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# Fecal neutrophil gelatinase-associated lipocalin as a biomarker for inflammatory bowel disease

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## Abstract

**Background and Aim:** Accurate, noninvasive biomarkers are needed to diagnose and monitor inflammatory bowel disease (IBD). Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin 2, is expressed in inflamed colonic epithelium and neutrophilic granulocytes. This study explores its properties as a biomarker in feces and plasma and, for the first time, compares fecal NGAL systematically with the existing fecal biomarker calprotectin.
**Methods:** Neutrophil gelatinase-associated lipocalin was measured in feces from 73 patients with IBD, 21 patients with infectious enterocolitis, 21 patients with irritable bowel syndrome, and 23 healthy subjects using ELISA. The results were correlated to calprotectin, clinical score, endoscopic score, and high-sensitive C-reactive protein. Plasma from 119 patients with IBD and 28 healthy controls was analyzed for NGAL.
**Results:** Fecal NGAL levels (median and interquartile range) were significantly elevated in active ulcerative colitis (UC) 6.05 (3.6–15.1) mg/kg and Crohn's disease (CD) 4.9 (1.5–7.7) mg/kg, compared with patients with inactive UC 1.3 (0.4–2.6) mg/kg, inactive CD 1.5 (0.5–1.7) mg/kg, irritable bowel syndrome 0.4 (0.2–0.6) mg/kg, and healthy controls (HC) 0.3 (0.1–0.4) mg/kg. Patients with infectious enterocolitis had significantly higher fecal-NGAL levels, 2.7 (1.4–5.6) mg/kg than HC. Sensitivity and specificity was 94.7% and 95.7%, respectively, for distinguishing between active IBD and HC. Stability of NGAL in stool was excellent for 7 days in room temperature. Plasma NGAL was significantly elevated in UC and CD compared with HC.
**Conclusions:** Fecal NGAL is a promising biomarker for IBD. As existing biomarkers are expressed mainly in granulocytes, NGAL's epithelial localization may give supplementary diagnostic information.

## Introduction

Modern treatment of inflammatory bowel disease (IBD) aims at achieving mucosal healing. For patients with colonic disease only, the gold standard for diagnosing and monitoring of IBD is colonoscopy with histology. For small-bowel Crohn's disease (CD), it is much more difficult to reach this level of diagnostic precision. Moreover, colonoscopy is an invasive, time-consuming, and expensive examination that is often not very well tolerated by the patients. Good, noninvasive methods for assessment of disease activity are thus needed.

One well-established IBD biomarker is fecal calprotectin. Calprotectin has been shown to have good correlation with inflammation in IBD and, important for clinical practice, is stable for 3–7 days in room temperature.[1–3] However, this test has some limitations, as variable sensitivity and specificity for the test has been reported in meta-analyses.[4–6] It is, thus, possible that other biomarkers alone or together with calprotectin may improve the accuracy in diagnosing and monitoring IBD.

One such potentially useful biomarker is neutrophil gelatinase-associated lipocalin (NGAL), a 25-kDa glycoprotein also known as lipocalin 2. NGAL is expressed in various cell types including neutrophilic granulocytes, adipocytes, and epithelium of the gastrointestinal, respiratory, and urogenital tracts.[7,8] It has been shown to be an iron-sequestrating antimicrobial protein with bacteriostatic effects.[9,10] NGAL is further shown to act as a growth and differentiation factor, and it stabilizes the proteolytic enzyme matrix metalloprotease-9 (MMP-9).[8,11]

*LCN2*, the coding gene for NGAL, is one of the most over-expressed genes in the colonic mucosa in ulcerative colitis (UC) and CD compared with healthy individuals.[12,13] Because of NGAL's small size (25 kDa), secreted nature, and relative stability, it has been investigated as a diagnostic and prognostic biomarker in several diseases including inflammatory disorders.[8,14] Urinary and serum NGAL have proven excellent biomarkers for early, acute renal failure.[15,16] NGAL has further been shown to be a sensitive biomarker for colitis in mouse models of IBD.[17]

The mucosal distribution of inflammation-induced NGAL makes this protein markedly different from the most studied fecal IBD biomarkers calprotectin and lactoferrin.[18–20] These proteins are mainly expressed in neutrophilic granulocytes that are shed to the bowel lumen during active inflammation, causing elevated values in feces. NGAL is, in addition to expression in granulocytes, strongly expressed in the intestinal epithelial cell layer during inflammation. It thus reflects a different aspect of the inflammatory process than neutrophil infiltration and may provide additional diagnostic information compared with the existing biomarkers. The role of serum NGAL in IBD has been studied to some degree previously,[21–25] while fecal NGAL remains largely unexplored as an IBD biomarker. One study from 1999 showed a potential role of fecal NGAL as a biomarker for IBD, but, to our knowledge, the present explorative study is the first to systematically compare this protein to calprotectin in feces.[26]

