Author's response to reviews

Title: Acoustic Radiation Force Impulse (ARFI) and Transient Elastography (TE) for evaluation of liver fibrosis in HIV-HCV co-infected patients

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Author's response to reviews: see over
Dear Editors,

We would like to submit our revised original research entitled “Acoustic Radiation Force Impulse (ARFI) and Transient Elastography (TE) for evaluation of liver fibrosis in HIV-HCV co-infected patients” for your consideration. We followed the constructive remarks made by the reviewers and the editors.

As requested by the Associate Editor, the authors added, in a new revised manuscript, the supplementary table untitled "Agreement between TE and ARFI fibrosis stage classification according to equivalent Metavir Fibrosis stage in genotype 1 and 4 patients" and referred to it in the text.

This manuscript has not been published elsewhere and is submitted exclusively to BMC Infectious Diseases.

This study showed promising results regarding non-invasive assessment of liver fibrosis with ARFI elastography in HIV-HCV patients providing a liver fibrosis staging similar to that obtained with transient elastography. Moreover this technique permits to perform in the same session, morphologic exam and fibrosis assessment.

We look forward to hearing from you,
Dr Nora Frulio and co-authors
ANSWERS TO THE EDITOR

The authors would like to thank the Associate Editor for appreciating that our manuscript was improved after our revisions.
Please find below the point by point response to the last minor comment detailing the modifications of the updated manuscript.
Please also find the answers to reviewers that have already been submitted which are now including the new modification concerning this minor revision. (table between agreement between TE and ARFI fibrosis stage classification according to equivalent Metavir Fibrosis stage in genotype 1 and 4 patients).

Editor:
Thank you for submitting your revised manuscript. The Associate Editor is requesting for a new revision of your manuscript and have the following minor comment that you should consider addressing prior to publication.
"The manuscript has improved a lot. Only one minor revision is needed. Please request the authors to add the supplementary table "Agreement between TE and ARFI fibrosis stage classification according to equivalent Metavir Fibrosis stage in genotype 1 and 4 patients" and refer to it in the text."

We have clarified this point and added a new table (table 3) in results section in order to demonstrate that agreement between TE and ARFI fibrosis stage classification according to equivalent Metavir Fibrosis stage in genotype 1 and 4 patients was not different than for any genotype.
Results are now given in the results section at the end of the paragraph untitled ‘Comparison between ARFI and TE according to equivalent Metavir fibrosis Score’:
“In the subgroup of patients with HCV genotypes 1 or 4, results were similar. Table 3 shows the agreement between TE and ARFI measurements according to equivalent Metavir fibrosis stage classification in genotype 1 and 4 patients. Agreement between the two methods was very good overall [concordance 65.6%, weighted Kappa= 0.84; 95%CI=[0.71-0.96], very good [concordance 93.8%, Kappa= 0.83, 95%CI=[0.61-1.00] for predicting severe fibrosis (≥ F3) and moderate [concordance 75%, Kappa= 0.51, 95%CI [0.22-0.79] for predicting significant fibrosis (≥F2).”

Table 3 untitled “Agreement between TE and ARFI fibrosis stage classification according to equivalent Metavir Fibrosis stage in genotype 1 and 4 patients” has been added at the end of the main document.
ANSWERS TO THE REVIEWERS

The authors would like to thank the 3 reviewers for their constructive comments. Please find below the point by point answer to each comment detailing the modifications of the updated manuscript.

Reviewer 1:
The authors would like to thank the reviewer for reading our article and finding an important level of interest in this field.

Reviewer 2

-Major Compulsory Revisions:

The aim in this study is very important to identify the reliability of ARFI as compared to TE, non-invasive methods, in co-infected HCV and HIV patients. However, some questions need to be evaluated, including R2-1. The cases numbers, only 46 co-infected patients, are enrolled in this study, which could not prove the reliability of ARFI even that there is a good agreement as compared to TE.

