Liver fibrosis, microbial translocation and immune activation markersin HIV and HCV infections and in HIV/HCV co-infection

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Abstract

Background: Liver fibrosis is accelerated in patients co-infected with human immunodeficiency virus and hepatitis C viruses.

Aims: We investigated the correlation between liver fibrosis, immune activation and microbial translocation.

Methods: This cross-sectional study included patients with hepatitis C virus (HCV) and human immunodeficiency virus (HIV) mono-infections, HIV/HCV co-infection, and healthy controls (20 subjects/group). Peripheral blood was analysed to determine the levels of Forkhead box 3 (Foxp3) T cells, TGF-β1, CD14 (soluble and surface isoforms), IL-17 and bacterial translocation products. These measurements were correlated to the severity of liver fibrosis, measured with the FIB-4 score and transient elastography.

Results: Foxp3T cell levels were significantly elevated in HIV mono-infected and co-infected groups ($p <$ 0.0005). FIB-4 and liver stiffness values inversely correlated with TGF-β1 ($p = 0.0155$ and $p = 0.0498$). Bacterial DNA differed significantly in the HIV-positive compared to the other groups: HIV/HCV co-infected subjects had significantly higher serum levels of bacterial translocation products, CD14, and IL-17 levels ($p < 0.001$).

Conclusions: Fibrosis stage in HIV/HCV co-infection may be influenced by immune activation due eitherby viral infections or to bacterial translocation.

Introduction

In prospective studies, co-infection of both human immunodeficiency virus (HIV) and hepatitis C virus (HCV) were associated witha higher cumulative incidence of end-stage liver disease and shortersurvival times compared to HCV mono-infection [1]. It has been well established that individuals with HIV/HCV co-infection exhibit accelerated rates

of liver fibrosis and progression to end-stage liver disease.

The accelerated course of HIV/HCV co-infection is mainly related to the persistence of HCV, which depends on alterations in cell-mediated immunity and activation of immune cells that secrete pro-inflammatory and pro-fibrotic cytokines [2]. Also, HIV plays a direct role by stimulating human stellate cells (HSC) through a C–C chemokine receptor-5 (CCR5)-dependent pathway [3]. Some authors have also postulated that the increased rate of fibrosis in individuals with HIV/HCV co-infection may be related to the loss or dysregulation of an immune response [4].

Regulatory T cells (Tregs) are a subset of T cells that express CD4 (CD4+) and the Forkhead-winged-helix transcription factor, Forkhead box 3 or Foxp3 [5]. Suchard et al. demonstrated that the level of Foxp3 expression in the CD4+ T cell population is a marker of the severity of HIV infection and a potential prognostic marker of disease progression [6].

Alterations in the cytokine network have important implications, on both global immune regulation and on the specific immunologic alterations that occur in subjects with HIV/HCV. In response to infection, the host immune response is mediated by inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6, and interferons (IFN) [7]. In addi-tion, alterations in Treg cells or the levels of transforming growth factor beta1 (TGF-β1), a regulatory cytokine released by numerous cell types during inflammation [8], might be involved in the acceler-ated course of liver fibrosis characteristically observed in individu-als with HIV/HCV co-infection. HIV/HCV co-infection induces a significant increase in TGF-β1, a central mediator of liver fibrogenesis, detected in the liver and serum of patients and in cell culture [9–12]. Microbial translocation is likely to promote liver fibrosis in patients with HIV/HCV co-infection via two main mechanisms that activate Kupffer cells and HSCs [13]. First, microbial products directly induce the secretion of pro-fibrotic cytokines, including IL-4, IL-5, IL-13, and TGF-β [14]. Second, microbial products [15] indirectly induce systemic immune responses and promote local, hepatocyte activation-induced apoptotic death, which results in collagen deposition [16].

Lipopolysaccharide (LPS) and toll-like receptor (TLR) 4, both products of microbial translocation, play key roles in the pathogenesis of HCV infections. Patients with chronic HCV infections display increased serum levels of LPS, even in the absence of significant fibrosis [17]. In HIV patients, the early damage of gut associated lymphoid tissue causes an increase of microbial translocation and the presence of increased LPS blood levels. Thus, in HIV/HCV co-infection, increased levels of LPS may act to drive the development of more frequent and more rapid progression of liver disease.

The primary aim of this study was to assess the correlations between liver fibrosis, microbial translocation, and immune-activation markers in subjects with HIV and HCV mono-infections and in HIV/HCV co-infection, compared to healthy controls.

A secondary aim was to investigate whether markers of immune activation, fibrogenesis, and microbial translocation were related to alterations in glucose metabolism, calculated with the homeostasis model assessment (HOMA) score.

