

Correlation of Fecal Markers with Magnifying Endoscopic Stratification in Patients with Ulcerative Colitis Who Are in Clinical Remission

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SM participated in the data collection, data analysis, and manuscript drafting. FT participated in the study design, data collection, data analysis, and manuscript drafting. YA participated in the data analysis and manuscript drafting. KM and HM participated in the data collection and data analysis. NY, KO, and HI participated in the data collection. KN gave final approval of the manuscript to be published. All authors read and approved the final manuscript.

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Key Words

Fecal marker • Calprotectin • Lactoferrin • Ulcerative Colitis Endoscopic Index of Severity • Magnifying endoscopic stratification • Mayo endoscopic subscore • Ulcerative colitis in clinical remission • Right colon inflammation

Abstract

Background: Maintenance of mucosal healing is recommended during the treatment of ulcerative colitis (UC). However, symptoms of UC often do not reflect mucosal disease activity. Fecal markers such as calprotectin, lactoferrin, and hemoglobin have been reported to correlate well with the Mayo endoscopic subscore (MES) and are being considered alternative monitoring tools in endoscopy. Ulcerative Colitis Endoscopic Index of Severity (UCEIS) is a new and more detailed endoscopic scoring system compared to MES. Furthermore, magnifying endoscopic stratification (ME) based on alterations in the mucosal surface pit patterns is noted in UC. However, the association between fecal markers and UCEIS and magnifying endoscopy is relatively unexplored.

Summary: This study investigated the association between the aforementioned fecal markers and MES, UCEIS, and ME in patients with UC in clinical remission. This prospective study included 60 patients with UC in clinical remission who underwent colonoscopy at the Nagasaki University Hospital between June 2015 and November 2016. A significant correlation was observed between MES and all fecal markers. Notably, the fecal markers correlated well with UCEIS (calprotectin Spearman's correlation coefficient $[r]=0.54$, $P<0.0001$; lactoferrin $r=0.56$, $P<0.0001$; and hemoglobin

r=0.43, P<0.001). Furthermore, ME findings correlated significantly with calprotectin (r=0.50, P=0.0002) and lactoferrin (r=0.46, P=0.0006) levels and slightly with hemoglobin (r=0.28, P=0.043) levels. Moreover, each cut-off level of fecal calprotectin, lactoferrin, or hemoglobin had a high sensitivity and specificity for the detection of MES=0, UCEIS=0, ME=A, for predicting mucosa healing.

Key Messages: Fecal markers correlated not only with MES but also with UCEIS and ME and should be useful for monitoring patients with UC in clinical remission.

Introduction

Ulcerative colitis (UC) is a chronic, idiopathic, inflammatory bowel disease that causes diffuse inflammatory mucosal damage, causing erosions and ulcers in the colon, especially in the rectum. The disease has a lifelong clinical course characterized by the repeat of relapse and remission. The main purpose of therapy in UC is to suppress mucosal inflammation and induce and sustain clinical remission. Recent evidence recommends sustained endoscopic remission, which is associated with improved outcomes for UC [1, 2]. Importantly, patient symptoms often do not reflect mucosal disease activity. Consequently, to evaluate disease activity and therapeutic efficacy, clinical symptoms and laboratory and endoscopic findings need to be monitored routinely in clinical practice [3]. Currently, colonoscopy is the most effective means of estimating of UC severity. However, colonoscopy is burdensome, invasive, and costly for patients, and requires the diagnostic skills of the endoscopist. Because frequent colonoscopy is not realistic for UC patients, the development of non-invasive surrogate markers is necessary.

Fecal markers such as calprotectin, lactoferrin, and hemoglobin have been reported to correlate well with the Mayo endoscopic subscore (MES) and are being considered alternative monitoring tools in endoscopy [4-8]. Calprotectin is a calcium-binding

protein and accounts for up to 60% of cytoplasmic proteins in neutrophils [6], and is mainly derived from neutrophils. Fecal calprotectin concentration is reported to be associated with the concentration of neutrophils in the colonic mucosa [9]. It is convenient to handle since calprotectin is stable in the feces for up to 1 week at room temperature [6, 10]. Lactoferrin is an iron-binding glycoprotein secreted from most mucosal membranes and is also a major component of neutrophils [5, 11, 12]. When inflammation occurs in the intestinal tract, leucocytes enter the mucosa and this may increase the excretion of lactoferrin in stool. Lactoferrin is also convenient to handle since it is relatively stable in the stool for up to 4 days [13, 14].

