ORIGINAL ARTICLE

Clinical utility of the assessment of fecal calprotectin in Leśniowski-Crohn's disease

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KEY WORDS

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ABSTRACT

INTRODUCTION From the epidemiological point of view, Leśniowski-Crohn's disease (CD) has become an important medical problem. It is essential to differentiate CD from functional disorders of the gastrointestinal tract, first of all, from irritable bowel syndrome (IBS). There are no simple, non-invasive tests available which could help to identify patients with common symptoms such as abdominal pain or diarrhea who should be referred for further evaluation, including endoscopy.

OBJECTIVES The aim of this study was to evaluate the diagnostic utility of the assessment of fecal calprotectin concentration in patients with CD.

PATIENTS AND METHODS Stool samples were taken from 31 patients of the Gastroenterology, Human Nutrition and Internal Diseases Department of Poznań Medical University who were diagnosed with CD. Patients suffering from IBS served as the control group. Calprotectin concentration was assessed by means of the immunoenzymatic ELISA method. Serum C-reactive protein (CRP) concentration and blood cell count were determined. The clinical activity of CD was assessed by means of Crohn's Disease Activity Index. An appropriate statistical analysis was performed.

RESULTS Mean calprotectin concentration in CD group was 32.01 ±22.58 mg/l and it was statistically higher ($p < 0.0003$) than among IBS patients. A concentration of 16.01 mg/l had 67.7% sensitivity and 66.7% specificity in distinguishing between CD and IBS. There was a positive correlation between calprotectin concentration and CRP, and negative – with hemoglobin concentration.

CONCLUSIONS The assessment of fecal calprotectin concentration may be useful in differential diagnoses of CD and monitoring patients with CD.

INTRODUCTION A number of subjects diagnosed with Leśniowski-Crohn disease (CD), one of nonspecific inflammatory bowel diseases (IBD), has become increasingly common.[1] The disease consists in chronic, segmental inflammation involving the whole wall of the digestive tract. The lesions are most commonly located in the distal segment of the ileum, but may affect each segment of the digestive tract. The disease is distinguished by periods of remission and relapse when symptoms like abdominal pain, diarrhea, fever and malaise occur. The disease is incurable and leads to several complications associated with inflammation in the digestive tract and to a number of extraintestinal symptoms. Etiopathogenesis of CD remains unknown.[2,3]

Both the diagnostics and monitoring of CD patients pose numerous problems. It often takes months or even years from the development of the first symptoms (e.g. chronic abdominal pain) to the final diagnosis. The most relevant stage of the diagnostic evaluation is gastrointestinal endoscopy along with histopathology of tissue specimens. It is frequently performed during the late stage of CD.[4] Moreover, the parameters routinely assessed in patients in remission, like erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) or complete blood count, have several drawbacks because they only indirectly reflect the intensity of the intestinal inflammation.[5] Therefore, new methods which could serve the purpose objectively and non-invasively are still sought.

Calprotectin, that represent >50% of neutrophil cytosol protein, is present in increased concentrations in inflamed tissues. It belongs to the S-100 protein family composed of two subunits

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TABLE 1 Characteristics of the study group

	Whole group	Females	Males
Group size	31	16	15
Age (years)	35.2 ±10.9	38.5 ±12.8	31.9 ±7.7
Disease duration (years)	4.9 ±4.3	4.7 ±3.9	5 ±4.8
Complain duration (years)	8.2 ±7.2	8.3 ±5.3	8.1 ±8.9

of molecular mass of 8 and 14 kDa. Calprotectin is released from leukocytes both due to cell death and in an active mechanism of secretion. Calprotectin is involved in inflammatory processes; its proapoptotic, antibacterial properties have been shown (probably by binding zinc and calcium ions essential for the development of individual microorganisms) and also its ability to inhibit in vitro the proliferation of proliferating cells, like bone marrow cells, cancer cells or stimulated lymphocytes. However, it should be noted that its significance in the development of inflammatory lesions in various diseases has not been fully understood.[6,7] Since one of the components of CD pathogenesis is a large inflammatory infiltrate in the intestinal wall, composed mainly of neutrophiles, it seems that calprotectin levels should be increased both within the affected segment of the digestive tract and in the patients' feces. The objective of the study was to assess the utility of fecal calprotectin determination in the diagnostic evaluation and monitoring of CD.