## Methods

***Clinical material.*** Patients with known IBD were recruited prospectively among individuals undergoing colonoscopy at the Department of Gastroenterology and Hepatology, St. Olav's University Hospital, or at gastroenterology units of three other hospitals in the Central Norway Regional Health Authority. Patients with infectious enterocolitis (IEC) had polymerase chain reaction-positive fecal samples for enteropathogenic bacteria or virus (11 for the bacteria species *Salmonella*, *Campylobacter*, enteropathogenic *Escherichia coli*, and *Yersinia*; 10 for the viruses norovirus and rotavirus). Patients with IBS fulfilled the Rome III criteria and had a negative colonoscopy. Patients with normal colonoscopies and not IBS were included as healthy controls. Seventeen of the controls were healthy volunteers. We did not have blood samples from all of the patients with available fecal samples and vice versa, and we therefore have separate, but overlapping, cohorts for fecal samples and plasma.

Clinical score, using partial Mayo score (UC) or Harvey–Bradshaw Activity Index (CD), was registered for all patients with IBD.[27] Endoscopic scoring was performed using Mayo score (UC) or simple endoscopic score for CD (SES-CD).[28,29] The endoscopies were performed by experienced gastroenterologists blinded to the results of the NGAL and calprotectin tests. Inactive UC was defined as an endoscopic Mayo score of ≤1. Inactive CD was defined as endoscopic SES-CD score of ≤2 (≤3 where there was only noninflammatory stenosis).

### Laboratory studies

*Feces analyses.* The stool samples were collected within 3 days before the examination and thereafter stored at −20 °C. For analysis, feces was diluted 1:5 (w/v) in a buffer with phosphate-buffered saline (PBS), 0.5% Tween, and 0.02% $NaN_3$, centrifuged at 2900 *g* for 10 min and the supernatant saved. Supernatant was further diluted in PBS with bovine serum albumin 1% w/v to a final concentration of 1:250 to 1:2500. The samples were analyzed for NGAL in duplicates using ELISA (BioVendor R&D, Brno, Czech Republic), blinded to endoscopic and clinical results.

Calprotectin in the same samples was analyzed using ELISA by Calpro AS, Lysaker, Norway. The samples were diluted 1:50 using Calpro EasyExtract and further diluted according to the manufacturer's protocol. Fifty-five of the samples were under the detection limit of 25 mg/kg and are shown with a value of 0 in the graphs.

To investigate the stability of fecal NGAL (fe-NGAL) and calprotectin, stool samples from 14 patients were split into four, with one sample frozen immediately at −80 °C and the remaining three samples kept at room temperature (+20 °C) for 1, 3, or 7 days, respectively, before frozen at −80 °C until analysis.

*Blood analyses.* In patients undergoing colonoscopy, blood was always drawn at the day of the examination. Plasma was kept frozen at −80 °C until analysis. The plasma was diluted 1:120 in PBS with bovine serum albumin 1% before measuring NGAL with the same ELISA method as described for fe-NGAL. Serum samples from the same patients were analyzed for high-sensitive C-reactive protein (hs-CRP) with an immunoturbidimetric assay (CRP vario; Abbott, Wiesbaden, Germany) at the Laboratory for Clinical Chemistry, Levanger Hospital HF, Levanger, Norway.

***Immunohistochemistry.*** Formalin-fixed, paraffin-embedded biopsies were cut into 4-μm-thick sections for routine histology and immunohistochemical examination. Colonic biopsies from patients with active and inactive UC, CD, and from healthy subjects ($n = 5$ in each group) were stained. Primary antibodies were rabbit polyclonal antihuman NGAL (antibody 41105, Abcam, diluted 1:50) and an antibody against the S100A8 subunit of the calprotectin heterodimer (Monoclonal Mouse IgG1 Anti-S100A8 (antibody MAB4570, R&D), diluted 1:8000). Secondary antibody was from Dako Real Envision (rabbit/mouse) and detection performed using diaminobenzidine + chromogen (Dako, Glostrup, Denmark).

***Statistics.*** As the data were not normally distributed, results are expressed as median (interquartile range) values and nonparametric tests used. The Kruskall–Wallis test was used for comparisons, and if significances were found, we used the Mann–Whitney *U*-test for comparisons among subgroups. Spearman's test was used for bivariate correlation studies. The statistical packages IBM SPSS STATISTICS 22, GRAPHPAD PRISM 6, and STATA 13.1 were used for data analysis. Significant values were defined as $P < 0.05$ (two-sided).

***Ethical considerations.*** The study was approved by the Regional Medical Research Ethics Committee of Central Norway (ref. no 2013/212). All participants gave informed consent.

