Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Differential Diagnosis Value of Neutrophil Gelatinase Associated Lipocalin as a Noninvasive Biomarker in Perianal Fistulizing Crohn's Disease

Kai Ma*, Yikun Li*, Jingwen Wu, Yi Fu, Lu Yin, Simin Xu, Feiyang Weng, Yibo Yao, Chen Wang

Department of Proctology, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, 200032, People's Republic of China

*These authors contributed equally to this work

Correspondence: Chen Wang; Yibo Yao, Department of Proctology, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, 200032, People's Republic of China, Email wangchen_longhua@163.com; elevenzoe@163.com

Background: Diagnosing perianal fistulizing Crohn's disease (pfCD) typically depends on costly and time-intensive endoscopic and radiographic procedures. Compelling evidence indicates that neutrophil gelatinase-associated lipocalin (NGAL) plays a role in the pathophysiology of Crohn's disease (CD) and may serve as a noninvasive biomarker for its diagnosis. This study aimed to evaluate NGAL's potential as a noninvasive diagnostic biomarker between pfCD and cryptoglandular (CG) perianal fistula, and its correlation with disease severity in pfCD.

Methods: Serum, fecal, and fistula tissue samples were collected from 96 patients with pfCD and 97 patients with CG perianal fistula as controls. Serum NGAL levels were quantified through ELISA and fistula tissue NGAL levels were quantified through immunohistochemical staining, while pfCD disease severity was evaluated using the Crohn's Disease Activity Index (CDAI) and Perianal Disease Activity Index (PDAI). Additional laboratory parameters, including NGAL, fecal calprotectin (FC), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR), were analyzed, and their correlations were assessed. Receiver operating characteristic (ROC) analysis was conducted to evaluate NGAL's diagnostic potential for pfCD.

Results: Levels of serum NGAL, FC, CRP, and ESR in patients with pfCD were significantly elevated compared to the control group ($p < 0.001$); Spearman correlation analysis indicated a positive correlation between serum NGAL and FC, CRP, ESR, CDAI, and PDAI scores. The area under the ROC curve (AUC) for serum NGAL in diagnosing pfCD was 0.927 (95% *CI*: 0.890–0.964). The AUC for FC in diagnosing pfCD were 0.887 (95% *CI*: 0.839–0.935). Additionally, serum and fistula tissue NGAL levels were positively correlated with disease complexity in pfCD according to the Montreal classification.

Conclusion: These findings suggest that serum NGAL is associated with pfCD severity and may offer a promising noninvasive biomarker for diagnosing and assessing pfCD.

Keywords: neutrophil gelatinase associated lipocalin, perianal fistulizing Crohn's disease, biomarker, diagnosis value

Introduction

Perianal fistulas are a prevalent medical and surgical concern, forming abnormal tracts between the anorectal canal (typically the anal canal or rectum) and the perianal skin.[1] Population-based studies indicate an incidence of perianal fistulas in Europe at approximately 2.5 per 10,000 individuals annually, with the highest incidence observed among those aged 30 to 50, and a higher prevalence in men than women.[2–4] Clinically, perianal fistulas are associated with significant symptoms, including anorectal pain, purulent discharge, recurrent abscess formation, and sometimes fecal incontinence; these manifestations severely impair patients' quality of life and impose substantial financial burdens.[5,6]

More than 80% of perianal fistulas are cryptoglandular (CG) anal fistulas, generally originating from infected anal crypts. Acute infection of these glands can lead to anorectal abscess formation, followed by perianal fistula development.[7] CG perianal fistulas typically occur in patients without Crohn's disease (CD) and are driven by infections

Received: 2 November 2024
Accepted: 4 March 2025
Published: 19 March 2025

in perianal crypt glands.[8] The second most prevalent form of perianal fistulas is associated with CD, a chronic and nonspecific inflammatory disorder of the gastrointestinal tract characterized by segmental, transmural, and recurrent intestinal inflammation, potentially affecting any part of the gut.[9] Perianal fistulas are a frequent and serious complication of CD; perianal fistulizing CD (pfCD) arises from chronic, transmural inflammation rather than gland infection.[10,11] In certain cases, the development of perianal fistulas may coincide with CD or precede the clinical onset of intestinal disease by several years.[12] Although CG perianal fistulas and pfCD share pathophysiological similarities, they have distinct etiologies, making the differentiation between CG anal fistulas and pfCD essential. Furthermore, the management of CG fistulas and pfCD is different, CG fistulas are primarily managed surgically, however, there is extensive evidence supporting that pfCD rely on the combination of surgical interventions and pharmacological therapies, especially using biological agents. Inadequate surgical managements may lead to significant morbidity including a risk of fecal incontinence, defunctioning stoma and proctectomy.[13] Currently, endoscopy and magnetic resonance imaging (MRI) are considered the gold standards for assessing pfCD severity; however, these methods are invasive, uncomfortable, time-consuming, and costly, which limits their accessibility and use.[14,15] Thus, given the burdens associated with endoscopy and MRI, there is a significant need for a biomarker to aid in diagnosing or monitoring patients at higher risk of pfCD, who would therefore require endoscopy and MRI.[16]

Neutrophil gelatinase-associated lipocalin (NGAL) is a secreted glycoprotein belonging to the lipocalin superfamily.[17] First identified in 1993, NGAL was isolated from neutrophil granules released at sites of human infection and inflammation and is regarded as proinflammatory, being secreted upon neutrophil activation.[18] Research has established NGAL's critical role in the progression of CD, highlighting its potential as a biomarker for predicting disease activity, assessing severity, monitoring prognosis, and evaluating therapeutic response.[19,20] Previous studies have highlighted that serum NGAL demonstrated the ability to distinguish active CD from irritable bowel syndrome patients and healthy subjects, with a sensitivity of 94% and specificity of 83%. Thorsvik S et al also found that fecal NGAL is a promising biomarker for distinguishing between active IBD and healthy subjects with the AUC was 0.987 (95% *CI*:0.97–1.0), Sensitivity and specificity was 94.7% and 95.7%, respectively.[21,22]

Previous studies have demonstrated that fecal calprotectin (FC) is an effective clinical tool for distinguishing active CD from CG fistulas in patients with perianal fistulas. Since both calprotectin and NGAL originate from various cell types, including neutrophils and macrophages, they are expected to reflect cellular leakage into the lumen.[23] Perianal fistulas are a frequent and serious complication of CD, however, to date, no data have been available on NGAL's diagnostic accuracy for mucosal inflammation in CD with perianal fistulas. This study aimed to evaluate NGAL's potential as a noninvasive diagnostic biomarker between pfCD and CG perianal fistula, and its correlation with disease severity in pfCD.

Materials and Methods

Patient Selection

This study retrospectively enrolled 193 patients with perianal fistulas from the Department of Anorectal Diseases at Longhua Hospital, Shanghai University of Traditional Chinese Medicine, between June 2021 and March 2024. Participants included 96 patients with pfCD and 97 gender- and age-matched patients with CG perianal fistulas who, in the absence of inflammatory bowel disease, underwent routine physical examinations. The diagnosis of pfCD adhered to expert consensus, based on clinical, radiological, laboratory, endoscopic, and histological criteria.[24] Data were retrospectively extracted by a single investigator from electronic medical records, using a standardized form from the center's database. A blinded reanalysis was conducted by an external statistician using raw data. Collected demographic data encompassed age, sex, weight, disease onset age, lesion location, disease duration, previous and current medical treatments, and fistula characteristics (number), as well as endoscopic and radiographic findings. Inflammatory markers, including NGAL, FC, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR), were recorded.

