PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Fecal calprotectin and rectal histologic inflammatory markers in cystic fibrosis - single centre study
AUTHORS	Roda, Juliana Maia, Carla Almeida, Susana Oliveira, Rui Caetano Ferreira, Ricardo Oliveira, Guiomar

VERSION 1 – REVIEW

REVIEWER	Reviewer name: Dr. Conrad Kabali Institution and Country: Spence Lane Burlington Ontario, Canada Competing interests: None
REVIEW RETURNED	23-Jan-2022

GENERAL COMMENTS	Page 4, line 54: Since you suggest that fecal calprotectin can be a good marker for rectal inflammation, I would advice that you report sensitivity and specificity so that readers can get a sense of its accuracy Page 9, lines 19-30: Please revise your stat section to reflect that you will be reporting the sensitivity and specificity of calprotectin as a marker for rectal inflammation Page 10, line 37: This looks more like a weak negative correlation. Large p-value may simply imply large sampling variability due to a small sample Tables 2 & 3: For clarity consider replacing "p" with "p-value"

REVIEWER	Reviewer name: Dr. Iram Haq Institution and Country: Great North Children's Hospital Paediatric Respiratory Medicine, United Kingdom of Great Britain and Northern Ireland Competing interests: None
REVIEW RETURNED	01-Mar-2022

GENERAL COMMENTS	This is interesting research, particularly as the authors have additionally investigated rectal biopsy material, which has previously been understudied. The authors recognise that a limitation of this work is the relatively small sample size and that the clinical meaning of the study findings remain to be explained. Nevertheless the findings are of interest. There are a few minor comments and suggestions: There is a useful and very recent review discussing gut inflammation in CF, that the authors have not referenced by Tam et al, J Clin Med 2022 11(3), 649; https://doi.org/10.3390/jcm11030649 and given its recency and relevance it is suggested that this reference is included.

	This review has discussed several studies which have investigated calprotectin levels in CF, and highlighted that one of the challenges relates to the cut-off values for elevated measurements (ranging from 50 µg/g to 250 µg/g) and therefore requires careful interpretation. It would be helpful for the authors to acknowledge this in their discussion and to discuss how their findings compare to other previous studies. For example, there is a similar recent paediatric study (Enaud et al, J Clin Med 2019 doi 10.3390/jcm8050645) which has investigated intestinal inflammation in children with CF including the microbiota of these children and should be referenced in this work. Aligned to this, are the authors able to include information about antibiotic use in their cohort of children and if there were any differences in those with greater levels of inflammation on biopsy or elevated calprotectin. Is has been suggested that older age may be related to gut inflammation. Are the authors able to perform any subgroup analyses relating to whether older children in their cohort had higher faecal calprotectin levels? One of the potential clinical relevance of intestinal inflammation is the development of GI cancers, which remains relatively poorly understood. This could also be discussed by the authors in their discussion. There has been some research to suggest that CFTR modulators may reduce intestinal inflammation (Safe, M et al Ann. Am. Thorac. Soc. 2016, 13, 297–298). Do the authors have data regarding modulator treatment for this cohort?

REVIEWER	Reviewer name: Dr. Praveen Unki Institution and Country: Adichunchanagiri Institute of Medical, Mandya Karnataka Competing interests: None
REVIEW RETURNED	28-Feb-2022

GENERAL COMMENTS	To the authors, I thank all the authors for their effort and valuable work. Overall instructions related to the structure of article well followed. Work is unique without plagiarism. Following minor changes have been recommended before considering for acceptance and publication. 1) Keywords mentioned while submitting to BMJ paediatrics open site are different from those submitted in abstract. Kindly update and correct the same. 2) Statistical method used in the study has not been mentioned in abstract (in methods). 3) In methods part of manuscript, kindly mention reference for such classification "semi quantitative approach: none, mild, moderate and severe". 4) Correct spelling mistake (significant instead sgnificant) in page 13 of 20 (page number 12 in manuscript) line 8. 5) Reference 18 is incomplete.

VERSION 1 – AUTHOR RESPONSE

15-Mar-2022

bmjpo-2022-001422 - "Fecal calprotectin and rectal histologic inflammatory markers in cystic fibrosis"

Dear Dr. Malcolm Brodlie and Prof. Imti Choonara,

The authors appreciated the constructive and concise comments that will definetly add value to this paper.
Responses to comments are described in full here and in the attached file. Changes in the main document were made and highlighted.

