Fecal Calprotectin Correlates More Closely With the Simple Endoscopic Score for Crohn's Disease (SES-CD) than CRP, Blood Leukocytes, and the CDAI

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Fecal Calprotectin Correlates More Closely With the Simple Endoscopic Score for Crohn's Disease (SES-CD) than CRP, Blood Leukocytes, and the CDAI

Abstract

OBJECTIVES: Studies evaluating the correlation between the widely used Simple Endoscopic Score for Crohn's disease (SES-CD) and noninvasive markers are scarce. The aim of this study was to evaluate the correlation between the SES-CD and fecal calprotectin, C-reactive protein (CRP), blood leukocytes, and the Crohn's disease activity index (CDAI).

METHODS: Crohn's disease patients undergoing complete ileocolonoscopy were prospectively enrolled and scored independently according to the SES-CD and the CDAI. SES-CD was defined as follows: inactive 0-3; mild 4-10; moderate 11-19; and high >/=20.

RESULTS: Values in CD patients (n=140 ileocolonoscopies) compared with controls (n=43) are as follows: calprotectin, 334+/-322 vs. 18+/-5 mug/g; CRP, 26+/-29 vs. 3+/-2 mg/l; and blood leukocytes, 9.1+/-3.4 vs. 5.4+/-1.9 g/l (all P<0.001). The SES-CD correlated closest with calprotectin (Spearman's rank correlation coefficient r=0.75), followed by CRP (r=0.53), blood leukocytes (r=0.42), and the CDAI (r=0.38). Calprotectin was the only marker that could discriminate inactive endoscopic disease from mild activity (104+/-138 vs. 231+/-244 mug/g, P<0.001), mild from moderate activity (231+/-244 vs. 395+/-256 mug/g, P=0.008), and moderate from high activity (395+/-256 vs. 718+/-320 mug/g, P<0.001). The overall accuracy for the detection of endoscopically active disease was 87% for calprotectin (cutoff 70 mug/g), 66% for elevated CRP, 54% for blood leukocytosis, and 40% for the CDAI >/=150.

CONCLUSIONS: Fecal calprotectin correlated closest with SES-CD, followed by CRP, blood leukocytes, and the CDAI. Furthermore, fecal calprotectin was the only marker that reliably discriminated inactive from mild, moderate, and highly active disease, which underlines its usefulness for activity monitoring.
Fecal calprotectin correlates closer with the Simple Endoscopic Score for Crohn's Disease (SES-CD) than CRP, blood leukocytes, and CDAI

Running head:

Correlation of SES-CD with noninvasive markers

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Abstract

Background: Studies evaluating the correlation between the widely used simple endoscopic score for Crohn’s disease (SES-CD) and non-invasive markers are scarce. Aim: To evaluate the correlation between a semi-quantitative SES-CD and fecal calprotectin, Crohn’s disease activity index (CDAI), C-reactive protein (CRP) and blood leukocytes.

Methods: CD patients undergoing complete ileo-colonoscopy were prospectively enrolled and scored independently according the SES-CD and CDAI. SES-CD was defined as follows: inactive 0-3; mild 4-10; moderate 11-19; high ≥20.

Results: Values in CD patients (n=140) compared to controls (n=43): Calprotectin: 334±322 vs. 18±5μg/g, CRP 26±29 vs. 3±2mg/L, blood leukocytes 9.1±3.4 vs. 5.4±1.9G/L (all P < 0.001). The SES-CD correlated closest with calprotectin (Spearman’s rank correlation coefficient r = 0.75), followed by CRP (r = 0.53), blood leukocytes (r = 0.42), and CDAI (r = 0.38). Calprotectin was the only marker that could discriminate inactive endoscopic disease from mild activity (104±138 vs. 231±244μg/g, P <0.001), mild from moderate activity (231±244 vs. 395±256μg/g, P =0.008), and moderate from high activity (395±256μg/g vs. 718±320μg/g, P <0.001). The overall accuracy for the detection of endoscopically active disease was 84% for calprotectin, 66% for elevated CRP, and 54% for blood leukocytosis, and 40% for CDAI ≥150.

