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TITLE: Bacterial DNA translocation and liver disease severity among HIV infected patients with chronic hepatitis C

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ABSTRACT

We carried out a cross-sectional study to explore whether bacterial 16S ribosomal DNA (bactDNA) show association with severity of liver disease among human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfected patients. Patients with advanced fibrosis (F3/F4), moderate activity grade (A2/A3), and high fibrosis progression rate (FPR>0.15) had higher values of plasma bactDNA levels than patients without these markers of liver disease ($p<0.05$). The chance of having a fibrosis stage or activity grade increased was 1.20 (95%CI=1.0–1.44), $p=0.045$) and 1.22 (95%CI=1.1–1.45), $p=0.029$) times greater for every 100 copies/µL of plasma bactDNA. Likewise, the odds of having values of FPR>0.15 was 1.18 (95%CI= 0.98–1.42), $p=0.089$). In addition, patients with high bactDNA levels (≥175 copies/µL) had the highest odds of having high values of Metavir score and FPR ($p<0.05$). Our data show that bacterial translocation is associated with severe liver disease among HIV infected patients with chronic hepatitis C.

Key Words: 16S rDNA; bacterial translocation; hepatitis C; HIV/AIDS; liver biopsy

INTRODUCTION

In the era of combination antiretroviral therapy (cART), chronic hepatitis C is a leading cause of death among HIV infected persons [1]. Coinfected patients are characterized by a higher rate of fibrosis progression, cirrhosis, and end-stage liver disease than HCV mono-infected patients [2]. However, the mechanisms through which HIV infection accelerates chronic hepatitis C progression are still unknown.

Bacterial translocation (BT) is a mechanism through which alcohol and some enteric conditions may cause liver disease [3,4]. HIV-infection related depletion of mucosal CD4+ lymphocytes has been linked to disruption of gut epithelial integrity and increased mucosal translocation of bacteria and bacterial products (bacterial DNA and endotoxin) from the intestinal lumen to the systemic circulation [5,6]. Lipopolysaccharide (LPS) is a component of the cell wall of gram-negative bacteria, and it is only present on a proportion of enteric bacterial microbiota. Furthermore, different markers from the LPS and of broader spectrum have been evaluated, such as plasma levels of the DNA sequences, encoding the well conserved 16S rRNA subunit (16S ribosomal DNA), common to most bacteria [7]. Detection of bactDNA in plasma is probably a better method for BT measure [8], and it has been proposed as an independent prognostic factor of poor prognosis in non-infected patients with cirrhosis [9].

The BT derived from the intestinal damage is a cause of systemic immune activation in chronic HIV infection [6,10], which can play a crucial role in the accelerated course of liver damage in HIV/HCV-coinfected patients [11,12]. Furthermore, hepatic macrophages or Kupffer cells are responsible for clearing BT products; however, these cells can be infected by HIV and it might result in their impaired ability to clear these potentially fibrogenic BT products [13]. However, nowadays, there is still scarce information on this topic in HIV/HCV-coinfected patients and there are no published studies with a large number of patients.

The aim of the present study was to investigate BT in HIV/HCV-coinfected patients and to explore potential associations between the magnitude of BT and the severity of liver disease.

PATIENTS AND METHODS

Patients

A cross-sectional study was performed on 255 HIV/HCV-coinfected patients of the Hospital Gregorio Marañón, in Madrid (Spain), who underwent a liver biopsy between May 2000 and May 2007. Additionally, 100 healthy blood donors from the "Centro de Transfusión de la Comunidad de Madrid" participated as a control group, all of which were negative for HCV, HBV, and HIV [14].

Liver biopsies were performed on patients who were potential candidates for anti-HCV therapy and had not received previous interferon therapy. The inclusion criteria were: no clinical evidence of hepatic decompensation, detectable HCV RNA by polymerase chain reaction (PCR), negative hepatitis B surface antigen, CD4+ lymphocyte count higher than 200 cells/µL, stable antiretroviral therapy or no need for antiretroviral therapy. The exclusion criteria were: active opportunistic infections or active drug or alcohol addiction.