All participants provided signed informed consent, and the study received approval from the ethics committee of Longhua Hospital of Shanghai University of Traditional Chinese Medicine, Shanghai, P. R. China (Ethics code: 2020LCSY032), adhering to the ethical guidelines of the Declaration of Helsinki.

Inclusion and Exclusion Criteria

Inclusion criteria for patients with pfCD were as follows: (a) a diagnosis of pfCD based on clinical assessment and a combination of endoscopic, histological, MRI, or biochemical evaluations; (b) the presence of at least one actively draining perianal fistula. Exclusion criteria for patients with pfCD were as follows: (a) anal strictures or abscesses requiring surgical intervention; (b) perianal fistulas with etiologies such as malignancy, trauma, or human immunodeficiency virus infection; (c) infectious gastroenteritis. For CG perianal fistulas,[25] inclusion criteria included: (a) at least one actively draining perianal fistula; (b) prior exclusion of CD through physical examination, endoscopy, and MRI. Exclusion criteria encompassed: (a) pilonidal disease; (b) perianal fistulas due to malignancy, trauma, or HIV infection; (c) infectious gastroenteritis; (d) recent trauma or surgery (within one month). A flowchart of patient selection is presented in Figure 1.

Clinical Evaluations

The Perianal Disease Activity Index (PDAI) and Crohn's Disease Activity Index (CDAI) were utilized to assess disease severity. The CDAI total score ranges from 0 to 600, with higher scores indicating greater disease severity: scores below 150 indicate clinical remission, while 150–220, 221–450, and above 450 correspond to mild, moderate, and severe clinical activity, respectively.[26] The PDAI, recognized as the gold standard for evaluating perianal disease severity, incorporates both patient-reported outcomes and physical assessments by the attending specialist. The PDAI consists of five items: discharge, pain/restriction of activities, restriction of sexual activity, perianal disease type, and degree of induration. Scores range from 0 to 20, where a score of ≤ 4 denotes inactive disease, and a score above 4 indicates active fistulizing disease.[27]

Sample Collection

All patients underwent blood analysis and provided fecal samples for FC evaluation. Perianal fistula tissues were collected and stored at −80°C for subsequent analysis. Fecal samples were placed in clean polypropylene tubes, reconstituted in PBS with 0.1% Tween 20 at a concentration of 100 mg feces/mL, and vortexed for 30 minutes to create a homogenous suspension. The samples were then centrifuged at 12,000 rpm at 4°C for 10 minutes, and the clear

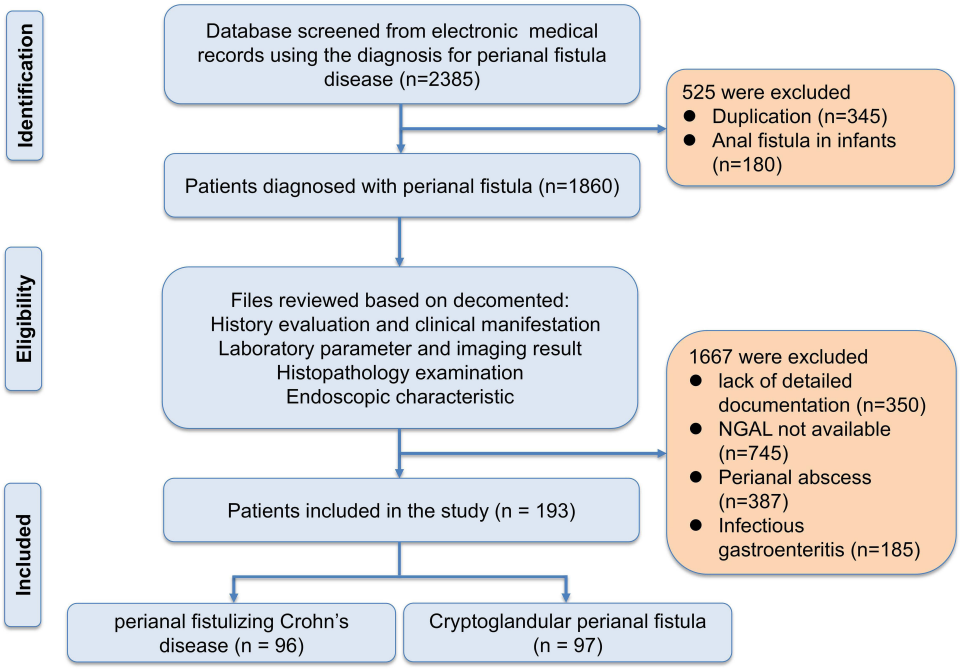

Figure 1 Flow diagram of patient selection in our study.

https://doi.org/10.2147/JIR.S504213

supernatant was transferred to clean polypropylene tubes and stored at −80°C until analysis. For serum samples, blood was collected in clot activator tubes (Terumo, Somerset, NJ), left at room temperature for 30 minutes, and centrifuged at 3000 rpm for 10 minutes. Serum samples were subsequently collected and stored at −80°C.

Measurement of Inflammatory Markers

Laboratory inflammatory biomarkers for pfCD diagnosis, including NGAL (upper limit of normal: 150 μg/L), FC (upper limit of normal: 60 μg/g), CRP (upper limit of normal: 5 mg/L), and ESR (upper limit of normal: 21 mm/h), were measured at sample collection. Serum NGAL levels were quantified using a quantitative enzyme immunoassay ELISA Kit (R&D Biosystems, Minneapolis, MN), following the manufacturer's instructions. FC levels were assessed using a quantitative immunochromatographic test (Buhlmann Laboratories AG, Schonenbuch, Switzerland). CRP was measured via the immunoturbidimetric method, while ESR was determined using the Westergren method.

Immunohistochemical Staining

Paraffin-embedded perianal fistula tissue sections (4–6 μm) were deparaffinized with xylene and dehydrated through a graded alcohol series. Antigen retrieval was performed by heating the sections in 0.1 M citrate buffer, followed by blocking with 5% H_2O_2. Sections were washed three times with PBS, then blocked with 5% non-fat dry milk at room temperature for 1 hour. The primary antibody against NGAL (1:500; Abcam, Cambridge, MA, USA) was diluted in antibody diluent (Agilent Dako) and incubated at 4°C overnight. Afterward, sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:100, Thermo Fisher Scientific, Waltham, MA, USA) in antibody diluent. Diaminobenzidine (DAB) (Agilent Dako) was applied for 5 minutes, and stained sections were imaged using a confocal laser scanning microscope. NGAL protein expression was quantified by calculating the integrated optical density using Image-Pro Plus 7.0 software (Media Cybernetics, Silver Spring, MD, USA), with results obtained by multiplying staining intensity by staining area.

