The aim of this work is to analyze the miRNA pattern in patients with cavitatory and non-cavitatory pulmonary tuberculosis, in order to find a biomarker that allows early and differential diagnosis of both modalities of the disease. I have several observations and questions about the project.

As mentioned in the last paragraph on page 5 of the manuscript, several miRNAs are already known that can serve as diagnostic markers in tuberculosis. Thus, the originality of this project would be in finding a pattern of miRNAs that would allow early distinction between cavitatory and non-cavitatory tuberculosis. However, I have two questions about this issue.

1. First: are 20 patients per group (cavitatory and non-cavitatory tuberculosis) sufficient to test the project hypothesis? On page 7 of the manuscript it is mentioned that no data from similar previously published work could be considered in calculating the sample size. So how was it established that each patient group should be 20 individuals, and that the control group should be 13 individuals?

   Response: Thank you very much for the query raised.

   This is a proof-of-concept study, so only a convenient sample has been chosen. miRNA will be altered in all the TB infected patients. So, the expected percentage of miRNA positivity among the cases is 100%. This sample size is calculated based on the expected percentage of miRNA positivity among the patients affected with Tuberculosis is 100%. Studies of microRNAs in tuberculosis are available. But it has not been associated with the type and extent of lesions in the lungs. The existing diagnostic tools have sensitivity and specificity upto 90%. The microRNA profiles could be better than the existing diagnostic tests, it has the potential to offer more than 90% sensitivity as well as specificity. However, no reproducible data is available in published literature on the difference in proportion of upregulation between cases and non-cases, as it is highly variable across published studies. So, computing sample size based on this might not be feasible.
   When it comes to sample size, there are studies saying minimum of 30 could be the adequate sample size. Some studies say 20 is adequate or 10 could be adequate; and there is no adequate information with regard to this sample size. However, the most important anticipated drawback could be bias. But the chances for bias in this study could be less since we are trying to identify profiles (set of upregulated and down regulated) of microRNAs specific to cavitatory and non-cavitatory tuberculosis. Moreover, we have stringent inclusion and exclusion for inclusion of study participants.
   We have considered geographical proximity, availability at a given time, or availability of financial support. Probably this study could facilitate us in conducting an in-depth study in future.

2. Second: What is the prognostic and/or treatment utility of finding a miRNA profile exclusive to each type of tuberculosis (cavitatory and non-cavitatory)? This issue should be discussed in the manuscript to further justify undertaking the project.

   Response: Thank you very much for the query raised.

   TB is ranked the second leading cause of mortality among all the infectious diseases. This could be due to inadequacy in performance of the existing biomarkers to differentiate the varied presentations of pulmonary tuberculosis. Therefore, circulating microRNA could be a promising diagnostic tool which shall address the different aspects of the disease.

It has been well established that the cavitatory tuberculosis have higher prevalence rates of multi drug resistant TB, higher relapse rates and more complications in the long run. Hence by identifying the microRNA specific for the group would help in prognosticating the patients.

3. Other observations are: The title does not reflect the content of the manuscript, as it does not refer to cavitatory and non-cavitatory tuberculosis.

   Response: Thank you very much for the query raised.

   We would to change the title as

   "Diagnostic utility of microRNA profiles in cavitatory and non-cavitatory Pulmonary Tuberculosis- Research Protocol"

4. Authors should define what Xpert MTB/RIF means the first time they mention it.

   Response: Thank you very much for the query raised.

   The Xpert MTB/RIF assay is a test that simultaneously detects *Mycobacterium tuberculosis* complex (MTBC) and resistance to rifampin (RIF), one of the most effective drugs used to treat tuberculosis (TB).

5. In the section of Ethic Statement is mentioned that: "The study is proposed to start by July 2022 and complete by June 2024". However, both dates have already passed.

   Response: Thank you very much for the query raised.

   The policies have changed in India. Most of the tuberculosis patients are being managed by the smaller government district hospitals. Hence, the number of TB patients approaching tertiary care hospitals like our institution is little lesser compared to scenario which existed few years back. Hence, the time taken to include participants also takes longer. That's why there is a discrepancy in the duration of the study being mentioned.

6. Why will interleukin-6 and matrix metalloproteinase-1 be analyzed in the subjects included in the study? The authors should explain this issue.

   Response: Thank you very much for the query raised.

   There is a chronic inflammatory state associated with tuberculosis which could be reflected in analysis of IL-6. One of the enzymes involved in cavity formation is matrix metalloproteinase-1, measurement of which could help in identifying early cases of cavitatory TB. MMP-1 levels can be associated with microRNA profiles.

7. The fourth primary objective is: "To compare the clinical course of the disease at the end of three months and six months with the baseline miRNA patterns in respective sub-groups of pulmonary tuberculosis". Will patients receive treatment during that time period? Would that treatment be the same for the two subgroups of patients?

   Response: Thank you very much for the query raised.

   Patients will receive treatment during the period. The treatment will be as per the standard of care decided by the treating physician. The microRNA results will not influence the treatment decisions. Probably this could give extra information for targeted treatment in future.

