Improvement of liver disease and inflammation in patients with advanced HCV-related cirrhosis after antiviral therapy

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Research

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Abstract

Eradication of hepatitis C virus (HCV) promotes an improvement in liver disease and deactivation of the immune system. Here, we aimed to evaluate the changes in liver disease scores and plasma biomarkers following HCV clearance with direct-acting antivirals (DAAs) in patients with advanced HCV-related cirrhosis. We performed a prospective study of patients with advanced cirrhosis (liver decompensation or liver stiffness measurement [LSM] ≥ 25 kPa or hepatic liver pressure gradient [HVPG] ≥ 10 mmHg or Child-Pugh-Turcotte (CTP) ≥ 7) who received DAA therapy. Variables were assessed at baseline and 48 weeks after HCV treatment completion. Statistical analyses were performed employing Generalized Linear Mixed Models (GLMM). In this study, 62 patients (12 HCV-monoinfected and 50 HIV/HCV-coinfected) who achieved SVR were included in the analysis. We found significant decreases in LSM, HVPG, LBP, IP-10, IL-8, IL-1 β , IL-18, IL-1RA, OPG, sVCAM-1, sICAM-1, TNFR-I, PAI-1, and VEF-A during follow-up. Variations in most outcome measures were similar as for HCV-monoinfected and HIV/HCV-coinfected patients. We found significant positive associations between changes in LBP, IP-10, MCP-1, IL-8, IL-1 β , IL-18, IL-6, OPG, sVCAM-1, sICAM-1, and PAI-1 and LSM values after HCV clearance. We found variations in LBP, IL-8, IL-6, IL-18, OPG, PAI-1, and D-dimer were positively associated with changes in HVPG values; and variations in IP-10, IL-1 β , IL-6, TNF- α , IL-1RA, OPG, and sICAM-1 were related to changes in CTP score. In conclusion, the eradication of HCV with all-oral DAAs promoted a decrease in the severity of advanced cirrhosis and plasma biomarkers (inflammation, coagulopathy, and angiogenesis), which were positively associated with each other.

Background

Hepatitis C virus (HCV) chronically infects about 71 million people in the world (1% prevalence) [1]. Chronic hepatitis C (CHC) causes progressive liver inflammation, which promotes the development of hepatic cirrhosis after 10 to 20 years of HCV infection, with a 3–6% annual risk for hepatic decompensation among cirrhotic patients [1]. HCV infection triggers an immune response against the viral infection, but it also promotes chronic inflammation, immune activation, and immune dysfunction that accelerate the development of the liver fibrosis and other comorbidities [2, 3]. Additionally, in advanced stages, cirrhosis-associated immune dysfunction (CAID) usually appears, characterized by higher levels of inflammation, immune activation, and immunosuppression in the liver and peripheral blood [4]. The extent of CAID is directly related to the severity of liver disease and plays a decisive role in its progression to hepatic decompensation [4]. Patients with hepatic decompensation may develop complications related to portal hypertension, such as ascites, jaundice, variceal bleeding, or hepatic encephalopathy; decreasing quality of life and survival rates [1]. The introduction of direct-acting antiviral agents against HCV (DAAs) has increased sustained virologic response (SVR) rates in patients with advanced HCV-related cirrhosis, which may improve quality of life and reduce morbidity from cirrhosis [5]. Additionally, significant decreases in liver disease scores [liver stiffness measurement (LSM), hepatic venous pressure gradient (HVPG) or Child-Pugh-Turcotte (CTP)] [6–14] and plasma biomarkers related to

inflammation and immune activation [15–20] have been described in HCV-monoinfected patients after SVR with all-oral DAAs.

The human immunodeficiency virus (HIV) co-infects a large number of patients infected with HCV since both viruses share the same routes of transmission [21]. HIV infection accelerates the course of CHC, resulting in higher rates of cirrhosis, end-stage liver disease, and death when compared to HCV-monoinfected patients [22]. In HIV/HCV-coinfected patients, HIV infection increases a series of negative factors such as HCV replication, HCV-induced hepatic inflammation, hepatocyte apoptosis, and microbial translocation, while it also leads to a deterioration of the specific immune responses against HCV [23, 24]. Antiretroviral therapy (ART) delays the progression of fibrosis and reduces clinical complications among HIV/HCV-coinfected patients, but despite suppressive ART, these patients still have abnormally high levels of plasma biomarkers related to bacterial translocation, immune activation, inflammation and coagulation [24], which are related to higher morbidity and mortality [25]. Similarly, DAA treatments against HCV infection also achieve elevated SVR rates [26, 27] and delay CHC progression in HIV/HCV-coinfected patients [28, 29]. Several reports have also shown a significant decrease in plasma biomarkers related to inflammation and immune activation after SVR with all-oral DAAs in HIV/HCV-coinfected patients [18, 30–34]. However, DAA therapy alone does not completely block uncontrolled inflammation and liver injury, particularly with advanced liver disease [35]. Thus, some cirrhotic patients who achieve SVR with DAAs remain at risk of cirrhosis progression and developing hepatocellular carcinoma [2].

