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OPEN PEER REVIEW

# Non-invasive Assessment of Liver Damage Degree in Patients with Viral Hepatitis: Is It Feasible and Acceptable?

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## About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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## 1 Original submission

4 comments · 30 Dec 2023 – 19 Jan 2024

### Referee 1 Reviewer

MAJOR REVISION 30 Dec 2023

Dear Editor
The manuscript has some major concerns that may have been neglected by the authors:
1- The design of the manuscript is not suitable. The clinical courses of HBV and HCV infections are different; however, after the development of cirrhosis or HCC, they may involve similar conditions that can be compared using the related laboratory or paraclinical tests.
2- The status of the cases was not well defined. In HCV or HBV infection, it is very important to check whether the case is new or resistant to antivirals, as well as the history of drugs used. This information is not provided in the manuscript.
3- The viral load of each viral disease is important too.
4- It is suggested that the HBV data be removed and the HCV data be compared using different categories of fibroscan results.
5- No patient history or background regarding HBV or HCV infection (serology and viral tests) was provided.

### Referee 2 Reviewer

MINOR REVISION 16 Jan 2024

Dear Authors,

Thank you for the opportunity to review your manuscript entitled "Non-invasive assessment of liver damage degree in patients with viral hepatitis: is it feasible and acceptable?". You have investigated an important topic regarding non-invasive markers of liver fibrosis in chronic hepatitis B and C. Overall, the study is well designed and the results provide useful insights.

I have a few suggestions that may help strengthen the manuscript:

1. In the introduction, it would be helpful to provide more context on the pathogenesis of liver fibrosis in hepatitis B and C and how fibrosis progression relates to disease outcomes. Briefly reviewing the stages of fibrosis (Fo-F4) may also help readers unfamiliar with liver histology.

2. Please clarify some methodological details, such as the timeframe over which patients were recruited, how liver biopsy samples were interpreted (e.g., who assessed them and whether the assessment was blinded), and the definition of "advanced fibrosis".

3. In the results, consider adding the median and IQR for continuous variables like age instead of just the mean, as the data may not be normally distributed.

4. The data could be further analyzed by HCV genotype and HBV e-antigen status, which are known to influence disease severity and response to treatment. Comparing outcomes between these groups may provide additional insights.

5. In the discussion, more emphasis could be placed on the clinical implications of these non-invasive markers, such as their potential role in treatment monitoring and cancer surveillance.

6. A couple of minor points—check for consistency of units of measurement (IU/L vs IU) and formatting of citations.

I hope these suggestions are helpful as you prepare your revised manuscript. The study addresses an important clinical issue and with some minor revisions the manuscript will make an excellent contribution to the literature. Please let me know if any part of the review requires clarification. I am happy to discuss further.

Sincerely,
MRE

**Referee 3** Reviewer

MAJOR REVISION 19 Jan 2024

Dear AE,
-The article fails to clearly state its research objectives and hypotheses. It is essential in scientific research to define what you intend to achieve and what you expect to find. Without clear objectives and hypotheses, the significance and relevance of the study become unclear.
-The interpretation of the results is somewhat superficial. The article describes correlations between various markers and fibrosis grades but does not delve into the clinical implications of these correlations. The discussion should provide more insight into how these findings can inform patient care and management.
-The article suggests that FIB-4 and the Forns index are reliable markers for liver fibrosis, but it also acknowledges inconsistencies and variations in predictive values. This calls for further validation studies to establish the robustness and generalizability of these markers.
-There are several grammatical and language issues in the article, which can hinder understanding and readability. Additionally, some technical terms and abbreviations are not adequately explained, making it challenging for non-experts to follow the content.
-Hepatitis B and C have multiple genotypes, and the progression of liver disease can vary depending on the genotype. The article does not discuss whether the genotypes of the patients were considered in the analysis.
-The article briefly mentions patient history and clinical examination as methods for diagnosing liver lesions. However, it lacks critical details about the patients' medical histories, disease duration, viral load, comorbidities, and other relevant factors that can significantly impact liver fibrosis and disease progression. Hepatitis B and C are complex diseases, and a comprehensive patient history is essential for accurate assessment.

**Associate Editor**

MAJOR REVISION 19 Jan 2024

Dear EIC,
Thanks to the authors and appreciation to the researchers. The manuscript is a valuable piece of research. But some revisions are needed. The authors should revise it based on the comments. Also, a minor change should be made in the abstract:
The study design should be mentioned in the first line of the methods section of the ABSTRACT.

