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Diagnostic accuracy of faecal calprotectin in patients with active perianal fistulas

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Abstract

Background: Faecal calprotectin (FC) is a marker of mucosal inflammation.

Objective: The aim of this study was to determine the diagnostic accuracy of FC to (a) differentiate between perianal fistulizing Crohn's disease (pCD) and cryptoglandular perianal fistulas; and (b) detect mucosal inflammation in pCD.

Methods: Patients with active perianal fistulas who had FC measured and a complete ileocolonoscopy within 10 weeks were retrospectively included.

Results: Fifty-six patients were included (pCD, $n = 37$) of whom 19 pCD patients exhibited ulcers. FC was significantly higher in pCD compared to cryptoglandular fistulas (μg/g) (708.0 (207.0–1705.0) vs 32.0 (23.0–77.0), $p < 0.001$). Area-under-the-curve (AUC) value for FC receiver operating characteristic (ROC) statistics was 0.900. Optimal FC cut-off was ≥ 150 μg/g. To differentiate pCD from cryptoglandular fistulas in the absence of luminal inflammation, optimal cut-off remained ≥ 150 μg/g (AUC = 0.857, sensitivity = 0.81, specificity = 0.89, positive predictive value (PPV) = 93.8% and negative predictive value (NPV) = 70.8%). In pCD, FC was significantly increased in the presence of ulcers (1672.0 vs 238.0, $p = 0.004$). Optimal cut-off was ≥ 250 μg/g (AUC = 0.776; sensitivity = 0.89, specificity = 0.56, PPV - 68.0% and NPV = 83.0%).

Conclusion: FC discriminates pCD from cryptoglandular fistulas, even in the absence of intestinal ulcers. In active pCD, an elevated FC does not accurately predict the presence of ulcers and should be interpreted with caution.

Keywords

Faecal calprotectin, biomarker, Crohn's disease, perianal fistula, diagnostic accuracy

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Key summary

1. Summarize the established knowledge on this subject
   - Cryptoglandular perianal fistulas and Crohn's disease perianal fistulas represent separate entities.
   - Differentiating Crohn's and cryptoglandular fistulas on clinical grounds can be difficult, and in a considerable number of patients endoscopy is required to rule out Crohn's disease.
   - In Crohn's disease, faecal calprotectin is a non-invasive surrogate marker of endoscopic inflammation.
2. What are the significant and/or new findings of this study?
   - Faecal calprotectin can discriminate between cryptoglandular and Crohn's perianal fistulas, even in the absence of endoscopic inflammation.
   - In Crohn's disease patients with an actively draining perianal fistula, specificity of faecal calprotectin to predict intestinal ulcers is low and faecal calprotectin values should be interpreted with caution.

Introduction

Perianal fistulas are tracts that connect the intestinal lumen, usually the anal canal or rectum, with the perianal skin.[1] Cryptoglandular (CG) perianal fistulas occur in patients without Crohn's disease (CD) and are caused by an infected perianal crypt gland. The incidence

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of CG perianal fistulas in Europe is reported to be around 2 per 10,000 per year.[2] CD is a chronic inflammatory condition affecting the gastrointestinal (GI) tract. The development of a perianal fistula is a common complication occurring in up to one-third of CD patients, depending on luminal disease location.[3–6] In CD, perianal fistulas develop due to transmural inflammation rather than gland infection.[7,8] Perianal CD constitutes a separate entity that warrants a comprehensive management strategy, often with combined medico-surgical treatment modalities.[9] Perianal fistulizing CD (pCD) is most frequently accompanied by typical GI symptoms such as diarrhoea and abdominal discomfort. However, in rare cases, perianal fistulas can also precede other clinical manifestations of CD.[6,10,11] Conversely, patients with CG perianal fistulas may exhibit (functional) GI complaints suspicious of CD. Differentiating pCD and CG fistulas on clinical grounds can therefore be difficult, and in a considerable number of patients endoscopy is required to rule out CD. Endoscopy is still the gold standard for diagnosing CD. However, given the burden of endoscopy, it would be useful to have a biomarker to help select patients that are more likely to have CD and therefore require endoscopy.