Despite many reports that have shown significant decreases in liver disease scores and plasma biomarkers after SVR with DAAs therapy, there still is a lack of consistency in the characteristics of these studies, such as the time points after completion of HCV treatment and statistical analyses used, as well as the biomarkers analyzed and identified to be statistically different and liver disease stage of the patients included.

In this study, we assessed changes in liver disease scores (LSM, HVPG, and CTP) and plasma biomarkers related to inflammation, coagulopathy, and angiogenesis in patients with advanced HCV-related cirrhosis following SVR with all-oral DAAs, as well as the association between the changes in the two set of markers during the follow-up.

Patients And Methods

Patients

We carried out a multicenter prospective study of 62 patients with advanced HCV-related cirrhosis who started anti-HCV therapy with all-oral DAAs, 12 HCV-monoinfected patients (HCV-group) and 50 HIV/HCV-coinfected patients (HIV/HCV-group). Patients were recruited at four tertiary referral hospitals in Madrid (Spain) between January 2015 and June 2016 (ESCORIAL study; see **Appendix**). This study was approved by the Research Ethics Committee of the Instituto de Salud Carlos III (CEI PI 41_2014) and was

conducted according to the Declaration of Helsinki. All participants gave their written consent before enrollment.

The inclusion criteria were: 1) active HCV infection at baseline defined by detectable plasma HCV-RNA; 2) advanced cirrhosis (prior history of liver decompensation (ascites, bleeding esophageal varices, hepatic encephalopathy), or LSM $\geq$ 25 kPa, or HVPG $\geq$ 10 mmHg or CTP $\geq$ 7); 3) starting anti-HCV therapy with all-oral DAAs (without interferon (IFN) or ribavirin) and achieving a SVR defined as an undetectable HCV-RNA load 12 weeks after finalization of anti-HCV therapy.

From 97 patients included in the ESCORIAL study and with plasma sample at baseline, 35 were lost to follow-up (2 deaths, 6 abandonment of the study, 2 virologic failure, and 25 unavailable at the end of follow-up (48 weeks after completing HCV therapy)). Therefore, 62 patients were included in the study: 12 HCV-monoinfected patients (HCV-group) and 50 HIV/HCV-coinfected patients (HIV/HCV-group).

Clinical data

Epidemiological and clinical data were collected through an online form, which fulfilled the confidentiality requirements. These data were monitored to verify that all the information in the database matched the patients' records.

The LSM was carried out by trained operators of transient elastography (FibroScan®, Echosens, Paris, France), as we previously described [36]. LSM values were reported in kilopascals (kPa) and range from 2.5 to 75 kPa. The CTP score was calculated from five factors (total bilirubin, albumin, international normalized ratio, ascites, and encephalopathy) and range from 5 to 15 points. Hemodynamic studies were performed after overnight fasting under light sedation with intravenous midazolam, as we previously described [37]. The HVPG was measured as the difference between wedged hepatic venous pressure and free hepatic venous pressure in millimeters of mercury (mmHg).

Enzyme-linked immunosorbent assays

The Spanish HIV Hospital Gregorio Marañón (HGM) BioBank was responsible for collecting the biological samples and storing them until use at -80°C. Plasma biomarkers were measured by ProcartaPlex™ multiplex immunoassay (Bender MedSystems GmbH, Vienna, Austria) using a Luminex 200™ analyzer (Luminex Corporation, Austin, TX, United States) according to the manufacturer's specifications. The plasma biomarkers measured by multiplex ELISA were: i) inflammation: IFN- γ -inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP1), interleukin (IL)-8; IL-1 β , IL-18, IL-6, tumor necrosis factor alpha (TNF- α), interleukin-1 receptor antagonist (IL-1RA), soluble receptor activator of nuclear factor-kappa B ligand (sRANKL) and osteoprotegerin (OPG); ii) endothelial dysfunction: soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular cell adhesion molecule 1 (sICAM-1); and soluble tumor necrosis factor receptor 1 (sTNFR-1); iii) coagulopathy: plasminogen activator inhibitor-1 (PAI-1)

and D-dimer; iv) angiogenesis: vascular endothelial growth factor A (VEGF-A) and soluble receptor 1 for vascular endothelial growth factor (sVEGF-R1). Because a high proportion of the analyzed samples were below the lower limit of detection, we used the raw fluorescence intensity values in arbitrary units, without subtracting blank, as a relative quantification of the analyte abundances [38].

