Manuscript ID: 40647

Title: "Spleen stiffness mirrors changes in portal hypertension after successful interferon-free therapy in chronic-HCV patients"

Article Type: Original Article

World Journal of Hepatology

Point-by-point response to reviewers

Dear Editor,

We would like to thank the editorial team of World Journal of Hepatology for the interest shown in our manuscript and the possibility of reconsidering its publication after revision.

Reviewer #1

1. Were patients co-infected with HBV or HIV excluded from the study?

We thank the reviewer for the annotation made, as co-infection with HBV or HIV might a be a confounding factor that should be considered. No patient with HIV co-infection was included in the study, whereas only 6 patients presented HBV co-infection. In a further analysis, no statistically significant difference was observed in liver (LSM) and spleen (SSM) between patients with only HCV infection, as opposed to others with HBV co-infection. At univariate analysis, HBV co-infection was not associated with the SSM reduction >20% (OR= 2.655, 95%IC = 0.469-15.019, *p = 0.270*).

2. Did oesophageal varices disappear after antiviral therapy?

We find the reviewer's remark important, as it would be very interesting to know whether major direct signs of portal hypertension (PH), such as esophageal varices (EV), would regress after therapy. However, our study was retrospective and repeated assessments with upper endoscopy (EGD) within 6-9 months were not scheduled, as they would not have been part of the routine management of patients with advanced liver chronic disease (ACLD).

However, we analysed the subgroup with patients presenting paired EGD. Out of 41 (61.2%) patients with EV at BL (*EGD available within 6 months from start of therapy in 67 patients*), 20 patients had undergone EGD also after achieving SVR. EV had disappeared only in 4 patients (20%), remained the same size in 13 (65%) and enlarged in 3 (15%).

Considering the limited number of patients with paired EGD and the different timepoints of the second EGD (after SVR), such results were not included in the final manuscript.

3. Did the authors investigate signs of portal hypertension (spleen stiffness) at the time-points 2, 4, 6, 8, and 12 weeks after antiviral therapy? Is there a kinetic?

Repeated measurements at the above-mentioned timepoints, as carried out by Pons et al (Therap Adv Gastroenterol, 2017), were not scheduled in our study. However, spleen (SSM) stiffness at end-of-treatment (EOT) was available in 49 patients, showing a median value of 43.5 kPa.

In this view, a numerical decrease in SSM can be observed: SSM at baseline (BL) 58.8 kPa, SSM at EOT 43.5 kPa and SSM at SVR24 38.2 kPa. However, this reduction was statistically significant only between SSM BL vs SSM EOT (*p= 0.0009*), but not between SSM EOT vs SSM SVR24 (*p= 0.109*).

Considering that this was a sub-analysis in a very limited number of patients (37%), this result was not included in the final manuscript.

4. Was genotype of hepatitis C of any Impact in this study?

Although such a result was not presented in included tables, HCV-genotype showed no significant association with any of the considered outcomes (SSM reduction >20%, CSPH persistence) at univariate analysis.

5. The authors should cite and discuss one of the latest manuscript by Buechter M. et al. investigating Spleen stiffness and HVPG after TIPS implantation.

The study by Buechter et al is now cited and discussed.

Reviewer #2

Major points:

1. The primary endpoint should be written in the Material and Methods section.

The aims of the study have now been described in the Material and Methods section.

2. As the conclusion in abstract, the authors described (p3), "SSM seems to reflect changes in PH after SVR better than other NITs." I cannot find the results supporting this opinion in the abstract. Please explain more clearly.

In our study we reported changes of all NITs after therapy with direct-acting antivirals (DAAs); however, only the different dynamics between SSM vs LSM decrease (and not other NITs) are described. Indeed, LSM decrease (shown in the majority of patients, due to reduction of necro-inflammation) was confronted with SSM decrease (direct surrogate of PH); with only the latter being similar to the decrease observed with the gold standard of PH assessment, hepatic venous gradient (HVPG) (Mandorfer et al, J Hepatol, 2017). In conclusion, since no direct comparison between HVPG and different NITs was presented in this study, thus, the concluding phrase was modified.

3. The explanation of Table 1 is not necessary in the main text, because many readers can understand by looking at table. (p10, lines 1-10)

The paragraph describing Table 1 has been reduced accordingly.

4. The context of Figure 2 is most important in this paper. Therefore, P values should be described in this figure (not use symbol mark).

We agree with the reviewer. The p-values have now been included in Figure 2.

5. I strongly recommend that the values of supplemental material 2 should be written in the Figure 2.

Median values of NITs have been written in Figure 2 and Supplemental Material 2 consequently deleted.

6. The authors described, "As presented in Supplemental Material 1, the baseline characteristics did not statistically differ between patients with paired TE measurements and those with only BL data, except of serum albumin levels (p= .042)." (p 10) But, I cannot find out this importance. Please describe the importance of this sentence.

