Level of Fecal Calprotectin Correlates With Severity of Small Bowel Crohn's Disease, Measured by Balloon-assisted Enteroscopy and Computed Tomography Enterography

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BACKGROUND & AIMS: **Previous studies have not found a correlation between fecal level of calprotectin and small bowel Crohn's disease (CD). However, these studies evaluated patients mainly by ileocolonoscopy, which views up to only the terminal ileum rather than entire small intestine. We investigated whether level of fecal calprotectin (FC) is a marker of active CD of the small bowel, identified by balloon-assisted enteroscopy and computed tomography enterography (CTE).**

METHODS: **We performed a prospective study of 123 patients with CD (35 with ileitis, 72 with ileocolitis, and 16 with colitis) evaluated by balloon-assisted enteroscopy from May 2012 through July 2015 at Toho University Sakura Medical Centre in Japan. Patients with strictures detected by balloon-assisted enteroscopy were evaluated by CTE (n = 17). Fecal samples were collected from each patient, and levels of calprotectin were measured; patient demographic variables and medical history were also collected. We developed a CTE scoring system for disease severity that was based on bowel wall thickness, mural hyperenhancement, and engorged vasa recta. The association between level of FC and simple endoscopic index for CD score or CTE was evaluated by using Spearman rank correlation coefficient.**

RESULTS: **Level of FC correlated with the simple endoscopic index for CD score ($r = 0.6362$, $P < .0001$), even in patients with only active disease of the small intestine ($r = 0.6594$, $P = .0005$). In the 17 patients with strictures that could not be passed with the enteroscope, CTE detected all lesions beyond the strictures as well as areas in the distal side of the strictures. Level of FC correlated with CTE score in these patients ($r = 0.4018$, $P = .0011$, n = 63). In receiver operating characteristic analyses, the FC cutoff value for mucosal healing was 215 μg/g; this cutoff value identified patients with healing with 82.8% sensitivity, 71.4% specificity, positive predictive value of 74.3%, negative predictive value of 80.6%, odds ratio of 12.0, and area under the receiver operating characteristic curve value of 0.81.**

CONCLUSIONS: **A combination of measurement of level of FC and CTE appears to be effective for monitoring CD activity in patients with small intestinal CD, including patients with strictures that cannot be passed by conventional endoscopy.**

Keywords: Active Small Intestinal Lesions; Balloon-assisted Enteroscopy; Crohn's Disease; Fecal Calprotectin; Computed Tomography Enterography.

Abbreviations used in this paper: **AUC, area under the curve; BAE, balloon-assisted enteroscopy; CD, Crohn's disease; CDAI, Crohn's disease activity index; CRP, C-reactive protein; FC, fecal calprotectin; CT, computed tomography; CTE, computed tomography enterography; IBD, inflammatory bowel disease; mSES-CD, modified simple endoscopic index for Crohn's disease; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; SES-CD, simple endoscopic index for Crohn's disease; TNF, tumor necrosis factor; UC, ulcerative colitis.**

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Crohn's disease (CD) is a relapsing and remitting inflammatory bowel disease (IBD) characterized by transmural inflammation, which frequently leads to surgery because of stenosis, abscess, bleeding, or perianal lesions.[1,2] Furthermore, since the introduction of anti–tumor necrosis factor (TNF)-α biologics such as infliximab and adalimumab into the CD clinical setting, endoscopic and histologic remission reflecting mucosal healing has become an accepted therapeutic goal.[3–6] This is because mucosal healing is closely associated with lower rates of hospitalization and surgery.[7,8] However, as yet, the precise definition of mucosal healing in CD has not been established, and no qualitative or quantitative endoscopic indices for mucosal healing have been widely used.[7] Furthermore, the evaluation of mucosal inflammation at the ileocolonoscopy alone in CD may be inadequate, because it is not unusual to find small intestinal CD lesions beyond the terminal ileum. In addition, lesions such as stenosis and intestinal adhesions make endoscopic examinations difficult in CD.

