

Metabolomics in Chronic Hepatitis C: Decoding Fibrosis Grading and Underlying Pathways, by Adriana C Ferrasi, Samara V G Lima, Aline F Galvani, Jeany Delafiori, Flávia L Dias-Audibert, Rodrigo R Catharino, Giovanni F Silva, Roberta R Praxedes, Driéle B Santos, Dayane T M Almeida, Estela O Lima

Dear Reviewers,

Thank you very much for your kind response regarding our manuscript. We very much appreciated reviewers' considerations, and thus we prepared a careful point-to-point response to all comments.

Kind regards,

Adriana Camargo Ferrasi
Corresponding Author

Reviewer 1:

Comments and suggestions:

It is an interesting manuscript about “Metabolomics in Chronic Hepatitis C: Decoding Fibrosis Grading and Underlying Pathways” My concern is determined in the following points. Some of the observed biomarkers, once validated, have the potential for application as prognostic biomarkers. Analyses based on liquid biopsy are quite less invasive, and blood plasma, once circulates through the whole body, contains biomarker representative of pathologies that have not yet manifested clinically. It is easier to understand if authors explain with a case example. Multisegment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS) and nuclear magnetic resonance (NMR) spectroscopy for characterizing the serum metabolome of patients with liver fibrosis in chronic hepatitis C virus (HCV) infection: both instrumental techniques enable rapid yet reliable quantification of serum metabolites in large-scale metabolomic studies with good overlap for biomarker replication. Advantages of MSI-CE-MS include greater metabolome coverage, lower operating costs, and smaller sample volume requirements, whereas NMR offers a robust platform supported by automated spectral and data processing software. Above mentioned methods associated with metabolomics should be referred to.

Response: We appreciate your observation, and we are grateful for the recognition of our job. We guarantee that this information and the following bibliographic references were already included in the revised version of the manuscript: Wang et al., 2023; Rattner et al., 2022; Shanmuganathan et al., 2021.

Reviewer 2:

Comments and suggestions:

Dear colleagues! I read with interest your manuscript "Metabolomics in Chronic Hepatitis C: Decoding Fibrosis Grading and Underlying Pathways", which is based on the original research. There are some major problems that does not allow to recommend the manuscript for publishing. 1. There is no description of the studied group (age, gender, BMI, disease duration, necroinflammatory activity, presence and grade of liver steatosis, habits of the subjects, their diet etc). As this information is lacking, the data cannot be reproducible. 2. There is a great number of factors (beside mentioned above) that can have an impact on the metabolite's profile in the subjects. A number of the confounders requires the greater sample size to enhance statistical power. 3. It is not clear, how fibrosis stage was established. In case of liver biopsy - what is the time between liver biopsy and taking a blood samples for metabolic profiling? 4. Selection criteria are not mentioned. Did you applied some? 5. The control group is lacking. This does not allow to judge whether variability may occur in "healthy" population. 6. Conclusions are too vague and are not based on the results of the study. As the number of flaws is too high and some of them may hardly be fixed, I cannot recommend this manuscript for publishing. However, I hope that my comments will help you to plan further studies.

Response: We appreciate the review of our manuscript and are very pleased with the level of attention and detail of the review. Below, we will clarify, point-by-point, the reviewer's notes and suggestions:

1. Thank you for your note. We recognize the lack of descriptive data about the casuistry, so we have included a table (Table 1) with the information in the manuscript.
2. The sample number was based on previous studies and published in scientific journals considered relevant by the scientific community. Additionally, the applied bioinformatics analyses result in highly reliable data and allow the use of a smaller number of samples in the assays.
3. Fibrosis was classified using the METAVIR score. Samples were collected by percutaneous biopsy before any treatment and then analyzed histologically. Peripheral blood was collected at the same time as the liver biopsy. This information will be included in the revised version of the manuscript.
4. We agree that the lack of selection criteria was a mistake. Thank you for highlighting it. This information was included in the revised version of the manuscript.
5. To ensure that the biomarkers found were exclusive to liver lesions (Chronic Hepatitis C – fibrosis, CHC), fifty healthy volunteer blood bank donors (Healthy Control Group - CG) were included in the study. The plasma samples from the two groups (CHC versus GC) were compared and this analysis showed that the fibrosis biomarkers (Table 2) were not detected

in the healthy control group (GC). This information, as well as the PLSDA and VIP Score graphs (Figures 3 and 4) comparing the groups, as well as the discussion of these data were included in the revised manuscript.

6. We thank you for the thorough review of our manuscript. We have modified the first version according to the reviewers' suggestions and included additional data. An improved version of the manuscript is now available, and we are confident that it will meet the necessary requirements for acceptance.

Kind regards,

Adriana Camargo Ferrasi, September 22, 2023

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Journal editor-in-chief review report

Comments: 1. Comments: "In the introduction section: any sentence including a number needs a reference after it." Response: Thank you for your note. In the introduction section, all the sentences that included a number were referenced. 2. Comments: "...The authors should state the absence of any comorbid liver diseases (e.g. SLDs) to the list of exclusion criteria." Response: Thanks for highlighting it. This information was added in the reviewed manuscript. 3. Comments: "In the discussion; at the end of the first paragraph; what is meant by "analytical bias of histological classification? Isn't it odd to degrade the gold standard to which you referred all the results of your manuscript???" Response: There was no intention to discredit any of the techniques currently in use. We only point out the possibility and relevance of sampling and/or inter-observer bias, a fact that is already widespread among pathologists and researchers in the field. However, we have modified the paragraph and included references. 4. Comments: "It is better to state points of weakness of the work." Response: This information was added in the reviewed manuscript. 5. Comments: "Authors can state that although the results of this work is encouraging; liquid biopsy could be used side by side with the other noninvasive tests (like elastography) for achieving more accuracy in predicting prognosis." Response: This information was added in the reviewed manuscript. 6. Comments: "Table 1: the last two cells are not clear! (what is 1 and No 1?)" Response: HCV genotype 1 is more frequent in the casuistry studied. Aiming to avoid multiple subgroups with small sample sizes, all HCV-infected patients were grouped as follows: HCV 1 (only HCV genotype 1) and HCV not 1 (HCV other genotypes). This information has been added to the legend in Table 1. We have modified the final version according to the Editors' suggestions. An improved version of the manuscript is now available, and we are confident that it will meet the necessary requirements for acceptance and publication. Kind regards, Adriana Camargo Ferrasi, October 16, 2023