Conclusions: Fecal calprotectin correlated closest with SES-CD, followed by CRP, CDAI, and blood leukocytes. Furthermore, fecal calprotectin was the only marker that reliably discriminated inactive from mild, moderate, and highly active disease which underlines its usefulness for activity monitoring.
Key words: fecal calprotectin, Crohn’s disease, biomarkers, CDAI, monitoring
INTRODUCTION

The assessment of Crohn’s disease (CD) activity is based on a combination of symptoms, clinical findings, and endoscopy. However, there is often an insufficient correlation between these diagnostic elements.\textsuperscript{1,2,3,4,5,6} Although considered the gold standard method for assessment of intestinal inflammation, ileo-colonoscopy has the disadvantage of being invasive, time-consuming, expensive, and sometimes uncomfortable for patients.

To overcome these limitations, several laboratory markers have been evaluated regarding their performance for monitoring Crohn’s disease activity. Active inflammation in CD patients is associated with an acute phase reaction and migration of leukocytes to the gut. Thereby, various proteins can be measured in serum and feces.\textsuperscript{7} C-reactive protein (CRP), produced by hepatocytes upon the stimulation by proinflammatory cytokines such as interleukins 1 and 6, has been found to be associated with clinical and endoscopic activity in IBD.\textsuperscript{8,9,10} Fecal calprotectin represents 60% of cytosolic proteins in granulocytes. The amount of calprotectin in feces is therefore proportional to the neutrophil migration to the gastrointestinal mucosa. Fecal calprotectin is stable against degradation for up to one week at room temperature.\textsuperscript{11} It accurately distinguishes inflammatory bowel disease (IBD) from non-inflammatory bowel diseases such as irritable bowel syndrome.\textsuperscript{12} Several publications in pediatric and adult cohorts have demonstrated a correlation of the amount of fecal calprotectin with the severity of mucosal inflammation.\textsuperscript{13,14,15} A good correlation between fecal calprotectin and the Crohn’s Disease Index of Severity (CDEIS) has already been demonstrated.\textsuperscript{16,17} However, the CDEIS is demanding to complete and time-consuming in clinical practice. Therefore, the simple endoscopic score for Crohn’s disease (SES-CD) was developed and validated in 2004.\textsuperscript{18} This score has a good correlation with the CDEIS, but is much easier to perform and therefore more frequently used in clinical practice. So far, data regarding the correlation of fecal calprotectin with SES-CD are scarce, and there is especially a paucity of studies evaluating the endoscopic disease activity with calprotectin in relation to other biomarkers.\textsuperscript{19,20} Knowledge about the test performance of different biomarkers to discriminate classes of inflammatory activity is important for the early detection of relapses.
and for tailoring the individual therapy. This appraisal is supported by recently published papers claiming that the optimal therapeutic target to modify disease course in IBD patients should not only be clinical remission, but also mucosal healing as this item has shown an association with reduced need for surgery and hospitalization in CD patients.\textsuperscript{21,22} We hypothesized, that fecal calprotectin would be superior to CDAI, CRP, and blood leukocytes to discriminate the endoscopic disease activity.

Thus, we aimed to answer the following questions in this study: first, is there any non-invasive marker able to discriminate between inactive, mild, moderate and severe endoscopic activity? And second, what is the overall test accuracy of the CDAI and the various biomarkers for detection of endoscopically active disease?

