

Faecal biomarkers for screening small bowel inflammation in patients with Crohn's disease: a prospective study

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Abstract

Background: The value of faecal biomarkers for screening small bowel inflammation in patients with Crohn's disease (CD) remains to be elucidated. This prospective study was to evaluate the utility of faecal biomarkers for detecting small intestinal inflammation.

Methods: A total of 122 consecutive patients with a diagnosis of CD in the small intestine were screened for eligibility. Computed tomography enterography (CTE) was undertaken to evaluate small bowel inflammation followed by colonoscopy to confirm no large bowel involvement. Seventy eligible patients with inflammation confined to the small intestine were included. Faecal samples were collected for assaying calprotectin, lactoferrin and haemoglobin. For assessing the degree of small bowel inflammation, a semi-quantitative scoring system (CTE0, normal; CTE1, mild; CTE2, moderate; CTE3, severe) was applied.

Results: The median calprotectin, lactoferrin and haemoglobin levels were significantly higher in patients with small bowel inflammation, CTE scores 1–3 ($n = 42$) versus 0 ($n = 28$): calprotectin, 330 versus 40 ng/ml, $p < 0.0001$; lactoferrin, 14 versus 3 ng/ml, $p < 0.0001$; haemoglobin, 29.5 versus 6.5 ng/ml, $p = 0.005$. There was a strong positive relationship between the faecal biomarkers and CTE score: calprotectin, $p < 0.0001$; lactoferrin, $p < 0.0001$; haemoglobin, $p = 0.0004$. A cutoff value of 140 ng/ml for calprotectin had a sensitivity of 69% and a specificity of 82% with an area under the receiver operating characteristic curve (AUC) of 0.82 to detect small bowel inflammation (CTE scores 1–3), while lactoferrin 6 ng/ml had a sensitivity of 69% and a specificity of 79% with an AUC of 0.83, and haemoglobin 9 ng/ml showed a sensitivity of 71% and a specificity of 39% with an AUC of 0.70.

Conclusions: Faecal calprotectin, lactoferrin, and to a lesser degree haemoglobin are relevant biomarkers for screening small bowel inflammation in CD patients without large bowel involvement. Further well-designed large-scale studies in this clinical setting should strengthen our findings.

Keywords: calprotectin, computed tomography enterography, Crohn's disease, faecal biomarkers, haemoglobin, lactoferrin, small bowel inflammation

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Introduction

Endoscopy is currently considered to be the gold standard for directly assessing mucosal inflammation in patients with inflammatory bowel diseases (IBD).^{1,2} Nevertheless, endoscopy is an unloved and frustrating examination for patients because it is time-consuming and invasive, and is associated with morbidity risks. A minimally invasive and

simple screening test should increase patients' compliance and lead to greater clinical outcomes.

A number of faecal biomarkers have been evaluated for their utility to diagnose and monitor IBD activities as alternative tests to endoscopy.^{3–7} Calprotectin and lactoferrin, both neutrophil-derived proteins, are frequently used faecal

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biomarkers in clinical practice settings.³⁻⁷ Faecal immunochemical test (FIT) for the detection of haemoglobin in the stool has been widely used in colorectal cancer screening, but it is currently being adopted for monitoring intestinal inflammation in IBD patients.^{8,9} These biomarkers are simple, convenient and low cost to assay, and can serve as non-invasive tests to assess intestinal inflammation.³⁻⁹

Several studies⁸⁻¹⁵ and a meta-analysis¹⁶ found that faecal calprotectin, lactoferrin and haemoglobin were relevant biomarkers in identifying mucosal inflammation in patients with IBD, which is confined mainly to the large intestine. Therefore, these biomarkers appeared to have greater utility to replace endoscopic activity in ulcerative colitis (UC) than in Crohn's disease (CD) patients.¹⁶ Further, there are published studies that failed to find any significant utility for faecal biomarkers in detecting ileal inflammation in patients with CD.^{11,17} Additionally, several studies suggest that faecal biomarkers are useful for the assessment of mucosal lesions in the neo-terminal ileum after ileocolonic resection for CD.¹⁸⁻²⁰ Therefore, the value of faecal biomarkers for the screening of small bowel inflammation in CD patients remains to be determined. However, it is not an easy task to evaluate the relationship between faecal biomarkers and small bowel inflammation, because an appropriate diagnostic method has not been validated for assessing small bowel CD. Additionally, the outcomes of faecal biomarkers may be affected by simultaneous inflammation in the large bowel. With these in mind, we undertook this prospective study with the aim of determining the value of faecal biomarkers for screening small bowel inflammation in patients with CD without large bowel involvement.