This was a pilot study of reliability that included a moderate, yet not unusual for this aim, number of patients. The two issues that may be discussed in relation with a small sample size are representativity and the precision of the estimation of the main outcome criterion. We agree that we cannot ensure the representativity by including 46 patients only, but this was a pilot study. By contrast, the estimation of our main measures, Intraclass correlation coefficients, are precise enough to draw conclusions by comparing the lower bound of the 95%CI to the 0.6 value, deemed to represent the lower limit of a good agreement. The agreement between ARFI and Fibroscan on fibrosis staging was very good overall and for predicting F≥3.

This point was discussed in the discussion section: “This study included a small number of patients (n=46). However, it is a pilot study and the estimation of our main measures, Intraclass correlation coefficients, are precise enough to draw conclusions in our sample by comparing the lower bound of the 95%CI to the 0.6 value, deemed to represent the lower limit of a good agreement.”

R2-2. Previous studies showed high accuracy between TE and ARFI in single HCV-infected patients. However, only F1 and F4 cases showed higher accordance rate than F2 and F3 cases in this study. However, the data in this study remain lower than that in single infection. Owing to the factor of co-infection or others? Which needs to be classified and liver biopsy also suggests correcting this difference.

Our study showed very good agreement between TE and ARFI for predicting overall stages and for predicting F≥3. A moderate agreement between the two techniques was found for predicting F≥2. Presently our accuracy is not inferior to that in single infection. Indeed, for predicting fibrosis in single infection either TE or ARFI are more accurate for predicting extreme stages of fibrosis (F1 or F4) than moderate fibrosis (F2) as shown by the AUROC in different publications. As an example, for TE, AUROC was calculated as 0.79; 0.83; 0.77 for F≥2 versus 0.97; 0.95; 0.97 for F=4 respectively for Zioll et al (hepatology 2005), Castera et al (Gastroenterology 2005), Sirli et al (Hepat Mon 2010). For ARFI, AUROC was at 0.84 and 0.79 for predicting F≥2 and 0.91 and 0.84 for predicting F=4 respectively for Friedrich Rust et al (Radiology 2009) and for Sporea et al (Eur J Radiol 2012). Similar results are obtained whatever the hepatopathy. (Friedrich Rust et al; Journal of Viral Hepatitis, 2012 and Sporea et Al Ultraschall Med 2010.)

By comparing the areas under the ROC curves for the prediction of each fibrosis stage in both ARFI and TE techniques, Lupsur et al demonstrated that there was a significant difference for predicting ≥F2 while in the case of severe fibrosis (F3) and cirrhosis (F4), there was no significant difference between the performances of the two methods (Lupsor et al. J Gastrointestin Liver Dis 2009). Friedrich Rust et al also showed a significant difference concerning the diagnostic accuracy of the two techniques for diagnosis of significant fibrosis (Friedrich Rust Journal of Viral Hepatitis, 2012)
We have clarified this point and added informations concerning comparison of our results to the literature in the discussion section:

“Our work reveals that ARFI has similar results to TE for the evaluation of liver fibrosis in our sample of coinfected HIV-HCV patients with a very good agreement between the 2 methods both for predicting overall stages and for predicting F≥3. Same results are observed in the literature for single infection or for others chronic liver disease. A meta-analysis of nine studies evaluating the diagnostic accuracy of ARFI imaging quantification for the staging of liver fibrosis in various chronic liver diseases, showed excellent diagnostic accuracy for the diagnosis of F≥3 and for the diagnosis of liver cirrhosis using liver biopsy as gold standard, with AUROC respectively at 0.91, and 0.93 [44]. Sporea et al in an international multicenter study including 911 HCV mono-infected patients, showed a good diagnostic accuracy for predicting F≥3 with AUROC at 0.83 [49].

In our study, a moderate agreement between the two techniques was found for predicting F≥2. Our data are not different from those obtained in the literature. Indeed, in some publications, either TE or ARFI are more accurate for predicting extreme stages of fibrosis (F1 or F4) than moderate fibrosis (F2). For example for ARFI, AUROC was estimated at 0.79; 0.77; 0.82 for F≥2 and 0.84; 0.95; 0.91 for F=4 respectively [30, 33, 49].”