Statistical Analysis

Statistical analyses were conducted using SPSS 25.0 (IBM Corp., Chicago, IL, USA), and data were visualized with GraphPad Prism version 7 (GraphPad Software Inc., California). Data distribution was assessed with the Shapiro–Wilk test. Measurement data with normal distribution were expressed as mean ± standard deviation (M ± SD) and analyzed between groups using the *t*-test. Non-normally distributed variables were presented as median and interquartile range (IQR) and compared using the Mann–Whitney rank-sum test. Correlation coefficients (Pearson or Spearman, as appropriate) were calculated to assess relationships between quantitative parameters, including associations between NGAL and various clinical and laboratory markers. Receiver operating characteristic (ROC) curves were generated, and sensitivity, specificity, and area under the ROC curves (AUCs) were compared to evaluate diagnostic performance and identify the optimal cut-off values for each marker. A p-value of < 0.05 was considered statistically significant.

Results

Patient Characteristics

No significant differences were observed between the pfCD (73 males, 23 females; mean age: 28.59 ± 1.13) and CG perianal fistulas (72 males, 25 females; mean age: 29.36 ± 1.62) groups in terms of gender, age, weight, lesion location, or disease duration ($p > 0.05$). According to the Montreal classification, 96 patients with pfCD were evaluated. Among these, the most common disease phenotype was A2L3B1 (24 cases; 25.0%). Age distribution included 11 patients (11.5%) in group A1 (under 17 years), 73 patients (76.0%) in group A2 (17–40 years), and 12 patients (12.5%) in group A3 (over 40 years). Regarding disease location, 30 patients (31.3%) had pure ileal disease (L1), 16 (16.7%) had colonic disease (L2), and 50 (52.0%) had ileocolonic disease (L3). In terms of disease behavior, 62 patients (64.6%) exhibited nonstricturing and nonpenetrating disease (B1), while 34 (35.4%) had stricturing disease (B2), with no cases of penetrating disease (B3). Levels of NGAL, FC, CRP, and ESR were significantly elevated in the pfCD group compared to the CG perianal fistulas group ($p < 0.001$). Demographic and clinical characteristics are presented in Table 1 and Supplementary Figure 1.

Table 1 Demographic and Clinical Parameters

Variable	pfCD (n=96)	CG Perianal Fistulas (n=97)	*p*
Age (years)	27.58 ± 0.87	28.18 ± 0.89	>0.05
Sex (Male/Female)	73/23	72/25	>0.05
Weight (kg)	64.48 ±0.90	63.69 ± 0.85	>0.05
Number of fistulas (IQR)	2.0 (2.00–3.00)	1.0 (1.00–2.00)	>0.05
Age at diagnosis			>0.05
A1 (<17 years)	11 (11.5%)	10 (11.2%)	
A2 (17–40 years)	73 (76.0%)	74 (75.5%)	
A3 (>40 years)	12 (12.5%)	13 (13.3%)	
Disease location			
L1 (ileum)	30 (31.3%)	-	
L2 (colon)	19 (19.8%)	-	
L3 (ileum-colon)	47 (51.1%)	-	
L4 (upper gastrointestinal)	0	-	
Disease behavior			
B1 (nonstenotic, nonpenetrating)	62 (64.6%)	-	
B2 (stenotic)	34 (35.4%)	-	
B3 (penetrating)	0	-	
p (perianal)	96 (100%)	97 (100%)	
CDAI median (IQR)	179.0 (132.00–261.75)	-	
PDAI median (IQR)	10.0 (7.00–13.00)	-	
NGAL median (IQR)	229.0 (179.00–285.00)	104.0 (85.00–124.00)	<0.001
FC median (IQR)	182.5(76.50–390.25)	32.0 (25.00–46.00)	<0.001
CRP median (IQR)	10.31 (4.86–15.57)	2.80 (1.00–4.60)	<0.001
ESR median (IQR)	32.0 (18.25–48.50)	12.0 (7.50–19.00)	<0.001

Abbreviations: pfCD, perianal fistulizing Crohn's disease; CG, cryptoglandular; CDAI, Crohn's Disease Activity Index; PDAI, Perianal Disease Activity Index; NGAL, neutrophil gelatinase-associated lipocalin; FC, fecal calprotectin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

NGAL Expression of Perianal Fistula Tissues in pfCD and CG Perianal Fistulas

To further verify NGAL expression in perianal fistula tissues between pfCD and CG perianal fistulas, immunohistochemistry was conducted. Results showed significantly elevated NGAL expression in pfCD tissues compared to CG perianal fistula tissues ($p < 0.001$). (Figure 2).

Correlation Between NGAL Expression in Serum and Clinical Parameters of pfCD

Given the non-normal distribution of quantitative parameter data, Spearman correlation analysis was used to examine relationships. Findings indicated that serum NGAL concentration correlated positively with FC ($r = 0.71$, $p < 0.001$), CRP ($r = 0.64$, $p < 0.001$), ESR ($r = 0.69$, $p < 0.001$), CDAI ($r = 0.66$, $p < 0.001$), and PDAI scores ($r = 0.65$, $p < 0.001$). (Figure 3).

The Diagnostic Value of NGAL, FC, CRP, and ESR in pfCD Based on Serological Testing

Based on serological testing, the AUC values for NGAL, FC, CRP, and ESR in diagnosing pfCD were 0.927 (95% *CI*: 0.890–0.964), 0.887 (95% *CI*: 0.839–0.935), 0.834 (95% *CI*: 0.776–0.892), and 0.820 (95% *CI*: 0.759–0.880), respectively. The optimal cut-off values were 175.5 μg/L for NGAL, 81.5 μg/g for FC, 5.7 mg/L for CRP, and 23.5 mm/h for ESR, with NGAL demonstrating notably higher diagnostic value than the other inflammatory markers for pfCD. (Figure 4 and Table 2).

https://doi.org/10.2147/JIR.S504213

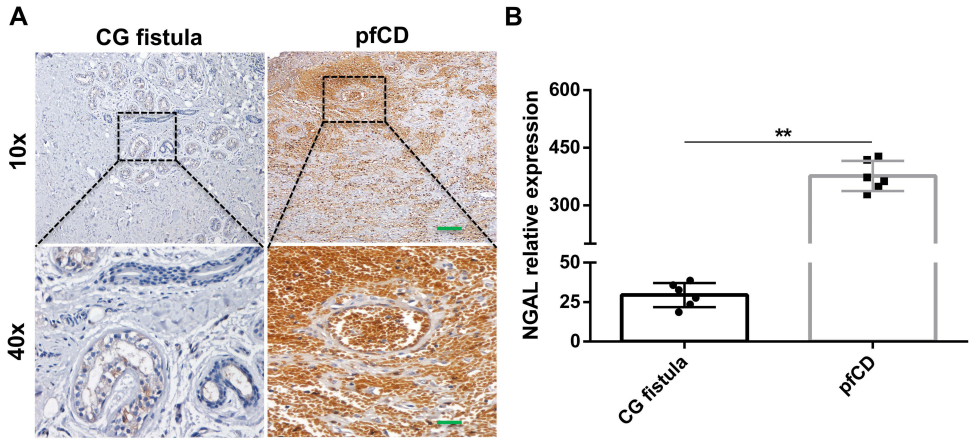

Figure 2 NGAL level in CG perianal fistulas and pfCD fistula tissue. (**A**) Representative immunohistochemical staining images. (**B**) Quantitative analyses for NGAL in CG perianal fistulas and pfCD fistula tissue sections. Scale bar: 100 μm (upper row), 25 μm (lower row), $^{**}p < 0.001$, n=6.