Recently, cross-sectional imaging including computed tomography enterography (CTE) and magnetic resonance enterography have been introduced into the CD clinical setting for the evaluation of small intestinal lesions. These imaging techniques allow accurate assessment of CD activity, extent, location of disease, extraluminal manifestations, and complications at a single examination.[9]

Fecal calprotectin (FC) is a calcium and zinc binding protein derived from neutrophils, monocytes/macrophages, which infiltrate the intestinal wall, and has emerged as a relevant and noninvasive surrogate marker of mucosal inflammation.[10–15] FC has shown positive correlation with endoscopic and histologic findings in IBD.[13] Therefore, in patients with IBD, FC is found to be a predictive marker of intestinal inflammation and clinical relapse.[15–18] However, currently it is not widely accepted that FC relates to disease activity in patients with small intestinal CD.[10,16] Likewise, the most appropriate cutoff value for FC has not been determined to identify a subgroup of patients in clinical remission but with endoscopically active CD or ulcerative colitis (UC).[16,17] With this background in mind, the present investigation was undertaken to evaluate disease activity and disease location by using balloon-assisted enteroscopy (BAE) together with CTE to see whether FC was a clinically relevant surrogate marker of disease activity with appropriate cutoff values in patients with small intestinal CD.

Methods

Patients

Between May 2012 and July 2015, patients with a diagnosis of CD[19] were enrolled into this prospective study at the Centre for Gastroenterology, Toho University Sakura Medical Centre. Patients who had very severe CD or needed immediate surgery were not included. The eligible patients (n = 89) underwent a total of 123 endoscopic examinations by BAE. Thirty-four of the 89 patients repeated endoscopy at a 6-month interval. In 82 patients, 100 BAE and CTE sessions were undertaken. From 70 patients who underwent BAE, 86 fecal samples were prepared for the assay of calprotectin. The patients' demographic variables, including CD activity index (CDAI), C-reactive protein (CRP), and current medications were recorded (Table 1).

Balloon-assisted Enteroscopy

Three experienced endoscopists carried out the BAE procedures by the retrograde approach. The endoscope was inserted into the small bowel as deep as possible. When difficulty was experienced because of CD-related stenosis, imaging with a contrast medium injected from the scope was used to assess the lesions in the deeper part of the small bowel (ileum). The distance from the ileocecal valve or the postoperative anastomosis at the deepest point where the scope had reached was recorded.[20] BAE was done after CTE on the same day. We calculated the simple endoscopic score for CD (SES-CD)[21] except when there was stricture, in which case it was defined as modified SES-CD (mSES-CD) and used for the evaluation of intestinal disease activity up to the rectum (Tables 1 and 2).

Assessment of Crohn's Disease Activity With Computed Tomography Enterography

One day before CTE, patients were asked to have a low residue lunch and dinner plus 20 mL 0.75% sodium picosulfate hydrate (Laxoberon; Teijin, Tokyo, Japan)

Table 1. Baseline Demographic Variables of CD Patients (n = 89) Included in This Study

Average age, *y* (range)	31.8 (15–69)
Gender: male/female	78 (87.6%)/11 (12.4%)
Average disease duration, *mo* (range)	108 (1–526)
Disease location	
Ileitis/ileocolitis/colitis	27/50/12
Perianal disease (%)	26 (29.2)
Previous surgery (%)	32 (36.0)
Surgical location (n = 32)	
Ileum/ileocecal/colon/perianal disease	18/20/2/3
Treatment	
Anti-TNF agents (%)	58 (65.2)
Immunomodulator (%)	15 (16.9)
Average CDAI (range)	120 (0–401)
Average CRP (*mg/dL*) (range)	1.09 (0.01–9.13)
Average FC ($\mu g/g$, n = 86) (range)	763.9 (0.75–5663.4)
Average CTE score (n = 100) (range)	5.5 (0–19)
Average mSES-CD (n = 123) (range)	7.7 (0–32)

NOTE. Different n values indicate that measurements were done on a fraction of the total patients or were repeated on some patients.