Calprotectin consists of the two damage-associated molecular pattern (DAMP) proteins S100A8/S100A9.[12] It is an abundant calcium-binding cytosolic protein complex and its concentration in stool reflects neutrophil trafficking through the inflamed bowel wall.[13,14] In CD, the diagnostic accuracy of faecal calprotectin (FC) to detect mucosal inflammation is high with an area under the curve (AUC) value varying between 0.79 and 0.94.[15–20] However, no data are available on the diagnostic accuracy of FC for mucosal inflammation in pCD patients with an active perianal fistula. Active fistula tracts drain acute (neutrophils) and chronic (lymphocytes) inflammatory cells.[21]

We hypothesized that due to distinct underlying aetiopathogenesis, FC could differentiate between pCD and CG perianal fistulas. Furthermore, we hypothesized that in pCD patients, due to the loss of mucus and pus into the stool, FC may not be an accurate marker of mucosal inflammation. Therefore, our aims were to determine the diagnostic accuracy of FC to (a) differentiate between pCD and CG perianal fistulas, and (b) detect mucosal inflammation in pCD patients with active fistulas.

Materials and methods

Study design and patient population

This study was a retrospective, observational, single-centre study, conducted at the Amsterdam University Amsterdam University Medical Centers (UMC), location AMC. Adult patients with active perianal fistulas between January 2009 and February 2018 were identified by an institutional database search using the diagnostic code for perianal fistula. The study is reported according to the STARD checklist for the reporting of studies of diagnostic accuracy.[22]

Inclusion and exclusion criteria. CD patients meeting all of the following criteria were included: (a) patients aged ≥ 17 years; (b) established CD, based on clinical evaluation and a combination of endoscopic, histological, radiological and/or biochemical tests;[23] (c) at least one actively draining perianal fistula, and (d) FC measurement and a complete ileocolonoscopy within a time window of 10 weeks. pCD patients meeting any of the following criteria were excluded: (a) signs of a perianal abscess upon physical examination; (b) known upper GI CD (L4) or small bowel CD proximal to the terminal ileum, and (c) infectious gastroenteritis. For CG perianal fistulas, inclusion criteria were: (a) patients aged ≥ 17 years old; (b) at least one actively draining perianal fistula; (c) available documentation of an active perianal fistula and an FC measurement within 10 weeks' time, and (d) CD previously ruled out by endoscopic and/or magnetic resonance imaging (MRI) examination of the GI tract. Exclusion criteria were: (a) hidradenitis suppurativa, (b) pilonidal disease, (c) a perianal abscess and (d) infectious gastroenteritis.

Definitions. With regard to the differentiation between pCD and CG perianal fistulas, the reference standard was clinical presentation (including GI symptoms, physical examination, family history and extraintestinal manifestations), and either an ileocolonoscopy and/or MRI. For the detection of intestinal ulcers in pCD, the reference standard was a complete ileocolonoscopy. Active fistulas were defined by the documentation of spontaneous drainage or drainage upon gentle finger compression, as reported by the patient or physician.[7] Active mucosal inflammation was defined by the presence of ulcers. An ulcer was defined as an ulcerative lesion ≥ 0.5 cm in diameter.[24] It was assessed by retrospective review of endoscopy images and reports by an experienced gastroenterologist (KG), who was not aware of related FC values. Scoring was limited to the presence or absence of ulcers as more elaborate activity grading was considered irrelevant for this study. For patients with CG perianal fistulas without an endoscopy, the absence of ulcers was assumed. If patients met pre-defined criteria at multiple time points, data where time points were closest to each other were chosen.

FC: the 'index test'. FC was measured by a quantitative enzyme-linked immunosorbent assay (ELISA)

(Bühlmann Calprotectin ELISA Kit, Bühlmann Laboratories AG, Schönenbuch, Switzerland). The upper detection limit of the test was 1800 µg/g. From January 2017, the Amsterdam UMC changed to a different quantitative ELISA (EliA, Calprotectin 2 test, Phadia AB, Freiburg, Germany). The upper detection limit of this test was 6000 µg/g. The upper limit of all FC values was set to 1800 µg/g for uniformity. For both assays, FC was measured according to the manufacturer's instructions and samples were processed within 3 days after collection.