The studies were conducted in accordance with the Declaration of Helsinki. All patients gave their written consent for the liver biopsy and the Institutional Ethics Committee approved the study.

Clinical and Laboratory data

The following information, on the date of liver biopsy, was obtained from medical records: age, gender, height, weight, risk category, Centers for Disease Control (CDC) clinical category, nadir CD4+, CD4+ T-cells, antiretroviral therapy, complete blood counts, HIV-RNA and HCV-RNA viral load, HCV-genotype, liver biopsy scores, and liver and basic metabolic panel.

Acquired Immune Deficiency Syndrome (AIDS) was defined according to CDC classification [15]. The duration of HCV infection for patients with a history of intravenous drug use (IDU) was estimated starting from the first year that needles and other injection paraphernalia were shared, which are the most important risk practices for HCV transmission[16]. For non-IDUs patients, we had information of the date of infection for 2 patients who were infected by blood transfusion, and for other 3 patients, who were infected by sexual contact, in which the HCV infection may be dated with certainty. Patients were questioned in relation to alcohol consumption. We considered the consumption of greater than 50 grams of alcohol per day for ≥12 months as a high intake. Liver biopsies were performed as previously described [17], and liver fibrosis and necroinflammatory activity were estimated according to Metavir score.

Quantitative Real-Time PCR for measurement of bacterial DNA

Plasma samples were obtained at the same time of the liver biopsy and stored at -80ºC. Next, DNA was extracted from 200 µl of plasma using the QIAamp® MiniElute® Virus Spin Kit (Qiagen) in a QIAcube automated extractor (Qiagen), as recommended by the manufacturer.
Real-time polymerase chain reaction (PCR) was performed in a LightCycler Instrument version 1.5 (Roche Molecular Biochemicals) to amplify DNA sequences encoding the well conserved 16S rRNA subunit (16S rDNA) as previously described [7]. The PCR protocol was as follows: 4 µl of TaqMan Master Mix (Roche Diagnostics), 0.5 µM each primer, 0.2 µM TaqMan probe and 5 µl DNA extract (or standard) in 20 µl total reaction volume. The conditions for amplification reaction of DNA were 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s, 60 °C for 1 min and at 72 ºC for 1 s. Primers sequence were as follows: 8F, (5`-AGT TTG ATC CTG GCT CAG-3`); 515R, (5`-GWA TTA CCG CGG CKG CTG-3`); and TaqMan probe, 338P, (5`-FAM-GCT GCC TCC CGT AGG AGT-BHQ1-3`) [7]. The limit of quantification in the bacterial DNA assay was 15 copies/µL.

Liver disease outcomes

Fibrosis was scored as follows: F0/F1, no fibrosis or portal fibrosis; F2, periportal fibrosis or rare portal-portal septa; F3/F4, fibrous septa with architectural distortion or cirrhosis.
Activity grade was scored as follows: A0/A1, no activity or mild activity; A2, moderate activity; A3, severe activity.
Fibrosis progression rate (FPR) was calculated dividing the fibrosis stage (0 to 4) by the estimated duration of HCV infection in years. Several cut-off were selected: low FPR (<0.075 ($P50^{th}$)), moderate FPR (0.075 ($P50^{th}$) to 0.15 ($P75^{th}$)), and high FPR (≥0.15 ($P75^{th}$)).

Statistic

Categorical data and proportions were analyzed by using the chi-squared test. The ANOVA test was used to compare the means between groups.

The liver disease outcomes studied are ordinal variables, and ordinal logistic regression (OLR) analysis was used to analyze the association between plasma bactDNA levels and liver disease in HIV/HCV-coinfected patients. One of the assumptions underlying OLR is that the relationship between each pair of outcome groups is the same and assumes that the coefficients that describe the relationship between, the lowest versus all higher categories of the response variable are the same as those that describe the relationship between the next lowest category and all higher categories.

