Severe Liver Fibrosis and Association with Plasma Inflammatory Biomarkers among HIV/HCV Co-infected Patients in China :a Cross-Sectional Study

Xiaochen Chen
Fudan University School of Public Health
Xing Liu
Fudan University School of Public Health
Song Duan
Dehong Prefecture Center for Disease Control and Prevention, Mangshi
Renhai Tang
Dehong Prefecture Center for Disease Control and Prevention, Mangshi
Sujuan Zhou
Fudan University School of Public Health
Runhua Ye
Dehong Prefecture Center for Disease Control and Prevention, Mangshi
Yuecheng Yang
Dehong Prefecture Center for Disease Control and Prevention
Jibao Wang
Dehong Prefecture Center for Disease Control and Prevention, Mangshi
Shitang Yao
Dehong Prefecture Center for Disease Control and Prevention, Mangshi
Meiyang Gao
Fudan University School of Public Health
Yingying Ding
Fudan University School of Public Health
Na He (nhe@shmu.edu.cn)
Fudan University School of Public Health

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Abstract

Background: Immune dysregulation among HIV/HCV co-infected patients with impaired liver function is common. Thus, this study aimed to evaluate the association of liver fibrosis with microbial translocation and related inflammation among HIV/HCV co-infected patients.

Methods: This cross-sectional study involved 343 HIV/HCV co-infected patients who received cART. All patients had current blood biochemical testing data. We measured sCD14 and 27 serum cytokines concentrations using the Hycult Biotech sCD14 ELISA kit and Bio-plex Human Cytokine 27-plex Assay, respectively. We compared the concentrations of each marker between severe liver fibrosis and mild liver fibrosis. Odds ratios (ORs) and 95% confidence intervals (95%CIs) for the association of each marker with severe liver fibrosis were estimated using logistic regression.

Results: Of the 343 HIV/HCV coinfect-ed patients enrolled, 188 (54.8%) had severe liver fibrosis (FIB-4 >3.25). Patients with higher FIB-4 score (>3.25vs. ≤3.25) had higher plasma level of IL-1β, IL-6, IL-7, IL-9, IL-12, IL-15, IL-17, GM-CSF, IFN-γ, TNF-α, IL-4, IL-10, IL-13, BasicFGF and MCP-1. Multivariate logistic regression analysis showed that increased plasma level of IL-1β, IL-6, IL-7, IL-12, IL-17, GM- CSF, IFN-γ, IL-4, IL -10, MCP-1, Eotaxin, BasicFGF and sCD14 were linked to severe liver fibrosis in our study.

Conclusions: Severe liver fibrosis are associated with increased microbial translocation plasma inflammatory biomarkers among HIV/HCV co-infected patients.

Background

Human immunodeficiency virus (HIV) and Hepatitis C virus (HCV) co-infection is a worldwide serious public health concern because of increased mortality and disease burden[1, 2]. Compared to HCV mono-infected patients, HIV/HCV co-infected patients have more frequent and accelerated progression to fibrosis, cirrhosis and end-stage liver disease (ESLD)[3]. Differ from western countries, direct-acting antivirals (DAAs) even peg-interferon plus ribavirin (PR) in China especially rural area cannot be widely used because of the high price[32].So the burden of disease caused by HIV/HCV may be heavier in China. Pathogenesis of progressive liver fibrosis in HIV/HCV co-infected is complex and multifactorial [4], and is mainly related to persistent HCV infection with altered cellular immunity as well as immune activation with elevated pro-inflammatory and profibrogenic cytokines[5]. More specifically, HIV infection induces immune suppression and leads to CD4 T-cell depletion, causes persistent innate and acquired immune activation, and directly stimulates Kupffer cells and hepatic stellate cells (HSCs) to secrete either pro-fibrotic cytokines or type 1 collagen through a C–C chemokine receptor–5 (CCR5)-dependent pathway[5]. In addition, the persistence of HCV infection due to weakened CD4 and CD8T cell responses caused by HIV[6] contributes to increased rates of liver fibrosis[7–9].

Moreover, microbial translocation (MT), which is thought to be associated with loss of mucosal barrier function and increased intestinal permeability secondary to immune dysregulation and/or alterations in the intestinal microbiome, has often been observed among HIV-infected individuals[10]. Blood microbial

components including peptidoglycan, lipoteichoic acid, lipopolysaccharide (LPS) and flagellin may directly promote liver fibrosis in HIV/HCV co-infected patients by stimulation of HSCs and/or Kupffer cells with LPS binding protein (LBP) and soluble CD14 (sCD14). These microbial components may enhance local hepatic inflammatory immune responses and activation-induced liver cell death[4].

Thus, the above-mentioned mechanisms jointly emphasize the important role played by inflammation in liver fibrosis among HIV/HCV co-infected patients. Cirrhosis-associated dysregulation of immune responses is reflected by increased production and elevated serum levels of pro-inflammatory cytokines and upregulated expression of cell activation markers[11]. Kupffer cells generate IL–1, IL–6, IL12, IL–18 and also release anti-inflammatory cytokines, including IL–10 after stimulation by LPS[12]. Upregulation of IL–4, IL13, TGF–1 and platelet-derived growth factor (PDGF) was observed during fibrogenesis, while among HIV-infected patients, gp120 may induce HSC accumulation by secretion of monocyte chemoattractant protein–1 (MCP–1) by HSCs[13]. Furthermore, HIV suppression by combination antiretroviral therapy (cART) among HIV/HCV co-infected patients may decrease inflammation and immune activation and slows down the progression of liver disease [14], whereas the levels of plasma inflammatory biomarkers remain abnormal in many individuals[15]. However, the inflammatory profile among HIV/HCV co-infected patients with different levels of liver fibrosis has not been well established. Up to now, studies of the role of microbial translocation and plasma inflammatory biomarkers in liver fibrosis are limited by small sample sizes and small number of examined biomarkers[16–18]. To fill this gap, the present study aimed primarily to examine the association of liver fibrosis with microbial translocation and related inflammation among HIV/HCV co-infected patients in the era of cART.