Materials and methods

Study design and patients

This cross-sectional study included 80 subjects at the Department of infectious Diseases of Policlinico San Matteo, Pavia University. The study population included patients with HIV mono-infection ($n = 20$), HCV mono-

infection ($n = 20$), HIV/HCV co-infection ($n = 20$), and healthy controls ($n = 20$). All subjects were over 18 years of age and had undergone a biochemical assessment. HCV infection was diagnosed based on the presence of serum antibodies against HCV and detectable serum HCV RNA (real time PCR). Patients in both mono- and co-infected HCV+ groups had chronic hepatitis. All patients with HIV infection were receiving anti-retroviral therapy. Healthy controls were seronegative for both HIV and HCV. Patients were excluded if they had been previously diagnosed with diabetes, hepatitis B, or cirrhosis, or if they had a history of excessive alcohol intake.

Biochemical assessment

All patients underwent a biochemical assessment to deter- mine serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelets, albumin, cholesterol, CD4, HCV-RNA, HIV-RNA, insulin, and glucose. The FIB4 score was calculated as follows: (age [years] AST [U/L])/(platelet count [10^9/L]) (ALT^2 [U/L]). A cut-off FIB4 value >3.25 is consistent with significant (F3–F4) fibrosis at a sensitivity of 70% and a specificity of 86–97% in individuals with HIV/HCV co-infection [18]. For each patient, insulin resistance was determined with the HOMA calculation: fasting plasma insulin (µU/ml) fasting plasma glucose (ml/L)/22.5 [19].

Liver stiffness measurement

Liver stiffness was measured with transient elastography, per- formed with a FibroScan® apparatus (Echosens, Paris, France) by a single operator (a staff physician), who had previously performed at least 100 determinations in patients with chronic liver disease. In each patient examination, at least ten valid measures of liver stiffness were obtained. To guarantee the validity of the transient elastography results, only examinations with an interquartile range (IQR) smaller than 30% of the median value and a success rate of acquisitions greater than 60% were analysed. The median value of all tests per patient was expressed in kilopascal (kPa) units. Advanced liver fibrosis (severe fibrosis or cirrhosis, corresponding to Metavir scores of F3 and F4) was defined as a liver stiffness 9.5 kPa, based on previous studies from patients with HCV mono- infection and HIV/HCV co-infection [20–22].

Flow cytometric analysis of $CD4^+CD25^+Foxp3^+$ T-cells

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. Then, 10^6 PBMCs were washed with phosphate-buffered saline (PBS) with 2% EDTA and stained for surface markers with a FITC-conjugated monoclonal antibody (MoAb) against Human CD4 (eBioscience) or a phycoerythrin- cyanine tandem (PE-Cy®)-conjugated mouse MoAb against human CD25 (BD Pharmingen). Cells were stained for 30 min at 4 °C according to the manufacturer's instructions. Cells were then washed with 2 ml PBS, resuspended, and incubated in 1 ml fixation/permeabilization solution (eBioscience) for 30 min at 4 °C.
The permeabilized cells were washed twice with 2 ml Perm/Wash Buffer (BD Biosciences) and stained for intracellular markers by incubating with the PE-conjugated MoAb, anti-human Foxp3 (eBioscience) at 4 °C for 30 min according to manufacturer's instructions. After intracellular staining, cells were washed with permeabilization

buffer and resuspended in 1% paraformaldehyde. Isotype-matched MoAbs were prepared for each sample. Cell acquisition and analysis were performed with a Cytomics FC 500 flow cytometer (Beckman Coulter). Colour compensation was achieved with an appropriate single fluorochrome-labelled sample. For each sample, a minimum of 50,000 CD4+ events were acquired.

Determination of monocyte subpopulations in peripheral blood with flow cytometry

For direct immunofluorescence labelling, 100 µl whole blood was incubated with 10 µl FITC-conjugated anti-human CD14 MoAb (BD Pharmingen) and with 10 µl PE Cy^{TM}-conjugated mouse anti- human CD16 MoAb (BD Pharmingen) for 15 min at room temperature. For erythrocyte lysis, we added 2 ml of lysis solution from the Immunoprep Reagent System (Beckman Coulter). Fixed cells were analysed by flow cytometry (Cytomics FC 500 Beckman Coulter).

TGF-˘1, sCD14, and IL-17

Biomarker levels were measured in all blood samples. Enzyme- linked immunosorbent assays (ELISA) were used to identify cells that expressed TGF-β1 (human TGF-β1, Quantikine ELISA kit, R&D
Systems Minneapolis, USA), sCD14 (human sCD14, Quantikine ELISA kit, R&D Systems), and IL-17 (human IL 17, Quantikine ELISA kit, R&D Systems).

DNA extraction and 23S rDNA real-time PCR

Total DNA was extracted from 200 µl whole blood with the DNeasy blood and tissue kit (Qiagen), according to the pre-treatment steps for bacteria suggested by the manufacturer. A further pre-treatment with Qiagen columns and sterile water pre- heated to 65 °C was performed to wash out contaminating bacterial DNA. Exogenous DNA (a quantified plasmid with a known insert) was spiked into each sample to normalize the efficiency of DNA extraction. Real-time PCR was performed with a 23S rDNA-targeted procedure previously described [23]. To normalize the samples for absolute quantification assays, we also amplified the spiked DNA.