\section*{METHODS}

\textbf{Participants}

Of 170 adult outpatients and inpatients with previously confirmed diagnosis of ulcerative colitis referred for colonoscopy to the Departments of Gastroenterology of the University Hospitals Bern and Basel between March 2006 and June 2008, 122 patients were included (72\%). They were diagnosed on the basis of standard clinical, endoscopic, and histologic criteria.\textsuperscript{23} The study was conducted with approval of the local ethics committees. Patients were first instructed by the local investigator, then they were provided with a fecal specimen collection set consisting of 2 fecal tubes (1 tube for 1ml, order number 55478, Sarstedt AG, Nümbrecht Germany, and one tube for stool culture and Cl. difficile toxin assay). Collection of the fecal specimens was performed by the patients themselves. The fecal specimens from the outpatients were shipped by mail to the laboratory. In inpatients, the fecal collection set was prepared by a trained nurse and then sent to the laboratory. The fecal samples for bacterial analysis were processed immediately after receipt whereas fecal samples for calprotectin were stored at -40°C until analysis. Blood samples for measurement of a full blood count and CRP were delivered by the patients within 3 days prior to endoscopy. Ten of
the included CD patients (8%) underwent an upper endoscopy (due to upper abdominal complaints) at the same day when ileo-colonoscopy was performed, no CD-related lesions or reflux-associated lesions could be detected in this subgroup.

Inclusion criteria: Disease duration > 3 months, complete ileocolonoscopy including biopsies (at least 2 biopsies from terminal ileum and 4 colonic biopsies from affected regions), age 18-85 years, fecal samples delivered from 3 to 1 days before ileocolonoscopy (bowel preparation was not started until the fecal specimen was delivered).

Exclusion criteria: Incomplete ileocolonoscopy (cecum not reached), infectious entero-colitis (positive stool culture for Salmonella, Shigella, Campylobacter, positive Cl. difficile Toxin A+B assay, cytomegalovirus positive in conventional histology or in immunohistochemistry), colorectal cancer, ulcerative colitis, indeterminate colitis, urinary incontinence (risk of contamination of fecal samples), inability to collect fecal samples, pregnancy, history of extensive bowel resection (ileosigmoidostomy, ileorectostomy), ostomy, symptoms related mainly to perianal fistulizing disease, known Crohn’s disease of esophagus or stomach or duodenum, regular intake of aspirin and/or NSAID (≥ 2 tablets/week). Of a total of 173 screened CD patients, 51 were excluded: 8 because of incomplete ileocolonoscopy (strictures that could not be passed after balloon dilation), 3 for not delivering fecal samples on time, 3 for known and active CD of upper gastrointestinal tract, one for intake of NSAID, one for positive Clostridium difficile toxin assay, and 35 for not being willing to participate in the study.

Endoscopic disease activity

During the study period, 18 patients underwent ileocolonoscopy twice, therefore 140 endoscopies were performed in 122 patients. Indications for endoscopy were clinically active disease (flare) (n=76, 54%), assessment of endoscopic activity after medical treatment (n=45, 32%), dysplasia surveillance for long-standing disease (n=15, 11%), and stricture dilation (n=4, 3%). Five experienced board certified gastroenterologists (AMS, CB, AS, SV,
FS), every of them with at least 5 years of experience in performing ileocolonoscopy, performed the endoscopies and graded the findings according to the SES-CD.

For calculating the SES-CD, the intestine was divided into five segments: ileum, right colon, transverse colon, left colon, and rectum. The degree of disease involvement in each of the five segments was determined by the assessment of four parameters: presence and size of ulcers (score 0-3), extent of ulcerated surface (score 0-3), extent of affected surface (score 0-3), and presence and type of narrowing (score 0-3). The sum of the score for each endoscopic variable ranges from 0 to 15, except for stenosis, where it varies between 0 and 11, because 3 represents a stenosis through which a colonoscope cannot be passed, and therefore can be observed only once. The lowest possible SES-CD was 0, representing an intestine without any lesions; the highest possible score was 56 points. The clinical disease activity was assessed by measurement of the CDAI by a physician not performing the colonoscopy. All gastroenterologists performing the endoscopies were unaware of the results of the CDAI, fecal calprotectin, CRP, and blood leukocytes to avoid bias.