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# Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor and Reviewers,

I would like to express our gratitude, on behalf of all co-authors of the manuscript, for your dedicated time and constructive comments. Your input has not only assisted us in improving the current study but has also provided guidance for future scientific endeavors within this topic. As a research team, we carefully reviewed all your observations and endeavored to make corrections to the manuscript to address your inquiries.

Reviewer 1:

1. *the design of the manuscript is not suitable. the clinical course of HBV and HCV infections are different however after cirrhosis induction or HCC they may have similar condition to be compare with their related laboratory or paraclinical tests.*
   - **Yes, we strongly agree that the clinical course of HBV and HCV infections differs, and this manuscript was designed to investigate whether we can use different serum markers to assess the degree of fibrosis in patients with HBV and HCV infections with the same level of confidence (Specificity and Sensitivity), taking into account the different etiopathogenesis of the resulting fibrosis.**
2. *the cases status was not defined well. in HCV infection or HBV infection being new case or resistant to antivirals and history of drug used are very important to be checked. it's not provided in the manuscript.*
   - **Due to the rules of the journal about limited word count on, some important data related to case status, among other things, were not included in the final version of the manuscript. Thank you for the suggestion, and we have revised the manuscript according to your recommendation. (All patients included in the study were antiviral-naive. Among both groups investigated, there were intravenous drug users, but only patients with confirmed stable abstinence for at least 6 months were included in the study.)**
3. *And 5.*
   *the viral load of each viral disease is important too.*
   *there were not any history and background of patients regarding to HBV or HCV infection (serology, and viral tests)*
   - **We strongly agree that various factors, such as viral load, HCV genotype, HBV e-antigen status, patients' medical histories, disease duration, comorbidities, lipid metabolism etc., have different degrees of influence on the course of chronic hepatitis underscores the complexity of the disease. Our primary goal was to determine whether we could uniformly utilize different serum markers as surrogate markers for liver fibrosis in patients with**

**chronic hepatitis C and chronic hepatitis B, irrespective of etiopathogenesis and other variables. Or it is crucial to consider the etiology of the disease when selecting serum markers for assessing liver fibrosis. Can these markers, easily calculable from basic blood biochemistry analyses, sufficiently correlate with the histopathological degree of fibrosis, or is it necessary to consider other variables related to hepatitis viruses in non-invasive assessment of liver fibrosis. Although all the mentioned factors undoubtedly impact the course and progression of the disease, they do not directly enter into the calculation of the investigated serum markers, and as such, they were not included in the database of this pilot study. Certainly, future directions in the search for an adequate non-invasive laboratory assessment of liver fibrosis must take into account other viral characteristics. This approach will likely enhance the sensitivity and specificity of the method, but at the same time, it may reduce the number of potential healthcare institutions that could evaluate and reevaluate this patient group during therapy.**

4. *It is suggested to remove the HBV data and HCV data compared by using different category of fibroscan results*
   - **In this study, we compared serum markers of patients with HBV and HCV with histopathological evaluation in a liver biopsy, using the Metavir scoring system. The results of FibroScan were not included in this study.**

Reviewer 2:

1. *In the introduction, it would be helpful to provide more context on the pathogenesis of liver fibrosis in hepatitis B and C and how fibrosis progression relates to disease outcomes. Briefly reviewing the stages of fibrosis (F0-F4) may also help readers unfamiliar with liver histology.*

- **Thank you for the suggestion. We agree that it would be helpful to provide more context on the pathogenesis of the disease, which is complex and crucial for understanding the disease itself, as well as for the assessment, management and prognosis of patients infected with hepatitis C or B viruses. Given the word limit for the manuscript, including the bibliography, is 3500, in the final version you had the opportunity to read, the pathogenesis was not covered, nor were the histological stages of fibrosis. We have revised the introduction of the manuscript with a brief overview of the pathogenesis of liver fibrosis, while the Materials and Methods**

**section has been enriched with a more detailed description of the METAVIR classification.**

2. *Please clarify some methodological details, such as the timeframe over which patients were recruited, how liver biopsy samples were interpreted (e.g. who assessed them and was it blinded), the definition of "advanced fibrosis".*

- **This retrospective single-center study included patients with chronic hepatitis C (CHC) and chronic hepatitis B (CHB) treated at the Clinic for Infectious Diseases, University Clinical Centre of Vojvodina, from January 2021 to September 2023. The liver biopsy was interpreted by the same specialist of pathology, since we have only one pathologist that is expert for liver diseases. Advanced fibrosis is considered fibrosis with METAVIR scores F3 and F4, and it was briefly mentioned in Results, subsection 3.3. We have revised manuscript with these information.**

3. *In the results, consider adding the median and IQR for continuous variables like age instead of just the mean, as the data may not be normally distributed.*

