INTRODUCTION
Crohn's disease (CD) is a chronic inflammatory disorder, which can involve each part of the gastrointestinal tract. The etiology and pathogenesis of CD are not well known. During the first phase of the disease, the inflammatory infiltration in the gut wall is present, mainly consisting of neutrophils, eosinophils, basophils, and lymphocytes. This leads to the impairment of the intestinal function, resulting in symptoms such as abdominal pain, diarrhea, or weight loss. With time, after repeated flares of the disease, the active inflammatory pattern of CD evolves into a more chronic one. During this phase of the disease, inflammatory infiltration mainly consists of lymphocytes and eosinophils; fibroblasts are also stimulated. This may lead to the development of fibrostenotic complications and fistulas.

The above phenomena define the so-called natural history of CD.

ABSTRACT
INTRODUCTION
Fecal calprotectin is a useful diagnostic marker in the assessment of Crohn’s disease (CD) activity. However, the clinical picture of CD is diverse and its phenotypes change with disease duration: in the early phase, an inflammatory activity dominates and, with time, an increasing percentage of patients develop strictures and fistulas.

OBJECTIVES
The aim of the study was to assess whether disease duration affects the diagnostic utility of fecal calprotectin measurement in patients with CD.

PATIENTS AND METHODS
A total of 150 patients with CD were prospectively enrolled into the study. CD activity was assessed by magnetic resonance enterography by calculating the Simple Enterographic Activity Score for Crohn’s Disease. Endoscopic activity was assessed using the Simple Endoscopic Score for Crohn’s Disease (SES-CD). The blood levels of inflammatory markers and the fecal calprotectin concentration were assessed using an enzyme-linked immunosorbent assay. Patients were divided into 2 subgroups depending on CD duration: less than 10 years and 10 years or longer from the diagnosis.

RESULTS
Patients with longer disease duration had lower inflammatory CD activity assessed by biochemical, endoscopic, and radiographic tests. Fecal calprotectin showed a tendency for lower concentrations in this subgroup (106.5 ±93.2 mg/l vs. 135.7 ±128.8 mg/l; P >0.05). A stricturing or penetrating CD phenotype was observed significantly more often in patients with long-lasting CD (P <0.04). Nonetheless, in both study subgroups, fecal calprotectin was significantly correlated with SES-CD, C-reactive protein levels, and platelet count.

CONCLUSIONS
Disease duration and time-dependent changes of the CD phenotype do not affect the diagnostic utility of the fecal calprotectin measurement. Reliability of this noninvasive biochemical method in the assessment of disease activity is similar in all patients with CD; therefore, it may be used independently of the time from diagnosis.
CD progresses with time from active inflammatory disease to a destructive and disabling condition, in which intestinal stenoses and fistulas are more commonly present. It was shown that after 10 years, half of the CD patients required surgery because of the intestinal complications of the disease. The measurement of fecal calprotectin levels is believed to be one of the most accurate tools for the estimation of CD activity. It correlates with biochemical findings and endoscopic severity of the disease. However, the diagnostic utility of this marker still needs to be confirmed. Considering the complexity and diversity of CD phenotypes, it has not been investigated whether fecal calprotectin is useful in the diagnostic evaluation of all CD patients. The main source of fecal calprotectin are neutrophils from the inflammatory infiltrates in the intestinal wall. It may be speculated that the time-dependent change in the CD pattern from an inflammatory to a strictureing and penetrating phenotype can result in a decrease of fecal calprotectin concentrations. The effect of this phenomenon on the diagnostic utility of fecal calprotectin has not been investigated so far. Therefore, the aim of the current study was to compare the usefulness of fecal calprotectin measurement for the estimation of CD activity in patients with different disease duration.

**Patients and Methods**

Patients with diagnosed CD were prospectively enrolled into the study. All patients were hospitalized at the Department of Gastroenterology, Human Nutrition and Internal Diseases of the Poznań University of Medical Sciences, Poznań, Poland, between 2009 and 2013 because of CD exacerbation or to perform follow-up investigations. The exclusion criteria were as follows: use of any nonsteroidal anti-inflammatory drugs (NSAIDs) during 4 weeks before the onset of the study, history of antitumor necrosis factor α (anti-TNF-α) therapy, and presence of any other concomitant organic gastrointestinal disease confirmed by colonoscopy or laboratory tests.

The study was approved by the Bioethics Committee at the Poznań University of Medical Sciences (Decision No 141/11).