The controls were healthy persons from the clinical and laboratory staff willing to provide blood and fecal samples. All healthy controls were free of symptoms and had no history of abdominal complaints. The control group did not undergo endoscopy. Except for birth control pills in some women, these persons were not on any regular medication.

So far there exists no definition on how to define endoscopic remission using the SES-CD. We defined a semiquantitative SES-CD as follows:

- inactive (remission) 0-3
- mild activity 4-10
- moderate activity 11-19
- high activity ≥20 points.
**Fecal Calprotectin**

Fecal calprotectin was measured by a quantitative enzyme linked immunosorbent assay (PhiCal™ Test, purchased from Medical Instrument Corporation, Solothurn, Switzerland, Art-No. 006, the test is delivered by CALPRO AS, Oslo, Norway). This sandwich ELISA measures quantitative calprotectin. Fecal specimens were diluted at 1:2500. The scientist (MT) performing the analyses was blinded to the patient names, the clinical and endoscopic findings. All fecal samples were processed within 72 hours after collection. The assays were performed according to the test instructions. ELISA plates were read on a Spectra mini reader (TECAN) at an OD of 450nm. According to the manufacturer, the calprotectin cutoff-level representing a positive value was $\geq 50 \mu g$ calprotectin/g feces.

**CRP, and Blood Leukocytes**

Blood leukocytes (normal range 2.6-7.8 G/L), hemoglobin (normal range for women 120-160g/L, for men 140-180g/L), a sedimentation rate (normal range for women and men up to 50 years up to 20mm/h and 15mm/h, normal range for persons older than 50 years up to 30mm/h and up to 20mm/h, respectively) as well as CRP (upper limit of normal <5mg/L) were determined as routine laboratory values within 3 days prior to endoscopy.

**Statistical Analysis**

Data were listed on an Excel sheet (Microsoft Excel 2003; Microsoft Switzerland Ltd Liab. Co., Wallisellen, Switzerland), statistical analyses were performed with a statistical package program (Stata Vs 9, College Station, Texas, USA). Results of numerical data are presented as mean ± standard deviation (SD), and range. Normal distribution of data was tested using a Normal-QQ-Plot. Fisher’s exact test (two-sided) or the Chi squared test was used to explore associations of categorical data in 2 independent groups. The Wilcoxon rank sum test was used to explore associations of numerical data in 2 independent groups. A $P < 0.05$ was considered statistically significant. A Bonferoni adjustment was performed in case of multiple testing. The association between endoscopic disease activity with CDAI, fecal
calprotectin, CRP, and blood leukocytes was assessed by determination of the Spearman’s rank correlation coefficient (r) for nonparametric correlations. The test characteristics are given as sensitivity, specificity, positive and negative predictive value (SENS, SPEC, PPV, NPV), and overall accuracy. The overall accuracy is calculated by addition of the true-positive and true-negative test results divided by all tests (a+d)/(a+b+c+d) and admits the comparative evaluation of the various tests.

A power analysis revealed that a sample size of 17 in each of the four subgroups of endoscopic disease activity (total n=68) would have 90% power to detect a difference in the mean calprotectin between the subgroups, using a Mann-Whitney rank-sum test with a 0.05 two-sided significance level.

RESULTS

Patient and Controls Characteristics

The clinical characteristics of the patients and healthy controls are shown in Table 1. Disease phenotypes were grouped according to the Montreal classification. As therapy regimens overlapped, the total counts for more than 100% (131%). The controls were healthy persons from the clinical and laboratory staff willing to provide blood and fecal samples.

The baseline clinical as well as laboratory characteristics of CD patients and controls are demonstrated in Table 2. Calprotectin, CRP, and blood leukocytes were significantly higher in CD patients compared to controls (all \( P <0.001 \)).

