Original paper

The concentration of calprotectin in the stools of children with diagnosed cystic fibrosis

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Abstract

Introduction: Calprotectin is a protein that plays a regulatory role in inflammatory reactions as an antibacterial and antiproliferative factor.

Aim: To assess the concentration of calprotectin in the stools of patients with diagnosed cystic fibrosis.

Material and methods: Forty-one patients were included in the study, 24 boys and 17 girls, aged from 7 weeks to 18 years. The concentration of calprotectin in stools was assessed with the ELISA method. The analysis included clinical symptoms and the results of laboratory tests and the type of mutation.

Results: An elevated level of calprotectin in the stool was observed in 4/41 (9.7%) patients, mainly in older children, and mainly delta F508/deltaF508 mutation. The correlation between the concentration of calprotectin and clinical symptoms, age, increased indicators of an inflammatory process, levels of protein and aminotransferases in blood serum and the values of acid steatocrit of the stool was not proven.

Conclusions: High concentrations of calprotectin in the stools of children with diagnosed cystic fibrosis do not correlate with the level of advancement of lesions within the gastrointestinal tract. Elevated concentrations of calprotectin in the stools of patients with cystic fibrosis may indicate inflammation of intestine and should be further scrutinised.

Introduction

In patients with cystic fibrosis an increased risk of inflammation within the gastrointestinal tract is observed. The pathomechanism of the process is complex and not entirely understood. Some of the causes include the impaired secretion of intestinal mucus and resulting impaired functions of CFTR protein and abnormal processes of glycosylation. The aetiopathogenesis also includes changes to the contents of the intestinal flora, bacterial overgrowth of the small intestine, chronic antibiotic therapy, recurrent infections of the respiratory tract, low pH of the intestinal contents, and intestinal motility disorders. The inflammatory process is stimulated by interleukin (IL)-1, IL-8, eosinophil cationic protein, and lipopolysaccharide [1, 2]. Smith showed an increased number of proteins of inflammation (albumins, α1-antitrypsyn, IgA, M, G and cytokine IL-8 and IL-1β) and eosinophil cationic protein in the liquid obtained by rinsing the colon of patients with cystic fibrosis [3]. Werlin, in his study using an endoscopic capsule, showed in 26/41 patients with cystic fibrosis inflammatory lesions in the form of swelling, redness, deficits of mucosa, and ulceration within the small intestine [4]. Calprotectin is a protein with a regulatory role in inflammatory processes, and which has antibacterial and proapoptotic properties, probably by binding the ions of calcium and zinc. It is produced by neutrophils, monocytes, and macrophages. The protein is resistant to intestinal bacteria. In inflammations of the gastrointestinal tract, there is an enlargement of mucous permeability and consequently leucocytes filter through the intestinal wall, and there is an increase in the secretion of calprotectin. Calprotectin is a marker acknowledged for the assessment of the activity of inflammatory bowel diseases. An increase in the concentration of faecal calprotectin is preceded by an increase in the acuteness of inflammatory bowel disease (IBD) without clinical symptoms. The test is non-invasive. It is

characterised by a high sensitivity and a low specificity. An increased level of calprotectin in stools is also observed in intolerances and food allergies, celiac disease, and infectious diseases of the gastrointestinal tract, especially of bacterial aetiology [5–8].

Aim

The aim of the study was to assess faecal calprotectin concentration as a marker of gut inflammation in patients with cystic fibrosis.

Material and methods

The study included a group of 41 patients, 24 (58.5%) boys and 17 (41.5%) girls, aged from seven weeks to 18 years (the average age was 4 years).

The following sub-groups were isolated from the patients:

– subgroup 1 (23/41 patients – 56.1%), with cystic fibrosis diagnosed based on newborn screening tests (the average age during the test was 11 months),
– subgroup 2 was composed of 18 children (18/41 – 43.9%) in which the neonatal screening tests for cystic fibrosis were not performed and the cystic fibrosis (CF) was diagnosed at a later age during diagnostic procedures in the Department of Paediatrics (the average age during the tests was 7 years).

The concentration of calprotectin was assessed in all children using the ELISA method and the Phical test (Calpro). The values of calprotectin are presented in ng/ml. The norm was less than 3000 ng/dl.

