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Fecal calprotectin in a sample of patients with Cystic Fibrosis and its relation to CFTR modulators

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Resumo

Introdução: A Fibrose Quística (FQ) é causada por mutações no gene *Cystic Fibrosis transmembrane conductance regulator* (CFTR) que levam à acumulação e obstrução por muco em múltiplos órgãos. No intestino, a inflamação crónica poderá ter impacto na morbilidade e mortalidade a curto e longo prazo, embora não exista terapêutica anti-inflamatória específica para doentes com FQ. Os fármacos moduladores da proteína CFTR provaram ter benefícios pulmonares significativos e melhorar a qualidade de vida dos doentes, mas ainda há poucos estudos relativos aos efeitos fisiológicos no intestino.

Objetivos: Estudar a inflamação intestinal em doentes com FQ sob tratamento modificador de doença com moduladores CFTR usando a calprotectina fecal como biomarcador.

Metodologia: Contactados 50 doentes seguidos num centro de referência de FQ, dos quais 30 aceitaram entrar no estudo. Os níveis de calprotectina fecal foram avaliados com recurso a *'Cobas Integra® 400 plus analyser'.* Do total de doentes que integraram a amostra final, 25 incluíam-se na idade pediátrica e 5 na idade adulta, com idades compreendidas entre os 7 meses e os 77 anos de idade, 18 do género feminino e 12 do masculino. 10 doentes estavam tratados com moduladores CFTR, e 20 doentes estavam sob tratamento convencional para a FQ. O peso, índice de massa corporal (IMC), elastase fecal, infeção pulmonar crónica, e função pulmonar também foram avaliados. As variáveis foram comparadas entre os grupos terapêuticos.

Resultados: Embora os resultados obtidos não sejam estatisticamente significativos (P = 0.54), as concentrações de calprotectina fecal eram mais elevadas em doentes sob tratamento convencional para a FQ (mediana [intervalo interquartil]: 94.5 μg/g [22.75-389.5]), quando comparados com doentes tratados com moduladores CFTR (76 μg/g [IQR: 28.25-149.5]). Adicionalmente, entre os doentes sob terapêutica moduladora, a diminuição da elastase fecal e percentagem do previsto para o volume expiratório forçado no primeiro segundo estavam correlacionadas com níveis aumentados de calprotectina fecal.

Conclusões: Apesar dos resultados obtidos não serem estatisticamente significativos, este estudo sugeriu benefícios na administração de moduladores CFTR em relação a terapêuticas convencionais quanto ao tratamento da inflamação intestinal na FQ. Adicionalmente, este estudo sugeriu a existência de associações entre os moduladores CFTR, insuficiência pancreática exócrina, inflamação intestinal e disfunção pulmonar.

Palavras-chave: Biomarcador; Calprotectina; Complexo antigénico leucocitário L1; Fibrose quística; Moduladores CFTR.

Abstract

Introduction: Cystic Fibrosis (CF) is caused by mutations in the Cystic Fibrosis transmembrane conductance regulator (CFTR) gene which leads to accumulated and plugged mucus in multiple organs. In the intestine, chronic inflammation may have short- and long-term impacts on morbidity and mortality; however, a specific anti-inflammatory treatment for patients with CF does not exist. Drugs that modulate the CFTR protein have proved to have significant pulmonary benefits and improve quality of life, but there are still few studies related to physiological effects in the intestine.

Objectives: Study intestinal inflammation in patients under disease-modifying treatment with CFTR modulators by using fecal calprotectin as a biomarker.

Methods: 50 patients treated at a reference center for CF were contacted, of which 30 accepted to take part in the study. Fecal calprotectin levels were evaluated using the 'Cobas Integra® 400 plus analyzer'. Among the total of patients that integrated the final sample, 25 were included within the pediatric age and 5 in the adult age, between the age range of 7 months and 77 years old, 18 females and 12 males. 10 patients were treated with CFTR modulators, and 20 patients were under conventional treatment for CF. Weight, body mass index (BMI), fecal elastase, lung function, and chronic airway infection were assessed as well. Variables were compared between treatment groups.

Results: Although the obtained results were not statistically significant ($P = 0.54$), fecal calprotectin concentrations were higher in CF patients under conventional treatment (median [interquartile range]: 94.5 μg/g [22.75-389.5]), compared with patients treated with CFTR modulators (76 μg/g [IQR: 28.25-149.5]). Additionally, among CF patients treated with CFTR modulators, decreased fecal elastase and percentage of predicted forced expiratory volume in 1 second were correlated with increased fecal calprotectin concentrations.

Conclusions: Although the obtained results were not statistically significant, this study suggested that CFTR modulation may show benefits over conventional treatments regarding anti-inflammatory effects in the gut in CF. Moreover, this study suggested the existence of possible associations between modulator therapy, gut inflammation, exocrine pancreatic insufficiency, and pulmonary dysfunction were observed.

Keywords: Biomarker; Calprotectin; CFTR modulators; Cystic fibrosis; Leukocyte L1 antigen complex.