Correlation of the SES-CD with CDAI, Fecal Calprotectin, CRP, and Blood Leukocytes

The Endoscopic Activity Index correlated significantly with levels of fecal calprotectin (Spearman’s rank correlation coefficient \( r = 0.75 \)), CRP (\( r = 0.53 \)), blood leukocytes (\( r = 0.42 \)), and CDAI (\( r = 0.38 \)). For all items a \( P < 0.01 \) was found. Figure 1 demonstrates the correlation between the SES-CD and fecal calprotectin with a scatter plot.
In Table 3, we present the relationship between the different subgroups of the SES-CD with their corresponding CDAI, fecal calprotectin, CRP, and blood leukocytes (values given as mean±SD and range). Calprotectin was the only biomarker able to discriminate the four subgroups of SES-CD (P <0.001 for discriminating inactive from mild disease, P = 0.008 for discriminating mild from moderate, and P <0.001 for discriminating moderate from high endoscopic activity). This finding is further illustrated by Figure 2.

The CDAI could neither discriminate inactive from mild nor mild from moderate endoscopic activity. The only significance in CDAI was found for discrimination of moderate versus highly active endoscopic disease (P <0.001). CRP was able to discriminate mild from moderate and moderate versus high endoscopic activity (P = 0.013 and P = 0.019), however, CRP failed to discriminate inactive from mildly active endoscopic disease. A difference in the blood leukocyte counts was detected only between moderate versus highly active endoscopic disease.

Test Characteristics of CDAI, Fecal Calprotectin, CRP, and Blood Leukocytes in Predicting Endoscopically Active Disease

The test performance (given by sensitivity/specificity/positive and negative predictive value in percent) of CDAI, fecal calprotectin with 2 cutoffs, CRP, and blood leukocytes in predicting endoscopically active disease (SES-CD ≥ 4) is demonstrated in Table 4.

In summary, calprotectin with a cutoff of ≥ 50μg/g had the best overall accuracy (84%) for the detection of endoscopically active disease, followed by elevated CRP (accuracy 66%), then blood leukocytosis (accuracy 54%), and finally CDAI ≥150 (accuracy 40%). Taking the higher cutoff of 100μg/g calprotectin did not improve the overall accuracy (82% vs 84% with 50μg/g as recommended by the manufacturer).

DISCUSSION
This study demonstrates that fecal calprotectin correlates very closely with the endoscopic disease activity and was the only biomarker that could discriminate inactive from mild, moderate, and highly active disease. Second, the CDAI (cutoff ≥150) presented the worst overall accuracy for detection of endoscopically active disease.

Several groups have already shown that fecal calprotectin is correlated to the endoscopic disease activity in pediatric and adult CD patients. However, studies assessing the correlation between a semiquantitatively captured endoscopic disease activity according the SES-CD and fecal calprotectin are scarce. Sipponen and coworkers evaluated the correlation of fecal calprotectin and lactoferrin with the SES-CD in a cohort of 61 CD patients (87 ileo-colonoscopies) and applied a semiquantitative assessment of endoscopic activity to a certain extent comparable to our classification (SES-CD: inactive 0-3, mild or moderate 4-14, severe ≥15). They found that fecal calprotectin could discriminate inactive from mild to moderate and highly active endoscopic disease. Our results are in accordance with their findings regarding the good performance of fecal calprotectin to discriminate between different inflammatory activities. Comparable to our findings, the correlation between SES-CD and calprotectin was closer than with CRP, blood leukocytes, and CDAI.