Statistical analysis

Continuous data were expressed as the mean and standard deviation (SD) or as the median and interquartile range (25th–75th); categorical data were expressed as the number of counts and percent. Comparisons between groups of patients were performed with the Kruskall–Wallis test or the Fisher exact test, respectively. Spearman's Rho correlation coefficient (Rho) and the 95% confidence interval (95% CI) were computed to determine associations between continuous variables and cytokines. A linear regression model was used to adjust for type of patient. Interactions between the type of patient and the several variables tested were analysed to assess whether a differential size of association was present within each group of patients. To verify model assumptions, we used a graphical check of residuals. Stata 12 (StataCorp, College Station, TX, USA) was used for all analyses. A 2-sided

p-value <0.05 was considered statistically significant. The Bonferroni correction was used for post hoc comparisons.

Ethics

The study was designed and conducted in accordance with the Helsinki declaration. The Ethics Committee of the IRCCS Fondazione Policlinico San Matteo approved the study, and written informed consent was obtained from all enrolled individuals.

Results

Study population

The baseline characteristics of the 80 patients included in the study are summarized in Table 1. All patients with HIV were receiving highly active anti-retroviral therapy (HAART) and 38/40 had an undetectable HIV viral load (95%).

The four groups were significantly different in the HOMA score, liver elastography, CD4 cell counts, and FIB4 score. Patients with HIV/HCV and HCV showed higher kPa levels than healthy controls and subjects with HIV. Similar results were observed for the FIB4 values. The HOMA scores were significantly higher in patients with HCV, HIV, and HIV/HCV compared to healthy controls.

Among patients with HIV infection CD4/CD8 ratio did not differ significantly between monoinfected and co-infected ones ($p = 0.1093$). Genotype distribution is outlined in Table 1. Because the small number of subjects in each group we could not establish a correlation between genotype and other parameters.

A total of 17 patients had received anti-HCV therapy: 8 were HCV monoinfected (4 non-responders, 2 partial responders and 2 relapsers) and were 8 HIV/HCV co-infected (7 non responders, 1 partial responder and 1 relapser).

Correlation between $CD4^+CD25^+Foxp3^+$ and TGF-˘1 in the four groups

The percentages of serum $CD4^+CD25^+Foxp3^+$ T-cells were significantly higher in patients with HIV and HIV/HCV than in those with HCV and the control group ($p < 0.0005$, Kruskal–Wallis test) (Fig. 1A and B). Alternatively, no statistically significant difference was observed between the HCV and the control groups or between the HIV and the HIV/HCV groups. $CD4^+CD25^+Foxp3^+$ % levels did not correlate either with duration of HIV infection or HIV-RNA undetectability.

The results showed no statistically significant differences in TGF-β1 levels among the four groups.

Correlation between $CD4^+CD25^+Foxp3^+$, TGF-˘1 levels, and liver fibrosis

In the HCV and the HIV/HCV co-infected groups, the FIB4 values inversely correlated with TGF-β1 levels (Rho: 0.38; $p = 0.0155$). However, in subjects with CD4$^+$CD25$^+$Foxp3$^+$ T-cells, there was no correlation between FIB4 and TGF-β1 (Rho: 0.1195; $p = 0.4625$).

Liver stiffness values were inversely correlated with TGF-β1 (Rho: 0.31; $p = 0.0498$). Similarly, TGF-β1 levels were significantly lower in patients with higher stages of fibrosis, assessed by transforming kPa values into Metavir scores (Fig. 2) [24]. How- ever, the CD4$^+$CD25$^+$Foxp3$^+$ T-cell counts were not associated with the fibrosis stage. There was no correlation between the number of CD4$^+$CD25$^+$Foxp3$^+$ T-cells and liver stiffness (Rho: 0.1780, $p = 0.2718$). We tried to define a cut off for TGF-β1 levels in relation to liver stiffness, graded according to kPa levels as reported by Tsochatzis et al. [22], but could not because only 6 patients had a stiffness level greater than 9.5 kPa.

Intergroup comparisons of soluble CD14, surface CD14, IL-17, and bacterial DNA levels

The levels of CD14 (soluble and surface) were significantly different between HIV and healthy controls, between HIV/HCV and healthy controls, and between HCV and HIV/HCV groups ($p < 0.0001$, Kruskal–Wallis test) (Fig. 3A and B).

