Serum ficolin-2 correlates worse than fecal calprotectin and CRP with endoscopic Crohn's disease activity

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Abstract

Background and aims: Ficolin-2 is an acute phase reactant produced by the liver and targeted to recognize N-acetyl-glucosamine which is present in bacterial and fungal cell walls. We recently showed that ficolin-2 serum levels were significantly higher in CD patients compared to healthy controls. We aimed to evaluate serum ficolin-2 concentrations in CD patients regarding their correlation with endoscopic severity and to compare them with clinical activity, fecal calprotectin, and CRP.

Methods: Patients provided fecal and blood samples before undergoing ileo-colonoscopy. Disease activity was scored clinically according to the Harvey–Bradshaw Index (HBI) and endoscopically according to the simplified endoscopic score for CD (SES-CD). Ficolin-2 serum levels and fecal calprotectin levels were measured by ELISA.

Results: A total of 136 CD patients were prospectively included (mean age at inclusion 41.5 ± 15.4 years, 37.5% females). Median HBI was 3 [2–6] points, median SES-CD was 5 [2–8], median fecal calprotectin was 301 [120–703] μg/g, and median serum ficolin-2 was 2.69 [2.02–3.83] μg/mL. SES-CD correlated significantly with calprotectin (R = 0.676, P < 0.001), CRP (R = 0.458, P < 0.001), HBI (R = 0.385, P < 0.001), and serum ficolin-2 levels (R = 0.171, P = 0.047). Ficolin-2 levels were higher in CD patients with mild endoscopic disease compared to patients in...
1. Introduction

Over the last couple of years increasing evidence has demonstrated that mucosal healing in Crohn’s disease (CD) is associated with less bowel damage and CD-related surgery. To monitor mucosal healing, either repetitive endoscopic examinations or surrogate markers for endoscopic activity can be used. To overcome the limitations of repetitive endoscopic exams which are costly and sometimes unpleasant for patients, considerable research efforts have been undertaken to evaluate different biomarkers in blood or feces regarding their correlation with endoscopic severity in CD. Fecal calprotectin has been shown to correlate better with endoscopic severity in CD compared to clinical activity or C-reactive protein (CRP).

Ficolin-2 is a soluble immune system, is produced in the liver and represents the lectin pathway of the serine proteases (MASP) MASP-1, MASP-2, MASP-3 and the ficolin-3 an independent family of lectins which can be found in bacterial and fungal cell walls. Ficolin-2 forms with ficolin-1 and glucosamine (GlcNAc) which can be found in bacterial and fungal cell walls. Ficolin-2 is a soluble pattern recognition receptor mainly binding to N-acetylglucosamine (GlcNAc) which can be found in bacterial and fungal cell walls. Ficolin-2 forms with ficolin-1 and ficolin-3 an independent family of lectins which can activate, in association with the so-called MBL-associated serine proteases (MASP) MASP-1, MASP-2, MASP-3 and the small non-protease Map19, the lectin pathway of the complement system.

Some patients may be reluctant to handle fecal material, thus, further research regarding serum biomarkers correlating with endoscopic disease severity is needed.

Inclusion criteria: Disease duration >3 months, complete ileo-colonoscopy including biopsies (at least 2 biopsies from terminal ileum and 4 colonic biopsies from affected regions), age 18–85 years, fecal samples delivered from 4 to 2 days before ileocolonoscopy.

Exclusion criteria: Incomplete ileo-colonoscopy (ileum not intubated), infectious enterocolitis (positive stool culture for Salmonella, Shigella, Campylobacter, positive Clostridium 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), symptoms related mainly to perianal penetrating disease, known Crohn’s disease of the esophagus, stomach, duodenum, or jejunum, regular intake of aspirin and/or NSAID (>2 tablets/week).

2. Methods

2.1. Patients

Between October 2011 and January 2013, adult CD patients undergoing ileo-colonoscopy at the Division of Gastroenterology and Hepatology at Tiefenau spitale Bern were included. The current work represents a project of the Swiss IBD Cohort Study which is supported by the Swiss National Science Foundation and approved by the local ethical committees of the participating centers. Patients provided written informed consent to participate in this study. Diagnosis of CD was based on standard clinical, endoscopic and histologic criteria. The description of disease location followed the Montreal classification.

