Title page

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Title: High plasma CXCL10 levels are associated with HCV-genotype 1, and higher insulin resistance, fibrosis, and HIV viral load in HIV/HCV coinfected patients

Running title: Plasma CXCL10 on chronic hepatitis C

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ABSTRACT

Background: CXCL10 may contribute to the host immune response against the hepatitis C virus (HCV), liver disease progression, and response to HCV antiviral therapy. The aim of our study was to analyze the relationship among virological, immunological, and clinical characteristics with plasma CXCL10 levels in human immunodeficiency virus (HIV)/HCV-coinfected patients.

Methods: We carried out a cross-sectional study on 144 patients. CXCL10 and insulin were measured using an immunoassay kit. The degree of insulin resistance was estimated for each patient using the homeostatic model assessment (HOMA) method. Insulin resistance was defined as a HOMA index higher than or equal to 3.8. Aspartate aminotransferase (AST) to platelet ratio (APRI), FIB-4, Forns index, HGM1, and HGM2 were calculated.

Results: The variables associated with $\log_{10}$ CXCL10 levels by univariate analysis were age (b=0.013; p=0.023), prior AIDS-defining condition (b=0.127; p=0.045), detectable plasma HIV viral load (b=0.092; p=0.006), $\log_{10}$ HOMA (b=0.216; p=0.002), HCV-genotype 1 (b=0.114; p=0.071), and liver fibrosis assessed by all non-invasive indexes ($\log_{10}$ APRI (b=0.296; p=0.001), $\log_{10}$ FIB-4 (b=0.436; p <0.001), $\log_{10}$ Forns index (b=0.591; p <0.001), $\log_{10}$ HGM1 (b=0.351; p=0.021), and $\log_{10}$ HGM2 (b=0.215; p=0.018). However, in multivariate analysis, CXCL10 levels were only associated with HOMA, detectable plasma HIV viral load, HCV-genotype 1 and FIB-4 (*R*-square=0.235; p<0.001).

Conclusion: Plasma CXCL10 levels were influenced by several characteristics of patients related to HIV and HCV infections, insulin resistance, and liver fibrosis, indicating that CXCL10 may play an important role in the pathogenesis of both HCV and HIV infections.

Key words: Chemokine, HOMA, AIDS, chronic hepatitis C, inflammation, fibrosis

SUBDIVISION - NUMBERED SECTIONS

1. INTRODUCTION

CXCL10 (also known as interferon (IFN)-γ-inducible protein 10 (IP-10)) is secreted by monocytes, endothelial cells, and fibroblasts in response to IFN-γ, attracting cells expressing CXCR3 receptor on the surface [1]. This chemokine may play an important role in the pathogenesis of chronic hepatitis C (CHC) through the recruitment of monocytes/macrophages, Th1 cells, NK cells, and dendritic cells to the liver parenchyma. Therefore, it potentially contributes to the host immune response against the hepatitis C virus (HCV), as well as to liver disease progression [1]. High levels of CXCL10 in plasma or serum of HCV-infected patients are associated to high activity grade and fibrosis stage in liver biopsy [2, 3]. Besides, plasma levels pre-treatment of CXCL10 are elevated in CHC patients infected by genotype 1 and without a sustained viral response (SVR) after completion of antiviral therapy [3-8].

The aim of our study was to analyze the relationship among virological, immunological, and clinical characteristics with plasma CXCL10 levels in HIV/HCV-coinfected patients.

2. PATIENTS AND METHODS

2.1. Study population

We carried out a cross-sectional study in a cohort of 144 HIV/HCV-coinfected patients without anti-HCV therapy. These patients are part of a cohort of HIV/HCV coinfected patients who were eligible to start anti-HCV therapy between September 2000 and July 2008, as it has been described previously [9]. Thus, the selection criteria for starting a HCV antiviral treatment was: A) Inclusion: CHC, no clinical evidence of hepatic decompensation, detectable HCV-RNA by polymerase chain reaction, negative for hepatitis B surface antigen, CD4+ lymphocyte ≥200 cells/mm^3, and stable highly active antiretroviral therapy (HAART). B) Exclusion: active opportunistic infections, active drug or alcohol addiction, and other concomitant diseases or conditions such as diabetes, nephropathies, autoimmune diseases, and neoplasia.