Methods

Study sample

The present cross-sectional study was conducted in Dehong Prefecture of Yunnan Province at China's southwest border, where the first China's indigenous HIV outbreak was reported in 146 infected heroin users in 1989, and injection drug use (IDU) had been the predominant mode of HIV transmission through the early 2000s and continues to be an important source of HIV infection[19, 20]. By March 2016, 1017 HIV/HCV co-infected patients with 170 (16.7%) deaths had been reported and registered in the Comprehensive Response Information Management System (CRIMS) for HIV/AIDS in China, which is a unified web-based national information system with the capacity of longitudinal assessment and monitoring of HIV-infected patients' health status[21]. Out of the remaining 847 patients, 463 (54.7%) were identifiable during the study period from April to October in 2016. Of them, 390 (84.2%) were receiving cART and gave informed consent to participate in the present study. Forty-seven patients were further excluded from the study due to missing data on biochemical measures about aspartate aminotransferase (AST), alanine aminotransferase (ALT), and platelet count (PLT) that are necessary to evaluate liver fibrosis status. Thus, a total of 343 HIV/HCV co-infected patients were included in the final analysis. These 343 participants had no significant differences with the 120 identifiable yet excluded

HIV/HCV co-infected patients in the distribution of age, sex, marital status, HIV transmission route, HBsAg serostatus, baseline CD4 cells count, years on cART, current HCV RNA and HIV RNA levels except for ethnicity (Table S1).

Data Extraction

Demographical and clinical epidemiological data were extracted from the CRIMS. The data included age, sex, marital status, ethnicity, HIV transmission route, date of cART initiation, antiretroviral regimen, and CD4 cell counts at cART initiation and follow-up visits.

Blood testing

Biochemical tests for liver fibrosis assessment

Biochemical measurements of AST, ALT and PLT were performed using an automatic biochemistry analyzer (Beckman Coultner, USA), according to the manufacturer's protocol. Liver fibrosis was assessed by FIB–4 score, using Sterling's formula calculated as (AST [IU/L] × age [years]) / (PLT [10^9/L] × ALT[IU/L]$^{1/2}$)[22]. This is an international recognized well-established noninvasive indicator of liver fibrosis in HIV/HCV co-infected patients[23]. FIB–4 is generally divided into three categories: no or mild liver fibrosis with FIB–4 <1.45, intermediate liver fibrosis with 1.45 ≤ FIB–4 ≤ 3.25, and severe liver Fibrosis with FIB–4 >3.25. In this study, we were more concerned with severe liver fibrosis (FIB–4 >3.25) [24].

HCV RNA Quantification and genotyping

Plasma HCV viral RNA was extracted (Roche diagnostic products (Shanghai) Co., Ltd., China) and quantified by a real-time polymerase chain reaction (RT-PCR) technique using commercially available kits for the quantification of HCV RNA (PCR-Fluorescent Probing, PG Biotech Ltd., Shenzhen, China). The limit of detection was 500 copies/ml, and the linear range of HCV RNA quantification was from 1.0×10^3 to 5.0×10^7 copies/ml.

Amplification was completed by a nested PCR with E1- or NS5B-specific primers. Splicing, proofreading, and aligning sample sequences were performed using ChromasPro 1.5 and BioEdit7.0.9.0 software. HCV genotype reference sequences were retrieved from the HCV database (http://hcv.lanl.gov/content/sequence/HCV/ToolsOutline.html). The phylogenetic tree was established by the Neighbor-joining method of MEGA 7.0 software. The Bootstrap repeat detection value was set to 1000 times, and the HCV gene subtype was further determined according to the phylogenetic tree[25].

Multiplex cytokine bead assay and ELISA

Twenty-seven cytokines, chemokines and growth factors cytokines in plasma specimens were quantified by a multiplex analysis performed on the BioPlex®200 Multiplex System platform using the Bio-Plex Human 27-plex panel of cytokines/chemokines/growth factors (Bio-Rad, Hercules, CA, USA), according to the manufacturer's instructions. These biomarkers included 1) proinflammatory biomarkers such as interleukin (IL)−1β, IL−2, IL−6, IL−7, IL−8, IL−9, IL−12, IL−15, IL−17, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), Interferon-γ (IFN-γ), and tumor necrosis factor (TNF-a); 2) anti-inflammatory biomarkers such as IL−1ra, IL−4, IL−5, IL−10, and IL−13; 3) chemokines such as IFN-γ-inducible protein (IP−10), monocyte chemoattractant protein−1 (MCP−1), macrophage inflammatory proteins−1alpha (MIP−1a), MIP−1b, and regulated upon activation normal T-cell expressed and secreted (RANTES); and 4) growth factors such as Eotaxin, fibroblast growth factor 2 (Basic FGF), platelet-derived growth factor (PDGF-BB), and vascular endothelial growth factor (VEGF). The detection limit for each molecule was determined by the recovery of the corresponding standard, and the lowest values with more than 70% recovery were set as the lowest detection limits. Measurements less than the lower limit of quantification (LLOQ) were assigned a value of half the LLOQ, and measurements more than the upper limit of quantification (ULOQ) were assigned a value of twice the ULOQ for each marker.

