THE VALUE OF FAECAL CALPROTECTIN ASSESSMENT IN CHILDREN WITH CHRONIC GASTROINTESTINAL SYMPTOMS

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Abstract

Background: Various studies have described fecal markers as powerful markers of inflammation of the intestinal mucosa in patients with inflammatory bowel disease (IBD). Calprotectin is a calcium-binding protein found in abundance in neutrophils, where it accounts for 60% of the protein in the cytosol. *Aim:* Several studies have compared fecal calprotectin with activity indexes and/or endoscopic/histological evaluation to confirm intestinal inflammation in IBD patients. This study proposed to assess the acuraccy of fecal excretion biomarker calprotectin in children with chronic gastrointestinal manifestations. The objective was to assess calprotectin as indicator of IBD activity using a commercial rapid semi-quantitative test (Cal Detect). *Material and methods:* 82 children (aged 18 months -18 years), presenting at least one of the following symptoms: diarrhea, rectal bleeding, recurrent abdominal pain, weight loss, constipation or alternative bowel habits were clinical and biological examined. Stool samples were collected from all of them and tested for calprotectin using a commercially available kit. In parallel, all patients were referred to colonoscopy. *Results:* 14 children were diagnosed with IBD (9 associating Crohn's disease – CD and 5 ulcerative colitis – UC). The remaining of 68 children formed the control lot, and their diagnoses included: chronic constipation, rectal and/or sigmoydian polyps, eosinophylic proctitis associated with cow's milk protein allergy or irritable bowel syndrome. The overall specificity (Sp) for IBD was 66% for a positive cut off point of 15 µg/g, and became 100% when raising the positive cut off point to 60µg/g (p= 0.0003). *Conclusions:* Raised fecal calprotectin more than 15 µg/g should prompt endoscopic assessment in children with chronic intestinal symptoms, since an organic bowel disorder is likely. Being an invasive method, colonoscopy can be avoided in children with gastrointestinal symptoms and low positive levels of fecal calprotectin between 0 and 15 µg/g. Fecal calprotectin detected by this rapid semi-quantitative test represents a sensitive and specific marker for detection of intestinal inflammation in children with CD and UC at a positive cut off point of 60 µg/g.

Key words: Crohn's disease, ulcerative colitis, calprotectin, inflammation, children

Introduction

Endoscopic evaluation with histopathological sampling is generally considered indispensable in the investigation of patients with suspected inflammatory bowel diseases (IBD). In a relatively large proportion of children with chronic gastrointestinal symptoms and suspected IBD, the results of endoscopy will be negative. Laboratory parameters such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum fibrinogen level and haematocrit, among others, are not specific to active IBD, so those can not be used routinely as markers of inflammatory activity in clinical practice (1), (2). In order to avoid invasive investigations, several noninvasive markers have been suggested to distinguish functional gastrointestinal disorders from organic diseases. Among these, fecal calprotectin concentrations have been shown to be a good marker of intestinal mucosal inflamation, being higher in patients with IBD than in controls (3). Calprotectin constitutes ~60% of the soluble cytosol proteins in neutrophil granulocytes and plays a central role in neutrophil defense. (4) Consequently, its concentration in stool correlates with the intensity of neutrophil infiltration of the intestinal mucosa and with the severity of inflammation. Furthermore, its in vivo and in vitro resistance to degradation allows fecal samples to be assayed for a reliable calprotectin determination (5). Faecal calprotectin is found elevated in adults and children with various gastrointestinal infections, but the concentrations are lower than in persons with IBD. (6), (7) Calprotectin is present in plasma, and the faecal calprotectin concentrations might be increased with any bleeding into the gastrointestinal tract (8). Elevated concentrations of faecal calprotectin have been described in cystic fibrosis, rheumatoid arthritis, Crohn's disease (CD), ulcerative colitis (UC) and bacterial infections, as well as neoplastic conditions (9). This new approach to the diagnosis of IBD - fecal calprotectin testing - is a useful tool for identifying patients who are most likely to need endoscopy for suspected IBD, thereby reducing the number of unnecessary endoscopies especially in children.

