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Girona, Spain, February 27th, 2020

To Jin-Zhou Tang, Science Editor and Editorial Officer of Baishideng Publishing Group Inc,

Subject: *Manuscript NO: 53498 can be accepted for publication in the World Journal of Gastrointestinal Pathophysiology*

After consideration with the co-authors, we accepted the offer to transfer our manuscript (**Manuscript ID: 53498** “*Evaluation of bacterial biomarkers to aid in challenging inflammatory bowel diseases diagnostics and subtype classification.*” by Lopez-Siles M et al) for publication in the World Journal of Gastrointestinal Pathophysiology.

As requested, we have fully considered the reviewers’ comments that were provided, and carefully revised manuscript accordingly. I would like to thank you and both anonymous reviewers for the constructive comments and detailed suggestions.

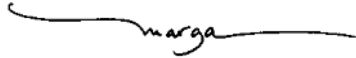
In addition to formatting issues and minor language editing, the concerns raised were addressed as summarised below:

Reviewer	Concern	Solved	Observations
R1	Indicate date of sample collection	Yes	Information included in the manuscript
R1	Specify if probiotics treatment was received	Yes	Information included in the manuscript
R1	Specify medical treatment for IBD patients	Yes	Information included in the manuscript
R1	Specify if CRC subjects undergo radiotherapy, chemotherapy or surgery	Yes	Information included in the manuscript
R1	Apply a newer version of SPSS	No	No license for newer version
R1&R2	Increase cohort studied	No	Retrospective cohort study, where data from a previous project has been re-analysed. The project is finished.

I thus enclose the revised manuscript and hope it will now be suitable for publication in World Journal of Gastrointestinal Pathophysiology. For the convenience of reviewer reading, the tracking-changes version is submitted for your reference. The detailed point-by-point responses to the referees' comments are also attached for your review.

I am looking forward to hear from you soon.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'marga', with a long, horizontal, slightly wavy line extending to the right.

Dr. Margarita Martínez Medina

Point-by-point responses to the referees' comments

The authors appreciate the Editor's comments and are grateful for the accurate review of the manuscript by the two anonymous experts.

Reviewer #1

Review info:

Conclusion: Accept (General priority)

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

We would like to thank the referee for his/her review and comments, since we believe they improve the manuscript. Changes in the manuscript are referenced in the responses by line (L) number in the revision of the manuscript with the tracked-changes version.

Specific Comments To Authors:

The application value of alterations in gut microbiota in the diagnosis of IBD is a research direction worth exploring. The research in this paper is more comprehensive and detailed.

Objections are:

A: Please indicate when biopsies and stool samples are collected. Was the patient received probiotics before taking the sample? What was the medical treatment for IBD patients? Did the CRC subjects undergo radiotherapy, chemotherapy or surgery?

Answer A) Thank you for pointing this missing information:

- Collection date for biopsies was already in the manuscript (L199-200) and data for faecal samples has been included (L208)
- None of the patients received probiotics. This information has been included in the manuscript (L226-227)
- IBD participants were either under moderate immunosuppressants, anti-TNF α treatment or not receiving medication. Medication at sampling for IBD patients has been included in Table 1.
- CRC subjects did not undergo radiotherapy, chemotherapy or surgery. This information has been included in the manuscript (L218-219)

B: increasing the number of newly diagnosed cases in recent years if possible.

Answer B) We appreciate the reviewer suggestion. We are presenting a retrospective cohort study, where data from a previous project has been re-analysed. We are not in position to include more subjects in the cohort as this project is finished. However, please note that the cohort of subjects that provided faecal samples is more recent than the one providing biopsies.

C: Please consider to apply a newer version of SPSS software for statistics.

Answer C) The SPSS version is the licensed at our University and therefore we are not in position of a newer version.

Reviewer #2

Review info:

Conclusion: Rejection

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing)

We would like to thank the referee for his/her review and comments. Changes in the manuscript are referenced in the responses by line (L) number in the revision of the manuscript with the tracked-changes version.

Specific Comments To Authors:

The classification of IBD and CRC by bacterial biomarkers is unique and seems useful in clinical site. However, the sample number is too small to be accepted. A minimum of 100 patients each is required.