Two blood samples were taken in the period of three to one days before endoscopy for CRP and ficolin-2 analysis (S-Monovettes, Sarstedt, Sevelen, Switzerland). The blood samples were processed by a trained nurse and the serum was frozen at –20 °C until analysis. Patients were provided with fecal collection tubes (Sarstedt, Sevelen, Switzerland) for fecal calprotectin measurement. Fecal specimens were collected by patients themselves in a period of four to two days before ileo-colonoscopy and sent directly to the laboratory for calprotectin analysis. The fecal samples for calprotectin were stored at 2–8 °C if processed within 24 h or frozen at –20 °C until analysis.

Some patients may be reluctant to handle fecal material, thus, further research regarding serum biomarkers correlating with endoscopic disease severity is needed.

Conclusions: Ficolin-2 serum levels correlate worse with endoscopic CD activity when compared to fecal calprotectin or CRP.

Inclusion criteria: Disease duration >3 months, complete ileo-colonoscopy including biopsies (at least 2 biopsies from terminal ileum and 4 colonic biopsies from affected regions), age 18–85 years, fecal samples delivered from 4 to 2 days before ileocolonoscopy.

Exclusion criteria: Incomplete ileo-colonoscopy (ileum not intubated), infectious enterocolitis (positive stool culture for Salmonella, Shigella, Campylobacter, positive Clostridium 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), symptoms related mainly to perianal penetrating disease, known Crohn’s disease of the esophagus, stomach, duodenum, or jejunum, regular intake of aspirin and/or NSAID (>2 tablets/week).

2.2. Endoscopic and clinical disease activity

No patient underwent ileo-colonoscopy twice. Indications for endoscopy were clinically active disease (flare) (n = 65, 47.8%), assessment of endoscopic activity after medical treatment (n = 58, 42.6%), and dysplasia surveillance for long-standing disease (n = 13, 9.6%). One board certified gastroenterologist (FS) with 19 years of experience in performing ileo-colonoscopy, performed the endoscopies and graded the activity according to the simplified endoscopic score for CD (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
Correlation of serum Ficolin-2 with endoscopic CD activity

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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).22 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. So far there exists no definition on how to define endoscopic remission and different levels of activity using the SES-CD. Similarly to a recently published paper we defined the SES-CD activity levels as follows: inactive (remission): 0–3 points; mild activity: 4–9 points; moderate activity: 10–17 points; severe activity: ≥18 points.23

The clinical disease activity was assessed by the measurement of the Harvey–Bradshaw Index (HBI).24 In 2006, using regression modeling, Best et al. reported that a one-point increase in a value of HBI corresponds to a 27-point increase in the value of CDAI.25 For the purposes of this manuscript, the following definitions were applied: a value of HBI from 0 to 4 points indicates clinical remission (corresponds to a mean ± SD CDAI values of 26 ± 26 to 134 ± 39, respectively); a value of HBI from 5 to 7 points indicates mild disease (corresponds to a mean ± SD CDAI values of 161 ± 42 to 216 ± 49, respectively); a value of HBI from 8 to 15 indicates moderate disease (corresponds to a mean ± SD CDAI values of 243 ± 52 to 432 ± 75, respectively); and a value of HBI ≥16 indicates severe disease (corresponds to a mean ± SD CDAI value of ≥459 ± 78). A flare-up episode in CD patients was defined as a rise in HBI value of ≥4 points, which corresponds to a CDAI increase of ≥108 points.