For plasma bactDNA levels, a cut-off near the 75^{th} percentile ($P75^{th}$) was selected because this cut-off point separates the 25% of patients with the highest values of plasma bactDNA. Thus, we are able to study the relationship between elevated levels of bactDNA and greater chance of having liver disease. For adjusted OLR, we included bactDNA along with epidemiological and clinical characteristics: age, gender, high alcohol intake, fasting glycaemia (mg/dL), CD4+ nadir, AIDS, CD4+ cell/mm3, undetectable HIV viral load, cART, HCV-genotype 1, and HCV viral load ≥500,000 IU/mL at biopsy date.

All tests were two-tailed with P values <0.05 considered significant. Statistical analysis was performed by SPSS 15.0 software (SPSS INC, Chicago, IL, USA).

RESULTS

Characteristics of HIV/HCV-coinfected patients at the same time than liver biopsy are shown in **Supplemental Digital Content 1 (SDC 1)**. The median age was 39.5 years, 76% were male, and 29% showed prior AIDS-defining conditions. On the date of liver biopsy, the median CD4+ count was 483 cells/mm^3, 70.6% had an HIV-RNA<50 copies/mL, 61.4% had HCV genotype 1, and 73.1% had an HCV-RNA>500,000 UI/mL. Overall, 84.7% patients were on cART: 23.5% with protease inhibitor based therapy, 47.8% with non-nucleoside analogue based therapy, and 13.3% with 3 nucleoside analogue based therapy. Furthermore, 53.3% patients showed significant fibrosis (F≥2), 26.6% advanced fibrosis (F≥3) and 55.1% moderate activity grade (A≥2).

The prevalence of positive PCR for plasma bactDNA was significantly higher in HIV/HCV-coinfected patients than in healthy blood donors (244/255 (95.7%) versus 7/100 (7%) respectively; p<0.001). Furthermore, patients with CD4+<350 cells/mm^3 had higher significantly plasma bactDNA levels than patients whose CD4+≥350 cells/mm^3 (157.1±28.2 versus 112.8±7.2 respectively; p=0.030). Moreover, bactDNA levels were not higher in patients who had detectable HIV-RNA and the level did not depend on the level of plasma HIV-RNA (*data not shown*).

Patients with markers of advanced liver disease (F3/F4, and A2/A3) and high FPR (≥0.15) had higher plasma values of bactDNA than patients without these markers of liver disease (**Figure 1A**). Note that A3 had a p-value close to statistical significance (p=0.083). In addition, it may be seen that patients with bactDNA values higher than 175 copies/µL had a tendency to liver disease, while others cut-offs did not show a trend so clear (**Figure 1B**).