We also used commercial simple ELISA assays for biomarkers that were not available by multiplex ELISA: Lipopolysaccharide binding protein (LBP) (R&D Systems, Minneapolis, USA), soluble CD14 (sCD14) and fatty acid binding protein 2 (FABP-2) (Raybiotech, Georgia, USA). The lipopolysaccharide (LPS; Hycult Biotech, Uden, The Netherlands) was evaluated by a Limulus amoebocyte lysate chromogenic endpoint ELISA.

Statistical analysis

Stata 15.0 (StataCorp, Texas, USA) and SPSS 22.0 (SPSS INC, Chicago, IL, USA) were used to perform the statistical analyses. All p -values were two-tailed and were corrected for multiple testing to reduce the risk of a spurious result by using the false discovery rate with Benjamini and Hochberg (q -values). The statistical significance was defined as $p \leq 0.05$ or $q \leq 0.05$, as appropriate.

Categorical variables were analyzed by the Chi-square test or Fisher's exact test, as required. Continuous variables were analyzed by Mann-Whitney U test (independent groups). Moreover, we used Generalized Linear Mixed Models (GLMM) with a gamma distribution (log-link) to evaluate repeated measurements. Gamma distribution in GLMM is recommended for modeling skewed continuous outcomes. The log-link was applied only to the outcome measure or dependent variable (y), which were the severity scores of liver cirrhosis and plasma biomarkers values. Our model only included two factors or independent variables (x), group (HIV/HCV-group vs HCV-group) that is the between-subject effect and time (basal vs final) that is the within-subject factor time. The interaction between group and time was taken into account. This analysis gives us the estimation of average increase ($\Delta x = \text{final value (x2)} - \text{baseline value (x1)}$) of each biomarker in each of the two study groups. GLMM models were also used to analyze the association between the change in plasma biomarkers (Δx) and the changes in severity scores of liver disease (Δy) during the follow-up. This analysis gives us the regression coefficient (β), which indicates the size and direction of the effect, according to your positive or negative sign.

Finally, GLMM was used to evaluate whether the change in outcome measures (liver disease scores and plasma biomarkers values) during the follow-up was similar between the HIV/HCV group and HCV group (impact of HIV infection). In this case, GLMM was adjusted by the most relevant patient's characteristics at baseline: gender, age, alcohol consumer, intravenous drug user, previous IFN therapy, HCV genotype, $\log_{10}$ HCV RNA, and statins. This analysis gives us an estimate of the difference between the increments for each group (Δx).

Results

Baseline characteristics of patients

Table 1 shows the epidemiological and clinical characteristics of the 62 patients with advanced HCV-related cirrhosis (12 HCV-monoinfected and 50 HIV/HCV-coinfected patients). The HIV/HCV-group were younger (p -value=0.002) and had less percentage of patients previously treated with IFN therapy (p -value=0.021), but had a higher percentage of intravenous drug users (p -value<0.001). All HIV/HCV-coinfected patients were on ART and plasma HIV viral load was undetectable (<50 copies/mL). None of the participants were actively using injection drugs.

Baseline values of the outcome measures

Table 2 shows the baseline values of the analyzed markers in this study. Regarding liver disease, the HIV/HCV-group had a lower percentage of patients with $CTP \geq 7$ (p -value=0.014) and lower values of sCD14 (p -value=0.001), FABP-2 (p -value=0.009), and sRANKL (p -value=0.029) than HCV-monoinfected patients. However, only the difference in sCD14 remained statistically significant after an adjustment for multiple comparisons (q -value=0.029).

Change in outcome measures after achieving SVR

The changes in the outcome measures during the follow-up for all patients are shown in **Figure 1** (full description in **Additional File 1 & 2**). We found significant decreases in severity scores of liver disease [LSM (q -value<0.001) and HVPg (q -value=0.005)] and plasma biomarkers [LBP (q -value<0.001), IP-10 (q -value<0.001), IL-8 (q -value<0.001), IL-1 β (q -value=0.035), IL-18 (q -value<0.001), IL-1RA (q -value=0.015), OPG (q -value<0.001), sVCAM-1 (q -value<0.001), sICAM-1 (q -value<0.001), TNFR-I (q -value=0.041), PAI-1 (q -value<0.001), and VEGF-A (q -value=0.002)].