Statistical analysis

Categorical variables were described through absolute (*n*) and relative (%) frequencies. Continuous numerical variables were described as mean and standard deviation, or median and interquartile range (IQR), depending on distribution. Normality of the data was explored with histograms. Distribution of numerical data between independent groups was assessed with the Mann–Whitney *U*-test or independent Student's *t*-test depending on the normality of the data. Distribution of categorical data between independent groups was assessed with a Chi-square test or Fisher's exact test as appropriate. Test characteristics are presented as sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), and overall accuracy. The overall accuracy was calculated by addition of the true-positive and true-negative test results divided by all tests. Diagnostic accuracy was assessed by plotting a receiver operating characteristic (ROC) curve and computing the AUC. Optimal FC cut-off was defined by the value that achieved the highest summation of sensitivity and specificity. Uncertainty was quantified by the 95% confidence interval (CI). An AUC of 1 represented a perfect test while an AUC of 0.5 represented no diagnostic value. Values 0.8–0.9, 0.7–0.8 and 0.6–0.7 represented a good, fair and poor diagnostic test, respectively. As a level of significance, an alpha of 0.05 was used. All data were analyzed by IBM Statistical Package for the Social Sciences (SPSS) version 24. Data were arranged and visualized in figures using Graphpad Prism version 7.

RESULTS

Patient characteristics

The inclusion flow diagram is presented in Figure 1. Using perianal fistula as a search term, 568 patient files were retrieved from the electronic database search. After removing duplicates, paediatric patients and patients that opted out, 356 patients were screened to meet all inclusion criteria. Finally, 56 (pCD, $n = 37$; CG perianal fistula, $n = 19$) patients met pre-defined

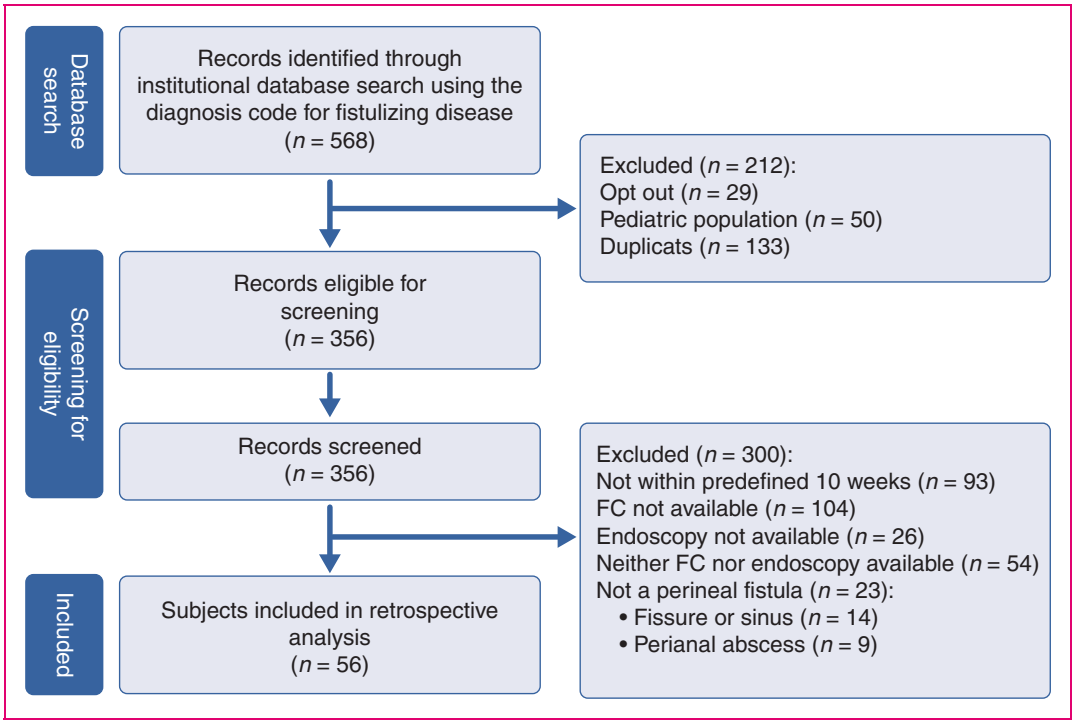

Figure 1. Flow diagram of patient selection.

criteria and were included in the analyses. Twenty-five were male (44.6%) with a mean age of 38.7 (range) (17–65) years. Mucosal inflammation was present in 19 (51.4%) of the pCD patients. Demographic and clinical characteristics are presented in Table 1.

Diagnostic accuracy of FC in differentiation between pCD and CG perianal fistulas

FC was significantly higher in patients with active CD perianal fistulas than in those with active CG perianal fistulas (median (IQR)) (708.0 (207.0–1705.0) vs 32.0 (23.0–77.0) μg/g, $p < 0.001$) (Figure 2(a)). The accuracy of FC to differentiate between the underlying cause of the active perianal fistula (pCD vs CG perianal fistula) was assessed by plotting a ROC curve. AUC value for FC ROC statistics was 0.900, 95% CI (0.818–0.982) (Figure 2(a)). The highest summation of sensitivity and specificity was achieved for an FC cut-off value of ≥ 150 μg/g (sensitivity 81%, specificity 89%, PPV 93.8% and NPV of 70.8%). Overall accuracy was 83.9% (Table 2).