The comparison was made to support the generalizability of our results: the included group of patients (with available paired SSM assessments) was not a result of a selection bias, as their baseline characteristics did not significantly differ from the findings in patients, in whom such variable, SSM, was not available.

However, in consideration also of remark #10, this table has been deleted.

7. Figure 3 is very confusing. Please simplify.

Figure 3 has been simplified and separated into two figures.

8. Please mention the reason of using cut-off value of SSM decrease > 20% in the table 3 with bibliographical consideration.

There is currently no specific data in the literature regarding to what extent the relative changes in LSM and/or SSM are to be considered significant, and therefore surrogates of therapy response. Because of that, a >20% decrease was used, as such cut-off is considered significant for HVPG values (Baveno VI Consensus Workshop). This explanation was also reported in the Material & Methods section.

9. In this paper, the diagnosis of CSPH was defined by LSM >21 kPa. Nevertheless, LSM value was analysed for the predictors of CSPH in the table 4. If the authors want to analyse the LSM value, CSPH should be defied by HVPG or other method.

We agree with the reviewer that use of LSM for both CSPH definition and prediction is suboptimal. However, HVPG values were not available in this study, and, despite several studies have shown accurate SSM cut-offs for CSPH prediction (i.e. Colecchia et al, Gastroenterology, 2012), no unified and overall accepted cut-off for SSM is yet available. Moreover, the use of the same variable as both outcome and predictor is not uncommon (i.e. LSM in the development of Boursier's criteria, Boursier J, Hepatology, 2013).

10. There are too many tables. Unnecessary tables should be deleted.

Supplemental Tables 1 and 2 have now been deleted, and Table 4 has been included in the Supplemental Material.

Minor points:

1. There is no figure legend.

Figure & tables legends have now been included. Moreover, abbreviations used in the figures were spelled out in the figure description, as suggested by Reviewer #3.

2. Please spell out about abbreviation (TE, EV, EBL, etc.). (p6, p7, p9)

The above-mentioned abbreviations are now correctly spelled out.

3. The description, "According to the Baveno VI Criteria [11], values of LSM > 10 kPa at TE were considered suggestive of having cACLD and whom with LSM ≥21 kPa were defined to rule-in CSPH as previously described [33,34]. At baseline, laboratory values, Model for End-Stage Liver Disease (MELD)

and Child-Turcotte Pugh (CTP) scores were also reported for each patient." should be moved to Introduction or Discussion section.

We believe that the study design section, describing variables considered for the study, i.e. LSM, SSM, MELD, CTP, belong in the Methods section. However, mention of LSM cut-off for compensated advanced chronic disease (cACLD) and clinically significant portal hypertension (CPSH) was made in the introduction section.

4. There are a lot of grammatical mistakes. You should get English proofreading for this paper.

The English has now been edited by a professional native-speaking editing agency.

Reviewer #3

1. The section "core tip" is missed.

As suggested by the reviewer, the core tip section has now been included.

2. (Introduction) It should be specified that the LSM values of 20-25 kPa recommended by the Baveno VI consensus are referred only to patients with chronic viral hepatitis and not to patients with other etiologies of liver disease.

The specification for viral etiology has been inserted in the sentence.

3. (M&M) In this study, only the M probe was used. It is recommended to use the XL probe when the skin-to-liver capsule distance is >25 mm. How it was dealt with this issue?

We agree with the reviewer that using the XL probe would be optimal in such patients. However, no severely obese patients were included in the study (BMI >40 kg/m2). In fact, the rate of unfeasible SSM assessments in our study is relatively low (7.6%).

4. (Results) How many patients with complete response to surgical resection or loco-regional ablation of previous HCC were included?

The patients with a complete response to surgical resection and loco-regional ablation were respectively 10 (7.5%) and 7 (5.2%). We conducted a further analysis that showed no statistically significant difference between baseline LSM and SSM values of these patients, as opposed to other patients with no history of HCC (p-values for LSM and SSM were 0.311 and 0.407, respectively).

5. (Discussion) The acronyms should be spelled out only at the first mention.

Corrections have been made where due.

6. (Discussion) Page 15: In this study a non-invasive assessment of HVPG was made, thus it is incorrect to state that SSM was compared to HVPG. It should be specified again that it is a surrogate of HVPG.

We agree with the viewer on the improper use of the "comparison between SSM and HVPG". A specification for SSM, a surrogate of PH assessment, has been inserted.

7. (References) Ref #18: there is a typing mistake. Please correct Ref #36 should be checked: it is incomplete, the name of some authors is not spelled correctly and the list of authors is not correct.

Corrections have been made where due.

8. (Figures) The acronyms should be spelled out because the reader may look at the figures without reading the text.

Acronyms have been spelled our also in the figures.