Plasma sCD14, as an indirect surrogate marker of microbial translocation (MT), was measured according to the manufacturer's protocol for commercial enzyme-linked immunosorbent assay (ELISA) (Hycult Biotech, Wayne PA, USA).

Other tests

Human hepatitis B virus surface antigen (HBsAg) was tested by ELISA technique (Wan Tai Biomedical Co. Ltd, Beijing, China). CD4 cell counts were assessed by FACSCount (Becton, Dickinson and Co., San Jose, CA, USA).

Statistical analysis

Group comparisons were assessed using chi-square test or Fisher's exact test for categorical variables and t-test or Mann-Whitney U test for continuous variable. Log10-transformation were conducted for variables of plasma cytokines levels for further statistical and regression analyses. A multiple logistic regression analysis with adjustment for age, sex, ethnicity, current HIV RNA, current HCV RNA, current CD4 cell counts, years since cART and ART regimen type was undertaken to explore the correlation of liver fibrosis with the plasma level of each of the twenty-seven cytokines and sCD14. The odd ratio (OR) and 95% confidence interval (95%CI) represent the risk of liver fibrosis per one log-unit change in plasma cytokine concentration. Spearman correlations were computed to explore associations between the

plasma inflammatory biomarkers. Statistical significance was defined as p <0.05 and Bonferroni p <0.002 (0.05/27≈0.002) for multiple comparison adjustment [26]. All statistical analyses were performed using R software (version 3.3.2).

Results

Demographical and clinical characteristics

The median (IQR) age at baseline was 35.4 (31.3–39.3) years old. The majority of the participants were male, injection drug users (IDUs), seronegative for hepatitis B surface antigen (HBsAg), detectable for plasma HCV RNA but undetectable for plasma HIV RNA. Around half of the participants were non-Han ethnicities, i.e., ethnic minorities (54.2%), currently married (51.9%), and of HCV subtype 3 (47%) (Table 1).

Prevalence of Severe Liver fibrosis

Among all participants, 75 (21.9%) had no or mild liver fibrosis, 80 (23.3%) had intermediate liver fibrosis, and 188 (54.8%) had severe liver fibrosis. Compared to patients without severe liver fibrosis, those with severe liver fibrosis were significantly different in age, education, cART regimen, and years on cART(Table 1).

Plasma levels of inflammatory biomarkers

Participants with sever liver fibrosis (FIB–4 3.25) had significantly higher plasma levels of IL–1β, IL–4, IL–6, IL–7, IL–9, IL–10, IL–12, IL–13, IL–15, IL–17, IFN-γ, TNF-α, MCP–1, GM-CSF, Basic FGF and VEGF than those with FIB–4≤3.25 （all p<0.005）(Table 2 and Figure 1). Such differences remained significant for IL–6, IL–10, IFN-γ, GM-CSF and Basic FGF after Bonferroni correction for multiple comparisons. No significant difference in sCD14 was observed between the two groups.

Correlations of inflammatory biomarkers and their associations with severe liver fibrosis

Most of the 27 cytokines were significantly correlated with each other with the exception of RANTES, which was not correlated with most of the other cytokines (Figure 2). The correlation coefficients are shown as a supplement in Table S2.

Given the high correlation between the 27 cytokines, separate multiple logistic regression models adjusting for potential confounders were performed to examine the association of sever liver fibrosis with

the each of the 27 cytokines and sCD14 levels. Severe liver fibrosis was shown to be associated with higher levels of eleven out of the twenty-seven cytokines with a significance level of 0.05, only Basic FGF was found to be positively and significantly associated with severe liver fibrosis after Bonferroni correction for multiple comparisons (aOR = 1.82; 95%CI: 1.26–2.66; p = 0.002). Furthermore, plasma sCD14 was significantly associated with severe liver fibrosis (aOR = 1.14; 95%CI: 1.01–1.30; p = 0.048. Figure 3).

Discussion

In the present study, 54.8% of HIV/HCV co-infected patients had severe liver fibrosis or cirrhosis. The prevalence was higher than studies conducted in western countries which range from 22.9% to 40.3% [27–30]. Among one study conducted in Southeast Asia including 380 HIV/HCV co-infected patients, 40.3% had liver fibrosis or cirrhosis[31]. The discrepancy is possibly attributed to the fact that these patients lack of antiviral therapy for viral hepatitis C.

HIV and HCV infection are both characterized by systemic inflammation with increased levels of blood inflammatory cytokines. HIV infection may alter the pattern of cytokine release and exacerbate immune-mediated inflammatory responses[33]. Although plasma inflammatory markers tend to decrease in HIV/HCV co-infected patients after cART, they still remain much higher than healthy controls [6, 34]. In the present study, we found that HIV/HCV co-infected patients with severe liver fibrosis were living with an elevated level of inflammatory cytokines including pro-inflammatory cytokines, anti-inflammatory cytokines, chemoattractant cytokines, growth factors and the microbial translocation surrogate marker sCD14. This indicates that progressive and advanced liver fibrosis in HIV/HCV co-infected is mainly an inflammatory syndrome [6,35].