R2-3 **In this study, there was no difference according genotype and also presented good agreement between the two methods for predicting overall stages. Data? And the author needs to classify the association between viral loads and genotype with both methods.**

Agreement between the 2 methods for all genotypes was studied: results are given in results section at the beginning of the paragraph untitled ‘Comparison between ARFI and TE according to equivalent Metavir fibrosis Score’:

In our study, ‘overall agreement between the 2 methods was very good [concordance 69.6%, weighted Kappa=0.82; 95% CI= [0.70-0.95]]. Agreement was also very good for predicting severe fibrosis (≥F3) [concordance 93.5%, Kappa=0.80, 95%CI= [0.59-1.00]]. Agreement was moderate for predicting significant fibrosis (≥F2) [concordance 76.1%, Kappa= 0.5, 95%CI = [0.25-0.75]].”

Additional analysis was performed limited to patients with HCV genotype 1 and 4 whose response to treatment is usually poorer than patients with genotype 2 and 3, and would therefore possibly need an assessment of their fibrosis stage before taking a treatment decision. We found no difference concerning agreement between the 2 techniques than for any genotype. Results are given in the results section at the end of the paragraph untitled ‘Comparison between ARFI and TE according to equivalent Metavir fibrosis Score’:

“Agreement between the two methods for genotype 1 and 4, was very good overall [concordance 65.6%, weighted Kappa= 0.84; 95% CI=[0.71-0.96]], very good [concordance 93.8%, Kappa= 0.83, 95%CI=[0.61-1.00]] for predicting severe fibrosis (≥ F3) and moderate [concordance 75%, Kappa= 0.51, 95%CI [0.22-0.79]] for predicting significant fibrosis (≥F2). “

Results concerning agreement between TE and ARFI fibrosis stage classification according to equivalent Metavir Fibrosis for genotype 1 and 4 are shown in the table below. As requested by the editors in last minor revision, we had added this table in the text and refer to it in the text (see answer to editors) Results are given in the results section at the end of the paragraph untitled ‘Comparison between ARFI and TE according to equivalent Metavir fibrosis Score’:

“Table 3 shows the agreement between TE and ARFI measurements according to equivalent Metavir fibrosis stage classification in genotype 1 and 4 patients. Agreement between the two methods for genotype 1 and 4, was very good overall [concordance 65.6%, weighted Kappa= 0.84; 95%CI=[0.71-0.96]], very good [concordance 93.8%, Kappa= 0.83, 95%CI=[0.61-1.00]] for predicting severe fibrosis (≥ F3) and moderate [concordance 75%, Kappa= 0.51, 95%CI [0.22-0.79]] for predicting significant fibrosis (≥F2). “
Agreement between TE and ARFI fibrosis stage classification according to equivalent Metavir Fibrosis stage in genotype 1 and 4 patients

<table>
<thead>
<tr>
<th>ARFI F1 F2 F3 F4 Total</th>
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<tbody>
<tr>
<td>Fibroscan</td>
</tr>
<tr>
<td>F1 n 13 5 1 19</td>
</tr>
<tr>
<td>F2 n 2 3 1 6</td>
</tr>
<tr>
<td>F3 n 2 2</td>
</tr>
<tr>
<td>F4 n 5 5</td>
</tr>
<tr>
<td>Total n 15 8 2 7 32</td>
</tr>
</tbody>
</table>

Overall proportion of agreement: 65.6% 95% CI [46.8-81.4]
Kappa: 0.84 95% CI [0.71-0.96]

R2-4. Additionally, some important data such as prolonged time and Child-Pugh Classification, and statistical analysis etc. are not enough, which need to be further corrected.

