January 07, 2021

To the Editor-in-Chief of the *World Journal of Gastroenterology*,

Dear Editor,

On behalf of my group, I have the pleasure to present this rebuttal version of the Manuscript NO.: 60647, (WJG) entitled “Serum 1,3-Beta-D-Glucan as a Noninvasive Test to Predict Histologic Activity in Patients with Inflammatory Bowel Disease ”, for your consideration.

We thank the reviewers for finding the results of our study interesting and for reading in detail our studies on a novel noninvasive approach for detecting mucosal inflammation and therapeutically monitoring patients with inflammatory bowel diseases.

We understand that the comments of the reviewers continue to support and confirm the previous findings, and basically support the manuscript structure.

In addition, we hereby certify that all authors contributed significantly to this manuscript, agree with the content and concur with the submission of this work. In addition, the authors declare no conflicts of interest regarding this work. Finally, none of the data presented here have been previously reported or are under consideration for publication elsewhere.

Thank you for your consideration,

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**Reply to reviewers:**

**Reviewer #1:**

**Scientific Quality:** Grade C (Good)

**Language Quality:** Grade A (Priority publishing)

**Conclusion:** Major revision

**Specific Comments to Authors:** This study is among the very few researches that addressed the correlation of BG with clinical, endoscopic and histological activity, as well as with some serological biomarkers and fecal calprotectin. The manuscript is original and represents a novelty, which, if replicated in other studies could represent a non-invasive test that could help monitoring IBD patients under different therapies. Very good impact on our practice and a cost-effective method! However, some points should be addressed/ clarified by the authors.

1. ABSTRACT:

Methods:

a. Please mention where and also the period the study was carried out.

R: We mentioned the period of time, as suggested (in Abstract, Methods).

b. Biomarkers should mention also IL-17 and IFN-gamma (since they appear in the Results).

R: We mentioned the cytokines, as suggested (in Abstract, Methods).

Results:

a. The sentence “Compared with endoscopic (AUC: 0.656; p=0.001) and histologic (AUC: 0.853; p<0.001) healing, no significant correlation was found between serum BG and transmural healing based on MRE (AUC: 0.576; p=0.192)” refers to CD I suppose. Since it mentions transmural healing! Then please clarify. Where is this in the main text?

R: This reviewer is correct. We apologize for the mistake (in Abstract, Results).

b. The sentence: “Performance analysis showed that the BG results were remarkably better for predicting histologic inflammation than FC and CRP”: Please mention the levels and correlations.

c. R: We understand this reviewer’s suggestion, and we managed to include more information. Several changes were made due to the new analysis, separating CD and UC (in Abstract, Results).

d. Also, the cut-off values for histological remission should be mentioned. In fact, this is the title of the manuscript.

R: We agree with this comment, and we managed to include the required information.

Conclusion: Should include histological remission (according to the title). Or, the title to be changed to “Serum 1,3-Beta-D-Glucan as a Noninvasive Test to Predict Histologic ACTIVITY in Patients with Inflammatory Bowel Disease” This would also be in accordance with the hypothesis of the study, as the authors wrote: “We

hypothesized that the serum levels of BG could reflect ACTIVE intestinal inflammation". Please decide and write accordingly.

R: We agree completely with this comment! It is obvious! We simply changed the title, according to his/her suggestion. New title is: "Serum 1,3-Beta-D-Glucan as a Noninvasive Test to Predict Histologic Activity in Patients with Inflammatory Bowel Disease"

2. INTRODUCTION:

a. Please add after references 3 and 4, the following references:

**1**. Peyrin-Biroulet L, Sandborn W, Sands BE, Reinisch W, Bemelman W, Bryant RV, D'Haens G, Dotan I, Dubinsky M, Feagan B, Fiorino G, Gearry R, Krishnareddy S, Lakatos PL, Loftus EV Jr, Marteau P, Munkholm P, Murdoch TB, Ordás I, Panaccione R, Riddell RH, Ruel J, Rubin DT, Samaan M, Siegel CA, Silverberg MS, Stoker J, Schreiber S, Travis S, Van Assche G, Danese S, Panes J, Bouguen G, O'Donnell S, Pariente B, Winer S, Hanauer S, Colombel JF. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. Am J Gastroenterol. 2015 Sep;110(9):1324-38. doi: 10.1038/ajg.2015.233. Epub 2015 Aug 25. PMID: 26303131.