## Results

***Fecal neutrophil gelatinase-associated lipocalin and calprotectin in ulcerative colitis, Crohn's disease, infectious enterocolitis, irritable bowel syndrome, and healthy controls.*** A total of 73 patients with IBD (44 UC and 29 CD), 21 patients with IEC, 21 patients with IBS, and 23 healthy controls were included in the study on fe-NGAL. Table 1 shows clinical and demographic characteristics of the participants. Fe-NGAL levels were significantly elevated in feces from patients with active UC 6.05 (3.6–15.1) mg/kg and active CD 4.9 (1.5–7.7) mg/kg compared with patients with inactive UC 1.3 (0.4–2.6) mg/kg ($P < 0.001$) or inactive CD 1.5 (0.5–1.7) mg/kg ($P < 0.005$), respectively. Significant differences were seen when comparing inactive UC or CD to healthy controls 0.3 (0.1–0.4) mg/kg ($P < 0.001$ for both) and to patients with IBS 0.4 (0.2–0.6) mg/kg ($P < 0.001$ and $P = 0.004$ respectively). Patients with IEC had significantly higher fe-NGAL-levels, 2.7 (1.4–5.6) mg/kg, than in HC or IBS ($P < 0.0001$). Calprotectin levels were significantly higher in bacterial than in viral enterocolitis ($P < 0.01$), but there was no such difference for fe-NGAL (data not shown). There was a tendency towards higher fe-NGAL-values in IBS-patients than for healthy controls, but the difference was not significant ($P = 0.06$). Individual values with median and significances are shown in Figure 1.

As shown in Figure 2, fe-NGAL was strongly correlated with calprotectin ($\rho = 0.82$). Table 2 shows correlations between fe-NGAL and calprotectin and between the two fecal biomarkers and hs-CRP, endoscopic score and clinical score, respectively.

In order to evaluate the performance of fe-NGAL in discriminating IBD-patients from healthy controls, receiver operating curve (ROC) analyses were performed. When comparing active IBD with healthy controls, the area under the curve (AUC) was 0.987 (95% CI 0.97–1.0) for fe-NGAL and 0.980 (0.95–1.0) for calprotectin. We found identical sensitivity of 94.7 and specificity of 95.7% for fe-NGAL and calprotectin using cut-off points of 0.81 mg/kg for fe-NGAL and 50 mg/kg for calprotectin.

In clinical practice, the most common differential diagnosis for IBD is IBS. When comparing IBD-patients with active disease with patients with IBS, the AUC was 0.968 (0.93–1.0) for fe-NGAL and 0.985 (0.95–1.0) for calprotectin. The sensitivity and specificity was 94.6% and 90.5% for fe-NGAL and 97.3% and 100% for calprotectin.

When it comes to distinguishing the IBD-patients with endoscopically active disease from inactive disease, higher cut-off values of 2.2 mg/kg for fe-NGAL and 150 mg/kg for calprotectin were used for optimal performance of the tests. This gave a sensitivity of 86.5% and a specificity of 77.8% for fe-NGAL and 91.9% and 80.6% for calprotectin. The AUC was 0.860 (0.77–0.95) and 0.908 (0.84–0.98), respectively (difference not significant, $P = 0.15$).

For 47 of the patients (25 CD and 22 UC) and for 21 healthy controls, we measured all of the parameters fe-NGAL, calprotectin, plasma NGAL, and hs-CRP and could relate these to endoscopic activity. Figure 3 shows ROC curves for the different parameters in discriminating between active IBD and HC and between active and inactive IBD. Contrasting active IBD and HC, AUC was significantly higher for fe-NGAL (AUC 0.990) compared with both hs-CRP (AUC 0.884) ($P = 0.04$) and plasma NGAL (AUC 0.801) ($P = 0.002$).

In CD, fe-NGAL-levels were significantly higher in patients with colitis than with ileitis ($P < 0.01$). In UC, there was a

**Table 1** Clinical and demographic characteristics of individuals in the study of fe-NGAL

| | UC | CD | IEC | IBS | HC |
|---|---|---|---|---|---|
| Subjects, *n* | 43 | 30 | 21 | 21 | 23 |
| Gender, female/male | 22/21 | 18/12 | 12/9 | 17/4 | 16/7 |
| Median age, years (range) | 43 (19–76) | 37 (17–69) | 41 (22–95) | 42 (16–69) | 40 (22–71) |
| Disease activity[†], *n* | | | | | |
| Active | 17 | 20 | — | — | — |
| Inactive | 26 | 10 | — | — | — |
| Endoscopic score[‡], *n* | | | | | |
| Quiescent | 15 | 7 | — | — | — |
| Mild | 11 | 13 | — | — | — |
| Moderate | 8 | 5 | — | — | — |
| Severe | 8 | 3 | — | — | — |
| Clinical score[§], *n* | | | | | |
| Quiescent | 24 | 17 | — | — | — |
| Mild | 6 | 5 | — | — | — |
| Moderate | 6 | 7 | — | — | — |
| Severe | 7 | 1 | — | — | — |
| Inflammation site[¶] | | | | | |
| Ileitis | — | 12 | — | — | — |
| Colitis | — | 7 | — | — | — |
| Colitis and ileitis | — | 5 | — | — | — |
| Total colitis | 9 | — | — | — | — |
| Left-sided colitis | 10 | — | — | — | — |
| Proctitis | 7 | — | — | — | — |
| Medications, *n* | | | | | |
| 5-ASA | 26 | 4 | — | — | — |
| Corticosteroids | 13 | 13 | — | — | — |
| Thiopurines | 2 | 7 | — | — | — |
| Anti-TNFα | 1 | 3 | — | — | — |