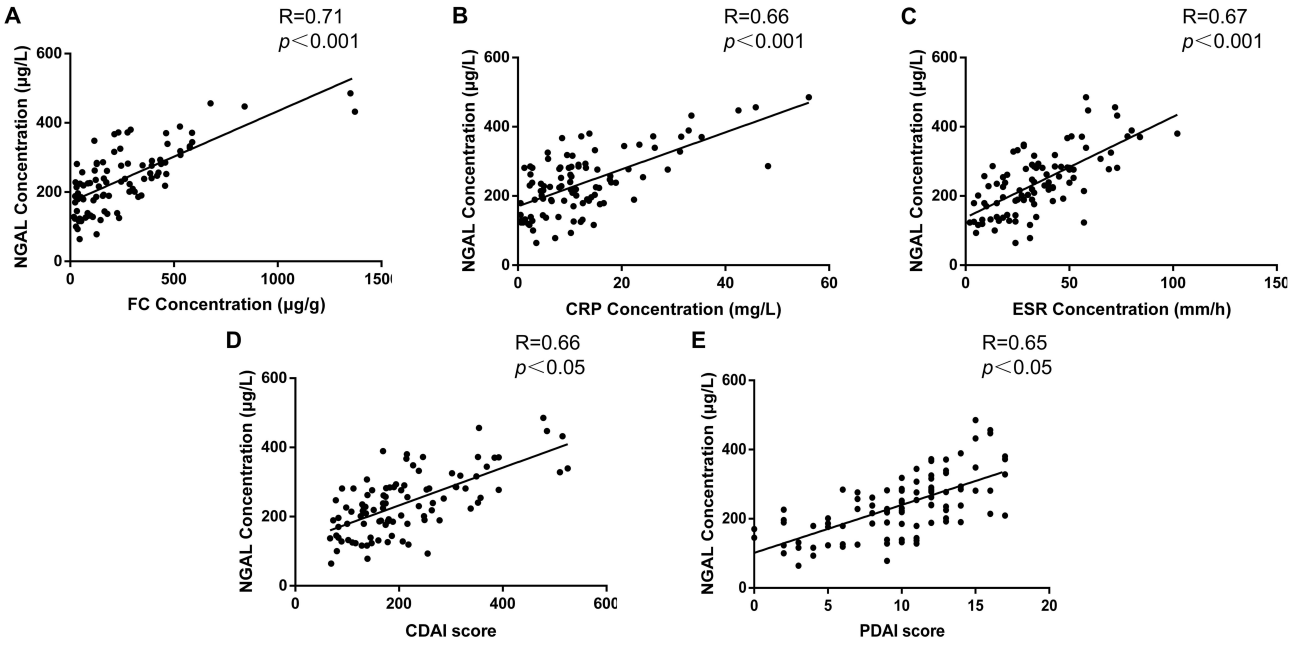

Figure 3 Correlation analysis between serum NGAL level and clinical parameters of pfCD. (**A**) FC. (**B**) CRP. (**C**) ESR. (**D**) CDAI. (**E**) PDAI.
Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; FC, fecal calprotectin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CDAI, Crohn's Disease Activity Index; PDAI, Perianal Disease Activity Index.

Association Between NGAL Level and Different Disease Ages in pfCD

An evaluation was conducted to determine if NGAL levels correlated with age at disease onset in patients with pfCD, categorized by the Montreal classification into group A1 (under 17 years), group A2 (17–40 years), and group A3 (over 40 years). Results showed that serum NGAL levels were slightly higher in group A2 (242.42 ± 11.08 μg/L) compared to group A3 (221.83 ± 22.41 μg/L) and group A1 (228.09 ± 20.28 μg/L); however, no significant differences were observed across age groups ($p > 0.05$). (Figure 5).

Association Between NGAL Level and Disease Locations in pfCD

Analysis was conducted to determine whether serum NGAL levels correlated with disease location in pfCD, categorized by the extent of CD lesions. Findings indicated significantly lower serum NGAL expression in pfCD individuals with isolated ileal disease (L1, 188.43 ± 12.95 μg/L) compared to those with pure colonic disease (L2, 249.05 ± 19.26 μg/L) ($p < 0.05$)

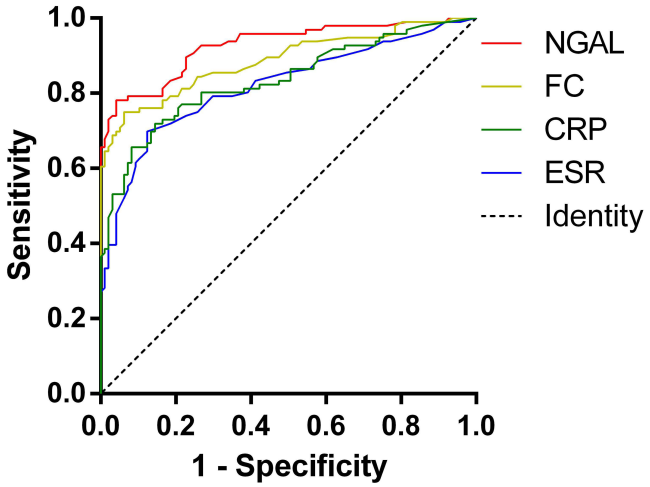

Figure 4 ROC curve analysis of NGAL, FC, CRP, ESR for the diagnosis value of pfCD.

and ileocolonic involvement (L3, 265.60 ± 13.22 μg/L) ($p < 0.001$) based on the Montreal classification. However, no significant difference was observed between pure colonic disease and ileocolonic involvement ($p > 0.05$). (Figure 6).

Association Between NGAL Level and Disease Behaviors in pfCD

The correlation between NGAL levels and disease behavior in pfCD was also assessed, revealing that serum NGAL expression was significantly elevated in patients with stenotic disease (B2, 282.79 ± 14.50 μg/L) compared to those with nonstricturing and nonpenetrating disease (B1, 213.76 ± 10.56 μg/L) ($p < 0.001$). (Figure 7).

Discussion

pfCD is recognized as a refractory and recurrent condition, imposing a substantial socioeconomic burden on the general population. The mechanisms underlying its development and persistence remain incompletely elucidated, pfCD results from a dysregulated, chronic, and transmural inflammatory response in the anal canal. Recent studies highlight the involvement of various factors, including upregulation of matrix metalloproteinases (MMPs), chronic intestinal inflammation, intestinal epithelial-mesenchymal transition, and dysbiosis of the gut microbiota.[28] This persistent inflammatory activity and compromised mucosal integrity in the gut and anal canal likely impede fistula healing.[29] One of the challenges in managing patients with perianal disease is distinguishing pfCD from CG perianal fistulas. pfCD rely on the combination of surgical and medical treatment, while CG perianal fistulas are mainly treated with surgery. Lee KY et al demonstrated that the healing rate and recurrence rate of surgery for CG perianal fistulas were 92.4% and 8.8%, respectively, during an average of 307 days of follow-up.[30] Long-term medical therapy remains a cornerstone of pfCD treatment, however, only 25% of patients with pfCD experience sustained perianal fistulas closure with biologic therapy.[31] pfCD is commonly associated with typical gastrointestinal symptoms like diarrhea and abdominal discomfort, individuals with CG perianal fistulas might also present with (functional) gastrointestinal issues that suspicious of CD.