Abbreviation list | Lista de abreviaturas

BMI – Body mass index

CF – Cystic fibrosis

CFTR – Cystic fibrosis transmembrane conductance regulator

IBD – Inflammatory bowel disease

IQR – Interquartile range

PERT – Pancreatic enzyme replacement therapy

ppFEV1 – Percentage of predicted forced expiratory volume in 1 second

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1. Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the gene coding for the CF transmembrane conductance regulator (CFTR) channel. The CFTR protein is a channel mainly for chloride and bicarbonate ions and is central in proper epithelial fluid secretion and intra-luminal hydration.[1] Most mutations cause reduced channel numbers or impaired channel function, leading accumulated and plugged mucus on the apical surface of epithelial cells in multiple organs.[2] In the intestine, this results in chronic inflammation, even in the absence of digestive symptoms.[3,4]

Gastrointestinal disease in CF likely results from a triad of obstruction, infection, and inflammation and may have a short- and long-term impact on morbidity and mortality in CF patients. Chronic inflammation, alongside pancreatic insufficiency, altered intestinal secretions, and dysmotility may contribute to malabsorption, which in turn can cause malnutrition and growth disorders in numerous CF patients, despite the use of treatments such as pancreatic enzyme replacement therapy (PERT) and high-fat calorie diets.[5,6] As many as 80% of patients experience gastroesophageal reflux, abdominal pain, bloating, constipation, and abnormal stools, impacting their quality of life.[7] Moreover, suboptimal nutritional status is recognized to be a predictor of respiratory function deterioration, which remains the main determinant of CF morbidity and mortality.[8]

The improvement in care for patients with CF has led to the need to address emerging comorbidities as patients are living longer. CF patients present a major risk of cancer, particularly of the small bowel and colon, at a younger age than the general population.[9,10] Chronic intestinal inflammation may play a role in the unclear pathophysiology of cancer in CF, as chronic inflammation constitutes a risk factor for colorectal cancer, associated with the degree and duration of inflammation in inflammatory bowel disease (IBD).[11,12,13]

Intestinal inflammation, therefore, needs to be targeted in CF clinical practice treatment; however, a specific anti-inflammatory treatment for patients with CF does not exist. Coffey M. et al. showed that there is a low level of evidence and significant inter-individual variability in treatment with probiotics,[14] despite previous promising studies.[15]

Nevertheless, other CF treatments have been developed during the last few years, with significant clinical benefits and impact on disease progression, substantially improving the quality of life of patients. While offering an alternative to previous treatments that only addressed patients' symptoms, CFTR modulating drugs increase efficacy and/or production of the CFTR channel. The initial therapeutic milestone was the approval for clinical use of ivacaftor, a potentiator that increases the function of CFTR proteins presenting with gating defect or residual function.[16,17,18,19,20]

Later, the development of lumacaftor and tezacaftor, correctors that improve the defective protein trafficking, showed beneficial clinical results when combined with a potentiator.[21,22,23,24] The approval for clinical use of highly effective modulator treatment (combination of elexacaftor, tezacaftor, and ivacaftor) has broadened access to disease-modifying treatment to up to 90% of individuals with CF.[25,26,27,28]

The beneficial effects of CFTR modulation on lung function, pulmonary exacerbations, and quality of life were the basis for its approval. However, studies have shown benefits on gastrointestinal manifestations of CF, such as improvement of the pH and histopathologic changes of inspissated mucin in the crypts of the small intestinal,[29,30] as well as clinically significant increases in weight and body mass index.[17,19,21,31] Nevertheless, there are still few studies related to physiological effects in the intestine.

Fecal biomarkers are increasingly being utilized to investigate gut physiology in CF. Calprotectin is a zinc and calcium-binding protein, formed by the heterocomplex of S100A8 and S100A9.[5] This protein is found in granulocytes, monocytes, and macrophages, and is released extracellularly when secreted by stimulated neutrophils or by cell disruption or death.[5,32] Its measure in feces quantitatively relates to neutrophil migration toward the intestine, making it a noninvasive biomarker of intestinal inflammation with high sensitivity and predictive value.[33] This protein is commonly used to diagnose and monitor IBD.[34] In patients with CF, it is significantly higher compared with healthy individuals, and studies have shown an association with clinical manifestations of intestinal inflammation.[4,5,15,35,36] Therefore, fecal calprotectin constitutes a promising noninvasive biomarker to predict gut response to treatment with CFTR modulators, measurable at the initial stages of chronic intestinal inflammation in CF.

We aim to study intestinal inflammation in CF patients under disease-modifying treatment with CFTR modulators by using fecal calprotectin as a biomarker. We seek to verify the existence of advantages in the treatment with CFTR modulators regarding intestinal inflammation, lung disease a,nd exocrine pancreatic disease. This study also aims to assess whether certain therapies with CFTR modulators display increased benefits compared to others regarding intestinal inflammation. Lastly, we seek to study if the intestinal response to treatment with CFTR modulators is associated with a response in other organs and systems.

2. Methods

2.1. Patient sample

In this observational study, 50 patients were contacted from the CF Reference Center of the Hospital and University Centre of Porto, between January and May 2022. All subjects with CF were diagnosed in accordance with the consensus diagnostic guidelines by Farrell P. et al.[37] A history of pulmonary transplant was considered a criteria of exclusion. 30 patients accepted to take part in the study.

The study was approved by the Hospital and University Centre of Porto's Ethics Committee. Informed consent was obtained from each patient or respective legal guardian. Data were obtained exclusively from patients' medical records and no interventions were performed. Collected data were preserved in a password-protected database and patient identification was encrypted via the use of a numbered code specific to each individual. The password and code key were known only to the study investigator to preserve anonymity. All information was kept for a period of 6 months after completion of the study.

