Intimate partner violence against young women: prevalence and associated factors in Europe.
Sanz-Barbero B, López Pereira P, Barrio G, Vives-Cases C.
J Epidemiol Community Health. 2018 Jul;72(7):611-616. doi: 10.1136/jech-2017-209701. Epub 2018 Mar 8.
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ORIGINAL ARTICLE

Title: Elevated liver stiffness is linked to increased biomarkers of inflammation and immune activation in HIV/HCV-coinfected patients

Running head: Liver and immune system

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Abstract

Objectives: Immune dysregulation is a hallmark of HIV and HCV infections. To evaluate the relationship between liver stiffness measure (LSM) and biomarkers of T cell activation, bacterial translocation, inflammation, endothelial dysfunction, and coagulopathy in HIV/HCV-coinfected patients.

Design: Cross-sectional study.

Methods: We studied 238 HIV/HCV-coinfected patients, 32 healthy controls and 39 HIV-monoinfected patients. Patients were stratified according to LSM into four groups: <12.5 kPa, 12.5 to 25 kPa, 25 to 40 kPa, and >40 kPa. T-cell subsets were measured using flow cytometry and plasma biomarkers using immunoassays.

Results: HIV/HCV-coinfected patients had higher biomarker levels of immune activation in peripheral blood [T cell activation ($CD4^+CD38^+$ and $CD8^+CD38^+$), bacterial translocation (sCD14), inflammation (IL-1b, IL-6, IL-8, IL-18, IP-10) endothelial dysfunction (sVCAM1, sICAM1, and sTNFR1), and coagulopathy (PAI-1)] than healthy controls and HIV-monoinfected patients. Moreover, in HIV/HCV-coinfected patients, a direct relationship between LSM and immune activation [T cell activation ($CD8^+CD38^+$ bacterial translocation (LPS), inflammation (IL-8, IP-10), endothelial dysfunction (sVCAM1, sICAM1, and sTNFR1), and coagulopathy (D-dimer)] was found. Subsequently, patients were stratified into different fibrosis stages, finding that patients with cirrhosis who had LSM≥40 kPa showed higher biomarker values of immune activation [T cell activation ($CD4^+CD38^+$ and $CD8^+CD38^+$), bacterial translocation (LPS), inflammation (IL-8, IL-6, IP-10), endothelial dysfunction (sVCAM1, sICAM1 and sTNFR1), and coagulopathy (D-dimer)] than patients from the other three groups (<12.5 kPa, 12.5-25 kPa, and 25-40 kPa).

Conclusion: T cell activation, bacterial translocation, inflammation, endothelial dysfunction, and coagulopathy increased with the severity of liver fibrosis in HIV/HCV-coinfected patients, particularly in patients who had LSM≥40 KPa.

Key Words

Chronic hepatitis C; HIV; cirrhosis; T cell activation; bacterial translocation; inflammation; coagulopathy

Introduction

Hepatitis C virus (HCV) infection is common among human immunodeficiency virus (HIV) - infected people due to similar routes of transmission [1]. Chronic hepatitis C (CHC) has been a leading comorbidity in HIV-infected patients since the introduction of combination antiretroviral therapy (cART) [2, 3]. The course of CHC may be accelerated in patients coinfected with HIV, resulting in higher rates of fibrosis progression, cirrhosis, and end-stage liver disease than HCV-monoinfected patients [4]. HIV infection leads to a gradual $CD4^+$ T cell count decline and causes persistent innate and acquired immune activation, which contributes to the pathogenesis of both AIDS and non-AIDS related diseases [5, 6]. Additionally, despite suppressive cART, HIV-infected patients show abnormally high levels of plasma biomarkers related to immune activation, inflammatory and coagulation markers that may predict increased morbidity and mortality [5]. This immune activation is related to several situations, such as the persistence of HIV replication, HCV coinfection, and bacterial translocation defined as the passage of bacteria or microbial products from the intestinal lumen to mesenteric lymph nodes or other extra-intestinal sites caused by HIV itself and CHC [6, 7].

Bacterial translocation is a key factor in the pathogenesis of both HIV and HCV infection, especially in the advanced stages of disease when bacterial translocation increases (AIDS [8] and cirrhosis [9]). During HIV infection, the depletion of $CD4^+$ T cells in gut-associated lymphoid tissue (GALT) compromises gut mucosal integrity, promoting increased intestinal permeability that leads to bacterial translocation [8]. In HCV infection, there is a pathological increase in bacterial translocation in liver cirrhosis due to changes and overgrowth of intestinal microbiota, an increase in intestinal permeability and the dysregulation of the immune response in GALT [9]. During this process, host immune cells are stimulated by bacterial pathogen-associated molecular patterns (PAMPs), such as LPS [10], which bind to the CD14/TLR4 complex activating the NF-κβ pathway and inducing the synthesis of proinflammatory cytokines such as TNF-α, IL-1, IL-6, etc., and overexpressing chronic activation markers [11-13].

Moreover, during CHC the massive destruction of liver cells leads to dramatic physiological and pathophysiological changes during advanced fibrosis and cirrhosis [14]. Furthermore, cirrhosis is linked to a dysregulation in the balance between activation and homeostasis of the immune system, leading to a state characterized by immune activation and inflammation [13]. In addition, most blood proteins are produced by hepatocytes and their concentration may be

altered by CHC progression, leading to an increased thrombotic risk [15].

The aim of our study was to evaluate the relationship between LSM and biomarkers of T cell activation, bacterial translocation, inflammation, endothelial dysfunction, and coagulopathy in HIV/HCV-coinfected patients.

Patients and methods

Patients

We carried out a cross-sectional study in 238 HIV/HCV-coinfected patients, who were selected from the cohort of "Grupo de Estudio del SIDA" (GESIDA 3603b study; see **Appendix**), which is composed of patients enrolled between February 2012 and February 2016 at 19 institutions in Spain. The GESIDA 3603b study is a cohort study that has the aim of evaluating the effect of HCV eradication on the immune system and the associated clinical outcomes. We selected HIV/HCV-coinfected patients at baseline of study.

The cohort included both naïve and anti-HCV therapy experienced patients, who were candidates to receive treatment with HCV therapy (peg-IFN-α/ribavirin or peg-IFN-α/ribavirin/direct-acting antivirals (DAAs)). Anti-HCV therapy in Spain is provided by hospital pharmacies and is covered by the National Health System. The selection criteria were: 1) detectable HCV RNA and HIV RNA by polymerase chain reaction (PCR); 2) availability of a valid baseline LSM; 3) availability of a valid sample of fresh blood to carry out immunological assays; 4) $CD4^+$ T cell count higher than 200 cells/μL; 5) stable cART for at least 6 months or no need for cART according to guidelines used in the study period. We excluded patients with acute hepatitis C, co-infection with hepatitis B virus, decompensated liver disease, or a prior diagnosis of hepatocellular carcinoma at the time of the transient elastography study.

We selected two control groups for evaluating differences of HIV/HCV-coinfected patients with respect to the normality in peripheral blood biomarkers. On the one hand, the healthy controls are negative subjects for HIV, HCV, and HBV infection. On the other hand, HIV-monoinfected patients with undetectable HIV viral load and $CD4^+$>500 cells/mm^3 represent the standard normality of HIV patient without HCV and HBV infection, and other severe comorbidities. The characteristics of both control groups are shown in **Supplemental Table 1**.

The study cohort received the approval of the ethics committees of the participating centers for analysis of anonymized routine clinical data with a view to scientific publication. This work was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board and the Research Ethic Committee of the Instituto de Salud Carlos III approved the study. All patients gave their informed consent for the study.

Clinical data

All the information was recorded at each institution using a common database via an online form, which satisfied local requirements of data confidentiality. This database included all demographic, clinical, virological, and laboratory data. All the centers included in the cohort were monitored to verify that all the information in the database was consistent with the patient's medical records.

We extracted the following baseline data from hospital records: (1) demographics; (2) HIV-related data (HIV transmission category, Centers for Disease Control and Prevention [CDC] clinical category, nadir $CD4^+$ T cell count, the most recent $CD4^+$ T cell count, the most recent HIV RNA load, and whether or not patients were receiving cART); (3) liver disease-related data (HCV genotype, HCV RNA load, hepatitis B surface antigen [HBsAg], and anti-HCV therapy); and (4) history of substance abuse including alcohol consumption >50 g/d.

The duration of HCV infections for patients with a history of intravenous drug use (IDU) was estimated starting from the first year they shared needles and other injection paraphernalia [16]. For non-IDU patients, we only calculated the time of infection for those patients that the initiation of HCV infection could be determined with certainty (acute hepatitis C, use of blood and blood products, needle piercing, identified sexual contact, etc.). The time of HIV infections was calculated from the date of HIV diagnosis.