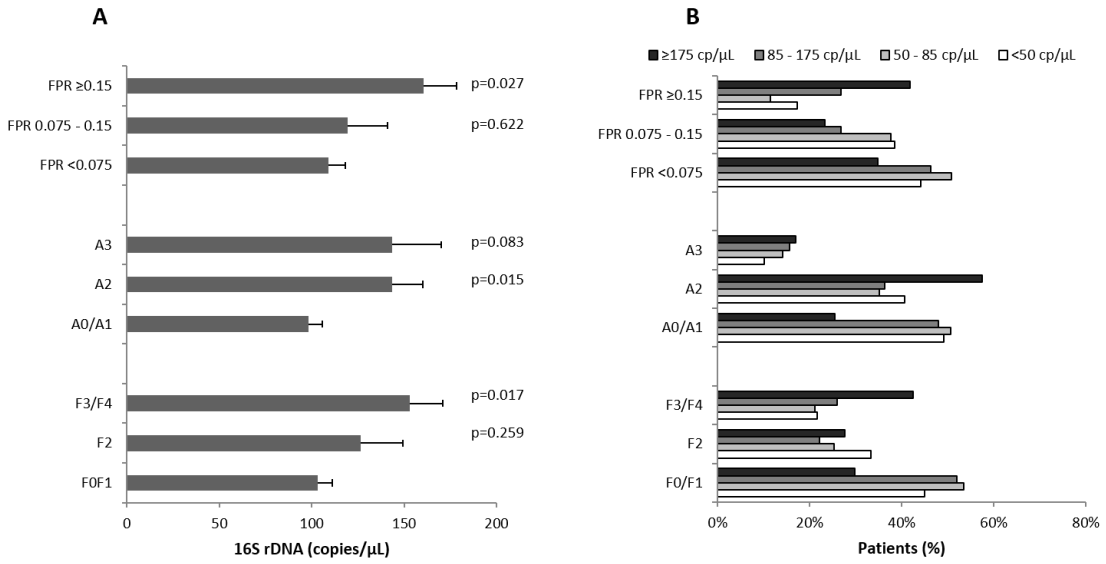

Figure 1. Summary of plasma 16S rDNA levels according to markers of liver disease in HIV/HCV coinfected patients.

(A), mean and standard error of plasma 16S rDNA levels. The statistical significance values were calculated with respect to the milder disease category.
(B), distribution of patients according to disease category and percentile of plasma 16S rDNA levels (P25th (50 copies/µL), P50th (85 copies/µL), and P75th (175 copies/µL)). The statistical significance values were calculated on all categories of the liver disease by linear-by-linear association chi-squared test.
Fibrosis was scored as follows: F0/F1, no fibrosis or portal fibrosis; F2, periportal fibrosis or rare portal-portal septa; F3/F4, fibrous septa with architectural distortion or cirrhosis. Activity grade was scored as follows: A0/A1, no activity or mild activity; A2, moderate activity; A3, severe activity. Fibrosis progression rate (FPR) was scored as follows: low FPR (<0.075 ($P50^{th}$)), moderate FPR (0.075 ($P50^{th}$) to 0.15 ($P75^{th}$)), and high FPR (≥0.15 ($P75^{th}$)).

Next to it, we analyzed the influence of plasma bactDNA on liver disease by OLR analysis. With regard to fibrosis, the chance of having an increased Metavir score was 1.20 (95% of confidence interval (95%CI)= 1.0–1.44), p=0.045) times greater for every 100 copies/µL of plasma bactDNA. For example, for every 100 copies/µL of plasma bactDNA, the odd of F3/F4 versus F2 and F0/F1 was 1.20 and the odds of having F2 versus F0/F1 was also 1.20. Likewise, the odds of having an increased activity grade was 1.22 (95%CI= 1.1–1.45), p=0.029) and of having values of FPR>0.15 or FPR 0.075–0.15 versus FPR<0.075 was 1.18 (95%CI= 0.98–1.42), p=0.089) times greater for every 100 copies/µL of plasma bactDNA. In addition, patients with high plasma bactDNA levels (≥175 copies/µL) had the highest prevalence and odds of having high values of Metavir score and FPR (**Table 1**).

Table 1. Prevalence of low (<p75th) and high (≥p75th) plasma 16S rDNA levels within each stage of the liver disease, and odds of finding liver disease and high fibrosis progression rate in patients with high (≥p75th) plasma 16S rDNA values.

	p75th of 16S rDNA			Unadjusted		Adjusted	
	<175 copies/µL	≥175 copies/µL	p-value (*)	OR (95%CI)	p-value (†)	OR (95%CI)	p-value (‡)
FPR							
<0.075	85 (47.2%)	15 (34.9%)					
0.075 – 0.15	61 (33.9%)	10 (23.3%)	0.009	2.44 (1.35; 4.41)	0.003	2.05 (1.09; 3.86)	0.026
≥0.15	34 (18.9%)	18 (41.9%)					
Activity grade							
A0/A1	102 (49.3%)	12 (25.5%)					
A2	77 (37.2%)	27 (57.4%)	0.017	2.13 (1.17; 3.88)	0.013	1.97 (1.04; 3.73)	0.038
A3	28 (13.5%)	8 (17%)					
Fibrosis							
F0/F1	105 (50.5%)	14 (28.8%)					
F2	55 (26.4%)	13 (27.7%)	0.013	2.26 (1.22; 4.21)	0.010	2.03 (1.04; 3.96)	0.037
F3/F4	48 (23.1%)	20 (42.6%)					

Abbreviations: OR, odds ratio; 95%CI, 95% of confidence interval.