Kind regards,
Juliana Roda

Editor in Chief Comments to Author:
1. Figure file format. Response: figure was changed and is now supplied as JPG file.
2. Title add "a single centre study" – Response: Title has been updated to include the recommended sentence.
3. Be cautious in your conclusions - your numbers are small – Response: limitations have been described including the small sample size. Adjectives like “important” have been removed from the sentence “Fecal calprotectin seems to be an important nonivasive biomarker” and replaced by “Fecal calprotectin may be considered a noninvasive biomarker of intestinal inflammation in CF patients
4. Discussion page 13, lines 36-39 Delete the sentence "However, to our knowledge, this is the first study to specifically look for histologic evidence of inflammation in CF patients and try to associate it to fecal calprotectin" Journal policy is for authors to avoid describing their study as the first. Response: sentence was replaced by “In this study we were specifically looking for histologic evidence of inflammation in CF patients and try to associate it to fecal calprotectin.”
5. What this study adds 2nd statement delete " a good" and replace with "an". Response: replacement has been made.

Associate Editor, Comments to the Author
- P10, L41: should this be median rather than mean (an IQR is quoted)? Response: yes, it is median, this was corrected.
- P11, L40: can you really conclude that the increased inflammation is due to the pancreatic insufficiency directly, many other factors correlate with pancreatic status too, including sweat Cl- concentration and level of CFTR function associated with a particular CFTR genotype. Response: The authors agree with the argument that was not accurately described. We included the following sentence in page 12: “Also, the pancreatic status is related to CFTR function (minimal or residual) that is determined by CFTR genotype and intestinal inflammation may be another manifestation of the multisystemic involvement of the disease and not only influenced by pancreatic function [22]”.

- was neutrophilic inflammation seen in the histology, especially since this is characteristic of CF lung disease and calprotectin is a neutrophil product? Response: Neutrophilic inflammation was not found in histology, we only found small lymphocyte and plasma cell infiltrates.

Reviewer: 1
Dr. Conrad Kabali
Comments to the Author
Page 4, line 54: Since you suggest that fecal calprotectin can be a good marker for rectal inflammation, I would advice that you report sensitivity and specificity so that readers can get a sense of its accuracy. Response: Thank you for this suggestion, we calculated sensitivity of 100% and

specificity of 86%.
Page 9, lines 19-30: Please revise your stat section to reflect that you will be reporting the sensitivity and specificity of calprotectin as a marker for rectal inflammation. Response: we included the sentence “Sensitivity and specificity of calprotectin as a marker of rectal inflammation was calculated.” in the statistical analyses of the methods.

Page 10, line 37: This looks more like a weak negative correlation. Large p-value may simply imply large sampling variability due to the small sample and interesting finding. Response: The authors agree this correlation can be interpreted as a weak negative correlation instead of no correlation because of the small sample size. This was also added to the discussion as a tendency towards lower values of calprotectin with increasing age in healthy children and adolescents has been discussed in this recent position paper: Koninckx CR, Donat E, Benninga MA, et al. The Use of Fecal Calprotectin Testing in Paediatric Disorders: A Position Paper of the European Society for Paediatric Gastroenterology and Nutrition Gastroenterology Committee. J Pediatr Gastroenterol Nutr. 2021;72(4):617-640. doi:10.1097/MPG.0000000000003046.
Tables 2 & 3: For clarity consider replacing “p” with “p-value”. Response: this was changed as suggested.

Reviewer: 2
Dr. Praveen Unki, Adichunchanagiri Institute of Medical Sciences

1) Keywords mentioned while submitting to BMJ paediatrics open site are different from those submitted in abstract. Kindly update and correct the same. Response: keywords were updated in the manuscript.
2) Statistical method used in the study has not been mentioned in abstract (in methods). Response: The following sentence was added to the methods section in the abstract. “Statistical analyses included Spearman´s correlation coefficient, Mann-Whitney and Exact Fisher tests. “

3) In methods part of manuscript, kindly mention reference for such classification “semi quantitative approach: none, mild, moderate and severe”. Response: Because there is no defined standard histological classification score in this case, the evaluation took into consideration the density of mononucleated inflammatory population on a semi quantitative approach, based on the pathologist experience.
4) Correct spelling mistake (significant instead sgnificant) in page 13 of 20 (page number 12 in manuscript) line 8. Response: spelling was corrected.
5) Reference 18 is incomplete. Response: This reference was replaced.

