Original Article

The diagnostic usefulness of fecal lactoferrin in the assessment of Crohn's disease activity

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ABSTRACT

Background: Diagnosis and monitoring of Crohn's disease (CD) is difficult and time-consuming. In recent years, diagnostic usefulness of fecal calprotectin has been proven. However, data on the utility of other fecal markers are scarce.
Aims: To evaluate the usefulness of fecal lactoferrin (FL) in the assessment of CD activity.
Methods: The group consisted of 101 CD patients (median age: 30 years, IQR: 24–37). FL was measured in a single stool sample by using the immunoenzymatic methods. The clinical activity of the disease was evaluated by using the Crohn's Disease Activity Index (CDAI). Depending on the location of the disease, either a colonoscopy or magnetic resonance enterography was performed or both in order to evaluate the disease activity by using appropriate endoscopic and enterographic scores.
Results: Median FL concentration was 84.14 (IQR: 36.4–302.9) μg/ml and it correlated with C-reactive protein concentration ($p = 0.0000001$, $r = 0.5$), CDAI ($p = 0.002$, $r = 0.3$) and colonic Simple Endoscopic Score for Crohn's Disease (SES-CD) ($p = 0.000004$, $r = 0.5$). Assuming endoscopic remission in the large intestine with colonic SES-CD ≤ 3 points, a ROC curve showed that FL concentration of 145.82 μg/ml had 84.6% sensitivity and 60.5% specificity in discriminating CD patients with endoscopically active and inactive disease [AUC: 0.676 (95% CI: 0.531–0.8), ($p = 0.0347$)]. The positive predictive value for this concentration was 42% and negative predictive value −92%.
Conclusions: FL is a sensitive marker of CD activity and it reliably reflects the mucosal inflammatory lesions in large intestine. Thus, it can be helpful in diagnostics and monitoring of CD.

1. Introduction

The incidence of Crohn's disease (CD), which belongs to the group of inflammatory bowel diseases (IBD), has been constantly increasing in recent years. Based on the most recent data, the incidence of CD in Greece is 1 case per 100,000 inhabitants/year, whereas in Scotland even up to 11.4 new cases per 100,000 inhabitants/year. This disease mostly affects teenagers and young adults. The initial symptoms of the disease are not specific. Most frequently patients complain about abdominal pain, weight loss and change in bowel movements. The diagnosis of IBD is not easy. It is assumed that the average time from the first symptoms to the full diagnosis is 4 years. In order to make a final diagnosis, a patient requires a full diagnostic work-up and endoscopic evaluation such as gastroscopy and ileocolonoscopy as well as other imaging studies, which prolongs the time to final diagnosis [1].

The assessment of fecal markers levels is considered to be new, innovative, non-invasive and reliable method to diagnose diseases of gastrointestinal tract [2,3]. Since the 90s there has been research on fecal markers as useful diagnostic tools in diarrhea [4,5]. The two representatives of the fecal markers are calprotectin and lactoferrin. It has been proven that these markers are useful in detection and differential diagnosis between functional and organic diseases of the gastrointestinal tract, including irritable bowel syndrome (IBS) and IBD [6–8]. The concentration of these markers is elevated in infectious diarrhea, especially those with bacterial etiology [9]. The role of calprotectin in the diagnosis of IBD is confirmed in numerous research studies [10–13]. Its usefulness has been proven in the evaluation of CD and ulcerative colitis activity [14,15]. Some data present a correlation of the concentration of this marker with endoscopic activity of IBD [16–19]. That is why calprotectin is used more and more common to monitor the activity of CD. It is believed that calprotectin concentration above 100 μg/g is associated with endoscopic recurrence of lesions and

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the exacerbation of the disease and is a signal for the clinicians to continue diagnostics in order to objectively confirm the disease flare or to intensify the treatment [20]. Fecal lactoferrin is believed to be another marker of CD activity, however data in this field are scarce [21,22].

Lactoferrin is a glycoprotein from the family of transferrins with a molecular mass of approximately 80 kDa. It is characterized by the ability to bind iron ions. It is produced by the secretory epithelium and its presence is observed in milk, sputum, cerebrospinal fluid, and seminal fluid. Its presence is also observed in secondary and tertiary granulation from which it is released directly to the bloodstream or inflammatory areas. Lactoferrin is characterized by extremely high stability [23,24]. It does not get digested in the gastrointestinal tract. At room temperature it does not get broken down for 7 days. At temperature up to 50 °C it does not undergo proteolysis even for up to 48 h. It remains stable during the process of freezing and defrosting, so it can be a good and stable research material. Lactoferrin is an immunomodulatory protein. It takes part in the innate immune system response. In numerous in vivo and in vitro studies its bacteriostatic and bactericidal functions have been proven [23,24]. It also has antiviral and antifungal properties. It is also interesting that lactoferrin has an anti-neoplastic function [23, 24]. As an easily accessible and stable fecal marker, lactoferrin can be a good indicator of CD activity. Because of the multiple locations of the disease, there is a question whether the concentration of lactoferrin depends on the localization of CD and whether we can estimate the concentration of lactoferrin above which we can suspect an exacerbation of disease. It would also be valuable to check the correlations between the lactoferrin concentration and the mucosal inflammatory lesions seen in the endoscopy. Thus, the goal of this study was to evaluate the usefulness of measuring lactoferrin concentration in stool in the assessment of CD activity.

