

June 29th, 2020

Na Ma

Company Editor-in-Chief

World Journal of Hepatology

We are pleased to submit our revised manuscript NO 56722, originally entitled “*Can gadoxetic acid-enhanced magnetic resonance imaging be used to avoid liver biopsy in patients with nonalcoholic fatty liver disease?*” for your consideration for publication as an original article in the *World Journal of Hepatology*.

We would like to thank the reviewers for the helpful comments and suggestions on our original manuscript. We believe our responses to these comments have resulted in a more scientifically robust article. We addressed all the reviewers’ comments and revised the manuscript accordingly based on the recommendations and suggestions. For the reviewers’ convenience, the revisions have been underlined in the manuscript. An annotated response to the reviewers’ comments is provided below. All authors approved the submission of the revised version of the manuscript.

Please feel free to contact us with any questions or concerns you may have. We look forward to hearing from you.

Sincerely,

Viviane Brandão Amorim, MD, PhD

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Responses to the comments from Reviewer #1 (02445646)

Comments:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: No comments to the authors.

Response:

We would like to thank you for your promptness and review. Thank you for accepting our manuscript.

Responses to the comments from Reviewer #2 (05040445)

Comments:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: The research is mainly about using gadoxetic acid-enhanced magnetic resonance imaging (GA-MRI) to differentiate NASH from simple liver steatosis and finally got to a conclusion about this GA-MRI could replace liver biopsy in many patients. The title reflects the main subject/hypothesis of the manuscript correctly and the abstract is properly described. The background reflects the significance of the study. However, the cases are very limited and the author could use a method with a sensitivity of 32% and a specificity of 94% to conclude such a conclusion. The biopsy is still the gold standard and a radiology method never replace it. It may be a potential method to help but with such a small number of cases, its hard to come to a conclusion. I

do suggest the author collect more cases to analyze and revise the conclusion with a more proper statement.

Response:

Thank you for the constructive comments on the manuscript.

We agree that the relatively small sample size ($n = 56$) is a limitation of our study. The sample was small due to the strict inclusion and exclusion criteria, especially the selection of patients who had undergone biopsy. However, our sample was larger than the optimal sample size ($n = 51$), calculated with a high significance level (0.01) and statistical power (0.99) to differentiate NASH from isolated steatosis. Furthermore, this is the first study that proposed cut-off points of CEI to identify and exclude NASH. We agree that larger sample sizes are recommended to suggest cut-off points. A comment about this limitation was included in Discussion section. Nevertheless, in the current global context of COVID-19 pandemic, the inclusion of new patients in the study has been struggling, especially in Brazil where the number of cases of people affected has been growing and the risk of collapse of the Health System is great. The research activities in our center remains temporarily suspended due to COVID-19. Several studies have been reporting that patients with metabolic syndrome (the vast majority of patients affected by NAFLD) is a risk factor for severe forms of COVID-19. In addition, the major international guidelines have been recommending postponing non-elective surgeries and/or procedures, such as liver biopsy or magnetic resonance imaging to assess liver steatosis/fibrosis, during the COVID-19 pandemic [Perazzo et al Clin Gastroenterol Hepatol 2020].

We entirely agree that biopsy is the gold standard method and will not be replaced by any radiological method. Our proposal is less ambitious, aiming to reduce the number of patients undergoing biopsy. We believe that biopsy the entire affected population is unattainable, since NAFLD is highly prevalent, affecting about 30% of the population.

The proposed method, using the contrast enhancement index (CEI) by MRI, showed low sensitivity, despite good specificity, as explained in your comment. Therefore, we suggest in this manuscript two other CEI cut-offs to optimize sensitivity and specificity. The combined use of these cut-offs appears to be more clinically useful for the identification of patients with greater probability of having NASH or isolated steatosis and to exclude NASH, which could prevent biopsy in 40% of patients.