Table 2. CTE Scores for CD

Intestinal segment	Bowel wall thickness	Mural hyperenhancement	Mural stratification	Engorged vasa recta (comb sign)
Ileum	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0
Right colon	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0
Transverse colon	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0
Sigmoid and left colon	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0
Rectum	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0	Yes/no = 1/0

before bedtime. In the morning, patients were to ingest 1350–1800 mL magnesium citrate solution (Magcorol P; Horii Pharmaceuticals, Osaka, Japan) during a period of 60 minutes before starting CTE. One hundred fifty milliliters Lohexol (Omnipaque 300; Daiich-Sankyo, Tokyo, Japan) was administered intravenously at 4 mL/s. In addition, 7.5 mg timepidium bromide hydrate (Sesden; Tanabe Mitsubishi Pharmaceuticals, Tokyo, Japan) was administered intravenously immediately before scanning. Computed tomography (CT) scanning at supine single phase was done at 50–60 seconds after intravenous contrast administration. For CT scanning, the slice thickness was set at 2 mm, and the reconstruction interval was set at 0.75 mm. Interpretation of CTE images was done by using a multi-planar reconstruction view at coronal, axial, or arbitrary cross sections. Two experienced radiologists blindly evaluated the CTE findings by using a novel CTE score (Tables 2 and 3). The CTE score had the following 4 variables: bowel wall thickness, mural hyperenhancement, mural stratification, and engorged vasa recta. The variables in the CTE score were evaluated in 5 predefined ileocolonic segments such as SES-CD, and each variable was scored from 0 to 4 per segment, with the total score being 20.

Assay of Fecal Calprotectin

Stool samples were stored at −80°C until the assay of FC in a specialized laboratory. Level of FC was measured by a Phical Calprotectin enzyme-linked immunosorbent assay kit (Immundiagnostik AG, Benshem, Germany) according to the package insert. The quantitative assay range for calprotectin was 0.65 μg/g–2100 μg/g stool. After appropriate dilution of fecal samples, the FC range was 1:50–1:10,000. The upper limit of normal range for FC in patients without inflammation has been found to be less than 50 μg/g.[15–18]

Statistics

Statistical analyses were undertaken by using the Excel Statistical 2012 (Social Survey Research Information, Tokyo, Japan). The Spearman rank correlation coefficient was applied for assessing the correlations between FC and CDAI, CRP, mSES-CD, and the CTE scores. All P values are 2-tailed, and $P < .05$ was considered significant. Receiver operating characteristic (ROC) analysis was applied for determining the optimal cutoff value of FC and the CTE score, with the sensitivity and specificity based on the endoscopic mucosal healing (mSES-CD = 0).

Ethical Considerations

Our study protocol was reviewed and approved by the ethics committee at the Toho University Sakura Medical Centre. Informed consent was obtained from all participating patients after explaining the purpose of the study and the nature of the procedures involved. Furthermore, the investigations were conducted with strict adherence to the Helsinki Declaration at all times.

Table 3. Scoring of Disease Activity According to Disease Location in Small Intestinal CD Patients, Subgrouped According to Disease Location

	Ileitis (n = 18)	Ileocolitis (n = 38)	Colitis (n = 7)	Ileitis vs ileocolitis (*P* value)	Ileitis vs colitis (*P* value)	Ileocolitis vs colitis (*P* value)
Average mSES-CD	4.6 [0–14]	9.8 [0–32]	7 [0–18]	.1109	1.0	1.0
Average CTE score	3.9 [0–9]	6.3 [0–19]	3.4 [0–13]	.2554	1.0	.4302
Average CDAI	101 [5–289]	135 [11–368]	60 [0–192]	.5463	.9086	.1299
Average CRP (*mg/dL*)	0.28 [0.01–1.28]	1.62 [0–10.31]	0.88 [0.01–4.43]	.0827	1.0	1.0
Average FC (*μg/g*)	679.39 [2.97–1887]	852.82 [3.00–5663.40]	435.23 [0.75–1990.75]	1.0	1.0	.9806

NOTE. Numbers in square brackets represent the range for the corresponding average measurement.

Results

Patients' Demographic Variables

A total of 89 patients, average age 31.8 years, were eligible for inclusion (Table 1). At the first endoscopy, the average CDAI was 120, range 0–401, and the average CRP was 1.09 mg/dL, range 0.01–9.13 mg/dL. Only 11 of the 89 patients (12.4%) were female. The average disease duration was 108 months. Twenty-seven patients (30.3%) had small bowel CD, 50 (56.2%) had ileocolonic CD, and 12 (13.5%) had colonic CD. Some patients had undergone multiple surgeries, which is common in CD patients experiencing disease-related complications, including ileocecal resection, right/left hemicolectomy, seton drainage for anal fistula, and fistulectomy. Therefore, at the first endoscopy, 26 of the 89 patients (29.2%) had perianal lesions, and 32 (36.0%) had undergone surgical intervention because of CD. In 18 of the 32 patients (56.3%), surgery had been done for small bowel lesions, in 20 (62.5%) for ileocecal lesions, in 2 (6.3%) for colonic lesions, and in 3 (9.4%) for perianal lesions. In the past, anti–TNF-α biologics such as infliximab or adalimumab were administered to 58 of the 89 patients (65.2%), and 15 patients (16.9%) had received azathioprine or 6-mercaptopurine.