Many studies have shown that inflammation plays an important role in the development of liver fibrosis among HIV/HCV co-infected patients[36, 37]. In this regard, levels of cytokines, chemokines and growth factors closely related to inflammation were altered. We found that patients with cirrhosis who had FIB–4 >3.25 showed a significantly higher plasma level of various inflammatory makers (IL–1β, IL–6, IL–7, IL–9, IL–12, IL–15, IL–17, GM-CSF, IFN-γ, TNF-α, IL–4, IL–10, IL–13, BasicFGF and MCP–1). Although the median plasma level of sCD14 was higher in the FIB–4 >3.25 group than FIB–4 ≤3.25, there was no significant difference.

Chronic inflammation and fibrosis are inextricably linked, and the interactions between immune effector cells, local fibroblasts and tissue macrophages at sites of scar formation determine the outcome of liver injury. With improved understanding of the processes that govern inflammation and fibrosis, it has become clear that both the adaptive and innate immune systems are involved in the regulation of fibrosis[38].

In our study, multivariate logistic regression analysis shows that increased plasma levels of IL–1β, IL–6, IL–7, IL–12, IL–17, GM- CSF, IFN-γ, IL–4, IL –10, MCP–1, Eotaxin, BasicFGF and sCD14 are linked to severe liver fibrosis. Our findings are in concordance with previous studies that show plasma levels of

proinflammatory, including TNF-a, IL−6, IL−8, IL−12, are profibrogenic and induce liver damage[39, 40]. In addition, these studies show that cirrhotic patients display increased production of IFN-γ, IL−4 and IL−13 [41]. However, the role of IFN-γ in liver fibrosis is controversial[42]. IFN-γ in these studies show that IFN-γ is significantly negatively correlated with liver fibrosis[39] [43], but IFN-γ also proves to have proinflammatory effects that can aggravate disease progression and organ dysfunction[44]. Our study shows a positive correlation between IFN-γ levels and liver fibrosis. The association between IL−17 and fibrosis is also controversial. Sheila et al found an inverse correlation between IL−17 and aminotransferase-to-platelet ratio index (APRI) [45]; to the contrary, Meng et al found that IL−17 could exacerbate liver fibrosis[46]. In our study, we found that increased IL−17 concentration is associated with higher FIB−4 score. Regarding IL−2, a previous study found a negative correlation of IL−2 with cirrhosis[47]. However, our data shows that IL−2 levels increase as fibrosis increases.

Anti-inflammatory cytokines, including IL−4、IL−10 and IL−13, are profibrogenic cytokines, and their levels increase in coinfected patients [48, 49], which is in accordance with our findings.

Chemokines may promote inflammation through the recruitment of lymphocytes to the liver parenchyma in chronic hepatitis C virus (HCV) infection [50]. MIP−1a is a profibrogenic chemokine. Tazi, et al found that cirrhotic monocytes spontaneously produce chemokines (MCP−1) unlike normal monocytes[51]. Although increased MIP−1a increased the risk of developing cirrhosis in our study, it was not statistically significant. Many studies have shown that plasma IP−10 levels increased as liver fibrosis increased in HCV or HIV / HCV co-infected [33, 52, 53, 54], but our data did not find this association.

As for now, studies on the involvement of growth factors other than TGF-β1 in liver fibrosis are rare. In our study, multivariate logistic models indicate that increased BasicFGF and Eotaxin levels persist as predictors for liver cirrhosis.

sCD14 is a significant biomarker related to microbial translocation since it reflects the host's response to microbial translocation [55]. Considering the limitations of the limulus test used to detect LPS, it is technically difficult to measure, and the results are often inconsistent [56]. Instead we can use sCD14 as an indirect biomarker of microbial translocation. Our study shows that increased sCD14 increases the risk of severe liver fibrosis, consistent with the findings of French et al[57].

Limitations

This study has several limitations. Firstly, due to cross-sectional design, this study fails to establish the direction of causality, e.g., inflammation may be a cause or response to liver cirrhosis. Secondly, we did not include factors known to influence the risk of liver fibrosis that could alter levels of immune/inflammatory markers such as alcohol abuse. Thirdly, we did not set a control group of HCV-monoinfected or HIV-monoinfected patients, and we are not able to know information of possible differential biomarkers from them.

Conclusions

In summary, severe liver fibrosis are associated with increased microbial translocation plasma inflammatory biomarkers among HIV/HCV co-infected patients. Our findings provide preliminary evidence that immune/inflammatory markers may be useful predictors of liver fibrosis. Further research using longitudinal design is warranted to determine the causal relationship between liver fibrosis and inflammation, in order to look for the optimal care and management of HIV/HCV co-infected patients in China.

Abbreviations

HIV:Human immunodeficiency virus;HCV: Hepatitis C virus; ORs: Odds ratios; 95%CIs: 95% confidence intervals; ESLD:end-stage liver disease; HSCs:hepatic stellate cells; CCR5: C–C chemokine receptor–5; LPS: lipopolysaccharide; LBP: LPS binding protein; sCD14: soluble CD14; cART: combination antiretroviral therapy; IDU: injection drug use; CRIMS: Comprehensive Response Information Management System; AST: aspartate aminotransferase; ALT: alanine aminotransferase; PLT: platelet count; IRB: Institutional Review Board; FIB–4:fibrosis index–4; MT: microbial translocation;

Declarations

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Author's contributions:

The study was conceived and supervised by HN.TRH, ZSJ, YRH, YYC, WJB, YST, GMY and DYY conducted the survey. CXC analyzed the data and drafted the manuscript. LX and HN critically reviewed and revised the manuscript. All authors commented on drafts and read an approved the final manuscript.