Liver stiffness measurement (LSM) was assessed by transient elastography (FibroScan®, Echosens, Paris, France) using a single machine. Results were expressed in kilopascals (kPa) with a range of 2.5 to 75 kPa. Trained operators performed all FibroScan® examinations. We considered 10 acquisitions with a success rate ≥60% and an interquartile range <30% of the median value as representative measurements of liver stiffness [17]. Fasting was not routinely required prior to the examination.

From these values of LSM, patients were stratified according to the following clinically relevant LSM cutoffs previously used: <12.5 kPa (non-cirrhosis, [17]), 12.5 to 25 kPa (non-risk of bleeding varices, [18]), 25 to 40 kPa (risk of bleeding varices, [18]), and >40 kPa (risk of hepatic decompensation, [19]).

Flow cytometry

The expression of CD38 was evaluated in $CD4^+$ and $CD8^+$ T-cell subsets by flow cytometry in 100µL fresh anticoagulated whole blood. The cells were labeled with the following antibodies: anti-CD38-APC-Cyanine 5.5 (APC-Cy5.5, clone HIT2, Invitrogen, Frederick, MD), anti-CD4-APC-

Cyanine 7 (APC-Cy7, clone OKT4, BioLegend, San Diego, CA), anti-CD8-Pacific Blue (PB, clone SK1, BioLegend, San Diego, CA), anti-CD3-Pacific Orange (PO, clone VCHT1, Invitrogen, Frederick, MD) and incubated for 20 min at room temperature in the dark. Next, the IMMUNOPREP Reagent System (Beckman Coulter, Mervue Galway, Ireland) was added to each sample using a Coulter MULTI-Q-PREP Lysing Workstation (Beckman Coulter, Miami, FL) to lyse and fixate them. Fluorescence was measured with a Gallios™ flow cytometer (Beckman Coulter, Miami, FL). The number of events was stopped at a minimum of 200,000 cells in the lymphocyte gate for each sample and flow cytometry data were analyzed using Kaluza™ acquisition software (version 1.5; Beckman Coulter, Miami, FL).

Multiplex assay and ELISA

We selected several biomarkers for each of the objectives we wanted to analyze: a) bacterial translocation [soluble CD14 (sCD14), lipopolysaccharide (LPS), fatty acid-binding protein 2 (FABP2), lipopolysaccharide binding protein (LBP)]; b) inflammation [interleukin (IL)-1b, IL-8, IL-6, IL-18, IFN-γ-inducible protein 10 (IP-10); c) endothelial dysfunction: soluble vascular cell adhesion molecule 1 (sVCAM1), soluble intercellular cell adhesion molecule 1 (sICAM1), soluble tumor necrosis factor receptor 1 (sTNFR1), monocyte chemoattractant protein-1 (MCP1)]; d) coagulopathy [D-Dimer, plasminogen activator inhibitor-1 (PAI-1)].

Plasma biomarkers were measured by ProcartaPlex™ multiplex immunoassay (ThermoFisher, USA) according to the manufacturer's specifications using a Luminex 200™ analyzer (Luminex Corporation, Austin, TX, United States) with the exception of sCD14 (Raybiotech, Georgia, USA), LPS (HycultBiotech, Uden, The Netherlands), LBP (R&D Systems, Minneapolis, USA) and FABP2 (Raybiotech, Georgia, USA), which were performed according to the manufacturer's procedure for each specific commercial ELISA.

Statistical analysis

The statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) 21.0 (SPSS INC, Chicago, IL, USA). Statistical significance was defined as $p<0.05$. All p-values were two-tailed.

For the descriptive study, values were expressed as absolute number (percentage) and median [25th; 75th percentile]. Categorical data and proportions were analyzed using the chi-squared test or Fisher's exact test as required. Kruskal-Wallis and Mann-Whitney tests were used to compare data among independent groups.

Generalized Linear Models (GLM), with a gamma distribution (log-link), was used to evaluate the association between LSM values (continuous variable and ordinal variable) and levels of biomarkers in peripheral blood. This test gives the differences between groups and the arithmetic mean ratio (AMR) or the ratio by which the arithmetic mean of the original outcome is multiplied. Each regression test was adjusted by age, gender, nadir $CD4^+$ T cells, baseline $CD4^+$ T cells, HIV viral load (>50 cp/mL), high alcohol intake, diabetes, $\log_{10}$ HCV RNA, HCV-GT1, previous HCV therapy (IFNα+ribavirin), and prior AIDS.

Results

Patients

The characteristics of the 238 HIV/HCV-coinfected patients are shown in **Table 1**. Overall, the median age was 49 years, 78.6% were males, 49.1% had high alcohol intake, 78.1% acquired HIV by IVDU, 26.9% had had prior aids-defining conditions, and 98% were on cART. Furthermore, the mean $CD4^+$ T cell count was 574 cells/mm^3, 12.6% had values of HIV RNA >50 copies/mL, 72.3% were HCV-GT1 and 71.4% had HCV RNA > 850,000 IU/mL. When patients were stratified by LSM values, we only found significant differences between groups in CD4+ T cells (p=0.005) and HCV-GT4 (p=0.005).

HIV/HCV-coinfected patients *vs*. control groups

HIV/HCV-coinfected patients had higher values of markers of T cell activation [CD4+CD38+ (p≤0.001), $CD8^+CD38^+$ (p≤0.001)] and plasma biomarkers of bacterial translocation [sCD14 (p≤0.010)], inflammation [IL-1b (p≤0.006), IL-8 (p≤0.001), IL-6 (p≤0.001), IL-18 (p≤0.001), IP-10 (p≤0.001), sVCAM1 (p≤0.001), sICAM1 (p≤0.001), sTNFR1 (p≤0.001)], and coagulopathy [PAI-1 (p≤0.001)] than healthy controls and HIV-monoinfected patients (**Table 2**). Additionally, HIV/HCV-coinfected patients had higher values of MCP1 than healthy controls (p=0.005).

Biomarkers of liver fibrosis

The relationship of liver stiffness measurements and biomarkers of activation, translocation, inflammation, and coagulopathy are shown in **Table 3**. In multivariate analysis, we found that the high LSM values were associated with high levels of $CD8^+CD38^+$ (aAMR=1.15; p=0.037), LPS (aAMR=1.18; p=0.009), IL-8 (aAMR=2.02; p<0.001), IP-10 (aAMR=1.27; p=0.006), sVCAM1 (aAMR=1.39; p<0.001), sICAM1 (aAMR=1.29; p=0.045), sTNFR1 (aAMR=1.48; p<0.001), and D-Dimer (aAMR=1.71; p<0.001).

Biomarkers of severe cirrhosis

Table 4 shows the univariate analysis of the biomarker values of activation, translocation, inflammation, and coagulopathy stratified by fibrosis/cirrhosis stages. Patients with >40 kPa had higher values of $CD8^+CD38^+$, IL-8, IL-6, and D-Dimer than patients with <12.5 kPa (p≤0.005), 12.5-25 kPa (p≤0.011), and 25-40 kPa (p≤0.027). Furthermore, patients with >40 kPa had higher values of IP-10, sTNFR1, and PAI-1 than patients with <12.5 kPa (p≤0.003)

and 12.5-25 kPa ($p \leq 0.004$). Additionally, patients with >40 kPa had higher values of sVCAM1 than patients with <12.5 kPa ($p \leq 0.001$).

These differences among groups were also analyzed by multivariate analysis with GLM tests, which showed significant values of aAMR >1 (patients with >40 kPa had higher values than other groups) for several biomarkers (**Table 5**). Patients with >40 kPa had higher values of $CD8^+CD38^+$, IL-6, sICAM1, and D-Dimer than patients with <12.5 kPa ($p \leq 0.05$), 12.5-25 kPa ($p \leq 0.05$), and 25-40 kPa ($p \leq 0.05$). Furthermore, patients with >40 kPa had higher values of LPS, IL-8, IP-10, and sTNFR1 than patients with <12.5 kPa ($p \leq 0.05$) and 12.5-25 kPa ($p \leq 0.05$). Additionally, patients with >40 kPa had higher values of sVCAM1 than patients with <12.5 kPa ($p \leq 0.001$) and $CD4^+CD38^+$ than patients with 25-40 kPa ($p = 0.038$).

