HCV clearance following treatment with direct acting antivirals in HIV-HCV co-infection modulates systemic immune activation and HIV transcription on ART.

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Article's main point: This article addresses the immediate and long-term effects of direct acting antiviral-mediated HCV clearance on the HIV reservoir dynamics and immune function, in a cohort of HIV/HCV co-infected individuals under antiretroviral treatment.

Conflict of interests: SRL has received financial support for investigator initiated industry funded research from Merck, Viiv Healthcare, Gilead Sciences and Leidos. The rest of the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Funding statement: This work was supported by grants from CONICET (Grant# PUE2016-INBIRS), from the *Agencia Nacional de Promoción Científica y Tecnológica* (ANPCyT, PICT2016, Grant #930), ViiV Healthcare Investigator Sponsored Studies (reference number 209424) and *Fundación Florencio Fiorini*.

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Abstract

Background: HCV coinfection among people living with HIV might perturb immune function and HIV persistence. We aimed to evaluate the impact of HCV clearance with direct acting antivirals (DAA) on immune activation and HIV persistence in HIV/HCV-coinfected individuals on antiretroviral therapy (ART).

Methods: In a prospective observational study, ART-treated participants with HIV/HCV coinfection received sofosbuvir/daclatasvir±ribavirin (n=19). Blood samples were collected before DAA therapy, at the end of treatment, and 12 months after DAA termination (12MPT). T and NK cell phenotype, soluble plasma factors, cell-associated (CA)-HIV DNA forms (total, integrated, 2LTR), CA-unspliced (US) and multiple-spliced (MS)-RNA and plasma HIV RNA were evaluated.

Results: HCV clearance was associated with a down-modulation of activation and exhaustion markers in CD4+, CD8+ T and NK cells; together with decreased plasma levels of IP-10, IL-8, sCD163 and sICAM. Cell-associated US HIV RNA was significantly higher at 12MPT compared to baseline with no change in HIV DNA or plasma RNA.

Conclusions: Elimination of HCV in HIV/HCV co-infected individuals alters immune function and the transcriptional activity of latently infected cells. This report provide insights into the effects of HCV coinfection in HIV persistence and regards coinfected subjects as a population where HIV remission might prove more challenging.

Keywords: Hepatitis C, direct antiviral agents, HIV reservoir, immune activation

Background

Antiretroviral therapy (ART) quickly and persistently suppresses viral replication resulting in improved quality of life for people living with HIV/AIDS (PLWHA), reduced AIDS-associated death rates, reduced morbidity events and longer life expectancy[1]. However, treatment is life-long with several limitations [2]. If treatment is interrupted, plasma viral load (VL) rapidly rebounds, due to the persistence of long lived and proliferating latently infected cells that persist on ART[3,4].

Because of overlapping pathways of transmission between HIV and Hepatitis C virus (HCV), approximately 2 to 5 million individuals worldwide are estimated to be coinfected[5,6]. In Argentina, coinfected individuals represent approximately 20% of the PLWHA[7]. HCV direct antiviral agents (DAAs), which target specific steps of HCV replication cycle, represent a major development in the treatment of HCV, with the possibility of >95% of cure and low rate of adverse events, even with advanced or decompensated cirrhosis[8]. DAA–mediated clearance of HCV is associated with loss of intrahepatic immune activation by IFN-α, recovery of T-cell proliferation, normalization of NK cell phenotype and function[9], and restoration of type I IFN response both in acute [10] and chronic [11] HCV infection, as well as enhanced HCV-specific $CD8^+$ T-cell responses [12].

Understanding the interaction between HCV co-infection prior to and following clearance of HCV on the HIV reservoir is important, because HIV-HCV co-infection is common and the high cure rate following DAAs allows for the opportunity to assess the impact of HCV on HIV persistence. HCV/HIV coinfection has been associated with increased levels of immune activation compared to HIV monoinfection including higher levels of microbial products[13,14], low-levels of detectable plasma HIV in PLWHA on ART[15,16] and increased risk of HIV

virological failure[17]. Early reports showed that cell-associated (CA) HIV RNA level decreased after HCV treatment with IFN-α plus ribavirin[18] while no effect[18] or a decrease[19,20] was observed in HIV DNA. More recent studies have reported an increase in $CD4^+$ T-cells harboring integrated HIV DNA in HIV/HCV even after spontaneous HCV clearance [21] whereas DAA-mediated HCV clearance was associated with stable or increased levels of HIV cell-associated DNA[22,23].