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Availability of data and materials

The datasets used during the study are available from the corresponding author and provided on a reasonable request.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB) of Fudan University, Shanghai, China. Written informed consent was obtained from all study participants.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Authors details

[1] Department of Epidemiology, School of Public Health, and the Key Laboratory of Public Health Safety of Ministry of Education, Fudan University, Shanghai, China, Na He, Xiaochen Chen, Xing Liu, Sujuan Zhou, Meiyang Gao, Yingying Ding, PhD;

[2] Key Laboratory of Health Technology Assessment of Ministry of Health, Fudan University, Shanghai, China, Na He, Xiaochen Chen;

[3] Dehong Prefecture Center for Disease Control and Prevention, Mangshi, Yunnan Province, China, Song Duan, Renhai Tang, Runhua Ye, Yuecheng Yang, Jibao Wang, Shitang Yao.

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Tables

Table 1. Demographic and clinical characteristics of HIV/HCV co-infected participants

	All No. (%)	FIB-4 score		*p**
		≤3.25 No. (%)	3.25 No. (%)	
N	343(100.0)	155(45.2)	188(54.8)	
Age, years				
Median (IQR)	35.4 (31.3-39.3)	34.6 (30.2-38.6)	36.0 (31.8-40.3)	**0.029**
Sex				
Male	336 (98.0)	151 (97.4)	185 (98.4)	0.796
Female	7 (2.0)	4 (2.6)	3 (1.6)	
Marital status				0.210
Unmarried	130 (37.9)	61 (39.4)	69 (36.7)	
Married	178 (51.9)	74 (47.7)	104 (55.3)	
Divorced/widowed	35 (10.2)	20 (12.9)	15 (8.0)	
Ethnicity				0.151
Han	157 (45.8)	62 (40.0)	95 (50.5)	
Dai	112 (32.6)	60 (38.7)	52 (27.7)	
Jingpo	62 (18.1)	28 (18.1)	34 (18.1)	
Others	12 (3.5)	5 (3.2)	7 (3.7)	
HIV transmission route				0.185
IDU	314 (91.5)	140 (90.3)	174 (92.6)	
Others	29 (8.5)	15 (9.7)	14 (7.4)	
Baseline CD4, cells/ul				0.618
<200	98 (29.0)	40 (26.3)	58 (31.2)	
200-349	139 (41.1)	65 (42.8)	74 (39.8)	
≥350	101 (29.9)	47 (30.9)	54 (29.0)	
Current CD4 , cells/ul				0.511
<200	21 (6.1)	7 (4.5)	14 (7.4)	
200-349	52 (15.2)	23 (14.8)	29 (15.4)	
≥350	270 (78.7)	125 (80.6)	145 (77.1)	
HCV RNA				0.234
Undetectable	105 (30.6)	53 (34.2)	52 (27.7)	
Detectable	238 (69.4)	102 (65.8)	136 (72.3)	
HIV RNA				0.053
Undetectable	264 (89.3)	126 (88.1)	138 (79.3)	
Detectable	53 (10.7)	17 (11.9)	36 (20.7)	
Years on cART	5.7 (4.2-8.4)	5.1 (3.9-7.6)	6.1 (4.4-8.9)	**0.001**
HBsAg positive				0.685
Yes	30 (8.7)	12 (7.7)	18 (9.6)	
No	313 (91.3)	143 (92.3)	170 (90.4)	
ART regimen, %				**0.012**
NVP (vs. EFV/RTV)	136 (39.7)	52 (33.5)	84 (44.7)	
TDF (vs. AZT/d4T/DDI)	147 (42.9)	80 (51.6)	67 (35.6)	
Others	60 (17.4)	23 (14.8)	37 (19.7)	
HCV genotype				0.182
1	46 (16.0)	23 (18.1)	23 (14.4)	
3	135 (47.0)	52 (40.9)	83 (51.9)	

6	106 (37.0)	52 (40.9)	54 (33.8)

[a]IDU, injection drug use;

[b]Chi-square tests or Mann-Whitney U test were performed wherever appropriate;