Table 2 The Diagnostic Value of NGAL, FC, CRP, ESR in pfCD

	Cut-off value	AUC	95% CI	Sensitivity	Specificity	Youden Index
NGAL	175.5 μg/L	0.927	0.890–0.964	0.781	0.959	0.740
FC	81.5 μg/g	0.887	0.839–0.935	0.750	0.938	0.688
CRP	5.7 mg/L	0.834	0.776–0.892	0.719	0.856	0.575
ESR	23.5 mm/h	0.820	0.759–0.880	0.700	0.876	0.574

Abbreviations: pfCD, perianal fistulizing Crohn's disease; NGAL, neutrophil gelatinase-associated lipocalin; FC, fecal calprotectin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate. AUC, Area under the receiver operating characteristic. 95% CI, 95% Confidence Interval.

https://doi.org/10.2147/JIR.S504213

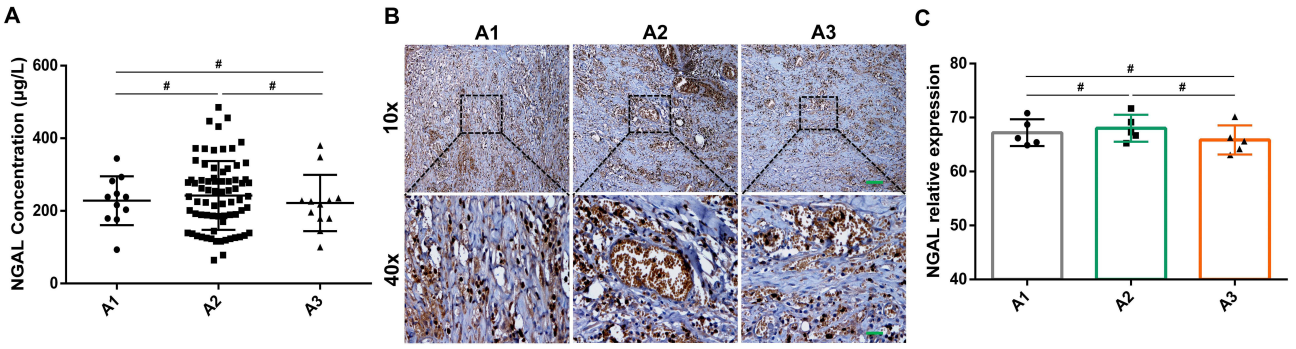

Figure 5 Association of NGAL levels with different disease ages in pfCD. (**A**) Serum NGAL expression in patients with pfCD across various disease ages. (**B**) Representative immunohistochemical staining images. (**C**) Quantitative analysis of NGAL markers in pfCD fistula sections. Scale bar: 100 μm (upper row), 25 μm (lower row). $^{\#}p > 0.05$, n=5.

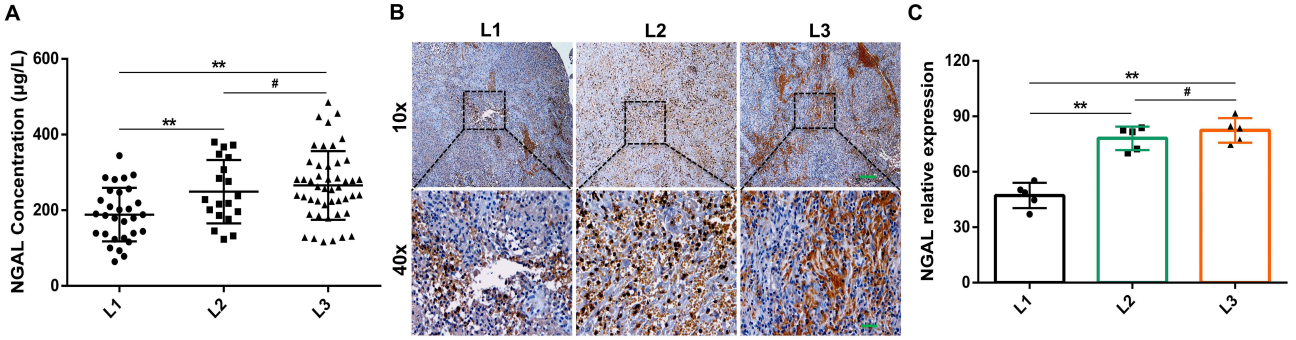

Figure 6 Association of NGAL levels with different disease locations in pfCD. (**A**) Serum NGAL expression in patients with pfCD across L1, L2, and L3. (**B**) Representative immunohistochemical staining images. (**C**) Quantitative analysis of NGAL markers in fistula sections across L1, L2, and L3. Scale bar: 100 μm (upper row), 25 μm (lower row). $^{*}p < 0.05$, $^{**}p < 0.001$; $^{\#}p > 0.05$, n=5.

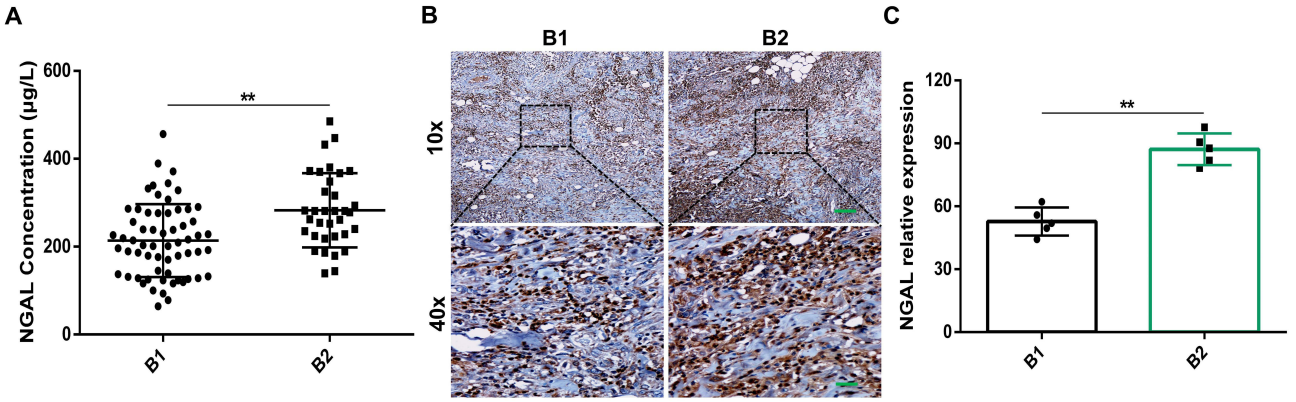

Figure 7 Association of NGAL levels with different disease behaviors in pfCD. (**A**) Serum NGAL expression in patients with pfCD exhibiting B1 and B2 disease behaviors. (**B**) Representative immunohistochemical staining images (**C**) Quantitative analysis for NGAL markers in pfCD fistula sections. Scale bar: 100 μm (upper row), 25 μm (lower row). $^{**}p < 0.001$, n=5.