We hypothesized that the elimination of HCV coinfection with DAAs would modulate the size and/or transcriptional activity of the HIV reservoir due to the restoration of HCV-driven immune dysregulation. We quantified the immediate and long-term effects of DAA-mediated HCV clearance in HIV/HCV-coinfected participants on multiple markers of immune function and on HIV persistence in blood. Overall, we observed a down-modulation of NK and T cell activation markers as well as of soluble plasma activation markers after treatment with DAAs. This was accompanied by an increase in peripheral cell-associated HIV US-RNA with no change in HIV DNA. These results suggest a relationship between HCV and transcriptional activity of the HIV reservoir.

Methods

This was a longitudinal, single-centre study approved by the local Ethics Committee of the *Huésped* Foundation (Buenos Aires, Argentina). All participants were included after signing the informed consent from March 17th to May 12th 2016, and samples were analyzed during 2017, 2018 and 2019. Sample sizes were determined using Harris, Horvitz, and Mood method in order to provide 80% power, at the 5% level of significance. Peripheral blood from 19 ART-treated participants with HIV/HCV coinfection were collected at baseline (BSL) before the start of

DAA, at the end of treatment (EOT) (either at completion of 12 or 24 weeks of DAA), and after 12 months of DAA termination (12MPT). At 12MPT, samples from two participants were not available due to lung cancer diagnosis and loss of follow up, respectively. All individuals received HCV treatment with sofosbuvir (SOF) and daclatasvir (DCV) and 12 participants also received ribavirin (RBV). Diagnosis of liver cirrhosis was made by liver biopsy or hepatic transient elastography (> 14 Kpa). No participants had signs of hepatic decompensation at the time of enrolment. Sustained virological response to DAA was defined by non-detectable HCV RNA (lower limit of detection- LOD-12 IU/mL) at 12 weeks after EOT. HCV RNA was also evaluated at 48 weeks after EOT and it was non-detectable in all cases. Inclusion criteria were successful ART with HIV viral load (VL) <40 copies/mL for more than 24 months. Peripheral blood mononuclear cells (PBMCs) were obtained from 60 ml of whole blood by Ficoll-Hypaque density gradient centrifugation (GE Healthcare, UK) and cryopreserved in liquid nitrogen.

Cell-associated (CA) HIV RNA (unspliced, US; and multiple-spliced, MS) and DNA forms (Total HIV, HIV-Integrated and 2LTR circles) were evaluated in sorted $CD4^+$ T-cells by quantitative real-time PCR, as described previously[24]. Ultrasensitive HIV plasma viral load was evaluated in all samples by replicate testing using the Aptima HIV-1 quant assay (Hologic). T-cell and NK-cell phenotyping was performed by flow cytometry. Soluble plasma factors were quantified by ELISA. Detailed methods are described as supplementary material S1.

Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software) and InfoStat (UNC, Argentina) and R project (R Foundation for Statistical Computing, Vienna, Austria) softwares. Data was analyzed using nonparametric methods. Longitudinal association between CA US-HIV RNA and plasma RNA was estimated by using a generalized linear mixed-effects regression model. Correlation analyses were performed using Spearman's rank test. For the

phenotypic analyses, SPICE 6.0 software (https://niaid.github.io/spice/) was used following the experimental and technical considerations published by the software developers[25]. All tests were considered significant when the *p*-value was <0.05.

Results

Study group: A total of 19 HIV/HCV coinfected individuals were enrolled. Most participants were male (74%), and the median age was 49 years (IQR 46-53). All participants were receiving ART with at least 2 years of documented undetectable HIV VL. During DAA, all individuals received integrase inhibitor based ART. SOF/DCV with RBV was prescribed for 12 weeks in 9 participants, for 24 weeks in three participants and seven participants received SOF/DCV for 24 weeks without RBV. The use of RBV depended on HCV genotype and drug tolerance. All participants achieved SVR. Further clinical details are summarized in table 1.

HIV persistence after DAA treatment: We quantified the frequency and the transcriptional activity of HIV-infected cells before (BSL sample) and after DAA treatment (EOT and 12MPT samples) as an indicator of HIV persistence. No statistically significant differences were observed in cell-associated viral DNA (total, integrated and 2LTR circles, Figure 1A), i.e. overall levels remained stable from BSL to 12MPT. No differences in plasma HIV RNA, measured by ultrasensitive single copy assay were found among time-points (Figure 1B).

