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Recife, November 04th, 2017.

Dear Science Editor, Editorial Office **Baishideng Publishing Group Inc**

Li-Jun Cui

We would like to thank the editor and reviewers for their comments that greatly helped us to improve the manuscript. Herein, we present our answers to each question and changes made in the paper.

Before answering the reviewers, we would like to inform that we changed the title of the article to "^{1}H NMR-based Metabonomic Models for Non Invasive Diagnosis of Liver Fibrosis in Chronic Hepatitis C: Optimizing the Classification of Intermediate Fibrosis" and the corresponding author email to adoria04@globo.com. In addition, we would like to inform you, the last author changed to Ricardo Oliveira Silva.

Reviewer #1

"If the authors expect the clinical use of these MMs, they should discuss their cost-effectiveness".

At the moment, it is difficult to estimate precisely the cost-effectiveness of the method, since ^{1}H NMR spectroscopy of biofluids is not commercially available yet, only at research centers. As there is no sample-processing step or use of reagents or dyes, for an individual ^{1}H NMR serum analysis the cost would be low, since only a 5mm glass tube and 400 microliters of deuterated water would be needed. In an automated NMR spectrometer, it is possible to analyze up to 300 samples per day, from different medical

centers, for example. Considering the non-invasive nature of the method and the fact that it does not require a medical professional to perform, the effectiveness must be relatively high during when compared to liver biopsy, which requires patient's hospitalization in a specialized center and a trained medical professional.

"In this study, the authors excluded HCV patients treated with antiviral agents. However, nowadays, sustained viral response can be achieved for most HCV patients treated with direct-acting antiviral agents. Studies have demonstrated that elastography can help monitor liver fibrosis stages after antiviral treatment. Are the MMs useful in this setting? Please discuss this point.¨

Liver fibrogenesis is a dynamic and potentially reversible process, at least in part, once the aggressor agent or condition is removed. Poynard et al. evaluated individual data from 3010 naive chronic hepatitis C patients with pretreatment and posttreatment biopsies and different regimens combining interferon and ribavirin. They showed that fibrosis stage was improved in 20% of patients with most of the differences of a one stage change by METAVIR, as well as a lower frequency of fibrosis progression among patients who achieved a virologic sustained response (7% in responders vs. 17% in relapsers and 21% in nonresponders) and reversion of cirrhosis in 49% of the patients with baseline cirrhosis (1).

Recently, it has been shown a significant reduction of liver stiffness measurement, by transient liver elastography, and of APRI and FIB-4 scores after achieving virologic sustained response with novel direct-acting antiviral agents targeting HCV. However, it remains to be examined whether this indicates a true regression of fibrosis or merely a resolution of chronic liver inflammation (2, 3).

We are not aware of studies evaluating the usefulness of metabonomics in this scenario. However, in an ongoing study of our group we will compare the metabonomics strategy to APRI and FIB-4 scores and to ARFI elastography, before and after 12 weeks of treatment with direct-acting antivirals agents in patients with chronic hepatitis C.

References:

1- **Poynard T**, Mc Hutchison J, Manns M, et al. Impact of Pegylated Interferon Alfa-2b and Ribavirin on Liver Fibrosis in Patients With Chronic Hepatitis C. *Gastroenterology* 2002; 122: 1303–1313

2**- Bachofner JA**, Valli PV, Kröger A, et al. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. *Liver International* 2017; 37 (3): 369-376

3- **Elsharkawy A**, Alem SA, Fauad R, et al. Changes in liver stiffness measurements and fibrosis scores following sofosbuvir based treatment regimens without interferon. *Gastroenterology and Hepatology* 2017; 32(9): 1624-1630

"The results of this study suggest that the MMs can more accurately distinguish intermediate fibrosis stages in HCV patients than the APRI and the FIB-4 index. However, the Reviewer has found a paper indicating that the measurement of serum osteopontin levels may be useful for this purpose (PLoS One, 10 : e0118744, 2015). Please cite this paper and discuss other measures to distinguish intermediate fibrosis stages."

We added this discussion to the paper on lines 310-329:

"The most widely indirect methods for the assessment of liver fibrosis in CHC patients in routine clinical practice are the non-commercial serological scores APRI and FIB-4, and the physical methods, such as the liver stiffness measurement, by elastography based on ultrasound (transient liver elastography, acoustic radiation force impulse elastography and 2D-shear wave elastography) or based on magnetic resonance imaging. In general, these methods do not distinguish well intermediate stages of fibrosis, although they are increasingly useful in the exclusion of significant fibrosis (<F2) and presence of advanced fibrosis (≥F3). The serum levels of the extracellular matrix protein osteopontin are promising for the diagnosis of intermediate fibrosis, with increasing concentration in different stages of fibrosis groups from F0 to F4, progressively and significantly different between the groups, and with an AUROC of 0.977 for the discrimination of F1/F2 from F3/F4 patients (24). Boursier et al. proposed the FibroMeter® + FibroScan® (FM+FS)

algorithm, based on two fibrosis indexes (significant and advanced fibrosis indexes), from a combination of these two methods by logistic regression. Reliable diagnosis intervals of these two indexes were determined, resulting in a noninvasive classification of fibrosis in six classes. This classification showed an accuracy of 86.7% and, using this algorithm, biopsy would be avoided in 100% of patients with significant and advanced fibrosis (25). However, these methods are based on high cost tests that are not always routinely available, especially in public health services in developing countries."