2. Material and methods

The studied group consisted of 101 CD patients—57 men and 44 women—from the Department of Gastroenterology, Human Nutrition and Internal Diseases of Poznan University of Medical Sciences. The inclusion criteria were as follows: CD confirmed clinically, endoscopically, and histopathologically; age ≥ 18 years; minimum 1 year duration of the disease. The presence of a stoma and pregnancy excluded patients from the study. The description of the studied group was performed using Montreal classification (Table 1).

Patients gave one stool sample that was stored at −20 °C. After defrosting the concentration of lactoferrin in stool was measured using an immunoenzymatic test IBDScan ELISA (TechLab, USA). Namely, from each stool sample 0.05 g amount of feces was manually prepared and diluted with 450 μl of diluent. Then further dilutions were prepared, and in the next step, together with standards, positive and negative controls, they were incubated for 30 min at the temperature of 37 °C. The detection of lactoferrin was performed by using rabbit polyclonal antibodies against lactoferrin which were conjugated to horseradish peroxidase. The final concentration was calculated after measuring the

spectrophotometric absorbance by using Sunrise spectrophotometer (Tecan, Austria).

All the patients got their blood sample taken in which the following parameters were measured: full blood count, fibrinogen, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). The clinical disease activity was evaluated using Crohn's Disease Activity Index (CDAI), stating that remission was a value less than 150 points, a mild activity— between 150 and 219 points, moderate activity—between 220 and 450 points, and a severe disease flare—above 450 points [1]. Depending on the localization of the disease, patients underwent a colonoscopy (81 patients) or magnetic resonance enterography (MRE) (89 patients). The disease activity in the large intestine was evaluated during colonoscopy using Simple Endoscopic Score for Crohn's Disease (SES-CD) (Table 2) [25]. Total score was the sum of all variables assessed in rectum, sigmoid and left colon, transverse colon, right colon and ileum. Remission of the disease was defined as colonic SES-CD ≤ 3 points. The disease activity in the small intestine was evaluated in MRE by using an authorized score—Simple Enterographic Activity Score for Crohn's Disease (SEAS-CD) (Table 3) [26]. Remission of the disease was present when the score was ≤ 6 points.

Detailed characteristics of the study group are presented in Table 4.

The statistical analysis was performed using the program Statistica 10.0 (StatSoft Poland). The evaluation of normal distribution measured in interval scale was performed using Kolmogorov–Smirnov test. Because the data was incongruent with the normal distribution, the results were presented as medians and interquartile ranges (IQR). For values measured in the interval scale with lack of normal distribution or values measured in ordinal scale, we used Wilcoxon's test for dependent variables or Mann–Whitney's test for independent variables.

In order to estimate the correlation for variables measured in interval scale or ordinal scale in case of lack of normal distribution we used Spearman's rank correlation coefficient. The statistical significance of a coefficient was analyzed using Student's t-test.

In order to estimate the cut-off point for lactoferrin to discriminate patients in remission and patients with disease flare, we used the receiver operating characteristic curve (ROC curve) analysis. In this analysis only patients with ileocolonic and colonic location were included, in whom a full endoscopic assessment of the large bowel was performed (n = 51). We assumed that the endoscopic remission in the large intestine was when the colonic SES-CD was ≤ 3 points. The optimal cut-off point was estimated using Youden's index. For the obtained cut-off point we presented the sensitivity and specificity and the evaluation of the diagnostic quality curve was based on the area under the curve (AUC). Positive predictive values (PPV) and negative predictive values (NPV) were also calculated.

Additionally, in order to assess, whether fecal lactoferrin is an independent predictor of CD activity, a stepwise multiple regression analysis was conducted. In this model the endoscopic assessment was considered to be the "gold diagnostic standard". In first step a multiple regression analysis was performed, in which the most important parameters assessing CD activity were analyzed (fecal lactoferrin, CRP, CDAI, white blood cell count—WBC, platelets—PLT, hemoglobin, fibrinogen, ESR). Then, in order to provide the most suitable statistical model predicting CD activity, multiple regression backward elimination method was used. In each case a standardized b coefficient was calculated, reflecting the strength of assessed parameters in predicting CD activity. For both models an adjusted coefficient of determination R^2 was calculated to assess the goodness of fit of the model.

The statistical significance was defined as $p < 0.05$.

The study was approved by the Bioethical Committee of Poznan University of Medical Sciences (No 335/14).

3. Results

The characteristics of the study group, together with the results of biochemical analyses, are presented in Table 4.

Table 1
Montreal classification of Crohn's disease.

Variable	Montreal Scale
Age at the time of diagnosis	A1 ≤ 16 years old
	A2 17–40 years old
	A3 ≥ 40 years old
Localization	L1 distal part of the ileum
	L2 colon
	L3 distal part of the ileum and colon
	L4 upper part of the gastrointestinal tract
Clinical course	B1 inflammatory presentation
	B2 strictures
	B3 transmural presentation
	p fistulas