Influence of Genetic Polymorphisms of Tumor Necrosis Factor Alpha and Interleukin 10 Genes on the Risk of Liver Cirrhosis in HIV-HCV Coinfected Patients

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Abstract

Objective: Analysis of the contribution of genetic (single nucleotide polymorphisms (SNP) at position -238 and -308 of the tumor necrosis factor alpha (TNF-α) and -592 of the interleukin-10 (IL-10) promoter genes) and of classical factors (age, alcohol, immunodepression, antiretroviral therapy) on the risk of liver cirrhosis in human immunodeficiency (HIV)-hepatitis C (HCV) virus coinfected patients.

Patients and Methods: Ninety one HIV-HCV coinfected patients (50 of them with chronic hepatitis and 41 with liver cirrhosis) and 55 healthy controls were studied. Demographic, risk factors for the HIV-HCV infection, HIV-related (CD4+ T cell count, antiretroviral therapy, HIV viral load) and HCV-related (serum ALT concentration, HCV viral load, HCV genotype) characteristics and polymorphisms at position -238 and -308 of the tumor necrosis factor alpha (TNF-α) and -592 of the interleukin-10 (IL-10) promoter genes were studied.

Results: Evolution time of the infection was 21 years in both patients’ groups (chronic hepatitis and liver cirrhosis). The group of patients with liver cirrhosis shows a lower CD4+ T cell count at the inclusion in the study (but not at diagnosis of HIV infection), a higher percentage of individuals with previous alcohol abuse, and a higher proportion of patients with the genotype GG at position -238 of the TNF-α promoter gene; polymorphism at -592 of the IL-10 promoter gene approaches to statistical significance. Serum concentrations of profibrogenic transforming growth factor beta1 were significantly higher in healthy controls with genotype GG at -238 TNF-α promoter gene. The linear regression analysis demonstrates that the genotype GG at -238 TNF-α promoter gene was the independent factor associated to liver cirrhosis.

Conclusion: It is stressed the importance of immunogenetic factors (TNF-α polymorphism at -238 position) above other factors previously accepted (age, gender, alcohol, immunodepression), on the evolution to liver cirrhosis among HIV-infected patients with established chronic HCV infections.

Introduction

Chronic infection with hepatitis C virus (HCV) is characterized by a broad spectrum of clinical manifestations that can culminate in decompensated cirrhosis. An estimated 20–30% of infected individuals will develop cirrhosis while others largely remain asymptomatic [1].

Liver fibrosis is the most important prognostic factor in chronic HCV-infected patients [2]. The hepatic stellate cell is the major cell responsible for fibrosis in the liver, with activation of these cells being a key fibrotic event [3,4]. The influence of inflammatory mediators in this liver process has been theorized [5]: impaired intestinal permeability and microbial translocation favour the presence of increased serum endotoxin or lipopolysaccharide (LPS) concentration in patients with chronic hepatopathies [6]. After been recognised by a toll-like receptor (toll-like receptor 4 – TLR4+), endotoxin signalling triggers a cascade that leads to proinflammatory cytokine production, including tumour necrosis factor (TNF)-α synthesis [7,8]. TLR4 can also detect endogenous ligands, many of which are abundant during tissue injury, such as hyaluronan, fibronectin and heat shock proteins [7].