[c]Bold signifies statistical significance as $p<0.05$

Table 2. Plasma level of inflammatory cytokines according to current FIB-4 score

	Overall	FIB-4 score		*p*
		≤3.25	3.25	
Pro-inflammatory				
IL-1β	9.8 (5.0 , 46.9)	8.0 (4.6 , 33.8)	11.3 (5.2 , 63.0)	**0.005**
IL-2	6.9 (0.50 , 87.0)	6.1 (0.5 , 63.4)	9.6 (0.5 , 95.6)	0.216
IL-6	19.8 (6.8 , 46.3)	16.3 (4.8 , 37.0)	23.6 (10.2 , 54.6)	**0.001**
IL-7	14.4 (6.2 , 29.8)	13.2 (5.3 , 24.6)	15.6 (6.4 , 35.0)	**0.038**
IL-8	63.6 (32.5 , 123.9)	56.0 (31.7 , 117.5)	73.0 (34.5 , 123.8)	0.166
IL-9	92.8 (39.4 , 267.5)	81.5 (32.7 , 183.4)	103.0 (50.0 , 337.1)	**0.009**
IL-12	27.0 (1.9 , 73.0)	20.9 (1.5 , 58.3)	32.4 (3.7 , 93.5)	**0.006**
IL-15	23.0 (5.3 , 112.1)	14.8 (5.3 , 86.4)	27.6 (5.3 , 127.0)	**0.046**
IL-17	92.2 (11.1 , 146.6)	46.0 (9.7 , 129.0)	106.3 (13.8 , 154.1)	**0.016**
G-CSF	52.0 (27.2-82.0)	44.5 (20.7 , 89.7)	54.8 (36.1 , 80.1)	0.278
GM-CSF	93.7 (0.3 , 238.3)	17.6 (0.2 , 196.2)	121.0 (2.2 , 269.3)	**<0.001**
IFN-γ	41.0 (13.8 , 72.4)	29.9 (5.0 , 65.7)	47.0 (26.9 , 75.2)	**0.002**
TNF-α	46.0 (31.1 , 70.0)	40.8 (27.1 , 70.3)	50.5 (35.6 , 68.3)	**0.015**
Anti-inflammatory				
IL-1rα	324.5 (141.0 , 755.4)	288.9 (124.1 , 722.8)	338.1 (156.0 , 801.7)	0.210
IL-4	5.6 (2.9 , 61.0)	5.1 (2.6 , 11.2)	5.9 (3.3 , 80.0)	**0.008**
IL-5	10.0 (4.5 , 22.0)	10.6 (4.3 , 20.0)	10.0 (4.7 , 23.0)	0.632
IL-10	9.9 (0.7 , 61.0)	7.4 (0.7 , 40.8)	11.8 (0.7 , 71.6)	**0.001**
IL-13	8.4 (3.0 , 32.0)	6.6 (2.1 , 20.9)	10.1 (3.7 , 34.1)	**0.006**
Chemoattractant				
IP-10	2390.3 (1331.0 , 4196.2)	2322.5 (1244.2 , 4004.6)	2565.7 (1492.8 , 4208.3)	0.335
MCP-1	46.1 (14.9 , 88.2)	32.9 (14.2 , 77.0)	53.2 (16.0 , 112.9)	**0.013**
MIP-1α	4.9 (3.0 , 37.0)	5.1 (2.6 , 19.2)	4.8 (3.4 , 42.3)	0.102
MIP-1β	132.2 (84.1 , 410.5)	126.0 (87.6 , 235.9)	142.5 (80.4 , 551.3)	0.229
RANTES	19871.0 (11759.0 , 69279.6)	19700.5 (9854.2 , 69279.6)	19979.5 (13163.8 , 69279.6)	0.881
Growth factor				
Eotaxin	182.7 (117.7 , 294.1)	179.1 (117.6 , 263.7)	193.0 (118.6 , 324.2)	0.126
Basic FGF	66.0 (9.8 , 104.5)	40.9 (4.35 , 97.1)	79.5 (30.6 , 107.0)	**0.001**
PDGF-BB	2840.7 (1266.9 , 4561.9)	2750.9 (1323.52 , 4295.5)	2924.1 (1231.3 , 4616.9)	0.978
VEGF	105.0 (27.05 , 215.1)	94.0 (7.6 , 196.9)	111.7 (47.0 , 230.5)	**0.050**
Microbial Translocation				
sCD14	3.0 (2.2 , 4.2)	2.9 (2.2 , 3.9)	3.2 (2.2 , 4.7)	0.094

data was presented as median and interquartile range (IQR), with μg/mL as the unit for sCD14 and pg/mL for all other cytokines.