All the work was conducted in accordance with the Declaration of Helsinki. All patients gave their written consent and the Institutional Ethics Committee approved the study.

2.2. Clinical and laboratory assessment

The following information was obtained from medical records: age, gender, height, weight, risk category, alcohol intake (consumption of more than 50 g of alcohol per day for at least 12 months as a high intake), Centers for Disease Control (CDC) clinical category, nadir CD4+, antiretroviral therapy, activity grade and fibrosis stage of liver biopsies according Metavir score, and HCV genotype. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

After an overnight fast, blood samples were collected in order to perform: a liver and basic metabolic panel with complete blood counts, CD4+ T-cells, plasma HIV viral load (HIV-RNA) and plasma HCV viral load (HCV-RNA).

Non-invasive fibrosis indexes were calculated according to the formula originally described for Aspartate aminotransferase (AST) to platelet ratio (APRI) [10], FIB-4 [11], Forns index [12], HGM1, and HGM2 [13]. The degree of insulin resistance was estimated for each patient using the homeostatic model assessment (HOMA) method, which was obtained using the following formula: fasting plasma glucose (mmol/L) times fasting serum insulin (mU/L) divided by 22.5. Insulin resistance was defined as a HOMA index higher than or equal to 3.8.

The plasma CXCL10 was measured using Multiplex kits (Panomics Afymetrix, Inc.; Procarta® Protein Profiling Assays, Fremont, California, United States) and insulin was measured using the Multiplex kit (LINCOplexTM; LINCO Research, St. Charles, Missouri, United States) employing the Luminex 100™ analyzer (Luminex Corporation, Austin, Texas, United States).

2.3. Statistics

Log transformation was performed for all variables that were not normally distributed. With the aim of analyzing the key factors that influence plasma CXCL10 levels, linear regression analyses were performed. First, we carried out univariate linear regression analysis to analyze the association of several epidemiological and clinical factors with plasma CXCL10 levels. The analyzed factors were gender, age, AIDS, CD4+/μL nadir, CD4+/μL, detectable HIV-RNA (≥50 copies/mL) or non-responders to HAART, HAART, BMI, HOMA, HCV-RNA, HCV-genotype 1 (genotype 1 versus non 1), advanced fibrosis (F≥3 or F3 to F4), moderate activity

grade (A≥2), and non-invasive fibrosis indexes (APRI, FIB4, Forns index, HGM1, and HGM2).
Those factors which showed a p-value lower than 0.1 in univariate analysis, were included in a multivariate linear regression analysis with the Enter (Forced Entry) algorithm. The final model included the factors considered to be independently important for plasma CXCL10 levels. The *R*-square value is an indicator of how well the *model* fits the data. Statistical analysis was performed by SPSS 15.0 software (SPSS INC, Chicago, IL, USA).

3. RESULTS

3.1. Patient characteristics

The characteristics of HCV/HIV-infected patients are shown in **Table 1**. Overall, mean age was 41.6 years, 88.7% acquired HIV infection by injection drug use and 53% had prior AIDS-defining conditions. Besides, 83.9% of the patients were on HAART, the mean CD4+ count was 517 cells/mm^3 and 71.3% had a plasma HIV-RNA<50 copies/mL. Regarding the HCV infection, 51.1% of the patients were infected by HCV-genotype 1 and 54.9% had plasma HCV-RNA>500,000 UI/ml. Moderate activity grade (A≥2) was found in 58.7% of the patients and advanced stage of fibrosis in 32.9%. The mean HOMA value was 5.42 and 31.7% of the patients had insulin resistance.

Table 1. Characteristics of 144 HIV/HCV co-infected patients without anti-HCV therapy.

