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Regular paper

Fecal lactoferrin, a marker of intestinal inflammation in children with inflammatory bowel disease

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The aim of this study was to analyze the usefulness of fecal lactoferrin in the diagnosis and monitoring of inflammatory bowel disease (IBD) in children. The study included 52 children with IBD (24 with Crohn's disease and 28 with ulcerative colitis) aged between 0.92 and 18 years, and 41 IBD-free controls of similar age. Fecal concentration of lactoferrin was determined with a quantitative immunoenzymatic test. Fecal concentration of lactoferrin in children with IBD was significantly higher than in the controls. The cut-off value of fecal lactoferrin concentration optimally distinguishing between the children with IBD and the controls was identified as 13 μg/g. The sensitivity and specificity of this cut-off value equaled 80.7% and 92.7%, respectively, and its positive and negative prognostic values were 96.8% and 63.3%, respectively. Patients diagnosed with moderate Crohn's disease had significantly higher fecal concentrations of lactoferrin than children with the mild or inactive disease. Similarly, children with moderate ulcerative colitis showed significantly higher fecal concentrations of lactoferrin than individuals with the mild condition. No significant relationship was found between the fecal concentration of lactoferrin and the severity of endoscopic lesions. Patients with IBD and a positive result of fecal occult blood test were characterized by significantly higher concentrations of lactoferrin than the individuals with IBD and a negative result of this test. In conclusion, fecal concentration of lactoferrin seems to be a useful parameter for diagnosis and monitoring of IBD in children.

Key words: fecal lactoferrin, inflammatory bowel disease, children

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INTRODUCTION

The group of chronic inflammatory bowel diseases (IBD) is comprised of Crohn's disease (CD) and ulcerative colitis (UC). An increase in the incidence of IBD has been recently observed, also in the pediatric population (Langholz *et al.,* 1997; Sawczenko *et al.,* 2001; Karolewska-Bochenek *et al.,* 2004; van der Zaag-Loonen *et al.,* 2004; Ravikumara & Sandhu 2006). IBD has a relatively complex etiology and is characterized by a relapsing-remitting course (Day *et al.,* 2012). Its principal clinical manifestations include abdominal pain, diarrhea and weight loss. Other common IBD-related complaints are recurrent fever, apathy, anorexia and paleness (Langholz *et al.,* 1997). Diagnosis of IBD is based on medical history, physical examination, laboratory testing, endoscopy and histopathological evaluation (Langholz *et al.,* 1997). Diagnostic value of fecal IBD markers is a subject of ongoing discussion. Some of these markers, such as calprotectin, alpha-1 antitrypsin, lysozyme, myeloperoxidase and neutrophil elastase, proved to be sensitive enough to accurately reflect the dynamics of intestinal inflammation and became an established component of clinical practice (Vermeire *et al.,* 2006).

Only few previous studies analyzed the diagnostic role of another fecal marker of gastrointestinal inflammation, lactoferrin (Kane *et al.,* 2003; Walker *et al.,* 2007). Lactoferrin is a 80-kDa transferrin characterized by high affinity to iron ions (Soerensen & Soerensen 1,939). It was first identified in bovine milk by Soerensen & Soerensen (1939). In 1960, three investigators have independently extracted lactoferrin from human breast milk (Groves 1960; Johanson, 1960; Montreuil *et al.,* 1960). This protein was shown to be an antibacterial and antiviral component of human innate immune system (Lu *et al.,* 1987; Valenti & Antonini, 2005). Moreover, lactoferrin may modulate inflammatory response to infectious factors and other antigens (Lu *et al.,* 1987). It is excreted by neutrophils, cells of mammary glands and other epithelial cells, and was isolated from blood, saliva, tears, semen, respiratory mucous and stool (Masson *et al.,* 1966; Levay & Viljoen, 1995; Baker & Baker, 2005). Importantly, lactoferrin can pass through the digestive tract in an unchanged form due to resistance to some proteases, including trypsin and its derivatives (Iyer & Lonnerdal, 1993). However, fecal concentration of this protein should be interpreted carefully in breastfed infants since, as mentioned above, this protein can be also synthesized in the mammary gland (Oberhelman *et al.,* 1999).