However, to date, endoscopy is still the gold standard for diagnosing CD.[32] The need for noninvasive biomarkers that accurately reflect endoscopic and histological activity in pfCD has renewed interest in serological and fecal markers for advancing diagnosis and disease monitoring. Our study suggest that serum NGAL may offer a promising noninvasive biomarker for diagnosing and assessing pfCD.

Among these biomarkers, the most commonly recognized biomarker for gastrointestinal inflammation currently is FC. FC has demonstrated the highest accuracy in detecting endoscopically active intestinal inflammation, followed by serum

CRP and ESR.[33] Calprotectin, a calcium-binding protein produced by neutrophils and monocytes during inflammation, has shown high sensitivity as a marker for gastrointestinal inflammation.[34] Studies support its role as a valuable predictor for diagnosing CD, correlating with endoscopic severity, evaluating therapeutic response, and predicting relapse risk.[35,36] Recent retrospective findings further establish FC as a precise and clinically essential marker to differentiate between pfCD and CG perianal fistulas, with a sensitivity of 0.81 and specificity of 0.89.[37] As both calprotectin and NGAL are inflammation-associated proteins expressed by inflammatory cells such as neutrophils and macrophages,[38] our results also showed significantly elevated FC levels in patients with pfCD compared to the CG perianal fistulas group. ROC analysis indicated an AUC of 0.887 for FC in diagnosing pfCD, reflecting its high sensitivity and specificity and underscoring its potential as a clinical diagnostic marker for pfCD. Although very useful, FC might not be as effective in certain situations. FC primarily is present in neutrophil granulocytes and in smaller amounts in monocytes and macrophages. NGAL expression is strongly regulated in the colonic epithelium during intestinal inflammation, alongside its presence in granulocytes. Consequently, NGAL could potentially offer greater sensitivity compared to calprotectin in chronic inflammatory conditions characterized by a low presence of infiltrating granulocytes.[39]

CRP and ESR are key acute-phase inflammatory proteins, with serum levels rising sharply in response to trauma, infection, and other inflammatory stimuli.[40] Research indicates that while CRP and ESR provide some diagnostic value, they are non-specific markers for CD and are typically not used independently for clinical diagnosis. Instead, they are best assessed in combination with other laboratory parameters and clinical findings to account for their limited specificity.[41] In our study, serum CRP and ESR levels in patients with pfCD were significantly higher than in the CG perianal fistulas group. ROC analysis demonstrated AUCs of 0.811 and 0.826 for CRP and ESR in diagnosing pfCD, showing relatively high diagnostic accuracy but lower than NGAL. This discrepancy likely arises from CRP and ESR's non-specificity, as these markers are elevated in various acute and chronic inflammatory conditions. To enhance diagnostic efficacy for pfCD, CRP and ESR should therefore be evaluated alongside other cytokines and clinical characteristics.

NGAL, also known as 24p3, has been investigated as a non-invasive marker for assessing disease activity in inflammatory bowel disease (IBD). NGAL is a 25-kDa secreted glycoprotein belonging to the lipocalin superfamily and is produced in response to inflammation. It is expressed in a variety of cell types, including neutrophilic granulocytes, adipocytes, macrophages, and epithelial cells in the gastrointestinal, respiratory, and urogenital tracts.[42] NGAL reflects neutrophil migration into the gut lumen, making it a sensitive marker of intestinal inflammation. Recent studies have reported elevated NGAL levels in both serum and feces of patients with IBD, suggesting its utility as a surrogate marker for mucosal inflammation in IBD.[43] Prior research has shown significantly higher NGAL expression in patients with active IBD compared to inactive cases, with positive correlations observed between NGAL levels and FC, PDAI scores, and endoscopic disease severity.[44] NGAL concentrations also rise across various pathological conditions, with studies confirming increased NGAL levels in sepsis and correlations with inflammatory markers such as IL-6, IL-10, TNF, CRP, and leukocyte counts.[45,46] In our findings, NGAL expression in pfCD was significantly higher than in the CG perianal fistulas group, with Spearman correlation analysis indicating positive associations between NGAL and FC, CRP, ESR, CDAI scores, and PDAI scores, suggesting NGAL as a potential biomarker for assessing clinical severity in patients with pfCD. ROC curve analysis demonstrated an AUC of 0.927 (95% *CI*: 0.890–0.964) for NGAL in pfCD diagnosis, which exceeded the diagnostic value of FC, CRP, and ESR. An NGAL cut-off of 175.5 μg/L was found to detect pfCD with 78.1% sensitivity and 95.9% specificity. The high sensitivity and specificity of NGAL levels, as well as the significant AUC values, emphasize its potential as discriminatory markers for pfCD. Furthermore, we also confirmed that serum NGAL levels were positively correlated with disease location and complexity, as assessed by the Montreal classification. The enrichment of NGAL at sites of active inflammation indicates its potential role as a biomarker for localized neutrophil activation and epithelial stress. The strong correlation between its expression levels and pathological scores further supports its potential as a noninvasive monitoring marker for pfCD.

NGAL is involved in diverse cellular processes, including immune regulation, cell proliferation, apoptosis, metabolism, iron transport, and tumor metastasis.[47] Previous studies have highlighted its role in the progression of various pathological disorders, particularly inflammatory diseases, renal dysfunction, neurodegenerative diseases, and cancers.[48] Research by Kim demonstrated that NGAL directly upregulates the NLRP3 inflammasome complex via NF-κB activation in response to LPS-stimulated macrophages, functioning as a proinflammatory regulator in macrophage activation

mediated by NF-κB, this finding suggests NGAL's potential as a target for IBD prevention and treatment.[49] In addition to its proinflammatory roles, NGAL helps control disease progression by inhibiting bacterial proliferation and reducing oxidative stress.[50] Toyonaga et al found that NGAL limits spontaneous intestinal inflammation in IL-10 deficient mice by enhancing bacterial clearance through macrophage phagocytosis.[51] Moreover, Bjornstad EC et al discovered that the urine NGALds dipstick performed similarly to the NGAL test in this low-resource setting and may be a useful tool to rule out Acute Kidney Injury.[52] A novel diagnostic test for pfCD that does not rely on radiological examination, invasive examination, or experienced laboratory technicians could be invaluable in this setting. This has inspired us to develop a diagnostic dipstick for NGAL in the future, which could evaluate the severity of pfCD by measuring serum or fecal supernatant. If it proves equally efficacious as the laboratory serum/fecal NGAL, it could be quite promising for improving pfCD diagnostics, it may also enable the differentiation of pfCD from CG perianal fistulas as early as possible.

However, several major limitations of the present study should be acknowledged. First, the modest sample size renders this study preliminary, necessitating further research to confirm the findings. Second, selection bias may be present, as patient samples were sourced from a single hospital. While NGAL shows promise as a biomarker, future research endeavors should focus on validating the diagnostic value of serum NGAL levels in pfCD through larger, multicenter cohorts studies with long-term follow-up data. Additionally, the exploration of potential therapeutic implications based on the integration of NGAL into clinical practice represents a promising avenue for future investigation.

Conclusion

In conclusion, this study identifies NGAL as a promising biomarker for differentiating between pfCD and CG perianal fistula, and we also found that NGAL levels were positively correlated with disease severity in pfCD. The findings offer a basis for developing a new non-invasive biomarker for pfCD.