Langhorst and coworkers evaluated in a cohort of 43 adult CD patients fecal calprotectin besides lactoferrin, PMN-elastase, CRP, and clinical indices. They calculated an endoscopic disease activity by means of a semiquantitative four-degree system (comparable to our arrangement with the SES-CD) and reported a significant correlation of calprotectin with the binary ordered (active vs. non-active) endoscopic disease activity. No correlation of calprotectin or any other fecal marker was reported with subclasses of endoscopic inflammatory activity. Comparable to our data, they documented significant correlations of SES-CD with fecal calprotectin, CRP, and CDAI. However, their SES-CD calprotectin correlation coefficient (r = 0.35) is lower than ours (r = 0.75) which is probably related to our larger patient cohort and our inclusion of a relatively high proportion of patients with high inflammatory activity. Canani and coworkers assessed in a pediatric cohort of 26 CD patients the correlation between fecal calprotectin, endoscopic activity, and mucosal inflammation.
using a histopathologic score. Fecal calprotectin showed a good correlation with the histologic and endoscopic grade of colonic inflammation. Again, a correlation of calprotectin with the different degrees of endoscopic activity is not reported.

Jones and coworkers evaluated in a cohort of 164 CD patients the relationship between endoscopic (measured by SES-CD) and clinical disease activity (CDAI) and serum (hsCRP, IL-6) and fecal biomarkers (calprotectin and lactoferrin). In accordance to the above cited studies, they reported significant correlations of SES-CD with fecal and serum biomarkers. However, and this stands in contrast to the above cited reports, they found no correlation between SES-CD and CDAI.

The question may arise if it is necessary at all to assess the extent of mucosal damage and if it is not sufficient to treat CD patients solely with the focus on clinical remission. First, CDAI has an insufficient ability to detect low-grade mucosal inflammation. We found that CDAI was not able to discriminate between inactive versus mild and mild versus moderate endoscopic activity; of note, the sensitivity of CDAI for detection of endoscopically active disease was only 33 percent. Similar findings were reported in the study of Sipponen and coworkers. Thus, caution should be exercised when interpreting CDAI data in relation to endoscopic activity. Furthermore, certain items of the CDAI vary because of individual particularities such as a variable weekly stool frequency if irritable bowel symptoms are superimposed on CD activity. Such IBS like symptoms have been reported in two-to three times higher frequency in IBD patients in remission compared to the normal population. Second, anti-inflammatory therapy is associated with a reduction of CD-related hospitalizations and surgical procedures. Thereby, achievement of mucosal healing may alter the natural course of the disease. The established symptom-based therapeutic concept (by measuring CDAI) should therefore be reconsidered. Whether mucosal healing is definitely a therapeutic goal in the treatment of CD remains to be proven in further prospective studies evaluating disease outcome, but at least, the assessment of mucosal healing should be included in clinical trials as an important outcome variable. Since fecal calprotectin closely correlates with endoscopically assessed mucosal damage and since patient’s acceptance for fecal sampling
is high\textsuperscript{29} this biomarker should be useful to monitor the extent of mucosal damage in Crohn’s disease.

It should be kept in mind that fecal calprotectin is an unspecific biomarker and may be elevated in non-IBD related inflammations such as bacterial or drug-induced enterocolitis.\textsuperscript{30} Endoscopy has an established role in CD assessment especially if the disease course deteriorates under therapy (search for CMV superinfection). However, when the patient improves under therapy and the extent of residual mucosal inflammation has to be assessed, non-invasive and cheap biomarkers such as fecal calprotectin seem preferable over logistically demanding endoscopic procedures.\textsuperscript{31}

Our results regarding the performance of elevated CRP to detect endoscopically active disease are comparable to an earlier publication of our group and compare also well to the results of Sipponen and coworkers.\textsuperscript{12,20} Polymorphisms in the CRP gene can be responsible for interindividual differences in CRP production in humans.\textsuperscript{32} Assessment of blood leukocytosis for detection of active endoscopic disease has obviously limitations. These may be explained by the fact that CD patients are usually under therapy with immunomodulators that can affect absolute leukocyte numbers.