2.3. Ficolin-2 ELISA

Ficolin-2 concentrations in serum were measured by an enzyme-linked immunosorbent assay (ELISA) developed in our lab. Nunc-Immuno MaxiSorp® 96-well plates (Nunc, Roskilde, Denmark) were coated overnight at 4 °C with 1 μg/mL monoclonal anti human ficolin-2 (clone GN4) antibody (Hycult Biotechnology, Uden, The Netherlands) in carbonate–bicarbonate buffer pH 9.6, 50 μL per well. The next day the plates were expelled and blocked for 5 h with phosphate-buffered saline (PBS) + 1% bovine serum albumin (BSA, Sigma, Buchs, Switzerland). Sera were 1:1000 diluted in PBS + 0.5% BSA (PBSB) and recombinant human ficolin-2 (R&D Systems, Minneapolis, MN, USA) in a serial dilution in PBSB was used as standard. After the plates were washed three times with 300 μL PBSB and 50 μL diluted sera or standard were added to the wells. Plates were incubated over night at 4 °C. Plates were then washed four times with PBSB + 0.05% Tween-20 (PBSBT) and 50 μL of polyclonal anti human ficolin-2 (R&D Systems) 0.1 μg/mL in PBSBT was added to the wells. Plates were incubated at room temperature (r.t.) for 2.5 h on an orbital shaker. Plates were then washed four times with 300 μL PBSBT and 50 μL Avidin-HRP (BD Biosciences, San Diego, CA, USA) 1/100 diluted in PBSBT was added per well. Plates were then washed four times with 300 μL PBSBT per well. Finally, plates were incubated with 50 μL 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate tablets (Sigma) dissolved in phosphate–citrate buffer pH5 for 20 min at r.t. in the dark. The reaction was stopped with 50 μL 0.5 M sulfuric acid and absorbance was measured at 450 nm on a BioTek EL800 microplate reader (BioTek Instruments, Winsloos, VT, USA). All incubations were performed in zip-lock bags.

2.4. Fecal calprotectin and CRP

Fecal calprotectin was measured by the quantitative enzyme linked immunosorbent assay RIDASCREEN® CALPROTECTIN, R-Biopharm AG, Darmstadt, Germany. This sandwich ELISA measures quantitative fecal calprotectin. Fecal specimens were diluted at 1:2500. The laboratory performing the analyses (Unilabs, Coppet, Switzerland) was blinded to the clinical and endoscopic findings. All fecal samples were processed within 72 h after collection. The assays were performed according to the test instructions of the manufacturer. ELISA plates were read at an OD of 450 nm and the concentrations were calculated using the RIDA®Soft Win program. According to the manufacturer, the calprotectin cutoff-level representing a positive value was ≥50 μg calprotectin/g feces. CRP (upper limit of normal <5 mg/L) was determined as routine laboratory value within 3 days prior to endoscopy.

2.5. Statistical analysis

Statistical analyses were performed with a statistical package program (Stata Vs 10, 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-Q–Q-plot. Fisher's exact test (two-sided) or the Chi squared tests were used to explore associations of categorical data in 2 independent groups. The Wilcoxon rank sum test was used to explore associations of non-parametric numerical data in 2 independent groups and the t-test was used for parametric numerical data. A P <0.05 was considered statistically significant. A Bonferroni adjustment was performed in the case of multiple testing. The association between endoscopic disease activity with HBI, ficolin-2, fecal calprotectin, and CRP was assessed by determination of the Spearman's rank correlation coefficient (R) for nonparametric correlations. An a priori power analysis revealed that a sample size of 23 in each of the four subgroups of endoscopic disease activity (total n = 92) would have 90% power to detect a difference in the mean ficolin-2 levels between the subgroups, using a Mann–Whitney rank-sum test with a 0.05 two-sided significance level.

3. Results

3.1. Patients characteristics

From a total of 154 screened CD patients, 18 were excluded for the following reasons: 12 for not delivering fecal samples
on time, 4 for known and active CD of the upper gastrointestinal tract, 1 for unwillingness to participate and one for intake of NSAID. The baseline characteristics of the patients are illustrated in Table 1. As use of medications could overlap the total accounts for 108.4%. We further evaluated the correlation between ficolin-2 serum levels and patient baseline characteristics using the Spearman’s rank correlation coefficient (R). No significant association of ficolin-2 serum levels was found with gender (R = 0.008, \( P = 0.923 \)), age at inclusion (R = 0.051, \( P = 0.558 \)), age at diagnosis (R = 0.083, \( P = 0.376 \)), disease location (R = 0.044, \( P = 0.628 \)), CD-related operations (R = 0.118, \( P = 0.172 \)), and extraintestinal manifestations (R = 0.046, \( P = 0.596 \)).