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Objectives of the study

Several studies have compared fecal calprotectin with activity indexes and/or endoscopic/histological evaluation to confirm intestinal inflammation in IBD patients. The results of these studies are promising, demonstrating that these markers are useful in detecting inflammation and differentiating it from other diseases as well as in predicting recurrence for periods of up to one year. This study proposed to assess the acuraccy of fecal excretion biomarker calprotectin in children with chronic gastrointestinal symptoms. The objective was to assess calprotectin as indicator of IBD activity using a rapid semi-quantitative test – Cal Detect.

Material and methods

The study was developed between October 2009 and December 2011 and included 82 consecutive patients presenting one or more of the following symptoms: diarrhea, rectoragia, recurrent abdominal pain, constipation, alternating constipation / diarrhea, fever, pallor, fatigue, weight loss, continuous or intermittent symptoms present at least one month before study entry. The patients' age varied between 1 year 6 months and 18 years old. One mandatory criteria for inclusion in the study was the written informed consent of children's legal tutors, accepting all the clinical and biological tests, including the lower ± upper digestive endoscopy followed by intestinal biopsy sampling.

Exclusion criteria were the presence of hepatitis B, C or HIV, bacterial or viral (Rotavirus or Adenovirus) diarrhea and intestinal parasitosis.

We performed to all children a complete initial clinical examination, followed by biological evaluation (CBC, CRP, ESR, fibrinogen, serum iron, blood glucose, liver and renal tests), assessment of total and specific IgE to food allergens - in some selected cases and viral markers such as HBsAg / HBsAb, HCVAb and HIV test for the exclusion of positive cases. Stool samples were taken in order to identify the Rotavirus and Adenovirus fecal antigens. Three consecutive coproparasitological exams were needed to exclude children with parasitic diarrhea from the study. Fecal calprotectin was assessed in all children. Colonoscopy ± colonic biopsy was performed after blood and stool sample examination to all patients who fulfilled the inclusion criteria in the study.

Calprotectin determination from feces was done using Cal Detect test, a rapid immuno-chromatographic semi-quantitativ test. Calprotectin (MRP 8 / 14) is a heterodimer of two proteins that are linked by calcium (MRP 8 and MRP 14), present in the cytoplasm of neutrophils and expressed by the membrane of monocytes. The physician can distinguish the value of calprotectin thanks to the presence of three strips proportional with the inflammatory level. Fecal sample should be placed directly on the foil tape included in the kit. According to the manufacture company instructions, the test shows a strip which represents the control. The first strip indicates that calprotectin concentration is less than 15 µg/g, value that is not representative in cases of intestinal inflammation and may indicate the presence of intestinal bacterias. The presence of a second strip indicates that calprotectin concentration is between 15 and 60 µg/g, which indicates an acute inflammation of the intestinal mucosa. The appearance of a third strip shows that the concentration of calprotectin is greater than 60 µg/g and displays a high degree of inflammation of the intestinal mucosa.

We performed to all children from our study a colonoscopy after a short-term sedation with Propofol and Midazolam. According to macroscopic appearance of mucosa we performed only sigmoidoscopy, partial, or total colonoscopy with the examination of the first 10 cm of terminal ileum. Endoscopic biopsies were taken in patients with affected areas. When the colonoscopic appearance was typical for Crohn's disease, upper (gastrointestinal) endoscopy was done to quantify the extension of lesions.

Statistical analysis was performed using SPSS 16 (Statistical Package for Social Sciences for Windows version 16).

Results and discussions

Out of the 82 patients, which were clinical, biological and endoscopic examined, 14 children were diagnosed with inflammatory bowel disease (IBD) based on endoscopic aspect and on the results of colonic biopsies. Of these, 9 patients had ileo-colic form of CD and the rest were diagnosed with hemorrhagic ulcerative colitis (UC).