2.2. Collected data

The collected demographic and clinical data included age, gender, genotype, treatment, weight, body mass index (BMI), fecal elastase, lung function, and chronic airway infection. Exocrine pancreatic insufficiency was defined as fecal elastase below 200 mg/g in accordance with the clinical guidelines from the Royal Brompton Hospital.[38] Values of forced expiratory volume in 1 second (FEV1) were expressed as percentages of predicted normal values. This value was missing in 8 subjects as they were too young to perform spirometry. Patients were divided into two groups according to CF treatment. The first group included patients under conventional CF treatment, defined as symptomatic care for respiratory, gastrointestinal, nutritional, as well as other non-pulmonary manifestations of CF.[38] The second group included patients additionally under CFTR modulator therapy for at least 3 months, which increases efficacy and/or production of the mutant CFTR channel.

2.3. Sample collection

A stool sample was collected from each patient. Samples were stored at a temperature of 2-8ºC within 48 hours of shipment to the laboratory.

2.4. Sample analysis

Calprotectin was extracted from the stools upon arrival at the laboratory using the 'Calex® Cap Device' (Bühlmann, Schönenbuch, Switzerland) and then stored at a temperature of 2ºC to 8ºC for 24 hours until quantification. Calprotectin was prepared for quantification using the 'Bühlmann fCAL® turbo' test (Bühlmann, Schönenbuch, Switzerland). Measurement of calprotectin was

performed using the 'Cobas Integra® 400 plus analyzer' (Roche Diagnostics, Mannheim, Germany). All tests were performed in accordance with the manufacturer's instructions. Calprotectin levels were expressed in µg/g. In order to identify patients with significantly increased fecal calprotectin, we applied a cut-off of 50 µg/g, predictive of endoscopically active intestinal inflammation.[39,40,41]

2.5. Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics (version 25). Continuous variables were presented as medians and interquartile ranges (IQR). Categorical variables were presented as frequency counts and percentages for categorical variables. Comparisons between groups of patients were made using the Mann-Whitney test for continuous variables and Fisher's exact test for categorical data. Pearson correlation tests were used to analyze the association between fecal calprotectin and other continuous variables. A *P* value of < 0.05 was considered statistically significant.

3. Results

3.1. Subject characteristics

Patient characteristics are summarized in Table I. Among the total of patients that integrated the final sample, 25 were included within the pediatric age and 5 in the adult age, between the age range of 7 months and 77 years old. The study included 18 females and 12 males. 10 patients were treated with CFTR modulators, and 20 patients were under conventional treatment for CF. The median body mass index (BMI) was 18.35 kg/m^2 (interquartile range (IQR): 15.925-21.35). The median percentage of predicted FEV1 (ppFEV1) was 84.95% (IQR: 66.875-95.775) among the 22 patients who had spirometry results. 14 patients (47%) had a chronic pulmonary infection. The lower limit of detection for fecal elastase was 15 µg/g. The median fecal elastase concentration was below the lower limit of detection, 15 µg/g (IQR: 15 – 398). Patient genotypes are presented in Table II.

3.2. Clinical characteristics in patients under CFTR modulator and conventional treatment

The clinical characteristics of patients are presented in Table III by treatment group. The median ppFEV1 was 65.75% (IQR: 44.35-88.18) in patients treated with CFTR modulators (10 patients), compared with 92.85% (IQR: 80.43-101.25) in patients under conventional therapy (12 patients, 8 patients were too young to perform spirometry). 90% (9/10) and 25% (5/20) had a chronic pulmonary infection, respectively. The median fecal elastase concentration was below the lower

limit of detection, 15 μg/g (IQR: 15 – 15), in patients treated with CFTR modulators, compared with 199 μg/g (IQR: 15-531.75) in patients under conventional therapy.

3.3. Fecal calprotectin levels

The median fecal calprotectin concentration was 89 μg/g (IQR: 24.25-262). The lower limit of detection for the assay was 10 μg/g. 17 patients (57%) had levels greater than 50 μg/kg. Fecal calprotectin concentrations were higher in CF patients under conventional treatment (94.5 μg/g, [IQR: 22.75-389.5]), compared with patients treated with CFTR modulators (76 μg/g, [IQR: 28.25-149.5]), although these results were not statistically significant (*P* = 0.54) (Figure 1). 60% of patients (6 patients) treated with CFTR modulator therapy had fecal calprotectin levels greater than 50 μg/kg, compared with 55% of patients (11 patients) treated with conventional therapy for CF. The median fecal calprotectin level was 90 μg/g (IQR: 21.5-168) in patients treated with lumacaftor/ivacaftor (5 patients), and 64 μg/g (IQR: 31.5-548.5) in patients treated with elexacaftor/tezacaftor/ivacaftor (5 patients). In this last group, 1 patient showed a 64% decrease in fecal calprotectin levels (from 136 to 90 ug/g), while another patient showed a 50% decrease (from 180 to 90 ug/g), after starting modulator treatment.

3.4. Fecal calprotectin concentrations and relation to other characteristics of patients treated with CFTR modulators

Among CF patients treated with CFTR modulators, females showed higher fecal calprotectin levels (89 μg/g, [IQR: 51.5-406]) than males (41 μg/g, [IQR: 15.75-110.5]), although these results were not statistically significant (*P* = 0.201). Females also included more subjects with increased fecal calprotectin concentrations (83.3%), compared with male patients (25%), although these results were not statistically significant (*P* = 0.19). Age was not statistically significantly positively correlated with fecal calprotectin levels (r = 0.211, *P* = 0.558) (Fig. 2). Decreased fecal elastase was not statisticallt significantly correlated with increased fecal calprotectin levels (r = -0,186, *P* = 0.607) (Fig. 3). Increased fecal calprotectin concentrations were not statistically significantly correlated with decreased ppFEV1 (r = -0.121, *P* = 0.738) (Fig. 4). Fecal calprotectin levels were not statistically significantly higher in patients with chronic pulmonary infection (64 μg/g, [IQR: 23.5-110.5]), compared with subjects with absent chronic airway infection (205 μg/g, [IQR: 205-205], *P* = 0.223).