[†]Active disease defined as Mayo endoscopic score ≥2 or SES-CD score ≥3.
[‡]UC, Mayo score: 1 quiescent, 2 mild, 3 moderate, and 4 severe. CD, SES-CD score: 0–2 quiescent, 3–6 mild, 7–15 moderate, and ≥16 severe.
[§]UC, Mayo partial score: 0–2 quiescent, 3–4 mild, 5–6 moderate, and 7–9 severe. CD, Harvey–Bradshaw: 0–4 quiescent, 5–8 mild, 9–15 moderate, and ≥16 severe.
[¶]Including patients with Mayo endoscopic score ≥1. Three patients were classified as active IBD because of symptoms and inflammation seen in abdominal CT scans.
anti-TNFα, anti-tumor necrosis factor α; CD, Crohn's disease; CT, computed tomography; fe-NGAL, fecal neutrophil gelatinase-associated lipocalin; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IEC, infectious enterocolitis; HC, healthy controls; 5-ASA, 5-aminosalicylate; SES-CD, simple endoscopic score for Crohn's disease; UC, ulcerative colitis.

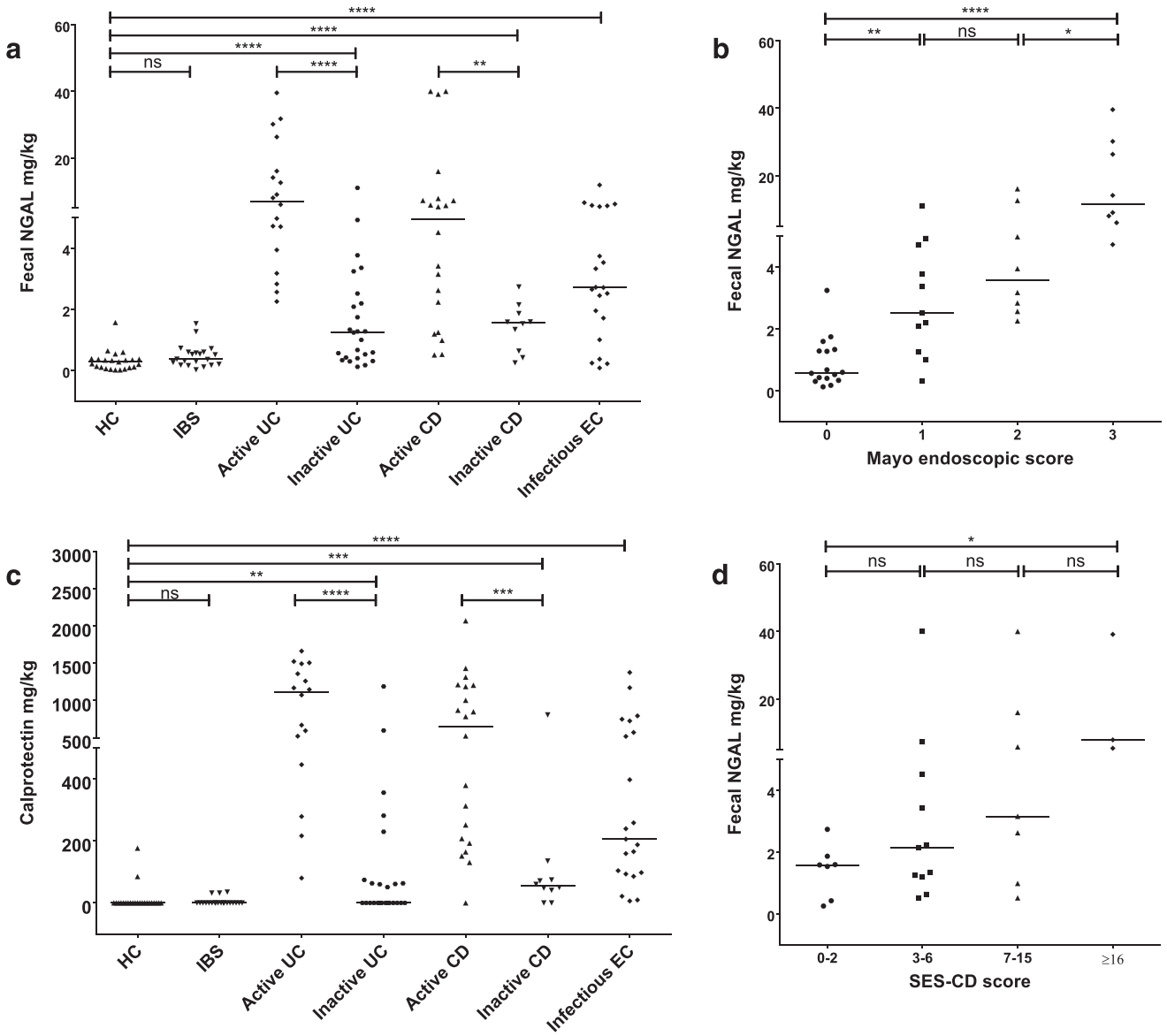


**Figure 1** Fecal neutrophil gelatinase-associated lipocalin (Fe-NGAL) (a) and calprotectin (c) in healthy controls (HC), irritable bowel syndrome (IBS), inactive and active Crohn's disease (CD), ulcerative colitis (UC), and infectious enterocolitis (IEC). Inactive CD defined as simple endoscopic score for Crohn's disease (SES-CD) endoscopic score ≤3. Inactive UC defined as Mayo endoscopic subscore ≤1. Individual values and medians are shown. (b) Fe-NGAL in UC sorted by Mayo endoscopic score. (d) Fe-NGAL in CD sorted by SES-CD endoscopic score. $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

tendency to higher fe-NGAL levels in patients with total colitis than with proctitis (not significant, $P = 0.10$).

***Stability of fecal neutrophil gelatinase-associated lipocalin.*** Figure S1 shows fe-NGAL and calprotectin levels measured after different storage time at ambient temperature. Both were very stable when considering patients with moderate disease, with no significant differences in fe-NGAL or calprotectin levels after storage up to 7 days. One patient with severe disease and very high levels of fe-NGAL and calprotectin had a significant decrease in the measured concentration of both proteins after storage. However, the level after 7 days still indicated severe disease.