Patients and methods

Study design and ethical considerations

This was a prospective, single-centre study undertaken at the Yokkaichi Hazu Medical Centre, a referral centre treating a large number of patients with IBD in the Mie Prefecture of Japan. Prior to initiating this study, our investigation protocol was reviewed and approved by the Institutional Review Board at our centre (approval number: 28). All included patients agreed to participate in this study after being informed of the study

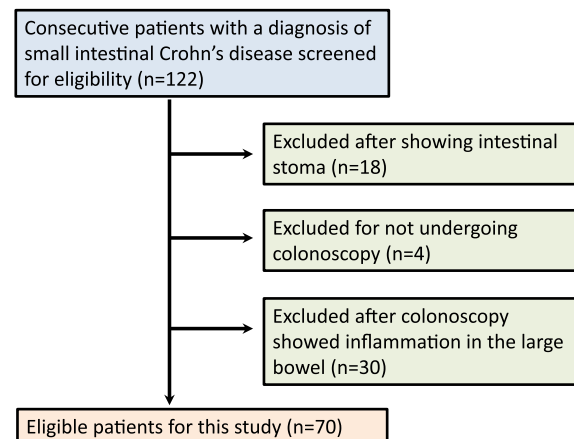


Figure 1. Flow chart summarizing the study design for the selection of eligible patients. In total 122 consecutive patients with a diagnosis of CD in the small bowel were screened. Another 52 patients were eventually excluded; 70 eligible patients were available for inclusion in this study.

purpose and the nature of the procedures involved. Written consent was obtained from participants. Further, all investigations were conducted in accordance with the Good Clinical Practice Guidelines for investigations involving human subjects. Likewise, the study adhered to the Helsinki Declaration at all times.

Selection of patients

As shown in Figure 1, consecutive patients with a diagnosis of CD were thoroughly screened to select patients who precisely met the study inclusion criteria, which included: (1) patient had a diagnosis of CD in the small bowel; (2) patient agreed to take and provide a stool sample for the assay of faecal biomarkers; (3) patient agreed to undergo computed tomography enterography (CTE) followed by colonoscopy at entry. Exclusion criteria were: (1) patients with an intestinal stoma including jejunostomy, ileostomy or colostomy; (2) patients with severe perianal disease; (3) patients with inflammation in the large bowel observed during colonoscopy at entry; (4) patients who were on nonsteroidal anti-inflammatory drugs at entry.

Clinical assessment

Patients were advised to record their symptoms in a diary every day. At entry, general wellbeing, stool frequency, stool consistency and presence or

absence of abdominal discomfort, tenderness, tenesmus, rectal bleeding and mucus in stool were recorded. Clinical disease activity was evaluated by the CD activity index (CDAI) score.²¹ Clinical remission was defined as CDAI < 150, mild activity as $150 \leq \text{CDAI} < 220$, moderate activity as $220 \leq \text{CDAI} < 450$, and severe activity as $450 \leq \text{CDAI}$. At entry, peripheral blood samples were collected for the measurement of white blood cell count (WBC), haemoglobin, platelet count, total protein, albumin, creatinine, urea, sodium, potassium, chloride, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactic dehydrogenase, total bilirubin, cholesterol, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR).

Measurements of faecal biomarkers

Patients were advised to collect a stool sample in the early morning within 5 days, and store at room temperature before their clinic visit. At the clinic, the sample was sent to our laboratory for the assays of calprotectin, lactoferrin and haemoglobin. Faecal calprotectin was measured by using a commercially available NS-Prime automatic analyser (Alfresa Pharma Corp., Osaka, Japan). A 0.01 ml sample plus 0.14 ml reaction buffer were pipetted into a cuvette at 37°C. After about 1 min, 0.05 ml of colloidal gold particles coated with the anti-calprotectin antibody was added and mixed. Reactions between the particles and any calprotectin in the sample resulted in the formation of agglutinates and a concomitant change in the absorbance signal. The change in the absorbance ratio at 660 nm and 546 nm, secondary and primary wavelengths respectively, was measured for about 6 min. The calprotectin concentration in the sample was determined by using a standard curve.²² This assay can provide results in about 10 min. Similar procedures were undertaken for the assays of faecal lactoferrin and haemoglobin. Briefly, lactoferrin was measured by a colloidal gold agglutination reagent (Auto Lf-Plus, Alfresa Pharma Corp., Osaka, Japan) by using a high-throughput discrete clinical chemistry analyser (Hemo Techt NS-Plus C, Alfresa Pharma Corp., Osaka, Japan). Similarly, haemoglobin was measured by a colloidal gold agglutination reagent (i-FOBT Haemoglobin NS-Plus, Alfresa Pharma Corp., Osaka, Japan) by using the aforementioned clinical chemistry analyser. Laboratory investigators were blinded to the clinical data and the results of CTE.