4. Discussion

Intestinal inflammation is a frequent manifestation of CF, as the CFTR channel regulates epithelial fluid secretion and subsequent luminal hydration in the gut.[1] Dysfunction of the CFTR

channel, therefore, leads to accumulated and plugged mucus in the lumen which, alongside dysmotility, chronic antibiotic use, and PERT,[4,42] may lead to bacterial growth,[43] likely promoting chronic intestinal inflammation. When neutrophils are activated, calprotectin is released into the intestinal lumen.[5,32] Fecal calprotectin, therefore, constitutes a highly sensitive non-invasive biomarker of intestinal inflammation,[5,33] with the potential to predict gut response to CFTR modulators, which there is still much to learn about. This study aimed to assess the potential intestinal anti-inflammatory effects of CFTR modulator treatment in patients with CF.

Previous studies have demonstrated a decrease in fecal calprotectin in patients treated with CFTR modulators.[44] We observed that fecal calprotectin concentrations were lower in patients treated with CFTR modulators (almost 20% lower), suggesting that CFTR modulators may have improved anti-inflammatory results compared with conventional treatments in patients with CF (Fig. 1). These results were, however, not statistically significant, but this could be due to intercurrent factors such as age and consequently prolonged duration of inflammatory gut disease in these patients. In fact, age was correlated with increased calprotectin concentrations in stool, although these results were not statistically significant (Fig. 2). 6 patients treated with CFTR modulators showed elevated fecal calprotectin levels. Interindividual variability in response to treatment[45] and intercurrent factors, such as antibiotic-related dysbiosis, may explain persistently elevated fecal calprotectin levels despite CFTR modulator therapy. Further studies are needed to investigate an eventual lack of intestinal response to treatment with CFTR modulators in certain patients.

Although all patients treated with CFTR modulators were pancreatic insufficient, exocrine pancreatic function and fecal calprotectin were associated (Fig. 3). This suggests a distinct intestinal response to treatment may exist between pancreatic insufficient and pancreatic sufficient patients. Although these results were not found to be statistically significant, previous studies have reported a possible correlation between increased pancreatic dysfunction and intestinal inflammation. An eventual variability in response to treatment may be explained by the fact that exocrine pancreatic dysfunction alters intestinal permeability,[46,47] which might influence the degree of inflammation in the CF gut. Other proposed mechanisms include a hyper acidic luminal environment due to pancreatic insufficiency and mucin glycolysis, combined with increased intestinal mucus viscosity.[12,48] This study did not identify such a correlation with statistical significance (Fig. 4), which could be due to factors such as PERT.[47]

To note, gut inflammation correlated with more severe pulmonary dysfunction (Fig. 4). Although these results were not statistically significant, an eventual association could be due to a

mutual influence between gut and lung disease evolution in CF. In fact, intestinal alterations seen in CF patients may cause malabsorption with subsequent malnutrition, which in turn compromises respiratory muscle function and innate pulmonary defenses. This in turn may allow for microorganism translocation to the lungs, which may result in the systemic release of pro-inflammatory mediators which will reach the intestine.[42] Additionally, studies have demonstrated that calprotectin levels directly varied according to the degree of airway inflammation,[47] suggesting that calprotectin may play a pro-inflammatory role in the lungs, which requires further investigation.[48]

We were unable to observe a statistically significant correlation between fecal calprotectin and nutritional status, despite descriptions in previous studies,[49] most likely due to the multifactorial nature of the nutritional status of patients with CF (Fig. 5 and 6). Studies have shown that treatment with ivacaftor increased intestinal pH and CFTR-mediated bicarbonate secretion.[50] The correlation reported between decreased fecal calprotectin and improved nutritional status may be explained by a combination of the latter with increased intestinal pH, leading to improved PERT results, prompting improved absorption of fat.[49]

This study has several limitations, including the limited size of the sample and the wide age range. The small sample size limits the study's power and requires larger-scale studies. Moreover, the inclusion of 5 patients younger than 4 years old limits the validity of the interpretation of fecal calprotectin concentrations as the biomarker is physiologically increased in this age group.[51]

Improvement of care for CF patients has led to a lengthening of patients' life expectancy. However, this sets the need to address the increasing incidence of gastrointestinal cancer in these patients.[9,10] Inflammation may play a role in the unclear pathophysiology of cancer in CF, as chronic intestinal inflammation has been shown to promote oncogenesis.[52,53] Future studies are needed to decipher the impact of CFTR modulation on the digestive mucosa. Studies suggest a link between gastrointestinal cancer risk, inflammation, and dysbiosis in CF.[12] The enteric microbiota and bacterial overgrowth, along with chronic use of antibiotics are, in fact, likely to play a role in intestinal inflammation in CF.[12,54] Large-scale longitudinal studies should be conducted to follow the evolution of microbiota and inflammation in patients treated with CFTR modulators.

