BMJ Paediatrics Open

Faecal calprotectin and rectal histological inflammatory markers in cystic fibrosis: a single-centre study

Juliana Roda ,[1,2] Carla Maia,[1] Susana Almeida,[1] Rui Caetano Oliveira,[3,4] Ricardo Ferreira,[1] Guiomar Oliveira[2,5]

To cite: Roda J, Maia C, Almeida S, *et al*. Faecal calprotectin and rectal histological inflammatory markers in cystic fibrosis: a single-centre study. *BMJ Paediatrics Open* 2022;**6**:e001422. doi:10.1136/bmjpo-2022-001422

Received 18 January 2022
Accepted 12 April 2022

[1]Pediatric Gastroenterology and Nutrition Unit, Centro Hospitalar e Universitario de Coimbra EPE Hospital Pediátrico de Coimbra, Coimbra, Portugal
[2]Clínica Universitária de Pediatria, Universidade de Coimbra Faculdade de Medicina, Coimbra, Portugal
[3]Pathology Department, Centro Hospitalar e Universitário de Coimbra EPE, Coimbra, Portugal
[4]Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment Genetics and Oncobiology (CIMAGO), Universidade de Coimbra Faculdade de Medicina, Coimbra, Portugal
[5]Centro de Desenvolvimento da Criança e Centro de Investigação e Formação Clínica, Centro Hospitalar e Universitario de Coimbra EPE Hospital Pediátrico de Coimbra, Coimbra, Portugal

Correspondence to
Dr Juliana Roda; juroda@hotmail.com

ABSTRACT

Objective To analyse the association of faecal calprotectin with the genetic and clinical characteristics of paediatric patients with cystic fibrosis (PwCF). In a subset of these patients, we aimed to associate histological inflammatory features of rectal mucosa to faecal calprotectin levels.

Methods In a prospective study, faecal calprotectin levels were collected in all 23 PwCF attending our paediatric centre, together with demographic and clinical data. Associations between faecal calprotectin and clinical features were determined. In 11 of these patients, endoscopic rectal biopsies were obtained and the association between faecal calprotectin and histological inflammatory markers was analysed. Statistical analyses included Spearman's correlation coefficient, Mann-Whitney U test and Fisher's exact test. Sensitivity and specificity was calculated.

Results Median age of PwCF was 12 years, 19 had pancreatic insufficiency (PI) (19/23). Seventeen (17/23) had elevated faecal calprotectin, and the median value was 88 µg/g (IQR=178 µg/g). Higher faecal calprotectin levels were observed in the PI group (101 vs 30 µg/g, p=0.027). No significant correlation between elevated faecal calprotectin level and body mass index z-score was found. Five patients (22%) reported abdominal pain, three (13%) complained of diarrhoea and three (13%) had constipation, but these symptoms were not associated with elevated faecal calprotectin.

Unspecific focal rectal inflammation was found in four patients (4/11). An association between rectal mucosa inflammation and elevated faecal calprotectin was found (p=0.015). Sensitivity was 100% and specificity was 86%.

Conclusions In our PwCF, elevated faecal calprotectin was frequent, particularly if PI, and it was not related to gastrointestinal symptoms or malnutrition. Elevated faecal calprotectin was present in patients with histological evidence of rectal inflammation. Faecal calprotectin may be an indicator of asymptomatic rectal inflammation in PwCF.

What is already known on this topic

- There is increasing evidence of intestinal inflammation in cystic fibrosis (CF).
- Elevated faecal calprotectin has been found in patients with CF.

What this study adds

- Elevated faecal calprotectin is frequent, particularly in pancreatic insufficient patients with CF.
- Focal inflammation in rectal biopsies was found in patients with CF and it is associated with elevated faecal calprotectin.

How this study might affect research, practice or policy

- Faecal calprotectin may be used as an indicator of rectal inflammation in CF.