Complete clinical data were obtained and the clinical activity of the disease was estimated using the Crohn’s Disease Activity Index (CDAI). Each patient underwent a colonoscopy performed by an experienced endoscopist, and the endoscopic CD activity was assessed by calculating the Simple Endoscopic Score for Crohn’s Disease (SES-CD). The severity of inflammatory lesions detected on magnetic resonance enterography (MRE) was evaluated using the Simple Enterographic Activity Score for Crohn’s Disease (SEAS-CD). The concentrations of blood inflammatory markers (full blood count, C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]) were also assessed. Before endoscopy or MRE, each patient provided a single stool sample. The samples were frozen and stored at –20°C until further tests. After thawing in room temperature, the concentration of calprotectin was estimated using PhiCal® Calprotectin ELISA (Immundiagnostik, Germany), according to the manufacturer’s recommendations.

The study group was divided into 2 subgroups, depending on the disease duration: less than 10 years from the diagnosis and 10 years or longer from the diagnosis of CD. Then we compared those subgroups in terms of the disease behavior, and the clinical, biochemical (together with the measurement of fecal calprotectin concentrations), endoscopic, and radiographic activity. We also performed correlation analyses between fecal calprotectin and the most important parameters defining the CD activity and compared the data between the 2 subgroups.

A statistical analysis was performed using the GraphPad Prism Version 4.0 (GraphPad Software, Inc., La Jolla, California, United States). Descriptive statistics were performed by calculating means with standard deviations or medians with 95% confidence intervals. The differences in the disease behavior between the study subgroups were assessed using the χ² test. To compare clinical, endoscopic, and biochemical data, the t test was used when conditions of normality and equal variance were met. The t test was used with the Welch correction when unequal variances were detected. When the normality test failed, 2-tailed and exact Mann–Whitney tests

**FIGURE**

Distribution of the phenotypes of Crohn’s disease in the study subgroups divided according to disease duration.

- **disease duration <10 years**
  - noninflammatory (penetrating and stricturing) phenotype: 75%
  - inflammatory phenotype: 25%

- **disease duration ≥10 years**
  - noninflammatory (penetrating and stricturing) phenotype: 57%
  - inflammatory phenotype: 43%
In patients with a disease duration of 10 years and longer, fecal calprotectin and CRP concentrations were insignificantly lower compared with the other subgroup. The differences in clinical, biochemical, endoscopic, and radiographic parameters between the study subgroups are presented in Table 1.

Fecal calprotectin concentration correlated significantly with CRP levels and platelet count in both subgroups. However, there was no correlation between the calprotectin concentration and cell blood cell counts. Moreover, there was no correlation between the calprotectin concentration and ESR. Endoscopic CD activity assessed by the SES-CD correlated significantly with fecal calprotectin in both subgroups. A correlation between fecal calprotectin and CDAI was observed only in patients with a disease duration of 10 years and longer ($r = 0.4$, $P < 0.0001$). The correlations are presented in Table 2.

### Table 1: Differences in selected clinical, biochemical, endoscopic, and radiographic parameters between the study subgroups

<table>
<thead>
<tr>
<th>Feature</th>
<th>Disease duration, y</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age, y</td>
<td>&lt;10, n = 99</td>
<td>≥10, n = 51</td>
</tr>
<tr>
<td></td>
<td>31 ± 12</td>
<td>37 ± 10</td>
</tr>
<tr>
<td>men/women</td>
<td>54/45</td>
<td>22/29</td>
</tr>
<tr>
<td>previous surgery, n (%)</td>
<td>26 (26)</td>
<td>24 (47)</td>
</tr>
<tr>
<td>calprotectin, mg/l</td>
<td>135.7 ± 128.8</td>
<td>106.5 ± 93.2</td>
</tr>
<tr>
<td>disease duration, y</td>
<td>4 ± 2</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>disease location, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ileal</td>
<td>33 (33.3)</td>
<td>18 (35)</td>
</tr>
<tr>
<td>ileocolonic</td>
<td>42 (42.5)</td>
<td>22 (43)</td>
</tr>
<tr>
<td>colonic</td>
<td>24 (24.2)</td>
<td>11 (22)</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>23.2 ± 32.7</td>
<td>20.7 ± 22.5</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>29 ± 22</td>
<td>28 ± 20</td>
</tr>
<tr>
<td>RBC count, mln/mm$^3$</td>
<td>4.4 ± 0.6</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>12.3 ± 1.9</td>
<td>13.1 ± 1.8</td>
</tr>
<tr>
<td>Ht, %</td>
<td>38 ± 5</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>WBC count, 10$^3$/mm$^3$</td>
<td>7.9 ± 3.4</td>
<td>7.3 ± 2.9</td>
</tr>
<tr>
<td>platelet count, 10$^3$/mm$^3$</td>
<td>367 ± 138</td>
<td>311 ± 91</td>
</tr>
<tr>
<td>CDAI</td>
<td>183 (164–206)</td>
<td>225 (197–253)</td>
</tr>
<tr>
<td>SES-CD</td>
<td>11 (9–15)</td>
<td>6 (5–11)</td>
</tr>
<tr>
<td>SEAS-CD</td>
<td>11 (10–13)</td>
<td>9 (6–10)</td>
</tr>
<tr>
<td>therapy, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aminosalicylates</td>
<td>90 (91)</td>
<td>46 (90)</td>
</tr>
<tr>
<td>steroids</td>
<td>33 (33)</td>
<td>18 (35)</td>
</tr>
<tr>
<td>thiopurines</td>
<td>81 (82)</td>
<td>39 (76)</td>
</tr>
<tr>
<td>antibiotics</td>
<td>26 (26)</td>
<td>12 (24)</td>
</tr>
<tr>
<td>probiotics</td>
<td>44 (44)</td>
<td>21 (41)</td>
</tr>
<tr>
<td>PPIs</td>
<td>8 (8)</td>
<td>6 (12)</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviations or as medians with 95% confidence interval.