***Plasma neutrophil gelatinase-associated lipocalin in ulcerative colitis, Crohn's disease, and healthy controls.*** A total of 119 patients (59 men and 60 women, age range 17–85 years) and 28 healthy controls (11 men and 17 women, age range 22–71 years) were included in the study of plasma NGAL. Of these, 65 had UC (37 inactive and 28 active disease) and 54 CD (13 inactive and 41 active disease). Plasma NGAL was elevated in UC 135.0 (87.2–153.8) ng/ml and CD 153.5 (93.4–194.5) ng/ml, compared with healthy controls 90.8 (69.1–112.8) ng/ml (Fig. 4). ROC curve analyses were made to test the ability of NGAL to discriminate between healthy subjects, inactive disease, and active disease. By setting a cut-off point of 108 ng/ml to distinguish active IBD from HC, we could determine a sensitivity of

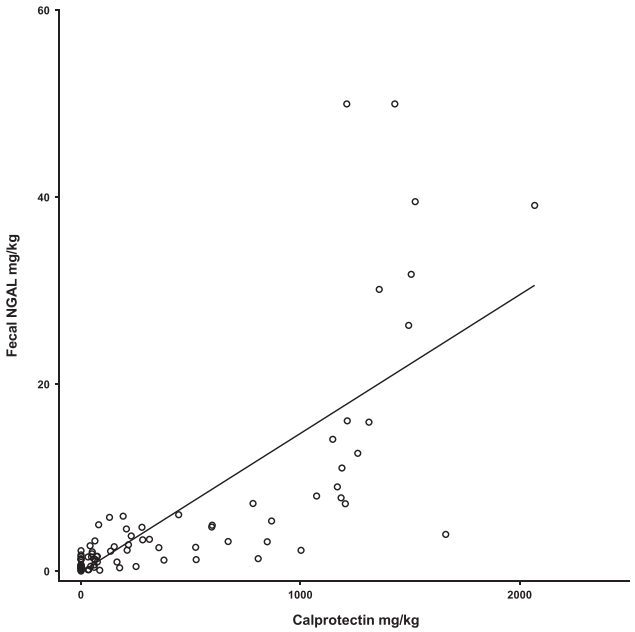


**Figure 2** Correlation plot between levels of fecal neutrophil gelatinase-associated lipocalin (fe-NGAL) and calprotectin. Rho, Spearman's rho coefficient.

**Table 2** Correlation between fe-NGAL and calprotectin related to clinical parameters and scores

| | *n* | Fe-NGAL | Calprotectin |
|---|---|---|---|
| Calprotectin | | | |
| All subjects | 138 | 0.82** | — |
| UC | 44 | 0.91** | — |
| CD | 30 | 0.71** | — |
| Endoscopic scores | | | |
| UC Mayo endoscopic score | 42 | 0.82** | 0.82** |
| CD SES-CD endoscopic score | 28 | 0.58** | 0.71** |
| Clinical scores | | | |
| UC Mayo partial score | 43 | 0.67** | 0.62** |
| CD Harvey–Bradshaw score | 30 | 0.55** | 0.33 ns |
| Hs-CRP | | | |
| UC | 29 | 0.35 ns | 0.29 ns |
| CD | 22 | 0.71** | 0.64** |

Spearman's test. *Significant at the 0.05 level (two-tailed).
**Significant at the 0.01 level (two-tailed).
CD, Crohn's disease; fe-NGAL, fecal neutrophil gelatinase-associated lipocalin; hs-CRP, high-sensitive C-reactive protein; SES-CD, simple endoscopic score for Crohn's disease; UC, ulcerative colitis.

