Optimizing anti-TNF therapy in inflammatory bowel disease
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CHAPTER 2

Impact of disease location on fecal calprotectin levels in Crohn’s disease

Fecal calprotectin in Crohn’s disease


ABSTRACT

Background and aim
The correlation between the Simple Endoscopic Index for Crohn’s Disease (SES-CD) and fecal calprotectin is well established in (ileo)colonic Crohn’s disease (CD). However, for ileal CD existing data are conflicting. The aim of this study was to evaluate the biomarker profile in CD patients with varying severity and location of mucosal ulceration.

Methods
An electronic patient database search identified CD patients in whom ileocolonoscopy, fecal calprotectin (CALPRO), serum C-reactive protein (CRP) and blood leukocyte counts (LEU) were measured within a four week interval without changes in medication. Ileocolonoscopies were scored for the presence of ulcers in each segment as defined by the SES-CD and the sum of segmental ulcer scores resulted in a partial SES-CD (pSES-CD).

Results
Fourty-four patients were identified of whom 9/44 had ileal CD, 20/44 colonic and 15/44 ileocolonic CD based on the Montreal classification. In the total study population CALPRO correlated best with pSES-CD (r=0.76, p<0.0001), followed by LEU (r=0.54, p=0.0004) and CRP (r=0.45, p=0.0026). Patients with ileal CD had significantly lower CALPRO level than those with (ileo)colonic disease even in the presence of large and/or very large ulcers (mean±SEM: 297±81µg/g vs. 1523±97µg/g, p<0.0001). LEU was also significantly lower in the presence of large and/or very large ulcers in ileal CD compared to those with (ileo)colonic disease (mean±SEM: 6.7±0.9×10⁹/l vs. 10.6±0.8×10⁹/l, p=0.02). A similar trend was identified regarding CRP levels.

Conclusions
Even in the presence of large or very large ulcers, patients with ileal Crohn’s may not have markedly elevated fecal calprotectin levels.
Introduction
The severity of mucosal damage in Crohn’s disease (CD) does not correlate well with clinical symptoms.\textsuperscript{1,2} To date, ileocolonoscopy is considered the gold standard to evaluate the severity of gut inflammation. To reduce costs and patient discomfort, several serum and fecal markers have been evaluated as tools to monitor CD activity. Of these biomarkers, fecal calprotectin has been shown to be the most accurate in the detection of endoscopically active disease, followed by serum C-reactive protein (CRP) and leukocyte count.\textsuperscript{3,4} Additionally, erythrocyte sedimentation rate, blood platelet count and serum albumin level are also commonly used, although are regarded as less accurate markers of inflammation. Active intestinal inflammation is associated with an acute phase reaction and migration of leukocytes to the gut. Calprotectin represents 60\% of the cytosolic protein concentration of neutrophil granulocytes and macrophages.\textsuperscript{5} Therefore its concentration in the stool is believed to be proportionate to neutrophil migration to the gastrointestinal tract, which makes it a sensitive marker of gut inflammation.\textsuperscript{6}

However, existing data are conflicting with regard to fecal calprotectin concentration and endoscopic mucosal damage in ileal as opposed to colonic or ileocolonic CD. Recently a fecal calprotectin cut-off of >250µg/ml was suggested to indicate endoscopically active disease, regardless of disease localization.\textsuperscript{1} In contrast, it was previously found that fecal calprotectin levels were significantly lower in endoscopically active ileal CD compared to CD with (ileo)colonic localization.\textsuperscript{3} This may suggest that the calprotectin cut-off used for active disease is influenced by disease localization. Therefore, the objective of this study was to evaluate the correlation between mucosal ulcerations and fecal as well as serum biomarkers and to examine possible differences between biomarker profiles of patients with ileal and (ileo)colonic CD.
Methods

Patients and study design

A retrospective search was carried out in the patient database of the Academic Medical Centre, Amsterdam, to identify CD patients seen between 2011 and 2013 who had undergone complete ileocolonoscopy and fecal calprotectin was measured within four weeks. Serum CRP and full blood count were additionally evaluated. Patients who had change in their IBD-related medication during the study period, patients with incomplete ileocolonoscopy, with history of aspirin or NSAID use, with erosive and/or ulcerative upper gastrointestinal disease, infectious gastroenteritis, colon polyp, colorectal cancer and total colectomy were excluded. The search identified 48 patients, of whom two were excluded due to incomplete endoscopy, one patient due to extensive bowel resection (total colectomy and ileal pouch anal anastomosis) and one patient due to NSAID use. Two patients who underwent subtotal colectomy were not excluded; in those patients the endoscopic score was only attributed to the residual segments. No patients were excluded due to change in medication during the study period.