Data Sharing Statement

Datasets of this work are available from the corresponding authors upon reasonable request.

Acknowledgments

We want to thank staff of the Department of Anal and Intestinal Diseases at Longhua Hospital, Shanghai University of Traditional Chinese Medicine, for including research subjects and providing clinical material.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This paper was published as part of a supplement financially supported by Shanghai Municipal Health Commission-Shanghai Sinus Fistula Disease Research Center (2023ZZ02003); Shanghai Hospital Development Center Foundation (SHDC22021316-A); High-level key discipline of Traditional Chinese Medicine (ZYYZDXK-2023064).

Disclosure

All authors declare they have no conflicts of interest in this work.

References

1. Meng ZW, Baumgart DC. Darvadstrocel for the treatment of perianal fistulas in Crohn's disease. *Expert Rev Gastroenterol Hepatol*. 2020;14(6):405–410. doi:10.1080/17474124.2020.1764349
2. Brochard C, Rabilloud ML, Hamonic S, et al. Natural history of perianal crohn's disease: long-term follow-up of a population-based cohort. *Clin Gastroenterol Hepatol*. 2022;20(2):e102–e110. doi:10.1016/j.cgh.2020.12.024

3. Wewer MD, Zhao M, Nordholm-Carstensen A, et al. The incidence and disease course of perianal crohn's disease: a Danish nationwide cohort study, 1997-2015. *J Crohn's Colitis*. 2021;15(1):5–13. doi:10.1093/ecco-jcc/jjaa118
4. García-Olmo D, Van Assche G, Tagarro I, et al. Prevalence of anal fistulas in Europe: systematic literature reviews and population-based database analysis. *Adv Ther*. 2019;36(12):3503–3518. doi:10.1007/s12325-019-01117-y
5. Furukawa S, Mizushima T, Nakaya R, et al. Darvadstrocel for complex perianal fistulas in Japanese adults with Crohn's Disease: a phase 3 study. *J Crohn's Colitis*. 2023;17(3):369–378. doi:10.1093/ecco-jcc/jjac144
6. Rizzo G, Rubbino F, Elangovan S, et al. Dysfunctional extracellular matrix remodeling supports perianal fistulizing crohn's disease by a mechanoregulated activation of the epithelial-to-mesenchymal transition. *Cell Mol Gastroenterol Hepatol*. 2023;15(3):741–764. doi:10.1016/j.jcmgh.2022.12.006
7. Iqbal N, Alrubaiy L, Hart A, et al. The development of a cryptoglandular anal fistula quality of life scale (AF-QoL). *Colorectal Dis*. 2024;26(4):716–725. doi:10.1111/codi.16917
8. Litta F, Papait A, Lucchetti D, et al. The pathogenesis of cryptoglandular anal fistula: new insight into the immunological profile. *Colorectal Dis*. 2022;24(12):1567–1575. doi:10.1111/codi.16290
9. Cao S, Colonna M, Deepak P. Pathogenesis of perianal fistulising Crohn's disease: current knowledge, gaps in understanding, and future research directions. *J Crohn's Colitis*. 2023;17(6):1010–1022. doi:10.1093/ecco-jcc/jjad008
10. Roda G, Chien Ng S, Kotze PG, et al. Crohn's disease. *Nat Rev Dis Primers*. 2020;6(1):22. doi:10.1038/s41572-020-0156-2
11. Petagna L, Antonelli A, Ganini C, et al. Pathophysiology of Crohn's disease inflammation and recurrence. *Biol Direct*. 2020;15(1):23. doi:10.1186/s13062-020-00280-5
12. McCurdy JD, Hartley I, Behrenbruch C, et al. Management of perianal fistulizing Crohn's disease according to principles of wound repair. *Aliment Pharmacol Ther*. 2025;61(4):600–613. doi:10.1111/apt.18466
13. Becker MAJ, Stevens TW, Voogd FAE, et al. Clinical relevance of calprotectin in patients with perianal fistulas in Crohn's disease and cryptoglandular fistulas. *United Eur Gastroenterol J*. 2024;16.
14. Parian AM, Obi M, Fleshner P, et al. Management of Perianal Crohn's Disease. *Am J Gastroenterol*. 2023;118(8):1323–1331. doi:10.14309/ajg.0000000000002326
15. Li J, Zhao X, Su W, et al. Magnetically guided capsule endoscopy and magnetic resonance enterography in children with Crohn's disease: manifestations and the value of assessing disease activity. *Front Pharmacol*. 2022;13:894808. doi:10.3389/fphar.2022.894808
16. Zhang H, Li W, Chen T, et al. Development and validation of the MRI-based deep learning classifier for distinguishing perianal fistulizing Crohn's disease from cryptoglandular fistula: a multicenter cohort study. *EClinicalMedicine*. 2024;78:102940. doi:10.1016/j.eclinm.2024.102940
17. Asaf S, Maqsood F, Jalil J, et al. Lipocalin 2-not only a biomarker: a study of current literature and systematic findings of ongoing clinical trials. *Immunol Res*. 2023;71(3):287–313. doi:10.1007/s12026-022-09352-2
18. Petrova I, Alexandrov A, Vladimirov G, et al. NGAL as biomarker of clinical and subclinical damage of kidney function after coronary angiography. *Diagnostics*. 2023;13(6):1180. doi:10.3390/diagnostics13061180
19. Bakke I, Walaas GA, Bruland T, et al. Mucosal and faecal neutrophil gelatinase-associated lipocalin as potential biomarkers for collagenous colitis. *J Gastroenterol*. 2021;56(10):914–927. doi:10.1007/s00535-021-01814-y
20. Thorsvik S, van Beelen Granlund A, Svendsen TD, et al. Ulcer-associated cell lineage expresses genes involved in regeneration and is hallmarked by high neutrophil gelatinase-associated lipocalin (NGAL) levels. *J Pathol*. 2019;248(3):316–325. doi:10.1002/path.5258
21. Oikonomou KA, Kapsoritakis AN, Theodoridou C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) in inflammatory bowel disease: association with pathophysiology of inflammation, established markers, and disease activity. *J Gastroenterol*. 2012;47(5):519–530. doi:10.1007/s00535-011-0516-5
22. Thorsvik S, Damås JK, Granlund A, et al. Fecal neutrophil gelatinase-associated lipocalin as a biomarker for inflammatory bowel disease. *J Gastroenterol Hepatol*. 2017;32(1):128–135. doi:10.1111/jgh.13598
23. Koon Siw KC, Engel J, Visva S, et al. Strategies to distinguish perianal fistulas related to Crohn's disease from cryptoglandular disease: systematic review with meta-analysis. *Inflamm Bowel Dis*. 2022;28(9):1363–1374. doi:10.1093/ibd/izab286
24. Geldof J, Iqbal N, LeBlanc JF, et al. Classifying perianal fistulising Crohn's disease: an expert consensus to guide decision-making in daily practice and clinical trials. *Lancet Gastroenterol Hepatol*. 2022;7(6):576–584. doi:10.1016/S2468-1253(22)00007-3
25. Reza L, Gottgens K, Kleijnen J, et al. European society of coloproctology: guidelines for diagnosis and treatment of cryptoglandular anal fistula. *Colorectal Dis*. 2024;26(1):145–196. doi:10.1111/codi.16741
26. Danese S, Panaccione R, Feagan BG, et al. Efficacy and safety of 48 weeks of guselkumab for patients with Crohn's disease: maintenance results from the Phase 2, randomised, double-blind GALAXI-1 trial. *Lancet Gastroenterol Hepatol*. 2024;9(2):133–146. doi:10.1016/S2468-1253(23)00318-7
27. Vuyyuru SK, Solitano V, Singh S, et al. Scoring indices for perianal fistulizing Crohn's disease: a systematic review. *J Crohn's Colitis*. 2023;21:jjad214.
28. Mcgregor CGC, Tandon R, Simmons A. Pathogenesis of fistulating Crohn's disease: a review. *Cell Mol Gastroenterol Hepatol*. 2023;15(1):1–11. doi:10.1016/j.jcmgh.2022.09.011
29. Zhou Z, Ouboter LF, Peeters KCM, et al. Crohn's disease-associated and cryptoglandular fistulas: differences and similarities. *J Clin Med*. 2023;12(2):466. doi:10.3390/jcm12020466
30. Lee KY, Lee JY, Han EC, et al. coring out fistulectomy for perianal cryptoglandular fistula: a retrospective cohort study on 20 years of experience as a single center. *Ann Surg Treat Res*. 2022;102(3):167–175. doi:10.4174/astr.2022.102.3.167
31. Chudy-Onwugaje KO, Christian KE, Farraye FA, et al. A state-of-the-art review of new and emerging therapies for the treatment of IBD. *Inflamm Bowel Dis*. 2019;25(5):820–830. doi:10.1093/ibd/izy327
32. Ouboter LF, Lindelauf C, Jiang Q, et al. Activated HLA-DR+CD38+ effector Th1/17 Cells distinguish Crohn's disease-associated perianal fistulas from cryptoglandular fistulas. *Inflamm Bowel Dis*. 2024;30(11):2146–2161. doi:10.1093/ibd/izae103
33. Sasidharan S, Sasson AN, Shannon KM, et al. Fecal calprotectin is a predictor of need for rescue therapy in hospitalized severe colitis. *Inflamm Bowel Dis*. 2022;28(12):1833–1837. doi:10.1093/ibd/izac011
34. Carnazzo V, Redi S, Basile V, et al. Calprotectin: two sides of the same coin. *Rheumatology (Oxford)*. 2024;63(1):26–33. doi:10.1093/rheumatology/kead405