The IL-17 levels were significantly different between the HCV group and the other three groups ($p < 0.0001$, Kruskal–Wallis test) (Fig. 4). IL-17 levels were significantly higher in HIV/HCV co-infected patients compared to HCV mono-infected ($p = 0.024$). The levels of bacterial DNA were significantly different between the HIV group and the other three groups ($p < 0.0001$, Kruskal–Wallis test). The translocation markers were correlated with FIB4 scores. In particular, FIB4 was correlated with monocyte levels, IL-17, and CD14 (soluble), when all subjects were evaluated; however, no correlation was found in the individual groups (Table 2). Similar results were found for associations between kPa and the translocation markers.

Bacterial 23S rDNA levels

The amount of bacterial DNA was calculated as the ratio of the number of 23S rDNA copies to the number of spiked DNA (reference) copies. Amplification efficiencies between 98% and 101% were achieved for primers that targeted both the 23S rDNA and the spiked DNA. No secondary peaks were observed.

The analysis of means of the ratio of 23S:ref DNA showed a significant statistical difference among the groups (interquartile range 25–75, $p = 0.001$). Bacterial 23S rDNA levels were significantly different between HIV-positive and HCV-positive groups ($p = 0.0003$), between HIV-positive and HIV/HCV co-infected groups ($p = 0.0001$), and between HIV-positive and healthy control groups ($p = 0.0002$).

Discussion

We evaluated serum levels of TGF-β1, CD4$^+$ CD25$^+$Foxp3$^+$ lymphocytes, IL-17, sCD14, and bacterial DNA products in patients with HIV, HCV, or HIV/HCV infections compared to a healthy con- trol group. We determined whether these serum factors were correlated to the severity of liver fibrosis (measured with two non-

invasive methods: Fibroscan and FIB4 score) or to alterations in glucose metabolism.

We found that the percent of $CD4^+CD25^+Foxp3^+$ lymphocytes

was significantly higher in patients with HIV and HIV/HCV infections compared to those with HCV and the control group. Also, patients with HCV had the same levels as healthy controls.

It is plausible that HIV might preferentially destroy or inactivate Tregs, which then leads to excessive immune activation [24]. Indeed, individuals with HIV infections have shown a significantly higher percentage of CD4+ T-cells that expressed Foxp3 compared to control individuals. Furthermore, the percentage of Foxp3 expressing CD4+ T-cells correlated negatively with the CD4 count and positively with the viral load. Our results confirmed these previous findings; thus, our results suggested that HIV, not HCV, was the major cause of immune activation.

$CD4^+CD25^+Foxp3^+$ levels did not correlate with FIB-4 or liver stiffness values. This result suggested that immune activation, mediated by CD4 subsets, was not a key factor involved in the fibrosis process.

Our findings were consistent with those from a similar study by Rallon et al. [25] That study also investigated TGF-β1 levels in individuals with HCV, those with HIV/HCV, and healthy controls, but they did not examine individuals with HIV mono-infections. They found that TGF-β1 levels were inversely correlated with liver stiff- ness. That finding was surprising, because TGF-1β is known to be a pro-fibrotic cytokine. Those authors suggested that their findings may depend on an up regulation caused by HIV replication, because they observed lower levels of TGF-β1 in patients with detectable HIV-RNA. In the present study, we found also that TGF-β1 levels were inversely correlated with fibrosis, assessed with a biochemical score or by measuring liver stiffness. However, this inverse correlation was independent of HIV replication, because our patients had nearly undetectable HIV-RNA.

Our findings that TGF-β1 levels were inversely correlated with fibrosis and directly correlated with the CD4 cell count suggested that immune status may have exerted a protective effect against fibrosis. In patients with either HIV infections or HIV/HCV co- infection, we observed a significant inverse correlation between the CD4 count and Treg proportions (Spearman's Rho = $-$0.54; $p < 0.0001$).

Fibrosis can also be caused by nonalcoholic steatohepatitis. This condition is associated with obesity, type 2 diabetes mellitus, and dyslipidemia, which are common comorbidities in patients with HIV [26]. This led to the notion that HIV/HCV co-infection may also induce additive or synergistic effects on glucose and lipid metabolism. Therefore, we investigated whether glucose metabolism disturbances (the HOMA score and BMI) might play a role in immune activation and cytokine release ($CD4^+CD25^+Foxp3^+$ lymphocytes and TGF-β1 levels). We found no correlation between the tested variables and cytokines levels, although there was a difference between patients and healthy controls. This finding sug- gested that, although both immunologic and metabolic alterations were present in patients with HIV/HCV co-infection, immune activation and cytokine release were independent of the metabolic

disorders and oxidative stress that were previously related to non- alcoholic steatohepatitis.