Discussion

In this study, HIV/HCV-coinfected patients were found to have higher biomarker levels of immune activation in peripheral blood [T cell activation ($CD4^+CD38^+$ and $CD8^+CD38^+$), bacterial translocation (sCD14), inflammation (IL-1b, IL-6, IL-8, IL-18, IP-10), endothelial dysfunction (sVCAM1, sICAM1, and sTNFR1), and coagulopathy (PAI-1)] than healthy controls and HIV-monoinfected patients. Moreover, in HIV/HCV-coinfected patients, we found a direct relationship between LSM values and biomarker values of immune activation [T cell activation ($CD8^+CD38^+$), bacterial translocation (LPS), inflammation (IL-8, IP-10), endothelial dysfunction (sVCAM1, sICAM1, and sTNFR1) and coagulopathy (D-dimer)]. Subsequently, patients were stratified at different fibrosis stages according to their LSM values. The resulting analysis found that patients with cirrhosis who had LSM≥40 kPa showed higher biomarker values of immune activation [T cell activation ($CD4^+CD38^+$ and $CD8^+CD38^+$), bacterial translocation (LPS), inflammation (IL-8, IL-6, IP-10), endothelial dysfunction (sVCAM1, sICAM1 and sTNFR1), and coagulopathy (D-dimer)] than patients from other groups (<12.5 kPa, 12.5-25 kPa, and 25-40 kPa). We also stratified by established cut-off points of LSM, such as <7.1 kPa (F0-F1), 7.1-9.4 kPa (F2; significant fibrosis), 9.5-12.4 kPa (F3; advanced fibrosis), and ≥12.5 kPa (F4; cirrhosis) [20]. However, although some significant differences were found, we did not find a clear trend in the relationship of LSM stages (ordinal variable) with markers of T cell activation and plasma biomarkers of bacterial translocation, inflammation, and coagulopathy in our HIV/HCV-coinfected patients (*data not shown*). To our knowledge, this is the first time that very high levels of these biomarkers of immune activation have been found in HIV/HCV-coinfected patients with compensated cirrhosis. Our results could shed light on the importance of hyperactivation of the immune system in the physiopathology of CHC in HIV/HCV-coinfected patients with compensated cirrhosis. However, longitudinal studies are necessary to confirm this hypothesis.

In our study, HIV/HCV-coinfected patients had higher plasma sCD14 values than control groups (healthy controls and HIV-monoinfected patients), which may indicate increased bacterial translocation in these subjects, which is usually accompanied by increased inflammation and liver disease severity [21-24], increasing the risk of developing non-AIDS-related complications and death [25]. However, we did not find any association between sCD14 values and LSM in HIV/HCV-coinfected patients. It is possible that the characteristics of our cohort may have played a part in this lack of association, resulting in the loss of a linear relationship between sCD14 and LSM. Moreover, plasma LPS levels were similar in HIV/HCV-coinfected patients and both control

groups, although elevated LPS levels have been previously observed in HIV/HCV-coinfected patients [26]. It is possible that the absence of significant differences may be influenced by the highest sCD14 values in our HIV/HCV-coinfected patients, which may capture and remove LPS from systemic circulation [27], reducing the plasma LPS concentration. However, LPS levels were related to LSM values and cirrhotic patients with LSM≥25 kPa had the highest LPS values. In accordance with our data, an association between plasma LPS levels and advanced stages of liver disease has been found in CHC patients [21, 22]. Thus, LPS may indicate liver disease severity and short-term survival of cirrhotic patients [28, 29]. However, there are also studies that did not find any such association [30, 31].

Regarding T cell immune activation, HIV/HCV-coinfected patients had higher percentages of $CD4^+CD38^+$ and $CD8^+CD38^+$ than healthy controls and HIV-monoinfected patients. Previous reports have shown that HCV infection may increase the percentage of $CD8^+CD38^+$ to above levels of healthy controls [32, 33], with a return to normal values after HCV elimination [33]. In addition, HIV/HCV-coinfected patients have higher levels of immune activation than HIV-monoinfected patients [34]. This immune activation may accelerate CHC [35, 36], and it has been linked to AIDS progression, onset of comorbidities and death in HIV-infected patients [25]. Moreover, high LSM values were linked to high $CD8^+CD38^+$ percentages, and cirrhotic patients with LSM≥40 kPa had the highest $CD8^+CD38^+$ percentages. The activation of the immune system is one of the main pathogenic mechanisms of liver disease, including CHC [9, 13]. Thus, the increased CD38 expression in T cells may indicate an increased risk of CHC progression in HIV/HCV-coinfected patients, as well as an increased risk of progression to AIDS.

Inflammation is characteristic of both HIV and HCV infection, and it plays a key role in the pathogenesis of liver disease in HIV/HCV-coinfected patients [25, 35, 36]. Additionally, inflammation is directly related to endothelial dysfunction, which is implicated in the development of liver diseases and increased cardiovascular risk [37]. In our study, among all biomarkers analyzed, the most relevant were IL-6, IL-8, IP-10, sVCAM1, sICAM1, and sTNFR1. These biomarkers showed higher values in HIV/HCV-coinfected patients and increased as liver stiffness increased, particularly in patients with advanced cirrhosis (LSM≥40 kPa). Overall, our results are in accordance with previous results described in the literature in HCV-infected patients. Firstly, plasma IL-6 levels are higher in patients with HCV infection, particularly in patients with advanced fibrosis or cirrhosis [23, 24, 38, 39]. Secondly, increased circulating IL-8 levels have been linked to CHC progression [40-45]. Thirdly, increased plasma IP-10 levels are related to liver disease severity in HCV-infected patients [26, 44, 46-49]. Fourthly, plasma sVCAM1 and sICAM1 are linked to liver disease severity in HCV-infected

patients [46, 50, 51] and HIV/HCV-coinfected patients [52, 53]. Fifthly, plasma sTNFR1 levels are increased in CHC patients with cirrhosis [54], are related to the influx of portal endotoxin [55] and liver disease severity [56-58], and predict death in patients with cirrhosis [59]. However, the excessive increase of inflammatory biomarkers in our compensated cirrhotic patients with LSM≥40 kPa should be highlighted, because this could indicate an increased risk of AIDS progression [25], development of decompensated cirrhosis and death [12, 35, 36].

Coagulopathy has been linked to an increased risk of progression and death in HIV-infected people [60] and CHC patients [61]. In our study, HIV/HCV-coinfected patients had higher PAI-1 values than healthy controls and HIV-monoinfected patients; whereas high LSM values were directly related to increased levels of D-Dimer with cirrhotic patients with LSM≥40 kPa having the highest D-Dimer levels. Previous studies have reported plasma PAI-1 values to be associated with liver disease severity in patients with non-alcoholic fatty liver disease (NAFLD) [62, 63], and D-Dimer levels are elevated in cirrhotic patients and gradually increase with increasing hepatic dysfunction [64, 65]. Additionally, D-Dimer is related to the development of portal venous thrombosis [66] and bleeding of esophageal varices [67, 68]. D-dimer also predicts in-hospital mortality in patients with hepatic cirrhosis [64]. Thus, coagulopathy biomarkers were also altered in HIV/HCV-coinfected patients, indicating a risk of clinical progression and death, particularly in cirrhotic patients with LSM≥40 kPa.

Limitations of study

Several aspects must be taken into consideration for the correct interpretation of our results. Firstly, this report has a cross-sectional design, which may entail a lack of uniformity, and the study has a limited number of patients in some of the study groups, which could limit the possibility of finding significance. Secondly, all selected patients met a set of criteria for starting HCV treatment (e.g., no alcohol abuse, $CD4^+$ cell counts >200 cells/mm^3, controlled HIV replication, and good treatment adherence), and this may have introduced a selection bias. Thirdly, we did not have a control group of HCV-monoinfected patients to provide information of possible differential biomarkers from HIV/HCV-coinfected patients. We also did not have any patients with decompensated cirrhosis to study the biomarker profile in end-stage liver disease. Fourthly, our results were not adjusted by multiple comparisons. In this regard, when clinical-orientated studies are not a random search of a meaningful result, it is not recommended adjusting the “p-value” after multiple tests because it can penalize significantly relevant results [67, 68]. Note that our hypothesis is supported by theory and previous reports in patients infected with HIV, HCV, or both, as discussed previously. Additionally, our data had a

clear interpretation since always pointed out in the same direction and the analyzed biomarkers cannot be considered completely independent. Fifthly, we have not used a fixable Live/Dead dye in our freshly whole blood samples, which may influence the results of flow cytometry. However, it is unlikely that there was a bias with respect to a group, since all the samples were processed in the same way. Finally, another limitation of the study is that TE was not always performed in the fasting state, as is currently recommended, since food intake increases liver stiffness in patients with HCV infection [71].

Conclusions

In conclusion, biomarker levels of T cell activation, bacterial translocation, inflammation, endothelial dysfunction, and coagulopathy increased with the severity of liver fibrosis in HIV/HCV-coinfected patients, particularly in patients who had LSM≥40 KPa. Further studies are needed to confirm our findings and evaluate whether these biomarkers in HIV/HCV-coinfected patients with compensated cirrhosis can serve as prognostic factors.