Figures

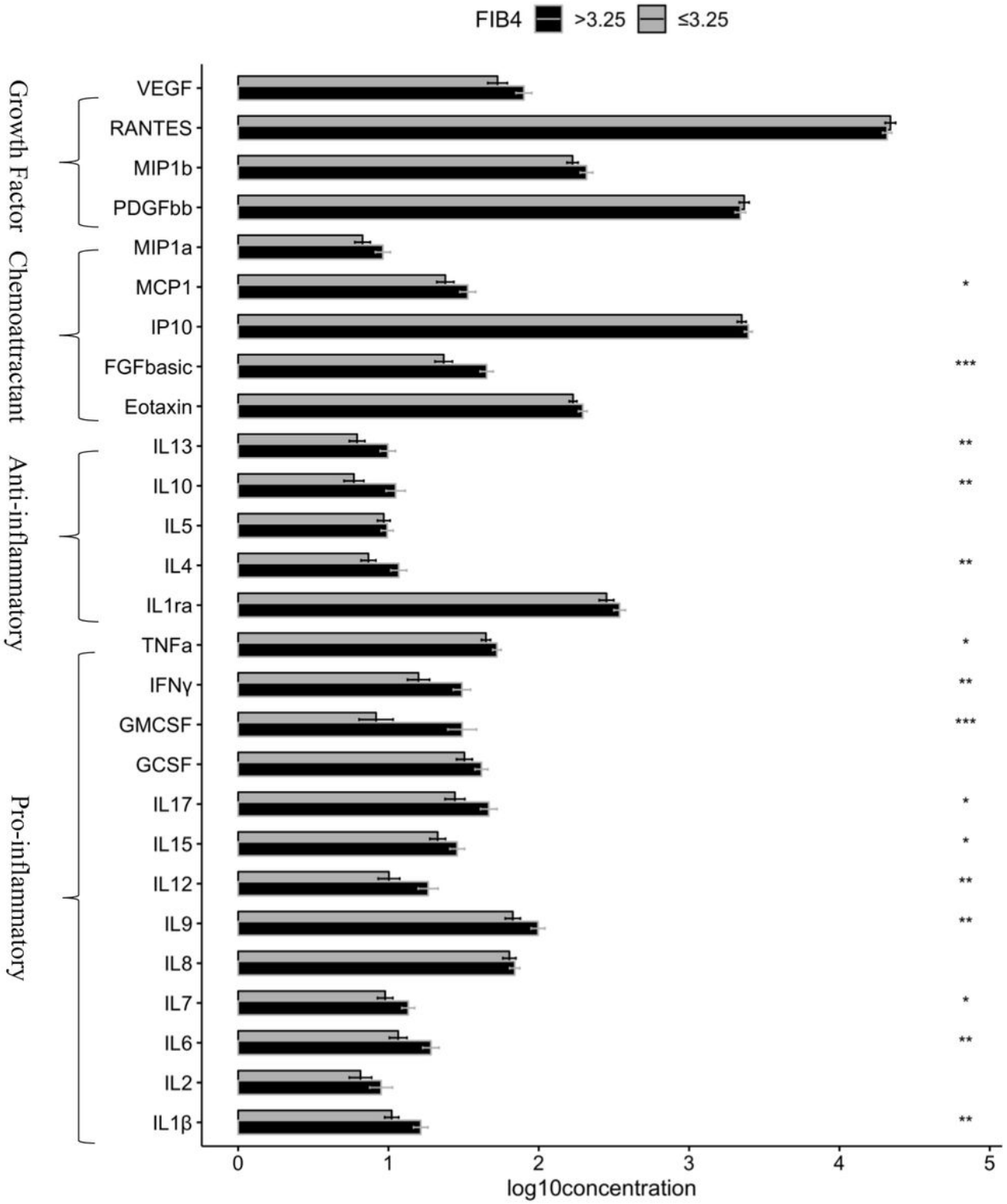

Figure 1

Comparison of log10 transformed plasma concentration of tested biomarkers between participants with different FIB-4 score. *p<0.05, **p<0.01, and ***p<0.001. All the p-values were calculated by Mann–Whitney tests.

