Author's response to reviews

Title: Is Fecal Calprotectin an Accurate Marker of Inflammation in Cystic Fibrosis?

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Author's response to reviews: see over
Dear Editors,

Thank you for considering our manuscript for your journal. We have made some revisions to the manuscript according to the reviewers’ comments. Please find below a point-by-point response to the concerns.

First Reviewer: Andrew S Day

Reviewer’s report:

This manuscript by Rumman and colleagues examines levels of faecal calprotectin (previously known as CF-related antigen) in individuals with CF.

There is increasing interest in the role that the CFTR may play in other organs, and the impact that gut inflammation may play in CF outcomes.

Specific Comments:

1. This work does not include age matched controls
   Lack of a control group is a limitation that we mentioned in the manuscript. The aim of the study was to look for an association between fecal calprotectin level and several CF characteristics. Therefore having a control group was not included. We do believe however that future studies need to have a larger cohort and a control group.

2. The researchers included patients with CF and another group with CRMS. The combination of these two groups needs better justification. Does one expect the same events to occur in the two groups? How do changes in CRMS relate to that of CF directly?
   We obtained the stool specimens from all patients attending the CF clinic at Children’s Hospital who agreed to participate regardless of pancreatic status or type of CF. Then we looked back for any associations. Therefore we did not exclude those with CRMS in order to see whether their levels would be any different from those with typical CF. Interestingly their levels were comparable to those with typical CF (both PI and PS) and this supports the idea that intestinal inflammation may be associated with the basic defect of CFTR, and the concept of CF enteropathy. However, given the small number of patients (only 7 with CRMS), it is difficult to draw conclusions from this observation.

3. There are a number of errors of grammar and English
4. Were concurrent enteric infections excluded in the subjects?
No, these were not excluded. Evaluation for small intestinal bacterial overgrowth was beyond the scope of this study.

5. Were other factors that can elevate calprotectin considered? (e.g. steroid use, NSAID exposure, IBD etc)
None of the CF patients was on steroids, NSAIDS, or has IBD.

6. The authors refer to "CF enteropathy", which implies small intestinal dysfunction. Is there any support for this?
There is extensive literature supporting the concept of CF enteropathy.
Examples:

7. Subsections within the Methods and Results sections would enhance clarity
The Methods and Results were revised.

8. The methods notes that three subjects were excluded because of incomplete data. Subsequently we learn that only some of the subjects had pulmonary
function testing data - why were those without lung function tests also excluded??

They were not excluded but in our clinic PFTS are not routinely performed in children less than 6 years of age. Those patients have never performed PFTs because they were young (less than 6 years).

9. Similarly, lung function testing is able to be completed from infancy - it is not clear why some patients did not have this assessment completed routinely

It is not a routine at our CF center to perform infant PFTs to all CF infants. We usually start PFT at the age of 5-6 years.

10. Although the median age was 8 years, the study included adults as well as children. The authors should present also the assessment of the children separate to the adults, as other bias could influence variation with age.

We do not think it is necessary to separate the statistics based on age. We already used a lower cut-off value of fecal calprotectin (normal being less than 50 mcg/gm) which makes it more sensitive to detect any associations for both adults and children.

11. The Discussion is too long, and should be revised to enhance flow and clarity.

The discussion has been shortened.

12. The Tables need extensive revision and reconsideration. The Legends/Titles are inadequate. table 3 is excessively long. Table is unhelpful and should be deleted.

The legends were reviewed.
Table 3 could be deleted or it could be provided as a supplement material.

13. Referencing is incomplete in some places (with incorrect placement of references (e.g. at the bottom of page 5)

Placement of references was adjusted.
Minor Comments:
1. One assumes that the ELISA was completed according to the instructions of the manufacturer? This should be stated.
   This was added to the manuscript.

2. Some sentences begin with numerals - these should be converted to letters.
   Numerals were converted to letters at the beginning of sentences.

Second Reviewer: George Vaos

Reviewer's report:

This manuscript raises the following comments: #

Minor essential revisions Headings of the tables are not presented properly.

Legends of the tables were reviewed.

Major compulsory revisions.

1. The title of the manuscript should be more specific for the question (Is fecal calprotectin an accurate inflammatory marker in cystic fibrosis?) posed.
   The title was changed.

2. ABSTRACT (conclusion). .... as it does in other inflammatory conditions of the intestines, to be omitted. This phrase is not supported by the data.
   The sentence was omitted.

3. The method is not quite appropriate as only one stool specimen (limitation) was used. Furthermore, the number of patients studied for each patient’s characteristics was relatively small.
   We mentioned these points as some of the limitations in our study. It
was very difficult to obtain more than one stool sample in order to follow the trends of fecal calprotectin. In addition the study was not designed to include an intervention and repeat measurements after this intervention. It was only looking for any associations between FC levels and CF characteristics.

4. Since fecal calprotectin concentration values have been reported to be different between children and adults, statistical analysis of data for both children and adults may lead to unreliable results. Besides, using two cut-off values to interpret the results may be not quite correct. The higher cut off value was omitted from the whole manuscript to avoid confusion. The study did not aim to look for differences between children and adult CF patients, therefore we used the lower cut-off value to obtain more reliable results.

5. A shortened Discussion section would be sufficient for this manuscript. Paragraph 4, line 6: “Canani showed……not included in that study.” and Paragraph 5. and 12 do not add to the manuscript and can be deleted

The paragraphs indicated above were deleted.

6. Conclusion section, line 1: “There is evidence……adequate PERT”, to be omitted as it is not adequately supported by the data.

There is considerable literature indicating that intestinal inflammation is part of the disease process in CF:


7. The sentence : Some of the limitations in our study include the …… of a control group belongs to the Discussion section.

Moved to discussion section
8. TABLE 1. Fecal calprotectin concentration values are lacking. Age at sample collection and at diagnosis should be in years(?)

These were corrected.

Thank you,

Nisreen Rumman, MD