List of abbreviations

Human immunodeficiency virus (HIV)

Hepatitis C virus (HCV)

Liver stiffness measures (LSM)

Chronic hepatitis C (CHC)

Combination antiretroviral therapy (cART)

Gut associated lymphoid tissue (GALT)

Direct-acting antivirals (DAAs)

Soluble CD14 (sCD14)

Lipopolysaccharide (LPS)

Fatty acid-binding protein 2 (FABP2)

Lipopolysaccharide binding protein (LBP)

Interleukin (IL)

IFN-γ-inducible protein 10 (IP-10)

Soluble vascular cell adhesion molecule 1 (sVCAM1)

Soluble intercellular cell adhesion molecule 1 (sICAM1)

Soluble tumor necrosis factor receptor 1 (sTNFR1)

Monocyte chemoattractant protein-1 (MCP1)

Plasminogen activator inhibitor-1 (PAI-1)

Generalized Linear Models (GLM)

Arithmetic mean ratio (AMR)

Non-alcoholic fatty liver disease (NAFLD)

Acquired immune deficiency syndrome (AIDS)

Non-nucleoside analogue HIV reverse transcriptase inhibitor (NNRTI)

Nucleoside analogue HIV reverse transcriptase inhibitor (NRTI)

Protease inhibitor (PI)

Integrase inhibitor (II)

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and patients gave their written consent. The Institutional Review Board and the Research Ethic Committee of the Instituto de Salud Carlos III (ISCIII) approved the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study may be available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Conceptualization: SR, JB, and JGG.

Data curation: JB, JGG, JMG, MC, CQ, JS.

Formal analysis: SR, LMM.

Funding acquisition: JB, JGG, and SR.

Investigation and methodology: LMM, PGB, and IC.

Project Administration: JB.

Supervision and visualization: SR.

Writing – original draft preparation: LMM, PGB, and SR.

Writing – Review & Editing: MAJS, JB.

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Authors' information

Not applicable

References

1. Koziel MJ, Peters MG. Viral hepatitis in HIV infection. *N Engl J Med* 2007,**356**:1445-1454.

2. Lewden C, May T, Rosenthal E, Burty C, Bonnet F, Costagliola D*, et al.* Changes in causes of death among adults infected by HIV between 2000 and 2005: The "Mortalite 2000 and 2005" surveys (ANRS EN19 and Mortavic). *J Acquir Immune Defic Syndr* 2008,**48**:590-598.

3. Graham CS, Baden LR, Yu E, Mrus JM, Carnie J, Heeren T*, et al.* Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. *Clinical Infectious Diseases* 2001,**33**:562-569.

4. Lo Re V, 3rd, Kallan MJ, Tate JP, Localio AR, Lim JK, Goetz MB*, et al.* Hepatic decompensation in antiretroviral-treated patients co-infected with HIV and hepatitis C virus compared with hepatitis C virus-monoinfected patients: a cohort study. *Ann Intern Med* 2014,**160**:369-379.

5. Hunt PW, Lee SA, Siedner MJ. Immunologic Biomarkers, Morbidity, and Mortality in Treated HIV Infection. *J Infect Dis* 2016,**214 Suppl 2**:S44-50.

6. Hunt PW. HIV and inflammation: mechanisms and consequences. *Curr HIV/AIDS Rep* 2012,**9**:139-147.

7. Wiest R, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. *Hepatology* 2005,**41**:422-433.

8. Dinh DM, Volpe GE, Duffalo C, Bhalchandra S, Tai AK, Kane AV*, et al.* Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection. *J Infect Dis* 2015,**211**:19-27.

9. Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* 2014,**60**:197-209.

10. Dirchwolf M, Ruf AE. Role of systemic inflammation in cirrhosis: From pathogenesis to prognosis. *World J Hepatol* 2015,**7**:1974-1981.

11. Sipeki N, Antal-Szalmas P, Lakatos PL, Papp M. Immune dysfunction in cirrhosis. *World J Gastroenterol* 2014,**20**:2564-2577.

12. Albillos A, Lario M, Alvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *J Hepatol* 2014,**61**:1385-1396.

13. Martinez-Esparza M, Tristan-Manzano M, Ruiz-Alcaraz AJ, Garcia-Penarrubia P. Inflammatory status in human hepatic cirrhosis. *World J Gastroenterol* 2015,**21**:11522-11541.

14. Asrani SK, Kamath PS. Natural history of cirrhosis. *Curr Gastroenterol Rep* 2013,**15**:308.

15. Gonzalez-Reimers E, Quintero-Platt G, Martin-Gonzalez C, Perez-Hernandez O, Romero-Acevedo L, Santolaria-Fernandez F. Thrombin activation and liver inflammation in advanced hepatitis C virus infection. *World J Gastroenterol* 2016,**22**:4427-4437.

16. Thorpe LE, Ouellet LJ, Hershow R, Bailey SL, Williams IT, Williamson J, *et al.* Risk of hepatitis C virus infection among young adult injection drug users who share injection equipment. *Am J Epidemiol* 2002,**155**:645-653.

17. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008,**48**:835-847.

18. Garcia-Tsao G, Abraldes JG, Berzigotti A, Bosch J. Portal hypertensive bleeding in cirrhosis: Risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. *Hepatology* 2017,**65**:310-335.

19. Perez-Latorre L, Rivero-Juarez A, Hontanon V, Diez C, Cuenca F, Martin-Carbonero ML, *et al.* Prognostic Value of Transient Elastography in Human Immunodeficiency Virus-Infected Patients With Chronic Hepatitis C. *Open Forum Infect Dis* 2016,**3**:ofw212.

20. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, *et al.* Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005,**128**:343-350.

21. Sandler NG, Koh C, Roque A, Eccleston JL, Siegel RB, Demino M, *et al.* Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. *Gastroenterology* 2011,**141**:1220-1230, 1230 e1221-1223.

22. Balagopal A, Philp FH, Astemborski J, Block TM, Mehta A, Long R, *et al.* Human immunodeficiency virus-related microbial translocation and progression of hepatitis C. *Gastroenterology* 2008,**135**:226-233.

23. French AL, Evans CT, Agniel DM, Cohen MH, Peters M, Landay AL, *et al.* Microbial translocation and liver disease progression in women coinfected with HIV and hepatitis C virus. *J Infect Dis* 2013,**208**:679-689.

24. Peters L, Neuhaus J, Duprez D, Neaton JD, Tracy R, Klein MB, *et al.* Biomarkers of inflammation, coagulation and microbial translocation in HIV/HCV co-infected patients in the SMART study. *J Clin Virol* 2014,**60**:295-300.

25. Younas M, Psomas C, Reynes J, Corbeau P. Immune activation in the course of HIV-1 infection: Causes, phenotypes and persistence under therapy. *HIV Med* 2016,**17**:89-105.

26. Shmagel KV, Saidakova EV, Shmagel NG, Korolevskaya LB, Chereshnev VA, Robinson J, *et al.* Systemic inflammation and liver damage in HIV/hepatitis C virus coinfection. *HIV Med* 2016,**17**:581-589.

27. Tapping RI, Tobias PS. Cellular binding of soluble CD14 requires lipopolysaccharide (LPS) and LPS-binding protein. *J Biol Chem* 1997,**272**:23157-23164.

28. Chan CC, Hwang SJ, Lee FY, Wang SS, Chang FY, Li CP, *et al.* Prognostic value of plasma endotoxin levels in patients with cirrhosis. *Scand J Gastroenterol* 1997,**32**:942-946.

29. Lin RS, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, *et al.* Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995,**22**:165-172.

30. Fukui H, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. *J Hepatol* 1991,**12**:162-169.

31. Kaser A, Ludwiczek O, Waldenberger P, Jaschke W, Vogel W, Tilg H. Endotoxin and its binding proteins in chronic liver disease: the effect of transjugular intrahepatic portosystemic shunting. *Liver* 2002,**22**:380-387.

32. Shen T, Zheng J, Xu C, Liu J, Zhang W, Lu F, *et al.* PD-1 expression on peripheral CD8+ TEM/TEMRA subsets closely correlated with HCV viral load in chronic hepatitis C patients. *Virol J* 2010,**7**:310.

33. Markowitz M, Deren S, Cleland C, La Mar M, Silva E, Batista P, *et al.* Chronic Hepatitis C Virus Infection and the Proinflammatory Effects of Injection Drug Use. *J Infect Dis* 2016,**214**:1376-1382.

34. Sajadi MM, Pulijala R, Redfield RR, Talwani R. Chronic immune activation and decreased CD4 cell counts associated with hepatitis C infection in HIV-1 natural viral suppressors. *AIDS* 2012,**26**:1879-1884.