The aim of this study was to analyze the usefulness of fecal lactoferrin in the diagnosis and monitoring of IBD in children.

MATERIALS AND METHODS PATIENTS

This study included a total of 93 children, among them 40 (43%) girls and 53 (57%) boys. The participants were divided into two groups. The group under investigation included 52 children with IBD (24 with CD and 28 with UC; 32 boys and 20 girls) aged between 0.92 and 18 years (mean 14.2 years, median 15.67 years). The control group was comprised of 41 IBD-free individuals

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Abbreviations: IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; PCDAI, pediatric Crohn's disease activity index; PPV, positive predictive value; NPV, negative predictive value; ROC, receiver operating characteristic

Table 1. Characteristics of the study participants

Group	n	%	Age (years)	Sex (f/m)
Inflammatory bowel disease	52	55.9%	0.92-18	20/32
– Crohn's disease	24	25.8%	0.92-18	11/13
– Ulcerative colitis	28	30.1%	1.92-18	9/19
Controls	41	44.1%	0.41-18	22/19

Table 2. Severity of macroscopic intestinal lesions documented on colonoscopy (Langhorst *et al.*, 2008)

Score	Description
0	Normal intestinal mucosa
1	Erythema, decreased vascular pattern, friability of mucosa, single aphthous lesions
2	Multiple aphthous lesions and erosions
3	Large ulcerous lesions, spontaneous bleeding, narrowing of the intestinal lumen

(23 healthy children and 18 patients diagnosed or treated due to non-inflammatory gastrointestinal conditions) (Table 1). The age of the controls ranged between 0.41 and 18 years (mean 9.78 years, median 9 years).

The protocol of the study was approved by the Local Bioethics Committee of the Medical University of Gdansk, and parents of all the children gave their informed consent for their participation in the project.

Medical histories were obtained from all children. Moreover, all the participants were subjected to physical examination, laboratory testing (erythrocyte sedimentation rate and complete blood count) and fecal occult blood testing.

A total of 57 colonoscopies were performed in 43 out of the 52 children with IBD (CD=25 and UC=32). All the colonoscopies were performed under general anesthesia with an aid of an OLYMPUS PCF-16OAL endoscope. The severity of inflammatory lesions was classified according to Langhorst and coworkers (2008) (Table 2). The results of endoscopic examination constituted the basis for determining the pediatric Crohn's disease activity index (PCDAI) scores and Truelove-Witts' severity index scores of ulcerative colitis.

Determination of fecal lactoferrin concentration. A total of 155 stool samples were obtained and subjected to analysis, including 114 samples from the children with IBD (CD n=56, UC n=58) and 41 samples from the controls (Table 3). The samples were placed in plastic containers, frozen and stored at –72°C until analysis. Fecal concentration of lactoferrin was determined with an IBD-SCAN™ quantitative immunoenzymatic test (catalogue no. 303511 TECHLAB, USA). The test uses antibodies to human lactoferrin. The samples were diluted at 1:100, 1:400, 1:1000 and 1:4000 and further handled according to the manufacturer's instructions. Absorbance of the samples was determined with an ElizaMat™ 3000 reader (DRG MedTek, Poland).

Table 3. Characteristics of the examined stool samples

Group	n	%
Crohn's disease	56	36.1
Ulcerative colitis	58	37.4
Controls	41	26.5
Total	155	100.0

Statistical analysis. As normal distribution of fecal lactoferrin concentration was not confirmed with the Kolmogorov-Smirnov test, statistical characteristics of this variable in the analyzed groups were presented as medians, lower and upper quartile values, and ranges. Mann-Whitney U-test and Kruskal-Wallis test with Scheffe's post-hoc test were used for intergroup comparisons. The cut-off value of fecal lactoferrin concentration optimally distinguishing between the children with IBD and the controls, as well as the sensitivity, specificity, positive and negative predictive values (PPV and NPV) of this parameter were identified by receiver operating characteristic (ROC) analysis. All the calculations were performed using Statistica 10 (StatSoft, Tulsa OK, United States) software, with statistical significance defined as $p \leq 0.05$.