TNF-α can potentially affect liver fibrogenesis by stimulating hepatic stellate cells [9]. The pathogenic importance of TNF-α in liver disease has been previously demonstrated: besides the increased concentration of TNF-α in the liver of patients with chronic hepatitis C [10], it has been observed that serum levels of this cytokine are correlated with histological grading score of
TNF is a pro-inflammatory cytokine that down regulates the synthesis of pro-inflammatory cytokines, including TNF, and has a modulatory effect on hepatic fibrogenesis [15]. IL-10 levels differ widely between individuals, possibly because of polymorphisms in the promoter region of the IL-10 gene [23]. IL-10 polymorphisms have been studied in the context of hepatic fibrosis, with controversial results [24,25,26,27,28]. Additionally to the possible contribution of genetic factors, evolution to cirrhosis in HCV-induced chronic hepatitis is dependent of the presence of coinfection by hepatitis B virus, alcohol ingestion, or immunodeficiency (immunomodulation to prevent graft rejection or HIV coinfection), among others [29]. Effectively, liver fibrosis progression to cirrhosis is faster in HIV-HCV coinfected individuals than in HCV-mono-infected subjects [30,31]. In fact, complications of the hepatitis C virus (HCV) infection are one of the main causes of death in human immunodeficiency virus (HIV)-coinfected patients [32]. Thus, we would like to address the question of whether the possible genetic contribution to liver fibrosis progression might be overruled by more vigorous stimuli, such as the HIV coinfection. With this objective, we have assessed the influence of TNF and IL-10 single nucleotide polymorphisms on the progression of HCV-induced chronic hepatitis to cirrhosis in HIV-coinfected patients. Likewise, an analysis of serum concentration of molecules related with pro-inflammatory (TNF, interleukin-6 –IL-6-) and anti-inflammatory (IL-10) and fibrogenic molecules (transforming growth factor beta 1 –TGF-B1-) was performed in healthy controls and patients with the diverse genotypes.

### Materials and Methods

#### Design

This was a cross-sectional population association study.

#### Patients

All subjects were consecutively recruited from a prospectively collected cohort of HIV-infected patients treated at the HIV outpatient’s clinics of an university hospital. Caucasian patients with chronic HIV and HCV infection were studied. Patients were classified in those with cirrhosis (n = 41) and those with chronic hepatitis (n = 50). For the control group we studied a sample of healthy subjects recruited from voluntary hospital workers (n = 55), whose age and gender were comparable with the patients.

Patients were classified in those with cirrhosis (n = 41) and those with chronic hepatitis (n = 50). For the control group we studied a sample of healthy subjects recruited from voluntary hospital workers (n = 55), whose age and gender were comparable with the patients.

All were 18–70 years old. All patients had serum negative for hepatitis B surface antigen and antinuclear, antismooth muscle antibody and antimitochondrial antibody. None had hemosiderosis [11]; moreover, patients with increased serum levels of TNF-α or their receptors showed a reduced survival [12].

A wide range of TNF-α production has been observed and can be attributed to polymorphisms in the TNF-α promoter and their corresponding extended HLA haplotypes [13]. In particular, two common biallelic variants at the -308 (G or A) and -238 (G or A) positions of the TNF-α promoter have been the first to receive attention [14]. The TNF-α polymorphism in -308 and -238 positions of the TNF promoter has been involved in the variability of the histological severity of chronic hepatitis C infection [15,16,17,18,19]. A possible explanation to the variable progression of liver fibrosis was provided by Wilson et al [20] with the demonstration that carriage of the -308 allele A, a much stronger functional consequence, is associated with higher constitutive and inducible levels of TNF-α. However, a metaanalysis of 11 different studies about this topic has not detected association between this polymorphism and the risk of liver cirrhosis [21]. The -238 allele A functional consequences are not yet clear compared with -238 allele G [22].

Other cellular cytokine genes in which genetic variation has been examined within the context of fibrotic disease include interleukin-10 (IL-10). IL-10 is an anti-inflammatory cytokine that down regulates the synthesis of pro-inflammatory cytokines, including TNF-α, and has a modulatory effect on hepatic fibrogenesis [15]. IL-10 levels differ widely between individuals, possibly because of polymorphisms in the promoter region of the IL-10 gene [23]. IL-10 polymorphisms have been studied in the context of hepatic fibrosis, with controversial results [24,25,26,27,28].

### Table 1. Single nucleotide polymorphism of TNF-α and IL-10 genes studied in healthy controls and patients with HIV-HCV coinfection.