Correlation of Fecal Calprotectin With Crohn's Disease Activity Index and C-Reactive Protein

The average level of FC was 763.9 μg/g stool, range 0.75–5663.40 μg/g. The level of FC showed significant correlation with CDAI ($r = 0.3510$, $P = .0009$, n = 86) or with CRP ($r = 0.2865$, $P = .0075$, n = 86). When the assessment was done in the patients with small bowel lesions but without any perianal involvement, again the level of FC showed significant correlation with CDAI ($r = 0.4280$, $P = .0004$, n = 64) and CRP ($r = 0.2981$, $P = .0167$, n = 64). Even though the disease location was based on both BAE and CTE instead of ileocolonoscopy alone, the location of CD lesions per se did not seem to affect the CDAI score or the CRP concentration.

Balloon-assisted Enteroscopy and Computed Tomography Enterography Findings

We introduced CTE to complement endoscopic observations, especially in patients in whom endoscopic observation was difficult because of stenosis. Before introducing CTE, we separately assessed its feasibility to evaluate CD activity by comparing the CTE findings with endoscopic findings. One hundred CTE sessions were performed in 82 patients who also underwent retrograde BAE on the same day or within a week after the CTE. Eighteen of 100 patients repeated both BAE and CTE at longer than 6 months apart. In 80 of the 100 BAE and CTE sessions (80%), endoscopic observations could be made beyond the terminal ileum, closer than 10 cm from the ileocecal valve or the postoperative anastomosis. The average for endoscopic observation was 59.1 cm, range 0–210 cm, from the ileocecal valve or the postoperative anastomosis.

Modified Simple Endoscopic Index for Crohn's Disease Findings

The average mSES-CD score in patients who underwent retrograde BAE was 7.7, range 0–32. In 100 patients who underwent both BAE and CTE, the average mSES-CD score was 8.45, range 0–32, and the average CTE score was 5.49, range 0–19. Within the range of endoscopic observations, the mSES-CD score was well-correlated with the CTE score ($r = 0.8177$, $P < .0001$, n = 100; Figure 1*A*). Furthermore, when we compared the mSES-CD score with the CTE score, there was significant correlation between the 2 scores ($r = 0.7676$, $P < .0001$, n = 473 segments; Figure 1*B*). Therefore, CTE could be an alternative to BAE for