Levels of CA MS-RNA remained low and stable along all the studied time points (Figure 1B). However, a statistically significant increase in CA US-RNA was observed between BSL and 12MPT (p=0,0203, Figure 1B). Of note, the higher increments in US-RNA were observed in those participants not receiving RBV within their regimens (Figure S1); US-RNA was higher at baseline in the RBV group, nevertheless no differences in any of the clinical or laboratory parameters were observed between both groups that could account for that disparity.

In order to provide further insight into the transcriptional activity of the infected cells, the ratio of CA US-RNA and both the integrated and total HIV DNA were calculated. Also, the ratio between CA MS- and US-RNA was obtained in order to estimate the relative efficiency of transcriptional elongation and splicing. No differences were found between BSL, EOT and 12MPT (Figure 1C). Finally, we hypothesized that increased US-RNA, if associated with increased viral transcription, would be correlated with plasma RNA. The longitudinal study of CA US-HIV RNA and plasma RNA across all three time-points evaluated, depicted a potential association between both variables (p= 0,002, Figure 2).

T-cell immune phenotyping: The profile of different memory subsets and the expression of different markers were studied on $CD8^+$ and $CD4^+$ T-cells (gating strategy is shown in Figure S2). We defined six $CD8^+$ or $CD4^+$ T-cell sub-populations: naïve ($T_{Naïve}$, $CCR7^+CD45RO^-CD28^+CD95^-$), stem memory ($T_{SCM}$, $CCR7^+CD45RO^-CD28^+CD95^+$), central memory ($T_{CM}$, $CCR7^+CD45RO^+CD28^+CD95^+$), transitional memory ($T_{TM}$, $CCR7^-CD45RO^+CD28^+CD95^+$), effector memory ($T_{EM}$, $CCR7^-CD45RO^+CD28^-CD95^+$) and terminal effector (T_{TE}, $CCR7^-CD45RO^-CD28^-CD95^+$) cells. The distribution of these subsets was analyzed as previously described [26]. The memory profile within $CD4^+$ T-cell compartment showed the following hierarchical distribution: T_{TM} cells represented the highest proportions, followed by T_{CM}, $T_{Naïve}$, T_{EM}, T_{SCM} and T_{TE} cells (Figure S3A). Regarding the $CD8^+$ T-cell compartment, the distribution was as follows: T_{EM} cells comprised the highest proportions, followed by $T_{TE,}$ T_{TM}, $T_{Naïve}$, T_{CM} and T_{SCM} cells (Figure S3B). Memory distribution observed on both T-cell compartments were conserved across the three time points analyzed (BSL, EOT and 12MPT) and no significant differences were observed between them.

We next quantified the expression of immune surface markers (such as CD38, HLA-DR, CD127, PD-1 and CD25) in both T-cell compartments. At BSL, elevated proportions of $CD4^+$/HLA-DR^+, $CD4^+$/$PD\text{-}1^+$, $CD4^+$/$CD38^+$/HLA-DR^+ and $CD4^+$/$CD38^+$/HLA-DR^+/$PD\text{-}1^+$ T cells were observed. These proportions were significantly reduced by 12MPT. (Figure S4A). The decay in activation markers observed from BSL to 12MPT was observed in all $CD4^+$ T cells subpopulations, with $CD4^+$ T_{TM} and T_{EM} having the largest and most significant changes.

A similar scenario was observed on $CD8^+$ T cells. At BSL, higher proportions of $CD8^+$ T-cells expressing CD38 and HLA-DR were recorded. After DAA treatment, the expression of these activation markers was significantly reduced. The proportions of double positive $CD8^+$/$CD38^+$/HLA-DR^+ and triple positive $CD8^+$/$CD38^+$/HLA-DR^+/$PD\text{-}1^+$ T-cells were significantly reduced at EOT and 12MPT, respectively (Figure S5A). Similar to the $CD4^+$ T-cell compartment, the decay in CD38, HLA-DR and PD-1 expression was observed in all $CD8^+$ T-cell subpopulations (Figure S5B). In this compartment the subpopulations that exhibited more pronounced modifications after HCV clearance were the more differentiated phenotypes ($CD8^+$ T_{TM}, T_{EM} and T_{TE}).