| Single nucleotide polymorphism | Genotype frequency (n, %) | Healthy controls (n = 55) | HIV-HCV coinfected patients (n = 91) | p |
|---|---|---|---|---|
| TNF rs361525 (- 238) | | | | |
| Genotype frequency | | | | |
| GG | 50 (93) | 74 (86) | 0,367 |
| GA | 4 (7) | 10 (12) | |
| AA | 0 (0) | 2 (2) | |
| Allelic frequency | | | | |
| G | 0,96 | 0,93 | 0,535 |
| A | 0,04 | 0,07 | |
| TNF rs1800629 (- 308) | | | | |
| Genotype frequency | | | | |
| GG | 43 (78) | 70 (81) | 0,527 |
| GA | 12 (22) | 15 (17) | |
| AA | 0 (0) | 2 (2) | |
| Allelic frequency | | | | |
| G | 0,88 | 0,90 | 0,821 |
| A | 0,12 | 0,10 | |
| IL10 rs1800872 (- 592) | | | | |
| Genotype frequency | | | | |
| CC | 24 (47) | 43 (49) | 0,758 |
| CA | 21 (41) | 38 (43) | |
| AA | 6 (12) | 7 (8) | |
| Allelic frequency | | | | |
| C | 0,69 | 0,70 | 1,000 |
| A | 0,31 | 0,30 | |

Note: In Genotype frequency, “n” expresses the number of patients in which the analysis of each polymorphism was successful. By that, it could be no coincidence among the sum of genotype frequencies and the absolute number of patients.

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matosis gene-related iron overload as assessed by serum iron markers, biopsy, and genotyping where indicated. Serum α1 antitrypsin and ceruloplasmin levels were normal.

### Definitions

Positive serum antibody to HIV was required for the diagnosis of HIV infection. Patients were classified according to the 1993 Centers for Disease Control and Prevention classification of HIV infection. Spanish Group for AIDS Study guidelines (www.gesida.es) were used to indicate the antiretroviral treatment (HAART). Plasma HIV RNA load was quantified by polymerase chain reaction (PCR) assay; a value lower than 50 copies/ml was considered as undetectable HIV load. CD4+ T cell counts were determined by standard flow cytometry; values obtained at diagnosis of HIV infection and at the time of inclusion were considered.

Positive serum antibody to HCV and persistent (more than 6 months) HCV RNA were required for the diagnosis of chronic HCV infection. Diagnosis of chronic hepatitis or cirrhosis was

### Table 2. Demographic, clinical, virological and immunological characteristics of HIV-HCV coinfected patients with chronic hepatitis or liver cirrhosis.

| General characteristics | HIV-HCV coinfected patients with chronic hepatitis (n = 50) | HIV-HCV coinfected patients with liver cirrhosis (n = 41) | p  |
|-------------------------|----------------------------------------------------------|----------------------------------------------------------|----|
| Age (years)             | 45 (40–49)                                               | 47 (44–49)                                               | 0.056 |
| Male sex (n, %)         | 39 (78)                                                  | 36 (88)                                                  | 0.275 |
| Drug use as risk factor (n, %) | 30 (60)                                                   | 27 (66)                                                  | 0.665 |
| Evolution of the infection (years) | 21 (19–24)                                               | 22 (19–25)                                               | 0.716 |
| Alcohol ingestion (≥ 50 g/day) (n, %) | 23 (49)                                                  | 32 (78)                                                  | 0.008 |
| HIV-related characteristics |                                                        |                                                         |    |
| CD4+ T lymphocytes/mm3 at HIV diagnosis | 188 (110–306)                                           | 146 (60–246)                                           | 0.116 |
| CD4+ T lymphocytes/mm3 at inclusion | 386 (276–574)                                           | 333 (156–392)                                           | 0.002 |
| CDC C stage (n, %)      | 20 (44)                                                  | 19 (49)                                                  | 0.667 |
| Patients in antiretroviral therapy (n, %) | 47 (94)                                                 | 39 (95)                                                  | 1.000 |
| Undetectable HIV viral load (<50 copies/ml) at inclusion | 39 (78)                                                  | 32 (78)                                                  | 1.000 |
| HCV-related characteristics |                                                        |                                                         |    |
| Diagnosis by liver biopsy (n, %) | 32 (64)                                                  | 31 (76)                                                  | 0.261 |
| Liver stiffness (Transient elastometry) (kPa) (median, range) | 8.4 (7.1 – 13.0)                                       | 28.4 (15.4 – 76.8)                                       | <0.001 |
| HCV genotype 1 or 4 (n, %) | 34 (68)                                                  | 23 (56)                                                  | 0.342 |
| HCV viral load (1000 x IU/l) at inclusion | 1192 (206–3133)                                        | 875 (288–2570)                                          | 0.906 |
| ALT (UI/l)              | 40 (31–56)                                               | 45 (33–85)                                               | 0.544 |