Compared to the other groups, patients with HIV/HCV co- infection had increased levels of bacterial DNA, CD14 (soluble and surface), and IL-17, which indicated enhanced bacterial translocation. The correlation between the translocation markers and FIB4 suggested that the fibrosis stage may depend on immune activation caused by bacterial translocation. The findings of our paper were consistent with those of other published studies, but we added some observations, particularly regarding the lack of HIV-RNA effect on TGF-β1 expression and the lack

of correlation between metabolic alterations and cytokine levels. In addition, our data on microbial translocation conflicted with previous stud- ies. The findings of our paper were inconsistent with those of Brenchley et al., who published a seminal paper on microbial translocation in HIV/SIV infections. There, the authors showed that microbial products derived from the gastrointestinal tract were among the causes of immune activation documented inpatients with HIV infections. The microbial products they measured were serum LPS in patients with chronic HIV infections and in rhesus-macaques infected with SIV [27]. In both groups of samples, the increased LPS levels were significantly related to elevated immune activation. On the other hand, another study focused on a cohort of patients with HIV/HCV co-infection that had never been treated with anti-retroviral therapy. Those patients had high CD4 T-cell counts, and they found that elevated sCD14 levels were independently associated with a decreased risk of liver disease progression, defined as the time to Fibrosis 4 (Fib-4) score of 1.45 or liver-related death [28]. They hypothesized that, because sCD14 prevents the interaction of LPS with membrane-bound CD14 on the surface of phagocytes, that high sCD14 would hamper the inflammatory response [29]. Thus, those authors proposed a model where elevated sCD14 levels preserved liver function by down regulating the LPS-induced inflammatory signal-transduction cascade [30].

Our results were similar to those of Marchetti et al. [29], because our results suggested that microbial translocation markers were increased in HIV/HCV co-infection, although they were not correlated with the extent of fibrosis. Furthermore, our finding that the extent of fibrosis was not associated with plasma sCD14 levels was consistent with other studies regarding the ability of inflam- matory markers to predict the degree of liver inflammation and its progression to cirrhosis in patients with HIV–HCV co-infection [15]. This, however, contradicted the results of Sandler et al., who found that sCD14 plasma levels were associated with cirrhosis and could predict progression to end-stage liver disease [31]. In conclusion, our results confirmed that HIV and HCV infections were linked to enhanced microbial translocation, but their correlation to liver fibrosis remain to be established.

One of the major limitations of our study was that the groups were not well matched in sex and age. Some data suggested a significant effect of age, but this should be confirmed in a larger sample size. As suggested by Rallon et al. [25], age may have lit- tle impact on $CD4^+CD25^+Foxp3^+$ levels, but it may influence the level of glucose metabolism alterations, because the frequency of glucose alterations has been shown to increase with age.

We did not find a correlation between immune cell expression and the level of pro-fibrotic cytokines, which suggested a protective role, meaning that preserving immune function may reduce the release of such cytokines Therefore, we speculated that the fibrosis processes associated with HIV infections may involve other factors. In a previous study, we demonstrated that HIV played a role in fibrosis through the gp120 pathway [32]. In addition, another recent study by our group showed that the HIV-gp120 complex, by activating CCR5, significantly increased the expression of components of the NALP3 inflammasome pathway in human HSCs and PBMCs. Those data identified a novel mechanism, where HIV-gp120 may directly influence hepatic necroinflammation and fibrosis in HIV/HCV co-infection. Moreover, those data established a direct link between the inflammasome complex, HIV proteins, and CCR5 [33].

Although the mechanisms underlying fibrosis progression in HIV–HCV co-infected patients is complex and multifactorial, immune mediated mechanism and microbial translocation result- ing by gut associated lymphoid damage play a key role in this process.

Our study confirms both these findings and enforces the suggestion that early treatment of both HIV and HCV

infections aimed at preserving immune control and eradicating HCV has a beneficial effect on the progression of fibrosis and could improve the outcome of these patients. We feel it is important to continue investigating chronic immune stimulation caused by microbial translocation and its relationship with immune cell subsets and pro-fibrotic cytokines.

Conflict of interests

None declared.

Acknowledgements

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Table 1

Characteristics of the study population.