INTRODUCTION

Cystic fibrosis (CF) is a severe autosomal recessive disease that results from mutations in a gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein, a chloride channel.[1] It is the most common life-limiting genetic disease among Caucasians, occurring in 1 of 2500 live births worldwide. In Portugal, a prevalence of 0.27 per 10 000 inhabitants and an incidence of 1 per 7500 newborns have been estimated.[2 3]

CF is also the most common cause of pancreatic insufficiency (PI) in children.[4] Historically, CF children died in infancy from severe malnutrition and later from respiratory failure, but due to improved clinical care, including pancreatic enzyme replacement therapy (PERT), currently, a majority of them reach adulthood.[5] Approximately 85% of patients with CF (PwCF) have impaired digestion due to PI and despite adequate PERT, several PwCF still have malabsorption, growth impairment and gastrointestinal problems, including abdominal pain, steatorrhoea and altered motility.[4 5] It has been suggested that digestive symptoms are attributable to PI and to intestinal inflammation. However, the

pathogenesis and nutritional implications of this finding remains unclear.[5]

Studies, in both animals and humans, have reported evidence of intestinal inflammation in CF. In the mouse model of CF, the most common manifestation is intestinal obstruction resulting from inflammatory enteropathy, leading to perforation.[6] Furthermore, abnormal mucus accumulation in the intestines of murine models predisposes them to gut dysmotility, creating a niche for bacterial overgrowth and dysbiosis.[7]

In humans, the presence of inflammatory biomarkers, including faecal calprotectin, oeosinophil cationic protein, interleukin-1B and interleukin-8, was reported, suggesting that intestinal inflammation is a feature of CF.[5 8] Videocapsule endoscopic studies elicited mucosal ulceration, erythema and mucosal breaks in the small bowel of PwCF, particularly those with PI.[4] Calprotectin is a neutrophil secretory product, and elevated faecal levels are well correlated with colonic inflammation in inflammatory bowel disease (IBD).[9–11] Dysbiosis may be associated with intestinal inflammation as reflected by increased levels of faecal calprotectin that respond to antibiotic treatment.[12] CFTR modulators may potentially improve dysbiosis and inflammation, for example, ivacaftor has been associated with a decrease in calprotectin levels.[13]

However, to our knowledge, there are no studies characterising intestinal histological inflammatory findings in PwCF and, particularly, studies analysing the relationship between elevated faecal calprotectin and these histological inflammatory findings.

The aim of the present study was to analyse, in a cohort of paediatric PwCF, the association between faecal calprotectin levels and genetic and clinical characteristics, including nutritional status and gastrointestinal symptoms. In a subset of this cohort, we also aimed to characterise histological inflammatory features of rectal mucosa and relate it to faecal calprotectin levels.

METHODS

This prospective study included children and adolescents aged 0–18 years followed in the Pediatric Unit of the Cystic Fibrosis Reference Centre of the Centro Hospitalar e Universitário de Coimbra, Portugal, in the year 2019. The criteria for the diagnosis of CF were: clinical characteristics compatible with CF, a positive sweat test and a genetic study with the identification of two disease-causing mutations, according to the latest consensus.[14] All patients willing to participate were included. Exclusion criteria included recent respiratory exacerbation/infection or ingestion of antibiotics, steroids or non-steroidal anti-inflammatory drugs, both in the previous 4 weeks.

In all participants, demographic data, genotype and clinical data, including nutritional status and PERT medication, were collected at the time of the appointment where stool sample was obtained. Nutritional status was assessed according to the z-score of weight, height and body mass index (BMI). Patients were asked whether they had experienced gastrointestinal symptoms (abdominal pain, constipation or diarrhoea) in the 2 weeks preceding the calprotectin measurement.

Exocrine pancreatic function was assessed using faecal elastase levels. PI was considered when the faecal elastase level was under 200 µg/g; above that, they were classified as pancreatic sufficient (PS).

Faecal calprotectin measurement

Stool samples were collected at home or at the CF Reference Centre. Calprotectin level in the faecal samples were measured using EliA Calprotectin (reagents from Thermo Fisher Scientific). Faecal calprotectin concentration was considered normal up to 50 µg/g stool, as was considered in previous studies with PwCF[4] and as it has been shown to be sensitive for detecting inflammation in children with IBD.[15]

Rectal biopsies

Rectal biopsies were obtained, from patients already enrolled in another study from our centre, which aimed to test CFTR modulator responses in intestinal organoids from patients with rarer mutations (data not yet published; *UID/MULTI/04046/2019*). As a consequence, most of these patients had less common mutations. Time between faecal calprotectin measurement and rectal biopsies was a maximum of 2 days.