### 3.2. Clinical, endoscopic, and biochemical (serum ficolin, fecal calprotectin, CRP) disease activity

The baseline data on the clinical activity, the endoscopic activity, and the biochemical disease activity, as measured by serum-ficolin-2 levels, fecal calprotectin, and CRP, are further depicted in Table 2. The patients had the following frequencies of clinical activity when evaluated categorically by the HBI: 72 (63.7%) were in clinical remission (HBI 0–4 points), 25 (22.1%) had mild clinical activity (HBI 5–7 points), 13 (11.5%) showed moderate clinical activity (HBI 8–15 points), and 3 (2.6%) had severe clinical activity (HBI ≥ 16 points). The proportions of patients per categorically ordered endoscopic activity were as follows: 51 (52.9%) were in endoscopic remission (SES-CD 0–3 points), 53 (23.5%) showed mild endoscopic activity (SES-CD 4–9 points), 25 (18.5%) had moderate endoscopic activity (SES-CD 10–17 points), and 7 (5.1%) showed severe endoscopic activity (≥ 18 points).

### 3.3. Correlation of endoscopic activity with HBI, serum ficolin-2 levels, fecal calprotectin and CRP

The correlation between endoscopic, clinical, and biochemical disease activity is illustrated by the Spearman’s rank correlation coefficient, including the corresponding P-values, in Table 3. The SES-CD correlated best with fecal calprotectin (R = 0.676, \( P < 0.001 \)), followed by CRP (R = 0.458, \( P < 0.001 \)) and ficolin-2 serum levels (R = 0.171, \( P = 0.047 \)). Serum ficolin-2 levels correlated best with CRP (R = 0.322, \( P < 0.001 \)) followed by HBI (R = 0.220, \( P = 0.019 \)) and fecal calprotectin (R = 0.185, \( P = 0.032 \)).

The correlation of the categorically ordered endoscopic activity according to the SES-CD, clinical activity and non-invasive biomarker is further illustrated in Table 4. In summary, ficolin-2 serum concentrations were significantly higher in CD patients with mild endoscopic activity compared to patients in endoscopic remission (P = 0.015), however, no difference regarding the serum levels could be detected between the patients with mild versus moderate or severe endoscopic severity. The findings regarding ficolin-2 serum levels and endoscopic severity are further illustrated in Fig. 1.

We further analyzed the correlation of binary ordered endoscopic activity (endoscopic remission vs. endoscopic activity) with HBI, serum ficolin-2 levels, fecal calprotectin, and CRP. Endoscopic remission could be discriminated from endoscopically active disease by HBI (2 [1–3] vs. 4 [2–7], \( P < 0.001 \)), serum ficolin-2 levels (2.38 [1.45–3.29] vs. 2.86 [2.23–3.86], \( P = 0.026 \)), CRP (2.4 [1–4.3] vs. 6 [2.1–16], \( P < 0.001 \)), and fecal calprotectin (108 [53–225] vs. 452 [249–855], \( P < 0.001 \)).

