

February 7, 2024

World Journal of Gastroenterology

Manuscript ID: 91855

Title: Washed microbiota transplantation for Crohn's disease: A metagenomic, metatranscriptomic, and metabolomic-based study

Dear Dr. Ma:

We would like to thank you and the reviewers for reviewing our work and providing us with very helpful comments. In the revised manuscript and this letter, we have made our best effort to address all the points that the reviewers raised. The major changes in the new version of the manuscript are in Yellow, so the reviewers and the editor can follow the modifications easier. Detailed revisions are as follows.

(91855-Answering Reviewers)

Reviewer #1:

The following revisions are made according to the comments of Reviewer #1.

1) I am not an expert in Crohn's disease. Could the authors please either explain the CD Activity Index (CAI), Harvey-Bradshaw Index (HBI), and Simple Endoscopy Score (SES) more (such as what cut-off values are meaningful and what they mean) or add references for articles that explain these?

Thanks for the reviewer's comment. The CD Activity Index (CAI) and Harvey-Bradshaw Index (HBI)s are the most common scoring systems used to measure clinical disease activity in Crohn's disease (reference: PMID: 28826742). Activity Index (CAI), a standardized instrument that incorporates both objective and subjective evaluations of disease activity. In most clinical trials active disease is defined as Clinical Disease Activity Index (CAI) > 220. (reference: PMID: 28826742; PMID:15131785). Remission is generally defined as a CAI score <150 or ≤150. Response is defined by a reduction in the CAI score exceeding a predefined quantity, usually 70 or 100 points. (reference: PMID:15131785; PMID: 31367877; PMID: 32014035). The HBI index score is easier to use than the CAI, and it correlates

well with the CDAI (reference: PMID: 16633052) and has been widely used in many studies on the assessment of Crohn's disease activity. (reference: PMID: 35631213; PMID: 35369995) . An HBI index score of ≥ 3 or >3 is usually defined as disease activity, and a reduction of ≥ 3 points from baseline is defined as clinical response (reference: PMID:31316785; PMID:32109634) . All patients included in our study had a CDAI score ≥ 150 , defining clinical response as a decrease in CDAI score greater than 100. Simple Endoscopic Score for Crohn's Disease is a simple, reproducible, and easy-to-use endoscopic scoring system for Crohn's disease. Selected endoscopic parameters (ulcer size, ulcerated and affected surfaces, stenosis) were scored from 0 to 3. It has been widely used in similar studies to assess intestinal mucosal healing. (reference: PMID:31316785; PMID: 15472670; PMID:37224198)

2) Could the authors please define in the methods what the Montreal classification is in Table 1?

Thanks for the reviewer's comment. We have added a definition of the Montreal classification in the methods section of the article. **Montreal classification was used to count the CD patients included in the study. The Montreal classification is based on the age at diagnosis (A1: <16, A2:17-40, A3: > 40), disease location (L1:Ileal, L2:colonic, L3:Ileocolonic, L4: upper gastrointestinal) and the disease behavior (B1:non-stricturing/non-penetrating, B2: stricturing, B3:penetrating, P: perianal disease modifier).** (reference: PMID: 28826742; PMID:16698746)(Table 1) Modification has been done in line 186-190.

Table 1 Montreal Classification

	Montreal Classification
Age at diagnosis(y)	A1: < 16 A2: 17–40 A3: > 40
Location	L1: ileal L2: colonic L3: ileocolonic L4: upper gastrointestinal

Behavior	B1: non-stricturing, non-penetrating B2: stricturing B3: penetrating P: perianal disease modifier
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3) At the beginning of the AE portion of the Results section, could the authors please explain what the number 22 is in reference to? In the methods, it was noted that there were 22 stool samples. However, it appears there were only 11 participants who received the treatment and would be expected to potentially experience an adverse event. The Results subsection on AEs also does not seem to add up overall. Perhaps I am misunderstanding the numbers but it looks like this section indicates there were two individuals who experienced AEs in the first sentence and yet the rest of the description makes it sound like there were four individuals.

Thanks for the reviewer's comment. A total of 11 patients with CD were included in the study. WMT was administered to each patient once a day for two consecutive days. So, a total of 22 WMT treatments were performed. A total of two adverse events (constipation in 1 patient and increased defecation in another). Two other patients experienced painful episodes of urticaria and gout after treatment, which resolved spontaneously within 24 hours. Because these patients had previously relapsed with similar conditions, the treating physicians did not consider them to be transplant-related. In summary, the incidence of AEs was 2/22 (9.1%).

4) Supplementary Figures 4, 5, and 6 appear to only be mentioned in the Discussion section. It appears there is quite a bit of text in the Discussion that describes results and should be moved up to the Results section. I do see that references to other studies are intertwined with this text though. I will leave the decision on the location of this text as a judgement call for the editors, but I think this seems like it should be included in the Results.

Thanks for the reviewer's comment. Supplementary figures 4, 5 and 6 have been placed in the main body of the results. Modification has been done in line 363-366, 410-411, 420-422.

We believe that we have addressed all the points raised by the reviewers. We sincerely hope that you would find the revised version satisfactory for publication in World Journal of Gastroenterology, and we look forward to your favorable reply.

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