Bogotá, March 31, 2021

World Journal of Gastroenterology
Editorial Committee

Dear Sirs,

We wish to thank the Editor and Reviewer for their fair review of our manuscript N. 63531 "Poor performance of anti-mitochondrial antibodies for the diagnosis of primary biliary cholangitis in Latin American patients", which we submitted to the Journal.

We have tried to address each of the reviewer´s comments one by one, our responses can be found below:

Round-1

Reviewer #1:
Specific Comments to Authors: General comments: Guatibonza-Garcia et al. conducted a retrospective, cross-sectional study to investigate the diagnostic yield of autoimmune antibodies including anti-mitochondrial antibodies for primary biliary cholangitis. This is a quite interesting and important study that suggests the possibility of different endotypes of PBC patients in Latin America compared to patients with other geographic background. However, there are many critical issues that need to be addressed/clarified, in order to validate the finding of the current report. Because the conclusion of this study may suggest the need of revision of the clinical procedures for diagnosis of PBC, the finding of the study needs careful validation.

Response: We thank the reviewer for his appreciation of our work, we have tried to address his comments to the best of our ability.

Comment: I am not sure what diagnostic criteria of PBC is used in the authors' country, but I believe the widely accepted criteria are at least two of the three below: 1) pathology; 2) autoimmune antibodies including AMA, AMA2 etc; and 3) elevation of serum liver enzymes, especially ALP. However, the authors' inclusion criteria for current study were solely pathology, which may not be accurate enough to include PBC subjects. The authors need to justify why they adopted pathology-based diagnosis for this study. Otherwise, it should be safe to include patients with pathology consistent with PBC AND elevation of serum ALP as PBC.

Response: Thank you for this important observation. Since we wanted to test the diagnostic performance of antibodies for PBC diagnosis, we could not use them to define the case/control status, and opted to use a more definitive and unequivocal method like pathology as gold standard for the purpose of our analyses. However, at the reviewer´s suggestion we have performed a sensitivity analysis in which PBC status was defined as pathology diagnosis of PBC **plus** elevation of alkaline phosphatase. The results are shown below, and have been incorporated to the last paragraph of the results section. In general, the results are almost exactly the same, except for a slightly better negative predictive value for AMA and slightly better sensitivity for IgM.

Sensitivity, specificity and predictive values of each antibody or antibody combination for the diagnosis of PBC, defined by pathology diagnosis + elevation of alkaline phosphatase

Ab or Ab combination	Sensitivity	Specificity	PPV	NPV
AMA (+)	44.4	76.1	59.3	63.6
ANA (+)	75.0	39.1	49.1	66.7
ASMA (+)	11.1	73.9	25.0	51.5
IgG (+)	8.33	66.7	20.0	42.1
IgM (+)	63.2	87.5	92.3	50.0
AMA (+), ANA (+)	25.0	84.8	56.3	59.1
AMA (+) , ANA (-)	19.4	91.3	63.6	59.2
AMA (-), ANA (+)	50.0	54.4	46.2	58.1
AMA (+), ASMA (+)	5.6	97.8	66.7	57.0
AMA (+), ASMA (-)	38.9	78.2	58.3	62.1
AMA (-), ASMA (+)	5.6	76.1	15.4	50.7
ANA (+), ASMA (+)	8.3	84.8	30.0	54.2
ANA (+) , ASMA (-)	66.7	54.4	53.3	67.6
ANA (-), ASMA (+)	2.8	89.1	16.7	54.0
AMA (+), IgG (+)	0.0	83.3	0.0	45.5
AMA (+), IgG (-)	50.0	75.0	66.7	60.0
AMA (-), IgG (+)	8.3	83.3	33.3	47.6
AMA (+), IgM (+)	57.9	87.5	91.7	46.7
AMA (+), IgM (-)	0.0	75.0	0.0	24.0
AMA (-), IgM (+)	5.3	100	100	30.8

Comment: It is hard to justify that the authors excluded one male PBC subject from the analysis in order to obtain more homogenous population. This is what you should decide as inclusion criteria IN ADVANCE when planning the study design. So, the reviewer suggests the authors include this male subject in the analysis. In another realistic alternative way, the authors may include only female subjects and modify the title of the manuscript so that it clearly shows that the study population was only female subjects. Further, it is unclear if the control group had only female or not. I suppose so based on the description on page 12, Line 5, but this should be stated clearly in the method section.