It has been shown that this marker correlates with biochemical, endoscopic, and MRE findings in both study subgroups. Moreover, patients with long-lasting CD have a history of surgical treatment significantly more often than those with shorter disease duration (figure). Moreover, patients with long-lasting CD have a history of surgical treatment significantly more often than those with shorter disease duration (table 1).

Another important finding is that patients with a disease duration of less than 10 years since diagnosis had higher inflammatory activity of CD, as confirmed by biochemical, endoscopic, and radiographic tests. Moreover, they had higher CRP and ESR levels as well as significantly higher platelet count. Anemia was also more frequent in this subgroup, which reflects a high inflammatory burden of the disease in its earlier phase. Active inflammatory lesions, detected on endoscopy and assessed by the SES-CD, were significantly more common in patients with shorter disease duration. The features of inflammatory activity in the small bowel observed on MRE and assessed by the SEAS-CD (bowel-wall thickening, contrast enhancement with the proliferation of mesenteric vasculature, ulcerations), were also more common in this subgroup of patients (P = 0.003). Of note, in contrast to those findings, the clinical activity tended to be higher in long-lasting CD (CDAI, 225 vs. 183 patients; nonsignificant). This shows that patients with the disabling phenotype, who more often suffer from stricturing or penetrating CD, can have more severe symptoms compared with patients with higher inflammatory burden.

A comparison analysis showed a higher concentration of fecal calprotectin in patients with shorter disease duration but the difference was not significant. As mentioned above, the main source of this protein in the intestines in CD are granulocytes and lymphocytes from the active inflammatory infiltration in the bowel wall. Lower fecal calprotectin levels in patients with long-lasting CD seem to be secondary to lower inflammatory burden at this stage of the disease, and this observation is in accordance with biochemical, endoscopic, and MRE findings in both study subgroups.

Our results provoke some important questions. Is fecal calprotectin equally useful in determining CD activity in all CD patients? Does disease duration (with the decrease of the inflammatory burden of the disease) affect the diagnostic accuracy of this noninvasive method? It is vital to answer these questions because the role of fecal calprotectin in diagnosing and monitoring CD is becoming increasingly important. It has been shown that this marker correlates more closely with CD endoscopic activity than CRP, blood leukocytes, and the CDAI do. In patients treated with anti-TNF-α agents, fecal calprotectin can probably serve as a surrogate marker of mucosal healing. Thus, it can help to rationalize and limit the need for invasive monitoring procedures in those patients. Sipponen et al. showed that fecal calprotectin correlated with histological findings in ileocolonic CD. Orlando et al. suggested that this biochemical test could be helpful in predicting the endoscopic postsurgical recurrence in asymptomatic CD patients. Tibble et al. and Gisbert et al. also confirmed the usefulness of fecal calprotectin assessment in the prediction of clinical CD relapse in patients in remission.
However, there are still questions to be answered. For example, Silponen et al.,17,18 studied the usefulness of noninvasive markers in inflammatory bowel diseases and raised the question of whether the disease location could affect the diagnostic accuracy of fecal calprotectin in CD. Indeed, there are data showing that the reliability of fecal calprotectin assessment seems to be lower in patients with small-bowel CD compared with those with ileocolonic or colonic CD.23 The use of drugs, such as NSAIDs or proton-pump inhibitors (PPIs) can also affect fecal calprotectin. However, in our study, the proportions of patients with various disease locations were similar in both groups. The use of any NSAIDs excluded patients from the study, and the statistical analysis revealed no differences in the use of PPI between the study groups. Thus, we considered our study groups to be homogeneous, which allowed us to perform further analyses, as the hypothesized effect of CD duration on the diagnostic utility of fecal calprotectin measurement has not been investigated so far.