CTE followed by colonoscopy

On the day before CTE and colonoscopy, patients were asked to eat a low-residue diet in the evening, and were given 20 ml of 0.75% sodium picosulfate hydrate (Laxoberon, Teijin Pharma Co. Ltd, Tokyo, Japan) before bedtime. The following morning, patients were asked to drink magnesium citrate solution 1350–1800 ml (Magcorol P, Horii Pharmaceutical Ind. Ltd, Osaka, Japan) over 60 min before the start of CTE. Hyoscine butylbromide 20 mg (Buscopan; Boehringer Ingelheim Japan, Inc., Tokyo, Japan) was administered intramuscularly 5 min before the scan. Iohexol 630 mgI/kg (Iopaque 300, Fuji Pharma Co. Ltd, Tokyo, Japan) was administered as a 30 s intravenous bolus injection. A single-phase CT scan was done in supine position at 50 s after the start of injecting the contrast medium. CTE images were generated at a slice thickness of 2 mm, and the images were reconstructed with an interval of 1 mm. For interpretation of the CTE images, a multi-planar reformatting technique was used to generate coronal, sagittal and bilateral oblique (30° angulation) views from a stack of axial slices.

In this study, colonoscopy was undertaken to support our inclusion criteria, to exclude patients with obvious inflammation in the large bowel (cecum, colon, rectum and perianal region). Following total evacuation of the bowel contents after a CTE, total colonoscopy was undertaken in all patients without intestinal stoma or anorectal strictures. Patients who showed mucosal inflammation in the large bowel were excluded. Likewise, any patient with an aphthous ulcer at the line of ileocolonic anastomosis was excluded.

Two radiologists who were blinded to clinical or laboratory data reviewed the CTE images to evaluate small bowel inflammation suggestive of CD. The number and location of lesions, and the presence or absence of mucosal irregularity, mucosal hyperdensity, stenosis, prestenotic dilatation, target sign, comb sign, abscess and fistulas were recorded. A semi-quantitative scoring system previously described by Minordi and colleagues²³ was applied for determining the overall degree of small bowel inflammation (Table 1).

Statistics

Differences between median values were compared by applying the Mann–Whitney *U* test or

Table 1. Scoring system for the assessment of small bowel inflammation in CTE.

Score	CTE findings
CTE 0	No findings
CTE 1	Minor mucosal irregularities with slight wall thickening and mural contrast enhancement
CTE 2	Mucosal hyperdensity with distinct bowel wall thickening, no stenosis or stenosis without prestenotic dilatation
CTE 3	Major mucosal abnormalities, distinct bowel wall thickening with target sign and extraviseral signs such as perienteric stranding, comb sign, fibrofatty proliferation, stenosis with prestenotic dilatation and/or the presence of complications

CTE, Computed tomography enterography.

the Kruskal–Wallis test if more than two groups were to be compared. Correlations were calculated by using the Spearman's rank correlation test; $p < 0.05$ was considered statistically significant. Additionally, to find an optimal cutoff value for the detection of small bowel inflammation, a receiver operating characteristic (ROC) curve was constructed. An ROC curve was to be a plot of the true-positive rate (sensitivity) against the false-positive rate ($1 - \text{specificity}$) for different cutoff values of a diagnostic test. In general, the closer the curve follows the left-hand border and then the top border of the ROC space, the more accurate is the test value. We defined the most optimal cutoff points by looking at the sensitivity and specificity for different cutoff values. The accuracy of the diagnostic test was determined by the area under the ROC curve (AUC).