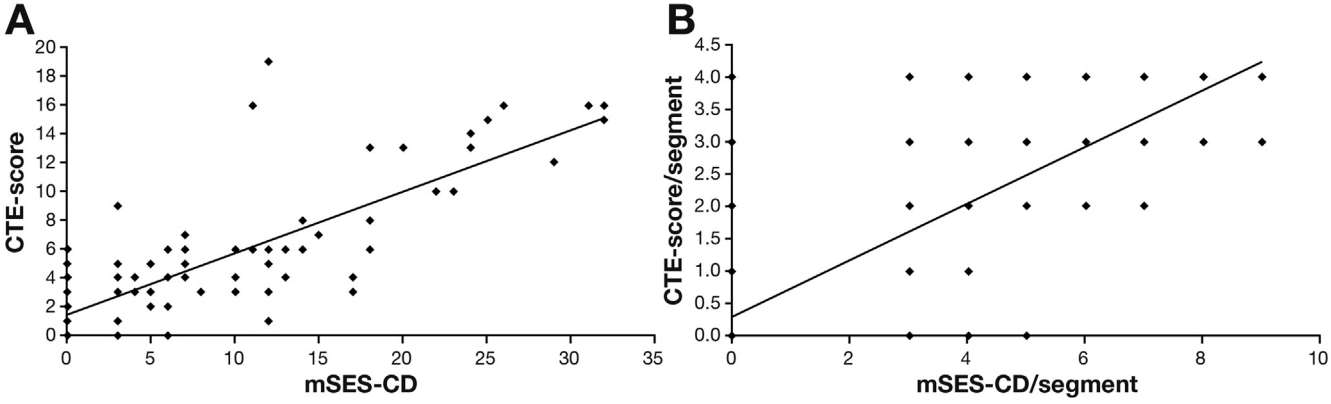

Figure 1. (*A*) Scatter plot demonstrating correlation of CTE score with mSES-CD. Spearman rank correlation coefficient *r* was 0.82 ($P < .0001$, n = 100). (*B*) Correlation between CTE score/segment and mSES-CD/segment. Spearman rank correlation coefficient *r* was 0.7676 ($P < .0001$, n = 473 segments).

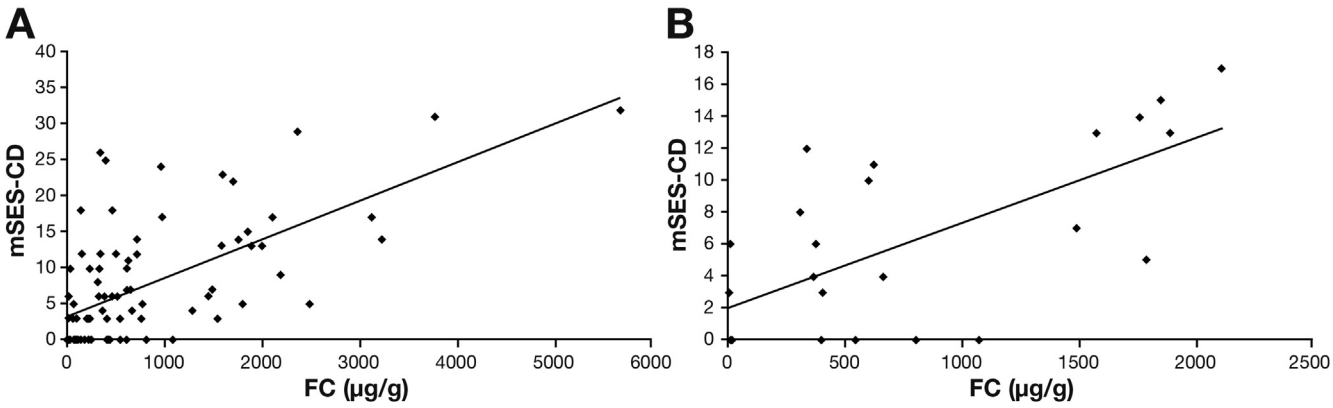

Figure 2. (*A*) Scatter plot showing correlation of mSES-CD with level of FC in all patients. Spearman rank correlation coefficient *r* was 0.6362 ($P < .0001$, n = 86). (*B*) Correlation between FC and mSES-CD in patients with small bowel lesion alone. Spearman rank correlation coefficient *r* was 0.6594 ($P = .0005$, n = 24).

the assessment of CD lesions, especially in intestinal segments unreachable by routine endoscopy.

Correlation of Fecal Calprotectin With Balloon-assisted Enteroscopy and Computed Tomography Enterography Findings

In this study, the level of FC showed better correlation with the BAE findings ($r = 0.6362$, $P < .0001$, n = 86; Figure 2*A*) than with the CDAI or CRP. Even in patients in whom the CD lesions were confined to the small bowel, FC showed good correlation with the mSES-CD ($r = 0.6594$, $P = .0005$, n = 24; Figure 2*B*). The correlation between FC and mSES-CD was significant even when patients with perianal lesions were excluded ($r = 0.6698$, $P < .0001$, n = 64) including patients in whom the CD lesions were confined to the small bowel ($r = 0.6723$, $P = .0022$, n = 18). Furthermore, in the ROC analyses, the FC cutoff value for endoscopic mucosal healing with mSES-CD = 0 was 215 μg/g, sensitivity 82.8%, specificity 71.4%, positive predictive value (PPV) 74.3%, negative predictive value (NPV) 80.6%, odds ratio 12.000, and area under the curve (AUC) 0.8091 (n = 86, Figure 3*A*). Among the patients who underwent both BAE and CTE, the FC assay was possible in 63. The FC concentration was well-correlated with the CTE score ($r = 0.4018$, $P = .0011$, n = 63), even when patients with perianal lesions were excluded ($r = 0.4543$, $P = .0013$, n = 47). In addition, when the endoscopic mucosal healing was defined as mSES-CD = 0/segment, the ROC analysis revealed a cutoff value of 2 for the CTE score/segment, with sensitivity of 74.1%, specificity of 89.9%, PPV 88.0%, NPV 77.6%, odds ratio 25.467, and AUC 0.8430 (n = 473, Figure 3*B*). In 17 of 63 patients who underwent BAE, CTE, and FC assay, it was difficult to reach the deepest end of the ileum by using the endoscope because of strictures. In 5 of these 17 patients, the active lesions could not be reached by BAE, because the lesions were beyond the range of endoscopic observation. In contrast, CTE could reach all the lesions, and 3 of the aforementioned 5 patients showed an FC level above 215 μg/g.