Rectal mucosa specimens (3–4 mm in diameter) were obtained from 11 patients, with or without sedation (depending on individual's will or collaboration) using a colonoscope and colon forceps (Endoflex, diameter 2.8 mm). Samples were immediately stored in formalin.

One pathologist with experience in gastrointestinal pathology from the Pathology Department, Centro Hospitalar e Universitário de Coimbra, Portugal performed the histological analysis. Samples were blinded to the clinical information and were analysed under an optical microscope (Nikon Eclipse 50i). Pictures were taken with a Nikon-Digital Sight DS-Fi1 digital camera.

Since the diagnosis of CF is based mainly on bioelectrical/biochemical analyses, there is no defined standard histological classification score. Therefore, the evaluation took into consideration the density of mononucleated inflammatory population, on a semi-quantitative approach: none, mild, moderate and severe.

Statistical analysis

Statistical analyses were performed with SPSS software (V.19; SPSS, Chicago, Illinois, USA), and a p value <0.05 was considered statistically significant. Descriptive statistics were presented according to the normality of the data distribution using Shapiro-Wilk test. Spearman's correlation coefficient, Mann-Whitney U test and Fisher's exact test were used between patient groups to evaluate correlation, differences and associations, respectively. Sensitivity and specificity of calprotectin as a marker of rectal inflammation was calculated.

Table 1 Genotype, BMI, gastrointestinal symptoms, faecal calprotectin and rectal histology of patients with cystic fibrosis

No.	Genotype	BMI z-score kg/m^2	Pancreatic function	Abdominal pain	Diarrhoea	Constipation	Calprotectin µg/g	Histology
1	F508del/R334W	−0.42	PS	Yes	No	No	19	Normal
2	F508del/R334W	1.18	PS	No	No	No	15	Normal
3	F508del/R334W	0.94	PS	Yes	No	Yes	79	Normal
4	F508del/711+1 G-T	−0.74	PI	No	No	Yes	9	Normal
5	F508del/711+1 G-T	−1.99	PI	No	No	No	19	Normal
6	F508del/2184insA	−4.95	PI	Yes	Yes	No	45	Normal
7	F508del/c.3321dup	−0.22	PI	No	No	No	341	Inflammation
8	F508del/P5L	−0.3	PS	No	No	No	40	Normal
9	F508del/F508del	−2.37	PI	No	No	No	223	Inflammation
10	F508del/F508del	−2.6	PI	No	No	No	62	Inflammation
11	F508del/F508del	−0.3	PI	Yes	No	Yes	63	Inflammation
12	F508del/F508del	−0.64	PI	No	No	No	55	–
13	F508del/F508del	0.3	PI	No	No	No	90	–
14	F508del/F508del	0.1	PI	No	No	No	352	–
15	F508del/F508del	−0.27	PI	No	Yes	No	347	–
16	F508del/F508del	−0.13	PI	No	No	No	116	–
17	F508del/F508del	0.1	PI	No	No	No	101	–
18	F508del/F508del	0.55	PI	Yes	Yes	No	104	–
19	F508del/F508del	1.42	PI	No	No	No	88	–
20	F508del/F508del	−1.16	PI	No	No	No	142	–
21	F508del/F508del	0.03	PI	No	No	No	55	–
22	F508del/F508del	1.1	PI	No	No	No	330	–
23	F508del/F508del	1.2	PI	No	No	No	289	–

BMI, body mass index; PI, pancreatic insufficiency; PS, pancreatic sufficiency.

Patient involvement

Patients were not directly involved in setting the research question, the design or in the implementation of the project.

RESULTS

Faecal calprotectin was measured in all 23 PwCF followed in our centre during 1 year. The median age was 12 years, aged from 2 months to 17 years. Twelve were male. All patients with PI (19/23, 83%) were taking PERT. The F508del mutation was present in all patients in at least one allele. Fifteen (65%) patients were F508del homozygous, and the others carried one of the following mutations: R334W (n=3), 711+1G->T (n=2) and 2184insA (n=1), P5L (n=1) and a novel mutation, c.3321dup (n=1) (table 1). Only two patients were taking CFTR modulator lumacaftor/ivacaftor (patient 15 and patient 20, presenting faecal calprotectin level of 347 and 142 µg/g, respectively). Sweat test of these patients did not improve with modulator drug.