- ***Thank you for this remark, we added median and IQR for continuous variables in the revised manuscript.***

4. *The data could be further analyzed by HCV genotype and HBV e-antigen status, which are known to influence disease severity and response to treatment. Comparing outcomes between these groups may provide additional insights.*

- **Thank you for the suggestion. This study represents our pilot study, through which we collected baseline data from drug-naive patients. We are still monitoring their response to the applied therapy, and one of the next steps will certainly be determining the predictive power of different surrogate markers regarding the therapeutic response, considering the etiology of the disease, the degree of liver fibrosis, viral load, viral genotype. We hope to obtain results in the near future that we can share with you.**

5. *In the discussion, more emphasis could be placed on the clinical implications of these non-invasive markers, such as their potential role in treatment monitoring and cancer surveillance.*

- **Thank you for the suggestion. In the final version of the manuscript, we focused almost exclusively on interpreting the obtained results and comparing them with the current literature, solely due to the strict word limit of 3500, including the bibliography, and not having enough space. We made a compromise, and in the introduction, we briefly mentioned their significance in monitoring post-therapy outcomes for chronic hepatitis C and B patients, identifying individuals at risk of hepatocellular carcinoma, and understanding underlying liver diseases sustaining fibrogenesis, enabling early planning of diagnostic procedures and HCC surveillance.**

6. *A couple minor points - check for consistency of units of measurement (IU/L vs IU) and formatting of citations.*
   -**Thank you very much. In the meantime, we have revised the manuscript, and I hope we have identified and corrected all the shortcomings.**

Reviewer3:

1. *The article fails to clearly state its research objectives and hypotheses. It is essential in scientific research to define what you intend to achieve and what you expect to find. Without clear objectives and hypotheses, the significance and relevance of the study become unclear.*

   - **After careful consideration of your comments, we realized that the research objectives and hypotheses were not clearly stated, which could potentially impact the significance and relevance of the study. Therefore, we have revised the manuscript accordingly. The study's objective was to explore the feasibility of using various serum markers to assess the extent of fibrosis in patients with both HBV and HCV infections equally, considering the distinct etiopathogenesis of the resulting fibrosis. Hypothetically, we posit that we can depend on some serum markers to evaluate advanced liver fibrosis, irrespective of whether it is induced by chronic HCV or HBV infection, and with the other consideration of chronic viral hepatitis etiology is needed.**

2. *The interpretation of the results is somewhat superficial. The article describes correlations between various markers and fibrosis grades but does not delve into the clinical implications of these correlations. The discussion should provide more insight into how these findings can inform patient care and management.*

   - ***Yes, in the discussion we were initially focused into interpretation of our results, and the comparison with newer literature. Since the journal have strict body count policy, that includes 3500 words as maximal limit, with section Literature included, we weren't able to provide more insight into how these findings can inform patient care and management.*** **However, after your observation, we have implemented more information in both section, Background and Discussion.**

***3.*** *The article suggests that FIB-4 and Forns index are reliable markers for liver fibrosis, but it also acknowledges inconsistencies and variations in predictive values. This calls for further validation studies to establish the robustness and generalizability of these markers.*

   - **We agree, and we consider this study as just one among the first steps, toward further validation of some serum markers as valuable surrogate markers for liver fibrosis.**

4. *There are several grammatical and language issues in the article, which can hinder understanding and readability. Additionally, some technical terms and abbreviations are not adequately explained, making it challenging for non-experts to follow the content.*
   - **We appreciate that you have carefully reviewed our manuscript, and highlighted any technical deficiencies for us. We hope that we have addressed all grammatical and linguistic issues in reviewed manuscript.**

5. *Hepatitis B and C have multiple genotypes, and the progression of liver disease can vary depending on the genotype. The article does not discuss whether the genotypes of the patients were considered in the analysis. The article briefly mentions patient history and clinical examination as methods for diagnosing liver lesions. However, it lacks critical details about the patients' medical histories, disease duration, viral load, comorbidities, and other relevant factors that can significantly impact liver fibrosis and disease progression. Hepatitis B and C are complex diseases, and a comprehensive patient history is essential for accurate assessment.*
   *The article briefly mentions patient history and clinical examination as methods for diagnosing liver lesions. However, it lacks critical details about the patients' medical histories, disease duration, viral load, comorbidities, and other relevant factors that can significantly impact liver fibrosis and disease progression. Hepatitis B and C are complex diseases, and a comprehensive patient history is essential for accurate assessment.*