By agreeing with the comments, some paragraphs were rephrased or some phrases were added in the revised version of the manuscript, in order to clarify these issues:

“The reference standard for differentiation of these two entities is based on liver histopathological findings.” [Introduction – page 6]

“The major limitation is the relatively small sample size.” [Discussion – page 14]

“Thus, this preliminary study suggests that GA-MRI may be an effective noninvasive method for the identification of patients for whom early intervention and more aggressive therapy should be implemented and those with low probability of having NASH, avoiding liver biopsy in up to 40% of the NAFLD population. Liver biopsy, the gold standard method, would still be required for cases in which GA-MRI findings are inconclusive. However, further studies with a larger sample size are warranted to validate the proposed cut-off points.” [Discussion - page 16]

Responses to the comments from Reviewer #3 (03478516)

Comments:

Scientific Quality: Grade E (Do not publish)

Language Quality: Grade B (Minor language polishing)

Conclusion: Rejection

Specific Comments to Authors: Potentially interesting study characterized by a very small population and consequently to be considered still preliminary. It lacks a cost-benefit analysis that is of paramount importance at the light of the high prevalence of NAFLD.

Response:

We would like to thank you for the comments.

We agree that the relatively small sample size ($n = 56$) is a limitation of our study. The sample was small due to the strict inclusion and exclusion criteria, especially the selection of patients who had undergone biopsy. However, our sample was larger than the optimal sample size ($n = 51$), calculated with a high significance level (0.01) and statistical power (0.99) to differentiate NASH from isolated steatosis. Furthermore, this is the first study that proposed cut-off points of CEI to identify and exclude NASH. We agree that larger sample sizes are recommended to suggest cut-off points. A comment about this limitation was included in Discussion section. Nevertheless, in the current global context of COVID-19 pandemic, the inclusion of new patients in the study has been struggling, especially in Brazil where the number of cases of people affected has been growing and the risk of collapse of the Health System is great. The research activities in our center remains temporarily suspended due to COVID-19. Several studies have been reporting that patients with metabolic syndrome (the vast majority of patients affected by non-alcoholic fatty disease) is a risk factor for severe forms of COVID-19. In addition, the major international guidelines have been recommending postponing non-elective surgeries and/or procedures, such as liver biopsy or magnetic resonance imaging to assess

liver steatosis/fibrosis, during the COVID-19 pandemic [Perazzo et al Clin Gastroenterol Hepatol 2020].

We consider that this study, although preliminary, is of great importance for the scientific community, opening up possibilities for a new clinical approach in patients with NAFLD with a good cost-benefit. Since NAFLD has a high prevalence affecting about 30% of the world population, performing liver biopsy in all patients is not feasible. It must be considered that liver biopsy presents risks inherent to an invasive procedure. In addition, liver biopsy is a high-cost procedure, requiring a day hospitalization, a specialist physician to perform the procedure, and a pathologist with expertise in hepatobiliary disease for histological sample analysis. In turn, the MRI scan has lower risk of complications, is cheaper, easier to perform and more widely available. The proposed calculation (contrast enhancement index - CEI) is easily performed by radiologists. Furthermore, whereas patients with NAFLD usually undergo imaging examinations for overall assessment of the hepatic parenchyma, the possibility of using GA-MRI as a noninvasive and comprehensive diagnostic modality holds great promise. Although the costs of medical procedures are highly variable in different countries, MRI scan seems to result in a more favorable cost-benefit ratio considering all the complexity of liver biopsy procedure. According to the reviewer suggestion, we included a comment about the cost-benefit of the MRI.

By agreeing with the comments, some paragraphs were rephrased or some phrases were added in the revised version of the manuscript, in order to clarify these issues:

“However, liver biopsy has several limitations, including its invasive nature, with rare but potentially life-threatening complications, poor patient acceptance, sampling error, and intra- and interobserver variability in findings. In addition, liver biopsy is a

high-cost procedure, requiring a day hospitalization, a specialist physician to perform the procedure and a pathologist with expertise in hepatobiliary disease for histological sample analysis. Given the high prevalence of NAFLD, liver biopsy is not a good option for routine clinical practice.” [Introduction – page 6]

“The major limitation is the relatively small sample size.” [Discussion – page 14]