Fibrosis was scored as follows: F0/F1, no fibrosis or portal fibrosis; F2, periportal fibrosis or rare portal-portal septa; F3/F4, fibrous septa with architectural distortion or cirrhosis. Activity grade was scored as follows: A0/A1, no activity or mild activity; A2, moderate activity; A3, severe activity. Fibrosis progression rate (FPR) was scored as follows: low FPR (<0.075 ($P50^{th}$)), moderate FPR (0.075 ($P50^{th}$) to 0.15 ($P75^{th}$)), and high FPR (≥0.15 ($P75^{th}$)).

(*), value of statistical significance was calculated by linear-by-linear association chi-squared test; (†), value of statistical significance was calculated by ordinal logistic regression test; (‡), value of statistical significance was calculated by adjusted ordinal logistic regression test.

DISCUSSION

We determined plasma levels of bactDNA in a group of 255 HIV/HCV-coinfected patients with compensated liver disease who underwent liver biopsy, in order to determine their suitability for undergoing interferon plus ribavirin therapy. HIV/HCV-coinfected patients had higher plasma levels of bactDNA than healthy controls, and patients with CD4+<350 cells/mm^3 had higher plasma bactDNA than those with CD4+≥350 cells/mm^3. In addition, an association between higher plasma bactDNA levels with advanced liver disease and more rapid progression to fibrosis was found.

The most likely source of these bacterial products is the gastrointestinal tract, in which mucosal defenses are profoundly disrupted by HIV infection [5]. Our results, as well as the emerging data from other investigators[7,11,15,18], indicate that bacterial products are often circulating in the plasma of HIV-infected and HIV/HCV-coinfected patients. Our finding of an association between lower CD4+ cells and higher bactDNA plasma values support the notion that in HIV-infected patients on cART bactDNA plasma levels correlate with CD4+ count, the magnitude of immune CD4+ restoration, and immune activation markers [7]. This is interesting because BT, CD4+ lymphocyte depletion and immune activation may contribute to liver disease progression among HIV/HCV-coinfected patients [11,19].

In our study, we confirmed the association between higher plasma levels of bactDNA and both higher grades of inflammation and higher stages of fibrosis in liver biopsies from HIV/HCV-coinfected patients. Furthermore, our data show a significant association between bactDNA levels and slow FPR in these patients. Most previous studies did not analyze the role of these bactDNA levels with FPR, which is a robust measure of the risk of cirrhosis progression. Nevertheless, we consider FPR as an indirect measure that has some limitations, such as the assumption of a constant progression rate. A direct method, using serial liver biopsies and the interval between two adjacent biopsies, has the ability to calculate stage-specific transition rates, but it is very difficult to compile patients with more than a single biopsy. Despite this limitation, we believe that bactDNA plasma levels might add valuable information to the factors influencing FPR and may be useful to implement targeted therapeutic interventions in HIV patients with HCV infection at risk of rapid liver disease progression.

Unfortunately, our study design does not permit to assess whether BT is either a cause or a consequence of liver disease progression. However, there are some evidences that BT promotes hepatic fibrogenesis, which ultimately increases portal systemic shunting (a well-known consequence of cirrhosis) and the abundance of circulating microbial products in a positive feedback loop [11]. In addition, presence of bactDNA in patients with cirrhosis is associated with hemodynamic consequences, such as aggravation of peripheral vasodilation and worsening of intrahepatic endothelial dysfunction [20].

In conclusion, although the differences observed among the patients with different stages and rates of disease progression are modest; our data show that BT was associated with severe liver disease among HIV infected patients with chronic hepatitis C. Future studies are needed to validate these results and to evaluate whether plasma levels of bactDNA are a predictive and/or surrogate marker of liver disease in HIV/HCV-coinfected patients.