PATIENTS AND METHODS The study group was composed of 31 persons aged 35 ±11 years, who were patients of Gastroenterology, Human Nutrition and Internal Diseases Department of Poznań Medical University, with CD confirmed clinically and in additional tests. The characteristics of the study group is presented in TABLE 1.

The control group involved 12 patients (9 females and 3 males) at a mean age of 45.5 ±21.2 with excluded organic background of gastrointestinal disorder and with irritable bowel syndrome (IBS) diagnosed using the Rome II criteria. The Bioethical Committee at Poznań Medical University approved the study.

A single stool sample was taken from the patients and then stored at a temperature of –20°C. After thawing, 100 mg portions were extracted from each sample using the Roche extraction method. Fecal calprotectin level was determined by enzyme-linked-immuno-sorbent-assay (ELISA) (Immundiagnostik, Bensheim, Germany), twice for each sample. CD patients were subjected to additional laboratory tests, like complete blood count, ESR and CRP level. Clinical progression of the disease was assessed by commonly used Crohn's Disease Activity Index (CDAI).[8] Remission is defined as CDAI <150, exacerbation: 150–450 and extremely serious flare-up: >450.

Statistical analysis Mean values, standard deviations, maximum and minimum values were calculated by Microsoft Excel XP. Statistical differences between the mean values in the study and control groups were calculated by Welch's unpaired t-test, assuming statistical significance of $p < 0.05$. Correlations between calprotectin levels and other results were tested by Pearson's linear correlation coefficient for variables of normal distribution and using Spearman's rank correlation coefficient for non-normal distribution. The assessment of diagnostic accuracy of the test, including sensitivity and specificity of calprotectin determination in stool in differentiation between CD and IBS, was tested by setting the receiver operating characteristic curve (ROC) and calculating the area under curve (AUC). For ideal ROC, AUC corresponds to 1.00. The closer to this value, the more precise the assessed method is.

RESULTS Mean fecal calprotectin level of CD patients was 32.01 ±22.58 mg/l. This level in the female group was estimated at 36.9 ±26.4 mg/l and was significantly higher than for the male group (27.1 ±17.5 mg/l, $p < 0.05$). In the IBS patient group, a calprotectin level amounted to 14.73 ±4.58 mg/l. The difference between the CD and IBS patients was statistically significant ($p < 0.0003$).

The value of AUC was 0.76 (95% CI 0.62–0.88; $p < 0.0002$). It was calculated for fecal calprotectin level equal to 16.01 mg/l; the test had sensitivity of 67.7% and specificity of 66.7% in differentiation between CD and IBS. Mean CDAI was 156 ±91. Other results obtained in the study group are presented in TABLE 2.

A statistically significant ($p < 0.05$) positive correlation was found between levels of protein, determined in the stool, and CRP, and a significant negative relationship between calprotectin and peripheral blood hemoglobin level. Other relationships, including a positive correlation with CDAI, were not significant.

DISCUSSION The results confirm that determination of fecal calprotectin levels in CD patients is useful at diagnosis. The receiver operating characteristic curve shows that the test may be useful in differentiation between CD and IBS. It is relevant inasmuch as IBS constitutes one of the most common diseases classified as functional intestinal disorders which slowly become lifestyle-related diseases.[9] Patients with those diseases are a great challenge for primary care physicians and gastroenterologists due to complex and still obscure etiology, chronic nature and frequent therapeutic failures.[10] In such a clinical setting, errors might be common especially while establishing diagnosis of chronic abdominal pain and defecation disorders; patients are sometimes prematurely classified as IBS and an appriopriate treatment based on the diagnosis is administered. However, it should be kept in mind that functional disorders sometimes mask inflammatory disease. Difficulties in diagnosing IBD encountered in the current study prove this observation (TABLE 1).