The rest of patients without inflammatory bowel disease that was excluded based on colonoscopy ± biopsies, were diagnosed as follow:

- 23 cases were diagnosed with irritable bowel syndrome (IBS) according to third Rome criteria,
- 31 children had functional constipation (FC) and some of them associated anal fissures
- 8 children had singular and benign sigmoid polyps (BSP)
- 6 cases presented eosinophilic proctitis (EP) in association with cow's milk protein allergy (Figure 1).

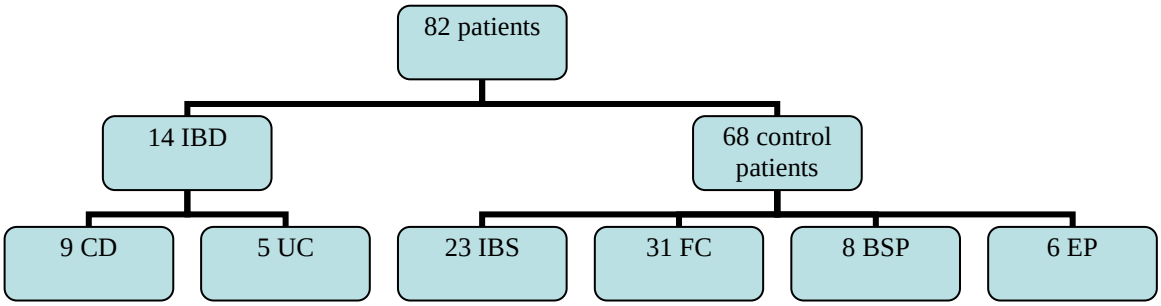

Figure 1: Distribution of diagnosis after colonoscopy was performed.

To assess the accuracy of calprotectin detected due to the intestinal inflammation at patients with CD and UC, sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (VNP) were calculated according to statistical formulas listed below (Table I).

Table I: Definition of statistical parameters used in this study.

	Present Disease (D+)	Absent Disease (D-)	Total
Positive Test (T+)	a	B	a+b
Negative Test (T-)	c	D	c+d
Total	a+c	b+d	a+b+c+d

a = true positive subjects (RP)
d = true negative subjects (RN)
b = false positive subjects (FP)
c = false negative subjects (FN)
Sn = a / a + c = RP / total B +
Sp = d / b + d = RN / total B –
PPV = a / a + b = RP / T + total
VNP d / c + d = NR / T-Total

Our study lot consisted in14 patients with IBD. It was analyzed in terms of the distribution by age and sex. The average age of patients was 14.5 years and sex ratio F / B was 5 / 9 (36% girls and 64% boys). The rest of 68 patients without endoscopic aspects and / or histological characteristics of CD or UC, were considered to be the control group. All 14 patients with histologic aspects characteristic for IBD showed positive values of calprotectin. In three cases the titer was between 15-60 µg/g, while the rest had values greater than 60 µg/g, according to cut off values established by the manufacture company of Cal-Detect kits (Figure 2). None of the 68 patients from the control group (without inflammatory bowel disease) had positive values of calprotectin greater than 60 µg/g. There were negative values of calprotectin (0-15 mg / g) in 45 patients, while in 23 of the children without inflammatory bowel disease it was detected, however, a positive value of calprotectin between 15 and 60 µg/g, underlining by the presence of inflammation in the gut due to other conditions than IBD (chronic constipation, anal fissures, polyps traumatized by sigmoid passage of faeces, eosinophilic proctitis) (Figure 3).