Seventeen of the 23 patients (74%) had an elevated faecal calprotectin measurement, and the median value was 88 µg/g (IQR=178 µg/g). Most patients (16/19) with PI had elevated faecal calprotectin (84%). The PI group had higher faecal calprotectin levels then the PS group (101 µg/g; IQR=234 vs 30 µg/g; IQR=53; p=0.0027).

A moderate positive correlation was found between faecal calprotectin and sweat test values (from the time of diagnosis) (r=0.46; p=0.029). A weak negative correlation was found between age and faecal calprotectin (r=−0.3; p=0.14). Also history of meconium ileus was not associated with elevated calprotectin (table 2).

Comparing the median BMI z-score in patients with normal and elevated faecal calprotectin, no significant difference was found (−0.58 kg/m^2; IQR=2.8 vs 0.1 kg/m^2; IQR=1.08; p=0.09). No significant correlation between faecal calprotectin level and BMI z-score was found (r=0.19; p=0.36).

All 15 patients homozygous for the F508del mutation had elevated faecal calprotectin. These PwCF have a statistically significant higher faecal calprotectin than F508del heterozygous patients (110 µg/g; IQR=217 vs 40 µg/g; IQR=54, p=0.003).

Some PwCF reported digestive symptoms: five patients (22%) reported abdominal pain, three (13%) complained of diarrhoea and three (13%) had constipation (table 1). Gastrointestinal symptoms were not associated with elevated faecal calprotectin (table 2).

Table 2 Association between gastrointestinal symptoms and history of meconium ileus and normal or elevated faecal calprotectin

	Normal calprotectin (n=6)		Elevated calprotectin (n=17)		
N=23	Yes	No	Yes	No	P value*
Abdominal pain	2	4	3	14	0.58
Diarrhoea	0	6	3	14	1
Constipation	2	4	1	16	1
Meconium ileus	1	5	4	13	1

*Fisher's exact test.

Histological features

Rectal biopsies were obtained from 11 of the 23 PwCF. Eight patients had the rarer mutations R334W (n=3), 711+1 G-T (n=2), P5L (n=1), c.3321dup (n=1) and 2184insA (n=1), and three patients were homozygous for the F508del mutation.

Unspecific mild focal inflammation was found in the three F508del homozygous patients, all of which had elevated faecal calprotectin (table 1). Inflammatory features were characterised as small lymphocyte and plasma cell infiltrates distributed in a vaguely nodular pattern between colonic crypts (figure 1).

Mild-to-moderate focal inflammation composed of plasma cells and small lymphocytes in the mucosa was found in the patient carrying F508del in one allele and the new mutation c.3321dup in the other allele (figure 1A). This patient had significantly elevated faecal calprotectin (341 µg/g) (patient 7; table 1).

There was an association between elevated faecal calprotectin levels and the presence of inflammation in rectal biopsies (table 3). In our study, sensitivity of calprotectin levels was 100% and specificity was 86%.

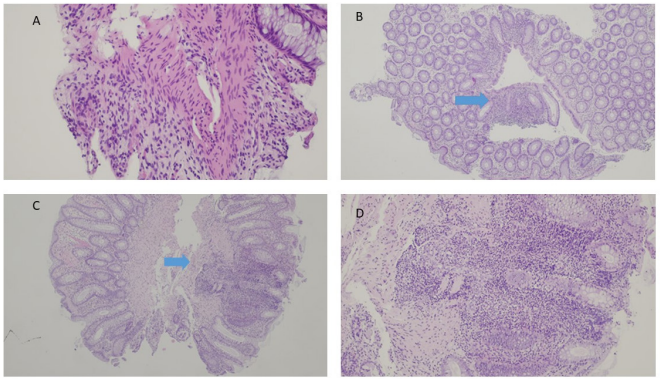

Figure 1 (A) Histological features of patient 7 (F508del/c.3321dup) showing mild-to-moderate focal inflammation, composed of plasma cells and small lymphocytes, in the mucosa, H&E 200×. (B) Histological features of patient 9 (F508del/F508del) showing mild focal inflammation of mononucleated cells of the mucosa in a vaguely nodular pattern (blue arrow), H&E 40×; (C) Histological features of patient 10 (F508del/F508del) showing mild focal inflammation of the mucosa, with small lymphocytes and plasma cells, between colonic crypts (blue arrow), H&E 40×, highlighted in higher magnification (D) H&E 200×.