TABLE 2 Results of laboratory tests of the study group – mean values

C-reactive protein	13.9 ±16.3 mg/l
Erythrocyte sedimentation rate	28.3 ±23.8 mm/h
Erythrocytes	4.4 ±0.6 x 10^6/mm^3
Leukocytes	7.1 ±1.9 x 10^3/mm^3
Platelets	297 ±94.5 x 10^3/mm^3
Hemoglobin	12.2 ±2.2 g/dl
Hematocrit	39.4 ±4.5%

In the group of CD patients, there were on average, approximately 4 years from the first gastrointestinal symptoms to establishing the definite diagnosis. It should be emphasized that in each case of suspected CD, it is important to make quick and appropriate diagnosis to forestall the occurrence of complications typical of this disease, like fistulas, intestinal strictures or intraabdominal abscesses.[3]

On the other hand, not every patient referred to a physician for abdominal pain, fatigue, or diarrhea is immediately subjected to examinations like colonoscopy, which is invasive and not free from the risk of complications, or radiological examination of the digestive tract, exposing the patient to ionizing radiation. Therefore, it is extremely important to take history and perform physical examination properly to differentiate between IBD and, first of all, functional disorders and infectious diseases of the digestive tract. A crucial stage in the diagnostics of the cases described is laboratory tests, including complete blood count, ESR or CRP level. Association of those symptoms with anemia, higher ESR or CRP level should encourage to expand diagnostic evaluation by specialized procedures. However, despite the intensified intestinal inflammatory lesions in CD, laboratory abnormalities are not always observed.[11] Also, positive fecal occult blood test is more typical of ulcerative colitis belonging to IBD than of CD where the most common location of the disease is the distal segment of ileum.[12] Therefore, it seems that the best diagnostic test would involve detecting inflammatory markers in stool. Calprotectin fulfils the criteria for a good marker due to several important reasons:

1 it is not decomposed by fecal bacterial microflora, being present up to 48 h for stool stored at room temperature and up to 3 months at a temperature of –20°C

2 it is a predominant neutrophil cytosolic protein, thus it well reflects the intensity of inflammatory infiltration in the intestinal wall whose the crucial component represents neutrophils

3 only a single stool sample is sufficient since it has been demonstrated that the determination of calprotectin level in such a sample correlates with the concentration of this protein in larger samples, including the daily stool collection[14]

4 the test is simple and non-invasive.

Unfortunately, the test has also its limitations. First of all, it is currently not universally available and, partly for this reason, expensive. Limited sensitivity and specificity in differentiation between CD and IBS, despite statistical significance, is satisfactory but insufficient since the values do not exceed 70% for fecal calprotectin level of 16 mg/l. Our data do not confirm the results obtained by Tibble et al., that showed that the sensitivity and specificity of this method for the level of calprotein of 30 mg/l is 100% and 94%, respectively.[13] It should, however, be mentioned that those numbers refer to differentiation between IBS, CD and ulcerative colitis together, whereas our study involved only CD patients. Nevertheless, despite some drawbacks of the method, determination of calprotectin level seems to be useful at the stage of differentiation between functional and organic bowel diseases, like CD. In particular, the test could help in making decisions whom to refer for additional diagnostic evaluation including endoscopy. This could reduce both the percentage of false IBS diagnoses and facilitate the process of referring for endoscopy.

The results also suggest that determination of fecal calprotectin could be useful during follow-up of patients with already diagnosed CD. Of substantial importance are the correlations with the most sensitive among commonly determined inflammatory markers, i.e. CRP and with hemoglobin level which to the largest extent (in comparison with RBC count and hematocrit value) reflects the degree of anemia.[5] In all probability the lack of a statistically significant relationship between CDAI and fecal calprotectin level results from the fact that this index is highly subjective and often poorly reflects the extent of inflammatory lesions in the digestive tract.

Some authors view calprotectin as a marker whose regular determination in patients being in the CD remission could help to predict exacerbation.[14] It results from the fact that the inflammatory process in the intestines is continuous and gradually exacerbates prior to the acute flare-up of the disease. These phenomena are asymptomatic. Only exceeding a peculiar critical point causes clinically overt relapse of the disease. The steadily rising fecal calprotectin level could suggest the intensification of inflammatory lesions in the intestines, and introduction of aggressive pharmacotherapy at this stage could prevent an acute flare-up or mitigate its course. But despite the presence of some premises, there are still no large studies confirming the usefulness of the analyzed test.

In conclusion, calprotectin may serve as a good inflammatory marker for the use in diagnostics and monitoring of CD patients. This protein is ranked among a even larger group of proteins determined in the stool in IBD patients, including among others lactoferrin, lipocalin, lysozyme or myeloperoxidase.[15,16] They are becoming still more popular due to high clinical usefulness, simplicity of determination and low invasiveness for patients. However, the ultimate position of those proteins in gastroenterology is unknown,

therefore there is a need to perform further studies in this respect.

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