**2**. Darr U, Khan N. Treat to Target in Inflammatory Bowel Disease: An Updated Review of Literature. Curr Treat Options Gastroenterol. 2017 Mar;15(1):116-125. doi: 10.1007/s11938-017-0130-6. PMID: 28161818.

**3**. Agrawal M, Colombel JF. Treat-to-Target in Inflammatory Bowel Diseases, What Is the Target and How Do We Treat? Gastrointest Endosc Clin N Am. 2019 Jul;29(3):421-436. doi: 10.1016/j.giec.2019.02.004. PMID: 31078245.

**4**. Colombel JF, D'haens G, Lee WJ, Petersson J, Panaccione R. Outcomes and Strategies to Support a Treat-to-target Approach in Inflammatory Bowel Disease: A

Systematic Review. J Crohns Colitis. 2020 Feb 10;14(2):254-266. doi: 10.1093/ecco-jcc/jjz131. PMID: 31403666.

R: All 4 references were included in the manuscript, where suggested (after references 3 and 4). Now the parenthesis includes references from 3 to 8.

b. The aim stated: "…to establish an optimal cut-off level of BG to predict mucosal healing". Please then present the optimal cut-off level that predicts MH also in the abstract.

R: We inserted the data in the abstract, as suggested (in Abstract, Results).

3. MATERIAL AND METHODS:

a. Study population:

1) Please mention whether patients under proton pump inhibitors previously were excluded as well (important, as they modify GI microbiota, levels of fecal calprotectin as well etc). What about those receiving probiotics prebiotics or synbiotics? They modify GI microbiota, as this is the reason they are used! Same for the control group (previous medication)! If not, this could introduce an important bias!

R: We understand this reviewer's concern. We included some more details regarding previous use of medications in our exclusion criteria, according to his/her suggestion (in Material and Methods, Study population, in the end of the first paragraph).

2) The number of patients with CD and UC should be mentioned here, as it is presented in the Abstract. Only the number of people in the control group is mentioned.

R: We agree with this comment, and we included the data, as suggested (in Material and Methods, Study population, first paragraph, lines 6-9).

3) One note here: Patients with IBS are also known to experience intestinal dysbiosis and it could influence the BG levels. It would have been better to include only healthy controls. This should be mentioned as a limit of the study. Another important bias!

R: We understand this reviewer's concern. We included a new paragraph of limitations (the last paragraph in the Discussion section), including several new references on the subject (refs 77-82).

Although we did not investigate the exact source of BG (which is beyond the scope of this study), we used the serum levels as a potential reflection of active mucosal inflammation, probably involving changes in permeability and dysbiosis, according to our hypothesis. BG is known to be present in several microorganisms, but it is mostly associated with fungal microorganisms, including *Candida* and *Aspergillus* spp. Nonetheless, in this study, the dramatic decrease in serum BG among the responders of therapeutic interventions, strongly indicate that the relative serum levels of BG might be even more important than the absolute values. Therefore, if BG levels are associated with intestinal dysbiosis (in addition to permeability), the study groups might have different types of dysbiosis (including patients with IBS). This potential association is interesting and should be investigated in a future study.