Results

The outcomes of screening for eligibility

Between December 2014 and December 2016, 122 consecutive patients with previously diagnosed CD in the small bowel were screened for eligibility to participate in this study (Figure 1). A total of 52 patients were eventually excluded, of whom 30 had large bowel inflammation detected during colonoscopy at entry. Eventually, 70 patients were included in this study; all had CD confined to the small intestine. The baseline

demographic features of the 70 eligible patients are presented in Table 2.

Faecal biomarkers versus CTE

The median levels of faecal calprotectin, lactoferrin and haemoglobin for all patients ($n = 70$) were 110 ng/ml (range, 0–7720 ng/ml), 5.5 ng/ml (range, 0–907 ng/ml) and 12 ng/ml (range, 0–1751 ng/ml), respectively. There was significant correlation between the levels of faecal biomarkers: calprotectin *versus* lactoferrin, $r = 0.819$, $p < 0.0001$; calprotectin *versus* haemoglobin, $r = 0.272$, $p = 0.02$; lactoferrin *versus* haemoglobin, $r = 0.259$, $p = 0.03$. The relationship between clinical disease activity and the levels of faecal biomarkers is presented in Figures 2a–c. There was a significant positive relationship between the levels of faecal calprotectin and lactoferrin, and clinical disease activity.

The results of CTE scanning are summarized in Table 3. The CTE score was 0 in 28 patients (40%), 1 in 15 patients (21%), 2 in 11 patients (16%) and 3 in 16 patients (23%). The relationship between the CTE findings and the levels of faecal biomarkers is presented in Table 4. Target sign and fistula were not included in this analysis because only a few patients ($n = 2$) had positive findings. There was a significant relationship between the levels of faecal biomarkers and almost all of the examined parameters, including the number and locations of lesions, mucosal irregularity and hyperdensity, stenosis, prestenotic dilatation and comb sign.

The median calprotectin, lactoferrin and haemoglobin levels were significantly higher in 42 patients with small bowel inflammation (CTE scores 1–3) than in 28 patients without small bowel inflammation (CTE score 0): calprotectin, 330 *versus* 40 ng/ml, $p < 0.0001$; lactoferrin, 14 *versus* 3 ng/ml, $p < 0.0001$; haemoglobin, 29.5 *versus* 6.5 ng/ml, $p = 0.005$. There was a significant and positive relationship between the faecal biomarkers and the CTE scores (calprotectin, $p < 0.0001$; lactoferrin, $p < 0.0001$; haemoglobin, $p = 0.0004$) (Figures 3a–c).

Faecal biomarkers for the detection of small bowel inflammation

ROC curves were constructed to determine an optimal cutoff value for faecal biomarkers to

detect small bowel inflammation (CTE scores 1–3). A cutoff value of 140 ng/ml for calprotectin had a sensitivity of 69% [95% confidence interval (CI): 55–83%], a specificity of 82% (95% CI:

68–96%), a positive predictive value (PPV) of 85% (95% CI: 73–97%) and a negative predictive value (NPV) of 64% (95% CI: 48–80%) with an AUC of 0.82 (95% CI: 0.70–0.90) to detect small bowel inflammation (Figure 4a). Likewise, a cutoff value of 6 ng/ml for lactoferrin had a sensitivity of 69% (95% CI: 55–83%), a specificity of 79% (95% CI: 63–94%), a PPV of 83% (95% CI: 70–95%) and an NPV of 63% (95% CI: 47–79%) with an AUC of 0.83 (95% CI: 0.72–0.90) (Figure 4b). A cutoff value of 9 ng/ml for faecal haemoglobin had a sensitivity of 71% (95% CI: 58–85%), a specificity of 39% (95% CI: 21–57%), a PPV of 64% (95% CI: 50–78%) and an NPV of 48% (95% CI: 27–68%) with an AUC of 0.70 (95% CI: 0.56–0.81) (Figure 4c). The AUC was higher in calprotectin and lactoferrin than in haemoglobin (calprotectin *versus* haemoglobin, $p = 0.07$; lactoferrin *versus* haemoglobin, $p = 0.04$). There was no significant difference in the AUC between calprotectin and lactoferrin ($p = 0.64$).

Table 2. Baseline characteristics of the 70 patients included in this study.