Possible associations between decreased gut inflammation and improved exocrine pancreatic and pulmonary functions supports the theory that CFTR modulation may act as a systemic anti-inflammatory in patients that respond to treatment. Longitudinal assessments of the evolution of fecal calprotectin, gastrointestinal symptoms, and systemic clinical outcomes in large patient samples, could be used to better understand the part of treatment in the evolution of inflammation

in CF. Long-term key clinical outcomes, such as a shift in gastrointestinal cancer risk, could signify the need to introduce CFTR modulation earlier in disease progression into CF patient care.

Gastrointestinal manifestations and complications of CF are detrimental to the quality of life of patients[7] and, as previously discussed, may have long-term implications for CF morbidity and mortality. The introduction of specific and perhaps systemic anti-inflammatory measures or drugs into patient's care could therefore positively impact the natural course of this disorder.

Another possible interpretation for the potencial association between fecal calprotectin and multi-systemic clinical outcomes, is that fecal calprotectin may act as a biomarker of systemic inflammation, therefore influenced by disease activity in other organs, as suggested by previous studies.[34,54] Further investigation is needed to assess whether this biomarker predicts long-term key clinical outcomes in CF. Fecal calprotectin could potentially constitute a noninvasive and inexpensive marker of systemic inflammation, and could, therefore, come to play a key role in the development of anti-inflammatory drugs for CF.

5. Conclusion

In summary, this study suggested in a sample of CF patients that CFTR modulation may show benefits over conventional treatments regarding anti-inflammatory effects in the gut. Despite the limitations related to a retrospective and small sample study, this study suggested the existence of possible associations between modulator therapy, gut inflammation, exocrine pancreatic insufficiency, and pulmonary dysfunction. Future studies are warranted to further investigate the extrapulmonary effects of CFTR modulator therapies and related disease outcomes, as well as the potential of fecal calprotectin as a marker of systemic inflammation in the development of anti-inflammatory therapies.

Appendix | Apêndice

Table I: Subject characteristics.

Abbreviations: BMI – Body mass index; CFTR – Cystic fibrosis transmembrane conductance regulator; IQR – Interquartile range; N – Number of patients; ppFEV1 – Percentage of predicted forced expiratory volume in 1 second.

	Patients
Number of patients	30
Females	18 (60%)
Males	12 (40%)
Age (years), median (IQR)	13.95 (6.075-16.550)
Patients < 18 years old	25 (83%)
Patients ≥ 18 years old	5 (17%)
Weight (kg), median (IQR)	42.2 (19.25-53.75)
BMI (kg/m^2), median (IQR)	18.35 (15.925-21.35)
Pancreatic insufficiency	21 (70%)
ppFEV1 (%), median (IQR)	84.95 (66.875-95.775) (N = 22)
Chronic airway infection	14 (47%)
Fecal calprotectin (µg/g), median (IQR)	89 (24.25-262)

Table II: Genotype of patients treated with CFTR modulator and conventional therapy.

Abbreviations: CFTR – Cystic fibrosis transmembrane conductance regulator.

CFTR modulators		Conventional treatment	
Mutation	Number	Mutation	Number
F508del/F508del	10	F508del/F508del	4
		F508del/R334W	2
		R1066C/R334W	2
		F508del/L163X	1
		F508del/c.743+1G>A	1
		F508del/P205S	1
		F508del/r.2620_2657delins2989_3139	1
		F508del/N1303K	1
		F508del/5T;TG13	1
		5T/R334W	1
		N1303K/N1303K	1
		G542X/c.3139+1G>T	1
		L206W/c.3140-26A>G	1
		V232D/R334W	1
		G576A/R668C	1

Table III: Characteristics of patients under CFTR modulator and conventional treatment.

Abbreviations: CFTR – Cystic fibrosis transmembrane conductance regulator; IQR – Interquartile range; N – Number of patients; ppFEV1 – Percentage of predicted forced expiratory volume in 1 second.

	CFTR modulators	Conventional treatment
Number of patients	10	20
Females	6 (60%)	12 (60%)
Males	4 (40%)	8 (40%)
Age (years), median (IQR)	15.65 (14.175-16.55)	9.35 (3.575-17.475)
Patients < 18 years old	10 (100%)	15 (75%)
Patients ≥ 18 years old	0 (0%)	5 (25%)
Pancreatic insufficiency	10 (100%)	11 (55%)
ppFEV1 (%), median (IQR)	65.75 (44.35-88.175)	92.85 (80.425-101.25) (N = 12)
Chronic airway infection	9 (90%)	5 (25%)
Fecal calprotectin (µg/g), median (IQR)	76 (28.25-149.5)	94.5 (22.75-389.5)