   - **We strongly agree that the progression of liver disease depends on the genotype, as well as on factors such as viral load, HBV e-antigen status, cholesterol metabolism impact, medical history, disease duration, etc. However, the goal of this pilot study was to investigate whether we can equally utilize serum markers in assessing the degree of liver fibrosis in patients with HCV or HBV infection, or whether it is necessary to consider the different etiopathogenesis of the disease when selecting a specific marker and interpreting it appropriately. Given that after the onset of liver cirrhosis or hepatocellular carcinoma, the laboratory and clinical presentation becomes similar, regardless of etiopathogenesis, we particularly focused on examining the correlation of these potential surrogate markers of fibrosis with histologically confirmed F3 and F4 fibrosis stages, representing advanced fibrosis. Certainly, future directions in the search for an adequate non-invasive laboratory assessment of liver fibrosis must take into account other viral characteristics. This approach will likely enhance the sensitivity and specificity of the method, but at the same time, it may reduce the number of potential healthcare institutions that could evaluate and reevaluate this patient group before and during therapy.**

-Thank you once again for your valuable contributions-

## 2 Revision 1

4 comments · 5 Feb 2024 – 8 Feb 2024

### Referee 2 Reviewer

ACCEPT 5 Feb 2024

Thank you for implementing the comments.

### Referee 1 Reviewer

MAJOR REVISION 7 Feb 2024

The manuscript is well written; however, some points should be added:
- Receiver Operator Characteristic (ROC) analysis should be presented as a figure in the results section.
- The cases are not matched, and the comparison between CHC and CHB with this sample size is not valid at all. The sample size in the two arms (groups A and B) should be justified by a hypothesis or formula. They should be analyzed separately unless they have at least a reasonable sample size.
- If patients have been treated, they could be called cases of resolved HBV or HCV infection, not CHC or CHB cases. This is not clear enough.
- It is indicated that "389 patients with chronic hepatitis C and B treated at the Clinic for..." but in the exclusion criteria, you exclude all cases with antiviral use! "prior antiviral or immune-suppressive medication use," Why? This is contradictory!
- In the discussion section, they should add a limitations section and explain the study limitations.
- As mentioned in the introduction section, "The AST/ALT ratio, APRI Score, FIB-4 Score, and Forns index are the most commonly used serum markers for assessing liver fibrosis (16-18). Notably, the FIB-4 Score is widely accepted as the most reliable marker for demarcating advanced liver disease (19)." What new finding do you add? We know these indices show liver dysfunction. What do you add? What is the novelty?

### Referee 3 Reviewer

ACCEPT 8 Feb 2024

Dear EIC.
Previous errors have been corrected, and there are no further recommendations.

### Associate Editor

MAJOR REVISION 8 Feb 2024

Dear EIC,
I would like to extend my gratitude to the authors for their contribution. Some revisions are still necessary, and I kindly request the authors to consider incorporating these suggested changes based on the provided comments.

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# Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor and Reviewers,

Again, we would like to express our great gratitude, for your dedicated time and constructive comments. As a research team, we carefully reviewed additional observations and endeavored to make corrections to the manuscript to address your inquiries. We hope that all changes we made in the manuscript, with the following explanations about certain aspects of our study will make our manuscript worthy of publication in your esteemed scientific journal.

Reviewer 3:

1. *Receiver Operator Characteristic (ROC) analysis should present as figure in the result section.*

- **Yes, we understand that will improve our manuscript, and we have made that adjustment. Since, by the Instruction for Authors section, we have to submit figures in different file than the manuscript, I will place them here as well, to make the reviewing process easier.**

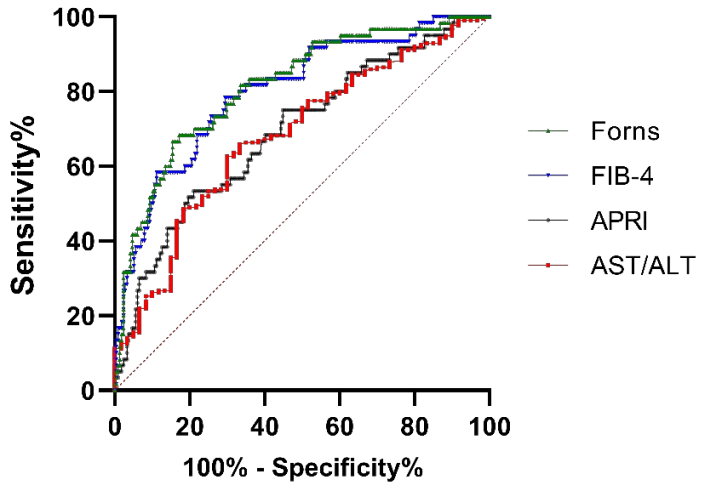



**Figure 1. ROC analysis of serological markers in predicting advanced liver fibrosis in patients with chronic hepatitis C**