“Also, this is the first study that proposed cut-off points of CEI to identify and exclude NASH, easily performed by radiologists.” [Discussion – page 15]

“Whereas patients with NAFLD usually undergo imaging examinations for overall assessment of the hepatic parenchyma, the possibility of using GA-MRI as a noninvasive and comprehensive diagnostic modality holds great promise. Furthermore, the MRI scan has lower risk of complications, is cheaper, easier to perform and more widely available when compared to liver biopsy. Although the costs of medical procedures are highly variable in different countries, MRI scan seems to result in a more favorable cost-benefit ratio considering all the complexity of liver biopsy procedure.” [Discussion – page 15]

“In conclusion, patients with NASH have significantly lower CEIs in the hepatobiliary phase of GA-MRI than do patients with isolated steatosis. Thus, this preliminary study suggests that GA-MRI may be an effective noninvasive method for the identification of patients for whom early intervention and more aggressive therapy should be implemented and those with low probability of having NASH, avoiding liver biopsy

in up to 40% of the NAFLD population. Liver biopsy, the gold standard method, would still be required for cases in which GA-MRI findings are inconclusive. However, further studies with a larger sample size are warranted to validate the proposed cut-off points."

[Discussion – page 16]

Responses to the comments from Science Editor

Comments:

1 Scientific quality: The manuscript describes a prospective study of the gadoxetic acid–enhanced magnetic resonance imaging be used to avoid liver biopsy in patients with nonalcoholic fatty liver disease. The topic is within the scope of the WJH. (1) Classification: Grade C, Grade C and Grade E; (2) Summary of the Peer-Review Report: The research is mainly about using gadoxetic acid–enhanced magnetic resonance imaging (GA-MRI) to differentiate NASH from simple liver steatosis and finally got to a conclusion about this GA-MRI could replace liver biopsy in many patients. However, it lacks a cost-benefit analysis that is of paramount importance at the light of the high prevalence of NAFLD. The questions raised by the reviewers should be answered; and (3) Format: There are 3 tables and 3 figures. A total of 25 references are cited, including 5 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Grade B, Grade B, and Grade B. A language editing certificate issued by Write Science Right was provided. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Clinical Trial Registration Statement, the signed Copyright License Agreement, the Institutional Review Board Approval Form, and the Written informed consent. The Conflict-of-Interest Disclosure Form is not right. No academic misconduct was found in the CrossCheck detection and Bing search. 4

Supplementary comments: This is an unsolicited manuscript. The study was supported by 1 grant. The topic has not previously been published in the WJH. 5 Issues raised: (1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and (3) The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text. 6 Re-Review: Required by Reviewer 05040445. 7 Recommendation: Conditional acceptance.

Response:

We sincerely appreciate the valuable comments and suggestions from the reviewers. The suggestions helped immensely to improve the manuscript. The suggestions and comments have been closely followed and revisions have been made accordingly.

We consider that this study is of great importance for the scientific community, opening up possibilities for a new clinical approach in patients with NAFLD with a good cost-benefit. Since NAFLD has a high prevalence affecting about 30% of the world population, performing liver biopsy in all patients is not feasible. It must be considered that liver biopsy presents risks inherent to an invasive procedure. In addition, liver biopsy is a high-cost procedure, requiring a day hospitalization, a specialist physician to perform the procedure and a pathologist with expertise in hepatobiliary disease for sample analysis. In turn, the MRI scan is easy to perform and widely available. The proposed calculation (contrast enhancement index - CEI) is easily performed by radiologists. Furthermore, whereas patients with NAFLD usually undergo imaging examinations for overall

assessment of the hepatic parenchyma, the possibility of using GA-MRI as a noninvasive and comprehensive diagnostic modality holds great promise.