### Table 3. Single nucleotide polymorphism of TNF-α and IL-10 genes studied in patients with HIV-HCV coinfection with chronic hepatitis or liver cirrhosis.

| Single nucleotide polymorphism | HIV-HCV coinfected patients with chronic hepatitis (n = 50) | HIV-HCV coinfected patients with liver cirrhosis (n = 41) | p  |
|--------------------------------|----------------------------------------------------------|----------------------------------------------------------|----|
| **TNF rs361525 (-238)**        |                                                           |                                                         |    |
| Genotype frequency (n, %)      |                                                          |                                                         |    |
| GG                             | 36 (77)                                                  | 38 (97)                                                  | 0.009 |
| GA                             | 10 (21)                                                  | 0 (0)                                                    | 0.939 |
| AA                             | 1 (2)                                                    | 1 (3)                                                    | 0.939 |
| **TNF rs1800629 (-308)**       |                                                           |                                                         |    |
| Genotype frequency (n, %)      |                                                          |                                                         |    |
| GG                             | 39 (80)                                                  | 31 (82)                                                  | 0.072 |
| GA                             | 9 (18)                                                   | 6 (16)                                                   | 0.072 |
| AA                             | 1 (2)                                                    | 1 (3)                                                    | 0.072 |
| **IL10 rs1800872 (-592)**      |                                                           |                                                         |    |
| Genotype frequency (n, %)      |                                                          |                                                         |    |
| CC                             | 29 (59)                                                  | 14 (36)                                                  | 0.072 |
| CA                             | 16 (33)                                                  | 22 (56)                                                  | 0.072 |
| AA                             | 4 (8)                                                    | 3 (8)                                                    | 0.072 |

Note: In Genotype frequency, “n” expresses the number of patients in which the analysis of each polymorphism was successful. By that, it could be no coincidence among the sum of genotype frequencies and the absolute number of patients.

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established according to histological criteria when liver biopsy was performed [33], or by transient elastography, performed according to a standardized technique by one trained operator (JAGG) (according to data validated in HIV-HCV coinfected patients using liver biopsy as reference, patients with a liver stiffness $14.6$ kPa were classified as individuals with cirrhosis [34].

Duration of hepatitis C infection was estimated using an interviewer-assisted questionnaire assessing risk factors for HCV infection. The earliest exposure was designated as the point of acquisition [35].

Alcohol abuse was considered when an ingestion higher than $50$ g/day during at least $5$ years occurred.

Laboratory determinations

HIV-1 infection was diagnosed using an ELA (Abbott Laboratories, North Chicago, IL, USA) and confirmed by New Lat Blot I (Bio-Rad, Marnes La Coquette, France). Plasma HIV-1 viral load was determined by the Cobas Amplicor HIV Monitor (Roche Diagnostics, Basel, Switzerland); the cutoff for undetectable viral load was $50$ copies/$mL$. Blood CD4$^+$ T-cell count were determined by flow cytometry (FAC Scan, Becton Dickinson Immunocytometry Systems, San Jose, CA, USA).

Antibodies anti-HCV were detected by 3rd generation ELISA (Abbott Diagnostics, Chicago, USA). Plasma HCV RNA was detected by quantitative PCR (Amplicor HCV Monitor test; Roche Diagnostics, Basel, Switzerland); the cutoff for undetectable viral load was $50$ copies/$mL$. Blood CD4$^+$ T-cell count were determined by flow cytometry (FAC Scan, Becton Dickinson Immunocytometry Systems, San Jose, CA, USA).

TNF-$\alpha$ and IL-10 polymorphisms

The $-308$ TNF-$\alpha$ polymorphism (rs1800629) consists of a G to A substitution at position $-308$ in the proximal promoter of the TNF-$\alpha$ gene. The $-238$ TNF-$\alpha$ polymorphism (rs361525) consists of a G to A substitution at position $-238$ in the proximal promoter of the TNF-$\alpha$ gene. The IL-10 polymorphism (rs1800872) consists of a C to A substitution at position $-592$ in the proximal promoter of the IL-10. Each polymorphism was genotyped by predesigned Taqman assays (Applied Biosystems, Foster City, CA, EEUU), following the instructions of the manufacturer’s.