We also analyzed the study population stratified by HIV coinfection (see **Additional File 3**). The HCV-group showed significant decreases only in IP-10 (q -value=0.001), sICAM-1 (q -value<0.001) and PAI-1 (q -value=0.019), while five other markers (LSM, IL-18, TNF- α , OPG, and sVCAM-1) were close to statistical significance (p -value $\leq$ 0.05 and q -value $\leq$ 0.1). The HIV/HCV-group showed significant decreases in LSM (q -value<0.001), HVPg (q -value=0.011), CTP (q -value=0.045), LBP (q -value<0.001), IP-10 (q -value<0.001), IL-8 (q -value<0.001), IL-18 (q -value<0.001), IL-1RA (q -value=0.013), OPG (q -value<0.001), sVCAM-1 (q -value<0.001), sICAM-1 (q -value<0.001), PAI-1 (q -value=0.001), and VEF-A (q -value=0.006)].

We used an adjusted GLMM to assess whether there were differences in the change of outcome measures during the follow-up between the two study groups (HIV/HCV-group vs HCV-group). In this case, significant differences between groups were found in CTP (p -value=0.002) and TNFR-I (p -value=0.012), but these differences disappeared after correcting for multiple testing (see **Additional File 4**). This indicates that changes during the follow-up in outcome measures were very similar between the HIV/HCV-group and the HCV-group for most markers.

Association between changes in plasma biomarkers and liver disease scores after reaching SVR

The summary of associations between the change in values of plasma biomarkers and severity scores of liver disease is shown in **Figure 2** (full description in **Additional File 5**). For LSM values (**Figure 2A**), we found a significant positive association with LBP (q -value<0.001), IP-10 (q -value<0.001), MCP-1 (q -value=0.016), IL-8 (q -value<0.001), IL-1 β (q -value=0.001), IL-18 (q -value<0.001), IL-6 (q -value<0.001), OPG (q -value<0.001), sVCAM-1 (q -value<0.001), and sICAM-1 (q -value<0.001), and PAI-1 (q -value=0.001). For HVPg values (**Figure 2B**), we found significant positive associations with LBP (q -value=0.048), IL-8 (q -value=0.006), IL-1 β (q -value=0.030), IL-18 (q -value=0.048), IL-6 (q -value=0.006), OPG (q -value=0.006), PAI-1 (q -value=0.030), and D-dimer (q -value=0.006). For CTP values (**Figure 2C**), we found significant positive associations with IP-10 (q -value=0.005), IL-1 β (q -value=0.009), IL-6 (q -value<0.001), TNF- α (q -value=0.011), IL-1RA (q -value=0.024), OPG (q -value=0.027), and sICAM-1 (q -value=0.015).

Discussion

In this study, the major findings in patients with advanced HCV-related cirrhosis were: i) HCV eradication with all-oral DAAs promoted an improvement in liver disease scores and plasma biomarkers (inflammation, coagulopathy, and angiogenesis); ii) HIV infection showed a slight impact on the evolution of outcome measures analyzed (liver disease scores and plasma biomarkers), since the evolution of the two study groups (HIV/HCV-coinfected and HCV-monoinfected patients) was similar; and iii) the decreases in plasma biomarkers (inflammation and coagulopathy) were associated with decreases in liver disease severity scores. Therefore, our data suggest HCV elimination with all-oral DAAs was linked to a significant improvement in liver disease scores and plasma biomarkers related to inflammation, coagulopathy, and angiogenesis in patients with advanced cirrhosis, with there being a relation between both changes. Our results could shed light on the evolution of patients with advanced HCV-related cirrhosis after HCV eradication with all-oral DAAs, irrespective of HIV infection.

Both HIV and HCV infection and advanced cirrhosis promote inflammation, immune activation, and dysfunction of the immune system, which are all linked to a greater severity of liver disease and the development of comorbidities [2–4]. Bacterial translocation in patients with advanced HCV-related cirrhosis is a key step in the pathogenesis of spontaneous bacterial peritonitis and bacteremia as well as a main factor that triggers the immune activation and inflammation, which in turn promotes hemodynamic changes and the development of decompensated cirrhosis [4]. Endothelial dysfunction is also promoted by inflammation and is linked to the progression of CHC and the development of cardiovascular events [39]. Moreover, hepatocytes release most of the proteins in the blood, where plasma levels can be altered by CHC progression, promoting an increase in thrombotic risk [40]. Coagulopathy is related to increased risk of disease progression and death in HIV-infected patients [41] and patients with advanced liver disease [42]. In a recent article on HIV/HCV-coinfected patients, we showed that plasma biomarkers of bacterial translocation, inflammation, endothelial dysfunction, and

coagulopathy increased with the increase of liver fibrosis severity, particularly in patients with LSM $\geq$ 40 KPa [36]. However, the eradication of HCV with antiviral therapy can stop this pathological process of the liver, at least almost entirely [35]. Additionally, the decrease in inflammation biomarker levels could also indicate a lower risk of developing comorbidities in cirrhotic patients who achieved SVR with DAA therapy [2, 25].