In order to clarify these issues, some paragraphs were rephrased or some phrases were added in the revised version of the manuscript:

“However, liver biopsy has several limitations, including its invasive nature, with rare but potentially life-threatening complications, poor patient acceptance, sampling error, and intra- and interobserver variability in findings. In addition, liver biopsy is a high-cost procedure, requiring a day hospitalization, a specialist physician to perform the procedure and a pathologist with expertise in hepatobiliary disease for sample analysis. Given the high prevalence of NAFLD, liver biopsy is not a good option for routine clinical practice.” [Introduction – page 6]

“Also, this is the first study that proposed cut-off points of CEI to identify and exclude NASH, easily performed by radiologists.” [Discussion – page 15]

“Whereas patients with NAFLD usually undergo imaging examinations for overall assessment of the hepatic parenchyma, the possibility of using GA-MRI as a noninvasive and comprehensive diagnostic modality holds great promise. Furthermore, the MRI scan has lower risk of complications, is cheaper, easier to perform and more widely available when compared to liver biopsy. Although the costs of medical procedures are highly variable in different countries, MRI scan seems to result in a more favorable cost-benefit ratio considering all the complexity of liver biopsy procedure.”

[Discussion – page 15]

The “Article Highlights” section (page 16) was also included in the revised version of the manuscript:

Research background

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, affecting up to 40% of the world population. It is characterized by fatty liver infiltration, and encompasses a wide clinical spectrum, ranging from a relatively benign isolated steatosis from potentially progressive nonalcoholic steatohepatitis (NASH), liver fibrosis and cirrhosis.

Research motivation

The diagnosis of NASH is crucial and has prognostic and therapeutic implications. Liver biopsy is currently the gold standard for diagnosing progressive NASH and has several limitations, such as sampling error, cost, and risk of complications.

Abundant research has been performed to develop noninvasive diagnostic methods for the early detection of NASH and its accurate differentiation from isolated steatosis, due to the utmost clinical importance of this diagnosis.

Research objectives

To evaluate the performance of gadoxetic acid–enhanced magnetic resonance imaging (GA-MRI) to differentiate NASH in patients with NAFLD using histopathology as the reference standard.

Research methods

In this prospective study, 56 patients with NAFLD (18 with isolated steatosis and 38 with NASH) underwent GA-MRI. Contrast enhancement index (CEI) was calculated as the rate of increase of the liver-to-muscle signal intensity ratio before and 20 min after intravenous GA administration. Between-group differences in mean CEI were tested with

the Student's t-test. Area under the receiver operator characteristic curve, and the diagnostic performance of GA-MRI were evaluated.

Research results

The mean CEI for all subjects was 1.82 ± 0.19 . The mean CEI was significantly lower in patients with NASH than in those with isolated steatosis ($p = 0.008$). Two CEI cut-off points were used: <1.66 (94% specificity) to characterize NASH and >2.00 (89% sensitivity) to characterize isolated steatosis. CEI values between 1.66 and 2.00 indicated liver biopsy, and the procedure could be avoided in 40% of patients with NAFLD.

Research conclusions

Patients with NASH have significantly lower CEIs in the hepatobiliary phase of GA-MRI than do patients with isolated steatosis. This study suggests that GA-MRI may be an effective noninvasive method for the identification of patients for whom early intervention and more aggressive therapy should be implemented, avoiding liver biopsy in up to 40% of the NAFLD population.

Research perspectives

The possibility of using GA-MRI as a noninvasive and comprehensive diagnostic modality holds great promise. As it is a preliminary study, further prospective studies with a larger sample size are warranted.

The Conflict-of-Interest Disclosure Form, the Grant Approval Form and the figures in PowerPoint were uploaded.

Responses to the comments from Editorial Office Director

Comments:

I have checked the comments written by the science editor.

Response:

We would like to thank you.

Responses to the comments from Company Editor-in-Chief

Comments:

I have reviewed the Peer-Review Report, the full text of the manuscript and the relevant ethics documents, all of which have met the basic publishing requirements, and the manuscript is conditionally accepted with major revisions. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report and the Criteria for Manuscript Revision by Authors.

Response:

We would like to thank you.

REFERENCE

Perazzo, Hugo, et al. "COVID-19: an overview of worldwide recommendations for management of patients with liver diseases or liver transplantation." *Clinical Gastroenterology and Hepatology* (2020).