Interestingly, in patients without mucosal inflammation, FC was still significantly higher in pCD compared to CG perianal fistulas (238.0 (75.8–795.0) vs 32.0 (23.0–77.0) μg/g, $p <$ 0.001) (Figure 2(b)). AUC for FC ROC statistics was 0.857, 95% CI (0.733–0.980) (Figure 2(b)). The highest summation of sensitivity and specificity was achieved for an FC cut-off value of ≥ 150 μg/g (sensitivity 0.67, specificity 0.90, PPV 85.7% and NPV of 73.9%). Overall accuracy was 78.4% (Table 3).

Diagnostic accuracy of FC in detection of mucosal inflammation in patients with pCD

In pCD, FC was significantly higher when ulcers were present (median (IQR)) (1672.0 (403.0–1800.0) vs 238.0 (75.8–795.0) μg/g, $p = 0.004$) (Figure 2(c)). The AUC value for FC ROC statistics was 0.776, 95% CI (0.622–0.931) (Figure 2(c)). The highest summation of sensitivity and specificity was achieved for an FC cut-off value of ≥ 250 μg/g (sensitivity 0.89, specificity 0.56, PPV 68.0% and NPV of 83.3%). Overall accuracy was 73.0% (Table 4). Varying cut-offs between 150–500 μg/g, specificity and concordant PPV remained low. Of the pCD patients with an FC above the determined optimal cut-off value of 250 μg/g ($n = 25$), 32% was a false-positive result and, in fact, had endoscopically quiescent disease.

Association between FC and CD location

Within the pCD population with luminal ulcers there appeared to be two groups, one with very high FC values (> 1600 μg/g) and one with considerably lower FC values (< 500 μg/g). Within the latter group, two-thirds of the patients had isolated ileal disease. Conversely, in the group with high FC values, 90% had either colonic or ileocolonic involvement, with only 10% with isolated ileal disease. Separating the group with regard to the presence of colonic inflammation, FC was significantly increased in pCD patients with active inflammation involving the colon (Montreal L2 or L3) compared to patients with isolated ileal disease (L1) (1746.0 (741.8–1800.0) vs 403.0 (140.5–1370.5), $p = 0.046$).

Discussion

In the present study, we have demonstrated that FC (a non-invasive surrogate marker of inflammation) can discriminate between CG and Crohn's perianal fistulas. Furthermore, this is the first study to report that the specificity of FC for mucosal inflammation is low in CD patients with an actively draining perianal fistula.

In this retrospective, observational, single-centre study, pCD was associated with elevated FC concentrations regardless of luminal Crohn's activity. Conversely, the presence of an actively draining CG perianal fistula was not associated with an increase in FC values. Nevertheless, three patients with an active CG perianal fistula had increased FC values of > 100 μg/g (132 μg/g, 203 μg/g and 365 μg/g). The two highest values were observed in patients using acetylsalicylic acid after myocardial infarction, and the patient with an FC of 365 μg/g also used rosuvastatin, a drug known to increase FC.[25] In these patients, CD was ruled out previously either by endoscopy or imaging in accordance with the study inclusion criteria. However, we cannot completely exclude the possibility that luminal CD had developed since the endoscopy was performed and that the perianal fistulas actually preceded other clinical manifestations of CD.[6,10,11] Although follow-up of these CG fistula patients with elevated FC values is currently limited to 12–24 months, the clinical suspicion of CD remained low. Differentiating Crohn's and CG fistulas on clinical grounds can be difficult, where FC appears to be a valuable additional tool.

Several studies have previously reported a strong correlation between FC and mucosal inflammation in CD.[15–20] In line with several landmark trials (ACCENT1, SONIC and EXTEND), using 'absence of ulcers' as the definition of mucosal healing in CD, an FC cut-off value ≥ 250 μg/g seemed most accurate to predict mucosal inflammation.[17,20,26–28] However, previous data have also demonstrated that the optimal cut-off value varies according to the clinical situation (e.g. differentiating somatic from functional bowel complaints, predicting active mucosal inflammation

Table 1. Clinical and demographic characteristics.