Patients’ demographic data, such as age, gender, Montreal classification, medication at the time of endoscopy, change in medication between endoscopy and biomarker measurement and previous history of IBD-related surgery were documented. The study complies with the principles laid down in the Declaration of Helsinki. The work was approved by the Ethical Committee of the Academic Medical Center in Amsterdam.

Biomarker measurement

Fecal calprotectin was measured by quantitative enzyme-linked immunosorbent assay (Bühlmann Calprotectin ELISA Kit, Bühlmann Laboratories AG, Schönenbuch, Switzerland) according to the manufacturer’s instructions. All fecal samples were processed within 72 hours after collection. The test had an upper detection limit of 1800 µg/g. Fecal samples of calprotectin levels above 1800 µg/g were not routinely further diluted for a second measurement and are therefore noted as 1800 µg/g. Blood samples were measured for serum CRP (upper limit of normal was 5 mg/l) and full blood count (blood leukocyte normal range 4.0-10.5x10⁹/l) as routine laboratory values. In patients, who had laboratory tests more than once within the four-week study interval, the measurements closest in time to endoscopy were used for analysis.
Scoring of mucosal ulceration
Ileocolonoscopies were scored for the presence of ulcers in each segment as absent (0), aphthous (1), large (0.5-2cm) (2) and very large (>2cm) (3), as defined by the Simple Endoscopic Score for Crohn’s Disease (SES-CD) in all five segments (i.e. terminal/neoterminal ileum, right colon, transverse colon, left colon and rectum). The sum of segment scores resulted in a partial SES-CD (pSES-CD) between 0-15 points. The scoring was based on videos where available, on photodocumentation and endoscopy reports. Photodocumentation of endoscopies was in line with the guidelines of the European Society of Gastrointestinal Endoscopy.

Statistical analysis
Statistical analysis was performed with Prism 5.01 (GraphPad Software Inc., CA, USA). Results of numerical data are presented as mean±SEM. Correlation between biomarkers (fecal calprotectin, serum CRP and blood leukocyte count) and pSES-CD was assessed by the Spearman’s rank correlation coefficient (r). Correlation between pSES-CD and blood leukocyte count was additionally separately evaluated in patients who received neither corticosteroids, nor azathioprine/6-mercaptopurine (AZA/6-MP) treatment during the study period.

The total study population and patients with ileal and (ileo)colonic localization were separately stratified according to the worst mucosal lesion present at endoscopy: no ulcers, aphthous lesions, large or very large ulcers. One-way analysis of variance was performed for fecal calprotectin followed by Tukey’s post-test for multiple comparison.

Fecal calprotectin, serum CRP and blood leukocyte count were all compared in ileal and (ileo)colonic disease in patients with large and/or very large ulcers by using the student’s t test. A p value of < 0.05 was considered statistically significant.
Results