35. Marquez M, Fernandez Gutierrez del Alamo C, Giron-Gonzalez JA. Gut epithelial barrier dysfunction in human immunodeficiency virus-hepatitis C virus coinfected patients: Influence on innate and acquired immunity. *World J Gastroenterol* 2016,**22**:1433-1448.

36. Lin W, Weinberg EM, Chung RT. Pathogenesis of accelerated fibrosis in HIV/HCV co-infection. *J Infect Dis* 2013,**207 Suppl 1**:S13-18.

37. Pasarin M, Abraldes JG, Liguori E, Kok B, La Mura V. Intrahepatic vascular changes in non-alcoholic fatty liver disease: Potential role of insulin-resistance and endothelial dysfunction. *World J Gastroenterol* 2017,**23**:6777-6787.

38. Marquez M, Romero-Cores P, Montes-Oca M, Martin-Aspas A, Soto-Cardenas MJ, Guerrero F, *et al.* Immune activation response in chronic HIV-infected patients: influence of Hepatitis C virus coinfection. *PLoS One* 2015,**10**:e0119568.

39. de Oca Arjona MM, Marquez M, Soto MJ, Rodriguez-Ramos C, Terron A, Vergara A, *et al.* Bacterial translocation in HIV-infected patients with HCV cirrhosis: implication in hemodynamic alterations and mortality. *J Acquir Immune Defic Syndr* 2011,**56**:420-427.

40. Zimmermann HW, Seidler S, Gassler N, Nattermann J, Luedde T, Trautwein C, *et al.* Interleukin-8 is activated in patients with chronic liver diseases and associated with hepatic macrophage accumulation in human liver fibrosis. *PLoS One* 2011,**6**:e21381.

41. Langhans B, Kramer B, Louis M, Nischalke HD, Huneburg R, Staratschek-Jox A, *et al.* Intrahepatic IL-8 producing Foxp3(+)CD4(+) regulatory T cells and fibrogenesis in chronic hepatitis C. *J Hepatol* 2013,**59**:229-235.

42. Shimoda K, Begum NA, Shibuta K, Mori M, Bonkovsky HL, Banner BF, *et al.* Interleukin-8 and hIRH (SDF1-alpha/PBSF) mRNA expression and histological activity index in patients with chronic hepatitis C. *Hepatology* 1998,**28**:108-115.

43. Asselah T, Bieche I, Laurendeau I, Paradis V, Vidaud D, Degott C*, et al.* Liver gene expression signature of mild fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2005,**129**:2064-2075.

44. Andersen ES, Ruhwald M, Moessner B, Christensen PB, Andersen O, Eugen-Olsen J*, et al.* Twelve potential fibrosis markers to differentiate mild liver fibrosis from cirrhosis in patients infected with chronic hepatitis C genotype 1. *Eur J Clin Microbiol Infect Dis* 2011,**30**:761-766.

45. Dirchwolf M, Podhorzer A, Marino M, Shulman C, Cartier M, Zunino M*, et al.* Immune dysfunction in cirrhosis: Distinct cytokines phenotypes according to cirrhosis severity. *Cytokine* 2016,**77**:14-25.

46. Patel K, Remlinger KS, Walker TG, Leitner P, Lucas JE, Gardner SD*, et al.* Multiplex protein analysis to determine fibrosis stage and progression in patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2014,**12**:2113-2120 e2111-2113.

47. Reiberger T, Aberle JH, Kundi M, Kohrgruber N, Rieger A, Gangl A*, et al.* IP-10 correlates with hepatitis C viral load, hepatic inflammation and fibrosis and predicts hepatitis C virus relapse or non-response in HIV-HCV coinfection. *Antivir Ther* 2008,**13**:969-976.

48. Harvey CE, Post JJ, Palladinetti P, Freeman AJ, Ffrench RA, Kumar RK*, et al.* 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.

49. Berenguer J, Fernandez-Rodriguez A, Jimenez-Sousa MA, Cosin J, Zarate P, Micheloud D*, et al.* High plasma CXCL10 levels are associated with HCV-genotype 1, and higher insulin resistance, fibrosis, and HIV viral load in HIV/HCV coinfected patients. *Cytokine* 2012,**57**:25-29.

50. Kaplanski G, Farnarier C, Payan MJ, Bongrand P, Durand JM. Increased levels of soluble adhesion molecules in the serum of patients with hepatitis C. Correlation with cytokine concentrations and liver inflammation and fibrosis. *Dig Dis Sci* 1997,**42**:2277-2284.

51. Bruno CM, Sciacca C, Cilio D, Bertino G, Marchese AE, Politi G*, et al.* Circulating adhesion molecules in patients with virus-related chronic diseases of the liver. *World J Gastroenterol* 2005,**11**:4566-4569.

52. de Castro IF, Micheloud D, Berenguer J, Guzman-Fulgencio M, Catalan P, Miralles P*, et al.* Hepatitis C virus infection is associated with endothelial dysfunction in HIV/hepatitis C virus coinfected patients. *AIDS* 2010,**24**:2059-2067.

53. Aldamiz-Echevarria T, Berenguer J, Miralles P, Jimenez-Sousa MA, Carrero A, Pineda-Tenor D*, et al.* Soluble Adhesion Molecules in Patients Coinfected with HIV and HCV: A Predictor of Outcome. *PLoS One* 2016,**11**:e0148537.

54. Tilg H, Vogel W, Wiedermann CJ, Shapiro L, Herold M, Judmaier G*, et al.* Circulating interleukin-1 and tumor necrosis factor antagonists in liver disease. *Hepatology* 1993,**18**:1132-1138.

55. Trebicka J, Krag A, Gansweid S, Appenrodt B, Schiedermaier P, Sauerbruch T*, et al.* Endotoxin and tumor necrosis factor-receptor levels in portal and hepatic vein of

patients with alcoholic liver cirrhosis receiving elective transjugular intrahepatic portosystemic shunt. *Eur J Gastroenterol Hepatol* 2011,**23**:1218-1225.

56. Moura AS, Carmo RA, Teixeira AL, Leite VH, Rocha MO. Soluble inflammatory markers as predictors of liver histological changes in patients with chronic hepatitis C virus infection. *Eur J Clin Microbiol Infect Dis* 2010,**29**:1153-1161.

57. Trebicka J, Krag A, Gansweid S, Schiedermaier P, Strunk HM, Fimmers R*, et al.* Soluble TNF-alpha-receptors I are prognostic markers in TIPS-treated patients with cirrhosis and portal hypertension. *PLoS One* 2013,**8**:e83341.

58. Kakumu S, Okumura A, Ishikawa T, Yano M, Enomoto A, Nishimura H*, et al.* Serum levels of IL-10, IL-15 and soluble tumour necrosis factor-alpha (TNF-alpha) receptors in type C chronic liver disease. *Clin Exp Immunol* 1997,**109**:458-463.

59. Grunhage F, Rezori B, Neef M, Lammert F, Sauerbruch T, Spengler U*, et al.* Elevated soluble tumor necrosis factor receptor 75 concentrations identify patients with liver cirrhosis at risk of death. *Clin Gastroenterol Hepatol* 2008,**6**:1255-1262.

60. Leeansyah E, Malone DF, Anthony DD, Sandberg JK. Soluble biomarkers of HIV transmission, disease progression and comorbidities. *Curr Opin HIV AIDS* 2013,**8**:117-124.

61. Sherman KE. Advanced liver disease: what every hepatitis C virus treater should know. *Top Antivir Med* 2011,**19**:121-125.

62. Jin R, Krasinskas A, Le NA, Konomi JV, Holzberg J, Romero R*, et al.* Association between plasminogen activator inhibitor-1 and severity of liver injury and cardiovascular risk in children with non-alcoholic fatty liver disease. *Pediatr Obes* 2016.

63. Ajmera V, Perito ER, Bass NM, Terrault NA, Yates KP, Gill R*, et al.* Novel plasma biomarkers associated with liver disease severity in adults with nonalcoholic fatty liver disease. *Hepatology* 2017,**65**:65-77.

64. Li Y, Qi X, Li H, Dai J, Deng H, Li J*, et al.* D-dimer level for predicting the in-hospital mortality in liver cirrhosis: A retrospective study. *Exp Ther Med* 2017,**13**:285-289.

65. Gram J, Duscha H, Zurborn KH, Bruhn HD. Increased levels of fibrinolysis reaction products (D-dimer) in patients with decompensated alcoholic liver cirrhosis. *Scand J Gastroenterol* 1991,**26**:1173-1178.

66. Dai J, Qi X, Peng Y, Hou Y, Chen J, Li H*, et al.* Association between D-dimer level and portal venous system thrombosis in liver cirrhosis: a retrospective observational study. *Int J Clin Exp Med* 2015,**8**:15296-15301.

