Peer Review File

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Reviewer A

This is a study of gut microbiome on fecal samples in 71 subjects with microscopic polyangiitis (MPA) to clarify the efficacy of gut microbiome profiles as biomarkers for disease activity. Authors demonstrated alterations of intestinal flora in MPA patients, and there were the several genera of bacteria were associated with disease activity or progression. Therefore, authors concluded that gut microbiome profiles were useful markers for disease activity in MPA.

The presented study was well performed and the manuscript is described in a reasonable manner. It seems that those biomarkers are not suitable for diagnosing MPA and predicting disease activity, because the examination of gut microbiome is not clinically easy to perform, in my opinion. However, it was interesting that genus Actinomyces and Streptococcus were increased in both active and inactive MPA. Therefore, authors had better re-analyze excluding factors other than MPA (such as CKD), and it may be better to lead to conclusions about the pathogenic mechanism.

I have minor concerns:
1. Had this study been cross-sectional or longitudinal? Were there the same patients in both active MPA and inactive MPA groups? Authors had better describe subjects in detail.
Reply 1: The study had been cross-sectional. There were no same patients in both active MPA and inactive MPA groups.
Changes in the text: We modified our text (see Page 6, line 98 and Page 10, line 186-187). We added a Table with detailed information on the clinical, laboratory and histopathological findings of all participants (see in Supplementary Appendix, Table S1).

2. Were there any patients treated with antibiotics? Patients with MPA are often treated with antibiotics because of inflammation.
Reply 2: Because antibiotics have an obvious influence on gut microbiome, we excluded patients who had received antibiotics within one month before enrollment and at sampling, as shown in Figure S1.
Changes in the text: We modified our text (see Page 7, line 113-114).

3. Were ANCA, urinary protein, serum CRP level not examined in healthy controls? Is it possible that CKD etc. were included? Authors had better describe about healthy volunteers in detail.
Reply 3: ANCA, urinary protein and serum CRP level were not examined in the healthy controls in this study. Although we excluded those with diagnosed CKD or elevated serum creatinine, we couldn't completely exclude the possibility of CKD in healthy individuals because we had no urine test results.
Changes in the text: We have described the clinical and laboratory findings of healthy controls in Supplementary Appendix, Table S1.

4. Although alterations of intestinal flora were demonstrated in MPA patients, which may be the pathogenic, the increasing or the decreasing microorganisms?
Reply 4: The changes of increasing and decreasing microorganisms may cause underlying mechanisms in the development of MPA. As Ooi and colleague's work(1) suggested that the increasing of *Staphylococcus aureus* will induce the molecular mimicry of the specific antigens to trigger immune response and inflammation in a murine model of anti-MPO glomerulonephritis. On the other hand, lots of decreasing bacteria were associated with the production of SCFAs, which possessed anti-inflammation function. In this study, we found the SCFA producing genera, including *Subdoligranulum*, *Eubacterium hallii*, *Ruminococcaceae UCG013*, *Eubacterium ventriosum*, *Dorea* and *Butyricicoccus*, were reduced in patients with active or inactive MPA. So, we supposed that it may be a mechanism to the development of MPA.
Changes in the text: We modified our text (see Page 19, line 367-369).

5. Authors described alterations of intestinal flora were associated with CKD. If so, were not patients with CKD or MPA without kidney involvement necessary as controls? Had Actinomyces and Streptococcus been increased in patients with CKD? Were those increased in MPA without kidney involvement?
Reply 5: We agreed that inclusion of CKD patients or MPA without kidney involvement as controls would make our conclusions more convincible. However, actually, the clinical manifestation of kidney involvement in MPA patients was variable, ranging from rapidly progressive glomerulonephritis (RPGN) to CKD, making a group of patients with CKD unsuitable as controls. MPA patients without kidney involvement were ideal controls, whereas it's difficult to recruit these patients due to the low incidence of MPA and a 90% rate of kidney involvement in MPA. To date, there is no report of increased intestinal *Actinomyces* in CKD, whereas elevated *Streptococcus* have been found in IgA nephropathy(2). To our knowledge, there is no reported study yet on the changes of the intestinal microbiota in MPA patients with or

without kidney involvement.
Changes in the text: We modified our text (see Page 18, line 352-355 and Page 18-19, line 360-367)

6. Was the correlation between genera of bacteria and clinical parameters analyzed in all subjects, in MPA patients, or active MPA patients?
Reply 6: The correlation was analyzed in all subjects of MPA patients.
Changes in the text: We modified our text (see Page 9, line 155).

Reviewer A advised us to re-analyze excluding factors other than MPA such as CKD. However, because of the varying manifestation of kidney involvement in patients with MPA as we mentioned in Reply 5, we may not re-analyze the data excluding CKD patients.

Reviewer B

This is a well-designed study investigating the gut microbiome in microscopic polyangiitis and renal disease. The methodology and design of the study is appropriate for the conclusion. I have only minor suggestions:
1. To better understand the cohort, a STROBE flow chart should be included.
Reply 1: We have fulfilled a STROBE flow chart as advised.
Changes in the text: see Reporting Checklist.

2. Additionally, a full table of the patient cohort with regard of clinical, laboratory and histopathological findings should be included.
Reply 2: We have fulfilled the table as advised.
Changes in the text: see in Supplementary Appendix, Table S1.

3. Finally, a table with information of co-medication (including antibiotics) should be included. This is of relevance since multiple drugs are known to alter gut microbiome.
Reply 3: The information of co-medicine was summarized in Table 1. Those who were treated with antibiotics or had been treated with antibiotics within one month before enrollment and at sampling were excluded in this study.
Changes in the text: We added a new table showing co-medication of every participants (see in Supplementary Appendix, Table S1).