Patient characteristics

Fourty-four patients (19 male, age 36.5±2.0 years) were studied, of which 9 patients were characterized as ileal (L1), 20 as colonic (L2) and 15 as ileocolonic (L3) according to the Montreal classification. Of the patients with ileal disease, one patient received oral prednisolone, one budesonide and two patients 6-MP at the time of the study period. Among patients with (ileo)colonic disease location none received oral prednisolone, four patients received budesonide and five patients were on AZA/6-MP. The patients’ baseline characteristics are demonstrated in Table 1.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>n=44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: female, n (%)</td>
<td>25 (57%)</td>
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<tr>
<td>Age (years): mean, range</td>
<td>36.5 (17-66)</td>
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<tr>
<td>Montreal classification, n (%)</td>
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</tr>
<tr>
<td>Age at diagnosis (years):</td>
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</tr>
<tr>
<td>A1 (≤16)</td>
<td>9 (21%)</td>
</tr>
<tr>
<td>A2 (17-40)</td>
<td>30 (68%)</td>
</tr>
<tr>
<td>A3 (≥40)</td>
<td>5 (11%)</td>
</tr>
<tr>
<td>Disease location:</td>
<td></td>
</tr>
<tr>
<td>L1 (ileal)</td>
<td>9 (21%)</td>
</tr>
<tr>
<td>L2 (colonic)</td>
<td>20 (45%)</td>
</tr>
<tr>
<td>L3 (ileo-colonic)</td>
<td>15 (34%)</td>
</tr>
<tr>
<td>+L4 (upper gastrointestinal)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>Behavior:</td>
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<tr>
<td>B1 (non stricturing, non penetrating)</td>
<td>21 (48%)</td>
</tr>
<tr>
<td>B2 (stricturing)</td>
<td>17 (39%)</td>
</tr>
<tr>
<td>B3 (penetrating)</td>
<td>6 (14%)</td>
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<tr>
<td>+p (perianal disease)</td>
<td>14 (32%)</td>
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<td>Medication at endoscopy, n (%):</td>
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</tr>
<tr>
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<td>17 (39%)</td>
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<tr>
<td>Thiopurines</td>
<td>9 (20%)</td>
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<td>Anti-TNF</td>
<td>8 (18%)</td>
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<tr>
<td>Corticosteroids</td>
<td>7 (16%)</td>
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<tr>
<td>5-ASA</td>
<td>4 (9%)</td>
</tr>
<tr>
<td>Vedolizumab</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Ustekinumab</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>History of previous bowel resection, n (%)</td>
<td>16 (36%)</td>
</tr>
</tbody>
</table>

Table 1. ASA: aminosalicylic acid; TNF-α: tumor necrosis factor α

In the total study population 14 (mean; range: 1-28; 95% CI: 12.8-16.3) days elapsed between endoscopy and fecal calprotectin measurement. There was no significant difference between the timing of endoscopy and fecal calprotectin measurement in patients with isolated ileal disease 16.3 (range: 7-25; 95% CI: 11.2-21.4) days and with (ileo)colonic disease 13.5 (mean; range: 1-28; 95% CI: 10.9-16) days. In the total study population 13.7 (mean; range: 0-28; 95% CI: 11.5-15.9) days elapsed between endoscopy and blood biomarker measurement.
Correlation of pSES-CD with biomarkers in the total study population

In the total study population the ulcer score pSES-CD correlated best with fecal calprotectin (n=44, r=0.76, p<0.0001), followed by blood leukocyte count (n=40, r=0.54, p=0.0004) and serum CRP (n=43, r=0.45, p=0.0026). Figure 1 demonstrates the correlation between pSES-CD and biomarkers on a scatter plot. Additionally, in patients who received neither corticosteroids nor AZA/6-MP, the pSES-CD also showed correlation with the blood leukocyte count (n=27, r=0.45, p=0.017).

**Figure 1** Correlation between pSES-CD and biomarkers in the total population
1a Correlation between pSES-CD and fecal calprotectin, 1b Correlation between pSES-CD and blood leukocyte count, 1c Correlation between pSES-CD and serum CRP.

Fecal calprotectin levels stratified according to the worst endoscopic lesion present

In the total population 3 patients had no ulcers, 9 patients had aphthous ulcers (<5mm), while 12 patients exhibited large (5-20mm) and 19 patients very large ulcers (>20mm) as defined by the SES-CD. In the total population a gradual increment in fecal calprotectin level was seen when stratified according to worst endoscopic lesion present as demonstrated by Figure 2a. Calprotectin level (mean±SEM) was 41.3±16µg/g in patients with no ulcers, 351.9±53µg/g in patients with aphthous ulcers, 992.8±212.3µg/g in patients with large and 1409±618.3µg/g in patients with very large ulcers.

In patients with (ileo)colonic disease localization (n=35) a significant difference was seen in fecal calprotectin levels between patients with no ulcers (n=3, 41.3±16µg/g) versus patients with large (n=7, 1447±615.1µg/g, p=0.0001) or very large ulcers (n=17, 1557±455.4µg/g, p<0.0001). Difference in fecal calprotectin level was also significant between patients with aphthous ulcers (n=7, 383.4±139.9µg/g) versus patients with large (p<0.0001) or very large ulcers (p<0.0001) (Figure 2b).
Due to the relatively low number of patients with ileal disease (n=9) no separate analysis was carried out for fecal calprotectin levels stratified for severity of endoscopic lesions. Notably, seven of the patients with ileal disease had large or very large ulcers whereas the fecal calprotectin was lower than 200µg/g in four of them.