Discussion

We found that in patients with small bowel CD, the level of FC was well-correlated with the CD activity when the latter was defined by both BAE and CTE. Neither the CDAI score nor serum CRP showed similar correlation with FC regardless of CD location. Furthermore, CTE could reach and evaluate CD lesions in the small intestine unreachable by endoscopy. A good correlation between FC and the CTE findings should mean that both FC and CTE may define endoscopic mucosal healing.

At present, CRP is considered a convenient, noninvasive biomarker of disease activity in patients with IBD. However, CRP is elevated by not only intestinal inflammation but also by systemic inflammation. In addition, it is known that a genetic heterogeneity exists in CRP generation; a CRP response does not occur in about 15% of normal, healthy individuals.[22,23] Furthermore, in CD patients, CRP has low sensitivity when disease location[24] or the degree of endoscopic severity is considered.[25] Therefore, more relevant biomarkers of CD activity are necessary. In line with this thinking, various IBD markers have emerged, among which FC has been most widely investigated in recent years. Calprotectin is a member of the S100 family of calcium-binding proteins and represents 40%–60% of the proteins in the neutrophil cytosol. The level of FC has been shown to reflect endoscopic disease activity in IBD.[10,14] However, it has been reported that FC has better specificity in UC than in CD.[26] This discordance of FC levels between UC and CD might be due to the involvement of intestinal segments that are not reached by ileocolonoscopy.[27]

An important approach toward better CD monitoring and disease control is more precise assessment of disease activity by both BAE and CTE, which we applied. It is believed that BAE is a better diagnostic strategy for assessing intestinal CD as compared with conventional