AUTHORS' CONTRIBUTIONS

Study concept and design: M García-Álvarez, S Resino.

Acquisition of data: J Berenguer, T Aldámiz-Echevarría; A Carrero, J Cosín, P Miralles.

Collection of samples: D Micheloud, J Berenguer.

Assessment of liver biopsy: E Álvarez

Administrative, technical, or material support: M García-Álvarez, M Guzmán-Fulgencio, MA Jimenez-Sousa, A Fernández-Rodríguez.

Analysis and interpretation of data: M García-Álvarez, M Guzmán-Fulgencio, S Resino.

Drafting of the manuscript: M García-Álvarez, S Resino

Critical revision of the manuscript for important intellectual content: J Berenguer.

Study supervision: S Resino.

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REFERENCES

1. The Data Collection on Adverse Events of Anti HIVDSG. Liver-Related Deaths in Persons Infected With the Human Immunodeficiency Virus: The D:A:D Study. *Arch. Intern. Med.* August 14, 2006 2006;166(15):1632-1641.

2. Graham CS, Baden LR, Yu E, et al. Influence of Human Immunodeficiency Virus Infection on the Course of Hepatitis C Virus Infection: A Meta-Analysis. *Clin. Infect. Dis.* August 15, 2001 2001;33(4):562-569.

3. Enomoto N, Yamashina S, Kono H, et al. Development of a new, simple rat model of early alcohol-induced liver injury based on sensitization of kupffer cells. *Hepatology.* 1999;29(6):1680-1689.

4. Thurman RG. II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. *American Journal of Physiology - Gastrointestinal and Liver Physiology.* October 1, 1998 1998;275(4):G605-G611.

5. Paiardini M, Frank I, Pandrea I, Apetrei C, Silvestri G. Mucosal immune dysfunction in AIDS pathogenesis. *AIDS Rev.* Jan-Mar 2008;10(1):36-46.

6. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* 2006;12(12):1365-1371.

7. Jiang W, Lederman MM, Hunt P, et al. Plasma Levels of Bacterial DNA Correlate with Immune Activation and the Magnitude of Immune Restoration in Persons with Antiretroviral-Treated HIV Infection. *J. Infect. Dis.* April 15, 2009 2009;199(8):1177-1185.

8. Francés R, González-Navajas JM, Zapater P, et al. Translocation of bacterial DNA from Gram-positive microorganisms is associated with a species-specific inflammatory response in serum and ascitic fluid of patients with cirrhosis. *Clin. Exp. Immunol.* 2007;150(2):230-237.

9. Zapater P, Francés R, González-Navajas JM, et al. Serum and ascitic fluid bacterial DNA: A new independent prognostic factor in noninfected patients with cirrhosis. *Hepatology.* 2008;48(6):1924-1931.

10. Ellis CL, Ma Z-MP, Mann SKMD, et al. Molecular Characterization of Stool Microbiota in HIV-Infected Subjects by Panbacterial and Order-Level 16S Ribosomal DNA (rDNA) Quantification and Correlations With Immune Activation. *JAIDS Journal of Acquired Immune Deficiency Syndromes.* 2011;57(5):363-370.

11. Balagopal A, Philp FH, Astemborski J, et al. Human Immunodeficiency Virus-Related Microbial Translocation and Progression of Hepatitis C. *Gastroenterology.* 2008;135(1):226-233.

12. Bruno R, Sacchi P, Puoti M, et al. Pathogenesis of liver damage in HCV-HIV patients. *AIDS Rev.* 2008;10(1):15-24.

13. Balagopal A, Ray SC, De Oca RM, et al. Kupffer cells are depleted with HIV immunodeficiency and partially recovered with antiretroviral immune reconstitution. *AIDS.* 2009;23(18):2397-2404 2310.1097/QAD.2390b2013e3283324344.