Intracellular expression and LPS-stimulated secretion of cytokines

Blood samples from healthy controls with genotype GG (n = 10) and with genotype GA (n = 10) at $-238$ of the TNF-promoter, collected in pyrogen-free heparinized tubes (Biofreeze, Costar,
PBMC were incubated with combinations of fluorescein-isothiocyanate (FITC), phycoerythrin (PE) and peridinin chlorophyll protein (PerCP)-labelled monoclonal antibodies (Becton-Dickinson, San Jose, CA, EEUU) and analysis of the unstimulated intracellular expression of TNF-α, IL-6, TGF-β1 and IL-10 was performed. Proportions of monocyte-expressing each cytokine were determined in PBMC by two-colour flow cytometry in a FACScalibur cytometer using Cell Quest and Paint-A-Gate software (Becton-Dickinson, San Jose, CA, USA).

In a separate set of experiments, whole-blood samples were mixed 1:1 with RPMI 1640 (Gibco, Germany) and lipopolysaccharide –LPS- (Escherichia coli 0111:B4, Difco, Detroit, MI) was added to the final concentrations of 1000 ng/mL; cells were stimulated for 4h at 37°C under 5% CO2 atmosphere. In addition, unstimulated baseline samples were obtained to act as a control. Concentration of TNF-α, IL-6, TGF-β1 and IL-10 in the supernatant of the cultures, after 24 hours incubation, was assayed.

Concentration of pro- and anti-inflammatory and of fibrogenic molecules

Serum and culture supernatants concentrations of TNF-α, IL-6 and IL-10 were analyzed using Milliplex MAP kit High Sensitivity Human Cytokine (Millipore, Billerica, MA, USA). Serum TGF-β1 levels were detected by Quantikine Human Immunoassay (R&D, Minneapolis, MN, USA).

Statistical analysis

Descriptive data were expressed as the median (25–75 interquartile range –IQR-) or as absolute number (percentage). Qualitative variables, including genotype distribution, were compared by the chi-square test or Fisher’s exact test when necessary. Quantitative variables were compared using the Mann-Whitney U test or ANOVA when necessary. The Pearson’s correlation coefficient analysed the association between quantitative variables. The independent predictive value of genotypes and of those other variables associated to liver cirrhosis in the bivariate analysis was analyzed by linear regression analysis. A p value <0.05 was considered significant. The statistical analysis was carried out using the SPSS 15.0 statistical software package (SPSS Inc., Chicago, IL, USA).

Ethical aspects

This study was performed according to the Helsinki Declaration. The project was approved by the hospital ethical research committee. Informed consent was obtained from each participant.

Results

Ninety one HIV-HCV coinfected patients and fifty five healthy controls were studied. Table 1 shows the genotype distribution and allele frequencies of the TNF-α -238 G to A, TNF-α -308 G to A and IL-10 -592 C to A gene promoter single nucleotide polymorphisms for the control group and the HIV-HCV coinfected patients. Observed genotypic frequencies approximated to those expected based on allele frequency calculations, and thus conformed to Hardy-Weinberg equilibrium.
Bivariate comparisons between HIV-HCV patients with chronic hepatitis and cirrhosis

The two patients’ groups, with and without cirrhosis, were comparable in terms of age, gender, risk factors for the infection, HCV viral load and viral genotypes. The frequency of alcohol use was significantly higher in patients with cirrhosis. More importantly, estimated duration of HCV infection was comparable in patients with chronic hepatitis and those with cirrhosis (Table 2). Analysis of HIV-related immune characteristics demonstrated that the CD4 T cell count at inclusion in the study was significantly lower in patients with cirrhosis, although CD4 T cell at diagnosis of HIV infection showed no significant differences. The percentages of patients with undetectable HIV viral load due to antiretroviral therapy were similar in both groups. HIV-related characteristics demonstrated that there were no significant differences between serum ALT concentration, HCV genotype, and HCV viral load of both groups.

Different distributions were noted in the single nucleotide polymorphisms among the patient groups (Table 3). Specifically, the TNF-α -238 A alleles were significantly more common in patients who suffered from chronic hepatitis than in those patients with cirrhosis. Differences in the distribution of IL-10 genotypes approach to significance. These differences persisted when only those patients diagnosed by liver biopsy (chronic hepatitis, n = 32; liver cirrhosis, n = 31) were considered (data not shown).