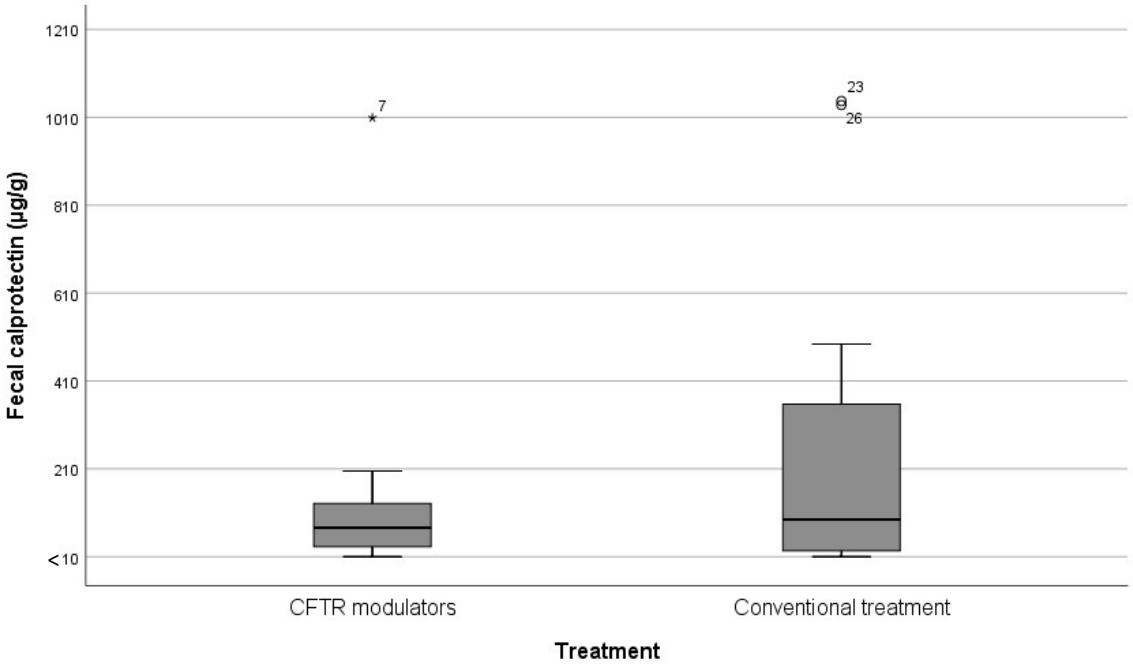

Figure 1: Fecal calprotectin levels in patients under CFTR modulator and conventional treatment.
<10 µg/g represents the lower limit of detection for fecal calprotectin. *P* = 0.54.

Abbreviations: CFTR – Cystic fibrosis transmembrane conductance regulator.

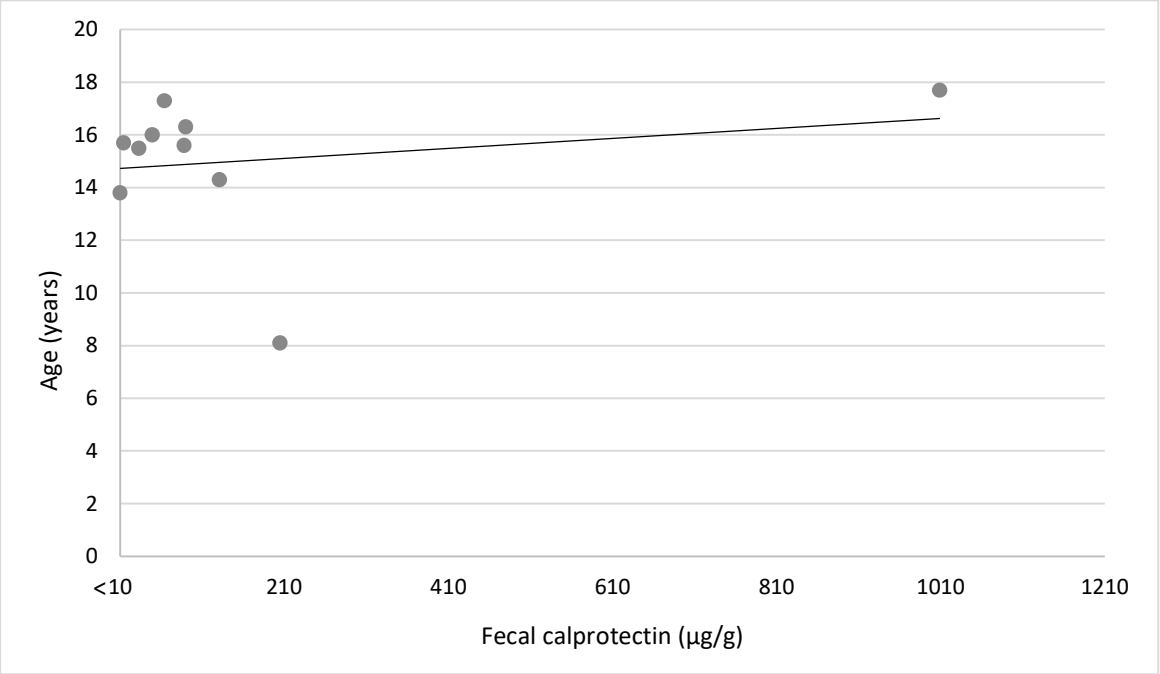

Figure 2: Correlation between fecal calprotectin and age in patients treated with CFTR modulators. <10 µg/g represents the lower limit of detection for fecal calprotectin. r = 0.211. *P* = 0.558.

Abbreviations: CFTR – Cystic fibrosis transmembrane conductance regulator.