Round 2

Dear reviewers

Thank you for critically reviewing our manuscript again and giving valuable inputs and suggestions.

We have taken utmost care in handling the queries raised in the manuscript and have given our responses to them.

1 **There are some specific comments to be modified in the second-round review. Please revise the manuscript according to its comments and make a point-to-point response to the review comments. Note that it is not my opinion, but the reviewer's opinion. Please see the attachment for the reviewer's opinion.**

The authors satisfactorily responded one by one to the comments I made on the original manuscript. Below I only point out some minor errors contained in the text: The period on line 5 of the "methods" section in the abstract should be removed. In some places, such as the core tip, M. tuberculosis is not written in italics. In some places in the manuscript "tuberculosis" is abbreviated as TB, but in other places it is not. In the "pulmonary tuberculosis" section of the introduction, Mycobacterium tuberculosis is abbreviated as Mtb, but this abbreviation is no longer used in the rest of the text. In some parts of the text "miRNA" is used and in others "microRNA" is used.

- The period on line 5 of the "methods" section in the abstract should be removed.

Response: Thank you very much for the query raised.

The fifth line in the methods section of the abstract has been removed. It is replaced with "The participants will undergo various laboratory and radiological investigations at the time of recruitment into the study."

- In some places, such as the core tip, M. tuberculosis is not written in italics.

Response: Thank you very much for the query raised.

M. tuberculosis has been rewritten in italics.

- In some places in the manuscript "tuberculosis" is abbreviated as TB, but in other places it is not.

Response: Thank you very much for the query raised.

In all the places in the manuscript, tuberculosis is uniformly mentioned as TB.

- In the "pulmonary tuberculosis" section of the introduction, Mycobacterium tuberculosis is abbreviated as Mtb, but this abbreviation is no longer used in the rest of the text.

Response: Thank you very much for the query raised.

Mtb has been changed to *Mycobacterium tuberculosis.*

- In some parts of the text "miRNA" is used and in others "microRNA" is used.

Response: Thank you very much for the query raised.

miRNA has been changed to microRNA in all the places.

2 **Audio core tip:** Please offer the audio core tip, the requirement are as follows: In order to attract readers to read your full-text article, we request that the first author make an audio file describing your final core tip. This audio file will be published online, along with your article. Please submit audio files according to the following specifications: Acceptable file formats: .mp3, .wav, or .aiff Maximum file size: 10 MB

3 Biostatistics Review Certificate: Please upload the PDF version of a statement affirming that the statistical review of the study was performed by a biomedical statistician to the system.

Response: Thank you very much for the query raised.

The sample collection is being done, but we have not completed the analysis. Hence, applying Biostatistics is not required at this stage of the study. Biostatistics will be used to obtain the data later. Therefore, the Biostatistics statement does not apply to this manuscript.

4 Clinical trial registration statement: Any research study (clinical trial) that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes must be registered. Authors have 6 mo from the first patient enrollment to register the trial, but BPG recommends registration prior to enrollment. This registration policy applies to prospective, randomized, controlled trials only. Authors must provide the registration identification number and the URL for the trial's registry. In addition, the registration information must be provided in a PDF format, and the registered URL and registration identification number must also be mentioned as a footnote in the manuscript text.

5 Conflict-of-interest statement: Please complete the Conflict of Interest (PDF), fill it in, and then upload the completed PDF version to the system.
Note: The Corresponding Author is responsible for filling out a Conflict-of-Interest Form.

https://www.f6publishing.com/Forms/Manuscript/public/checkcopyright.aspx?msid=C39D7D6331FF97F9&aid=C5AB10580DADDA28

6 Please click on the link to sign the copyright agreement.

https://www.f6publishing.com/Forms/Manuscript/public/checkcopyright.aspx?msid=C39D7D6331FF97F9&aid=5F7E9279EFE53B90

https://www.f6publishing.com/Forms/Manuscript/public/checkcopyright.aspx?msid=C39D7D6331FF97F9&aid=0FA8E3F2A931D7B5

7 Informed consent statement: Please provide the primary version (PDF) of the Informed Consent Form that has been signed by all subjects and investigators of the study, prepared in the official language of the authors' country to the system; for example, authors from China should upload the Chinese version of the document, authors from Italy should upload the Italian version of the document, authors from Germany should upload the Deutsch version of the document, and authors from the United States and the United Kingdom should upload the English version of the document, etc.

8We are very pleased to receive your revised manuscript (No. 97460). However, after our verification, we found that there are still major language problems in the revised manuscript you submitted. The manuscript will require further language polishing, to fix all grammatical, syntactical, formatting and other related errors, in order to meet the publication requirement (Grade A).

Now, you are requested to send the revised manuscript to a professional English language editing company or a native English-speaking expert to polish the language further. When you submit the subsequent polished manuscript to us, you must provide a language certificate along with it. Once this step is completed, your manuscript will be quickly accepted and published online. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240