RESULTS

Fecal concentration of lactoferrin in children with IBD (both in the whole IBD group and in the subsets of patients with CD and UC) was significantly higher than in the controls ($p < 0.001$ for all comparisons; Fig. 1 and Table 4).

The cut-off value of fecal lactoferrin concentration optimally distinguishing between the children with IBD and the controls was identified as 13 μg/g. The sensitivity and specificity of this cut-off value equaled 80.7% and 92.7%, respectively, and its PPV and NPV were 96.8% and 63.3%, respectively.

Patients diagnosed with moderate CD on the basis of PCDAI scores had significantly higher fecal concentrations of lactoferrin than children with mild or inactive CD ($p < 0.05$ for either comparison). The latter two groups did not differ significantly in terms of their fecal lactoferrin concentrations ($p > 0.05$; Fig. 2).

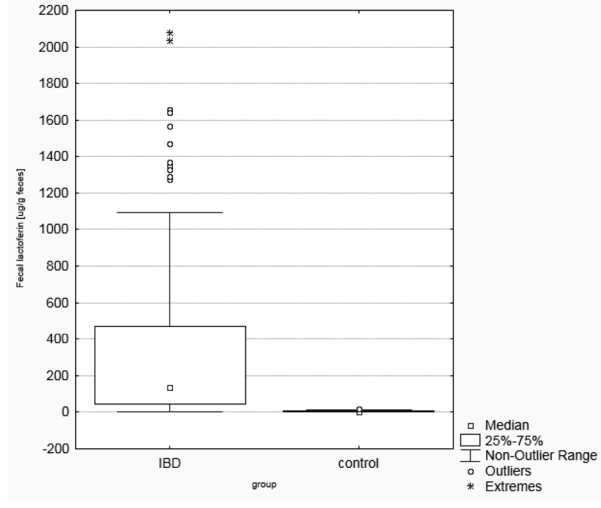

Figure 1. Fecal concentrations of lactoferrin in children with inflammatory bowel disease and in the controls.

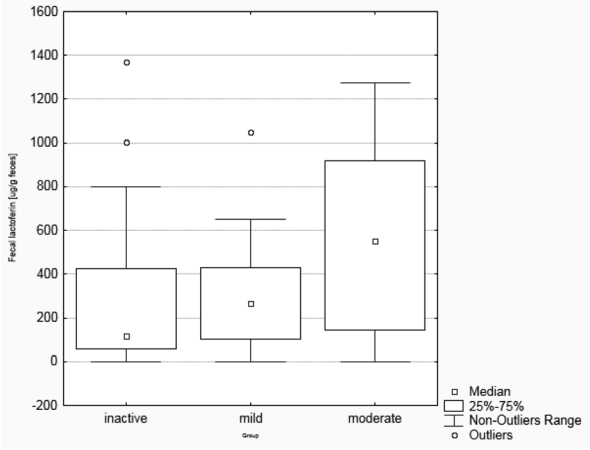

Figure 2. Fecal concentrations of lactoferrin in patients with various activity of Crohn's disease scored according to the pediatric Crohn's disease activity index.

Table 4. Fecal concentrations of lactoferrin in children with Crohn's disease and ulcerative colitis

Group	n	Median (range) of fecal lactoferrin concentration (μg/g)
Crohn's disease	56	173.96 (0.01–1368.80)
Ulcerative colitis	58	104.31 (0.55–2080)
Controls	41	1.43 (0.26–71.26)

Children with moderate UC according to the Truelove-Witts' index values showed significantly higher fecal concentrations of lactoferrin than individuals with mild UC ($p<0.05$; Fig. 3). The analysis did not include patients with severe UC due to a too small size of this subset.

We did not find a significant relationship between the fecal concentration of lactoferrin and the severity of endoscopic lesions classified according to Langhorst *et al.,* (2008) ($p>0.05$; Fig. 4).