Median calprotectin was higher in PwCF with histological inflammatory alterations compared with PwCF with normal histology (143 µg/g; IQR=249 vs 19 µg/g; IQR=30; p=0.024). None of the mucosal samples had architectural changes, which are a major sign of chronic inflammation.

DISCUSSION

Most patients from this study (74%) had an elevated faecal calprotectin level, some reaching a maximum level of >300 µg/g. This is consistent with previous studies and suggests the presence of intestinal inflammation in PwCF.[4 5 16] The pathophysiology of intestinal inflammation may be explained by the same triad of obstruction by mucus accumulation, inflammation and infection that causes disease in the airways of PwCF.[17] The *CFTR* gene is strongly expressed all along the intestinal tract in a cephalad-caudal gradient, *CFTR* messenger RNA levels are highest in the duodenum and levels decrease distally along the small intestine to the large intestine.[17] This 'CF enteropathy' may be an independent entity in the disease process or may be due to other factors. High doses of PERT can cause inflammation and fibrosing colonopathy.[18] Inspissated intestinal secretions, mucus accumulation, constipation, slow intestinal motility, the use of proton pump inhibitors and frequent courses of antibiotics are multiple risk factors for small bowel bacterial overgrowth in PwCF, which can cause inflammation, mucosal damage and aggravate maldigestion.[16] An unfavourable intestinal microbiome may also be a stimulus for inflammation.[19 20] One trial with probiotics supported this hypothesis, as the use of *Lactobacillus rhamnosus GG* reduced calprotectin concentrations in

Table 3 Comparison of histological rectal inflammation presence between patients with normal or elevated faecal calprotectin

	Normal calprotectin n=6		Elevated calprotectin n=5	
N=11	Yes	No	Yes	No
Mucosal inflammation	0	6	4	1

Fisher's exact test p=0.015.

children with CF.[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 with intestinal inflammation in PwCF similar to changes found in patients with Crohn's disease.[20]

In our study, lower faecal calprotectin levels were found in PS patients. Elevated faecal calprotectin only in PI patients has also been reported by Dhaliwal *et al*.[5] On the other hand, 16 of 19 patients with PI had elevated faecal calprotectin, and the difference in faecal calprotectin levels between the PI and PS groups was impressive (101 vs 30 µg/g). This means that either PI by itself or PERT may be responsible for intestinal inflammation in these patients.[21] However, a lack of correlation has been reported between PERT and faecal calprotectin.[22] As reported by Dumoulin *et al*, calprotectin is subject to proteolysis by tripsin activity, which is virtually absent in PI PwCF. As a result, calprotectin proteolysis is also reduced. Therefore, calprotectin levels detected in stools of PI patients may be higher, and this may not be exclusively attributed to intestinal inflammation.[23] Perhaps, in PI PwCF, the upper limit of the considered 'normal' faecal calprotectin should be higher than the value considered for IBD, as no association was found with digestive symptoms.[23 24] Some recent studies suggest an upper limit of >50 µg/g or 250 µg/g and it remains unclear whether reference ranges that are useful in IBD are equally applicable in CF.[20 24] Also, the pancreatic status is related to CFTR function and genotype and intestinal inflammation may be another manifestation of the multisystemic involvement of the disease and not only influenced by pancreatic function.[25] Another interesting finding was that PwCF F508del homozygous have significantly higher calprotectin levels in comparison to heterozygotes. This genotype may be associated with an increased risk of more significant intestinal inflammation.

Overall, only a small number of patients complained of gastrointestinal symptoms, and no association between elevated calprotectin and digestive symptoms could be found. The same conclusion has been reported even in studies where digestive symptoms were much more frequent.[22]

In contrast to previous studies,[5] no relationship could be found between faecal calprotectin and nutritional status or growth parameters. However, interestingly, a positive correlation was found between faecal calprotectin and sweat test values, which may be indicative of the presence of significant intestinal inflammation in patients with a more severe phenotype. The negative correlation found between faecal calprotectin and age is in line with the reported tendency towards lower values with increasing age in healthy individuals, even though there are no well-established cut-off levels for specific age ranges.[26] However, it is in contrast with some studies that found an increase in calprotectin with age in PwCF, particularly in those with severe disease.[27–29]

Historically, distinct histological changes, which have been interpreted as signs of mucus hypersecretion, have been reported in light microscopic studies of large intestinal mucosa from PwCF. 'Hypertrophic' or enlarged goblet cells and crypts distended by accumulated mucus were described, and these changes were considered useful in the diagnosis of CF by some authors.[30] In this study, we were specifically looking for histological evidence of inflammation in PwCF and try to associate it with faecal calprotectin.

