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Liver Fibrosis Hinders Normalization of Systemic Inflammation After HCV Eradication

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I. Background

- HCV co-infection significantly impacts on inflammation, endothelial activation and coagulation function parameters, leading to several co-morbidities among people living with HIV (PLWH).
- The new direct-acting antivirals (DAAs) therapy achieves eradication of HCV in the majority of patients. However, the early effect of HCV eradication on these parameters in PLWH has been scarcely explored.
- We have analyzed the effect of HCV on systemic inflammation and endothelial/coagulation function in PLWH and its early evolution after HCV eradication with DAAs.

II. Methods

- Twenty five HIV/HCV co-infected (HIV/HCV group), 25 HIV mono-infected (HIV group) patients and 20 healthy controls participants (HC group) were included in the study.
- All patients were on cART and HIV suppressed. Fourteen parameters of systemic inflammation, endothelial activation and coagulation were measured on plasma samples using ProcartaPlex multiplex immunoassay (Invitrogen, Thermo Fisher Scientific) and acquired in a Luminex 200 analyzer (Luminex Corporation, Texas).
- Cross-sectional and longitudinal (comparing baseline vs. 12 weeks post end of DAAs treatment in HIV/HCV group) analyses were performed. Non-parametric tests were used to establish inter and intra-group differences.

III. Results

- The clinical characteristics at baseline of HIV/HCV and HIV groups are shown in **Table 1**. There were no significant differences between patient's groups for age, gender, time since HIV diagnosis, time on cART, CD4 count, CD4/CD8 ratio, and HIV transmission route.
- All HIV/HCV patients were on INSTI-based cART regimen, whereas this was the case in 56% of HIV patients (p= 0.001).
- The liver fibrosis stage assessed by transient elastography was F0-1 (Kpa<7.6, absent to minimal liver fibrosis) in 60% and F≥2 (Kpa≥7.6, significant liver fibrosis) in 40% of HIV/HCV co-infected patients.
- As expected, HIV/HCV co-infected patients showed higher levels (UI/L) of ALT (80 [51-195]) and AST (60 [40-136]) than HIV mono-infected patients (32 [24-37] and 27 [22-31] respectively; p<0.0001 for both comparisons). The levels of total cholesterol and LDL (mg/dL) were lower in HIV/HCV co-infected (158 [123-184] and 85 [71-104] respectively) compared to HIV mono-infected patients (194 [162-227], p<0.0001 and 119 [93-146], p=0.001; respectively) (**Table1**).

Table 1. Baseline characteristics of patients included in the study

Characteristic	HIV group (n=25)	HIV/HCV group (n=25)	p-value
Age (years)	48 [42 – 55]	44 [39 – 48]	0.11
Gender (% of males)	88%	100%	0.24
Time since HIV diagnosis (years)	9 [6 – 14]	7 [2 – 10]	0.07
Time since HCV diagnosis (years)	NA	2 [1.5 - 5]	NA
Time on cART (years)	5 [3.5 – 7.5]	4 [2 – 9]	0.63
CD4 count (cells/μL)	816 [605 – 992]	735 [577 – 902]	0.31
CD4/CD8 ratio	0.84 [0.56 – 1.34]	0.75 [0.60 – 1.09]	0.49
ALT level (IU/L)	32 [24 – 37]	80 [51 – 195]	**<0.0001**
AST level (IU/L)	27 [22 – 31]	60 [40 – 136]	**<0.0001**
GGT level (IU/L)	36 [22 – 54]	65 [25 – 133]	0.06
Total cholesterol level (mg/dL)	194 [162 – 227]	158 [123 – 184]	**<0.0001**
HDL level (mg/dL)	42 [37 – 50]	43 [33-49]	0.815
LDL level (mg/dL)	119 [93 – 146]	85 [71 – 104]	**0.001**
HCV-RNA (log copies/mL)	NA	6.1 [5.8 – 6.4]	NA
HIV transmission route (%)			1
Sexual	100%	96%	
Parenteral	0%	4%	
cART regimen			**0.001**
INSTI-based regimen (%)	56%	100%	
NNRTI-based regimen (%)	36%	0%	
PI-based regimen (%)	8%	0%	
HCV genotype (%)			NA
1a	NA	60%	
1b	NA	12%	
4	NA	28%	
Liver stiffness (elastography) (%)			NA
F0-F1	NA	60%	
F≥2	NA	40%	

cART: combination antiretroviral therapy; **ALT**: alanine aminotransferase; **AST**: aspartate aminotransferase; **GGT**: gamma glutamil transferase; **HDL**: high density lipoprotein; **LDL**: low density lipoprotein; **HCV**: hepatitis C virus. **NNRTI**: nonnucleoside reverse transcriptase inhibitor; **INSTI**: integrase strand transfer inhibitor; **PI**: protease inhibitor. Data for continuous variables are given as median [interquartile range]. **NA**: do not apply.