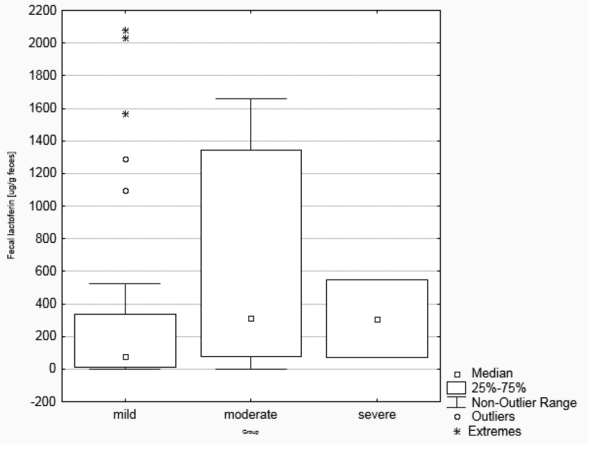

Figure 3. Fecal concentrations of lactoferrin in patients with various activity of ulcerative colitis scored according to the Truelove-Witts' index.

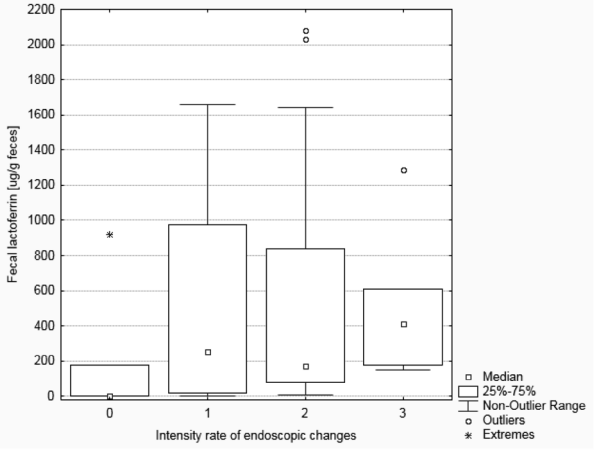

Figure 4. Fecal concentrations of lactoferrin in inflammatory bowel disease patients with various severity of endoscopic lesions (0 – no changes, 1 – mild, 2 – moderate, 3 – severe lesions).

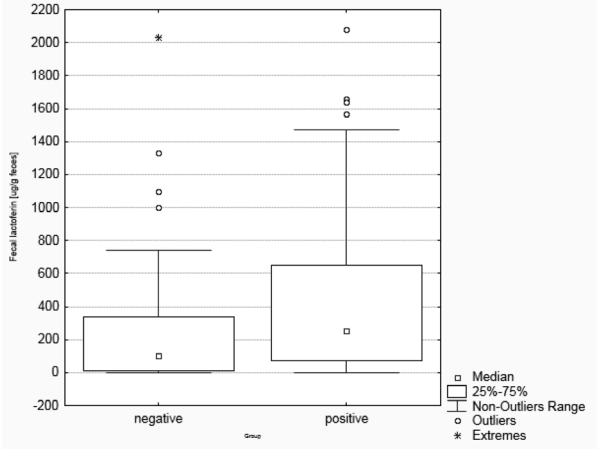

Figure 5. Fecal concentrations of lactoferrin in inflammatory bowel disease patients with positive and negative results of fecal occult blood test.

Patients with IBD and a positive result of fecal occult blood test were characterized by significantly higher concentrations of lactoferrin than the individuals with IBD and a negative result of this test ($p<0.005$; Fig 5).

DISCUSSION

Endoscopic examination and histopathological analysis of intestinal mucosal specimens still constitute the gold standard in the diagnosis and evaluation of IBD. As colonoscopy is an invasive procedure and needs to be performed under general anesthesia in pediatric patients, clinical research focuses on identification of fecal markers of IBD that could be used in the diagnosis and monitoring of this condition. One such potential marker is fecal lactoferrin; according to literature, determination of this parameter may serve as a prescreening test in qualification for endoscopy, especially in children (Sudo *et al.,* 1993; Sugi *et al.,* 1996; Fine *et al.,* 1998; Walker *et al.,* 2007; Pfefferkorn *et al.,* 2010). Kane *et al.,* (2003) were the first to show that patients with IBD had a significantly higher fecal lactoferrin levels than the individuals with irritable bowel syndrome. Although the usefulness

of this marker of gastrointestinal inflammation was confirmed in further studies, most of them dealt solely with adult patients with IBD (Schroder *et al.,* 2007; Schoepfer *et al.,* 2008; Sipponen *et al.,* 2008; Sidhu *et al.,* 2010). The first sparse reports on the diagnostic value of fecal lactoferrin in children (Walker *et al.,* 2007; Pfefferkorn *et al.,* 2010) were published no earlier than in 2007.