The analysis included the following criteria: sex, clinical symptoms, type of CFTR mutation, results of laboratory tests (the levels of protein, aminotransferases, C-reactive protein, and the index of acid steatocrit of the stool).

Statistical analysis

Statistical analysis of the data was performed using the procedures of MedCalc v.11.0.1.0 software (MedCalc, Belgium). For the purpose of the description of quantitative variables, average values and their standard deviations were calculated. The normality of distribution of variables was verified using the Smirnov-Kołmogorov test. To describe the qualitative variables, the frequency of their occurrence as absolute values and/or the percentage is given. A *t*-Student test was used to assess the inter-group differences for quantitative variables and the analysis of variance (for the variables fulfilling the criteria) or their non-parametric equivalents; the *U* Mann-Whitney test and Kruskal-Wallis test was used if the distribution was abnormal. The χ^2 test was used for qualitative variables. The criterion of statistical significance was $p < 0.05$.

Results

The clinical picture of the analysed patients is shown in Table I.

Table I. Clinical picture of the analysed patients

Clinical picture	The group of analysed patients (n = 41)	Subgroup 1 – patients subject to the screening test (n = 23)	Subgroup 2 – patients not subject to the screening test (n = 18)
Average AGE	4 years	11 months	7 years
Mutation:			
F508del/F508del	18/41 (43.9%)	8/23 (34.8%)	10/18 (55.6%)
F508del/other	16/41 (39%)	10/23 (43.5%)	6/18 (33.3%)
Other	7/41 (17.1%)	5/23 (21.7%)	2/18 (11.1%)
Distribution of sexes (F/M)	17/24	9/14	8/10
Pancreatic failure	41/41 (100%)	23/23 (100%)	18/18 (100%)
Deficiency of body mass	15/41 (36.6%)	9/23 (39.1%)	6/18 (33.3%)
Symptoms from the respiratory tract	23/41 (56.1%)	9/23 (39.1%)	14/18 (77.7%)
Liver damage	4/41 (9.8%)	1/23 (4.3%)	3/18 (16.6%)
History of meconium ileus	5/41 (12.2%)	3/23 (13.1%)	2/18 (11.1%)
Electrolyte disturbances	3/41 (7.3%)	2/23 (8.7%)	1/18 (5.5%)
Schwachman-Kulczycki score (average) [points]	78.6	81.5	71.9
Average level of calprotectin	1390.8	1670.7	1171.78
Elevated level of calprotectin (number of patients)	4/41 (9.7%)	1/23 (4.3%)	3/18 (16.6%)

An elevated level of calprotectin in stools was observed in 4/41 (9.7%) patients with cystic fibrosis. There were no statistically significant differences between the concentrations of calprotectin in the stools and the patients' sex.

The statistical significance between the concentration of calprotectin and diarrhoea in children with diagnosed cystic fibrosis was shown. The values were statistically significantly higher in children with diarrhoea ($p < 0.05$) (2206.7 vs. 1302). The co-existence of acute infectious diarrhoea in those patients was ruled out during the determination of the level of calprotectin in stools.

The correlation between the concentration of calprotectin and other clinical symptoms, including the level of malnutrition, symptoms from the respiratory tract, undergone meconium ileus, age of when cystic fibrosis was diagnosed, body mass at birth, and the Apgar score was not observed (Tables II, III).

The analysis of the concentration of calprotectin in stools was performed depending on when the cystic fibrosis was diagnosed: based on the screening newborn test (subgroup 1) vs. during the differential diagnostic procedures of the clinical symptoms (Table IV). Higher average concentrations of calprotectin in the stools of patients subject to screening test were proven, despite the earlier diagnosis and treatment.

All patients with high concentrations of calprotectin in stools carried the CFTR gene as delta F508/delta F508 homozygote. However, average values of calprotectin in stools were not statistically different between patients with various mutations (Table V).