NK-cell immune phenotyping: Compared to BSL, CD38 expression on NK cells was significantly reduced at EOT, and continued at this lower level throughout the study (Figure S6A). The frequency of HLA-DR-expressing NK cells was significantly reduced at EOT (Figure S5B) while there was a slight increase by 12MPT levels but still remaining at lower levels than those from BSL (Figure S6B). When analyzing the distribution of NK cell subsets defined by the expression of both HLA-DR and CD38, we found a significant reduction in the frequency of HLA-DR^+/$CD38^+$ NK cells, with a concomitant increase in the frequency of cells negative for both markers at EOT and 12MPT (Figure S6C). Additionally, the apoptosis-inducing receptor

CD95 was studied. Percentages of CD95-expressing cells at EOT were lower than those from BSL; however, CD95 levels rebounded at 12MPT reaching similar levels observed at BSL (Figure S6D). Also, a trend towards a decreased frequency of both CD25 and $CD69^+$ NK cells at EOT and 12MPT was observed, however neither CD25 nor CD69 expression differed among time points (Figures S6E and F). Interestingly, and similar to our previous results, the frequency of $CD25^+/CD69^+/CD95^+$ NK cells was reduced at EOT and 12MPT, while percentages of triple-negative NK cells were significantly augmented (Figure S6G). Finally, expression of NK cell activating receptor NKG2D, and natural cytotoxic receptors (NCRs) NKp30 and NKp46 were evaluated. As shown in Figure S5H, the percentage of NKG2D-expressing NK cells was significantly decreased at EOT, compared to BSL. When relative fluorescence intensities (RFI) were examined, a significant reduction in NKG2D expression was also obtained at 12MPT (Figure S6H, right panel). Regarding NCRs, while NKp46 expression was not differentially modulated, we observed a trend towards a reduction in the expression of NKp30 at EOT and 12MPT. No differences were found when analyzing RFI (Figure 6H).

Soluble factors: Then, plasma concentration of different soluble factors, frequently associated with markers of immune activation and inflammation, were evaluated. IL-17 and IL-1β were below the limit of detection so they could not be quantified. For IL-6, IL-2, sCD14, IFN-γ, TNF-α and sCD23 no differences were observed along the different time-points evaluated (Figure S7A-F). Conversely, IP-10, IL-8 sICAM-1 and sCD163 were elevated at BSL but were significantly reduced at EOT ($p<0.0001$, $p=0.0015$, $p<0.0001$ and $p=0.0002$, respectively) and remained low (always compared to BSL) at 12M-PT ($p=0.0107$, $p=0.0426$, $p=0.0001$ and $p=0.002$, respectively) (Figure S7G-J).

Association between immune status and HIV persistence. Finally, we assessed the relationship between the different immune parameters and HIV viral persistence. All immune measurements (T and NK phenotype and plasma soluble factors) evaluated at 12MPT were compared with US-RNA levels at 12MPT, the US-RNA fold-up between 12MPT and BSL (12MPT/BSL) and the US-RNA change (delta) between 12MPT and BSL (12MPT-BSL). For simplicity, results are shown in heat-maps denoting r values. Significant correlations are highlighted and the corresponding y vs. x plot is shown. No statistically significant correlations were found between US-RNA at 12MPT, US-RNA fold-increase or delta US-RNA with parameters evaluated on $CD4^+$ or NK cells (not shown). Conversely, US-RNA fold-increase was negatively associated with the proportions of CD8 T_{TM} cells and positively with %CD8 T_{EM} cells (Figure 3A). Levels of most cytokines at 12MPT showed positive r values with the three virological parameters evaluated, only sCD163 showed a statistically significant correlation with delta US-RNA (Figure 3B).

Discussion

HIV remission and cure clinical research has largely occurred in high-income settings where most participants are men who have sex with men, where subtype B is the most prevalent viral variant, malnutrition is not a regular finding and there exists a low burden of coinfections. All these factors may be important determinants of the size and transcriptional activity of the HIV reservoir, and cure strategies might need to be different for high and low-middle income settings, especially if the intervention is dependent on a change in immune function[27]. Here, we found that DAA-mediated HCV clearance in HIV/HCV coinfected subjects is associated with: i) a clear improvement in liver functionality and a tendency to improved $CD4^+$ T cells counts, ii) a

decrease in the surface expression of activation and exhaustion markers in $CD4^+$, $CD8^+$ T cells and also in NK cells; iii) lower plasma levels of IP-10 and IL-8 and of indicators of macrophage and monocyte activation, such as sCD163 and sICAM and iv) higher levels of US-RNA at 12 months post-DAA treatment.