Characteristics	Values
No. HIV patients	144
Gender (Male)	108 (76.2%)
Age (years)	41.6±0.5
Epidemiological history	
Injection drug users	126 (88.7%)
High alcohol intake	74 (51.3%)
CDC category C	76 (53.5%)
Viral markers	
Nadir CD4+ (cells/mm^3)	235±15
CD4+ (cells/mm^3)	517±22
Log$_{10}$ HIV RNA (copies/mL)	2.19±0.07
HIV RNA < 50 copies/mL	102 (71.3%)
HCV genotype 1	72 (51.1%)
Log$_{10}$ HCV RNA (IU/ml)	5.74±0.05
HCV RNA >500,000 UI/mL	78 (54.9%)
Antiviral therapy	120 (83.9%)
Metabolic markers	
BMI (Kg/m^2)	22.8±0.30
BMI ≥25	30 (21%)
HOMA-IR	5.42±0.92
HOMA-IR ≥3.8	51 (35.7%)
Metavir fibrosis stage (n=120)	
Significant fibrosis (F≥2)	95 (66.4%)
Advanced fibrosis (F≥3)	47 (32.9%)
Cirrhosis (F4)	16 (11.2%)
Metavir activity grade (n=117) *	
Moderate activity (A≥2)	84 (58.7%)
Severe activity (A≥3)	24 (16.8%)
ALT (IU/L)	100.1±5.7
ALT ≥110 IU/L	97 (67.3%)

Plasma chemokine	
CXCL10 (pg/mL)	719.7±59.7
Fibrosis indexes	
FIB-4	2.78±0.29
Forns index	4.84±1.36
APRI	1.60±0.17
HGM1	0.66±0.02
HGM2	0.31±0.02

Categorical variables are expressed in absolute numbers (%) and continuous variables in mean and standard error of mean. Abbreviations: HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; CDC, Centre of Diseases Control; HAART, highly active antiretroviral therapy; BMI, body mass index; HOMA, homeostatic model assessment method; HCV RNA, HCV plasma viral load; HIV RNA, HIV plasma viral load; APRI, Aspartate aminotransferase to platelet ratio; ALT, alanine aminotransferase.

3.2. Factors associated with increased plasma CXCL10 levels

The variables significantly associated with log_{10} CXCL10 levels by univariate analysis were age (Pearson correlation coefficient (r)= 0.190; p= 0.023), prior AIDS-defining condition (r= 0.168; p= 0.045), detectable plasma HIV viral load (r= 0.165; p= 0.006), log_{10} HOMA (r= 0.255; p= 0.002), and liver fibrosis assessed by all non-invasive indexes (log_{10} APRI (r= 0.355; p= 0.001), log_{10} FIB-4 (r= 0.270; p <0.001), log_{10} Forns index (r= 0.312; p <0.001), log_{10} HGM1 (r= 0.196; p= 0.021), and log_{10} HGM2 (r= 0.205; p= 0.018). Besides, HCV-genotype 1 was close to statistical significance (b= 0.152; p= 0.071) (**Table 2**).

We carried out a multivariate analysis in which we included factors that showed significant correlation (p<0.1) in previous univariate analysis, but considering only FIB-4 as non-invasive index because it had the best association with CXCL10 (high correlation coefficient, narrow confidence interval, and low p-value). In this analysis, the variables significantly associated with plasma CXCL10 levels were HOMA (regression coefficient (b)=0.188; p= 0.008); detectable HIV-RNA (≥50 copies/mL) or non-responders to HAART (b=0.077; p= 0.021), HCV-genotype 1 (b=0.166; p= 0.050), and FIB-4 (b=0.271; p= 0.011) (*R*-square= 0.235; p<0.001) (**Table 2**).

Table 2. Summary of the association among HIV/HCV coinfection disease progression markers (x_1, x_2, x_3, ...x_n) and CXCL10 levels (y).