	pCD population ($n = 37$)			CG perianal fistula ($n = 19$)	
Variables	pCD with ulcers ($n = 19$)	pCD without ulcers ($n = 18$)	p value		p^* value
Male, n (%)	10 (52.6)	5 (27.8)	NS	10 (52.6)	NS
Mean age, years (range)	37.1 (17.0–55.0)	37.7 (21.0–62.0)	NS	41.3 (25.0–65.0)	NS
Montreal classification			NS	NA	–
A1 (<17 years old)	6 (31.6)	4 (22.2)			
A2 (17-40 years old)	13 (68.4)	14 (77.8)			
A3 (>40 years old)	–	–			
L1 (ileal disease)	5 (26.3)	5 (27.8)			
L2 (colonic disease)	7 (36.8)	6 (33.3)			
L3 (leocolonic disease)	7 (36.8)	7 (38.9)			
B1 (non-stricturing, non-penetrating)	12 (63.2)	11 (61.1)			
B2 (stricturing)	1 (5.3)	4 (22.2)			
B3 (penetrating)	6 (31.6)	3 (16.7)			
Median CD disease duration, years (range)	10.0 (0.0–33.0)	10.5 (0.0–52.0)	NS	NA	–
Mean time between visit, FC and endoscopy, weeks (range)	4.7 (0.0 - 10.0)	5.4 (1.0–10.0)	NS	3.1 (0.0–10.0)	0.031
Median number of active fistulas, n (range)	1.0 (1.0–5.0)	1.0 (1.0–2.0)	NS	1.0 (1.0–2.0)	NS
Median FC value, μg/g [IQR]	1672.0 [403.0–1800.0]	238.0 [75.8–795.0]	0.004	32.0 [23.0–77.0]	<0.001
Endoscopy					
Present, n (%)	19 (100)	18 (100)	–	7 (36.8)	–
Active disease, n (%)	19 (100)	0 (0)	–	–	–
Crohn's disease active medication			NS	NA	–
Infliximab	4 (21.1)	7 (38.9)			
Adalimumab	4 (21.1)	2 (11.1)			
Thiopurines	4 (21.1)	8 (44.4)			
Methotrexate	2 (10.5)	–			
Corticosteroids (any kind)	4 (21.1)	2 (11.1)			
Vedolizumab	2 (10.5)	–			
5-aminosalicylic acid	–	1 (5.6)			
Other active medication			NS		NS
Anti-microbial treatment	2 (10.5)	2 (11.1)		3 (15.8)	
NSAIDS	1 (5.3)	–		1 (5.3)	

(continued)

Table 1. Continued

Variables	pCD population ($n = 37$)			CG perianal fistula ($n = 19$)	
	pCD with ulcers ($n = 19$)	pCD without ulcers ($n = 18$)	*p* value		*p** value
Other anti-inflammatory drugs	–	–		1 (5.3)	
Other relevant drugs	2 (10.5)	1 (5.6)		4 (21.1)	
Medical history present, n (%)	4 (21.1)	8 (44.4)	NS	10 (52.6)	NS
Cardiovascular disease	1 (5.3)	2 (11.1)		4 (21.1)	
Respiratory disease	1 (5.3)	1 (5.6)		2 (10.5)	
GI disease (not IBD)	1 (5.3)	4 (22.2)		–	
Other autoimmune disease	1 (5.3)	3 (16.7)		3 (15.8)	
Relevant (non-GI) infectious disease	–	2 (11.1)		1 (5.3)	
Other relevant history	2 (10.5)	1 (5.6)		4 (21.1)	
Surgical history (not CD or CG related), n (%)	3 (15.8)	5 (27.8)	NS	2 (10.5)	NS
Surgical history (CD or CG related), n (%)	17 (89.5)	15 (83.3)	NS	19 (100.0)	NS

Testing for demographic and clinical characteristics was performed with the Mann–Whitney *U*-test, independent Student's *t*-test or Chi-square/Fisher's exact test when appropriate.
CD: Crohn's disease; CG: cryptoglandular; FC: faecal calprotectin; GI: gastrointestinal; IBD, inflammatory bowel disease; IQR: interquartile range; *n*: number; *NA*: not applicable; NS: not statistically significant; NSAID: non-steroidal anti-inflammatory drug; *p**: *p* values generated from the comparison between the cryptoglandular group with the complete Crohn's disease group. PAF: perianal fistula; pCD: perianal fistulizing Crohn's disease.