In our study overall, HCV clearance after DAA therapy promoted a significant improvement in severity scores of liver cirrhosis and many plasma biomarkers linked to inflammation (bacterial translocation, inflammatory response, and endothelial dysfunction) and coagulopathy. Our data are in concordance with a large number of previous studies that found a significant decrease in liver disease scores of HIV/HCV-coinfected patients [6, 7, 32, 43–45] and HCV-monoinfected patients [6–14] after HCV eradication with DAA therapy; and also in plasma biomarkers of HIV/HCV-coinfected patients [18, 30–34] and HCV-monoinfected patients [15–20]. However, there is an important lack of consistency in these previous publications regarding the plasma biomarkers and liver severity scores evaluated, time-points used to take data or samples after the end of HCV treatment, statistical analysis used, and liver fibrosis stages included. In our study, a high number of markers were found to be significant despite the small sample size, and others might be masked due to limited statistical power. In addition and notably, other factors provided robustness to our study, such as its prospective design (repeated measures), the evaluation of changes in markers at 48 weeks after treatment completion (medium-term), the higher suitability of the statistical analysis (GLMM), and the fact that all the patients had advanced cirrhosis. Our analysis of VEGF-A, which plays a key role in liver cancer angiogenesis [46], revealed an interesting observation. Previous reports did not find any change in plasma VEGF-A values [43, 47] or showed a significant increase [48] after HCV eradication with DAA therapy. However, our data suggest a discontinuation in the process of angiogenesis and a shift towards an "antiangiogenic" profile, since we observed a significant decrease in VEGF-A levels after HCV therapy with DAAs therapy.

Previously published studies do not usually have a control group of HCV-monoinfected patients, or if they do, they do not analyze the impact of HIV infection on the evolution of plasma biomarkers and liver disease scores after HCV eradication with all-oral DAAs [18, 30–34]. In our study, baseline values of outcome measures (plasma biomarkers and liver disease scores) and their subsequent evolution after DAA therapy were very similar in HIV/HCV-coinfected and HCV-monoinfected patients. Therefore, HIV infection only showed a slight impact on the outcome measures in our cohort of patients with advanced HCV-related cirrhosis, both at baseline and during follow-up after HCV eradication with AAD. This fact is remarkable because HIV infection promotes a faster progression of chronic hepatitis C [22] and higher plasma levels of markers linked to bacterial translocation, immune activation, inflammation and coagulation, despite suppressive ART [24]. It is probable that the absence of relevant differences between groups may be due to the fact that all of our patients had advanced stages of cirrhosis, where CAID is usually found, characterized by elevated immune activation, inflammation, and dysregulation of the immune system [4]. Additionally, Corma-Gómez et al. [49] have recently reported that HIV coinfection is not related to a higher risk for developing liver complications in HCV-infected patients with advanced fibrosis, who achieved SVR with IFN-free regimens. Malin et al [50] observed that the LSM regression after

successful DAA therapy did not differ in patients with HCV/HIV coinfection and those with HCV monoinfection.

The relationship between plasma biomarkers (bacterial translocation, inflammatory response, endothelial dysfunction, coagulopathy, and angiogenesis) and liver disease scores (LSM, HVPG, and CTP) has been scarcely explored in patients with advanced cirrhosis after HCV eradication with DAAs. Laursen et al. [20] reported that the levels of macrophage activation markers (sCD163 and soluble mannose receptor) correlated with LSM values during follow-up in HCV-monoinfected patients. Kostadinova et al. [32] showed that an improvement in LSM values correlated with a decrease in sCD14 levels after IFN-free HCV therapy. Additionally, plasma levels of sCD163, IL-6, and Mac2-binding protein correlated with changes in AST level and APRI score. In our study, the decreases in several plasma biomarkers levels linked to bacterial translocation, inflammatory response, endothelial dysfunction, and coagulopathy were directly associated with the decreases in liver disease scores (LSM, HVPG, and CTP) after HCV eradication with DAAs. Therefore, our data suggest a relation between improvement in liver cirrhosis and resolution of inflammation after HCV eradication with all-oral DAAs.

Conclusions

In conclusion, eradication of HCV with all-oral DAAs in patients with advanced HCV-related cirrhosis promoted an improvement in the severity of advanced cirrhosis and plasma biomarkers (inflammation, coagulopathy, and angiogenesis), in both HIV/HCV-coinfected and HCV-monoinfected patients. Changes in these plasma biomarkers related to inflammation were positively associated with an improvement in liver disease scores.