Characteristics	Healthy controls $n = 20$	HCV-monoinfected $n = 20$	HIV-monoinfected $n = 20$	HIV/HCV-coinfected $n = 20$	p
Median age (years)	37.75	56.25	46.55	48.6	0.0001
Male gender	5 (25%)	8 (40%)	16 (80%)	19 (95%)	*
Median HOMA	20.39	36.09	42.29	65.27	0.0001§
Median BMI	23.54	25.10	24.90	23.94	0.4679
Median CD4+ T-cell count (cells/mmc)	744.3	757.25	421.8	508.4	0.0001
Median FIB4	0.67	1.88	0.98	2.46	0.0001#
Median liver stiffness (kPa)	3.1	8.46	3.9	9.16	0.0001**
Median CD4+CD25+Foxp3+	1.07	1.4	2.89	3.14	0.0001
Median TGF-beta1 concentration (ng/ml)	1.041	0.896	0.935	0.935	0.6328
Median serum HCV-RNA (IU/ml)	0	6,303,071.1	0	1,216,752	0.523
Patients receiving HAART	0	0	20	20	Not applicable
Median plasma HIV RNA (copies/ml)	0	0	<40***	<40***	Not applicable
Median AST level (IU/L)	18.4	48.1	26.8	55.7	Not applicable
Median ALT level (IU/L)	17.1	61.2	30.35	73.8	Not applicable
Median duration HIV infection (years)	0	0	10.55	19.35	0.004
Median duration HCV infection (years)	0	10.95	0	12.45	Not applicable
Genotype 1a	0	3 (15%)	0	7 (35%)	****
Genotype 1b	0	5 (25%)	0	3 (15%)	****
Genotype 1a/1b	0	2 (10%)	0	0	****
Genotype 2a	0	3 (15%)	0	0	****
Genotype 3	0	1 (5%)	0	3 (15%)	****
Genotype 4	0	1 (5%)	0	1 (5%)	****
Genotype 2a/2c	0	4 (20%)	0	2 (10%)	****
Genotype 1a ± 2a/2c	0	1 (5%)	0	0	****
Genotype 1a ± 4c/4d	0	0	0	1 (5%)	****
Genotype 1b ± 4c/4d	0	0	0	1 (5%)	****
Genotype 4c/4d	0	0	0	2	****

HOMA, the homeostasis model assessment; BMI, body mass index; TGF-beta1, transforming growth factor-ß1; HIV, human immunodeficiency virus; HCV, hepatitis C virus; HIV/HCV, human immunodeficiency virus/hepatitis C virus; HAART, highly active anti-retroviral therapy; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Post hoc comparison: HOMA: HCV+/healthy controls, $p = 0.0013$; HIV+/healthy controls, $p = 0.0016$; HCV+/HIV+/healthy controls, $p = 0.0021$; HCV+/coinfected, $p = 0.0087$, HIV+/coinfected, $p = 0.0373$.

FIB4: HCV+/healthy controls, $p = 0.0001$; HIV+/healthy controls, $p = 0.0063$; HCV+/HIV+/healthy controls, $p = 0.0001$; HCV/HIV, $p = 0.0004$, HIV/coinfected, $p = 0.0231$.

* Comparison for gender distribution among groups Fisher's exact, $p = 0.000$.

** kPa: HCV+/healthy controls, $p = 0.0001$; HIV+/healthy controls, $p = 1.0$; HCV+/HIV+/healthy controls, $p = 0.0001$; HCV+/HIV+, $p = 0.0001$; HIV+/coinfected, $p = 0.0001$.

*** Two patient for groups have the viral load >40 copies/ml.

FOR HCV/HIV group 6006 copies/ml, 123 copies/ml.

For HIV group 15,528 copies/ml, 145 copies/ml.

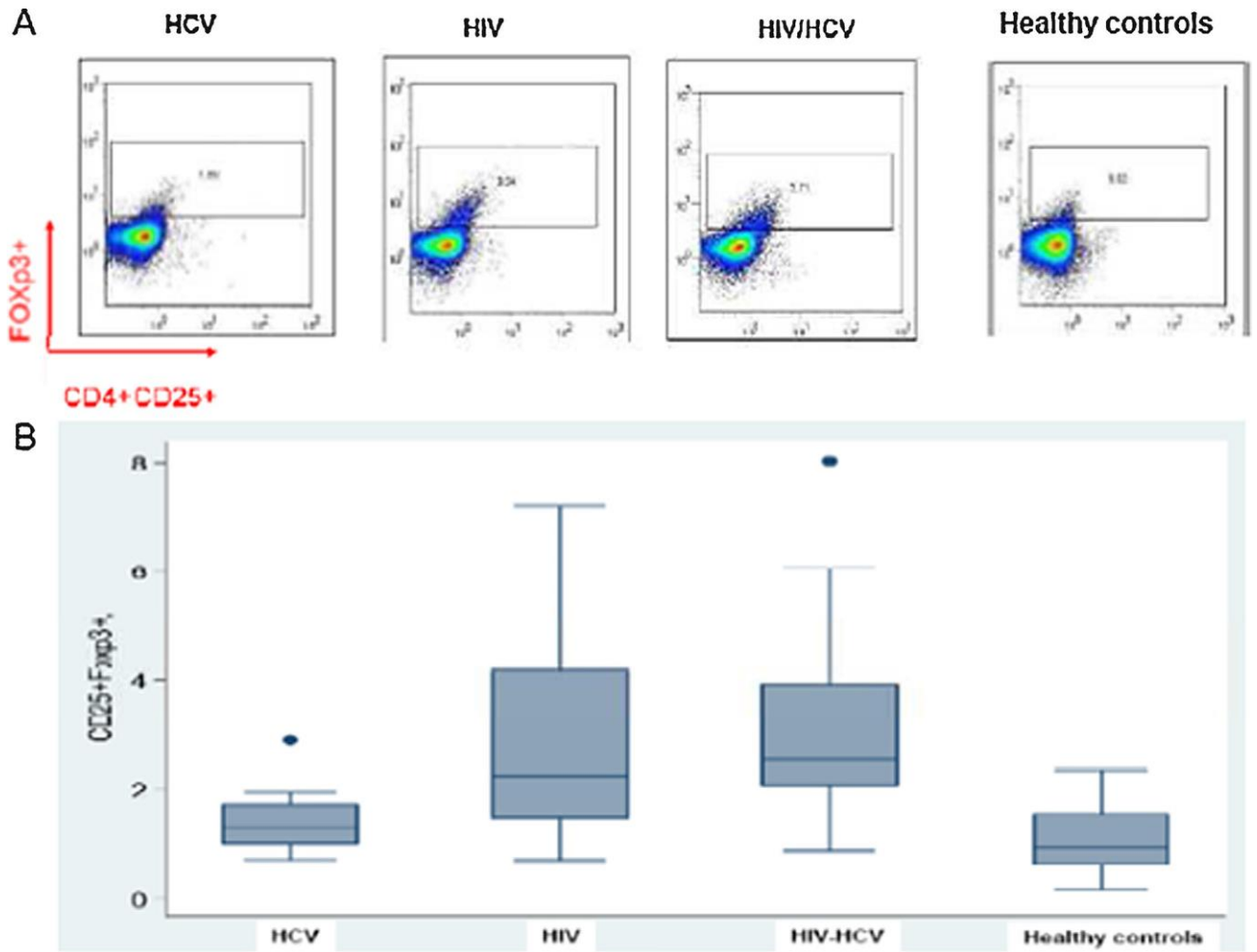*** Comparison for genotype distribution among groups Fisher's exact, $p = 0.238$.