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**Table 1** Clinical characteristics of the CD patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>136</td>
<td>100.0%</td>
</tr>
<tr>
<td>Females</td>
<td>51</td>
<td>37.5%</td>
</tr>
<tr>
<td>Age at inclusion (mean ± SD) (years)</td>
<td>41.5 ± 15.4</td>
<td></td>
</tr>
<tr>
<td>Disease duration (mean ± SD) (years)</td>
<td>11.6 ± 8.9</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 16 years</td>
<td>13</td>
<td>9.6%</td>
</tr>
<tr>
<td>Between 17–40 years</td>
<td>99</td>
<td>72.8%</td>
</tr>
<tr>
<td>&gt; 40 years</td>
<td>24</td>
<td>17.6%</td>
</tr>
<tr>
<td>Disease location at diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileal</td>
<td>18</td>
<td>13.2%</td>
</tr>
<tr>
<td>Colonic</td>
<td>57</td>
<td>41.9%</td>
</tr>
<tr>
<td>Ileocolonic</td>
<td>61</td>
<td>44.9%</td>
</tr>
<tr>
<td>Behavior (at inclusion)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-stricturing, non-penetrating</td>
<td>114</td>
<td>83.8%</td>
</tr>
<tr>
<td>Ileal</td>
<td>12</td>
<td>8.9%</td>
</tr>
<tr>
<td>Colonic</td>
<td>57</td>
<td>41.9%</td>
</tr>
<tr>
<td>Ileocolonic</td>
<td>61</td>
<td>44.9%</td>
</tr>
<tr>
<td>CD-related operation(s)</td>
<td>32</td>
<td>23.5%</td>
</tr>
<tr>
<td>Extraintestinal manifestations</td>
<td>27</td>
<td>19.9%</td>
</tr>
<tr>
<td>Medication at inclusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>7.4%</td>
</tr>
<tr>
<td>5-ASA</td>
<td>4</td>
<td>2.9%</td>
</tr>
<tr>
<td>Budesonide orally</td>
<td>13</td>
<td>9.6%</td>
</tr>
<tr>
<td>Budesonide rectally</td>
<td>1</td>
<td>0.7%</td>
</tr>
<tr>
<td>Systematic steroids</td>
<td>16</td>
<td>11.8%</td>
</tr>
<tr>
<td>Azathioprine/6-MP</td>
<td>35</td>
<td>25.7%</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>6</td>
<td>4.4%</td>
</tr>
<tr>
<td>TNF antagonist</td>
<td>63</td>
<td>46.3%</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
<td>2.9%</td>
</tr>
</tbody>
</table>

**Table 2** Clinical, endoscopic, and biochemical activity. Abbreviations: HBI = Harvey–Bradshaw Index; IQR = interquartile range.

<table>
<thead>
<tr>
<th>Item</th>
<th>Median</th>
<th>IQR</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBI</td>
<td>3</td>
<td>2–6</td>
<td>0–20</td>
</tr>
<tr>
<td>SES-CD</td>
<td>5</td>
<td>2–8</td>
<td>0–22</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4</td>
<td>2–12</td>
<td>1–198</td>
</tr>
<tr>
<td>Fecal calprotectin (µg/g)</td>
<td>301</td>
<td>120–703</td>
<td>15–2651</td>
</tr>
<tr>
<td>Serum ficolin-2 (µg/mL)</td>
<td>2.69</td>
<td>2.02–3.83</td>
<td>0.24–16.53</td>
</tr>
</tbody>
</table>
We performed a ROC analysis to evaluate the accuracy of serum ficolin-2 levels, fecal calprotectin, CRP, and HBI to predict mucosal healing which was defined as an SES-CD from 0–3 points. The results are further illustrated in Fig. 2. The area under the curve (AUC) for serum ficolin-2 was 0.614 (standard error [SE] 0.052, 95% confidence interval [95%-CI] 0.513–0.716), compared to an AUC of 0.826 (SE 0.038, 95%-CI 0.751–0.901) of fecal calprotectin, an AUC of 0.691 for CRP (SE 0.046, 95%-CI 0.602–0.780), and an AUC of 0.737 for HBI (SE 0.047, 95%-CI 0.644–0.829). A fecal calprotectin value of \( N > 250 \) μg/g had a 75.3% sensitivity and 76% specificity to detect endoscopically active disease. A CRP of \( N > 5 \) mg/L had a sensitivity of 52.9% and specificity of 84.3% for detection of endoscopically active disease. A serum-ficolin-2 level of \( N > 2.404 \) μg/mL had a sensitivity of 68.2% and specificity of 51% for detection of endoscopically active disease. A serum-ficolin-2 level of \( > 2.404 \) μg/mL had a sensitivity of 68.2% and specificity of 51% for detecting endoscopically active disease and an HBI \( > 3 \) had a sensitivity of 61.2% and a specificity of 76.1% to detect endoscopically active disease. In conclusion, fecal calprotectin had the best accuracy to predict mucosal healing, followed by CRP, HBI, and serum ficolin-2 levels. Adding ficolin-2 serum levels to fecal calprotectin or CRP did not improve the test performance of either fecal calprotectin (AUC 0.812, 95% CI 0.719–0.894) or CRP (AUC 0.702, 95% CI 0.571–0.792) to detect endoscopically active disease.