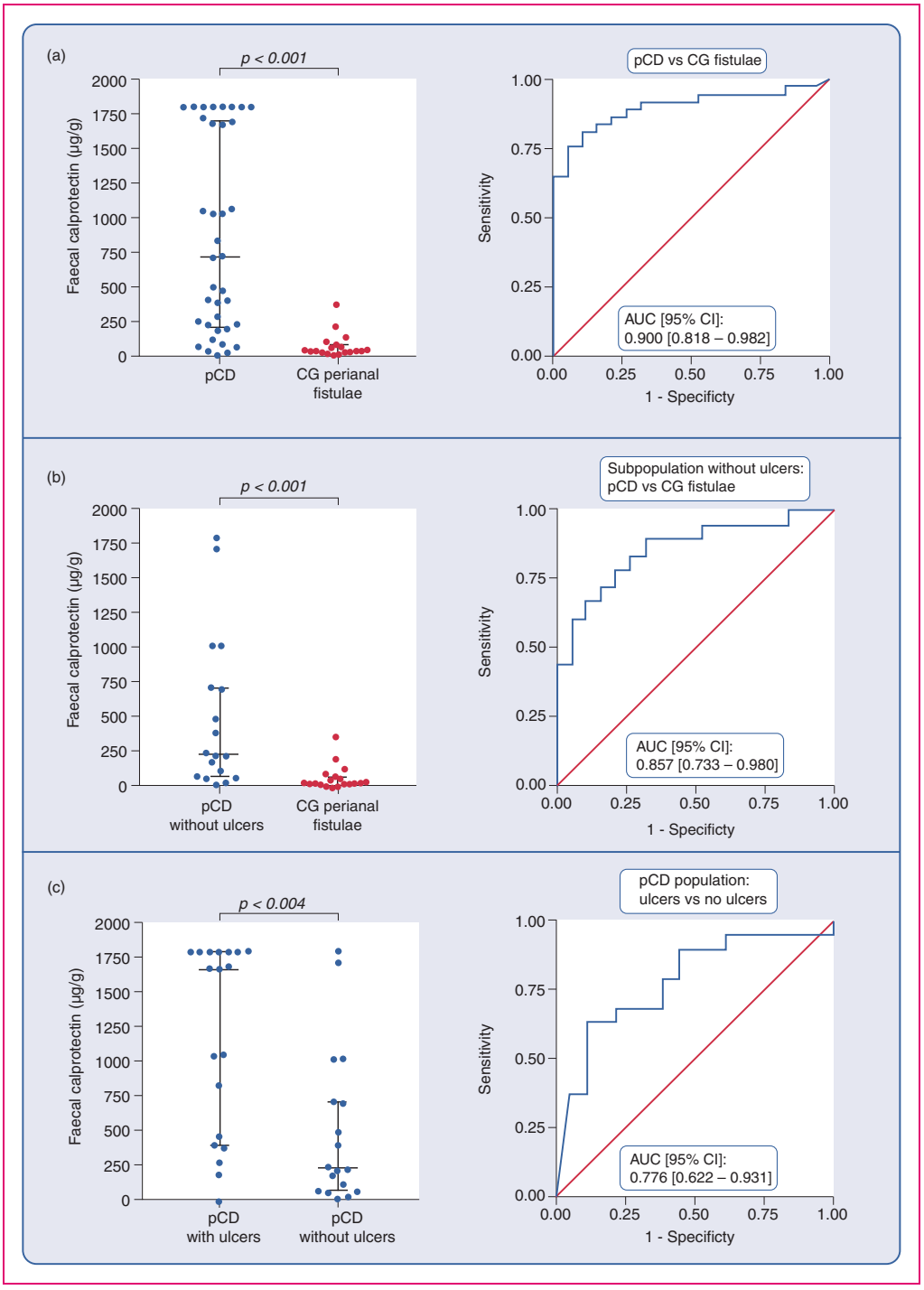

Figure 2. Diagnostic accuracy of faecal calprotectin in patients with an active perianal fistula. (a) Left: individual faecal calprotectin levels in the two groups of active perianal fistulas (perianal fistulizing Crohn's disease, $n = 37$; cryptoglandular fistulas, $n = 19$); right: receiver operating characteristic curve for the ability of faecal calprotectin to differentiate between perianal fistulizing Crohn's disease and cryptoglandular perianal fistulas. (b) Left: individual faecal calprotectin levels in the two groups of active perianal fistulas (perianal fistulizing Crohn's disease, $n = 18$; cryptoglandular fistulas, $n = 19$) within a subpopulation of patients without ulcers; right: receiver operating characteristic curve for the ability of faecal calprotectin to differentiate between perianal fistulizing Crohn's disease and cryptoglandular perianal fistulas within a subpopulation without ulcers. (c) Left: individual faecal calprotectin levels in perianal fistulizing Crohn's disease patients according to the presence of ulcers (perianal fistulizing Crohn's disease with ulcers, $n = 19$; perianal fistulizing Crohn's disease without ulcers, $n = 18$); right: receiver operating characteristic curve for the ability of faecal calprotectin to discriminate between the presence or absence of intestinal ulcers in perianal fistulizing Crohn's disease patients with an active perianal fistula. AUC, area under the curve; CG: Cryptoglandular; CI, confidence interval; pCD: perianal fistulizing Crohn's disease; ROC: receiver operating characteristic.

