression, we would expect this chemokine to have higher capacity to predict future fibrosis among white patients.

In conclusion, although peripheral CXCL10 levels do have a potential to predict present and future fibrosis in patients with chronic hepatitis C, this ability is most likely dependent on the patients' race. To determine if CXCL10 could be developed as a marker of fibrosis or fibrosis progression, additional analysis in larger, racially diverse populations is needed. Identification of race-specific CXCL10 cutoff values might be necessary to precisely identify HCV-infected patients most likely to have mild/moderate or advanced stages of fibrosis at the present or in the future. This information could be considered when making treatment decisions.

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