Median (range) age at entry	38 (15–68) years
Male : female (n)	47 : 23
Median (range) duration of CD before entry	118 (19–353) months
Non-smoker : ex-smoker : smoker (n)	59 : 8 : 3
Previous bowel resection (n)	38 (54%)
Medications at entry (n)	
Mesalazine	63 (90%)
Elemental diet	34 (49%)
Corticosteroids	4 (6%)
Azathioprine	11 (16%)
Biologics (infliximab : adalimumab)	33 : 17
Clinical disease activity at entry (n)	
Remission (CDAI < 150)	35 (50%)
Mild (150 ≤ CDAI < 220)	21 (30%)
Moderate (220 ≤ CDAI < 450)	11 (16%)
Severe (450 ≤ CDAI)	3 (4%)
CD, Crohn's disease; CDAI, Crohn's disease activity index.	

Correlation between the faecal and blood biomarkers

The correlation between the levels of faecal and blood biomarkers is shown in Table 5. There was a significant correlation between WBC count and faecal haemoglobin. A significant negative correlation was found between blood albumin and faecal calprotectin or haemoglobin. The levels of the three faecal biomarkers significantly and positively correlated with CRP. Further, there was a significant positive correlation between ESR and faecal calprotectin or haemoglobin.

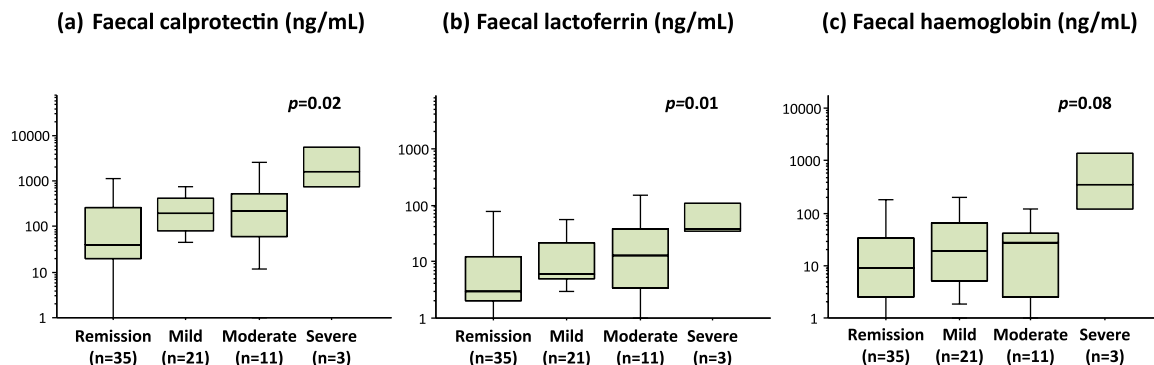


Figure 2. There was a significant positive relationship between the levels of faecal calprotectin ($p = 0.02$) (a) and lactoferrin ($p = 0.01$) (b), and clinical disease activity. Boxes indicate interquartile ranges, with horizontal lines indicating medians and whiskers indicating the upper and lower limits.

Table 3. Summary of the CTE results.

Number of lesions suggestive of CD (<i>n</i>)	
0	28 (40%)
1, 2	28 (40%)
≥3	14 (20%)
Location of lesions suggestive of CD (<i>n</i>)	
No lesions	28 (40%)
Jejunum alone	0
Ileum alone	28 (40%)
Jejunum and ileum	14 (20%)
Mucosal irregularity (<i>n</i>)	42 (60%)
Mucosal hyperdensity (<i>n</i>)	30 (43%)
Stenosis (<i>n</i>)	13 (19%)
Prestenotic dilatation (<i>n</i>)	8 (11%)
Target sign (<i>n</i>)	2 (3%)
Comb sign (<i>n</i>)	13 (19%)
Abscess (<i>n</i>)	0
Fistula (<i>n</i>)	2 (3%)
CTE score (<i>n</i>)	
CTE 0	28 (40%)
CTE 1	15 (21%)
CTE 2	11 (16%)
CTE 3	16 (23%)
CD, Crohn's disease; CTE, computed tomography enterography.	

Discussion

This study was prospectively designed and diligently conducted with a relatively large number of patients. Strict adherence to our pre-set criteria meant that only 70 patients were found to be eligible from a population of 122 patients with CD. To see the relevance of these faecal biomarkers to small intestinal inflammation, we had to be sure that patients did not have mucosal inflammation in the large bowel. Additionally, the investigators were blinded to the patients' other clinical or laboratory data. We found that the levels of faecal calprotectin, lactoferrin and haemoglobin were elevated in CD patients with small bowel inflammation. A significant and positive correlation between the faecal biomarkers and the CTE scores was found. Likewise, there was a significant association between the faecal biomarkers

and features of the CTE images including mucosal irregularity and hyperdensity, stenosis, prestenotic dilatation and comb sign. Calprotectin and lactoferrin showed a higher accuracy than haemoglobin for the detection of small bowel inflammation. These observations indicate that faecal calprotectin and lactoferrin are favourable biomarkers of small bowel inflammation in patients with CD, while haemoglobin is a weak biomarker in this clinical setting.