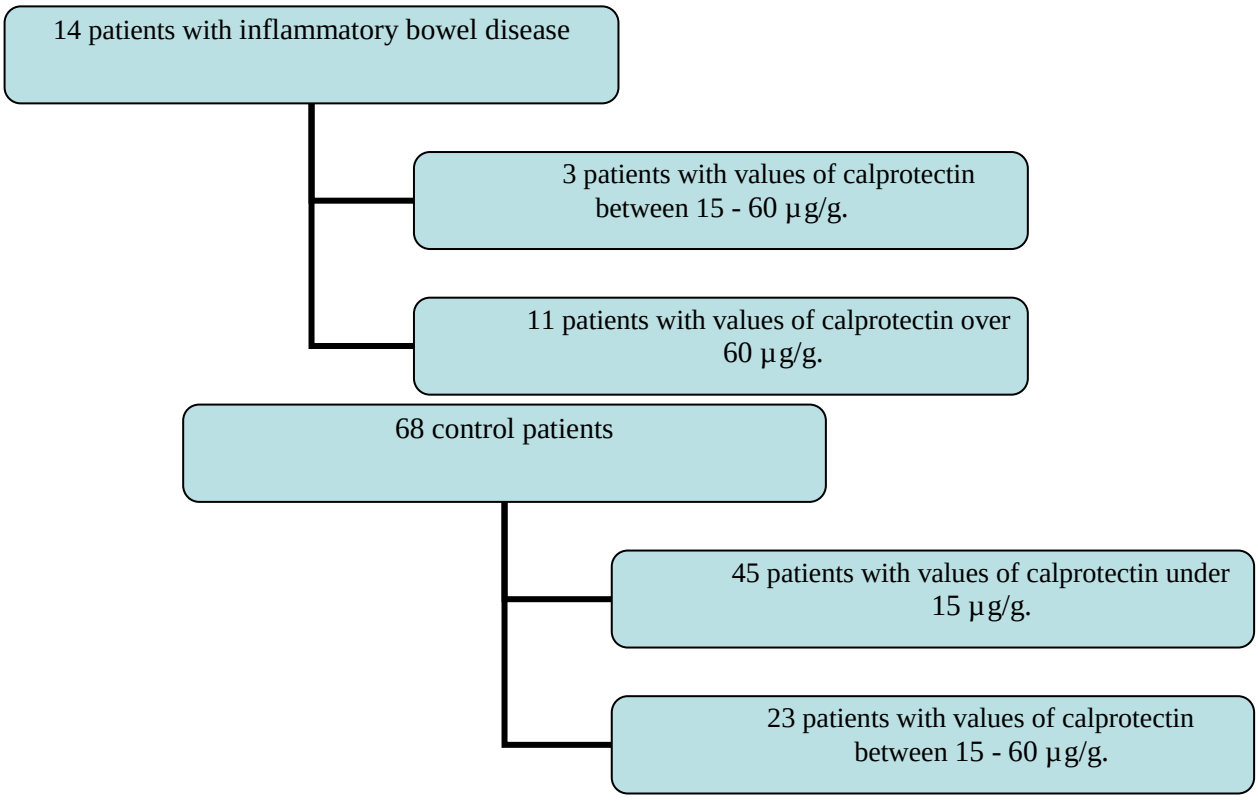

Figure 2: Semi-quantitative assessment of fecal calprotectin determined by Cal Detect kits in patients with inflammatory bowel disease and in control group.

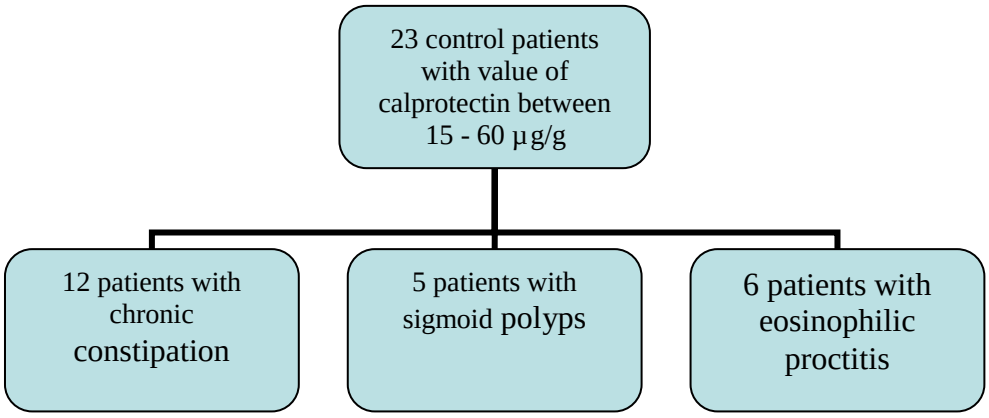

Figure 3: Distribution of diagnoses in children from the control group with positive fecal calprotectin.