	Univariate				Multivariate		
	r	**b**	**95% CI**	**p-value**	**b**	**95% CI**	**p-value**
Age (years)	0.190	0.013	0.002; 0.023	**0.023**	0.009	-0.001; 0.020	0.092
CDC category C	0.168	0.127	0.003; 0.251	**0.045**	0.038	-0.080; 0.157	0.516
Detectable HIV-RNA (≥ 50 cp/mL)	0.165	0.092	0.026; 0.158	**0.006**	0.077	0.012; 0.142	**0.021**
Log_{10} HOMA	0.255	0.216	0.080; 0.353	**0.002**	0.188	0.049; 0.316	**0.008**
HCV-genotype 1	0.152	0.114	-0.010; 0.239	0.071	0.116	0.001; 0.231	**0.050**
Log_{10} FIB-4	0.355	0.436	0.243; 0.629	**<0.001**	0.271	0.064; 0.477	**0.011**
Log_{10} APRI	0.270	0.296	0.118; 0.473	**0.001**	-	-	-
Log_{10} Forns	0.312	0.591	0.286; 0.896	**<0.001**	-	-	-
Log_{10} HGM1	0.196	0.351	0.055; 0.647	**0.021**	-	-	-
Log_{10} HGM2	0.205	0.215	0.037; 0.392	**0.018**	-	-	-

Values expressed as Pearson correlation coefficient (r), regression lineal coefficient (b), and standard error of mean (s.e.m.). Abbreviations: HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; HAART, highly active antiretroviral therapy; HCV-RNA, HCV plasma viral load; HIV-RNA, HIV plasma viral load; HOMA, insulin resistance score; BMI, Body mass index; CDC, Centre of Diseases Control.

4. DISCUSSION

In the present study, we have assessed the CXCL10 plasma levels and its relation with different virological, immunological, and clinical parameters in coinfected HIV-HCV patients. Our results have shown that plasma CXCL10 levels were significantly high in patients with high HIV viral load, insulin resistance, non-invasive fibrosis indexes, and HCV genotype 1 infection.

Regarding the association between HIV-RNA viral load and plasma CXCL10 level, previous studies in HIV-monoinfected patients on HAART have reported higher CXCL10 levels in non-responders than in HAART responders (undetectable plasma HIV-RNA) [14]. In fact, HAART also improves T-cell number and its function, which might result in a significant decrease of immune activation on T-cell [15], and inflammatory responses in liver biopsy [16].

Insulin resistance and type-2 diabetes, common comorbidities in CHC patients, are proinflammatory conditions, which are associated with steatosis, advanced fibrosis and poor response to HCV antiviral therapy [9, 17]. Besides, HCV or HIV infection is characterized by an increased production of proinflammatory cytokines, which is one way to develop insulin resistance [18, 19]. In our study, HOMA and CXCL10 were strongly associated in HIV/HCV coinfected patients. Interestingly, our data suggest that there is a link between metabolic and immunologic perturbations, matching up with the hypothesis proposed by Casrouge *et al*.; in which the plasma CXCL10 of CHC patients is enzymatically processed by the dipeptidyl peptidase IV (DPP4), producing an antagonist form that may contribute to the inability to clear HCV infection [20]. This study also suggests a possible link among liver inflammation, metabolism and immune system in CHC patients via DPP4 [20]. Thus, the inflammation promotes an increased DPP4 activity that might catabolize the glucagon-like peptide–1 (GLP-1) and CXCL10, disturbing glucose homeostasis and the removal of HCV infection [21].