III. Results

- There were no significant differences between HIV mono-infected patients and HC participants for any of the plasma markers evaluated.
- However, at baseline (before DAAs therapy), levels of IL-18 (p=0.028), IP-10 (p<0.0001), VCAM-1 (p<0.0001), and ICAM-1 (p=0.045) were significantly different between HIV/HCV, HIV and HC study groups, being higher in HIV/HCV patients compared to either HIV patients and/or HC participants (**Figure 1**).
- Interestingly, the highest levels of of IL-18 (p=0.005), IP-10 (p<0.0001), VCAM-1 (p<0.0001) and ICAM-1 (p=0.004) were observed in HIV/HCV patients with significant liver fibrosis (F≥2) compared with the rest of groups (**Figure 2**). A detailed analysis revealed the existence of two different patterns: IL-18 and ICAM-1 were significantly increased with respect to HIV and/or HC groups only in HIV/HCV patients with significant fibrosis (F≥2); in contrast IP-10 and VCAM-1 were significantly increased in both groups of HIV/HCV patients (F0-1 and F≥2 groups) with respect to HIV and/or HC groups. Moreover, levels of IL-18, IP-10 and ICAM-1 were higher in HIV/HCV patients with (F≥2) compared to those without (F0-1) significant liver fibrosis (p=0.007 for IL-18, p=0.015 for IP-10 and p=0.053 for ICAM-1) (**Figure 2**).

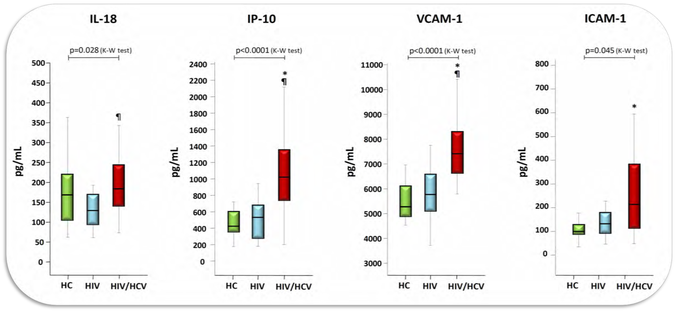

Figure 1. Box-plots graphs showing levels (median pg/mL, range) of IL-18, IP-10, VCAM-1 and ICAM-1, in HC participants, HIV mono-infected, and HIV/HCV co-infected patients at baseline. p-values for the global comparison between groups (Kruskal-Wallis test, K-W) are shown. (*): p<0.05 with respect to HC group (Mann-Whitney U test). (¶): p<0.05 with respect to HIV group (Mann-Whitney U test).

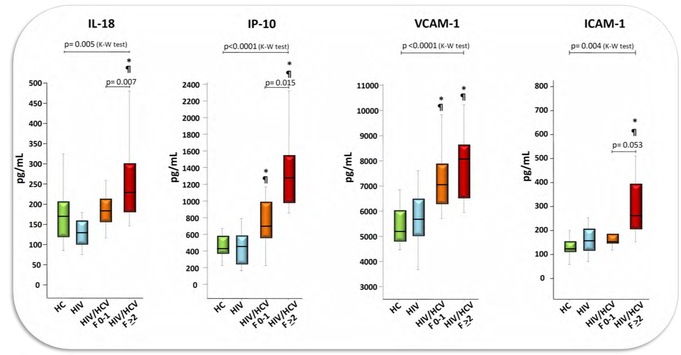

Figure 2. Box-plots graphs showing levels (median pg/mL, range) of IL-18, IP-10, VCAM-1 and ICAM-1 in HC participants, HIV patients, and HIV/HCV patients according to their liver fibrosis stage (without [F0-1] or with [F≥2]) significant fibrosis). p-values for the global comparison between groups (Kruskal-Wallis test, K-W) and for the comparison between subgroups of HIV/HCV patients (Mann-Whitney U test) are shown. (*): p<0.05 with respect to HC group (Mann-Whitney U test). (¶): p<0.05 with respect to HIV group (Mann-Whitney U test).

- At baseline, levels of IL-18 were positively correlated with HCV-RNA load (rho=0.542 p=0.005), with ALT levels (rho=0.539, p=0.005) and with AST levels (rho=0.461, p=0.02). Moreover, the IP-10 levels were positively correlated with ALT (rho=0.575, p=0.003), with AST (rho=0.516, p= 0.008) and with GGT (rho=0.465, p=0.019) levels. Similarly, VCAM-1 was positively correlated with ALT (rho=0.641, p=0.001) and with AST levels (rho=0.562, p= 0.003) and negatively with CD4/CD8 ratio (rho= - 0.496, p=0.012) (**Figure 3**).
- All 25 HIV/HCV co-infected patients achieved sustained virological response (undetectable HCV RNA at 12 weeks after the end of DAAs therapy). After HCV eradication, levels of IL-18 and ICAM-1 in HIV/HCV patients were comparable to those observed in HIV patients and in HC participants, whereas VCAM-1 levels remained significantly increased in HIV/HCV patients compared to HC participants (Mann-Whitney U test p=0.001) and levels of IP-10 showed a clear trend (Mann-Whitney U test p=0.07) (**Figure 4**).
- Interestingly, after HCV eradication, increased levels of VCAM-1 (compared to HC group) and of IP-10 (compared to both HC and HIV groups) were observed in the subgroup of HIV/HCV patients with significant liver fibrosis (F≥2) at baseline. In contrast, only levels of VCAM-1 were significantly increased (compared to HC group) in HIV/HCV patients without significant liver fibrosis (F0-1) at baseline (**Figure 5**).