Table 2. Diagnostic test characteristics of faecal calprotectin to discriminate between cryptoglandular perianal fistulas and perianal fistulizing Crohn's disease.

AUC		95% confidence interval			*p* value	
0.900		(0.818–0.982)			< 0.001	
FC (μg/g)	Sensitivity	Specificity	Summation of scores	PPV (%)	NPV (%)	Accuracy (%)
50	0.92	0.63	1.55	82.9	80.0	82.1
75	0.86	0.74	1.60	86.5	73.7	82.1
100	0.84	0.84	1.68	91.2	72.7	83.9
150	**0.81**	**0.89**	**1.70**	**93.8**	**70.8**	**83.9**
200	0.76	0.89	1.65	93.3	65.4	80.4
250	0.68	0.95	1.63	96.2	60.0	76.8

AUC: area under the curve; FC: faecal calprotectin; NPV: negative predictive value; PPV: positive predictive value.
Bold values represent the optimal faecal calprotectin cut-off values with concurrent test characteristics.

Table 3. Diagnostic test characteristics of faecal calprotectin to discriminate between cryptoglandular perianal fistulas and perianal fistulizing Crohn's disease in the absence of intestinal ulcers.

AUC		95% confidence interval			*p* value	
0.857		(0.733–0.980)			< 0.001	
FC (μg/g)	Sensitivity	Specificity	Summation of scores	PPV (%)	NPV (%)	Accuracy (%)
50	0.89	0.63	1.52	69.6	85.7	75.7
75	0.78	0.74	1.52	73.7	77.8	75.7
100	0.72	0.84	1.56	81.3	76.2	78.4
150	**0.67**	**0.90**	**1.57**	**85.7**	**73.9**	**78.4**
200	0.61	0.89	1.50	84.6	70.8	75.7
250	0.44	0.95	1.39	88.9	64.3	70.3

AUC: area under the curve; FC: faecal calprotectin; NPV: negative predictive value; PPV: positive predictive value.
Bold values represent the optimal faecal calprotectin cut-off values with concurrent test characteristics.

or predicting post-operative recurrence).[12] However, the diagnostic accuracy of FC to detect luminal inflammation has not previously been studied in patients with CD with actively draining perianal fistulas. We hypothesized that an actively draining perianal fistula, via the loss of mucus and pus into the stool, can increase FC independent of intestinal inflammation. Indeed, specificity of FC to predict intestinal ulcers was low, as one-third of patients with Crohn's fistulas had an elevated FC value (>250 mg/g) despite endoscopically quiescent disease. Considering the high variability of FC, high specificity with high positive predictive value should be considered an important property of the test to minimize the number of false-positive tests, rule-in mucosal inflammation and thereby minimize the number of unnecessary ileocolonoscopies. Sensitivity of FC (>250 mg/g; 0.83) to detect mucosal inflammation was not influenced by the presence of an active perianal fistula, as also illustrated by the NPV (83.3%). However, other potential reasons for elevated FC, independent of an actively producing perianal fistula, need to be considered. Firstly, FC could be elevated due to the presence of inflammation without ulcers. Indeed, a few patients had signs of mild inflammation (hyperaemia and mucosal oedema) upon endoscopy; however, neither the magnitude nor the number of patients with major FC elevations could be explained solely by the presence of mild mucosal inflammation. Secondly, as small bowel imaging was not performed in the majority of patients and an ileocolonoscopy is limited to evaluating the terminal ileum, proximal small bowel inflammation cannot be excluded. However, none of these patients were previously diagnosed with proximal small bowel CD. The use of specific drugs (e.g. non-steroidal anti-inflammatory drugs, acetylsalicylic acid, statins

Table 4. Diagnostic test characteristics of faecal calprotectin for the presence of ulcers in perianal fistulizing Crohn's disease with an active fistula.