Our data show that longer disease duration does not affect the diagnostic utility of fecal calprotectin measurement in CD. Most importantly, we showed a correlation between calprotectin and endoscopic CD activity (SES-CD). Endoscopy seems to be the most important diagnostic investigation in CD from the clinical point of view. Data from clinical studies concerning anti-TNF-α agents have shown that mucosal healing observed on endoscopy is a sensitive predictor of long-term remission in CD, and it is associated with lower hospitalization and surgery rates.24 In both study subgroups, the correlation between SES-CD and fecal calprotectin was of key importance. This allows us to conclude that fecal calprotectin is a reliable marker of the endoscopic severity of CD not only in patients with high inflammatory activity, but also in those with long-lasting CD presenting more discrete endoscopic lesions.

Another important finding is the correlation between fecal calprotectin, CRP, and platelets. CRP is believed to be the most accurate biochemical marker of inflammation in inflammatory bowel diseases. A decrease or normalization of the CRP concentration was one of the main endpoints in several clinical studies on, for example, biological agents in CD.25 Platelets are another sensitive inflammatory marker. Various hemostatic disturbances and alterations of the platelet function in inflammatory conditions in CD have been reported.26,27 Thus, the diagnostic role of platelet count still seems to be underestimated in inflammatory bowel diseases. Our study revealed significant correlations between fecal calprotectin and CRP, and between fecal calprotectin and platelets in both study subgroups. This observation shows that fecal calprotectin is a useful marker of active inflammation, independently of the disease duration and phenotype.

Our study provided conflicting results concerning the correlation between fecal calprotectin concentrations and clinical CD activity (CDAI). The CDAI reflected clinical disease activity in patients with long-lasting CD but not in those with a disease duration of less than 10 years. However, the usefulness of the CDAI in discriminating patients with active from those with non-active CD is controversial. For example, Lahiff et al.28 showed that the CDAI can be similarly elevated in patients with irritable bowel syndrome and in CD. These limitations of the CDAI should be considered when analyzing data on the correlation between fecal calprotectin and the clinical status of the patients.

In conclusion, we have shown for the first time that disease duration and time-dependent changes of the CD phenotype do not affect diagnostic utility of the fecal calprotectin measurement in CD. Therefore, this noninvasive biochemical marker may be considered as reliable and may be used in all CD patients, independently of the disease duration.

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Związek między czasem trwania choroby a przydatnością diagnostyczną oznaczania kalprotektyny w stolcu u pacjentów z chorobą Leśniowskiego i Crohna

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STRESZCZENIE

Badanie kalprotektyny w stolcu jest przydatną metodą oceny aktywności choroby Leśniowskiego i Crohna (Crohn’s disease – CD). Niemniej jednak CD charakteryzuje się dużą różnorodnością kliniczną, a jej fenotyp zmienia się wraz z czasem trwania choroby: w początkowym okresie dominuje komponenta zapalna, z czasem u coraz większego odsetka chorych pojawiają się zwężenia przewodu pokarmowego oraz przetoki.

Celem badania była ocena, czy czas trwania choroby wpływa na przydatność diagnostyczną oznaczania kalprotektyny w stolcu w CD.

Do badania włączono prospektywnie 150 chorych. Aktywność CD w enterografii rezonansu magnetycznego oceniano, obliczając Simple Enterographic Activity Score for Crohn’s Disease. Endoskopowa aktywność CD oceniano za pomocą skali Simple Endoscopic Score for Crohn’s Disease (SES-CD). Oceniono stężenia biochemicznych markerów zapalenia we krwi oraz stężenie kalprotektyny w stolcu za pomocą metody ELISA. Pacjentów podzielono na dwie podgrupy w zależności od czasu trwania choroby: <10 lat oraz ≥10 lat od diagnozy.

Chorzy z dłuższym czasem trwania choroby charakteryzowali się mniejszą aktywnością zapalną CD ocenianą w badaniach biochemicznych, endoskopowych i obrazowych. Kalprotektyna w stolcu wykazywała tendencję do niższych stężeń w tej podgrupie chorych (106,5 ±93,2 mg/l vs 135,7 ±128,8 mg/l; p >0,05). Chorzy, u których choroba trwała dłużej, istotnie częściej mieli postać CD z obecnością zwężen i przetok (p <0,04). Niemniej jednak w obu podgrupach kalprotektyna w stolcu istotnie korelowała z SES-CD, stężeniem białka C-reactywnego i płytkami krwi.

Czas trwania choroby oraz związane z tym zmiany w fenotypie CD nie wpływają na przydatność diagnostyczną oznaczania kalprotektyny w stolcu. Wiarygodność tego badania biochemicznego w ocenie aktywności choroby jest podobna u wszystkich chorych z CD, dlatego może ono być używane niezależnie od czasu, jaki minął od rozpoznania choroby.

SŁOWA KLUCZOWE
choroba Leśniowskiego i Crohna, czas trwania choroby, enterografia rezonansu magnetycznego, ileokolonoskopia, kalprotektyna

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