Analyses of the association between epidemiological, clinical, HIV- or HCV-related characteristics and TNF-α -238 phenotypes according to polymorphisms did not show any significant differences. In brief, age, sex, alcohol ingestion, CD4+ T cell count at diagnosis and at inclusion, percentage of patients with undetectable HIV load, HCV genotypes, HCV load or serum ALT concentration were similar in patients with genotype GG or GA/AA of TNF-α -238 (p > 0.05 in each case).

Relation among the detected polymorphisms and the serum concentrations of pro- and anti-inflammatory and profibrogenic molecules

Serum concentrations of TNF-α, IL-6, IL-10 and TGF-β1 were measured, separately, in healthy controls and in patients with HIV-HCV coinfection, distributed in two groups, those without and those with liver cirrhosis. In healthy controls, increased serum concentrations of TGF-β1 were detected in individuals with genotype GG at -238 position of the TNF-α promotor gene with reference to those with genotype GA or AA. The rest of comparison showed no significant differences among individuals with the different genotypes, both in healthy controls and in HIV-HCV coinfected patients (Figure 1).

Due to the relation between polymorphism at -238 position of the TNF-α promotor gene and serum concentration of TGF-β1, stimulation experiments were performed. Ten healthy controls with the genotype GG at -238 position of the TNF-α promotor gene and ten with the genotype GA were selected. No significant differences were detected when compared intracellular expression of TNF-α, IL-6, IL-10 and TGF-β1 in peripheral blood monocytes from both groups. LPS-stimulated secretion of these cytokines was analyzed in the culture supernatants. Peripheral blood mononuclear cells from healthy controls with the genotype GG at -238 position of the TNF-α promotor gene synthesized significantly higher concentrations of TGF-β1 than those of those with the genotype GA. Concentrations of TNF-α, IL-6 and IL-10 were similar in the culture supernatants from patients with genotype GG and GA (Table 4).

Multivariate analysis of parameters associated with cirrhosis

We evaluated, using a linear regression analysis, those factors independently associated to cirrhosis. The evaluated parameters were those whose differences among patients with chronic hepatitis and those with liver cirrhosis were lower than 0.1 (age, alcohol abuse, CD4 T cell count at inclusion, TNF-α -238 and IL-10 -592 polymorphism) in the bivariate analysis and those other with possible clinical significance (CD4 T cell count at diagnosis of HIV infection) (Table 5). Continuous variables were categorized in function of their median values. In the resultant model, the polymorphism of TNF-α at -238 was the only factor associated with cirrhosis. Kaplan-Meier curves in function of the TNF-α -238 polymorphism are shown in Figure 1.

When the patients diagnosed by liver biopsy were exclusively considered, we also detect that the polymorphism of TNF-α at -238 was the only factor associated with cirrhosis (standardized coefficient beta (IC 95%), 0.395 (0.156–0.534), p = 0.001).

Discussion

Several factors, including age, alcohol abuse, duration of the HCV infection or the presence of immunodepression, influence the natural history of HCV-induced chronic hepatitis to cirrhosis [1,2,29,30,31]. However, the contribution of genetic factors to this evolution has been rarely examined. Moreover, the independent contribution of genetic modifications to the evolution to liver cirrhosis in HIV-HCV coinfected patients has not been practically studied, with the exception of the IL28 B polymorphism [36].

The present work has analyzed the influence of genetic parameters on the risk of cirrhosis in a series of HIV-HCV coinfected patients. Selected genetic markers were those influencing the expression or secretion of molecules implicated in innate or acquired immunity, such as the TNF-α and IL-10. In this cohort of Caucasian patients, we found that TNF-α -238 polymorphism was involved in the risk of liver cirrhosis. Our data also indicated that differences in disease outcome among HIV-infected patients with established chronic HCV infections could have more to do with immunogenetic factors than with other factors previously accepted (age, gender, alcohol consumption, immunodepression), although several of them were associated with cirrhosis in the univariate analysis. This association persisted when only those patients with histologic diagnosis of chronic hepatitis or liver cirrhosis were considered.

