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Title: Relationship between mucosa-associated microbiota and pouchitis following restorative proctocolectomy for ulcerative colitis

Dear Dr. Wen and dear Prof. Ma,

My co-authors and I are now submitting the revised version of our manuscript entitled "Relationship between mucosa-associated microbiota and pouchitis following restorative proctocolectomy for ulcerative colitis" for your and the reviewers' evaluation. As you can see in the enclosed point-by-point response, we have attempted to address all of your and the reviewers' questions and comments. The outcome, we feel is a clearer, more focused manuscript.

We would like to take this opportunity to thank you for helping us to improve the manuscript and trust that in its present form it will be deemed suitable for publication in your journal. The revisions/corrections, which have been read and approved by all of us, are explained in detail here below.

Needless to say, we will be looking forward to receiving your comments in due course.

Sincerely yours,

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Point by point reply

REV 1

This review examines the impact of the flora and host responses in pouchitis

Specific Comments:

1. There are a number of errors of English grammar and/or construction that require correction

- *The entire manuscript has been revised by a mother tongue language consultant.*

2. In the Abstract, what is meant by RPC? Abbreviation should be explained here/in intro

- *OK. This and all other abbreviations have been explained.*

3. Another reason for "pouchitis" is that the disease is actually CD not UC. Crohn's disease is another disease and it must do a differential diagnosis with chronic pouchitis.

- *Your observation is a good one, and we were aware of the need to make a differential diagnosis between the two diseases. In the Search Strategy Section we point out: "..."*

4. In the Methods, what is meant by pouch affection?

- *It is a mistake, we meant disease.*

5. With regards to the flora and pouchitis, the authors could explain a little more on the impact of pouchitis therapies upon the flora.

- *This is indeed a very interesting point, but, as we pointed out in the discussion: "Unfortunately for the moment no studies in the literature have attempted to analyze the direct impact of pouchitis therapy on pouch microbiota"*

6. What is meant by "chronic pouchitis off"? (page 8) 7. The Table needs to be reformatted so that words are complete

- *OK for the first point - Chronic off = asymptomatic, and we have reformatted the table.*

REV 2

The authors made a well written, interesting review about pouch bacteriota and its pathogenic role. The used references are up-to-date. Though I was not able to open Figure 1, the content of the manuscript is such good that I suggest to accept it for publication in WJG.

- *We reformatted the figure*

REV 3

Thank you for the opportunity of reviewing this interesting manuscript. It is indeed a comprehensive review on pouch bacteriology. It is nicely written yet it is not easy to read; the paragraphs are somewhat long and sometimes repetitive. Try breaking the text into shorter paragraphs. Figure 1 is not available for review.

We reformatted the figure

1. There are numerous errors of language in tenses, plurals, wording, etc. This needs to be carefully edited by a native English speaker.

The entire manuscript has been revised by a mother tongue language consultant.

2. There are several instances where acronyms are either not defined or are defined later than first use, such as with RPC, TRFLP, OTU, CD, NP, CP-off, and CP-on. In addition, the authors use RPC, IPAA, pouch, ileo-anal pouch, ileal pouch, etc. interchangeably. The authors should pick one and be consistent.

- *All the abbreviations have been explained. We would like to point out to you that we avoided using abbreviations in the “core tip” so that anyone not reading the entire manuscript could nevertheless understand everything. We have done our best to be consistent in a situation in which various authors use a variety of names and terms.*

3. There are confusing or poorly worded sentences. The definition of pouchitis by Mahadevan and Sandborn can be clarified. In the methods section, second sentence, the word “affection” is likely wrong. It makes the intent of the authors unclear. Similarly, in the sentence beginning “A manual cross reference...” the meaning of “originally resulting compatible” is unclear.

- *Please note that the entire manuscript has been revised by a native speaker.*
- *With regard to Mahadevan and Sandborn’s definition, we have outlined their definition in the revised text.*
- *Affection is a mistake.*
- *That sentence has been revised.*

4. The table, as presented to the reviewer, was poorly constructed and difficult to read. Perhaps a landscape format, instead of letter format, as well as a smaller font, would make this more clear.