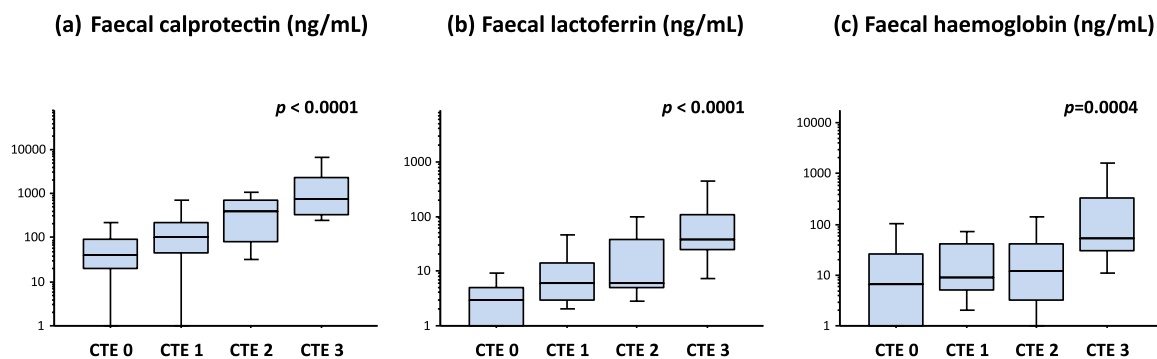
In recent years there has been a growing interest in faecal biomarkers of small bowel CD activity. The strength of our findings might be that we diligently screened the subjects and excluded all patients with any obvious inflammation in the large bowel, which is known to affect the levels of faecal biomarkers.^{10–16} At entry, total colonoscopy was undertaken in all patients to detect large bowel inflammation. In the previous studies,^{24–32} the methods of investigating large bowel inflammation and the timing of investigation were not recorded in detail. It is therefore unclear whether there was no large bowel inflammation at the time of collecting a stool sample. Another strength of our study is the use of three biomarkers – calprotectin, lactoferrin and haemoglobin. Most previous studies investigated the value of faecal calprotectin alone.^{24–27,30–33} To our knowledge, this is the first study to assess the value of the three major faecal biomarkers simultaneously using the same stool sample. Further, previous studies^{24–29,31} included patients with suspected (unestablished) CD. In contrast, we included only patients with established CD confined to the small bowel.

Currently, an appropriate diagnostic technology or a validated scoring system for assessing small bowel inflammation has not been established. Capsule endoscopy has been commonly used in several studies.^{24–31} Magnetic resonance enterography (MRE)³² and balloon-assisted enteroscopy³³ are alternative techniques. We used CTE as a cross-sectional imaging technique for the assessment of small bowel inflammation because it is the most common and widely available diagnostic technology in our institute. CTE and MRE showed comparable diagnostic accuracy to detect small bowel CD.^{34–37} However, one group reported that CTE provided better image quality and inter-observer agreement than MRE.³⁵ CTE appears to be more cost-effective for the long-term assessment and follow-up of patients, particularly

Table 4. The relationship between the CTE findings and the levels of faecal biomarkers.