The A allele at -238 position of the TNF-α promotor gene has been associated with a more strict virological control in HIV-infected patients [37]. Moreover, several studies in HCV-monoinfected patients have reported that the A allele at -238 position of the TNF-α promotor gene was associated with development of chronic active hepatitis C, advanced fibrosis progression or higher risk of cirrhosis [16,17], although others had no demonstrated any association [18]. Our study in HIV-HCV coinfected patients has demonstrated precisely the opposite finding: those patients with a genotype GG shows an increased association with cirrhosis. The discrepancy could be explained by the consideration in our study, and not in the others, of the several factors which can be implicated in the evolution (age, sex, alcohol abuse), those related with the immunodepression (CD4 T cell count at diagnosis or at inclusion), as well as the evaluation of the independent influence of each one in a multivariate analysis.

The A allele at -238 position of TNF-α promotor gene has been associated with a more intense inflammatory activity [22]; however, data about its influence on parameters associated to fibrosis are lacking. After TNF-α activation, Kupffer cells secrete...
The gene encoding TNF-α is located within the HLA-region, the relation between HLA-haplotypes and TNF-α polymorphisms, including the influence on disease severity or chronic hepatitis evolution to cirrhosis, is possible. As a factor implicated in the evolution of inflammatory diseases, it can be hypothesized the influence of TNF-α polymorphism in a complex inflammatory and immune cascade inducing an increased ability to secrete TGF-β1. In each case, the more intense fibrogenic activity, detected in patients with genotype GG, could be influencing the collagen secretion by hepatic stellate cell, favoring the fibrosis progression in the liver.

The influence of the GG genotype at -238 position of TNF-α promoter gene was observed after a prolonged period: in fact, Kaplan Meier analysis demonstrated that curves of development of cirrhosis in function of the presence of genotypes GG or GA and AA at -238 position of TNF-α promoter gene begin to be clearly different after 20 years of evolution (Figure 2).

IL-10 is a cytokine which down-regulates the inflammatory response and modulate the liver fibrogenesis [42]. The polymorphism at -592 position of the IL-10 promoter gene has been associated with more accelerated progression of HIV infection [43] and with persistent HCV infection [28]. Data about influence on liver fibrosis progression are controversial [24,25,26,27]. In our series, the genotype GA at -592 position of the IL-10 promoter gene was associated with a near significant higher frequency of liver cirrhosis in the bivariate analysis. This haplotype has been correlated with a decreased synthesis of IL-10 [23], suggesting that a lower anti-inflammatory activity could be implicated in the progression of liver fibrosis. However, serum concentrations of IL-10 were similar in healthy controls with the different IL-10 genotypes in our study. Moreover, the polymorphism at -592 position of the IL-10 promoter gene was not associated with cirrhosis in the multivariable analysis.

Several characteristics support the validity of our results: a) No significant difference existed between the distribution of TNF-α and IL-10 genotypes in the healthy control and HIV-HCV groups. b) Populations of Spanish healthy controls and HIV- or HCV-infected patients shows similar frequency of the genotypes tested [37,44]. c) Data were similar when only those patients diagnosed of chronic hepatitis or cirrhosis by liver biopsy were considered, d) The evolution time from the infection until the moment of the inclusion was similar in HIV-HCV patients with chronic hepatitis and those with liver cirrhosis, excluding the possibility that cirrhosis developed exclusively as a consequence of a higher time with the infection. e) Patients were carefully evaluated for every one of the different known factors influencing the evolution to cirrhosis (age, sex, ethanol abuse, CD4+ T cell count at diagnosis and at inclusion, antiretroviral therapy or undetectable HIV viral load).

In conclusion, a GG genotype at -238 position in the TNF-α promoter gene influences the risk of liver cirrhosis in HIV-HCV infected patients, even more than those classically accepted factors influencing the progression of liver fibrosis. The implications of the influence of this polymorphism include the need of a more strict vigilance and counseling about other risk factors in them.

**Author Contributions**

Conceived and designed the experiments: JAGG SC. Performed the experiments: SC MM MMi0 PR-C CF-G JAGG. Analyzed the data: JAGG SC. Contributed reagents/materials/analysis tools: SC MM CF-G JAGG. Wrote the paper: JAGG SC.
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