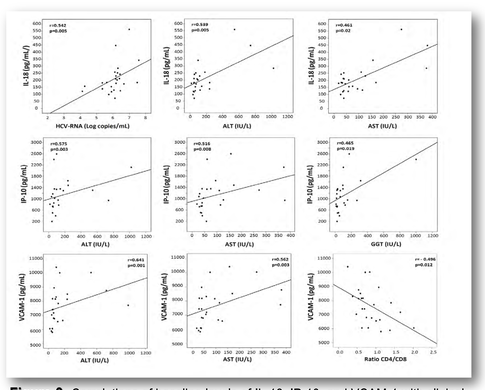

Figure 3. Correlations of baseline levels of IL-18, IP-10, and VCAM-1 with clinical and immunological parameters in HIV/HCV patients. Spearman correlation analysis was used to estimate the level of correlation.

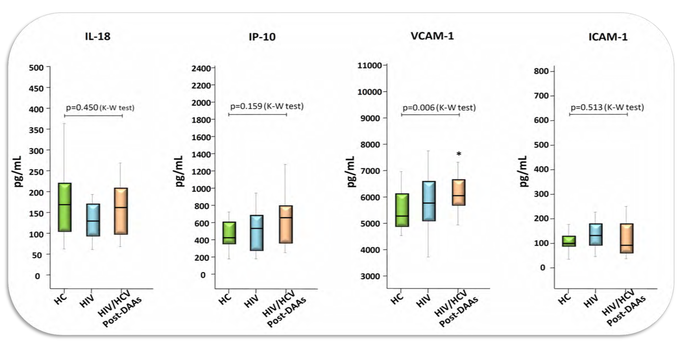

Figure 4. Box-plots graphs showing levels (median pg/mL, range) of IL-18, IP-10, VCAM-1 and ICAM-1, in HC participants, HIV mono-infected and HIV/HCV co-infected patients at 12 weeks after the end of DAAs therapy (HIV/HCV post-DAAs). p-values for the global comparison between groups (Kruskal-Wallis test; K-W) are shown. (*): p<0.05 with respect to HC group (Mann-Whitney U test).

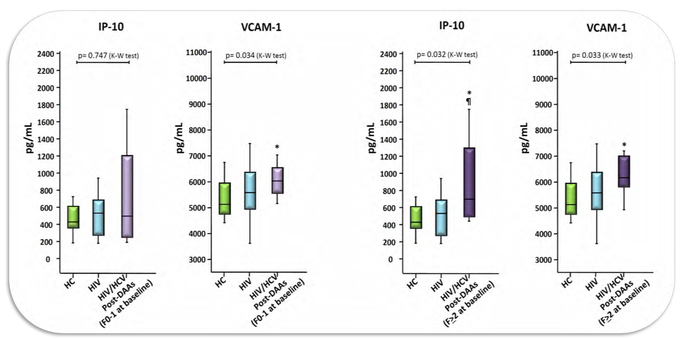

Figure 5. Box-plots graphs showing levels (median pg/mL, range) of IP-10 and VCAM-1 in HC participants, HIV mono-infected patients and HIV/HCV co-infected patients at 12 weeks after the end of DAAs therapy without significant liver fibrosis (F0-1) at baseline (left graphs) or with significant liver fibrosis (F≥2) at baseline (right graphs). p-values for the global comparison between groups (Kruskal-Wallis test, K-W) are shown. (*): p<0.05 with respect to HC group (Mann-Whitney U test). (¶): p<0.05 with respect to HIV group (Mann-Whitney U test)

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V. Conclusions

- Our study provides evidence that both HCV co-infection and liver fibrosis significantly impact on markers of systemic inflammation and endothelial activation in cART treated PLWH.
- The existence of a correlation between live injury (AST, ALT enzymes) and systemic inflammation suggests a role for hepatic tissue damage in the induction and/or maintenance of a state of systemic inflammation.
- Normalization of these inflammatory and endothelial activation markers is not completely achieved after HCV eradication, especially in patients with significant liver fibrosis. This data prompts anti-HCV treatment in al HCV co-infected PLWH at the earliest stages of liver damage to enhance normalization of systemic inflammation and endothelial activation.