Our study has several potential limitations. The first may be our decision not to assess further fecal leukocyte markers besides calprotectin. We voted against inclusion of lactoferrin because we have assessed this marker in two recent studies and found the results to compare well with calprotectin.\textsuperscript{12,29} We further omitted the determination of PMN-elastase in feces because a well conducted trial again demonstrated similar results with calprotectin and lactoferrin for detection of active endoscopic disease.\textsuperscript{15} Second, the topic of mucosal healing is controversially discussed and available data on the significance in Crohn’s disease are so far limited. Despite that, our results provide evidence that mucosal healing can non-invasively be measured by calprotectin as surrogate marker. Third, an ileo-colonoscopy may not be sufficient to detect the whole extent of CD. In the absence of symptoms, we did not perform a systematic search for CD involvement of the upper gastrointestinal tract and small bowel because this would have represented an imbalance between diagnostic expenses on
the one and expected yield on the other hand. We tried to reduce the inevitable diagnostic blurring by offering upper endoscopies to CD patients with symptoms compatible of upper gastrointestinal tract CD involvement.

In summary, we demonstrated that fecal calprotectin was the only marker that could reliably discriminate inactive from mild, moderate, and severe endoscopic activity. Therefore, fecal calprotectin has the potential to replace endoscopy in the disease monitoring of Crohn’s disease. Measuring CDAI is not appropriate for assessment of endoscopic inflammatory activity.
**Tables**

**Table 1.** Clinical characteristics of the CD patients’ cohort according the Montreal classification.

**Abbreviations:** CD = Crohn’s disease. HC = healthy controls.

**Behaviour:** B1 = non-stricturing, non-penetrating; B2 = stricturing, B3 = penetrating; p = perianal disease.

**Location:** L1 = ileal; L2 = colonic, L3 = ileocolonic, L4 = isolated upper disease (L4 is added to L1-L3 when concomitant upper disease is present).

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
<th>HC</th>
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<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>122</td>
<td>43</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>71 (58%)</td>
<td>32 (74%)</td>
</tr>
<tr>
<td><strong>Age: mean±SD, range</strong></td>
<td>42±16 (19-83)</td>
<td>37±11 (25-61)</td>
</tr>
<tr>
<td><strong>Mean disease duration (years)</strong></td>
<td>13.2±6.1</td>
<td>-</td>
</tr>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- A1 (&lt;16yrs)</td>
<td>13 (11%)</td>
<td>-</td>
</tr>
<tr>
<td>- A2 (17-40yrs)</td>
<td>92 (75%)</td>
<td>-</td>
</tr>
<tr>
<td>- A3 (≥40yrs)</td>
<td>17 (14%)</td>
<td>-</td>
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<tr>
<td><strong>Disease phenotype</strong></td>
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<tr>
<td>- B1:</td>
<td>54 (44%)</td>
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</tr>
<tr>
<td>- B1p:</td>
<td>7 (6%)</td>
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<tr>
<td>- B2:</td>
<td>31 (25%)</td>
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<td>- B2p:</td>
<td>7 (6%)</td>
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<tr>
<td>- B3:</td>
<td>18 (15%)</td>
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<td>- B3p:</td>
<td>5 (4%)</td>
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<tr>
<td><strong>Disease location</strong></td>
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<tr>
<td>- L1:</td>
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<tr>
<td>- L2:</td>
<td>20 (16%)</td>
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<td>- L3:</td>
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<td><strong>Prior CD related surgery</strong></td>
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<tr>
<td><strong>Medication at endoscopy</strong></td>
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<tr>
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<td>5-ASA</td>
<td>6 (5%)</td>
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<tr>
<td>Corticosteroids</td>
<td>19 (16%)</td>
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<tr>
<td>Azathioprine</td>
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<td>6-Mercaptopurine</td>
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<td>Methotrexate</td>
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<tr>
<td>TNF-alpha Inhibitor</td>
<td>21 (17%)</td>
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Table 2. Baseline clinical and laboratory characteristics of CD patients and healthy controls. Calprotectin, CRP, and blood leukocytes were significantly higher in CD patients compared to controls (all $P <0.001$).