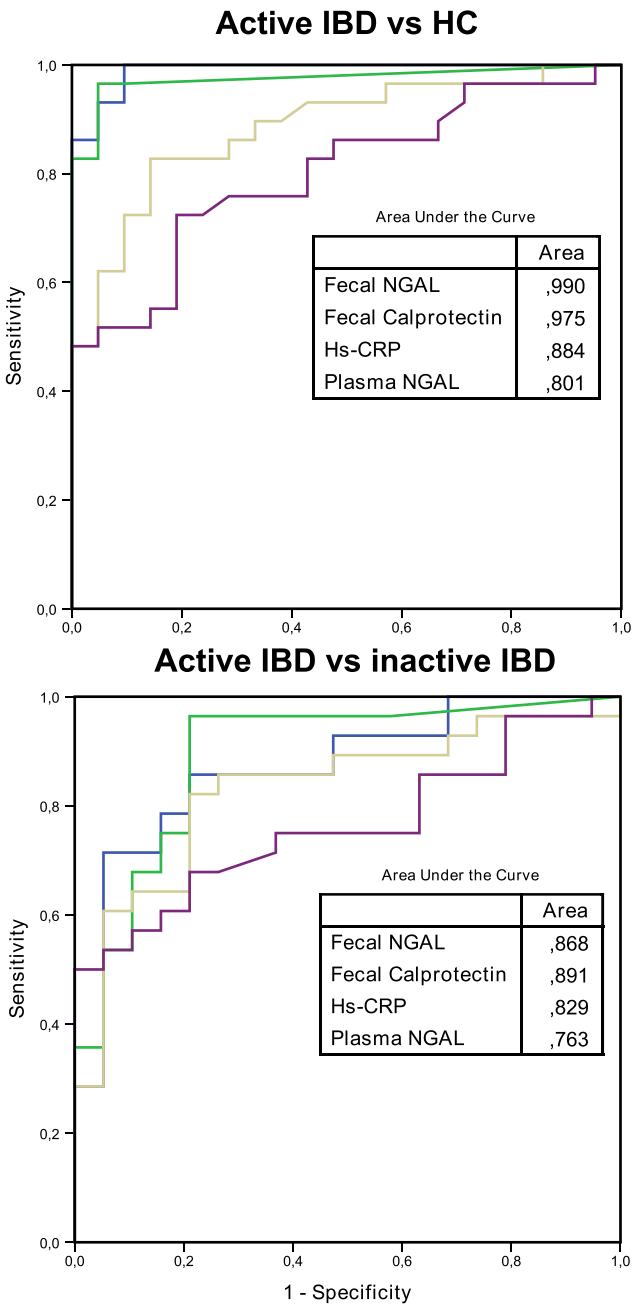


**Figure 3** Receiver operating curves (ROCs) for all 68 subjects (25 UC, 22 CD, and 21 HC) with available measurements of fecal neutrophil gelatinase-associated lipocalin (fe-NGAL), calprotectin, high-sensitive C-reactive protein (hs-CRP), and plasma NGAL, exhibiting the comparative performance in distinguishing patients with active inflammatory bowel disease (IBD) from healthy controls and patients with active IBD from patients with inactive IBD.

75.3% and a specificity of 67.9%. AUC was 0.79 (95% CI 0.71–0.88). When performing the same analysis for hs-CRP, AUC was 0.87 and sensitivity and specificity were 82% and 75%, respectively (cut-off point of 1.7 mg/l). For distinguishing between inactive and active IBD AUC was 0.72, and we found a sensitivity and specificity for NGAL of 78.3% and 50%, respectively, while hs-CRP had a sensitivity of 85.5% and a specificity of 50%.

***Immunohistochemistry.*** Figure 5 shows NGAL expressed in the colonic epithelium and in inflammatory cells in endoscopic pinch biopsy from active UC and minimal staining of the epithelium in a biopsy from a healthy control individual. Biopsies from inactive IBD showed staining similar to the controls. Calprotectin was expressed in inflammatory cells, and we saw no significant staining in epithelial cells.