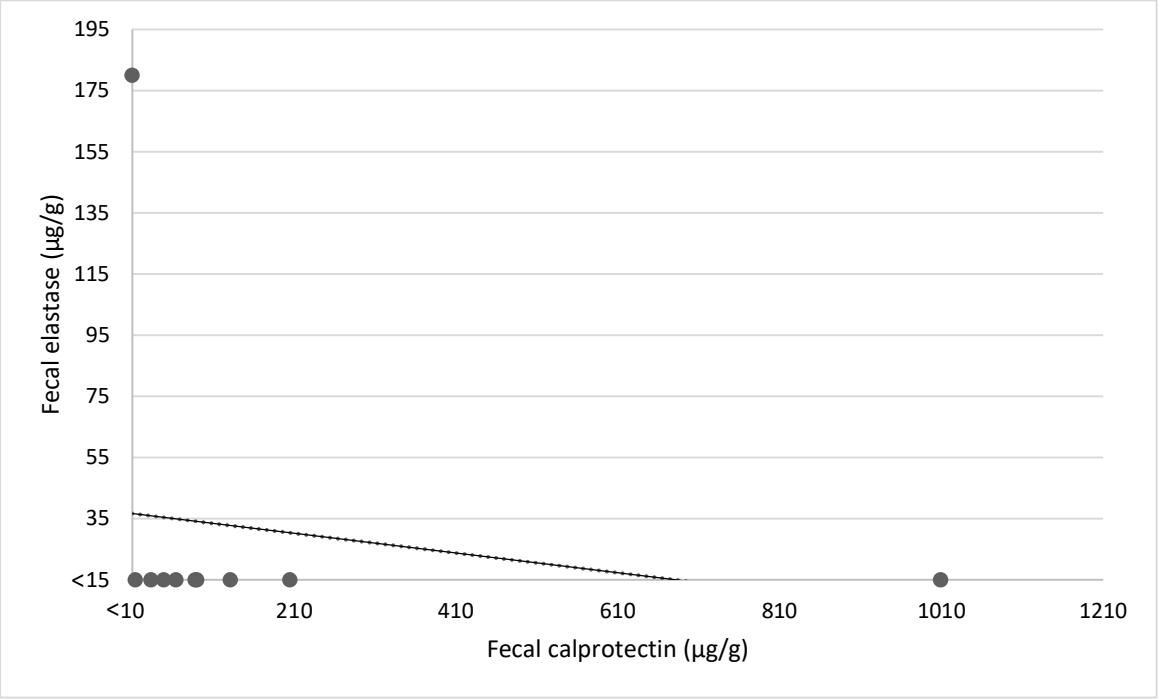

Figure 3: Correlation between fecal elastase and fecal calprotectin in patients treated with CFTR modulators. <10 µg/g represents the lower limit of detection for fecal calprotectin. < 15 µg/g represents the lower limit of detection for fecal elastase. r = -0.186. *P* = 0.607.

Abbreviations: CFTR – Cystic fibrosis transmembrane conductance regulator.

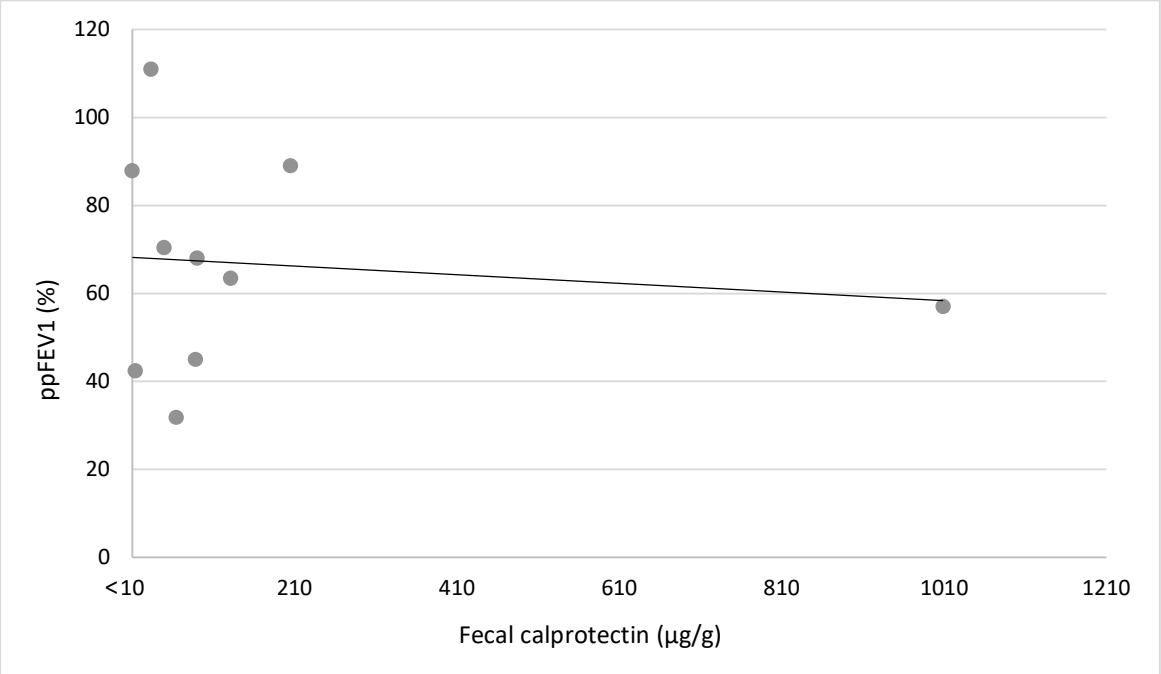

Figure 4: Correlation between fecal calprotectin and ppFEV1 in patients treated with CFTR modulators. <10 µg/g represents the lower limit of detection for fecal calprotectin. r = -0.121. *P* = 0.738.

Abbreviations: CFTR – Cystic fibrosis transmembrane conductance regulator; ppFEV1 – Percentage of predicted forced expiratory volume in 1 second.

