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Milan, July 12th 2022

Dear Editor,

Thank you for considering this work for publication in *Viruses*.
As requested, we are submitting a revised version of the manuscript that addresses the points raised during the peer-review process, with amendments to the original version highlighted in yellow.
You can also find below the responses to each point raised.

Reviewer #1

The paper of Bono V et al focused on DAA therapy impact on some immune parameters, that were reported to impact hepatic disease progression, in HIV/HCV co-infected as compared to HCV mono-infected subjects. By this paper the authors try to elucidate the impact of DAA on some immune cell populations involved in fibrogenesis. They have found significant differences in gamma/ delta T cells and B cells activation after HCV cure in both groups. They found that the ratio between Vδ2 and Th17 cells inversely correlated with liver transaminases level. However the importance of these results is poorly discussed, moreover HIV impact is not discussed.

Major points:

The paper show only Vδ2 of γδ T cells and no data on Vδ1, which was previously reported to be increased in the circulation HIV/HCV coinfected subjects and contribute to liver disease progression. (AIDS Res Hum Retroviruses (2001) 17(14):1357–63.); HIV infection induces a Vδ1 mobilization from tissue to the peripheral blood and depletion of Vδ2. I think if you want to have a better image for liver damage in HIV/HCV co-infection, this population should be evaluated.

We thank the reviewer for this comment. We agree that studying Vδ1 cells in the pathogenesis of liver damage in HIV/HCV co-infected individuals and have cited as well as discussed relevant literature accordingly (Agrati et al. AIDS Res Hum Retroviruses (2001) 17(14):1357–63; ref 20),

(page 2, line 48 and page 8, lines 240-244). Unfortunately, PBMCs from study participants are no longer available, therefore we are unable to perform experiments on the Vδ1 cell subset.

Another major point is the correlation between transaminases level at SVR12 and Vδ2/Th17 ratios. The authors suggest that is negatively linked to liver damage; however the AST and ALT levels at SVR 12 (table 1) seems to be in normal range, they should discuss more on this. ***We thank the reviewer for this comment and have discussed it "Further, in keeping with the concept suggested by Wu et al. [28] of the interaction between the two cell subsets in driving liver inflammation and together with the notion of low-level inflammation in treated HIV subjects [23], our findings may suggest subclinical evidence of hepatic damage in the setting of HIV-HCV co-infection." (page 8, lines 257-260).***

The flow cytometry gating strategy should be included in the figures (at least as supplemental material if not allowed by journal guidelines).

As suggested by the referee, we have now added the gating strategy used as Supplementary Figure S1, S2, S3 (page 2, lines 90-91).

The conclusions are not supported by the results:

lane 25-27: "DAA had a limited effect on the phenotype and function of γδ T cells, frequency of Treg and Th17 cells as well as B cells activation in HIV-HCV co-infection." - only the statistically significant results should be considered.

We agree with the reviewer and have rephrased to: "In HIV-HCV-coinfected and HCV-monoinfected participants the effect of DAA was limited to γδ T and B cell activation as well as γ-globulin concentrations and the Vδ2/Th17 ratio, with no changes in γδ T cell function and Treg frequencies. Importantly, γδ T and B cell activation remained at higher levels in coinfected individuals than in those with HCV moninfection alone. The persistence of such alterations within these cell subsets may associate with the risk of hepatic and extrahepatic complications" (page 1, lines 25-29)

lane 238-242: "In conclusion, we show a partial benefit of HCV eradication mediated by DAA treatment on the phenotype and function of γδ T cells as well as Treg and Th17 frequencies in the

setting of HIV-HCV co-infection. These findings shed light onto the possible immunopathogenic mechanisms underlying the improvement of liver function following HCV clearance." - same here

We agree with the reviewer and have rephrased to: "In conclusion, we show a benefit of HCV eradication mediated by DAA treatment in HIV-HCV and HCV monoinfected subjects, with decreased activation of γδ T and B-cells as well as increases in the Vδ2/Th17 ratio and reduced γ-globulin concentrations. These findings shed light onto the possible immunopathogenic mechanisms underlying the improvement of liver function following HCV clearance. However, γδ T and B cell activation remained at higher levels in HIV-HCV coinfection than that measured in HCV monoinfection alone" (page 9, lines 270-276).

In addition the differences between groups, such as the grB+γδ T cells, should be given a proper discussion***. We thank the reviewer for this comment. We have elaborated this finding in the results section of text (page 4, line 132).***

Minor:

In the methods section for B cell activation markers should be CD3-CD19+CD69+/CD86+

We have corrected the text accordingly.

It would be nice to have the Figures as dots not bars. In the axes title (or in the figure legend) should be noted to which cell population is the percentage reported (ex: % γδ T cells of CD3+ T cells) . ***We have changed the figures from bars to dot plots and noted the percentage of the total cell population.***

Check the number of subjects to be consistent, at figure 1D.

We have corrected figure and legend.

Other points:

Are there any correlations at baseline with liver damage (Fibrosis, ALT, AST) - if performed should be mentioned at least in the discussion.

At baseline, correlations between* Vδ2/Th17 *ratio and AST/ALT and liver stiffness were non significant in both study groups. In particular:

-HIV-HCV coinfection: AST: r=0.2, p=0.1; ALT: r=0.2; p=0.09; stiffness: r=0.06, p=0.7

- HCV monoinfection: AST: r= 0.1, p=0.4; ALT: r=0.1; p=0.5; stiffness: r=0.19, p=0.3

We have included a sentence in the results section (page 6, lines 165-168)