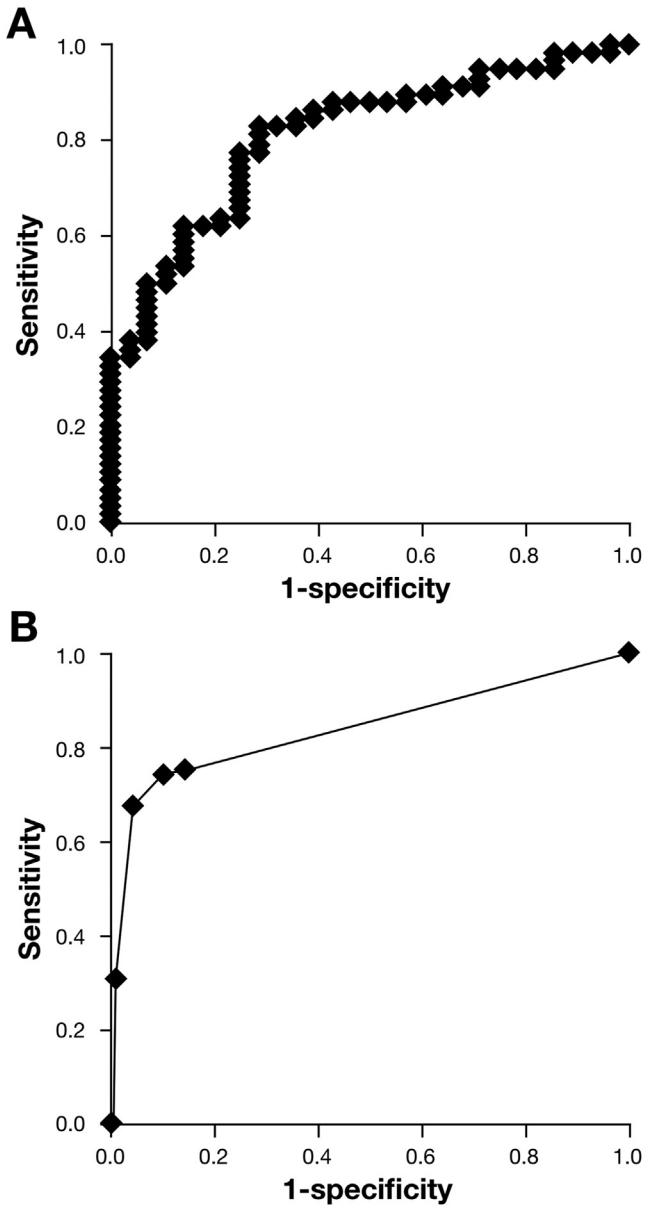

Figure 3. (*A*) Predictive values of FC for endoscopic mucosal healing in CD. ROC curve analyses revealed cutoff value of 215 μg/g FC to indicate mucosal healing with sensitivity of 82.8%, specificity of 71.4%, PPV 74.3%, NPV 80.6%, odds ratio 12.000, and AUC 0.8091 (n = 86). (*B*) CTE score/segment to indicate endoscopic mucosal healing. ROC curve analyses revealed cutoff value of 2 for CTE score/segment marking endoscopic mucosal healing in CD patients, with sensitivity of 74.1%, specificity of 89.9%, PPV 88.0%, NPV 77.6%, odds ratio 25.467, and AUC 0.8430 (n = 473 segments).

ileocolonoscopy.[28] However, in patients with small bowel CD, endoscopic observation is often complicated by intestinal stenosis, strictures, and adhesions. Therefore, CTE as cross-sectional imaging should complement BAE. With this in mind, we introduced a new CTE scoring method that factored mucosal hyperenhancement, mural thickening, stratification, and engorgement of vasa recta (comb sign), because these findings in CTE are closely related to inflammation.[9] We found a strong correlation between the CTE scores and mSES-CD values. However, because a cutoff value of CTE score/segment 2 for mucosal healing defined no ulcerative lesions, its sensitivity, 76.2%, was lower than its specificity, 91.0%. The relatively low sensitivity for mucosal healing might be explained by the detection limit of CTE at sites of aphthous ulcers in CD patients.

Sipponen et al[13] reported that in the colon, level of FC showed better correlation with the SES-CD score ($r = 0.642$, $P < .001$) than ileal SES-CD score did, with or without upper gastrointestinal involvement ($r = 0.317$, $P > .05$). However, the authors used ileocolonoscopy for CD diagnosis, and the endoscopic findings were scored according to the original SES-CD, which includes the stenosis factor. Furthermore, when the results were grouped according to CD location, the median FC concentration in the inflammatory phase (n = 50) was higher than in the stricturing or penetrating CD phase (n = 37; FC, 565 μg/g vs 118 μg/g).[13] Accordingly, in the inflammatory phase, the total SES-CD score was 10, range 0–32, the colon SES-CD score was 7, range 0–26, and the ileal SES-CD score was 0. In the stricturing or penetrating CD, the total SES-CD score was 6, range 0–26 ($P = .051$), the colon SES-CD score was 1, range 0–26 ($P < .001$), and the ileal SES-CD score was 4, range 0–10 ($P < .001$).[13] We found that in ileocolonic CD, there was a significant correlation between FC concentration and mSES-CD score. Likewise, there was significant correlation between the FC concentration and CTE score. It is interesting that in patients with ileocolonic CD, the correlation between FC and CTE was lower than between FC and the mSES-CD score.

In conclusion, the level of FC parallels disease activity when the latter is evaluated by BAE in combination with CTE. However, the rise in FC was independent of CD location. We believe that FC could be a relevant surrogate marker of disease activity in small bowel CD. Furthermore, assessment of CD activity that was based on CTE showed good correlation with the BAE findings. Likewise, CTE score/segment <2 was associated with endoscopic mucosal healing. Therefore, CTE could be a reliable alternative to endoscopy when endoscopic observation is complicated by intestinal strictures or adhesion, or when CD lesions are unreachable by an endoscope.

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Conflicts of interest
The authors disclose no conflicts.