Regarding the composition of the control group, we attempted to work with a group, which could reflect more accurately a real world setting. In fact, our results show that the overall variability within the control group is very small compared to the dispersion among patients with IBD. However, the absolute levels of serum BG

among patients in remission (CD and UC) were not clearly distinguishable from controls. This has been attributed to changes in the inflammatory activity among the IBD patients. Therefore, we believe that serum BG should be used preferentially as an inflammatory indicator, a biomarker for IBD activity, and not for screening purposes. Based on our study results, we cannot recommend using serum BG as a primary diagnostic tool to replace endoscopy/histopathology or imaging exams.

b. Assessment of disease activity:

1) For SES-CD and respectively Mayo endoscopic subscore the original references should be used, instead of some papers that cited them (to replace the present 36, 37 and 38): for SES-CD: Daperno M, D'Haens G, Van Assche G, Baert F, Bulois P, Maunoury V, Sostegni R, Rocca R, Pera A, Gevers A, Mary JY, Colombel JF, Rutgeerts P. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointest Endosc. 2004 Oct;60(4):505-12. For MES: Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. 1987;317:1625–9.

R: We agree with this comment, and the references were included in the manuscript replacing the previous ones, as suggested. Previous references 36-38 were replaced by the news references 40 and 41;

2) Regarding the criteria for the presence of inflammatory activity at MRE, parietal thickness of > 3 was not included and this was the most universally accepted parameter (as mentioned in many papers – one example - Maconi G, Armuzzi A. Beyond remission and mucosal healing in Crohn’s disease. Exploring the deep with cross sectional imaging. Dig Liver Dis 2017; 49: 457-458 [PMID: 28449813 DOI: 10.1016/j.dld.2017.04.009]. Besides, wall thickness is included in most-used magnetic resonance scoring systems: MaRIA, London, Nancy and Clermont.

R: We again agree with this reviewer's comment, and apologize for the omission. More details were included in the manuscript text, and the new reference 46, as suggested.

3) The authors wrote: "Patients with IBD were selected consecutively depending on the presence of active disease based on both clinical and endoscopic evaluation." Why not histologic activity as well? Since it was presented.

R: We agree with this comment, and the correction was performed, as suggested.

4) Another aspect that should be further discussed is that the population of patients with IBD is very heterogenous, including various therapies.

R: This reviewer is correct and we agree with his/her comment. We added some more text and references (71-73) in the Discussion section, in the penultimate paragraph, on limitations.

4. RESULTS:

a. In the sentence, "One hundred fifteen patients with CD (sixteen with ileal [L1], thirty-nine with colonic [L2], and sixty with ileocolonic [L3] CD, and fifty-one patients with UC), fourteen", please insert the parenthesis before "Fifty-one patients with UC", as they do not have CD.

R: We agree with this comment, and we made the correction according to his/her observation.

b. It appears that not all patients underwent ileocolonoscopy, as in the sentence about histology, ileocolonoscopy is mentioned in only 71 in CD and 29 in UC) – "Histological activity was detected in 33 of 71 (46.5%) patients with CD and 9 of 29 (31%) patients with UC WHO UNDERWENT ILEOCOLONOSCOPIES". Or, the histological activity was not measured in all patients that underwent ilocolonoscopies? Please correct/clarify if this is just the result of the translation.

R: This reviewer is correct and we agree with his/her observation. We made the changes in the text for clarity. All patients underwent ileocolonoscopies, but biopsies were obtained from 92.2% of CD and 82.8% of UC patients, regarded as endoscopically active.

c. Why did the authors choose to analyse BG in both UC and CD together and not in each disease? (Figure 3)

R: We did not want to end up with small numbers for subgroup analysis. But, we understand this reviewer's concern, and we separated CD from UC (creating new Figures 3 and 4).

d. Same for Table 2. It would be crucial to know cut-off, sensitivity, specificity, PPV, NPV and accuracy separately in UC and CD. I suggest to present also a separate analysis.

R: Exactly as the above question and response, we thought of combining CD and UC because of the sample size. Again, we managed to separate CD from UC and we prepared two different new Tables 2 and 3.

OTHER COMMENTS: There are no Conflict-of-Interest Disclosure Form and Copyright License Agreement. Please insert.

R: We inserted the documents, as observed by this reviewer.

Finally, we thank this reviewer very much for such an attentive reading and important suggestions and corrections, which support our work and clearly contribute for critical improvements to the manuscript.