- *A new table in ppt landscape format has been constructed.*

5. The title speaks specifically to the mucosa-associated microbiota. However, the majority of the review is on luminal microbiota. If it is the intent of the authors (and I believe it was) to emphasize this point, then the section on luminal microbiota can be shortened and more emphasis on mucosa-associated microbiota be placed. And along this line, the conclusion does not specifically address this issue as stated in the title. If it was not the authors’ intention to differentiate the mucosa associated microbiota from luminal microbiota, then the title should be adjusted accordingly. Overall, I believe the authors should make a major effort to shorten this paper - it is way too long

- *The new title is: “Relationship between pouch microbiota and pouchitis following restorative proctocolectomy for ulcerative colitis”*
- *A linguistic revision was made with the intention of producing a more readable, shorter manuscript*

REV 4

A comprehensive review on the importance of microbial factors in the development of pouchitis
Comments;

1. Please rename patients and methods to search strategy

- *We did it*

2. Include an additional Table on how authors have identified the studies included

- *We did it*

REV 5

This is an interesting topic for review and the manuscript is well written.

- *Thank you*

REV 6

This is a succinct well written and informative review on the analysis of the microbiota in the ileal anal pouch after ulcerative colitis and FAP. The subject matter is of high interest and is timely. It provides state of the art information on the subject. However, the table that is included is difficult to assess due to the format in which it was presented.

- *The table has been reformatted*

Some spelling revisions may be needed. For instance, the last name of an author referred to in the text and in the table is spelled differently.

- *The manuscript has been revised for spelling/grammar errors by a mother tongue language consultant and we have checked the bibliography for spelling errors.*

REV 7

This paper is a review of studies showing that dysbiosis of the ileal pouch microbiota has a role in the pathogenesis of pouchitis by impairing the regulation of mucosal immune system. In the paper, intestinal microbiota is classified into 2 groups: mucosal-adherent microbiota and luminal microbiota. Although the authors mentioned on these in the manuscript, there is a need for an in-depth discussion of these 2 concepts of microbiota. The authors should refer to the methods of culturing and showing the presence of these 2 groups of microbiota. Methodological differences between the published literature and the ideal method should be discussed in the manuscript. Also, it should be noted if mucosal-adherent microbiota can be affected by the treatment with antibiotics.

- *As we explained in the text, most authors directly analysed microbiota adherent to the mucosa since it is the one that directly interacts with the host immune system. We debated this concept in the following paragraph: “It is well established that within the first year after ileostomy closure, the overall composition of microbiota shows similarities with the colonic one [40,41]. A number of studies using faecal cultures to evaluate the microbiota of pouches in UC and FAP patients [42,43] produced conflicting results with regard to the ratio of anaerobic bacteria to aerobic bacteria, total bacterial counts, and sulphate-reducing bacteria [44] in the pouchitis and non-pouchitis patients. The high grade of variability in these kind of studies may be due to the daily variability of stool composition in relation of diet. Moreover, the high frequency of bowel movement in IPAA patients may enhance this variability.*

The mucosal-adherent microbiota, which is in close contact with the gut mucosa, has recently been shown [45,46] to be distinct from the luminal and faecal ones, which are made up of free-living or particle-attached cells. The differences in community structure are probably linked to a number of factors such as differential substrate availability (mucus vs undigested dietary residues), oxygen levels, and host-microbe interactions. In particular, mucosa adherent microbiota may be influenced by drugs. The close proximity of the mucosal-adherent microbiota to the gut epithelium suggests that these bacteria may be more relevant than the luminal microbiota in the pathogenesis of inflammatory bowel disease (IBD) since they, as well as their excreted products, probably have direct contact with the host [46]. Moreover, since they live in a mucous environment their population are more protect and thus more constant. The ideal microbiota analysis probably would take in exam both faecal and mucosal-adherent ones. In fact, faecal microbiota may give a rough but more complete idea of whole bowel microbiota while mucosa adherent bacteria are those that directly cross-talk with the host and that more likely are involved in the pathogenesis of pouchitis.”.

- *We added the sampling technique in table 1.*

Is microbiota of patients with refractory pouchitis different from the patients with responsive?

- *This is an interesting point but, as far as we know, there are no studies in the literature that have examined this possibility.*

Are there any evidence showing this possible difference is related to mucosal-adherent microbiota?

- *Again, this is an interesting point but, as far as we know, there are no studies in the literature that have examined this possibility*
- With the clarification of these questions, the results of the study will become more apprehensible and efficient.