4. Discussion

We present the first data evaluating serum ficolin-2 concentrations regarding their correlation with endoscopic disease activity in CD patients. Our findings demonstrate that serum ficolin-2 concentrations are inferior regarding their correlation with endoscopic severity compared to fecal calprotectin and CRP. We therefore believe that serum ficolin-2 levels do not possess the required test performance to qualify as a biomarker to monitor endoscopic CD severity.

Our group recently demonstrated that serum ficolin-2 concentrations in CD patients were significantly higher compared to healthy controls (1.78 ± 0.88 μg/mL vs. 1.17 ± 0.77 μg/mL, \( P < 0.001 \)). We therefore decided to evaluate serum ficolin-2 levels regarding its correlation with endoscopic disease severity.

No literature exists so far having evaluated ficolin-2 serum concentrations in IBD patients. Clearly, serum ficolin-2 performed worse than CRP or fecal calprotectin regarding its correlation with endoscopic disease severity.
Numerous prospective studies have already demonstrated that fecal calprotectin correlates closely with the endoscopic severity in CD.5,8,23,26–28 Compared to markers measured in serum, which may reflect an inflammatory response to several locations in the body, fecal calprotectin has the advantage to be specific for the gut. Similar to fecal calprotectin, CRP has demonstrated a fair to good correlation with endoscopic severity in CD patients, especially in patients with moderate to severe endoscopic activity.23 Based on the results of this study we do not believe that serum ficolin-2 levels will have a future place as a biomarker for non-endoscopic monitoring of endoscopic disease severity. Besides serum ficolin-2 levels we also evaluated the serum levels of ficolin-3.9 In contrast to ficolin-2, ficolin-3 levels in CD patients did not differ from healthy controls (22.7 ± 10.6 μg/mL vs. 24.3 ± 9.7 μg/mL, P = 0.645).

Therefore, we did not evaluate ficolin-3 levels as a potential biomarker for monitoring endoscopic severity in CD patients. Our study has strengths and weaknesses. We present the first data evaluating serum ficolin-2 concentrations regarding a potential correlation with the endoscopic disease severity in CD patients. The data stem from the well-established SIBDCS and the recruited sample size is appropriate to support the conclusions. The drawback lies in the fact that serum ficolin-2 levels perform worse than fecal calprotectin or CRP regarding the correlation with endoscopic severity. Nevertheless, we think that this "negative" result deserves to be communicated to a broader audience. A second drawback may lie in the fact that not a systematic small bowel workup was performed in every patient to evaluate CD activity proximally to the terminal ileum. Given the fact that a small bowel involvement proximally to the terminal ileum is reported in about 10% of CD patients we do not think that this might have majorly affected our results. A third drawback may be that the number of patients with severe endoscopically active disease is limited. We think that this fact may explain why in this cohort fecal calprotectin did not discriminate between moderate and severe endoscopic disease activity in contrast to our formerly published findings.23 We did not evaluate serum ficolin-1 levels as a potential surrogate marker for endoscopic disease activity as knowledge about this molecule was limited at the time this study was planned.29

In summary, we found that serum levels of ficolin-2 did not correlate with endoscopic severity in CD and confirmed earlier published data on the good correlation of fecal calprotectin and CRP with endoscopic severity.

**Disclaimers for all authors**

All authors have no conflicts of interest or financial ties relevant to the manuscript to disclose.

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We thank all the members of the SIBDCS group involved in the acquisition of data:


Specific author contributions:

(1) Study concept and design; (2) acquisition of data; (3) statistical analysis; (4) analysis and interpretation of data; (5) drafting of the manuscript; (6) critical revision of the manuscript for important intellectual content; (7) obtained funding; (8) technical or material support; (9) study supervision.

Thomas Schaffer: 1, 2, 3, 4, 5, 6; Alain M. Schoepfer: 1, 2, 3, 4, 5, 6, 7, 8, 9.
Figure 2  Receiver operator curves (ROCs) for serum ficolin-2 levels, fecal calprotectin, CRP, and Harvey–Bradshaw Index (HBI) to detect endoscopically active disease (SES-CD ≥ 4 points). The best test performance to detect endoscopically active disease is shown by fecal calprotectin, followed by HBI, CRP, and serum ficolin-2 levels.
References