**Reviewer #2:**

**Scientific Quality:** Grade C (Good)

**Language Quality:** Grade A (Priority publishing)

**Conclusion:** Accept (General priority)

**Specific Comments to Authors:** It was novel and well presented study. I have no addittional recommendation.

R: We thank this reviewer for supporting our work and for finding the results of our study interesting.

**Reviewer #3:**

**Scientific Quality:** Grade B (Very good)

**Language Quality:** Grade A (Priority publishing)

**Conclusion:** Major revision

**Specific Comments to Authors:** In the present prospective study Farias et al investigated serum levels of 1, 3 beta-D-glucan (BG) in patients with inflammatory bowel disease (IBD) and found that BG correlated with clinical, endoscopic and laboratory investigations suggestive of active inflammation. Additionally, improvement of IBD disease activity paralleled with declining BG levels.

Main comments:

1) My most relevant point concerns the fact that BG is a component of bacterial cell wall. Therefore, were conditions that may interfere with correct BG estimation (SIBO, Salmonella, Shigella infection) ruled out in IBD group?

R: We understand this reviewer's concern. In fact, we did not look specifically for SIBO, Salmonella, or Shigella among our patients. However, as we state in our exclusion criteria, we ruled out patients with "acute or chronic enteric infection (e.g., *Clostridioides difficile*), and individuals who had received concomitant antibiotics and/or nonsteroidal anti-inflammatory agents in the previous 3 months". This was performed on clinical and laboratory grounds, based on our routine follow-up of patients. Being aware of the exploratory nature of this study, we thought of working with all variables and the heterogeneity that IBD imposes, attempting to simulate the actual practice.

Although BG is present in several microorganisms, it is mostly associated with fungal microorganisms, including *Candida* and *Aspergillus* spp. Nonetheless, in this study, the dramatic decrease in serum BG among the responders of therapeutic

interventions, strongly indicate that the relative serum levels might be even more important than the absolute values. Therefore, although we did not investigate the exact source of BG (which is beyond the scope of this study), we used the serum levels as a reflection of active mucosal inflammation, probably involving changes in permeability and dysbiosis.

2) Do not report interquartile range in square brackets (it seems to be a reference list).

R: This reviewer is correct. We apologize for the inconvenience. We replaced the brackets with parenthesis.

3) It would have been interesting to calculate ***r*** correlation with ***scatterplots*** between BG, FC and CRP.

R: We agree with this reviewer and we were also very excited with all the results we achieved. However, we tried to adjust to the limitations imposed by the journal. It was a difficult choice in terms of tables, figures and types of graphs. Therefore, we decided to present only the ones we evaluated to have a greater impact. The scatterplots would take more space in the article (we already have several Supplementary materials). In fact, we did not find a significant correlation of serum BG with CRP or ESR, and the correlation with FC was weak, and only marginally significant. Anyway, we managed to add the information to the text in first paragraph of the Results section.

4) Table 3: you can not compare by a statistical test disease location of UC and CD, these data are not comparable.

R: This reviewer is correct. We apologize for the mistake!

5) Sample size calculation is absent.

R: We understand this reviewer's concern. Nevertheless, the test for serum beta-glucan (BG) has been designed basically to identify systemic fungal infections. In this study, according to our hypothesis, we proposed a completely different utilization for the BG kit, therefore entering into a new field. The only previous paper studying BG in Crohn's disease (Guo Y et al, Mediators Inflamm 2015; 2015:843089), analyzed only 46 patients with active disease and 24 patients in remission, and 20 healthy controls. Here, we analyzed a greater number of participants, including a group of patients with ulcerative colitis. In addition, our study counted with additional analyses, with more laboratory tests, and including endoscopic, histologic, and imaging exams. Moreover, in terms of control group, we included non-IBD patients and healthy controls (total of 82). Finally, our total sample size was of 248 individuals. According to this reviewer's request, we present our preliminary calculation using the *G*Power software*, which we managed to insert in the section of Methods, of the manuscript.