Therapies with DAAs are very effective oral and short-term treatments, with more than 90% of SVR, even in HIV coinfected individuals[28]. This has been reflected in our study, where all participants achieved viral clearance and improved liver function parameters, despite the presence of advanced hepatic disease. In addition, a recovery in both $CD4^+$ and $CD8^+$ T cell phenotype (in terms of the expression of activation and exhaustion markers) was registered after DAA treatment. These findings are in line with previous reports following DAA for HIV/HCV co-infection describing improved HCV-specific $CD8^+$ T-cell functionality and lower PD-1 expression, recovery of the $CD4^+$ T-cell compartment and a replenishment of T cells with memory/effector phenotype[29-31]. Similarly, a decrease in the proportion of activated NK cells was found after DAA treatment, in agreement with previous publications describing reduced expression of activation markers and cytolytic activity[9,29,31,32]. Although others have observed an increase in NK cell frequency after DAA treatment[33-37], this was not found in our study. It might be due to the fact that in this study all individuals presented with end-stage liver fibrosis which is linked to low NK cell frequencies[34]. In these individuals, liver damage could be a stronger factor modifying NK cell population than HCV clearance. Finally, lower levels of soluble inflammatory factors, such as IP-10, were previously reported[38], and recapitulated in our study.

The first reports regarding the impact of HCV on HIV persistence on ART, described that IFN-α/ribavirin treatment was associated with decreased levels of total and integrated HIV DNA in $CD4^+$ T cells[20] and 2LTR circles from PBMC[19], as assessed by RT-PCR; and also, $CD4^+$ T cell

HIV RNA reduction[18]. In this latter report, no differences were found regarding $CD4^+$ T cell proviral HIV DNA, 2LTR circles, and replication competent reservoirs measured with qVOA (quantitative viral outgrowth assay). Nevertheless, it is important to highlight that those therapies were based on two drugs (IFN-α and ribavirin) that could also have a direct effect on HIV replication[39,40]. More recently, three studies have evaluated the levels of CA HIV DNA in PBMCs before and after DAAs for HCV treatment. Parisi *et al* described that there was an increase or decrease in total HIV DNA after DAA treatment depending on the magnitude of HIV viremia (low-level versus undetectable) before starting DAAs[22]. In other works, HIV DNA remained stable before and after DAA treatment[23,41]. All the three forms of HIV DNA measured here (total, integrated and 2LTR) remained stable during follow up. The differential findings might be explained by the use of purified $CD4^+$ T-cells instead of total PBMCs to quantify HIV DNA and a more rigorous criteria regarding HIV undetectable VL before enrollment. It is also worth noting here that, although extensively used, PCR-based techniques for measuring both total and integrated HIV DNA face considerable limitations, since they tend to overestimate the size of the reservoir due to the high prevalence of defective proviruses. Thus, these results should not be interpreted as if there was no effect on the size of the competent reservoir[42].

We also studied the transcriptional activity of infected cells by measuring US and MS-RNA and plasma RNA. A significant increase in US-RNA was found 12 months after HCV clearance with no increase in CA MS-RNA or plasma RNA levels. Correlation analysis indicated that the elevation of US-RNA was accompanied by diminished proportions of $CD8^+$ T_{TM} cells and higher proportions of $CD8^+$ T_{EM} cells. This could be an indicator that higher transcription might be accompanied by production of at least some viral proteins which might be priming memory $CD8^+$ T-cell responses. On the other hand, the steady-state of MS-RNA and plasma RNA levels

could be reflecting a block in viral cycle termination. This raises different hypotheses. Higher levels of US-RNA might represent higher rates of genuine HIV transcripts but also host-HIV read-through transcripts[43,44]. However, these latter transcripts have been shown to contribute poorly to the bulk of HIV RNA, so this hypothesis seems unlikely[44]. Yukl et al showed that non-activated latently-infected $CD4^+$ T-cells show substantial transcription initiation which is afterwards blocked at the elongation, polyadenylation and splicing steps[43]. Also, evidence indicate that unstimulated naïve and memory $CD4^+$ T-cell subsets support transcription initiation and elongation with different capacity; but upon stimulation T_{EM} cells are the cell subset that more efficiently achieve transcript elongation[45]. Although we did not observe differences in the bulk distribution of $CD4^+$ T-cell subsets from BSL to 12MPT, we cannot exclude modifications within particular T *helper* subsets that might justify the higher frequency of US-RNA positive cells in periphery. The potential variations in T *helper* subsets could contribute to our findings as a consequence of increased transcription initiation, enhanced blockade or even reduced trafficking of these cells to the tissues secondary to the changes of chemokines and chemokine receptors expression after HCV clearance. In other line, HCV has been shown to replicate in lymphocytes[46] thus HCV elimination might modify signalling pathways leading to increased HIV transcription in these cells. Finally, it has been demonstrated that DAA-mediated viral clearance was accompanied by a lower activity of type I IFN (α y β) receptors, meaning a downregulation of interferon stimulated genes, in HCV monoinfected individuals[11]. This might impact the transcriptional activity of HIV latently infected cells. Recently, it was shown *in vitro* that, once latency is stablished, IFNα could act as a reversal agent promoting viral replication[47]. *A priori*, this result contrasts our findings. However, it should be considered the establishment

and maintenance of latency is a complex multifactorial phenomenon governed by mixed, sometimes opposed, mechanisms and the net result of this is what is observed *ex vivo*.