	Faecal calprotectin (ng/ml)	Faecal lactoferrin (ng/ml)	Faecal haemoglobin (ng/ml)
Number of lesions	$p < 0.0001$	$p < 0.0001$	$p = 0.02$
0 ($n = 28$)	40 (20–100)	3 (1–5)	6.5 (1–28)
1, 2 ($n = 28$)	160 (60–640)	9.5 (3–46)	18 (7–67)
≥ 3 ($n = 14$)	700 (74–1350)	35.5 (8–64)	38.5 (5–215)
Location of lesions	$p < 0.0001$	$p < 0.0001$	$p = 0.02$
No lesions ($n = 28$)	40 (20–100)	3 (1–5)	6.5 (1–28)
Ileum alone ($n = 28$)	310 (80–820)	14 (5–40)	24.5 (7–144)
Jejunum and ileum ($n = 14$)	330 (30–1160)	21.5 (3–78)	35 (4–70)
Mucosal irregularity	$p = 0.0004$	$p < 0.0001$	$p = 0.01$
Presence ($n = 42$)	250 (60–740)	12 (4–39)	24 (6–72)
Absence ($n = 28$)	40 (10–160)	3 (1–6)	6.5 (1–28)
Mucosal hyperdensity	$p < 0.0001$	$p < 0.0001$	$p = 0.003$
Presence ($n = 30$)	520 (190–1350)	34.5 (6–80)	37 (9–201)
Absence ($n = 40$)	60 (20–140)	3 (2–6)	7 (2–28)
Stenosis	$p = 0.002$	$p = 0.004$	$p = 0.06$
Presence ($n = 13$)	500 (250–1160)	34 (9–39)	38 (9–227)
Absence ($n = 57$)	80 (20–280)	5 (2–13)	10 (4–43)
Prestenotic dilatation	$p = 0.003$	$p = 0.01$	$p = 0.04$
Presence ($n = 8$)	580 (260–1500)	33.5 (11–38)	39.5 (19–213)
Absence ($n = 62$)	80 (20–360)	5 (2–15)	10 (4–54)
Comb sign	$p = 0.001$	$p = 0.0003$	$p = 0.002$
Presence ($n = 13$)	460 (250–2270)	38 (15–105)	45 (16–259)
Absence ($n = 57$)	80 (30–250)	5 (2–11)	9 (4–38)

Data represent the median (interquartile range). Bold values represent statistically significant differences.

**Figure 3.** There was a significant and positive association of the faecal biomarkers with the CTE scores: (a) calprotectin, $p < 0.0001$; (b) lactoferrin, $p < 0.0001$; (c) haemoglobin, $p = 0.0004$. Boxes indicate interquartile ranges, with horizontal lines indicating medians and whiskers indicating the upper and lower limits. CTE, computed tomography enterography.

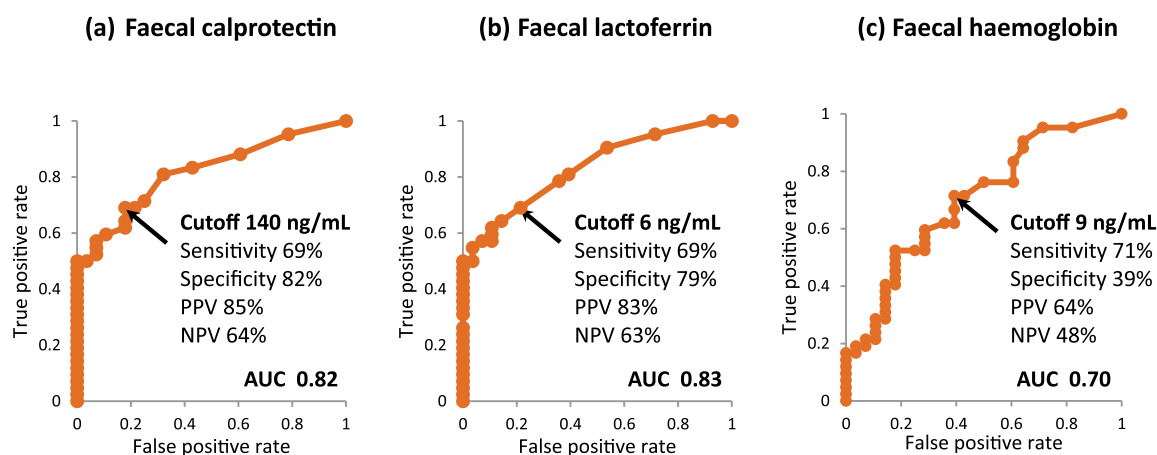


Figure 4. ROC curves showing the sensitivity and specificity of faecal biomarkers for the detection of small bowel inflammation [CTE scores 1–3] at different cutoff values. An optimal cutoff value to detect small bowel inflammation was determined to be 140 ng/ml for calprotectin (a), 6 ng/ml for lactoferrin (b) and 9 ng/ml for haemoglobin (c).

AUC, area under the ROC curve; NPV, negative predictive value; PPV, positive predict value.

Table 5. The correlation between the levels of faecal and blood biomarkers.