Regarding fibrosis, we have found a significant association between plasma CXCL10 and non-invasive fibrosis indexes, although METAVIR scores or serum ALT levels were not significant. This discrepancy may be due to differences in the quantification methods of CXCL10, as well as in protocols used for scoring liver-biopsy samples in our study. It has to be noticed that there is a considerable controversy regarding the association of CXCL10 and activity grade, fibrosis stage, or serum transaminase levels. Regarding HCV-monoinfected patients, positive results have been found, but they were controversial [2-4, 22, 23]. On the one hand, Harvey *et al.* found that the expression of CXCL10 mRNA correlates with lobular necroinflammatory activity but not with inflammation or fibrosis in the portal tracts [2]. On the other hand, Diago *et al.* reported lack of association with necroinflammatory activity, but could find elevated levels of CXCL10 in advanced fibrosis [22]. Conflicting associations were also described by Reiberger *et al.* and Romero *et al.* who found that serum CXCL10 levels were both correlated with fibrosis stage and with necroinflamatory activity, but with discrepant data for ALT levels [3, 4]. Finally, Roe *et al*. found that liver fibrosis scores, liver enzyme levels such as ALT and AST levels, were positively correlated with CXCL10 levels in HCV-monoinfected patients but not in HCV/HIV-coinfected patients [23]. On the other hand, the non-invasive fibrosis indexes (APRI, FIB-4, Forns index, HGM1, and HGM2) include laboratory parameters that are routine parameters in the evaluation of CHC [10-13]. These indexes have shown an acceptable diagnostic performance for hepatic fibrosis in HIV/HCV-coinfected patients [24]. In our study, high levels of plasma CXCL10 might indicate an increased migration rate of inflammatory Th1 cells to the liver, causing liver injury; and conversely, HAART and control of HIV replication may down-regulate CXCL10 levels and delay liver damage. On the other hand, CXCL10 may be catabolised by DPP4, producing the short form of CXCL10 (CXCR3 antagonist) which might dissuade CXCR3+ T cells to fight against HCV infection in the liver [20]. In any case, our data indicate that CXCL10 may play an important role in the progression of liver fibrosis in HIV infected patients with CHC. Future studies are needed to clarify the entire role of CXCL10 in the progression of liver fibrosis.

Finally, we also found by the multivariate regression, that CXCL10 levels were significantly higher in HCV-genotype 1 patients than in HCV-genotypes 2/3/4. These results are directly in agreement with previous studies in HCV monoinfected patients [22], and indirectly with previous reports where HCV-genotype 1 also showed an association with an elevated inflammation [25], apoptosis [26], and activation of the endogenous IFN system [7]; and may influence the natural history of HCV and HIV-1 disease [27].However, this finding generates controversy, due to the fact that many of the published studies about circulating CXCL10 levels in

HCV-infection did not find any association with HCV-genotype [3, 4]. In addition, HIV coinfected patients with HCV-genotype 1 showed higher levels of CD81 in peripheral blood lymphocytes [28]; a protein that plays a main role in reducing the cell activation threshold and in promoting cell proliferation [29]. Furthermore, HCV-genotype 1 is associated with a significantly higher oxidative damage [30], which could induce a high rate of inflammation and further explain the high plasma CXCL10 levels. Therefore, HCV-genotype 1 might contribute to liver and systemic inflammation, which might explain the CXCL10 elevation in CHC patients.

Moreover, we have recently reported that non-responders HIV/HCV coinfected patients with HCV-genotype 1 showed significantly higher baseline CXCL10 levels than those who responded to HCV treatment [6]. In this study, we included 68 patients with HCV-genotype 1 (that were also included in the present study) where similar results have been observed (*data not shown*). On the one hand, high CXCL10 levels may indicate a pre-activation of the IFN system in liver [7], which indicates some possible defects at steps downstream of IFN-stimulated gene (ISG) expression, making these patients refractory to both endogenous IFN-α and IFN-α therapy [7]. On the other hand, the antagonist form of CXCL10 in the plasma of CHC patients with HCV-genotype 1 is also capable of binding CXCR3 as CXCL10 does, but in this case do not induce signalling, contributing therefore this inability to clear HCV infection.

In our study, we did not have the possibility of measuring both the complete (agonist) and truncated (antagonist) form of CXCL10 because the method used was not designed to distinguish between both forms. In addition, since CXCL10 levels seem to depend on the degree of inflammation and levels of DPP4, it is quite likely that some of the CXCL10 detected in our immunoassays, in our patients with high values of HOMA or non-invasive indices of fibrosis or HCV genotype 1, might be the antagonist form of CXCL10. This form may be implicated in the pathogenesis of HCV infection, liver damage, and HCV-antiviral therapy response via hindering the immune response against HCV infection [20, 21]. For this reason, in future studies, it would be interesting to analyze both the agonist and antagonist form of CXCL10 which could provide valuable information about the mechanisms in HCV disease pathogenesis, liver damage and response to treatment.