Fig. 1. FoxP3 levels in patients with HIV, HCV, HIV/HCV, and controls. (A) Flow cytometry analysis for each of the four groups. The squared area shows the percentage of CD4+CD25+Foxp3+ lymphocytes. X = patient groups, Y = Foxp3%. (B) Foxp3 levels in the four groups. p = 0.0001. FoxP3, T cells expressing the Forkhead-winged-helix transcription; HIV, human immunodeficiency virus; HCV, hepatitis C virus; HIV/HCV, co-infection human immunodeficiency virus/hepatitis C virus; CD4+CD25+Foxp3+ lymphocytes, T cells expressing the Forkhead-winged-helix transcription factor, Forkhead box 3; FoxP3, T cells expressing the Forkhead-winged-helix transcription.
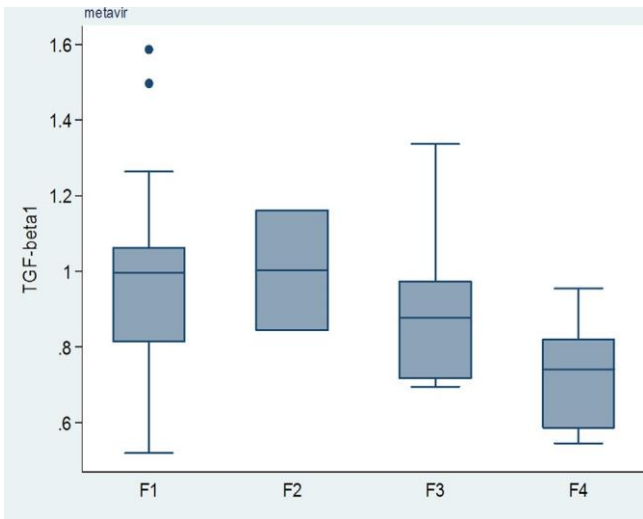
Fig. 2. Correlation between Metavir score categories and TGF-β1 (*p* = 0.006). TGF-β1, transforming growth factor-β1.
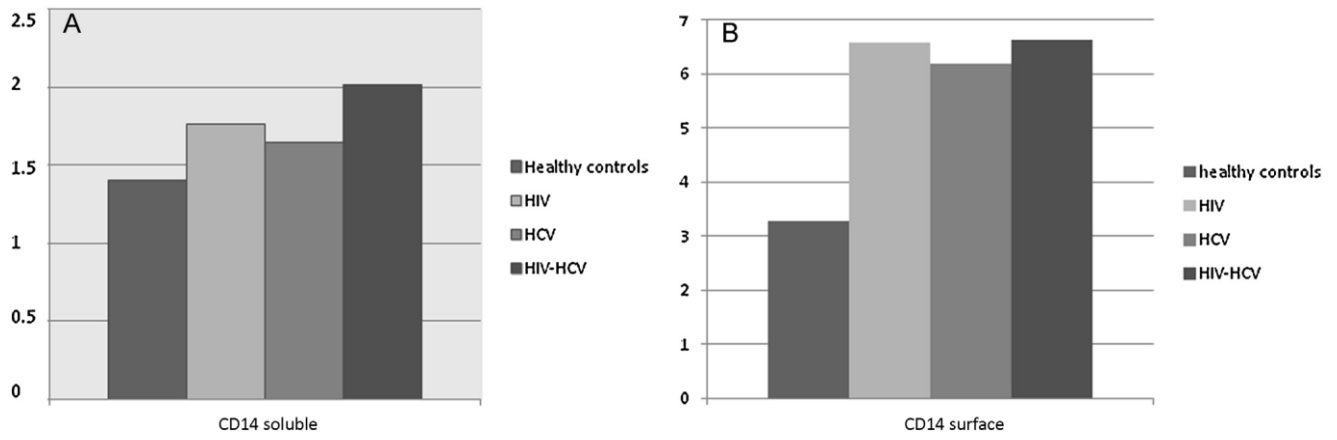
Fig. 3. CD14 levels in patients with HIV, HCV, HIV/HCV, and controls. (A) Levels of soluble CD14 in the blood in the four groups, $p = 0.0100$; (B) levels of surface-bound CD14 in the blood in the four groups, $p = 0.0016$. CD14, T cell with cluster of differentiation 14; HIV, human immunodeficiency virus; HCV, hepatitis C virus; HIV/HCV, co-infection human immunodeficiency virus/hepatitis C virus; CD14, soluble T cell with cluster of differentiation 14.