We calculated the values of Sn, Sp, PPV and NPV of calprotectin in patients diagnosed with IBD, with a positive cut-off set at 15 µg/g first and then with a positive cut-off set at 60 µg/g and then we compared the datas.

Table II: Sn, Sp, PPV and NPV values of calprotectin using Cal Detect kits for the diagnosis of IBD at a positive cutt-off set at 15 µg/g

Test with positive cutt-off > 15µg/g	Sn	Sp	VPP	VPN
Calprotectin	100%	66%	37%	100%

Table III: Sn, Sp, PPV and NPV values of calprotectin using Cal Detect kits for the diagnosing of IBD at a positive cutt-off set at 60 µg/g

Test with positive cutt-off > 60 µg/g	Sn	Sp	VPP	VPN
Calprotectin	78%	100%	100%	95%

Statistic analyze was performed using SPSS 16 programme. We used chi-square test for comparison of specificity and positive predictive values of calprotectin considered positive first at a cut-off of 15 µg/g, and then 60 µg/g. We obtained a statistically significant difference p = 0.0003.

Although this study evaluated calprotectin at a positive cut-off of 15 µg/g, it showed a good sensitivity. The lower value of its specificity at this cut-off was responsable for a significant lower PPV.

None of the 68 patients from the control group showed positive values of faecal calprotectin in titers greater than 60 µg/g, but, however a moderate value of calprotectin between 15 and 60 µg/g was detected in 23 of the children without IBD due to the presence of inflammation in the gut because the underlying disease. This fact lowers the specificity of Cal-Detect kit in diagnosis of IBD at moderate values between 15 and 60 µg/g. If the threshold is increased and we consider values of calprotectin greater than 60 µg/g significant for IBD, the specificity of this test will increase to 100%. So, we can diagnosed pediatric patients with inflammatory bowel based on clinical and biological picture suggestive for this disease, which associates calprotectin values greater than 60 µg/g. In children with clinical and biological data characteristics for IBD, calprotectin values between 15 and 60 µg/g, colonoscopy and serial biopsies are needed for the diagnosis. Colonoscopy ± upper digestive endoscopy investigations are mandatory in all children with IBD, because it helps to establish the extension of the disease, makes the differences between CD and UC and contribute to the pursuit of therapeutic effectiveness (10). Semi-quantitative rapid Cal- Detect test for detection of calprotectin in the faeces has a high negative predictive value of great importance in the selection of pediatric patients with intestinal symptoms (abdominal pain, diarrhea, rectoragia, stagnation weight, etc.) for colonoscopy. Tests with values below 15 µg/g can exclude the colonoscopy in children, sparing pediatric patients of the discomfort of an invasive, traumatic exploration.

Conclusions

Fecal calprotectin detected by a rapid semi-quantitative test using Cal Detect kits represents a sensitive and specific marker for detection of intestinal inflammation in children with CD and UC at a positive cut off point of 60 µg/g. Raised faecal calprotectin more than 15 µg/g should prompt endoscopic assessment in children with chronic intestinal symptoms, since an organic bowel disorder is likely. Being an invasive method, colonoscopy can be avoided in children with gastrointestinal symptoms and low positive levels of fecal calprotectin between 0 and 15 µg/g. Recent studies reported an increasing of IBD prevalence in children. A lot of other bowel disorders can present with similar clinical manifestations (cow's milk protein allergy, eosinophylic

proctitis, anal fissure, chronic constipation etc) (11). Therefore, it is useful to promptly detect intestinal inflamation by rapid fecal markers, in order to select the referral to endoscopy of pediatric patients. Due to its high negative predictive value, fecal calprotectin assessment using quick test Cal Detect can avoid unnecessary colonoscopy in children, making this a cost/efficient test in pediatric digestive pathology.

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