14. Álvarez do Barrio M, González Díez R, Hernández Sánchez J, Oyonarte Gómez S. Residual risk of transfusion-transmitted viral infections in Spain, 1997-2002, and impact of nucleic acid testing. *Euro Surveill.* 2005;10(2):20-22.

15. CDCP. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep.* 1992;41(RR-17):1-19.

16. Thorpe LE, Ouellet LJ, Hershow R, et al. Risk of hepatitis C virus infection among young adult injection drug users who share injection equipment. *Am. J. Epidemiol.* Apr 1 2002;155(7):645-653.

17. Berenguer J, Bellon JM, Miralles P, et al. Identification of liver fibrosis in HIV/HCV-coinfected patients using a simple predictive model based on routine laboratory data. *J Viral Hepat.* Dec 2007;14(12):859-869.

18. Montes-de-Oca M, Blanco MJ, Marquez M, et al. Haemodynamic derangement in human immunodeficiency virus-infected patients with hepatitis C virus-related cirrhosis: the role of bacterial translocation. *Liver Int.* Jul 2011;31(6):850-858.

19. Caradonna L, Mastronardi ML, Magrone T, et al. Biological and Clinical Significance of Endotoxemia in the Course of Hepatitis C Virus Infection. *Curr. Pharm. Des.* 2002;8(11):995-1005.

20. Bellot P, García-Pagán JC, Francés R, et al. Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology.* 2010;52(6):2044-2052.

SUPPLEMENTAL DIGITAL CONTENT

Supplemental Digital Content 1. Characteristics of 255 HIV/HCV coinfected patients, who underwent a liver biopsy.

	All patients
No. HIV-1 patients *	255
Gender (male) *	194 (76.07%)
Age (years) #	39.52 (37.1; 43.6)
Epidemiological history	
Injection drug users *	219 (85.8%)
Years since HCV infection #	21.4 (17.3; 24.5)
High alcohol intake *	135 (53.4%)
CDC category C *	74 (29.02%)
Antiretroviral therapy *	
Non treated	39 (15.3%)
PI-based	60 (23.5%)
NNRTI-based	122 (47.8%)
3 NRTI-based	34 (13.3%)
Metavir fibrosis stage *	
No fibrosis (F0)	24 (9.4%)
Portal fibrosis (F1)	95 (37.2%)
Periportal fibrosis (F2)	68 (26.7%)
Bridging fibrosis (F3)	43 (16.9%)
Cirrhosis (F4)	25 (9.8%)
Metavir activity grade (no.=254)*	
No activity (A0)	6 (2.3%)
Mild activity (A1)	108 (42.5%)
Moderate activity (A2)	104 (40.9%)
Severe activity (A≥3)	36 (14.2%)
HIV markers	
Nadir CD4+ T-cells #	203 (88; 329)
CD4+ T-cells/µL #	483 (360; 680)
HIV-RNA < 50 cp/mL *	180 (70.58)
HCV markers *	
HCV-genotype 1	154 (61.4%)
HCV RNA >500,000 UI/mL	177 (73.1%)
Biochemical parameters #	
Glucose (mg/dL)	86 (77; 94)
ALT (UI/dL)	74 (47; 114.2)
AST (UI/dL)	54 (35.7; 82.2)
GGT (UI/dL)	92 (52; 182.2)
AST/ALT	0.78 (0.62; 1.00)
Cholesterol	175 (146; 202)

*Absolute number (percentage). #Median (percentile 25; percentile 75).
Abbreviations: ALT: alanine aminotransferase. AST: aspartate aminotransferase. GGT: gamma glutamyl transpeptidase. HAART: highly active antiretroviral therapy. HCV: Hepatitis C virus. HCV-RNA: HCV plasma viral load. HIV-1: Human immunodeficiency virus type 1. HIV-RNA: HIV plasma viral load. NNRTI: non-nucleoside analogue HIV reverse transcriptase inhibitor. NRTI: nucleoside analogue HIV reverse transcriptase inhibitor. PI: protease inhibitor.