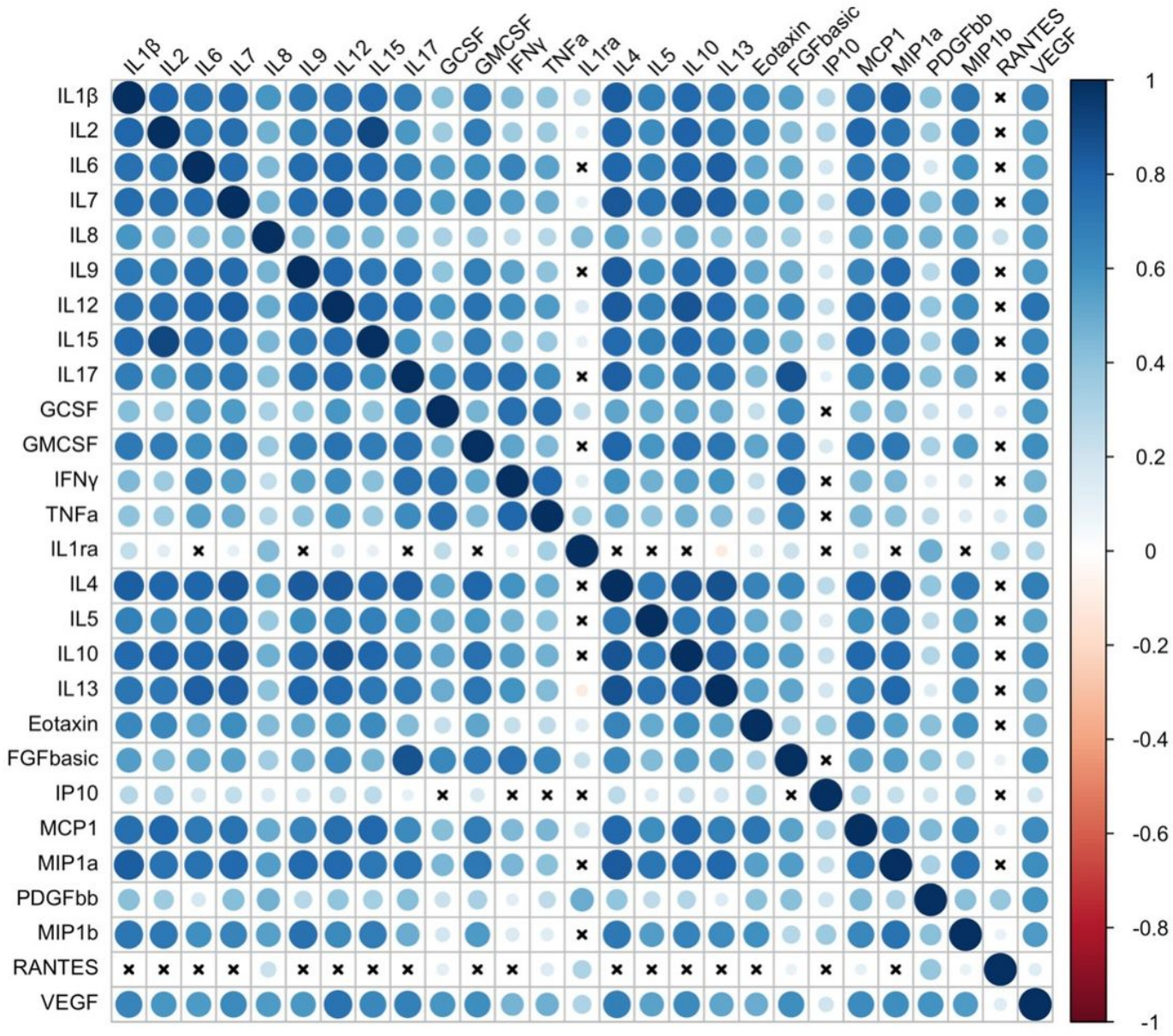

Figure 2

Spearman correlations between plasma inflammatory biomarkers. “x” represents p≥0.05. Blue and red indicate that the two variables were positively and negatively correlated, respectively. The darker the color (the larger the circle), the greater the correlation of the variables.

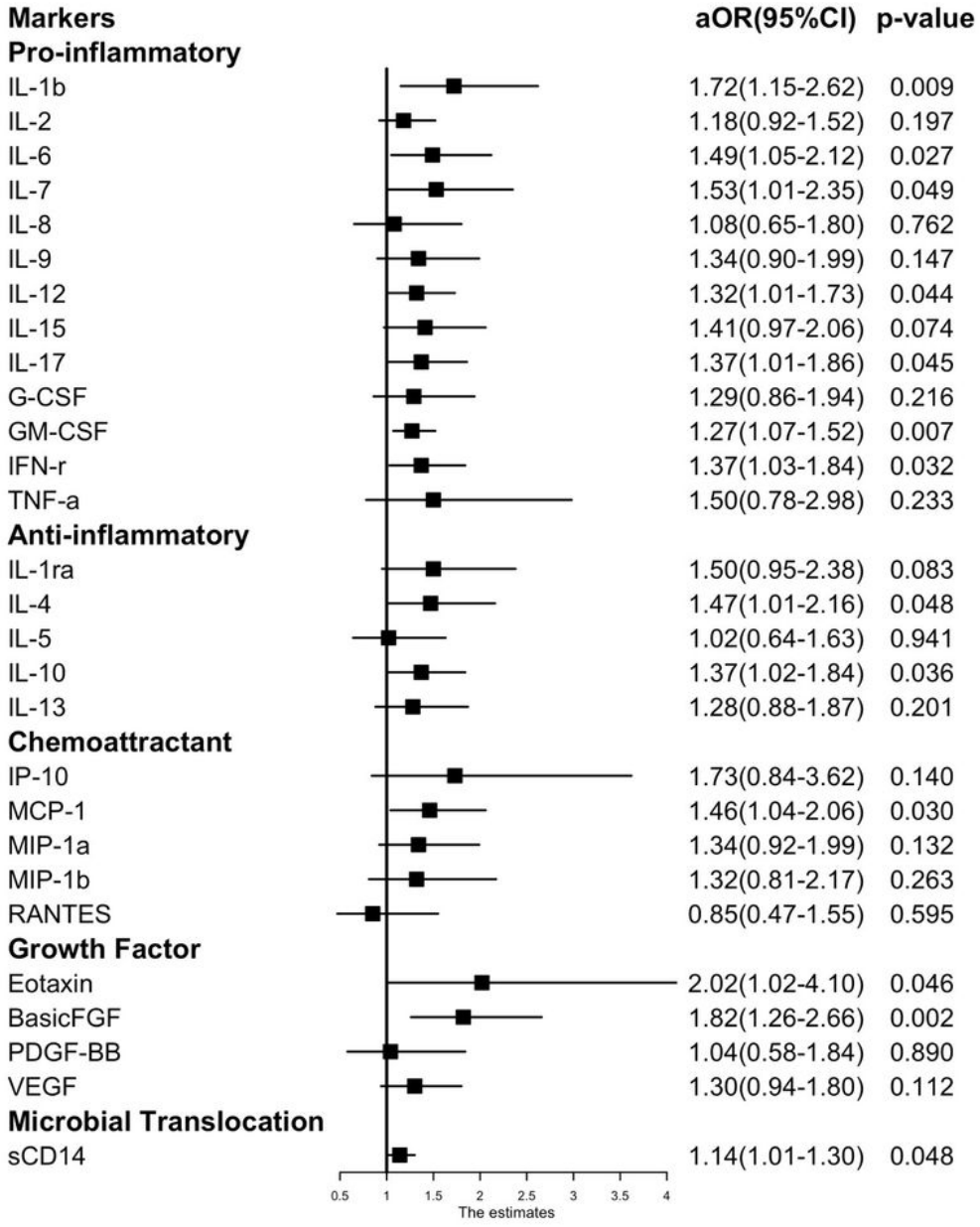

Figure 3

adjusted Odds ratios and 95% confidence intervals for the associations between plasma cytokine levels (log10 transformed) and serve liver fibrosis among HIV/HCV co-infected patients. Each variable was assessed in separate multivariable logistic regression adjusting for age, sex, ethnicity, current HIV RNA, current HCV RNA, current CD4 count, years on cART, and ART regimen.

Supplementary Files

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