We should also underline that our study has some limitations that have to be taken into account for a correct interpretation of our findings: a) it was a cross-sectional design in a reduced number of patients, b) it was limited to patients with well-preserved immune function and the extrapolation to individuals with more marked immune suppression would require further study, c) as with any study measuring plasma levels of cytokines and/or chemokines, any additional unknown infection(s) could potentially influence our results, although we have no evidence. Indeed, we have checked that none of the patients had hepatitis B virus infection or active opportunistic infections.

In conclusion, plasma CXCL10 levels were influenced by several characteristics of patients related to HIV and HCV infections, insulin resistance, and liver fibrosis; indicating that CXCL10 may play an important role in the pathogenesis of both HCV and HIV infections.

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6. REFERENCES

[1] Heydtmann M, Adams DH. Chemokines in the immunopathogenesis of hepatitis C infection. Hepatology. 2009;49:676-88.

[2] 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;74:360-9.

[3] 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;13:969-76.

[4] Romero AI, Lagging M, Westin J, Dhillon AP, Dustin LB, Pawlotsky JM, et al. Interferon (IFN)-gamma-inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and ribavirin for chronic hepatitis C virus infection. J Infect Dis. 2006;194:895-903.

[5] Zeremski M, Markatou M, Brown QB, Dorante G, Cunningham-Rundles S, Talal AH. Interferon gamma-inducible protein 10: a predictive marker of successful treatment response in hepatitis C virus/HIV-coinfected patients. J Acquir Immune Defic Syndr. 2007;45:262-8.

[6] Vargas A, Berenguer J, Ryan P, Catalan P, Lopez JC, Cosin J, et al. Plasma interferon-gamma-inducible protein-10 can predict virologic response to hepatitis C virus therapy in HIV/HCV-coinfected patients with HCV genotype 1. J Acquir Immune Defic Syndr. 2010;54:219-20.

[7] Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, et al. Interferon signaling and treatment outcome in chronic hepatitis C. Proc Natl Acad Sci U S A. 2008;105:7034-9.

[8] Askarieh G, Alsio A, Pugnale P, Negro F, Ferrari C, Neumann AU, et al. Systemic and intrahepatic interferon-gamma-inducible protein 10 kDa predicts the first-phase decline in hepatitis C virus RNA and overall viral response to therapy in chronic hepatitis C. Hepatology. 2010;51:1523-30.

[9] Ryan P, Resino S, Miralles P, Cosin J, Lopez JC, Moreno S, et al. Insulin resistance impairs response to interferon plus ribavirin in patients coinfected with HIV and hepatitis C virus. J Acquir Immune Defic Syndr. 2010;55:176-81.

[10] Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518-26.

[11] Sterling RK, Chiu S, Snider K, Nixon D. The Prevalence and Risk Factors for Abnormal Liver Enzymes in HIV-Positive Patients without Hepatitis B or C Coinfections. Dig Dis Sci. 2007.

[12] Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology. 2002;36:986-92.

[13] Berenguer J, Bellon JM, Miralles P, Alvarez E, Sanchez-Conde M, Cosin J, et al. Identification of liver fibrosis in HIV/HCV-coinfected patients using a simple predictive model based on routine laboratory data. J Viral Hepat. 2007;14:859-69.

[14] Stylianou E, Aukrust P, Bendtzen K, Muller F, Froland SS. Interferons and interferon (IFN)-inducible protein 10 during highly active anti-retroviral therapy (HAART)-possible immunosuppressive role of IFN-alpha in HIV infection. Clin Exp Immunol. 2000;119:479-85.