Response: We have modified the title in order to express that the study included only female participants.

Comment: I cannot understand why you mentioned ROC analysis of AMA and ANA titers. Please clearly state that the titers shown in this section (1:50 for AMA and 1:120 for ANA) are

threshold used for the positivity of AMA/ANA if this is the case. As long as I know (at least in my country (Japan) and the US), the threshold for AMA is 1:20. Is this different from the threshold used in your country and/or the current study? If the threshold was different, the positivity and specificity observed in the current study must be affected, which ruins the compatibility of the results in the current study and previous reports. Related with this, what is the definition of positive/negative IgM/IgG? The authors should state the threshold for all the antibodies stated in the study.

Response: Thanks for the opportunity to clarify this. We performed two separate analyses using AMA and ANA. In the study´s primary analysis, AMA and ANA were classified as positive or negative according to current established thresholds (more than 1:20 for AMA and more than 1:40 for ANA). The reported diagnostic performance parameters for AMA and ANA positivity are those found employing these cutoffs.

The exploratory ROC analysis was performed using the *plain* reported AMA and ANA titer, in order to explore which hypothetical cutoff would best identify patients with confirmed PBC. In this exercise, the best combination of sensitivity and specificity were provided by a cutoff 1:50 for AMA and 1:120 for ANA.

Comment: Based on a previous report that PBC patients in Mexico, the genetic background of which should be similar to the current study, showed high AMA/ANA positivity, the result obtained by the authors needs to be validated by independent cohorts.

Response: We acknowledge the importance of replicating our results. Nonetheless, it is currently unfeasible for us to repeat all analyses in an entirely new and different cohort. We touch upon possible explanations for the differences between our study and prior reports in the discussion section.

Comment: The authors need to pay careful attention to the reliability of the AMA/ANA tests. Were these tests performed in your hospital or a third-party laboratory?

Response: Although biopsies were mostly taken at our institution, the histological analyses of slides and immunological tests were performed by external laboratories and by different pathologists and technicians.

Comment: I strongly suggest adding an independent cohort (e.g. patients from other hospital in Latin America) to validate the findings. If the authors cannot agree with this, the authors should modify the title of the manuscript so that it clearly shows that this study is a SINGLE CENTER study for COLOMBIAN population, but not for Latin American population.

Response: Thank you, we agree with the reviewer on this comment, and have modified the title accordingly.

Round-2

Comment: The authors have revised their manuscript and it reads better than the initial manuscript. The reviewer still thinks they need to further discuss why their finding was different

from previous reports from other populations including Mexicans, but this is a sort of limitation of a single center, retrospective study and it is feasible to accept this if the title and main text clearly mentioned this limitation. The reviewer hopes future studies will validate the current report.

Response: We have modified the title in order to express that this was a single center study among female Colombian patients. We have tried to provide a hypothetical explanation of the difference between our results and those of the previous Mexican study. This paragraph appears highlighted in the discussion section. The references to support this hypothesis have been included and highlighted in the reference list, and cites and references have been re-numbered accordingly.

One of such factors may be polymorphisms in genes for proteins involved in antigen presentation at the bile ducts. It has been demonstrated that cholangiocytes express not only MHC class I molecules, but also surface markers found on antigen presenting cells including MHC class II and co-stimulatory molecules (CD80, CD86, CD40) [23]. Since the distribution of HLA alleles differs substantially across Latin America [24] , it is conceivable that individuals from certain populations and better presenters of ductal self-antigens, and elicit a stronger humoral self-immunity in the context of PBC. This may constitute a potential explanation for the stark contrast between our results and those from the prior Mexican study.

23. Chuang YH, Lan RY, Gershwin ME. The immunopathology of human biliary cell epithelium. Semin Immunopathol 2009;**31**:323-31 [PMID: 19533127 DOI: 10.1007/s00281-009-0172-5]

24. Arrieta-Bolaños E, Madrigal JA, Shaw BE. Human leukocyte antigen profiles of latin american populations: differential admixture and its potential impact on hematopoietic stem cell transplantation. Bone Marrow Res 2012;**2012**:136087 [PMID: 23213535 DOI: 10.1155/2012/136087]