Abbreviations

Hepatitis C virus (HCV)

Chronic hepatitis C (CHC)

Cirrhosis-associated immune dysfunction (CAID)

Direct-acting antiviral agents (DAAs)

Sustained virologic response (SVR)

Liver stiffness measurement (LSM)

Hepatic liver pressure gradient (HVPG)

Child-Pugh-Turcotte (CTP)

Human immunodeficiency virus (HIV)

Combination antiretroviral therapy (ART)

Kilopascals (kPa

Millimeter of mercury (mmHG)

Soluble CD14 (sCD14)

Lipopolysaccharide (LPS)

Fatty acid-binding protein 2 (FABP2)

Lipopolysaccharide binding protein (LBP)

IFN- γ -inducible protein 10 (IP-10)

Monocyte chemoattractant protein-1 (MCP1)

Interleukin (IL)

Tumor necrosis factor alpha (TNF- α)

Interleukin-1 receptor antagonist (IL-1RA)

Soluble receptor activator of nuclear factor-kappa B ligand (sRANKL)

Osteoprotegerin (OPG)

Soluble vascular cell adhesion molecule 1 (sVCAM1)

Soluble intercellular cell adhesion molecule 1 (sICAM1)

Soluble tumor necrosis factor receptor 1 (sTNFR1)

Plasminogen activator inhibitor-1 (PAI-1)

Vascular endothelial growth factor A (VEGF-A)

Soluble receptor 1 for vascular endothelial growth factor (sVEGF-R1)

Generalized Linear Mixed Models (GLMM)

Interferon (IFN)

Appendix

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Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Instituto de Salud Carlos III (CEI PI 41_2014).

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Author contributions

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Writing – original draft preparation: MAJS and SR.

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Tables

Table 1. Summary of characteristics of patients with advanced HCV-related cirrhosis at baseline.

	All	HCV	HIV/HCV	<i>p</i>
No.	62	12	50	
Gender (male)	47 (75.8%)	8 (66.7%)	39 (78%)	0.411
Age (years)	52.9 (48.9; 55.8)	59.9 (54.3; 71.3)	52.2 (48.8; 54.1)	0.002
Smoker				
Never	10 (16.4%)	4 (33.3%)	6 (12.2%)	0.111
Previously (≥ 6 months)	16 (26.2%)	4 (33.3%)	12 (24.5%)	
Currently	35 (57.4%)	4 (33.3%)	31 (63.3%)	
Alcohol intake				
Never	29 (46.8%)	8 (66.7%)	21 (42%)	0.232
Previously (≥ 6 months)	28 (45.2%)	4 (33.3%)	24 (48%)	
Currently	5 (8.1%)	0 (0%)	5 (10%)	
IVDU				
Never	24 (38.7%)	12 (100%)	12 (24%)	<0.001
Previously (≥ 6 months)	38 (61.3%)	0 (0%)	38 (76%)	
Currently	0 (0%)	0 (0%)	0 (0%)	
Treatments				
Previous IFN α therapy	33 (53.2%)	10 (83.3%)	23 (46%)	0.021
Statins	9 (14.5%)	1 (8.3%)	8 (16%)	0.498
Antiretroviral therapy				
NRTI+NNRTI-based	-	-	7 (14.3%)	
NRTI+II-based	-	-	24 (49%)	
NRTI+PI-based	-	-	6 (12.2%)	
PI+II+others-based	-	-	4 (8.2%)	
Others	-	-	8 (16.3%)	
HIV markers				
Prior AIDS	-	-	18 (36%)	
Nadir CD4+ T-cells (cells/mm ³)	-	-	114.7 (70; 182)	
Nadir CD4+ <200 cells/mm ³	-	-	35 (76.1%)	
CD4+ T-cells (cells/mm ³)	-	-	439 (234; 721)	
CD4+ <500 cells/mm ³	-	-	30 (60%)	
HCV markers				
HCV genotype				0.231
1	42 (68.9%)	10 (83.3%)	32 (65.3%)	
3	9 (14.8%)	2 (16.7%)	7 (14.3%)	
4	10 (16.4%)	0 (0%)	10 (20.4%)	
Log ₁₀ HCV-RNA (IU/mL)	6.2 (5.7; 6.7)	6.2 (5.6; 6.4)	6.2 (5.7; 6.7)	0.569
HCV-RNA ≥ 850.000 IU/mL	41 (66.1%)	8 (66.7%)	33 (66%)	0.965

Statistics: Values are expressed as absolute number (percentage) and median (interquartile range). *P*-values were calculated by Chi-square, Fisher's exact test, and Mann-Whitney tests, as required.