- We clarified the point concerning Child Pugh classification, and added the Child Pugh classification in table1 for patients with cirrhosis. As all cirrhotic patients were Child Pugh A, the influence of Child Pugh stage in the analysis has not been studied. However cirrhosis has been studied as a potential factor associated with discordance between the two elastography methods (cf results section, paragraph entitled ‘Comparison between ARFI and TE according to equivalent Metavir fibrosis score’):
  “Among the 46 patients, 14 (30%) were discordant according to Metavir score between the 2 methods especially for predicting ≥F2 (n=11) or ≥F3 (n=3). In these discordant cases, ARFI had a higher score of fibrosis in 11/14 cases. As the discordance was more important for predicting F≥F2, an analysis of factors possibly associated with these discordances (age, sex, BMI, alcohol consumption, plasma HCV-RNA levels, absolute CD4+ (/mm³) cells rate, HCV genotype, increase of AST, ALT, ALP, GGT (>2N), or bilirubin blood level, steatosis, cirrhosis, HIV and HCV treatment, HCV treatment response), was performed.”

- For the point concerning statistical analysis, the objective of the study was to determine agreement between “equivalent Metavir” fibrosis stages established by ARFI and TE. That was assessed by simple Kappa coefficients for prediction of F≥3 or F≥2, and F=4, and weighted Kappa coefficients for prediction of F1 to F4 Metavir scores as recommended in literature. Ninety-five per cent two-sided confidence intervals of Kappa coefficients were also estimated.
Factors associated with discordances between ARFI and TE staging were described and tested by Wilcoxon test (quantitative variables) or Fisher’s exact test (qualitative variables). Only univariate analyses were conducted because of the small number of cases.
Statistik analysis is detailed in the text materials and method section, in the paragraph untitled ‘Statistical Analysis’.

- Discretionary Revisions

R2-5. HCC patients need to be excluded in this study.

The presence of HCC does not influence ARFI measurement in non tumoral liver because measurements has been performed at distance.
On contrary, one of the advantages of ARFI technique in comparison to Fibroscan® is the possibility to detect tumor especially HCC for patient with hepatopathy in the same examination with the same ultrasound device.
This point has been clarified in the discussion section:
“Note that, the presence of hepatocellular carcinoma (HCC) does not influence fibrosis evaluation because ARFI measurement in non tumoral liver was performed at a distance from the tumor (more than 3cm). Indeed, thanks to a visual control in B mode, the location to position the ROI could be freely chosen and it was possible to avoid specific area such as tumor, or vessels.”
Reviewer 3:

Major Compulsory Revisions:

R3-1. Please provide the information concerning the treatment to both HCV and HIV in the text as well as in Table 1. Any of these received antiviral therapy for HCV? What kind of therapy (e.g. SOC or DAA)? Was there any effect concerning antiviral therapy, treatment response and the performance TE/ARFI as well as the discordance between them? Similarly were all these patients receiving HAART? If not was there any effect concerning HAART and the performance of TE/ARFI?

We agree with this comment.

1- We have clarified this point in the result section. We added information concerning both HCV and HIV treatment in table 1 and also in the text (cf. results section, paragraph 'Characteristics of the study population'):

“Among the 46 patients, 28 did not receive any treatment for HCV. The 18 patients treated for HCV, received Peg-Interferon-Ribavirine (SOC). Among them, at the time of ARFI, 10 were in treatment failure and the 8 others were cured. No patients received Direct-acting antiviral agents (DAA). Moreover, all of the 46 patients of the study received highly active antiretroviral therapy (HAART). 28 patients received as third agent protease inhibitors (PIs), 3 patients received Integrase inhibitors (INIs), 8 patients received non-nucleoside reverse transcriptase inhibitors (NNRTIs), the seven other patients received other combinations of treatment. Details are given table 1”

2- The effect of HCV and HIV treatment on the discordance between the 2 methods has been studied as suggested by the reviewer.

In order to study the effect of HCV and HIV treatment on the discordance between the 2 methods, patients were divided in groups.

Concerning HCV treatment, 3 groups were designed (cf. table 1): the first one included patients who did not receive HCV treatment (n=28), the second group included patients treated for HCV with treatment failure (n=10), and the third one included patients with HCV treatment and cured (n=8).

Concerning HIV treatment 4 groups were designed according to different drug combinations (cf. table 1): the first one included patients treated by PIs (n=28), the second group corresponded to patients with INIs (n=3), the third group included patients treated by NNRTs (n=8), and the last one included patients treated by other combinations.