We added two references to the paper (references 24 and 25):

24. **Matsue Y**, Tsutsumi M, Hayashi N, Saito T, Tsuchishima M, Toshikuni N, et al. Serum Osteopontin predicts degree of hepatic fibrosis and serves as a biomarker in patients with hepatitis C virus infection. *PLoS One*. 2015;10(3):1–15.
25. **Boursier J**, de Ledinghen V, Zarski JP, Fouchard-Hubert I, Gallois Y, Oberti F, et al. Comparison of eight diagnostic algorithms for liver fibrosis in hepatitis C: New algorithms are more precise and entirely noninvasive. *Hepatology*. 2012;55(1):58–67.

Reviewer #2

"I carefully reviewed the manuscript."

Thank you for your attention.

"The authors should show diagnosis criteria of clinical cirrhosis and Child-Pugh score of cirrhosis patients, because the cirrhosis group mostly consists of clinically diagnosed cirrhosis patients."

This information was added to the section Patients and Methods, on lines 150-156: " The clinical diagnosis of cirrhosis were based on characteristic symptoms and signals and/or according to evidence of chronic liver disease and/or portal hypertension on ultrasound (US), such as liver parenchymal heterogeneity, straight borders, reduced liver size, enhanced portal vein dimensions, presence of collateral vessels, splenomegaly,

and/or signals of portal hypertension observed on upper gastrointestinal endoscopy, such as the presence of esophageal/gastric varices and/or hypertensive gastropathy."; and on lines 236-237: "… thus classified according to the Child-Pugh score: 10 patients Child-Pugh A and 5 Child-Pugh B."

"Although, in the manuscript, the results of the LDA MM for SF were compared to the APRI score, the comparison between the LDA MM and the FIB4 index for SF was not shown. How was the result of comparison between LDA MM and the FIB4 index? Similarly, the authors should show the result of comparison between LDA MM and the APRI score for AF."

We chose to compare the LDA MM for SF to the APRI score and the LDA MM for AF to the FIB-4 score, considering the fibrosis group for which each index was created and validated, with the APRI score being validated for SF and cirrhosis, and the FIB-4 score for AF. Therefore, this is the reason we did not compare the LDA MM for SF to the FIB-4 score and the LDA MM for AF to the APRI score.

"The authors showed high accuracy and performance of the MM to diagnose liver fibrosis. But, external validation of the models has not been yet conducted. Regarding this points, I think that the comparison between the MM, APRI score and FIB4 index may be premature. The authors should describe the accuracy and performance of the MM without comparisons with other models."

Indeed, the MMs were tested using cross-validation in this population. However, in an ongoing study of our group, which is now in the final phase, we performed an external validation of MMs for SF and AF using patients from another region of Brazil. The performance of the MMs was compared to the APRI and the FIB-4 scores and it showed preliminary results very similar to the results presented here.

"I hope these comments will be helpful."

We sincerely appreciate your comments in reviewing our article. They were very helpful.

Reviewer #3

"The manuscript deals with an interesting aspect of staging of chronic liver disease in patients with viral hepatitis C."

Thank you.

"Transient elastography was not used / mentioned as a tool for staging chronic liver disease in these patients. Authors should comment on their choice of FIB4 and APRI without including transient elastography,"

At the time of patient's selection for this study, liver elastography was not available in our outpatient clinic. For this reason, we decided to use the APRI and FIB-4 serological scores, widely used in our country. However, as answered to reviewer #1, in an ongoing study of our group we will compare the metabonomics strategy to the APRI and FIB-4 scores and to ARFI elastography, currently available in our outpatient clinic, before and after 12 weeks of treatment with direct-acting antivirals agents in patients with chronic hepatitis C in the city of Recife/PE, Brazil.

"Liver biopsy was performed in 54 out of 69 patients. How did the remaining patients who did undergo liver biopsy have their liver disease staged and how accurate could that staging be?"

This information was added the section Patients and Methods, on lines 150-156: " The clinical diagnosis of cirrhosis were based on characteristic symptoms and signals and/or according to evidence of chronic liver disease and/or portal hypertension on

ultrasound (US), such as liver parenchymal heterogeneity, straight borders, reduced liver size, enhanced portal vein dimensions, presence of collateral vessels, splenomegaly, and/or signals of portal hypertension observed on upper gastrointestinal endoscopy, such as the presence of esophageal/gastric varices and/or hypertensive gastropathy".

"The results of MM seem very good. I believe however, that this study would be much more useful with an external validation."

We acknowledge that external validation make the results more robust. However, as answered to reviewer #2, in an ongoing study of our group, which is now in the final phase, we performed an external validation of MMs for SF and AF using patients from another region of Brazil. The performance of the MMs was compared to the APRI and the FIB-4 scores and it showed preliminary results very similar to results presented here.

"Severity of cirrhosis was not mentioned. Were these all Child Pugh A patients?"

This information was added to the section Patients and Methods, on lines 236-237: "… thus classified according to the Child-Pugh score: 10 patients Child-Pugh A and 5 Child-Pugh B."

Again, we appreciate all comments. We tried our best to be responsive to them. Thank you to help us improve the paper.

Best regards,

Andrea Dória Batista
Corresponding Author