Figure 2 Fecal calprotectin levels stratified according to the worst endoscopic lesion present 2a Total population, 2b Patients with (ileo)colonic disease localization

Figure 3 Biomarker levels compared in ileal and (ileo)colonic Crohn’s disease in patients with ulcers larger than 0.5cm 3a Fecal calprotectin, 3b Blood leukocyte count, 3c Serum CRP

Biomarker levels compared in ileal and (ileo)colonic Crohn’s disease in patients with ulcers larger than 0.5cm

Patients with ileal CD (n=7) had a significantly lower fecal calprotectin level than those with (ileo)colonic disease (n=25) in the presence of large and/or very large ulcers (297±81µg/g vs. 1523±97µg/g, p<0.0001, Figure 3a). Blood leukocyte count was also significantly lower in the presence of large and/or very large ulcers in ileal CD compared to patients with (ileo)colonic disease (6.7±0.9x10^9/l vs. 10.6±0.8x10^9/l, p=0.02, Figure 3b). A similar trend was identified for serum CRP levels (5.3±2.2mg/l vs. 39.9±13.4mg/l, p=0.17, ns, Figure 3c).
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Discussion

This study evaluated the fecal as well as serum biomarker profile of Crohn’s disease patients. CD patients with large and/or very large ulcers confined to the ileum had significantly lower fecal calprotectin levels compared to patients with similar ulcerations in the colon. We found that patients with normal fecal calprotectin level may even have large or very large ileal ulcerations. Therefore, disease location should be taken into consideration when interpreting fecal calprotectin values in clinical practice.

Consistent with previous findings, our results show that fecal calprotectin, blood leukocyte count and serum CRP correlate well with endoscopic disease activity in CD.\(^3\) Furthermore, our data also confirms that fecal calprotectin shows the closest correlation with endoscopic mucosal damage.\(^3\) However, previous reports are conflicting about the sensitivity of fecal calprotectin in CD with different disease localization.\(^1,3,5-11\) In a large cohort of Crohn’s patients, Schoepfer et al. showed that ileocolonic CD was associated with significantly higher fecal calprotectin level compared to CD localized to the terminal ileum.\(^3\) Fecal calprotectin was also shown to correlate with histological findings in ileocolonic and colonic CD, but not in ileal disease localization.\(^12\)

In the present study the extent of small bowel disease localization was assessed by ileocolonoscopy; hence, evaluation was limited to the terminal ileum. Therefore, active inflammation of proximal small bowel segments could not be excluded. Small bowel lesions evaluated by MR follow-through was shown to correlate with fecal calprotectin concentration.\(^13\) In contrast, others reported that fecal calprotectin levels poorly correlate with active small bowel CD as detected by capsule endoscopy.\(^14\)

Indications to use fecal calprotectin as a biomarker in clinical practice are steadily expanding. As a non-invasive marker it is not only used to estimate endoscopic activity at a given time point, but also to predict relapse or response to therapy.\(^15-17\) Recently, a rapid point-of care test has been shown to reliably predict recurrence in post-operative CD.\(^18\) With this rising interest several groups came up with proposed cut-off levels as an indicator of significant mucosal inflammation (i.e. large ulcers) in CD patients, generally ranging between 200-300µg/g.\(^1,19,20\) Notably, in our study four out of seven patients with ileal disease exhibiting large or very large ulcerations (in the absence of colonic disease activity) had fecal calprotectin levels below 200µg/g. However, variability between fecal samples and different assays could considerably influence these outcomes.\(^21-23\) To overcome this variability, two consecutive measurements, within a one-month interval, preferably from morning
stool samples, have been proposed to be more reliable than one single measurement to predict relapse in ulcerative colitis.\textsuperscript{24}

A possible explanation of lower fecal calprotectin values found in active ileal CD compared to colonic CD is that the total ulcerated surface could be lower in the ileum. This could result in a lower mucosal and systematic inflammatory load in patients with ileal ulcerations, compared to patients with similar colonic ulcers.

In conclusion, our results suggest that disease location should be taken into account when interpreting fecal calprotectin levels. Patients with ileal CD may have large or very large ulcers even in the absence of markedly elevated fecal calprotectin levels. Consequently, cut-off values for ileal CD may differ from those with (ileo)colonic disease.
REFERENCES


