Reviewer’s report

Title: Characterization of gut microbiota in children with pulmonary tuberculosis

Version: 1 Date: 13 Aug 2019

Reviewer: Saurabh Mehta

Reviewer's report:

Summary:

This research used a case-control study design to explore composition of the gut microbiota in n=18 pulmonary tuberculosis pediatric patients and n=18 healthy controls. Patients were recruited from a hospital in China between June 2016-17. Next generation sequencing to amplify the 16S rRNA gene. No differences in species richness, but diversity as measured by the Simpson Index was lower in PTB patients. There was no difference in community structure (weighted UniFrac) between groups. Relative abundance comparisons showed differences in bacterial proportions at the family, genus and species level. Notably, species often associated with pathogens were enriched in the PTB group compared to healthy controls, while commensals previously associated with nutrient metabolism and other beneficial functions were at higher proportions in healthy controls.

Strengths:

* First study to analyze the gut microbiome in pediatric PTB patients.
* Used NGS to analyze gut microbiota.
* Thorough review of previous literature in the discussion section.

Concerns - in the order as they appear in the manuscript:

Overall, it will be helpful to include the STROBE checklist for case control studies to help improve the manuscript as well as to facilitate review.

Abstract:

* Please include the age range of the participants in your abstract text or reference the participants as "pediatric patients with PTB" instead of only "PTB patients."
* Please note misspelled F. prausnitzii (pg 3, line 14). Also, please use proper scientific nomenclature for referencing bacteria at the genus (and species) level (see further comments).

Background:

* Please use proper scientific nomenclature when referencing bacteria (pg 4, line 1): Mycobacterium tuberculosis (genus is capitalized).

* Please consider including a reference for your statement, "…pediatric populations who are deemed as a high-risk group" (pg 5, line 10-11)

Methods:

* Please use proper scientific nomenclature when referencing bacteria (pg 6, line 4): Mycobacterium tuberculosis (genus is capitalized).

* Please use the proper format for referencing the Bacillus Calmette-Guérin vaccine (capitalize, use hyphen).

* Please include the corresponding hypervariable region in the 16S rRNA that is isolated using the primers you specified (338F, 806R).

* In your abstract you specified that the Illumina HiSeq platform was used. In the methods in the paper this is referenced as Illumina MiSeq. Please check which instrument was used.

* Please include which version of QIIME you used. Also, please cite this using the correct reference paper (see the QIIME website for how to cite their materials)
  o QIIME 1: http://qiime.org/1.4.0/
  o QIIME 2: https://docs.qiime2.org/2019.7/citation/

* Please note which version of the Greengenes reference database was used.

* Please fix misspellings:
  o "microbota" (pg 4, line 22) → microbiota
  o "Bata" (pg 8, line 1) (pg 7, line 1,7) → beta
Results

* Please correct "weight Unifrac" (it is called "weighted UniFrac") and cite accordingly. (pg 8, line 2) (pg 9, line 15). This is correctly referenced in your figure legend 1.

* Page 11, line 8 - why is "aerosolized Mycobacterium tuberculosis" capitalized?

* Consider also presenting the relative abundance of phyla in each group, as this measure is commonly reported in microbiome papers and would enable comparison with other research.

* The paper mentions the Shannon Index but did not report associations with the Shannon Index, only Simpson index. Please consider at least mentioning that no associations with the Shannon Index were observed if that is the case.

Discussion

* Page 11, line 15: Please consider elaborating on the differences between Luo et al's study and yours (e.g., age, sex ratio, etc).

* Please correct misspelling - pg 12, line 5, "Prevolla"

* Please use proper scientific nomenclature when referencing bacteria (pg 13, line 19) - species names are not capitalized. Consider rephrasing (note underlined edits as well) to: "In the present study, F. ruminococcaceae and F. prausnitzii were significantly lower in the PTB group compared to healthy controls. Previous studies have described that F. ruminococcaceae and F. prausnitzii may exert…"

* Please note, Bifidobacteriaceae is not a phylum, it is a family (pg 14, line 12-13).

* Please consider discussing that many pathogens are strain-specific. For example, though Enterococcus was increased in PTB patients, many harmless commensal bacterial species/strains are contained in this genus (namely, E. faecalis and E. faecium) and resolution to the species or strain level presents a future direction or limitation of the study.

* You build a case for Prevotella as a pro-inflammatory, immune-regulating genus of bacteria, which is accurate, but species of Prevotella have also been found in populations consuming more carbohydrates, namely fiber. Do you have data on the dietary intakes of the cases and controls? This may also be contributing to the differences observed.

* Please consider giving examples of microbiota interventions in your conclusion (pg 15, line 16).
Figure legends

Conclusions:

Overall, this study requires major revisions. From a patient population perspective, the criteria used to determine active TB disease are somewhat different from international guidelines and some of the included patients may be more appropriately categorized as probable TB rather than suspected TB. Do you have a breakdown of how many participants were included based on criteria A alone versus others?

On an analysis level, some additional ideas: Are there other clinical characteristics of the participants that you may wish to analyze with parameters of the gut microbiota? (For example, signs or self-reported symptoms, etc.). Though sample size may present a challenge, it would be interesting to determine if there are any differences in gut microbiome metrics by sex, age/age group, or weight within cases and controls, separately. Is there information on the duration or specific treatment that PTB cases were given (or if no treatment given then please state)?

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics
Quality of written English
Please indicate the quality of language in the manuscript:

Not suitable for publication unless extensively edited

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