No correlation between the concentration of calprotectin and the parameters of functions and dam-

Table II. Analysis of the concentrations of calprotectin in stools depending on clinical symptoms

Clinical symptom	Average concentration of calprotectin [ng/ml]	Number of patients with elevated concentration of calprotectin	Statistical significance, *p*
Pancreatic failure *n* = 41/41	1276.0	4/41	NS
Recurrent diarrhoea *n* = 4/41	2206.7	2/4	< 0.05
Deficiency of body mass *n* = 15/41	1444.1	2/15	NS
Low concentration of protein and/or albumins *n* = 14/41	1109.14	2/14	< 0.05
Symptoms from the respiratory tract *n* = 23/41	1417.5	2/23	NS
Liver damage *n* = 4/41	1263.0	1/4	NS
History of meconium ileus *n* = 5/41	1372.3	1/5	NS
Electrolytes disturbances *n* = 3/41	1562.5	1/3	NS

Table III. Concentrations of calprotectin in stools depending on the age of patients with cystic fibrosis

Parameter	Age < 12 months	Age 12 months – 6 years	Age > 6 years
Average	1073.2	1588.3	1835.5
Min.	355.9	493.6	757.8
Max.	3612.3	2893.7	3878.5
Median	873.3	1357.1	1411.6
Elevated levels *n* (4/41)	1/21	0/9	3/11
Statistical significance	NS	NS	$p < 0.005$

Table IV. The comparison of the concentrations of calprotectin in subgroup 1 of patients subject to the screening test and subgroup 2

Parameter	All analysed patients (n = 41)	Subgroup 1 (cystic fibrosis diagnosed in the screening test) (n = 23)	Subgroup 2 (patients not subject to the screening test) (n = 18)
Min.	355.9	493.6	355.9
Max.	3878.5	3878.5	3612.33
Average	1390.8	1670.7	1171.78
Median	1276.0	1384.39	875.1
Values over the norm	4/41 (9.7%)	1/23 (4.3%)	3/18 (16.6%) $p < 0.05$

Table V. Concentrations of calprotectin in stools depending on the mutation of the CFTR gene

Parameter	Type of mutation		
	Homozygote delta F508	Heterozygote delta F508/other	Other mutations
Min.	493.68	355.98	536.01
Max.	3878.5	2377.6	1979.82
Average	1554.2	1333.0	1102.6
Median	1260.4	1352.0	1025.1
Values over the norm	4/41 $p < 0.005$	– NS	– NS

age to liver cells and the secretion of fat in stools was proven.

Discussion

Cystic fibrosis is a destructive inflammatory disease of a largely diverse clinical expression. In many studies a more frequent than populational occurrence of inflammatory lesions in the intestine, mainly in older children, was observed. Calprotectin is a marker of inflammation within the intestine. It has a regulatory role in inflammatory reactions as an antibacterial and antiproliferative factor. It inhibits bacterial enzymes, induces the apoptosis of cells, and stimulates neutrophils and the production of IL-8 [1, 2, 4].

Among our patients an elevated level of calprotectin was observed in 4/41 patients (9.7%). The patients were delta F508/delta F508 homozygotes. In the course of CF a more frequent occurrence of respiratory infections, bacterial and fungal colonisations (especially *Pseudomonas aeruginosa*), which may theoretically induce a elevated level of calprotectin, are more common [9–11]. The clinical pictures of our patients were dominated by symptoms from the respiratory tract (over 56%) and deficiency of body mass of a varied degree. We proved that there was a statistically significant difference between the concentration of calprotectin and diarrhoea in children with diagnosed cystic fibrosis (2206.7 ng/ml vs. 1302 ng/ml). The concentration of calprotectin was not affected by the degree of malnutrition, abnormalities within the respiratory tract, or the history of meconium ileus. The patient with the highest concentrations of calprotectin in their stools, due to respiratory failure, was qualified for a lung transplant. However, reports about correlations between faecal calprotectin concentrations and parameters of inflammation in children with cystic fibrosis are divergent. Even though the studies of other authors showed a correlation between the concentration of calprotectin in the stool and other parameters of inflammation (the C-reactive protein and the erythrocyte sedimentation rate (ESR)), those correlations were not concluded in our patients. In patients with CF Grey a correlation between pulmonary exacerbations and the concentration of calprotectin in the sputum and blood serum was seen [2, 4, 9].