67. Primignani M, Dell'Era A, Bucciarelli P, Bottasso B, Bajetta MT, de Franchis R*, et al.* High-D-dimer plasma levels predict poor outcome in esophageal variceal bleeding. *Dig Liver Dis* 2008,**40**:874-881.

68. Drolz A, Horvatits T, Roedl K, Rutter K, Staufer K, Kneidinger N*, et al.* Coagulation parameters and major bleeding in critically ill patients with cirrhosis. *Hepatology* 2016,**64**:556-568.

69. Perneger TV. What's wrong with Bonferroni adjustments. *BMJ* 1998,**316**:1236-1238.

70. Sterne JA, Davey Smith G. Sifting the evidence-what's wrong with significance tests? *BMJ* 2001,**322**:226-231.

71. Mederacke I, Wursthorn K, Kirschner J, Rifai K, Manns MP, Wedemeyer H*, et al.* Food intake increases liver stiffness in patients with chronic or resolved hepatitis C virus infection. *Liver Int* 2009,**29**:1500-1506.

Table 1. Clinical and epidemiological characteristics of HIV/HCV-coinfected patients.

		Patients stratified by LSM				
	All patients	**<12.5 kPa**	**12.5-25 kPa**	**25-40 kPa**	**>40 kPa**	***p***
No.	238	119	73	28	18	-
Age (years)	49 (46; 52)	48 (45; 52)	49 (46; 51)	49.5 (46; 53)	50 (47; 52)	.482
Gender (male)	187 (78.6%)	96 (80.7%)	54 (74%)	23 (82.1%)	14 (77.8%)	.694
BMI (kg/m^2)	24.4 (21.8; 26.9)	23.8 (21.4; 26.3)	24.6 (22.7; 28.1)	24.7 (21.9; 26.5)	24.6 (21.6; 25.9)	.247
BMI ≥25 (kg/m^2)	98 (41.2%)	46 (38.6%)	33 (45.2%)	12 (42.8%)	7 (38.9%)	.693
Diabetes	20 (8.4%)	8 (6.7%)	6 (8.2%)	4 (14.3%)	2 (11.1%)	.600
High alcohol intake	117 (49.1%)	56 (47.1%)	36 (49.3%)	16 (57.1%)	9 (50%)	.876
HIV acquired by IVDU	186 (78.1%)	92 (77.3%)	56 (76.7%)	24(85.7%)	14 (77.7%)	.773
Prior AIDS	64 (26.9%)	27 (22.7%)	23 (31.5%)	8 (28.6%)	6 (33.3%)	.374
Years since HIV diagnosis	23 (18; 26)	22 (17; 26)	24 (20; 27)	21 (18; 26)	23 (19; 27)	.194
Years since HCV infection	21 (16; 24)	21 (13; 24)	21 (18; 25)	19 (17; 21)	22 (15; 26)	.575
Previous HCV therapy (IFNα+rib)	114 (47.9%)	38 (31.9%)	53 (72.6%)	17 (60.7%)	6 (33%)	**.001**
Antiretroviral therapy						
Non-treated	5 (2.1%)	2 (1.7%)	1 (1.4%)	2 (7.1%)	0 (0%)	.242
PI-based	35 (14.7%)	19 (15.9%)	12 (16.4%)	2 (7.1%)	2 (11.8%)	.627
2NRTI+II-based	59 (24.8%)	31 (26.1%)	18 (24.7%)	6 (21.4%)	4 (23.5%)	.944
2NRTI+PI-based	47 (19.7%)	25 (21%)	10 (13.7%)	6 (21.4%)	6 (35.3%)	.220
2NRTI+NNRTI-based	70 (29.5%)	31 (26.1%)	26 (35.6%)	10 (35.7%)	3 (17.6%)	.344
Others	22 (9.2%)	11 (9.2%)	6 (8.2%)	2 (7.2%)	2 (11.8%)	.274
HIV markers						
Nadir CD4+ T cells	172 (84; 254)	196 (78; 277)	167 (87; 234)	160 (85; 251)	116 (95; 198)	.345
Nadir CD4+ T cells<200 cells/mm^3	131 (55.1%)	58 (48.7%)	42 (57.5%)	17 (60.7%)	14 (77.7%)	.100
CD4+ T cells	547 (394; 803)	603 (436; 832)	511 (339; 736)	570 (395; 828)	364 (243; 520)	**.005**
CD4+ T cells<500 cells/mm^3	100 (42%)	41 (34.4%)	35 (47.9%)	13 (46.4%)	11 (61.1%)	.065
HIV-RNA >50 cp/mL	30 (12.6%)	16 (13.4%)	7 (9.6%)	4 (14.3%)	3 (16.7%)	.808
HCV markers						
HCV genotype (n=235)						
1	170 (72.3%)	78 (66.7%)	58 (80.6%)	20 (71.4%)	14 (77.8%)	.205
2	5 (2.1%)	3 (2.6%)	1 (1.4%)	1 (3.6%)	0 (0%)	.915
3	39 (16.6%)	18 (15.4%)	12 (16.7%)	7 (25%)	2 (11.1%)	.599

4	21 (8.9%)	18 (15.4%)	1 (1.4%)	0 (0%)	2 (11.1%)	**.005**
Log_{10} HCV-RNA (IU/mL)	6.3 (5.87; 6.74)	6.32 (5.83; 6.83)	6.36 (6.04; 6.69)	6.36 (5.79; 6.52)	6.16 (5.78; 6.59)	.692
HCV-RNA > 500,000 IU/mL	191 (80.2%)	92 (77.3%)	63 (86.3%)	23 (82.1%)	13 (72.2%)	.495
HCV-RNA > 850,000 IU/mL	170 (71.4%)	80 (97.2%)	58 (79.4%)	19 (67.9%)	13 (72.4%)	.608

Statistics: Values expressed as absolute number (percentage) and median (interquartile range). *P*-values were calculated by Chi-square tests and Mann-Whitney tests in HIV/HCV-coinfected patients stratified by LSM (<12.5 kPa, 12.5-25 kPa, 25-40 kPa, and >40 kPa).
Abbreviations: HCV, hepatitis C virus; HCV-RNA, HCV plasma viral load; HIV-1, human immunodeficiency virus type 1; LSM, liver stiffness measure; HIV-RNA, HIV plasma viral load; IVDU, intravenous drug user; AIDS, acquired immune deficiency syndrome; IFNα+rib, interferon-alpha plus ribavirin; NNRTI, non-nucleoside analogue HIV reverse transcriptase inhibitor; NRTI, nucleoside analogue HIV reverse transcriptase inhibitor; PI, protease inhibitor; II, integrase inhibitor; FIB-4, noninvasive test for liver fibrosis based on AST/ALT ratio and platelet count.

Table 2. Summary of markers of T cell activation and plasma biomarkers of bacterial translocation, inflammation, and coagulopathy in healthy controls, HIV-monoinfected and HIV/HCV-coinfected patients.

	Healthy controls (0)	HIV-monoinfected (1)	HIV/HCV-coinfected (2)	*p* (0-1)	*p* (0-2)	*p* (1-2)
T cells (%)						
$CD4^+CD38^+$	3.7 (2.5; 5.8)	2.8 (2; 5.6)	6.8 (3.8; 14.7)	.206	**.000**	**.000**
$CD8^+CD38^+$	6.3 (5.1; 8.5)	7.1 (4.6; 10.7)	11.6 (7.1; 20)	.504	**.000**	**.001**
Bacterial translocation						
sCD14 (µg/mL)	3.3 (2.3; 3.9)	3.7 (1.9; 5.4)	5.1 (3.3; 7.4)	.370	**.000**	**.010**
FABP2 (ng/mL)	0.5 (0.3; 0.7)	0.6 (0.4; 1.3)	0.7 (0.3; 1.6)	.051	.050	.822
LPS (UE/mL)	1.3 (0.9; 4.7)	1.2 (0.9; 2.1)	1.4 (1; 1.9)	.654	.765	.483
LBP (µg/mL)	0.7 (0.2; 1.4)	0.8 (0.4; 1.4)	0.9 (0.6; 1.5)	.599	.088	.294
Inflammation						
IL-1b (pg/mL)	0.6 (0.2; 1.5)	0.6 (0.3; 1)	1.2 (0.5; 2.3)	.705	**.006**	**.000**
IL-8 (pg/mL)	1.2 (1.2; 2.8)	1.9 (1.2; 3.5)	5.2 (3.6; 11.3)	.197	**.000**	**.000**
IL-6 (pg/mL)	2 (1.4; 3.6)	3.4 (2.4; 4)	5.4 (3.7; 8.1)	**.014**	**.000**	**.000**
IL-18 (pg/mL)	87.4 (55.9; 147.3)	122.8 (87.9; 194.4)	247.7 (128.2; 507.2)	.077	**.000**	**.000**
IP-10 (pg/mL)	26.6 (20.1; 50.5)	28.4 (17.5; 35.4)	203.7 (111.3; 350.6)	.704	**.000**	**.000**
Endothelial dysfunction						
sVCAM1 (µg/mL)	0.3 (0.2; 0.5)	0.3 (0.2; 0.6)	1.6 (0.8; 3.2)	.154	**.000**	**.000**
sICAM1 (µg/mL)	0.4 (0.1; 0.9)	0.6 (0.3; 1.2)	2.1 (1.1; 3.9)	.084	**.000**	**.000**
sTNFR1 (ng/mL)	1.5 (0.2; 2.2)	1.4 (0.3; 2.1)	2.3 (1.3; 3.6)	.917	**.000**	**.000**
MCP1 (pg/mL)	12.5 (9.2; 21.7)	29.5 (17.7; 38.4)	20.7 (11; 41.2)	**.000**	**.005**	.173
Coagulopathy						
D-Dimer (ng/mL)	23.5 (10.8; 62.2)	28.2 (12.7; 50)	33.3 (12.4; 77.8)	.898	.293	.253
PAI-1 (ng/mL)	4.9 (3.7; 8.6)	6.8 (5.3; 8.9)	10 (7; 12.8)	**.035**	**.000**	**.000**