	Faecal calprotectin	Faecal lactoferrin	Faecal haemoglobin
WBC	$r = 0.032, p = 0.80$	$r = 0.098, p = 0.42$	$r = 0.261, p = 0.03$
Haemoglobin	$r = -0.120, p = 0.32$	$r = 0.086, p = 0.48$	$r = -0.173, p = 0.15$
Platelet	$r = 0.174, p = 0.15$	$r = 0.130, p = 0.28$	$r = 0.196, p = 0.10$
Albumin	$r = -0.455, p < 0.0001$	$r = -0.214, p = 0.07$	$r = -0.254, p = 0.03$
CRP	$r = 0.552, p < 0.0001$	$r = 0.387, p = 0.0008$	$r = 0.552, p < 0.0001$
ESR	$r = 0.461, p < 0.0001$	$r = 0.232, p = 0.053$	$r = 0.329, p = 0.005$

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell count. Bold values represent statistically significant differences.

those with established CD. Although MRE is currently being used more frequently because of lack of radiation exposure, it has serious limitations including high cost, longer examination time and inferior spatial resolution, which make it an unfavourable choice for many adult patients.³⁷

Both faecal calprotectin and lactoferrin are neutrophil-derived proteins that are stable, and can be detected mainly by quantitative enzyme-linked immunosorbent assay using small stool samples. These two faecal biomarkers provide a unique, inexpensive and non-invasive method for testing intestinal inflammation.^{5–7} FIT can also quantify the levels of haemoglobin in stool samples, and was originally used for screening colorectal cancer. Researchers in Japan recently reported that faecal haemoglobin detected by immunochemical

test is also valid as a biomarker in patients with IBD.^{8,9,38} FIT has several advantages over faecal calprotectin testing in terms of user-friendliness, lower cost, simplicity, clean handling and the ability to carry out rapid assays by using an automated measurement system.^{8,9,38} A direct comparison between the value of faecal calprotectin and haemoglobin indicated that both biomarkers are equally useful for the assessment of mucosal inflammation in patients with UC.³⁸ However, in patients with CD, faecal haemoglobin was less sensitive for reflecting CD lesions in the small bowel compared with calprotectin.⁹ Likewise, in our study, we found that the diagnostic value (specificity, PPV, NPV and AUC) of faecal haemoglobin for small bowel inflammation was much lower than calprotectin or lactoferrin. These findings might suggest that FIT is more suitable for

the detection of blood in the colon and rectum, and should be mainly used for screening large bowel inflammation in IBD patients. Further, between calprotectin and lactoferrin, sensitivity, specificity, PPV and NPV were similar, and the AUC was not significantly different. Thus, the values of faecal calprotectin and lactoferrin for the detection of small bowel inflammation appeared to be similar.

At this stage, we should acknowledge that our findings have certain limitations that need to be considered in the interpretation of the results. First, the small bowel abnormalities detected by CTE were not verified by endoscopy. Therefore, we are not sure if mucosal irregularities were due to CD or other inflammatory events, especially when the lesions are mild without typical features suggestive of CD. Second, in this study the proportion of patients with moderately to severely active CD was relatively low, and half of the patients were in clinical remission at entry. Patients with more severely active CD should be included in future studies.

In conclusion, in this study faecal calprotectin, lactoferrin and, to a lesser degree, haemoglobin appeared to be relevant biomarkers for screening small bowel inflammation in CD patients who showed no obvious inflammation in the large bowel. To our knowledge, this is the first time that three biomarkers have been measured in the same stool samples. To ensure that the faecal biomarkers we aimed to measure relate to small bowel inflammation, we had to adhere to a rigorous patient-selection criteria, which allowed 70 patients from a population of 122 to be eligible for inclusion. Our findings suggest that in patients showing low levels of faecal biomarkers, unnecessary time-consuming or invasive examinations by endoscopy or radiology can be avoided. Additionally, this study should provide valuable insight into faecal biomarkers for the detection of intestinal inflammation in patients with CD. Further well-designed, large-scale studies in this clinical setting should strengthen our findings.

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and in drafting/editing the manuscript. Takahiro Shimoyama and Satoru Umegae were involved in the planning, collection and interpretation of data for this study, and in drafting the manuscript. Koichi Matsumoto was involved in editing the manuscript. All the above-mentioned authors have approved the final draft submitted.

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Conflict of interest statement

The authors declare that there is no conflict of interest.


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