Four out of the 11 patients to whom rectal biopsies were performed had histological inflammatory alterations. Interestingly, all the three patients homozygous for F508del mutation had histological signs of inflammation and this may be related to the severe phenotype associated with this common mutation.

Elevated calprotectin level was associated with histological inflammation in the rectal mucosa (table 3) and PwCF with rectal inflammation had a significantly higher calprotectin level. High sensitivity and specificity allow us to conclude that faecal calprotectin may be a good indicator of rectal inflammation in PwCF. However, the clinical meaning of this finding remains to be explained, as this did not translate into more frequent gastrointestinal symptoms or influenced nutritional status. However, as the life expectancy of PwCF is increasing an increased risk of gastrointestinal malignancies has been reported. Chronic intestinal inflammation is a risk factor for cancer development and this should probably be addressed early in life.[24]

We are aware that our study had several limitations: it is a small and unicentric study, and biopsies were performed on a subset of our patients and limited to the rectum. Larger multicentre studies with the aim of determining serial and longitudinal studies of calprotectin levels and biopsies of the upper and lower gastrointestinal tract may help to determine clinical relevance.

However, the finding of abnormal calprotectin levels and inflammatory alterations in the intestinal mucosa in the paediatric population raises questions about the early detection of CF enteropathy.

In conclusion, there is increasing evidence that intestinal inflammation is part of CF and is present early in life, particularly in childhood and adolescence. The additional contribution of low trypsin activity, chronic enzyme dosage, dysmotility, bacterial overgrowth, dysbiosis and other unidentified factors may play a role in its multifactorial cause. Faecal calprotectin may be considered a non-invasive biomarker of intestinal inflammation in PwCF since a relationship with histological evidence of rectal mucosa inflammation was found. Further and larger studies need to be performed to confirm and explain the mechanisms and clinical relevance of these findings.

Contributors JR conceived and designed the study, collected, analysed and interpreted the data, wrote the first draft of the manuscript and is responsible for the overall content as guarantor. CM interpreted the data and critically reviewed

the manuscript. SA interpreted the data and critically reviewed the manuscript. RCO prepared figure 1 and analysed and interpreted the data. RF analysed and interpreted the data and critically reviewed the manuscript. GO critically reviewed the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval The present study was performed in accordance with the Declaration of Helsinki and approved by the board of the Centro Hospitalar e Universitário de Coimbra (Portugal) after a favourable report by the Health Ethics Committee (Ref. CHUC-080-16). Informed consent was obtained by all participants aged over 16 years or by their parents or legal guardians if under 16 years old.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iD