Statistics: Values expressed as median (interquartile range). *P*-values were calculated by Mann-Whitney tests.
Abbreviations: HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; CDXX, cluster of differentiation; sCD14, soluble CD14; LPS, lipopolysaccharide; FABP2, fatty acid-binding protein 2; LBP, lipopolysaccharide binding protein; IL, interleukin; IP-10, IFN-γ-inducible protein 10; sVCAM1, soluble vascular cell adhesion molecule 1; sICAM1, soluble intercellular cell adhesion molecule 1; sTNFR1, soluble tumor necrosis factor receptor 1; MCP1, monocyte chemoattractant protein-1, PAI-1, plasminogen activator inhibitor-1.

Table 3. Association of liver stiffness measurements (continuous variable) with markers of T cell activation and plasma biomarkers of bacterial translocation, inflammation, and coagulopathy in HIV/HCV-coinfected patients.

	AMR (95%CI)	*p*	aAMR (95%CI)	*p*
T cells (%)				
$CD4^+CD38^+$	0.9 (0.78;1.04)	.150	0.97 (0.83;1.14)	.734
$CD8^+CD38^+$	1.14 (1;1.29)	**.048**	1.15 (1.01;1.31)	**.037**
Bacterial translocation				
sCD14 (µg/mL)	1.04 (0.89;1.22)	.628	1.12 (0.93;1.3)	.226
FABP2 (ng/mL)	1.09 (0.9;1.3)	.376	1.14 (0.93;1.40)	.205
LPS (UE/mL)	1.19 (1.06;1.34)	**.003**	1.18 (1.04;1.34)	**.009**
LBP (µg/mL)	1.02 (0.89;1.18)	.742	1.03 (0.88;1.21)	.679
Inflammation				
IL-1b (pg/mL)	0.91 (0.75;1.11)	.355	1.01(0.81;1.24)	.975
IL-8 (pg/mL)	1.99 (1.72;2.3)	**.000**	2.02 (1.74;2.35)	**.000**
IL-6 (pg/mL)	1.06 (0.92;1.22)	.415	1.14 (0.98;1.34)	.089
IL-18 (pg/mL)	0.96 (0.78;1.2)	.743	1.04 (0.83;1.29)	.748
IP-10 (pg/mL)	1.34 (1.14;1.57)	**.000**	1.27 (1.07; 1.51)	**.006**
Endothelial dysfunction				
sVCAM1 (µg/mL)	1.5 (1.29;1.75)	**.000**	1.39 (1.18;1.65)	**.000**
sICAM1 (µg/mL)	1.14 (0.9;1.43)	.275	1.29 (1.01;1.66)	**.045**
sTNFR1 (ng/mL)	1.51 (1.3;1.75)	**.000**	1.48 (1.27;1.74)	**.000**
MCP1 (pg/mL)	1.15 (0.98;1.35)	.098	1.14 (0.96;1.36)	.139
Coagulopathy				
D-Dimer (ng/mL)	1.88 (1.54;2.3)	**.000**	1.71 (1.38; 2.12)	**.000**
PAI-1 (ng/mL)	1.05 (0.94;1.18)	.365	1.05 (0.93;1.18)	.429

Statistics: *P*-values were calculated by Generalized Linear Models (GLM) with a gamma distribution (log-link).
Abbreviations: HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; AMR, arithmetic mean ratio; aAMR, adjusted AMR; CDXX, cluster of differentiation; sCD14, soluble CD14; LPS, lipopolysaccharide; FABP2, fatty acid-binding protein 2; LBP, lipopolysaccharide binding protein; IL, interleukin; IP-10, IFN-γ-inducible protein 10; sVCAM1, soluble vascular cell adhesion molecule 1; sICAM1, soluble intercellular cell adhesion molecule 1; sTNFR1, soluble tumor necrosis factor receptor 1; MCP1, monocyte chemoattractant protein-1, PAI-1, plasminogen activator inhibitor-1.

Table 4. Summary of T cell activation and plasma biomarkers of bacterial translocation, inflammation, and coagulopathy in HIV/HCV-coinfected patients according to fibrosis/cirrhosis stages.

	<12.5 kPa (0)	12.5-25 kPa (1)	25-40 kPa (2)	>40 kPa (3)	*p* (0-3)	*p* (1-3)	*p* (2-3)
T cells (%)							
$CD4^+CD38^+$	7.5 (4.5; 17.6)	6 (3.3; 14.1)	5.3 (2.6; 7.6)	10.9 (5.5; 24.8)	.268	.080	**.015**
$CD8^+CD38^+$	11.2 (6.3; 18.7)	10.9 (7.1; 19.2)	12.7 (6.3; 17.7)	21 (10; 39.9)	**.005**	**.011**	**.013**
Bacterial translocation							
sCD14 (µg/mL)	4.8 (3.2; 7.1)	5 (3.2; 7.5)	4.9 (3.5; 6.6)	7 (4.4; 10.6)	.081	.154	.100
FABP2 (ng/mL)	0.6 (0.3; 1.4)	0.6 (0.3; 1.5)	1 (0.4; 2.2)	1.2 (0.4; 2.5)	.082	.149	.529
LPS (UE/mL)	1.3 (1; 1.7)	1.3 (0.9; 2)	1.7 (1.3; 2.2)	1.5 (1.1; 2.3)	.121	.245	.761
LBP (µg/mL)	0.9 (0.5; 1.4)	0.9 (0.5; 1.8)	0.8 (0.5; 1.3)	1.1 (0.8; 1.4)	.412	.914	.184
Inflammation							
IL-1b (pg/mL)	1.3 (0.7; 2.5)	0.9 (0.5; 1.9)	1.6 (0.5; 2.8)	0.5 (0.2; 2.1)	.087	.375	.192
IL-8 (pg/mL)	4.2 (2.8; 6.4)	5.6 (4.1; 10)	11.9 (6.3; 20.9)	18.8 (9.1; 31.5)	**.000**	**.000**	**.027**
IL-6 (pg/mL)	4.6 (3.3; 7.7)	5 (3.6; 6.9)	6.7 (4.6; 12.1)	9.1 (6; 20)	**.000**	**.000**	**.025**
IL-18 (pg/mL)	231.1 (121.2; 569.9)	301.4 (145.6; 612.4)	254.3 (129.5; 465.4)	254 (155.1; 377.7)	.828	.508	.787
IP-10 (pg/mL)	171.3 (110.4; 258.3)	189.2 (79.1; 329)	336.1 (146.2; 455)	407 (203.7; 689.5)	**.000**	**.003**	.283
Endothelial dysfunction							
sVCAM1 (µg/mL)	1.2 (0.7; 2.1)	2.1 (0.9; 4.7)	1.4 (0.9; 4.2)	3.5 (1.1; 5.5)	**.000**	.214	.079
sICAM1 (µg/mL)	1.9 (1; 3.3)	2.2 (1.1; 4.1)	2.4 (1.2; 4.2)	3.9 (1.1; 12.1)	.070	.126	.188
sTNFRI1 (ng/mL)	1.9 (1.2; 3.3)	2 (1.2; 3.1)	2.6 (1.3; 5.4)	3.5 (2.3; 4.5)	**.003**	**.003**	.498
MCP1 (pg/mL)	18.2 (10; 39.3)	23.2 (12.6; 39.2)	25.3 (11.3; 43.5)	27.9 (10.6; 52)	.234	.750	.787
Coagulopathy							
D-Dimer (ng/mL)	26.6 (11.4; 64.7)	32.9 (13.6; 79.8)	23.5 (8.4; 68.2)	132.2 (59.5; 280.1)	**.000**	**.000**	**.002**
PAI-1 (ng/mL)	9.7 (7; 12.1)	8.8 (5.8; 12.8)	10.7 (7.7; 14.4)	13.2 (10.1; 15.5)	**.003**	**.004**	.121