The Ulcerative Colitis Endoscopic Index of Severity (UCEIS) is a newly proposed and more detailed endoscopic scoring system compared to MES [15]. In addition to the more detailed scoring of the extent of ulcers compared to MES, UCIES includes the degree of vascular appearance. Indeed, it has been reported that UCEIS precisely reflects clinical outcomes and predicts the medium-to-long-term prognosis in UC patients on induction therapies [16]. However, only few studies have examined the association between fecal markers and UCEIS [17]. Furthermore, we recently proposed a magnifying endoscopic stratification (ME) based on alterations in the mucosal surface pit patterns, which are associated not only with the Matts endoscopic score but also

with pathognomonic microscopic features of mucosal inflammation [18]. Notably, we also demonstrated its utility in predicting relapse in patients with UC [18]. However, the association between fecal surrogate markers and UCIES or ME has not been studied.

The aim of our study was to investigate the correlation of fecal calprotectin, lactoferrin, and hemoglobin levels with MES, UCEIS and ME in patients with UC in clinical remission.

Material and Methods

Study Design and Patients

This prospective study included 60 patients with UC in clinical remission who underwent colonoscopy at the Nagasaki University Hospital between June 2015 and November 2016. The inclusion criteria were as follows: UC patients diagnosed with established criteria, aged ≥ 20 years, and in clinical remission. The exclusion criteria were active UC, pregnancy, liver, kidney, and heart disease, and other severe complications. Clinical remission was defined as a partial UC disease activity index of ≤ 2 and a bloody stool score of 0 [19].

Measurement of Fecal Calprotectin, Lactoferrin, and Hemoglobin Levels

Stool samples were collected before colonoscopy on the same day. The collected feces were stored at room temperature and immediately analyzed at the Kyoto Medical Science Laboratory (Kyoto, Japan). Fecal lactoferrin was measured using sandwich enzyme-linked immunosorbent assay, previously described in detail [20]. Fecal calprotectin level was measured using an enzyme-linked immunosorbent assay kit (Immunodiagnostik AG, Benshem, Germany). Hemoglobin level was measured using the Nescauto hemo plus (Alfresa Pharma Corporation, Japan).

Ulcerative Colitis Endoscopic Index of Severity

MES, UCEIS, and ME were evaluated during the same session of colonoscopy. Scores and classifications were determined by the agreement of two experienced endoscopists.

The UCEIS score is based on a visual analog scale represented by incorporating a combination of 3 elements: vascular pattern (2 levels), bleeding (3 levels), and erosions and ulcers (3 levels). Via colonoscopy, the worst state of the colon is identified, and the score may be calculated by adding the scores from each section ranging from 0 (normal) to 8 (worst) [17].

Magnifying Endoscopic Stratification

ME observations were performed at the location where the most severe inflammation was detected during white light examination. For cases without apparent inflammation,

ME was performed in the rectal mucosa. The endoscopy system consisted of a light source (CLV-260SL; Olympus Optical Co., Tokyo, Japan), a colonoscope (PCF-Q260AZI; Olympus), and a processor (CV-260SL; Olympus). On insertion of the colonoscope, luminal cleansing was performed using water with dimethicone and pronase. After standard observation, in the rectal mucosa, ME was performed following vital staining including 0.05% crystal violet. ME findings were classified into 4 grades: ME-A, regular arrangement of normal round-to-oval pits; ME-B, irregular arrangement of the round-to-oval pits with/without enlarged spaces between the even crypts; ME-C, irregular pits in size and shape with a more irregular arrangement of the pits compared to ME-B; and ME-D, destruction and disappearance of pits, as previously described by Isomoto et al [18].

Statistical Analysis

Statistical analyses were performed using JMP version 11.0 for Windows (SAS Institute, Cary, NC, USA). Sensitivity and specificity with 95% confidence intervals for finding MES, UCEIS, and ME levels were established according to calprotectin, lactoferrin, and hemoglobin results. We analyzed the receiver operating characteristic (ROC) curve and the area under the curve (AUC) to evaluate the appropriate cut-off values for calprotectin, lactoferrin, and hemoglobin. Moreover, we conducted the Spearman's rank

correlation test to clarify the correlation between calprotectin, lactoferrin, and hemoglobin levels and MES, UCEIS, and ME. p-values < 0.05 were considered statistically significant.

Ethical Considerations

The study was approved by the Nagasaki University Ethics Committee (Number : 16020830) and was conducted in accordance with the Helsinki Declaration. Informed consent was acquired from all patients in advance regarding the use of their fecal samples and clinical data for this study.

Results

Clinical Characteristics of Patients (Table 1)

The total number of 60 patients consisted of 35 men and 25 women. The median patient age was 51. Among the 60 UC patients, 40 (67%) had extensive colitis, 13 (22%) had left-sided colitis, and 7 (12%) had proctitis. For remission maintenance therapy, salazosulfapyridine was used in 5 patients, mesalamine in 43 patients, azathioprine/mercaptopurine in 13 patients, infliximab/adalimumab in 5 patients, and 6 patients received no medication.