Juliana Roda http://orcid.org/0000-0001-8990-779X

REFERENCES

1 Elborn JS. Cystic fibrosis. *The Lancet* 2016;388:2519–31.
2 Marcão A, Barreto C, Pereira L, *et al*. Cystic fibrosis newborn screening in Portugal: Pap value in populations with stringent rules for genetic studies. *Int J Neonatal Screen* 2018;4:22.
3 Farrell PM. The prevalence of cystic fibrosis in the European Union. *J Cyst Fibros* 2008;7:450–3.
4 Werlin SL, Benuri-Silbiger I, Kerem E, *et al*. Evidence of intestinal inflammation in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 2010;51:304–8.
5 Dhaliwal J, Leach S, Katz T, *et al*. Intestinal inflammation and impact on growth in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 2015;60:521–6.
6 Norkina O, Kaur S, Ziemer D, *et al*. Inflammation of the cystic fibrosis mouse small intestine. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G1032–41.
7 De Lisle RC. Altered transit and bacterial overgrowth in the cystic fibrosis mouse small intestine. *Am J Physiol Gastrointest Liver Physiol* 2007;293:G104–11.
8 Bruzzese E, Callegari ML, Raia V, *et al*. Disrupted intestinal microbiota and intestinal inflammation in children with cystic fibrosis and its restoration with Lactobacillus GG: a randomised clinical trial. *PLoS One* 2014;9:e87796.
9 Summerton CB, Longlands MG, Wiener K, *et al*. Faecal calprotectin: a marker of inflammation throughout the intestinal tract. *Eur J Gastroenterol Hepatol* 2002;14:841–5.
10 Montalto M, Santoro L, Curigliano V, *et al*. Faecal calprotectin concentrations in untreated coeliac patients. *Scand J Gastroenterol* 2007;42:957–61.
11 García-Sánchez V, Iglesias-Flores E, González R, *et al*. Does fecal calprotectin predict relapse in patients with Crohn's disease and ulcerative colitis? *J Crohn's Colitis* 2010;4:144–52.
12 Schnapp Z, Hartman C, Livnat G, *et al*. Decreased fecal calprotectin levels in cystic fibrosis patients after antibiotic treatment for respiratory exacerbation. *J Pediatr Gastroenterol Nutr* 2019;68:282–4.
13 Stallings VA, Sainath N, Oberle M, *et al*. Energy balance and mechanisms of weight gain with ivacaftor treatment of cystic fibrosis gating mutations. *J Pediatr* 2018;201:229–37.
14 Farrell PM, White TB, Howenstine MS, *et al*. Diagnosis of cystic fibrosis in screened populations. *J Pediatr* 2017;181S:S33–44.
15 Sands BE. Biomarkers of inflammation in inflammatory bowel disease. *Gastroenterology* 2015;149:1275–85.
16 Rumman N, Sultan M, El-Chammas K, *et al*. Calprotectin in cystic fibrosis. *BMC Pediatr* 2014;14:133.
17 De Lisle RC, Borowitz D. The cystic fibrosis intestine. *Cold Spring Harb Perspect Med* 2013;3:a009753.
18 Sankararaman S, Schindler T, Sferra TJ. Management of exocrine pancreatic insufficiency in children. *Nutrition in Clinical Practice* 2019;34:S27–42.
19 Bruzzese E, Raia V, Gaudiello G, *et al*. Intestinal inflammation is a frequent feature of cystic fibrosis and is reduced by probiotic administration. *Aliment Pharmacol Ther* 2004;20:813–9.
20 Enaud R, Hooks KB, Barre A, *et al*. Intestinal Inflammation in Children with Cystic Fibrosis Is Associated with Crohn's-Like Microbiota Disturbances. *J Clin Med* 2019;8:645.
21 Brennan GT, Saif MW. Pancreatic enzyme replacement therapy: a Concise review. *JOP* 2019;20:121–5.
22 Rumman N, Sultan M, El-Chammas K, Goh V, *et al*. Calprotectin in cystic fibrosis. *BMC Pediatr* 2014;14.
23 Dumoulin EN, Van Biervliet S, Delanghe JR. Trypsin is a potential Confounder in calprotectin results. *J Pediatr Gastroenterol Nutr* 2015;61:e19.
24 Tam RY, van Dorst JM, McKay I, *et al*. Intestinal inflammation and alterations in the gut microbiota in cystic fibrosis: a review of the current evidence, pathophysiology and future directions. *J Clin Med* 2022;11:649.
25 Ahmed N, Corey M, Forstner G, *et al*. Molecular consequences of cystic fibrosis transmembrane regulator (CFTR) gene mutations in the exocrine pancreas. *Gut* 2003;52:1159–64.
26 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;72:617–40.
27 Parisi GF, Papale M, Rotolo N, *et al*. Severe disease in cystic fibrosis and fecal calprotectin levels. *Immunobiology* 2017;222:582–6.
28 Więcek S, Woś H, Kordys-Darmolińska B, *et al*. The concentration of calprotectin in the stools of children with diagnosed cystic fibrosis. *Prz Gastroenterol* 2017;12:38–43.
29 Garg M, Leach ST, Coffey MJ, *et al*. Age-Dependent variation of fecal calprotectin in cystic fibrosis and healthy children. *J Cyst Fibros* 2017;16:631–6.
30 Neutra MR, Trier JS. Rectal mucosa in cystic fibrosis. morphological features before and after short term organ culture. *Gastroenterology* 1978;75:701-10.