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; IVDU, intravenous drug user; IFN α , interferon-alpha; NRTI, nucleoside analogue HIV reverse; NNRTI, non-nucleoside analogue HIV reverse transcriptase inhibitor; PI, protease inhibitor;

II, integrase inhibitor; AIDS, acquired immune deficiency syndrome;; HCV-RNA, HCV plasma viral load.

Table 2. Summary of severity scores of liver disease and plasma biomarkers in patients with advanced HCV-related cirrhosis at baseline.

	All	HCV	HIV/HCV	p-values	q-values
Liver disease markers					
LSM (kPa)	31.6 (23.4; 40.7)	29.9 (27; 66.4)	32.8 (22.3; 39.3)	0.293	0.397
<25 kPa	16 (26.7%)	2 (18.2%)	14 (28.6%)	0.582	0.703
25-40 kPa	29 (48.3%)	5 (45.4%)	24 (49%)		
≥40 kPa	15 (25%)	4 (36.4%)	11 (22.4%)		
CTP	5 (5; 6)	5 (5; 7)	5 (5; 5)	0.107	0.255
CTP ≥7	6 (10.3%)	3 (33.3%)	3 (6.1%)	0.014	0.135
HVPG (mmHg)	16 (11; 18)	17.8 (13.5; 20.5)	15 (11; 17)	0.123	0.255
<16 mmHg	15 (48.4%)	2 (25%)	13 (56.5%)	0.117	0.255
16-20 mmHg	11 (35.5%)	3 (37.5%)	8 (34.8%)		
≥20 mmHg	5 (16.1%)	3 (37.5%)	2 (8.7%)		
Plasma biomarkers					
Bacterial translocation					
LPS (EU/ml)	0.9 (0.7; 1.2)	0.8 (0.5; 0.9)	0.9 (0.7; 1.4)	0.058	0.240
LBP (µg/ml)	1 (0.6; 1.4)	0.7 (0.6; 1.1)	1.1 (0.7; 1.5)	0.087	0.255
sCD14 (µg/ml)	2.1 (1.6; 3.2)	3.2 (2.5; 4.9)	2 (1.6; 2.3)	0.001	0.029
FABP-2 (ng/ml)	0.4 (0.1; 0.7)	0.8 (0.2; 1.6)	0.3 (0.1; 0.5)	0.009	0.131
Inflammation					
IP-10 (a.u.)	1221.5 (865.3; 1799.5)	1413.5 (1117; 2083.8)	1180.3 (807.8; 1706)	0.209	0.319
MCP-1 (a.u.)	493.8 (276.1; 684.1)	548.3 (211; 750.5)	481.5 (289.6; 684.1)	0.845	0.904
IL-8 (a.u.)	117.8 (74.6; 185.8)	84.5 (63.3; 158.1)	127 (80.8; 188.4)	0.196	0.316
IL-1β (a.u.)	15 (13; 27.6)	16.3 (12.1; 63.5)	15 (13; 20.4)	0.809	0.904
IL-18 (a.u.)	988.5 (573.1; 1670.9)	710 (461.4; 1333.8)	1116.3 (584.8; 1697.9)	0.301	0.397
IL-6 (a.u.)	65.3 (33.8; 156.3)	156.8 (48; 417.4)	59 (32.9; 114.5)	0.052	0.240
TNF-α (a.u.)	9.8 (7.4; 12)	10 (7.9; 15.5)	9 (7; 12)	0.381	0.480
IL-1RA (a.u.)	18 (13; 25.9)	18.5 (14.1; 22.3)	18 (13; 27.5)	0.865	0.904
sRANKL (a.u.)	24.3 (19; 36.4)	31 (24.6; 39)	22.5 (18.4; 36.4)	0.029	0.168
OPG (a.u.)	217.5 (158.5; 307)	282.5 (220; 334.8)	195.5 (151.5; 286.1)	0.069	0.250
Endothelial dysfunction					
sVCAM-1 (a.u.)	10747 (8616.8; 12142.3)	9285 (8580.8; 11099.6)	10916 (9008.3; 12352.1)	0.159	0.288
sICAM-1 (a.u.)	107.8 (63.5; 163.1)	71.5 (43.6; 139.9)	111.3 (68.8; 165.1)	0.134	0.259
TNFR-I (a.u.)	32 (20.4; 57.8)	24.5 (16.8; 38)	33.5 (20.9; 76.5)	0.101	0.255
Coagulopathy					
PAI-1 (a.u.)	1054.8 (808.6; 1315.3)	1132.5 (628; 1463.1)	1036 (833.4; 1315.3)	0.915	0.915

D-dimer (a.u.)	1767.5 (653.4; 4289.9)	2738 (924.6; 9896.5)	1717 (559.8; 3627.6)	0.187	0.316
Angiogenesis					
VEGF-A (a.u.)	76 (57.6; 90.8)	72 (54.5; 111)	76 (57.6; 90)	0.873	0.904
	44.5 (32.4; 69.5)	59.5 (40.4; 83.1)	42 (31.6; 66.9)	0.107	0.255
sVEGF-R1 (a.u.)					