The analysis of HIV or HCV treatment, and treatment response did not reveal any significant association. Only age was associated with discordance between the two methods, with older patients in the discordant group (p =0.047).

Precisions have been given in the results section, paragraph ‘Comparison between ARFI and TE according to equivalent Metavir fibrosis Score’:

“As the discordance was more important for predicting F≥F2, an analysis of factors possibly associated with these discordances (age, sex, BMI, alcohol consumption, plasma HCV-RNA levels, absolute CD4+ (/mm³) cells rate, HCV genotype, increase of AST, ALT, ALP, GGT (>2N), or bilirubin blood level, steatosis, cirrhosis, HIV and HCV treatment, HCV treatment response), was performed. Only age was associated with discordance between the two methods with older patients in the discordant group (p =0.047). Moreover, there was a trend for a lower percentage of patients with overweight, cytolyis, or cholestasis and a higher percentage of patients with hepatomegaly, in the discordant group. No significant association was found with HIV and HCV treatment (p=0.22 and 0.55 respectively).”

However, the study size was not powered for this analysis (cf. discussion section)

R3-2. Please provide the data concerning TE and ARFI (e.g. median LSM, IQR etc) in Table 1.

We have added in table 1 data concerning ARFI and TE including median (Q1 and Q3), min, max for the 46 patients.

Data (median; Q1 and Q3) concerning each fibrosis stage, are described in the text for both ARFI and TE in results section, paragraph ‘Evaluation of liver fibrosis’:

“46 patients underwent TE and ARFI. Median delay between ARFI and TE was 10 days (0-25). The median liver stiffness value was 6.1 kPa (range 3.4-35.3) for TE and the median shear wave velocity (SWV) was 1.29m/s (range 0.93-2.86) for ARFI. The distribution of median LS measurements was as follows: 5.4 kPa (4.1-6.1) for stage F1; 7.2 kPa (7.1-7.8) for stage F2; 11.6 kPa (10.8-12.3) for stage F3; and 24.6 kPa (18.0-27.7) for...
F4, for TE. For ARFI, the distribution of median SWV was 1.15m/s (1.06-1.25) for stage F1; 1.44m/s (1.40-1.47) for stage F2; 1.60m/s (1.58-1.61) for stage F3; and 2.25m/s (2.12-2.52) for stage F4.”

**R3-3. Please provide a scatterplot and perform Pearson’s correlation to demonstrate the relationship between the raw results of TE and ARFI.**

We agree with this comment and added a scatterplot in this article in order to demonstrate the relationship between results of TE and ARFI (cf. Results section, paragraph ‘Evaluation of liver fibrosis’):

“The figure 2 represents a scatterplot to demonstrate relationship between median results of TE and ARFI”

**Minor Essential Revisions:**

**R3-4 Abstract:** "Agreement was moderate for predicting significant fibrosis (≥F2).» Please also provide the Kappa of this.

We agree with this comment. These informations are now given in the abstract.

**R3-5 Results:** "Ultrasound analysis allowed the detection of focal liver lesions in 5 cases, including 2 HCC (hepatocellular carcinoma). For the 3 other cases the liver lesions were benign.” How were the natures of these few lesions confirmed? Please describe.

We agree with this comment. Precisions have been added in results section at the end of the paragraph ‘Characteristics of the study population’:

“The 2 HCC presented typical imaging criteria with arterial hypervascularity and wash out in venous and delayed phase, on dynamic enhanced MRI according to AASLD 2011 recommendation [45]. Moreover, diagnosis was confirmed by histology after biopsy performed just before liver nodule radiofrequency. The diagnosis of the 3 benign liver lesions was made on the basis of tumor specific vascularity pattern in the arterial, portal, and late phases on dynamic enhanced MRI as described previously [46-48]. Imaging features were typical and no liver biopsy was necessary to confirm the diagnosis.”

**R3-6. Please spell out all abbreviations in the footnote of Table 1 and 2.**

All abbreviations (including those in table 1 and 2) are now spelled and listed in the beginning of the main document in the dedicated section.