Reviewer: 3
Dr. Iram Haq, Great North Children's Hospital, Newcastle University

There is a useful and very recent review discussing gut inflammation in CF, that the authors have not referenced by Tam et al, J Clin Med 2022 11(3), 649; https://doi.org/10.3390/jcm11030649 and given its recency and relevance it is suggested that this reference is included. Response: The authors agree this is a very interesting and recent review and appreciated the comment. Changes in discussion have been made to include this reference.

This review has discussed several studies which have investigated calprotectin levels in CF, and highlighted that one of the challenges relates to the cut-off values for elevated measurements (ranging from 50 µg/g to 250 µg/g) and therefore requires careful interpretation. It would be helpful for the authors to acknowledge this in their discussion and to discuss how their findings compare to other

previous studies. Response: these suggestions have been included in the discussion and reference added.

For example, there is a similar recent paediatric study (Enaud et al, J Clin Med 2019 doi 10.3390/jcm8050645) which has investigated intestinal inflammation in children with CF including the microbiota of these children and should be referenced in this work. [19, 20]. Response: this was included in the discussion “One trial with probiotics supported this hypothesis, as the use of Lactobacillus rhamnosus GG reduced calprotectin concentrations in CF children [19]. Another study, found increased abundances of Staphylococcus, Streptococcus,and Veillonella dispar, along with decreased abundances of Bacteroides, Bifidobacterium adolescentis, and Faecalibacterium prausnitzii to be associated to intestinal inflammation in PwCF in similarity to changes found in patients with Crohn´s disease [20]. “ and reference was added.

Aligned to this, are the authors able to include information about antibiotic use in their cohort of children and if there were any differences in those with greater levels of inflammation on biopsy or elevated calprotectin. Response: Patients did not take antibiotics on the 4 weeks previously to the fecal sample collection and rectal biopsy and this was included in exclusion critera.

Is has been suggested that older age may be related to gut inflammation. Are the authors able to perform any subgroup analyses relating to whether older children in their cohort had higher faecal calprotectin levels? Response: As suggested by another reviewer we could find a weak negative correlation between fecal calprotectin and age and this is in line with what is discussed in this recent position paper (doi:10.1097/MPG.0000000000003046) where it is discussed a tendency towards lower values of calprotectin with increasing age in healthy children and adolescents.
We also tried to find a difference comparing age subgroups and no difference was found.

One of the potential clinical relevance of intestinal inflammation is the development of GI cancers, which remains relatively poorly understood. This could also be discussed by the authors in their discussion. “Response: However, as the life expectancy of PwCF is increasing there has been reported an increased risk of gastrointestinal malignancies. Chronic intestinal inflammation is a risk factor for cancer development and this should probably be addressed early in life[24]”.

There has been some research to suggest that CFTR modulators may reduce intestinal inflammation (Safe, M et al Ann. Am. Thorac. Soc. 2016, 13, 297–298). Do the authors have data regarding modulator treatment for this cohort? Response: Only two patients were taking CFTR modulators at the time of the study, but we have no previous calprotectin measurements in order to compare and conclude if there was a benefit.

VERSION 2 – REVIEW

REVIEWER	Reviewer name: Dr. Iram Haq Institution and Country: Great North Children's Hospital Paediatric Respiratory Medicine, United Kingdom of Great Britain and Northern Ireland Competing interests:
REVIEW RETURNED	29-Mar-2022

GENERAL COMMENTS	The authors have addressed the reviewer and editorial comments.

REVIEWER	Reviewer name: Institution and Country: Competing interests:
REVIEW RETURNED	

GENERAL COMMENTS	

REVIEWER	Reviewer name: Institution and Country: Competing interests:
REVIEW RETURNED	

GENERAL COMMENTS	

VERSION 2 – AUTHOR RESPONSE

VERSION 3 – REVIEW

REVIEWER	Reviewer name: Institution and Country: Competing interests:
REVIEW RETURNED	

GENERAL COMMENTS	

REVIEWER	Reviewer name: Institution and Country: Competing interests:
REVIEW RETURNED	

GENERAL COMMENTS	

REVIEWER	Reviewer name: Institution and Country: Competing interests:
REVIEW RETURNED	

GENERAL COMMENTS	

VERSION 3 – AUTHOR RESPONSE