Statistics: Values are expressed as absolute number (percentage) and median (interquartile range). *P*-values were calculated by Chi-square, Fisher's exact test, and Mann-Whitney test, as required. *P*-values, raw *p*-values; *q*-values, *p*-values corrected for multiple testing using the false discovery rate (FDR) with Benjamini and Hochberg procedure. The statistically significant differences are shown in bold.

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; LSM, liver stiffness measure; kPa, kilopascal; HVPG, Hepatic Venous Pressure Gradient; mmHg, millimetre of mercury; CTP, Child-Pugh-Turcotte; a.u., arbitrary units of fluorescence; sCD14, soluble CD14; LPS, lipopolysaccharide; FABP2, fatty acid-binding protein 2; LBP, lipopolysaccharide binding protein; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; TNF- α , tumor necrosis factor alpha; IP-10, IFN- γ -inducible protein 10; MCP1, monocyte chemoattractant protein-1; OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor-kappa B ligand; sVCAM-1, soluble vascular cell adhesion molecule 1; sICAM-1, soluble intercellular cell adhesion molecule 1; sTNFR-1, soluble tumor necrosis factor receptor 1; PAI-1, plasminogen activator inhibitor-1; VEGF-A; vascular endothelial growth factor A; sVEGF-R1, soluble receptor1 for vascular endothelial growth factor.

Figures

Figure 1

Change in severity scores of liver disease and plasma biomarkers during follow-up in patients with advanced HCV-related cirrhosis. Statistics: Values are expressed as the estimated average of the increase ($\Delta x = x_2 - x_1$) and 95% of confidence interval (95%CI). P-values were calculated by GLMM models. P-values, raw p-values; q-values, p-values corrected for multiple testing using the false discovery rate (FDR) with Benjamini and Hochberg procedure. The statistically significant differences are shown in bold. Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; LSM, liver stiffness measurement; CTP, Child-Pugh-Turcotte; HVPG, hepatic venous pressure gradient; mmHg, millimeter of mercury; kPa, kilopascals; a.u., arbitrary units of fluorescence; sCD14, soluble CD14; LPS, lipopolysaccharide; FABP2, fatty acid-binding protein 2; LBP, lipopolysaccharide binding protein; IL,

interleukin; IL-1RA, interleukin-1 receptor antagonist; TNF- α , tumor necrosis factor alpha; IP-10, IFN- γ -inducible protein 10; MCP1, monocyte chemoattractant protein-1; OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor-kappa B ligand; sVCAM-1, soluble vascular cell adhesion molecule 1; sICAM-1, soluble intercellular cell adhesion molecule 1; sTNFR-1, soluble tumor necrosis factor receptor 1; PAI-1, plasminogen activator inhibitor-1; VEGF-A; vascular endothelial growth factor A; sVEGF-R1, soluble receptor1 for vascular endothelial growth factor.

Figure 2

Association between the change in values of plasma biomarkers and severity scores of liver disease during HCV treatment in patients with advanced HCV-related cirrhosis. Statistics: Values are expressed as regression coefficient (β) and 95% of confidence interval (95%CI). P-values were calculated by GLMM models. P-values, raw p-values; q-values, p-values corrected for multiple testing using the false discovery rate (FDR) with Benjamini and Hochberg procedure. The statistically significant differences are shown in bold. Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; LSM, liver stiffness measures; CPT, Child-Pugh-Turcotte; KVPg, hepatic venous pressure gradient (mmHg); a.u., arbitrary units of fluorescence; sCD14, soluble CD14; LPS, lipopolysaccharide; FABP2, fatty acid-binding protein 2; LBP, lipopolysaccharide binding protein; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; TNF- α , tumor necrosis factor alpha; IP-10, IFN- γ -inducible protein 10; MCP1, monocyte chemoattractant protein-1; OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor-kappa B ligand; sVCAM-1, soluble vascular cell adhesion molecule 1; sICAM-1, soluble intercellular cell adhesion molecule 1; sTNFR-1, soluble tumor necrosis factor receptor 1; PAI-1, plasminogen activator inhibitor-1; VEGF-A; vascular endothelial growth factor A; sVEGF-R1, soluble receptor1 for vascular endothelial growth factor.

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