Lisowska and Walkowiak showed more frequent occurrence of elevated concentrations of calprotectin in stools of children with cystic fibrosis compared with

their concentrations in the stools of healthy children. Also in our studies the average values of calprotectin were higher in the group of infants [12].

The analysis of the concentration of calprotectin with regard to age showed more frequent occurrence of its elevated levels in older children. Three of 4 of patients with elevated levels of calprotectin were over 6 years old. In the course of cystic fibrosis the abnormalities within the respiratory tract, especially the lungs, intensify with age. On the other hand, according to literature on the subject, the concentrations of calprotectin are usually higher in the youngest patients, which require further studies.

Lisowska and Walkowiak, similarly to us, did not show a correlation between the concentrations of calprotectin in stools with the level of malnutrition and the occurrence of bronchopulmonary abnormalities [12, 13]. Werlin concluded a significantly higher concentration of calprotectin in stools of patients with cystic fibrosis accompanied by pancreatic failure, which was not confirmed in our studies [4].

The correlation between the CFTR genotype and the clinical symptoms is incomplete, especially in terms of the pulmonary manifestation. As molecular studies suggest, the symptoms of cystic fibrosis are observed when the activity of the CFTR protein does not exceed 10% of the reference value. In our patients, the elevated levels of calprotectin were observed only in patients with delta F508/delta F508 mutation, which may indirectly confirm potential inflammation within the mucous membrane of the intestine.

No correlation was concluded between the level of calprotectin in stools and the concentrations of the parameters of an inflammation within the blood serum (the C-reactive protein, leukocytes). Also, Golden in his studies did not show such a correlation, and he claimed that the concentration of calprotectin in the sputum is a much better marker of inflammation within the respiratory tract [1].

In inflammation of the intestine, especially in IBD, the correlation between an increased concentration of calprotectin and the intensification in the acuteness of the inflammation resulting from an intensified migration of neutrophils has been proven. The correlation between the concentration of calprotectin in stools and intensification of inflammatory lesions was concluded following colonoscopy and histopathology tests. The determination of the concentration of calprotectin in stools helps distinguish between the organic causes of chronic diarrhoea and irritable bowel syndrome. Consequently, this may be a promising, non-invasive, and cheap diagnostic method used in screening tests for organic diseases of the bowels. It seems that due to an increase in the frequency of inflammatory bowel disease in patients with cystic fibrosis compared with the population of healthy children, these patients should be further observed for other coexisting diseases [3, 8].

There are studies confirming higher levels of calprotectin in stools with recently diagnosed celiac disease. Positive results for antibodies for tissue p/transglutaminase IgA in blood serum were not observed in any of our patients with elevated levels of calprotectin in their stools, and at the same time the concentrations of A immunoglobulin in the blood serum were normal. Montalto *et al.* in their study did not conclude a correlation between elevated levels of calprotectin in stools and the atrophy of intestinal villi in patients with untreated celiac disease [14].

There are studies showing a correlation between elevated levels of calprotectin in stools and bacterial overgrowth of the small intestine. Bacterial overgrowth of the small intestine is very often observed in patients with cystic fibrosis and may affect as much as 30–50% of patients. The causes may involve a long antibiotic therapy and disorders in the motor activity of the gastrointestinal tract [13, 15, 16]. In patients with cystic fibrosis an increased production of methanol in the colon is also reported more often. In our 4 patients with an elevated concentration of calprotectin we did not observe the characteristics of bacterial overgrowth of the small intestine in the hydrogen breath test. On the other hand, Bruzzese concluded higher average concentrations of calprotectin in the stools of patients with diagnosed cystic fibrosis compared to their healthy peers, and positive results of including probiotics in the treatment (*Lactobacillus casei* strain GG), which resulted in a decrease in the concentration of calprotectin in the patients' stools, and which may indirectly confirm a reduction in the inflammation [17].

Conclusions

Concentrations of faecal calprotectin in children with diagnosed cystic fibrosis do not correlate with the level of pancreas failure and increased parameters of liver damage and cholestasis. Elevated concentrations of calprotectin in the stools of patients with cystic fibrosis may indicate inflammation of the intestine, and require further detailed observation.

Conflict of interest

The authors declare no conflict of interest.

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