**F tests** - ANOVA: Fixed effects, special, main effects and interactions

| | | | |
|---|---|---|---|
| **Analysis:** | A priori: Compute required sample size | | |
| **Input:** | Effect size f | = | 0.25 |
| | α err prob | = | 0.05 |
| | Power (1-β err prob) | = | 0.95 |
| | Numerator df | = | 1 |
| | Number of groups | = | 3 |
| **Output:** | Noncentrality parameter λ | = | 13.1250000 |
| | Critical F | = | 3.8867744 |
| | Denominator df | = | 207 |
| | Total sample size | = | 210 |
| | Actual power | = | 0.9501203 |

6) Please report the cut-off for BG that was used for ROC analysis.

R: We thank this reviewer for his/her observation. We managed to add text reporting the cut-off levels of BG in the Results section.

7) Demographic characteristics of the whole control group should be reported in a table and compared with the IBD group.

R: We understand this reviewer's concern and agree with the comment. We managed to add data from the whole control group to Table 1.

8) Authors affirmed that, in control group, some patients with diverticular disease were enrolled. What about the BG levels in this subgroup of patients compared to IBD? Indeed, FC may be high in diverticulosis, and if this phenomenon does not occur for BG, it could be very handful for clinical practice.

R: We thank this reviewer for supporting our work and for reading in detail all aspects of our study. In fact, we thought of using a control group, which could reflect more accurately a real world setting. In our Unit for Intestinal Diseases, we see hundreds of patients with IBD monthly, but also patients with other intestinal diseases, mostly chronic conditions, including diverticulosis. Although we also believe that the question is extremely interesting and relevant, unfortunately, we did not have patients with active inflammatory forms of diverticulosis during the study period. Moreover, another subgroup analysis with small numbers would render data difficult to interpret. We understand that this should be actively investigated in another study, in the future. In addition, we believe that serum BG should be investigated also in other inflammatory diseases affecting the GI tract.

**4 LANGUAGE QUALITY**

Please resolve all language issues in the manuscript based on the peer review report. Please be sure to have a native-English speaker edit the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability, so that the manuscript's language will meet our direct publishing needs.

**5 EDITORIAL OFFICE'S COMMENTS**

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

**(1)** ***Science editor:***

1 Scientific quality: The manuscript describes a observational study of the Serum 1,3-Beta-D-glucan as a noninvasive test to predict histologic remission in patients with inflammatory bowel disease. The topic is within the scope of the WJG.

(1) Classification: Grade B, Grade C and Grade C;

(2) Summary of the Peer-Review Report: The study is among the very few researches that addressed the correlation of BG with clinical, endoscopic and histological activity, as well as with some serological biomarkers and fecal calprotectin. The manuscript is original and represents a novelty. However, some points should be addressed by the authors. The questions raised by the reviewers should be answered; and (3) Format: There are 3 tables and 4 figures. A total of 69 references are cited, including 13 references published in the last 3 years. There are no self-citations.

R: After responding to all queries and making all the amendments, we now have 4 tables, 5 figures, and 82 references. In addition, we included a new supplementary Figure S1.

2 Language evaluation: Classification: Grade A, Grade A and Grade A. A language editing certificate issued by AJE was provided.

3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the STROBE Statement, and the Institutional Review Board Approval Form. Written informed consent was waived. The authors need to provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. No academic misconduct was found in the Bing search.

R: Here, we provide both Conflict-of-Interest Disclosure Form and Copyright License Agreement, as required.

4 Supplementary comments: This is an unsolicited manuscript. The study was supported by 3 grants. The topic has not previously been published in the WJG.

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);

R: We are presenting the 3 approved grant application forms related to the manuscript.

(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

R: We are presenting the original figures in a PowerPoint file.

(3) The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text.

R: We are presenting the Article Highlights, as required.

6 Re-Review: Required.

7 Recommendation: Conditional acceptance.

**(2) *Editorial office director:***

**(3) *Company editor-in-chief:*** I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office’s comments and the Criteria for Manuscript Revision by Authors.

R: Thank you very much for your support.