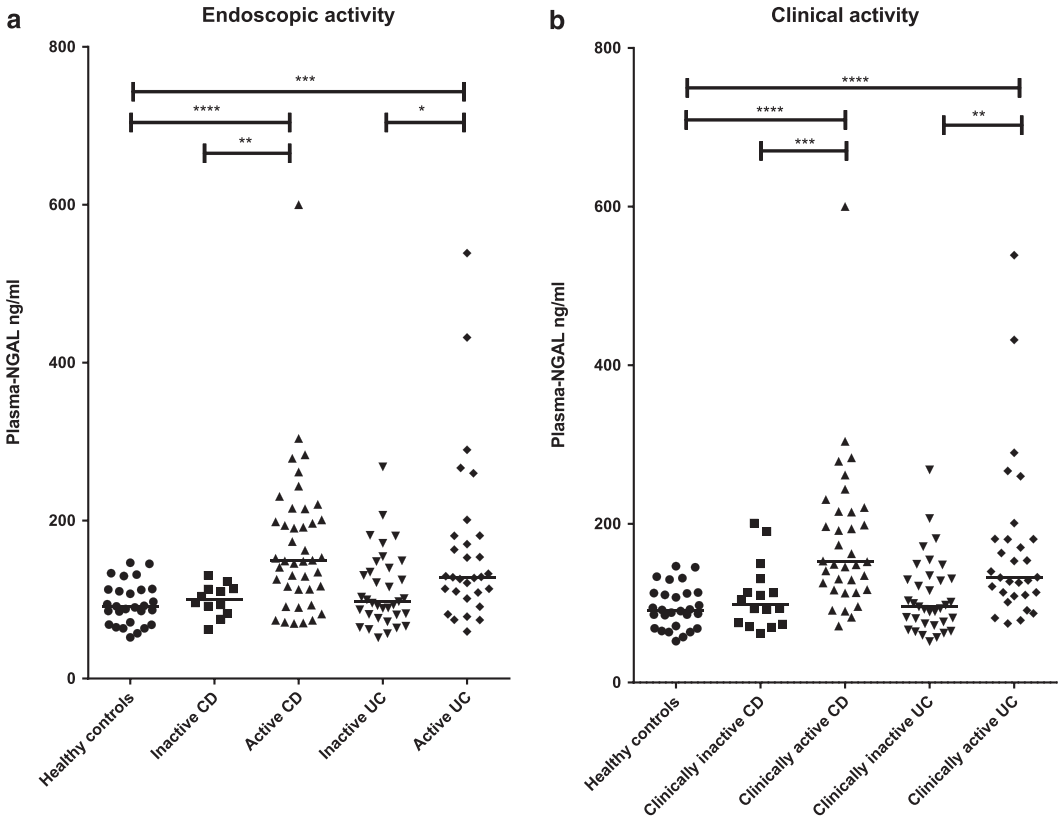


**Figure 4** Plasma neutrophil gelatinase-associated lipocalin (NGAL) in healthy controls (HC), inactive and active Crohn's disease (CD), and ulcerative colitis (UC). Individual values and medians are shown. (a) Activity level based on endoscopic score. (b) Activity level based on clinical score. Active disease is defined as Mayo partial score >2 or Harvey–Bradshaw Index >5. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$.

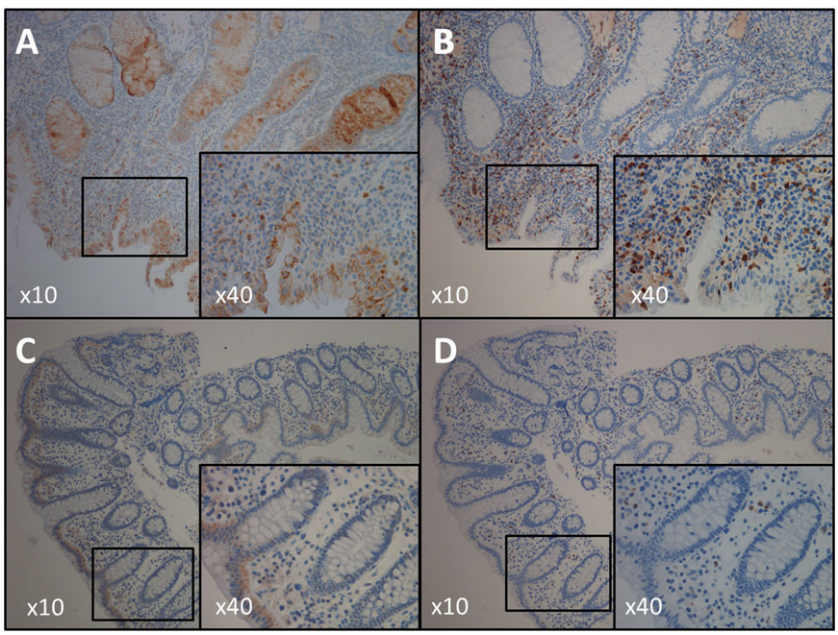


**Figure 5** Immunohistochemistry of serial biopsies from active ulcerative colitis (upper panel) (representative of $n = 5$) and healthy control (lower panel) stained for (a,c) neutrophil gelatinase-associated lipocalin (NGAL) and (b,d) calprotectin. During inflammation, NGAL is located to epithelial cells and granulocytes; calprotectin solely to granulocytes.