Certainly, this work opens new perspectives that should be addressed. First, we used PCR based techniques to measure viral reservoirs. These assays tend to overestimate the frequency of replication competent virus, since both defective and non-defective viral strains are detected. Future studies will be aimed at including assays aimed at identifying the translation-competent reservoir[48] as well as qVOA (quantitative viral outgrowth assay), TILDA (Tat/rev Induced Limiting Dilution Assay) and IPDA (intact proviral DNA assay) assays to address this limitation[49,50]. Second, longer time of follow up would provide more information regarding the long-term effect of HCV clearance in HIV transcriptional activity. Also, it would be relevant to evaluate the magnitude and quality of HIV-specific immune response after HCV clearance and its association with HIV persistence, since it could be hypothesized that the increase in HIV transcriptional activity might be associated with a boosting of HIV specific T cells. Third, all the individuals included in the present study presented advanced liver fibrosis; it will be important to evaluate if the results found are reproducible in a cohort of participants with low to mild liver fibrosis. Finally, results could not be extended to the intrahepatic CD4+ T-cells but a similar o higher increase in HIV transcription may be expected. There is certain evidence to suggest that hepatocytes and hepatic stellate cells support HIV infection[51]. Thus, the modification in the liver environment, including decreased HCV-specific immune surveillance, could lead to a relapse in HIV transcription in these cells. Nevertheless, results presented here provide an important insight into the effects of chronic HCV infection on virus persistence, confirming HCV coinfection as a relevant factor imposing an extra challenge in HIV remission studies, even after HCV clearance.

Acknowledgements

Authors specially acknowledge study participants for agreeing to collaborate in this study and to provide blood samples. We thank Drs. Michael Busch and Mars Stone from Vitalant Research Institute for scientific input; Mrs. Sabrina Azzolina for technical help during sample processing; Dr. Carla Pascuale and Dr. Virginia Polo for technical assistance in multiparametric flow cytometry; and Andrea Peña Malavera for statistical advice. SRL is supported by the National Health and Medical Research Council of Australia and the National Institutes for Health Delaney AIDS Research Enterprise (DARE) Collaboratory [U19 A1096109, UM1AI126611]

Figure legends:

Figure 1: HIV reservoir dynamics in HIV/HCV-coinfected individuals treated with DAA. Cell-associated (CA) HIV DNA and RNA, as well as plasma HIV RNA were evaluated in coinfected individuals before treatment initiation (baseline, BSL), at the end of treatment (EOT), and 12 months after finalizing DAA therapy (12MPT). CA total HIV DNA, Integrated DNA and 2LTR **(A)**, CA multiple spliced (MS)-RNA, unspliced (US)-RNA, and plasma RNA **(B)**, and US-RNA/Integrated DNA, US-RNA/Total HIV DNA and MS/US-RNA ratios **(C)** are shown. Viral DNA and RNA copies were calculated relative to 10^6 cell equivalents (CE). Individual values, median and 25th and 75th percentiles are indicated. Statistical comparisons were performed using Wilcoxon test, $p<0,05$.

Figure 2: Association analysis between cell-associated unspliced (US) and plasma HIV RNA in HIV/HCV-coinfected individuals treated with DAA. Cell-associated (CA) unspliced (US) RNA as well as plasma HIV RNA were evaluated in coinfected individuals before treatment initiation (baseline, BSL), at the end of treatment (EOT), and 12 months after finalizing DAA therapy (12MPT). Relationship between variables was measured by applying a generalized linear mixed-effects model with plasma RNA as the independent variable, and CA US-RNA and time as fixed-effect predictors; *p* value for the CA US-RNA coefficient is shown. White, gray and black filled-dots represent individual measures belonging to BSL, EOT and 12MPT subgroups, respectively.