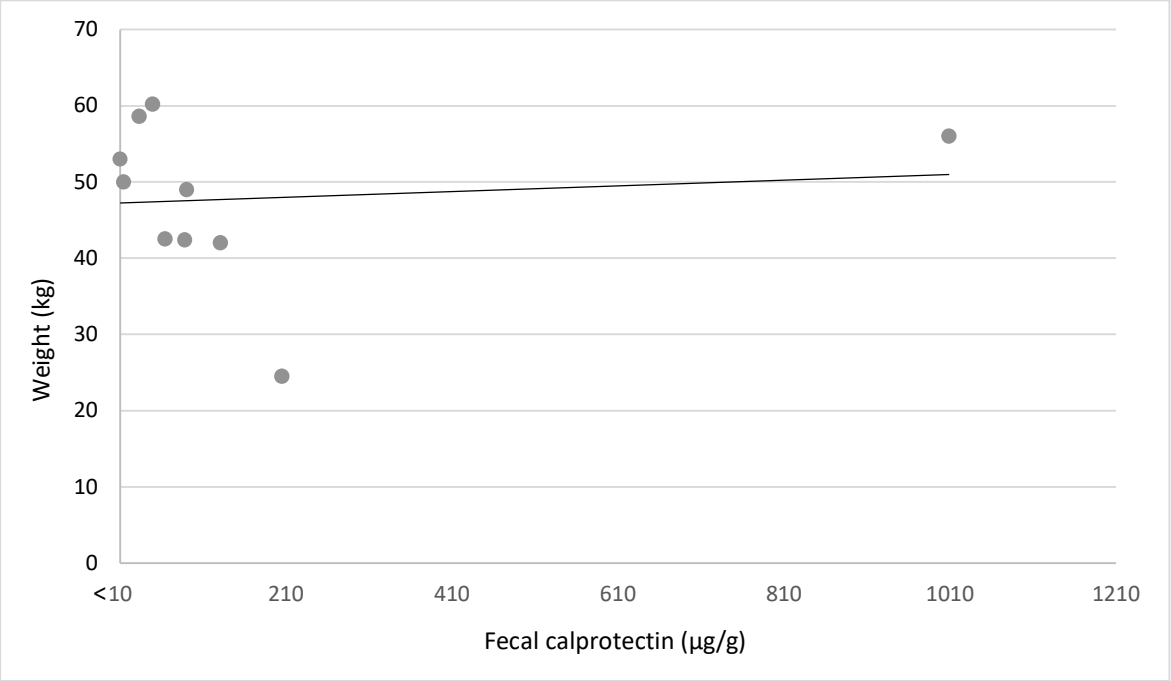

Figure 5: Correlation between fecal calprotectin and weight in patients treated with CFTR modulators. <10 µg/g represents the lower limit of detection for fecal calprotectin. r = 0.106. *P* = 0.770.

Abbreviations: CFTR – Cystic fibrosis transmembrane conductance regulator.

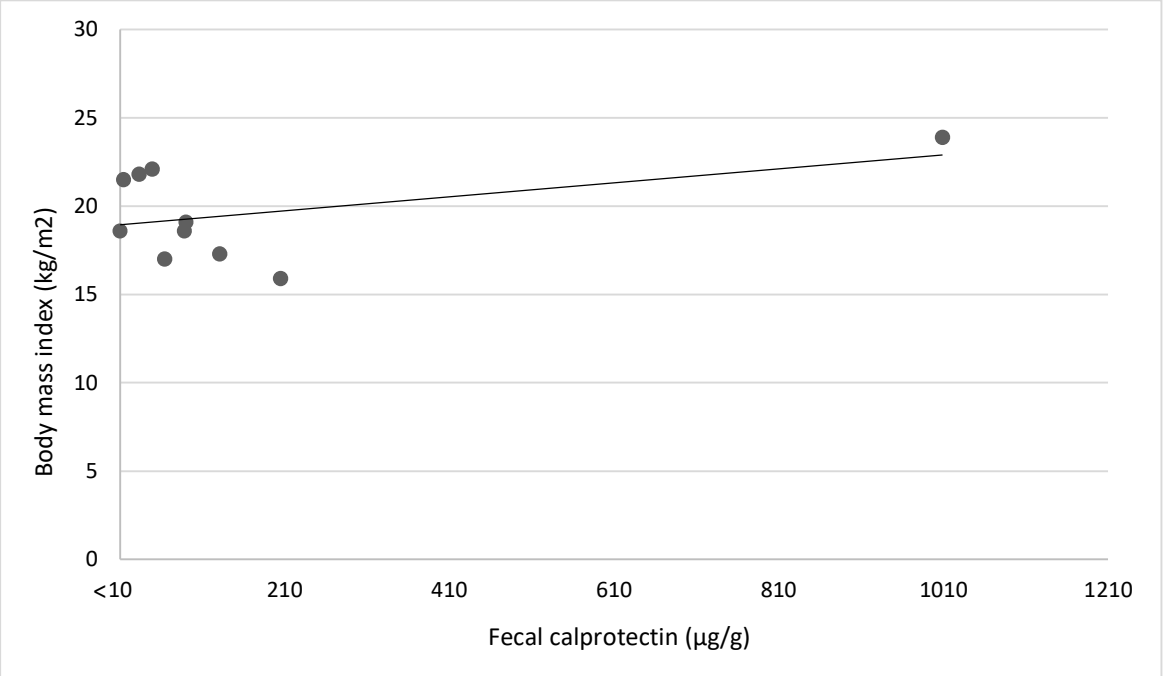

Figure 6: Correlation between fecal calprotectin and BMI in patients treated with CFTR modulators. <10 µg/g represents the lower limit of detection for fecal calprotectin. r = 0.457. *P* = 0.184.

Abbreviations: BMI – Body mass index; CFTR – Cystic fibrosis transmembrane conductance regulator.

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