## Discussion

The present study shows that fe-NGAL is markedly raised in active UC and CD compared with IBS and healthy controls and compared with inactive disease. In an ROC analysis of our material, we found AUC 0.987 and a sensitivity and specificity for fe-NGAL of 95.7% and 94.7%, respectively, for distinguishing between active IBD and

HC. Of further importance for clinical practice, we found the stability of fe-NGAL in room temperature to be excellent for up to 7 days. Altogether, these findings show that fe-NGAL is a very promising biomarker of IBD. The use of fe-NGAL as a biomarker for IBD was suggested by Nielsen *et al*. [26] however, the present study further explores its potential as a useful method in clinical practice. While Nielsen *et al*. found fe-NGAL to be valuable only in UC, the present study shows that fe-NGAL is significantly increased also in patients with active CD. This discrepancy is probably explained by the fact that the 14 CD patients in the study by Nielsen *et al*. were classified based on clinical score and proctoscopy only, while the CD group in the present study underwent full ileocolonoscopy as well as being assessed with a clinical score.

In this direct comparison of fe-NGAL with the generally accepted fecal biomarker calprotectin, we find a strong correlation between these with a rho coefficient of 0.82. Fe-NGAL has excellent correlation with endoscopic Mayo score in UC patients with $\rho = 0.82$. The correlation coefficient for SES-CD score is somewhat weaker for fe-NGAL ($\rho = 0.58$) than for calprotectin ($\rho = 0.71$), but the difference is not significant. Seven of our patients had a relatively low level of fe-NGAL despite moderately high endoscopic SES-CD scores and high calprotectin levels. All of these patients had stenotic CD and the high SES-CD score was due to intestinal stenosis with macroscopic ulcerations. One possible explanation for this discrepancy between calprotectin and fe-NGAL could by bleeding from ulcerations without concomitant inflammation, giving increased calprotectin and relatively low NGAL in feces. The fe-NGAL method should thus be further assessed and compared with calprotectin in a large CD patient population with optimal characterization (computed tomography and/or magnetic resonance imaging scans, capsule enteroscopy) of the extent and degree of small intestinal inflammation to potentially confirm this observation. Like calprotectin, fe-NGAL is not a specific marker for IBD, as it also increases in other inflammatory conditions such as IEC.

A blood marker for IBD activity is of obvious interest; thus, we included a study on plasma NGAL. For plasma NGAL, we found significantly elevated levels in active CD and UC compared with healthy controls, but with a wide distribution of NGAL-levels within the group of patients with active IBD. Using plasma NGAL to discriminate active IBD from inactive IBD, we found a sensitivity and specificity for NGAL of 78.3% and 50%, respectively, not obviously superior to hs-CRP. Dividing the patients in CD and UC did not markedly improve the performance of the test. These findings are partly in line with the report of Nielsen *et al*. and Yesil *et al*., showing no significant correlation between serum NGAL and endoscopic inflammatory activity.[23,26] A recent study of Stallhofer *et al*. found a sensitivity of 83% and a specificity of 63% for distinguishing between active and inactive UC, and there seemed to be considerable overlap between the groups. In yet another study, however, Oikonomou *et al*. found a markedly better correlation of serum NGAL with clinical IBD activity level, but they did not have endoscopic results to confirm inflammation.[21] Studies on NGAL in plasma and serum to diagnose IBD thus show conflicting results; however, when looking at the individual NGAL measurements in our 147 IBD patients and healthy controls, these values overlap considerably, and it is difficult to see how this analysis can provide a robust assessment of ongoing bowel inflammation.

The most widely accepted fecal biomarker for inflammation today is calprotectin. Although very useful, calprotectin may perform less well in some situations. A meta-analysis of van Rheenen *et al*. found a pooled sensitivity of 0.93 and a specificity of 0.96 in adults and 0.92 and 0.76 in children/teenagers for fecal calprotectin when used as screening tool in patients with suspected IBD.[4] Another meta-analysis by Waugh *et al*. found a sensitivity of 93% and specificity of 94% in adults and 95–100% sensitivity and 44–93% specificity in children for differentiating between inflammatory and non-IBD.[5] These results indicate that there is a need for additional biomarker(s) to improve noninvasive IBD screening and diagnostics.

The existing fecal biomarkers calprotectin and lactoferrin are molecules mainly found in granulocytes.[18] We suggest that an additional biomarker should reflect different aspects of the inflammatory process than the existing ones. In this respect, fe-NGAL could be a good candidate marker, as shown in Figure 5 and previous studies from our laboratory.[12] The expression of NGAL is potently regulated in the colonic epithelium during inflammation in addition to being expressed in granulocytes. Thus, fe-NGAL may be a more sensitive test than calprotectin in a more chronic inflammatory setting with low numbers of infiltrating granulocytes. Moreover, fe-NGAL should be studied in pediatric IBD, where meta-analyses show that calprotectin may have a relatively low specificity.[4,5]

The major limitation of the present study is a relatively low number of patients. However, the study subjects are well characterized, the results are highly significant, and fe-NGAL emerges favorably in this first comparison with the established fecal marker calprotectin. The different expression pattern of NGAL and calprotectin in the inflamed gut makes it possible that these two disease markers can yield complementary information, this could however not be penetrated in the present work. In our opinion, the findings presented here warrant further large-scale studies of NGAL in the diagnosis of IBD and in the follow-up and care of these patients.

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