**Abbreviations:** SD = standard deviation; CI = confidence interval; HC = healthy controls; SES-CD = simple endoscopic score for Crohn’s disease; CDAI = Crohn’s disease activity index; CRP = C-reactive protein.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CD patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>95%-CI</td>
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<tr>
<td>SES-CD</td>
<td>14±13</td>
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</tr>
<tr>
<td>CDAI</td>
<td>160±99</td>
<td>12-402</td>
</tr>
<tr>
<td>Calprotectin (μg/g)</td>
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<td>15-1083</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>26±29</td>
<td>3-121</td>
</tr>
<tr>
<td>Leukocytes (G/L)</td>
<td>9.1±3.4</td>
<td>2.9-17.8</td>
</tr>
</tbody>
</table>
Table 3. Correlation of the Simple Endoscopic Score for Crohn’s Disease (SES-CD) subgroups with fecal calprotectin, CRP, CDAI, and bloek leukocytes.

Numbers are presented as mean±SD and range. The corresponding P-values for discrimination between the different subgroups are listed in grey.

Abbreviations: NS = not significant. CDAI: Crohn’s Disease Activity Index

<table>
<thead>
<tr>
<th>Endoscopic Activity</th>
<th>Inactive (0-3)</th>
<th>Mild (4-10)</th>
<th>Moderate (11-19)</th>
<th>High (≥20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>26</td>
<td>40</td>
<td>27</td>
<td>47</td>
</tr>
<tr>
<td>CDAI</td>
<td>79±86 (-13-281)</td>
<td>85±70 (14-297)</td>
<td>116±47 (44-323)</td>
<td>218±75 (86-417)</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.739</td>
<td>0.201</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Calprotectin µg/g</td>
<td>104±138 (10-725)</td>
<td>231±244 (12-1009)</td>
<td>395±256 (68-912)</td>
<td>718±320 (93-1327)</td>
</tr>
<tr>
<td>P-Value</td>
<td>&lt;0.001</td>
<td>0.008</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>CRP mg/L</td>
<td>12±19 (3-94)</td>
<td>8±10 (3-53)</td>
<td>23±31 (3-172)</td>
<td>40±28 (5-121)</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.349</td>
<td>0.013</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>Leukocytes G/L</td>
<td>7.7±3.1 (4-17.9)</td>
<td>7.6±2.8 (3.7-13.6)</td>
<td>8.8±3.1 (1.4-15.8)</td>
<td>11.1±3.5 (2.9-18.6)</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.903</td>
<td>0.117</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Sensitivity (SENS), Specificity (SPEC), Positive (PPV), Negative Predictive Value (NPV), and the overall Accuracy of fecal calprotectin, CRP, CDAI ≥ 150, and blood leukocytes in predicting endoscopically active disease (SES-CD ≥4).

<table>
<thead>
<tr>
<th></th>
<th>SENS (%)</th>
<th>SPEC (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calprotectin ≥ 50μg/g</td>
<td>89</td>
<td>59</td>
<td>89</td>
<td>61</td>
<td>84</td>
</tr>
<tr>
<td>Calprotectin ≥ 100μg/g</td>
<td>84</td>
<td>74</td>
<td>83</td>
<td>77</td>
<td>82</td>
</tr>
<tr>
<td>CRP ≥ 5mg/L</td>
<td>68</td>
<td>58</td>
<td>88</td>
<td>29</td>
<td>66</td>
</tr>
<tr>
<td>Blood Leukocytes ≥ 7.9G/L</td>
<td>55</td>
<td>50</td>
<td>83</td>
<td>21</td>
<td>54</td>
</tr>
<tr>
<td>CDAI ≥ 150</td>
<td>33</td>
<td>68</td>
<td>80</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>
Figures

Figure 1  Correlation of the SES-CD with fecal calprotectin
**Figure 2**  Correlation between SES-CD activity and fecal calprotectin.

Abbreviations: SES-CD = Simple Endoscopic Score for Crohn’s Disease
References