[15] Molina-Pinelo S, Vallejo A, Diaz L, Soriano-Sarabia N, Ferrando-Martinez S, Resino S, et al. Premature immunosenescence in HIV-infected patients on highly active antiretroviral therapy with low-level CD4 T cell repopulation. J Antimicrob Chemother. 2009;64:579-88.

[16] Kuntzen T, Tural C, Li B, Feldmann G, Kupfer B, Nischalke HD, et al. Intrahepatic mRNA expression in hepatitis C virus and HIV/hepatitis C virus co-infection: infiltrating cells, cytokines, and influence of HAART. AIDS. 2008;22:203-10.

[17] Negro F, Sanyal AJ. Hepatitis C virus, steatosis and lipid abnormalities: clinical and pathogenic data. Liver Int. 2009;29 Suppl 2:26-37.

[18] Gonzalez VD, Landay AL, Sandberg JK. Innate immunity and chronic immune activation in HCV/HIV-1 co-infection. Clin Immunol. 2010;135:12-25.

[19] Lecube A, Hernandez C, Genesca J, Simo R. Proinflammatory cytokines, insulin resistance, and insulin secretion in chronic hepatitis C patients: A case-control study. Diabetes Care. 2006;29:1096-101.
[20] Casrouge A, Decalf J, Ahloulay M, Lababidi C, Mansour H, Vallet-Pichard A, et al. Evidence for an antagonist form of the chemokine CXCL10 in patients chronically infected with HCV. J Clin Invest. 2011;121:308-17.
[21] Charles ED, Dustin LB. Chemokine antagonism in chronic hepatitis C virus infection. J Clin Invest. 2011;121:25-7.
[22] Diago M, Castellano G, Garcia-Samaniego J, Perez C, Fernandez I, Romero M, et al. Association of pretreatment serum interferon gamma inducible protein 10 levels with sustained virological response to peginterferon plus ribavirin therapy in genotype 1 infected patients with chronic hepatitis C. Gut. 2006;55:374-9.
[23] Roe B, Coughlan S, Hassan J, Grogan A, Farrell G, Norris S, et al. Elevated serum levels of interferon-gamma -inducible protein-10 in patients coinfected with hepatitis C virus and HIV. J Infect Dis. 2007;196:1053-7.
[24] Resino S, Seoane JA, Bellon JM, Dorado J, Martin-Sanchez F, Alvarez E, et al. An artificial neural network improves the non-invasive diagnosis of significant fibrosis in HIV/HCV coinfected patients. J Infect. 2010;62:77-86.
[25] Zeremski M, Dimova R, Brown Q, Jacobson IM, Markatou M, Talal AH. Peripheral CXCR3-associated chemokines as biomarkers of fibrosis in chronic hepatitis C virus infection. J Infect Dis. 2009;200:1774-80.
[26] Panasiuk A, Parfieniuk A, Zak J, Flisiak R. Association among Fas expression in leucocytes, serum Fas and Fas-ligand concentrations and hepatic inflammation and fibrosis in chronic hepatitis C. Liver Int. 2010;30:472-8.
[27] Yoo TW, Donfield S, Lail A, Lynn HS, Daar ES. Effect of hepatitis C virus (HCV) genotype on HCV and HIV-1 disease. J Infect Dis. 2005;191:4-10.
[28] Micheloud D, Gonzalez-Nicolas J, Berenguer J, Lorente R, Miralles P, Lopez JC, et al. CD81 expression in peripheral blood lymphocytes before and after treatment with interferon and ribavirin in HIV/HCV coinfected patients. HIV Med. 2010;11:161-9.
[29] Levy S, Todd SC, Maecker HT. CD81 (TAPA-1): a molecule involved in signal transduction and cell adhesion in the immune system. Annu Rev Immunol. 1998;16:89-109.
[30] Cardin R, Saccoccio G, Masutti F, Bellentani S, Farinati F, Tiribelli C. DNA oxidative damage in leukocytes correlates with the severity of HCV-related liver disease: validation in an open population study. J Hepatol. 2001;34:587-92.