Figure 3: Correlation analyses between cellular and soluble markers of immune activation and inflammation, and HIV reservoirs in HIV/HCV-coinfected individuals treated with DAA. Heat map representation of Spearman rank correlation coefficients computed for the expression of $CD8^+$ T-cell immune markers at 12MPT **(A)** and plasma levels of soluble factors of immune activation and inflammation at 12MPT **(B)** versus HIV unspliced (US)-RNA at 12MPT (US-RNA 12 MPT), US-RNA fold up between 12MPT and BSL (Fold-up US-RNA 12MPT/BSL) and differences between US-RNA at 12MPT minus levels at BSL (Delta US-RNA 12MPT-BSL). The colors denote both the correlation direction and strength of association, ranging from -1 (blue) to 1 (red). Statistical significant associations are further shown below each panel in individual x vs. y plots. Spermans's r and p values are shown.

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Table 1. Subjects characteristics.

Characteristics	BSL n=19	EOT n=19	12MPT n=17	p value [a/b]
Age (years)[1]	49 (46-53)	--	49 (45-52)	--
male sex (n,%)[2]	14 (73.4)	--	13 (76.4)	--
CD4 count (cells/μL)[1]	291 (231-776)	460 (205-692)	506 (233-1058)	>0.999/0.455
CD8 count (cells/μL)[1]	849 (498-1263)	917 (407-1293)	1041 (503-1389)	0.851/0.216
NK cells (%)[1]	8.9 (5.6-16.2)	8.1 (5.7-39.6)	9.5 (4.2-28.2)	0.903/0.845
CD4/CD8 ratio [1]	0.56 (0.33-0.78)	0.52 (0.39-0.78)	0.51 (0.39-0.71)	0.025/0.397
Time of HCV infection (years)[1]	13 (11-22)	--	--	--
Time of HIV infection (years)[1]	19 (12-21)	--	--	--
HCV viral load (log_{10} copies)[1]	5.89 (5.56-6.13)	All < 1.3	All < 1.3	<0.001/<0.001
Time of ARV (years)[1]	10.5 (4-16.5)	--	--	--
Routes of transmission (n,%)[2]				
IDU	14 (73.7)	--	--	--
Heterosexual	5 (21.1)	--	--	--
MSM	1 (5.3)	--	--	--
HCV genotype (n,%)[2]				
1a	12 (63.2)	--	--	--
1b	1 (5.3)	--	--	--
1	3 (15.8)	--	--	--
3	3 (15.8)	--	--	--
Liver stiffness (Kpa)[1]	22.2 (17.9-32.2)	ND	ND	--
APRI score[1]	1.16 (0.58-2.09)	0.76 (0.29-0.84)	0.55 (0.29-1.03)	0.0084/0.0004
ALT (IU/L)[1]	67.5 (46-82)	27 (17-49)	33 (24-49)	<0.001/<0.001
AST (IU/L)[1]	78.5 (68.2-93.7)	33 (26-45)	38 (32-51)	<0.001/<0.001
Albumin (g/dl)[1]	4.2 (3.6-4.5)	4.2 (3.9-4.4)	4.4 (4.1-4.5)	0.382/0.020
Platelets (x10^3/mm^3)[1]	111 (98-213)	121 (87-	121 (77-193)	0.922/0.130

		229)		
Total bilirubin (µg/dl)[1]	0.85 (0.72-1.1)	0.80 (0.55-1.42)	0.80 (0.70-1.0)	0.183/0.450
Prothrombin time (%)[1]	74 (63-93)	71 (63-81)	76 (69-85)	0.531/0.867

Abbreviations: BSL: baseline. EOT: end of treatment. 12MPT: 12 month pos-treatment. ARV: antiretroviral therapy, ALT: alanine aminotransferase, AST: aspartate transaminase, nd: not determined. 1- Median (IQR). 2-Number of cases (number/total in %). a: BSL vs. EOT and b: BSL vs.12MPT, Wilcoxon test. IDU: Injecting drug user. MSM: men who have sex with men.