In our study, the sensitivity and specificity of fecal lactoferrin as a diagnostic marker of IBD were 80.7% and 92.7%, respectively. These figures point to potential usefulness of this parameter for clinical practice and are consistent with the previously published data on the sensitivity (78–97%) and specificity (74–100%) of fecal lactoferrin in adults and children (Fine *et al.,* 1998; Kane *et al.,* 2003; Schoepfer *et al.,* 2007; Walker *et al.,* 2007; Langhorst *et al.,* 2008; Pfefferkorn *et al.,* 2010). The value of this marker would be even greater if it accurately reflected the severity of intestinal lesions. Unfortunately, contrary to previous studies (Walker *et al.,* 2007; Langhorst *et al.,* 2008), we did not find a significant association between the fecal concentration of lactoferrin and the severity of endoscopic lesions graded according to Langhorst and coworkers (2008). Nevertheless, we showed that fecal concentration of lactoferrin increases proportionally to the activities of UC and CD assessed on the basis of the Truelove-Witts' index and PCDAI scores, respectively. Significant relationships between the fecal concentration of lactoferrin and the activity of IBD determined on the basis of clinical symptoms and various scoring systems were observed in several previous studies (Kane *et al.,* 2003; Walker *et al.,* 2007; Schoepfer *et al.,* 2008; Sipponen *et al.,* 2008), albeit not universally (Schroder *et al.,* 2007; Schoepfer *et al.,* 2008). These discrepancies with regards to the role of fecal lactoferrin as a marker of IBD's activity substantiate further research involving larger subsets of patients with this condition.

Interestingly, we showed that fecal concentrations of lactoferrin in children with mild UC and mild/inactive CD were higher than in the controls. Similar phenomenon was previously reported by other authors who identified a subset of patients with non-active IBD and elevated fecal concentration of lactoferrin (Oberhelman *et al.,* 1999; Kane *et al.,* 2003; Dai *et al.,* 2007; Schoepfer *et al.,* 2008; Sipponen *et al.,* 2008). The fact that at least a fraction of individuals whose IBD was classified as mild/inactive had elevated levels of the established inflammatory marker suggests that the existing scoring systems may not accurately reflect the activity of this condition. However, this hypothesis needs to be verified objectively as, at least in younger pediatric population, fecal concentration of lactoferrin may, to a certain degree, reflect the breast milk intake of this protein (Oberhelman *et al.,* 1999).

One potential limitation of fecal lactoferrin as a diagnostic marker of IBD stems from the fact that the determination of this protein is costly and time-consuming. According to the manufacturer of commercially available test for fecal lactoferrin, the stool samples should be examined at least at two different dilutions (1:100 and 1:1000). Unfortunately, the data on the dilutions of stool samples examined in the previous studies of fecal lactoferrin concentrations are limited. D'Inca *et al.,* (2007) and Schoepfer *et al.,* (2007) determined concentrations of lactoferrin in stool samples diluted at 1:400. In this study, we examined stool samples at up to four various dilutions (1:100, 1:400, 1:1000 and 1:4000 in some cases) which significantly increased the cost of testing. Perhaps qualitative or semi-quantitative determination of fecal lactoferrin would be sufficient for the screening purposes, especially if examined as a component of a larger panel of established fecal markers (Chung-Faye *et al.,* 2007; Kaiser *et al.,* 2007; Turner *et al.,* 2010).

CONCLUSION

Fecal concentration of lactoferrin seems to be a useful parameter for diagnosis and monitoring of IBD in children. However, these preliminary findings need to be confirmed in a larger group of patients with this condition.

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