Statistics: Values expressed as median (interquartile range). *P*-values were calculated by Mann-Whitney test.
Abbreviations: HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; kPa, kilopascal; CDXX, cluster of differentiation; sCD14, soluble CD14; LPS, lipopolysaccharide; FABP2, fatty acid-binding protein 2; LBP, lipopolysaccharide binding protein; IL, interleukin; IP-10, IFN-γ-inducible protein 10; sVCAM1, soluble vascular cell adhesion molecule 1; sICAM1, soluble intercellular cell adhesion molecule 1; sTNFR1, soluble tumor necrosis factor receptor 1; MCP1, monocyte chemoattractant protein-1, PAI-1, plasminogen activator inhibitor-1.

Table 5. Summary of values of adjusted arithmetic mean ratio (aAMR) for <12.5kPa, 12.5-25kPa and 25-40kPa, compared to HIV/HCV-coinfected patients with >40 kPa, for T-cell subsets and plasma biomarkers.

	<12.5 kPa		**12.5-25 kPa**		**25-40 kPa**	
T cells (%)	**aAMR (95%CI)**	***p***	**aAMR (95%CI)**	***p***	**aAMR (95%CI)**	***p***
$CD4^+CD38^+$	1.35 (0.89;2.07)	.159	1.55 (1;2.4)	.051	1.68 (1.03;2.75)	**.038**
$CD8^+CD38^+$	1.66 (1.16;2.36)	**.005**	1.49 (1.03;2.16)	**.036**	1.62 (1.06;2.49)	**.026**
Bacterial translocation						
sCD14 (µg/mL)	1.18 (0.77;1.81)	.449	1.05 (0.67;1.66)	.830	1.16 (0.71;1.9)	.548
FABP2 (ng/mL)	1.36 (0.82;2.26)	.230	1.47 (0.86;2.52)	.155	1.07 (0.6;1.91)	.824
LPS (UE/mL)	1.61 (1.18;2.17)	**.003**	1.45 (1.04;2.04)	**.027**	1.05 (0.74;1.49)	.788
LBP (µg/mL)	1.09 (0.76;1.56)	.653	1.02 (0.69;1.5)	.937	1.23 (0.8;1.89)	.338
Inflammation						
IL-1b (pg/mL)	0.74 (0.45;1.23)	.245	0.91 (0.53;1.58)	.745	0.52 (0.29;1.01)	.073
IL-8 (pg/mL)	3.35 (2.31;4.86)	**.000**	2.34 (1.55;3.54)	**.000**	1.33 (0.86;2.07)	.201
IL-6 (pg/mL)	1.65 (1.12;2.44)	**.012**	2.23 (1.46;3.4)	**.000**	1.69 (1.08;2.64)	**.022**
IL-18 (pg/mL)	0.77 (0.48;1.24)	.280	0.67 (0.4;1.13)	.135	0.74 (0.42;1.29)	.284
IP-10 (pg/mL)	1.78 (1.15;2.75)	**.010**	1.72 (1.06;2.8)	**.029**	1.04 (0.62;1.75)	.885
Endothelial dysfunction						
sVCAM1 (µg/mL)	2.14 (1.45;3.17)	**.000**	1.15 (0.75;1.77)	.515	1.3 (0.81;2.07)	.276
sICAM1 (µg/mL)	1.84 (1;3.35)	**.048**	2.26 (1.17;4.36)	**.015**	2.06 (1.02;4.19)	**.045**
sTNFR1 (ng/mL)	1.56 (1.08;2.26)	**.017**	1.64 (1.1;2.45)	**.015**	0.98 (0.63;1.52)	.913
MCP1 (pg/mL)	1.52 (0.99;2.32)	.056	1.23 (0.76;1.98)	.399	1.38 (0.82;2.31)	.228
Coagulopathy						
D-Dimer (ng/mL)	4.06 (2.32;7.11)	**.000**	4.34 (2.36;7.97)	**.000**	3 (1.55;5.8)	**.001**
PAI-1 (ng/mL)	1.23 (0.9;1.67)	.189	1.31 (0.94;1.84)	.112	1.13 (0.79;1.63)	.503

Statistics: *P*-values were calculated by Generalized Linear Models (GLM) with a gamma distribution (log-link).
Abbreviations: HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; aAMR, adjusted AMR; kPa, kilopascal; CDXX, cluster of differentiation; sCD14, soluble CD14; LPS, lipopolysaccharide; FABP2, fatty acid-binding protein 2; LBP, lipopolysaccharide binding protein; IL, interleukin; IP-10, IFN-γ-inducible protein 10; sVCAM1, soluble vascular cell adhesion molecule 1; sICAM1, soluble intercellular cell adhesion molecule 1; sTNFR1, soluble tumor necrosis factor receptor 1; MCP1, monocyte chemoattractant protein-1, PAI-1, plasminogen activator inhibitor-1.

Supplemental Table 1. Characteristics of HIV/HCV co-infected patients.

	Healthy control	HIV	HIV/HCV
No. *	32	39	238
Gender (male)	17 (53.1%)	24 (61.5%)	187 (78.6%)
Age (years) #	49.5 (47; 53)	51 (46; 53)	49 (46; 52)
BMI (kg/m²) #	24.9 (23.1; 27.1)	25.3 (23.5; 26.6)	24.4 (21.8; 26.9)
BMI ≥25 (kg/m²)*	14 (43.7%)	21 (55.3%)	98 (41.2%)
Diabetes	-	6 (15.8%)	20 (8.4%)
High alcohol intake	-	1 (3.1%)	117 (89.1%)
HIV acquired by IVDU *	-	-	186 (78.1%)
Prior AIDS *	-	13 (33.3%)	64 (26.9%)
Years since HIV infection #	-	-	23 (18; 26)
Years since HCV infection #	-	-	21 (16; 24)
Antiretroviral therapy *			
Non treated	-	-	5 (2.1%)
PI-based#	-	10 (25.6%)	35 (14.7%)
2NRTI+II-based *	-	4 (10.2%)	59 (24.8%)
2NRTI+PI-based *	-	-	47 (19.7%)
2NRTI+NNRTI-based *	-	23 (64.1%)	70 (29.5%)
Others	-	2 (5.1%)	22 (9.2%)
HIV markers			
Nadir CD4+ T-cells #	-	215 (107; 343)	172 (84; 254)
Nadir CD4+ T-cells<200 cells/mm³ *	-	14 (38.9%)	131 (55.1%)
CD4+ T-cells #	-	832 (685; 1036)	547 (394; 803)
CD4+ T-cells<500 cells/mm³ *	-	0 (0%)	100 (42%)
HIV-RNA >50 cp/mL*	-	0 (0%)	30 (12.6%)
Non-invasive indexes			
APRI #	-	-	0.98 (0.57; 1.70)
APRI >1.5*			65 (27.3%)
FIB-4 #	-	-	2.33 (1.45; 3.59)
FIB-4 >3.25*			64 (26.9%)
Forns index #	-	-	4.94 (3.67; 6.31)
Forns index >6.9*			36 (15.1%)
HCV markers			
HCV genotype (n=235)*			
1	-	-	170 (72.3%)
2	-	-	5 (2.1%)
3	-	-	39 (16.6%)
4	-	-	21 (8.9%)
Log10 HCV-RNA (IU/ml) #	-	-	6.3 (5.87; 6.74)
HCV-RNA > 850,000 IU/ml *	-	-	170 (71.4%)

*Absolute number (percentage).
Mean and mean standard error.
HCV: Hepatitis C virus; HCV-RNA: HCV plasma viral load; HIV-1: Human immunodeficiency virus type 1; HIV-RNA: HIV plasma viral load; IVDU: intravenous drug user; NNRTI: non-nucleoside analogue HIV reverse transcriptase inhibitor; NRTI: nucleoside analogue HIV reverse transcriptase inhibitor; PI: protease inhibitor; II: integrase inhibitor; APRI: Aminotransferase-to-platelet ratio index; FIB-4: noninvasive test for liver fibrosis based on AST/ALT ratio and platelet count; FORNS: test for liver fibrosis based on age, GGT and platelet count.