35. Singh S, Ananthakrishnan AN, Nguyen NH, et al. AGA clinical practice guideline on the role of biomarkers for the management of ulcerative colitis. *Gastroenterology*. 2023;164(3):344–372. doi:10.1053/j.gastro.2022.12.007
36. Murray J, Kok KB, Ayling RM. Fecal calprotectin in gastrointestinal disease. *Clin Chem*. 2023;69(7):699–710. doi:10.1093/clinchem/hvad051
37. Stevens TW, D'Haens GR, Duijvestein M, et al. Diagnostic accuracy of fecal calprotectin in patients with active perianal fistulas. *United Eur Gastroenterol J*. 2019;7(4):496–506. doi:10.1177/2050640619834464
38. Thibault MP, Tremblay É, Horth C, et al. Lipocalin-2 and calprotectin as stool biomarkers for predicting necrotizing enterocolitis in premature neonates. *Pediatr Res*. 2022;91(1):129–136. doi:10.1038/s41390-021-01680-7
39. Foell D, Wittkowski H, Roth J. Monitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage. *Gut*. 2009;58(6):859–868. doi:10.1136/gut.2008.170019
40. Jiang Y, Wang W, Zhang Z, et al. Serum amyloid a, C-reactive protein, and procalcitonin levels in children with Mycoplasma pneumoniae infection. *J Clin Lab Anal*. 2022;36(3):e24265. doi:10.1002/jcla.24265
41. Li J, Xu M, Qian W, et al. Clinical value of fecal calprotectin for evaluating disease activity in patients with Crohn's disease. *Front Physiol*. 2023;14:1186665. doi:10.3389/fphys.2023.1186665
42. Bonnard B, El Moghrabi S, Ueda K, et al. NGAL is a novel target in hypertension by modulating the NCC-mediated renal na balance. *Hypertension*. 2023;80(9):1860–1870. doi:10.1161/HYPERTENSIONAHA.123.21156
43. Korkmaz H, Asıl M, Temel T, et al. Evaluation of serum neutrophil gelatinase-associated lipocalin (NGAL), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) levels, and their relations with disease type and activity in inflammatory bowel diseases. *Turk J Med Sci*. 2021;51(5):2403–2412. doi:10.3906/sag-2103-220
44. Zollner A, Schmiderer A, Reider SJ, et al. Fecal biomarkers in inflammatory bowel diseases: calprotectin versus lipocalin-2-a comparative study. *J Crohn's Colitis*. 2021;15(1):43–54. doi:10.1093/ecco-jcc/jjaa124
45. Zhan J, Chen J, Deng L, et al. Exploring the ferroptosis-related gene lipocalin 2 as a potential biomarker for sepsis-induced acute respiratory distress syndrome based on machine learning. *Biochim Biophys Acta Mol Basis Dis*. 2024;870(4):167101. doi:10.1016/j.bbadis.2024.167101
46. Luo LX, Deng LY, Chen YT, et al. Identification of lipocalin 2 as a ferroptosis-related key gene associated with hypoxic-ischemic brain damage via STAT3/NF-κB signaling pathway. *Antioxidants (Basel)*. 2023;12(1):186. doi:10.3390/antiox12010186
47. Marakala V. Neutrophil gelatinase-associated lipocalin (NGAL) in kidney injury-A systematic review. *Clin Chim Acta*. 2022;536:135–141. doi:10.1016/j.cca.2022.08.029
48. Romejko K, Markowska M, Niemczyk S. The review of current knowledge on Neutrophil Gelatinase-Associated Lipocalin (NGAL). *Int J mol Sci*. 2023;24(13):10470. doi:10.3390/ijms241310470
49. Bjornstad EC, Muronya W, Kamija M, et al. Validity of Urine NGALds dipstick for acute kidney injury in a Malawian trauma cohort. *Kidney Int Rep*. 2020;5(10):1791–1798. doi:10.1016/j.ekir.2020.07.019
50. Schreiber A, Rousselle A, Klocke J, et al. Neutrophil gelatinase-associated lipocalin protects from ANCA-induced GN by inhibiting TH17 immunity. *J Am Soc Nephrol*. 2020;31(7):1569–1584. doi:10.1681/ASN.2019090879
51. Toyonaga T, Matsuura M, Mori K, et al. Lipocalin 2 prevents intestinal inflammation by enhancing phagocytic bacterial clearance in macrophages. *Sci Rep*. 2016;6(1):35014. doi:10.1038/srep35014
52. Kim SL, SHin MW, Kim SW. Lipocalin 2 activates the NLRP3 inflammasome via LPS-induced NF-κB signaling and plays a role as a pro-inflammatory regulator in murine macrophages. *Mol Med Rep*. 2022;26(6):358. doi:10.3892/mmr.2022.12875