Colonoscopic Findings

Of the 60 cases, 18 cases (30%) had a MES score of 0, 27 cases (45%) had a MES score of 1, and 15 (25%) cases had a MES score of 2. Seventy percent (42/60) of patients in clinical remission had ongoing endoscopic inflammation ($MES \geq 1$).

Of the 60 cases, 16 cases (27%) had a UCEIS score of 0, 20 cases (33%) had a UCEIS score of 1, 7 (11%) cases had a UCEIS score of 2, 8 (13%) cases had a UCEIS score of 3, 7 (12%) cases had a UCEIS score of 4, 1 (1.7%) case had a UCEIS score of 5, and 1 (1.7%) case had a UCEIS score of 6.

Of the 60 cases, 52 cases underwent ME. According to ME stratification, there were 21 cases of ME-A, 17 cases of ME-B, 12 cases of ME-C, and 2 cases of ME-D (Table 2).

Relationship between Calprotectin, Lactoferrin, Hemoglobin, and Colonoscopic Findings

A significant correlation was observed between MES and all fecal markers (Spearman's correlation coefficient [r] for calprotectin = 0.34, $P < 0.01$; lactoferrin $r = 0.45$, $P < 0.01$; and hemoglobin $r = 0.38$, $P < 0.01$) and between UCEIS and all fecal markers (calprotectin $r = 0.54$, $P < 0.0001$; lactoferrin $r = 0.56$, $P < 0.0001$; and hemoglobin $r = 0.43$, $P < 0.001$) (Figure 1a). Furthermore, ME findings correlated significantly with calprotectin and lactoferrin levels and slightly with hemoglobin levels (calprotectin $r = 0.50$, $P < 0.001$; lactoferrin $r = 0.46$, $P < 0.001$; and hemoglobin $r = 0.28$, $P = 0.043$)

(Figure 1b). In addition, a significant correlation was observed between ME and UCEIS ($r = 0.74$, $P < 0.0001$). These results suggest that fecal markers correlate not only with MES but also with UCEIS and ME.

Receiver Operating Curve Analysis and the Predictive Ability of Fecal Markers

Calprotectin, at a cut-off of 201 $\mu\text{g/ml}$, had a sensitivity of 71% and specificity of 78% for the detection of MES=0 (prediction of mucosal healing) (AUC 0.77). Lactoferrin (cut-off 78.3 ng/ml) had a sensitivity of 60% and specificity of 89% for the detection of MES=0 (AUC 0.73). Hemoglobin (cut-off 34 ng/ml) had a sensitivity of 64% and specificity of 89% for the detection of MES=0 (AUC 0.76). Calprotectin, at a cut-off of 201 $\mu\text{g/ml}$, had a sensitivity of 70% and specificity of 81% for the detection of UCEIS=0 (prediction of mucosal healing) (AUC 0.76). Lactoferrin (cut-off 78.3 ng/ml) had a sensitivity of 57% and specificity of 88% for the detection of UCEIS=0 (AUC 0.71). Hemoglobin (cut-off 31 ng/ml) had a sensitivity of 64% and specificity of 88% for the detection of UCEIS=0 (AUC 0.74) (Figure 2a). Calprotectin, at a cut-off of 280 $\mu\text{g/ml}$, had a sensitivity of 68% and specificity of 86% for the detection of ME=A (prediction of mucosal healing) (AUC 0.78). Lactoferrin (cut-off 43.6 ng/ml) had a sensitivity of 71% and specificity of 76% for the detection of ME=A (AUC 0.77). Hemoglobin (cut-off 34 ng/ml) had a sensitivity of 71% and specificity of 86% for the detection of ME=A (AUC 0.78) (Figure 2b). These

results suggest that fecal markers are useful for monitoring patients with UC in clinical remission.

Detection of the Inflammation in Right-Side Colon

In 10 of the 40 patients with extensive colitis, endoscopic inflammation remained only in the right side of the colon. In these 10 cases, calprotectin, lactoferrin, and hemoglobin, at a cut-off value (for detecting UCEIS=0 i.e. the prediction of mucosal healing) of 201 µg/ml, 78.3 ng/ml, and 31 ng/ml, respectively, had a sensitivity of 100% (10/10), 70% (7/10), and 50% (5/10), respectively. No significant difference was found between the markers. This result suggests that calprotectin could be a highly sensitive tool for detecting inflammation in the right side of the colon.

Discussion

In this study, although all the patients were in clinical remission, 70% (42/60) of patients had ongoing endoscopic inflammation, including 25% cases with a MES score of 2. Our results suggest that patients with active intestinal inflammation occasionally have no symptoms or may fail to report symptoms. Therefore, monitoring symptoms alone lead to under-treatment of the disease and a higher likelihood of clinical recurrence. Recently, the importance of achieving mucosal healing, rather than

symptoms elimination alone, has been recognized [21]. Moreover, it has been reported that patients who achieve mucosal healing have lower rates of hospitalization and a lower risk of colectomy [1, 2]. Furthermore, in UC patients, a prolonged disease course and immunosuppressive therapy increase the risk for emerging malignancies compared to those who have a normal colon [22-24].