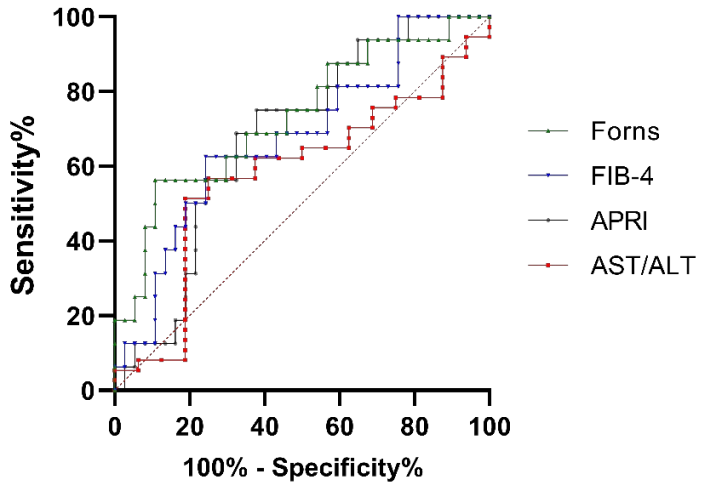



**Figure 2. ROC analysis of serological markers in predicting advanced liver fibrosis in patients with chronic hepatitis C**

2. *the cases not match and comparison between CHC and CHB by this sample size is not true at all. sample size in two arms (group A and B) should claim by a hypothesis or formula. They should analyse separately, unless at least they have reasonable sample size.*

- **We are completely aware of the difference between sample size of the CHC and CHB groups. That is one of the reasons we provided both means and SDs, as well as medians and IQRs in Table 1, along with p-values for tests comparing these two groups. Due to the size difference between them, we used Mann-Whitney´s U test as a non-parametrics alternative to the t-test. One of the criteria necessary for the t-test was not met (equality of groups), so we opted for Mann-Whitney´s U test to avoid making a type I error. All p-values presented in the table are the results of Mann-Whitney´s U test, as indicated in the reviewed version of the manuscript.**

**3.** *if patients are treated, they could call resolved HBV or HCV infection not CHC or CHB cases. its not clear enough.*

- **The patients were not treated, at the moment they entered the study they were antiviral drug naive.**

4. *its indicated "389 patients with chronic hepatitis C and B treated at the Clinic for..." but in the exclusion criteria you reject all cases with antiviral usage! " Prior antiviral or immune-suppressive medication use," why? it is the controversial!*

- **Thank you for bringing this to our attention. We recognize that the wording we initially used may have been confusing, particularly regarding the phrase "... hepatitis C and B treated at the Clinic..." The term "treated" was intended to encompass all procedures conducted at the Clinic, not specifically antiviral treatment. We have revised this in the manuscript to clarify that all patients are antiviral drug-naive.**

5. in discussion section they should add a limitation section and explain the study limitations.

- **Due to the word count policy, we have not included a limitations section. We apologize for this and have enriched the discussion with these aspects.**

6. as in the introduction section mentioned "The AST/ALT ratio, APRI Score, FIB-4 Score, and Forns index are the most commonly used serum markers for assessing liver fibrosis (16-18). Notably, the FIB-4 Score is widely accepted as the most reliable marker for demarcating advanced liver disease (19)." what is the new finding that you add? we know these indices show the liver disfunction, what you add? whats the novelty?

- **Using the aforementioned serological surrogate markers of liver fibrosis in patients with chronic hepatitis B and C infection, we aimed to verify their uniformity in clinical practice. Given the usage of gold-standard techniques for liver assessment (hepatic biopsy) in all participants, we were able to assess the specificity and sensitivity of serological markers in evaluating liver damage in both groups of patients. Our findings led us to conclude that FIB-4 and Forns index provide accurate confirmation or exclusion of advanced liver fibrosis in viral chronic liver disease, assisting in severity assessment, where FIB-4 serves as a universal marker, while interpreting Forns index requires consideration of the etiology of chronic viral hepatitis.**

-Thank you once again for your valuable contributions-

## 3 Revision 2

2 comments · 27 Feb 2024 – 1 Mar 2024

### Referee 1 Reviewer

ACCEPT 27 Feb 2024

There are valuable data; however, the two arms of the study are still not sufficiently matched.

### Associate Editor

ACCEPT 1 Mar 2024

Dear EIC,
I read the manuscript, and the authors corrected the requested items. The article could be accepted at this stage.
Sincerely;