AUC		95% confidence interval			*p* value	
0.776		(0.622–0.931)			0.004	
FC (μg/g)	Sensitivity	Specificity	Summation of scores	PPV (%)	NPV (%)	Accuracy (%)
150	0.95	0.33	1.27	60.0	85.7	64.9
200	0.89	0.39	1.28	60.7	77.8	64.9
250	**0.89**	**0.56**	**1.45**	**68.0**	**83.3**	**73.0**
300	0.81	0.56	1.39	61.9	76.9	67.7
500	0.68	0.67	1.34	68.4	66.7	67.6

AUC: area under the curve; FC: faecal calprotectin; NPV: negative predictive value; PPV: positive predictive value.
Bold values represent the optimal faecal calprotectin cut-off values with concurrent test characteristics.

and corticosteroids[25]) could also increase FC concentration. No patients in the pCD group without mucosal inflammation used non-steroidal anti-inflammatory drugs, acetysalicylic acid, statins or corticosteroids. One patient with markedly elevated FC was known to be infected with Human Immunodeficiency Virus, for which anti-retroviral therapy had been initiated several years earlier. However, to the best of our knowledge, anti-retroviral therapy is not associated with increased FC concentration. Furthermore, the high inter- and intraindividual variability of FC could also be a potential confounding factor.[29,30] Finally, it has been reported that up to one-third of CD patients in mucosal healing had ongoing histological inflammation.[31,32] Some studies have shown an acceptable correlation between FC and histological disease activity in ulcerative colitis, and one in CD.[33] Unfortunately, histological examination for inflammation was only available in a minority of patients. However, it appears unlikely that ongoing histological inflammation by itself is responsible for such major FC elevations.

Interestingly, in the small subgroup of patients with active luminal CD, we confirmed the association between the impact of disease location on FC concentrations.[18,34,35] Patients with active inflammation involving the colon (either L2 or L3) had significantly higher median FC concentrations than patients with isolated ileal disease.

There are a number of limitations of this study that need to be recognized. Firstly, this was a single-centre retrospective study with the inevitable limitations of its design and patient number. We used strict inclusion and exclusion criteria in order to be able to adequately interpret results. However, several potentially eligible subjects did not meet the 10 week timeframe for FC and ileocolonoscopy, and were thereby excluded. This increases the risk of selection bias and potentially influences the external validity of our results. In the CG population, 12 patients (63.2%) did not undergo an endoscopy at time of the FC measurement. In these patients, the absence of ulcers was assumed. Finally, the association between active fistulizing disease and decreased accuracy of FC is not confirmed as being causal, as FC was not measured in the drainage of the fistula tracts. Hence, to confirm the generalizability of the attained results, these findings should be prospectively validated in larger populations.

In conclusion, FC is an accurate and clinically useful tool to distinguish between active pCD and CG perianal fistulas, even in the absence of intestinal ulcers. In patients with active fistulizing pCD, the diagnostic accuracy of FC to detect mucosal inflammation was limited as one-third of patients had an increased value despite endoscopically quiescent disease. Consequently, in patients with active fistulizing pCD, elevated FC values should be interpreted with caution when the absence or presence of luminal inflammation influences management strategy. Future prospective validation of attained results is required in order to truly elucidate their generalizability.

Acknowledgements

TS and KG conceived the study. TS and CB collected the data. TS, KG, CB, GD and WB analysed and interpreted the data. TS, MD and KG drafted the manuscript. GD, CB and WB critically reviewed the data and first drafts. All authors critically reviewed and approved the final manuscript. TS is guarantor of this article.

Declaration of conflicting interests

TS, MD, CB and WB declare that there is no conflict of interest. KG has served as speaker and/or advisor for Amgen, AbbVie, Boehringer Ingelheim, Ferring, Hospira, MSD, Pfizer, Samsung Bioepis, Sandoz, Takeda and Tigenix; GD has served as an advisor for Abbvie, Ablynx, Amakem, AM Pharma, Avaxia, Biogen, Bristol Meiers

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Informed consent

A waiver for individual patient informed consent was granted by the local Institutional Review Board of the Amsterdam UMC (W17_357#17.417). Via an opt-out consent method, eligible patients were given the opportunity to refuse the retrospective analysis of their electronic medical charts.

Ethics approval

A waiver for formal approval was granted by the local Institutional Review Board of the Amsterdam UMC (W17_357#17.417). The study was conducted in accordance with local ethical and legal requirements, and in line with the principles of the Declaration of Helsinki.

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