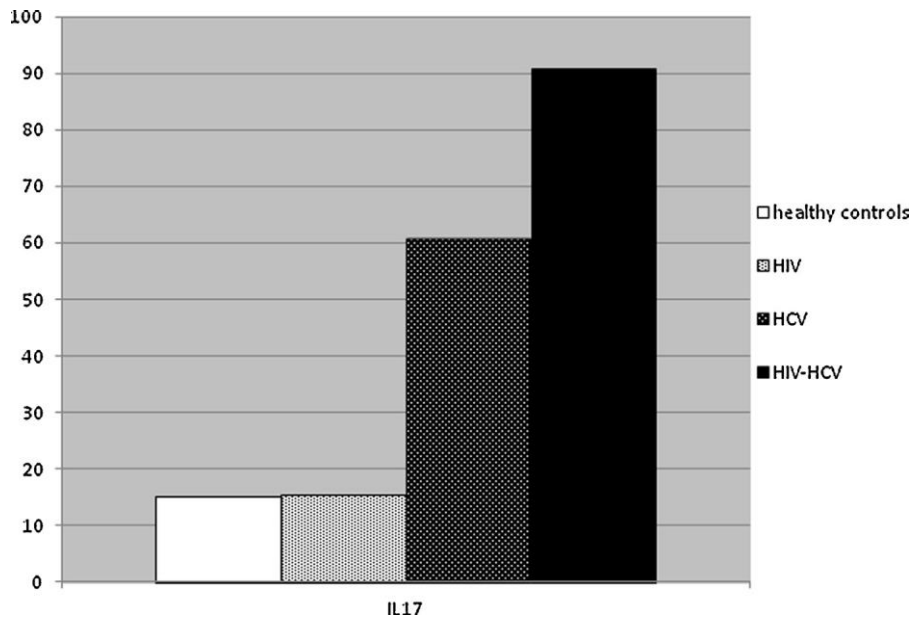
Fig. 4. IL-17 levels in patients with HIV, HCV, HIV/HCV, and controls. *p* = 0.0003. IL-17, interleukin-17; HIV, human immunodeficiency virus; HCV, hepatitis C virus; HIV/HCV, co-infection human immunodeficiency virus/hepatitis C virus.

Table 2
Correlation between FIB4 and index of translocation.

	R	95% CI	p	HCV+	HIV+	COINF	Healthy
Bacterial DNA	16%	−6% to 36%	0.1600	32.0	21	−11	6
CD14 (surface)	34.6%	13% to 52%	0.0016	40.5	−15.5	14.4	−3
Monocytes	44.52%	25% to 60%	0.0000	49.3	14	25.3	4
CD 14 (soluble)	27.79%	6% to 46%	0.0100	−5	4	20	6
CD 4	−2	−24% to 2%	0.8500	41	22	−35	9
IL 17	39%	19% to 56%	0.0003	−10	6	−38	9

CD14, T cell with cluster of differentiation 14; CD4+, T cells expressing the Forkhead-winged-helix transcription factor; IL-17, interleukin-17.

	R	95% CI	p	HCV+	HIV+	COINF	Healthy
Bacterial DNA	16%	−6% to 36%	0.1600	32.0	21	−11	6
CD14 (surface)	34.6%	13% to 52%	0.0016	40.5	−15.5	14.4	−3
Monocytes	44.52%	25% to 60%	0.0000	49.3	14	25.3	4