We agree with the reviewer that a larger cohort would allow us to obtain more solid results. In addition of the information provided to Reviewer 1 in Answer B, we would like to point out the following considerations:

1. There are many published studies that present results with a similar cohort size. In our study, sample size was defined taking into account the number of patients analysed in similar published studies of bacterial abundance in patients suffering of these conditions. These papers are key studies, known for setting milestones in the field of gut microbiota and in characterising IBD dysbiosis in the past, but still nowadays studies with similar cohorts are published. For example:
 - Frank D.N et al Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases, PNAS, 2007 Aug 21;104(34):13780-5. **(CD=36, UC=43, H=40)**.
 - Sokol H et al Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. Inflamm Bowel Dis. Aug;15(8):1183-9. **(CD=32, UC=17, H=27)**.
 - Willing B et al, Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. Inflamm Bowel Dis. 2009 May;15(5):653-60. **(CD=10)**.
 - Kowalska-Duplaga K et al, Differences in the intestinal microbiome of healthy children and patients with newly diagnosed Crohn's disease. Sci Rep. 2019; 9: 18880. **(CD=64, H=18)**.
 - Fernandes MA et al, Enteric Virome and Bacterial Microbiota in Children With Ulcerative Colitis and Crohn Disease. J Pediatr Gastroenterol Nutr. 2019 Jan;68(1):30-36. **(CD=7, UC=5, H=12)**.
2. It should be taken into account not only the quantity of samples (n) but also the quality of them, and the information that can provide. This work is based on biopsies, therefore the cohort studied is suitable. On the one hand, this is an invasive sample to obtain, thus from an ethical point of view reduction of number of samples should be applied as much as possible, and we have based our study in the principle to keep necessary samples to a minimum. On the other hand, given that we are studying mucosa-associated microbiota, the effect of hosts and disease in it is expected to be more clearly elucidated, with less effect of confounding factors (for example such as diet if faeces would have been

analysed). This has allowed to reduce the number of samples to be tested without compromising obtaining relevant results.

3. We have published previous results in several peer-review journals from this cohort which has been considered valid. References are:
 - a. Lopez-Siles,M.; et al. Mucosa-associated *Faecalibacterium prausnitzii* and *Escherichia coli* co-abundance can distinguish Irritable Bowel Syndrome and Inflammatory Bowel Disease phenotypes Int J Med Microbiol. 2014 May;304(3-4):464-75.
 - b. Lopez-Siles,M.; et al. Mucosa-associated *Faecalibacterium prausnitzii* phylotype richness is reduced in patients with inflammatory bowel disease Appl Environ Microbiol. 2015 Nov; 81(21): 7582–7592.
 - c. Lopez-Siles,M.; et al. Changes in the Abundance of *Faecalibacterium prausnitzii* Phylogroups i and II in the Intestinal Mucosa of Inflammatory Bowel Disease and Patients with Colorectal Cancer Inflamm Bowel Dis. 2016 Jan;22(1):28-41.
 - d. Lopez-Siles,M.; et al. Alterations in the Abundance and Co-occurrence of *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* in the Colonic Mucosa of Inflammatory Bowel Disease Subjects Front Cell Infect Microbiol. 2018 Sep 7;8:281.
4. Some of the previous results obtained with our cohort were later replicated by other studies. Examples include:
 - a. Pascal V, et al A microbial signature for Crohn's disease. Gut. 2017 May;66(5):813-822.
 - b. Jia W, et al Is the abundance of *Faecalibacterium prausnitzii* relevant to Crohn's disease? FEMS Microbiol Lett. 2010 Sep 1;310(2):138-44
5. In the article submitted for evaluation, further validation of the results in the IBD cohort is performed with independent patients, which gives robustness to the results.
6. Finally, considering our cohort, and estimating that disease incidence in Spain ranges between 1.9-3.8%, and that Girona has 7500.00 habitants, we have estimated the confidence interval (i.e margin of error, MOE) with a confidence level of 95%:

Group of subjects	n	MOE
CD	45+19	15%-12.25%
UC	25+10	20%-16.56%
CRC	20	22%

Altogether, these considerations support that our cohort is not that limited for providing meaningful results, and is worth to be shared with the scientific community.

Besides, information regarding sample size determination has been included in the manuscript (L267-270).