Figure 1

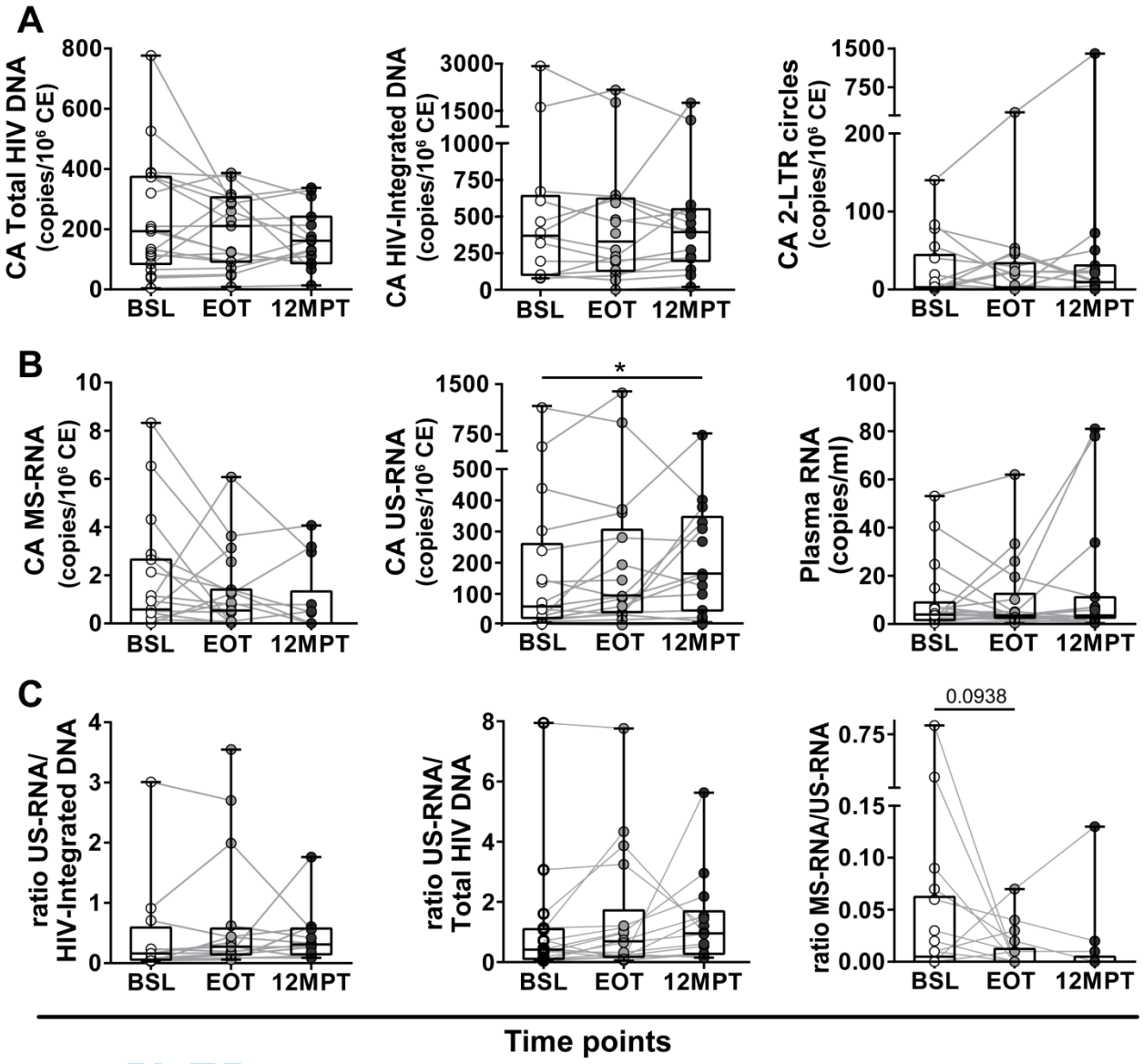

Figure 2

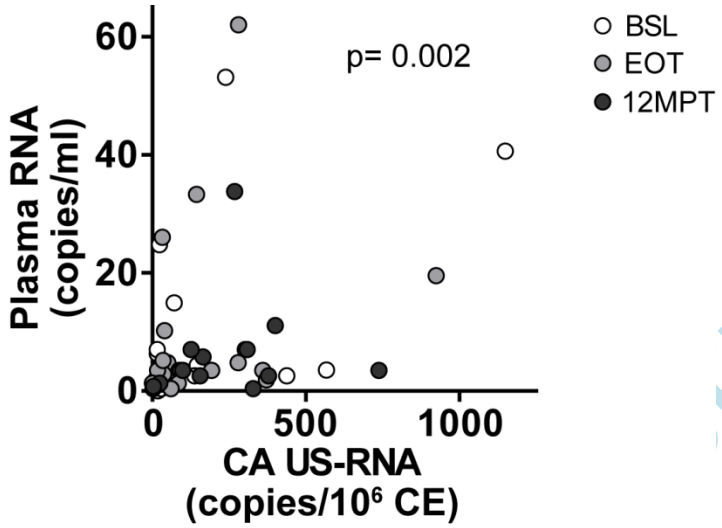

Figure 3