Recently, fecal markers have received attention as means for the noninvasive monitoring of disease activity. Several studies have reported that fecal markers such as calprotectin, lactoferrin, and hemoglobin correlate well with MES [4, 25, 26], but only few studies have examined the association between fecal markers and UCEIS [17]. In this study, we confirmed the correlation of fecal calprotectin, lactoferrin, and hemoglobin with MES or UCEIS in patients with UC in clinical remission. Surface pit pattern classification with ME is known to have a good association with the histopathological diagnosis of colorectal tumor [18]. Similarly, recent studies have shown that ME findings are significantly correlated with histological grades [27, 28]. Of note, it is reported that ME could be useful in assessing microscopic inflammatory features of UC that cannot be determined by white light colonoscopic examination [27, 28]. The use of ME in UC is relatively new and different ME classifications have been proposed to date [18, 27, 28]. Further studies are required to determine the

classifications that most accurately represent and predict the patient's clinical status and outcome. This study is the first to report that fecal calprotectin and lactoferrin levels are significantly correlated with ME grade in patients with UC in clinical remission. Moreover, the cut-off levels of fecal calprotectin, lactoferrin, or hemoglobin had a high sensitivity and specificity for the detection of MES=0, UCEIS=0, ME=A. Therefore, fecal markers should be considered as alternative monitoring tools in endoscopy and could provide an opportunity for recommending colonoscopy to patients with UC in clinical remission.

In the 10 cases with extensive colitis involving macroscopic inflammation on the right side of the colon alone, fecal calprotectin, lactoferrin, and hemoglobin, at a cut-off value for detecting UCEIS=0, had a sensitivity of 100% (10/10), 70% (7/10), and 50% (5/10), respectively. It is reported that hemoglobin tends to lose antigenicity because of its degradation by fecal bacteria and digestive enzymes [29]. Moreover, the stability of calprotectin in the feces is also reported to be greater than that of lactoferrin [6, 14]. Our results suggest that calprotectin could have a superior sensitivity, compared to other fecal markers, for the detection of inflammation on the right side of the colon, although the small sample size may have masked the true differences.

Reports show that in patients with UC, regulation of the 5-aminosalicylic acid

(ASA) dose, with the monitoring of fecal calprotectin levels, resulted in significantly lower relapse rates compared to the control group [30, 31]. Isomoto et al. demonstrated significant differences in the percentages of relapse of UC among the ME grades retrospectively. Our prospective study is ongoing, to analyze whether ME findings can predict relapse in UC. When future relapse of UC is predicted by fecal markers and colonoscopy findings, it may be possible to take preventive measures such as increasing 5-ASA dosage before symptoms appear and, thus, exclude complications and the progression to intractable cases.

In conclusion, our analysis demonstrated that fecal markers correlated not only with MES but also with UCEIS and ME and may be useful for monitoring patients with UC in clinical remission. Moreover, we suggest that calprotectin is a highly sensitive tool for detecting macroscopic inflammation on the right side of the colon. Additional prospective studies are required to ascertain whether fecal markers can be used as an alternative to endoscopy with biopsy for improving treatment optimization and outcomes.

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Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this manuscript.

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Figure legends

Figure 1a. Correlation between UCEIS and fecal markers

The relationships between calprotectin, lactoferrin, hemoglobin, and UCEIS were analyzed. A significant correlation was observed between UCEIS and all fecal markers (Spearman's rank correlation coefficient for calprotectin $r = 0.54$, $P < 0.0001$; lactoferrin $r = 0.56$, $P < 0.0001$; and hemoglobin $r = 0.43$, $P < 0.001$). FC: fecal calprotectin; Lf: lactoferrin; UCEIS: Ulcerative Colitis Endoscopic Index of Severity

Figure 1b. Correlation between ME and fecal markers

The relationship between calprotectin, lactoferrin, hemoglobin, and ME were analyzed. ME correlated significantly with calprotectin and lactoferrin levels but slightly with hemoglobin levels (calprotectin $r = 0.50$, $P < 0.001$; lactoferrin $r = 0.46$, $P < 0.001$; and hemoglobin $r = 0.28$, $P = 0.043$). FC: fecal calprotectin; Lf: lactoferrin; ME: magnifying endoscopic stratification

Figure 2a. Receiver operating curve analysis for the fecal markers (for differentiating between UCEIS=0 and ≥ 1)

Calprotectin, at a cut-off of 201 $\mu\text{g/ml}$, had a sensitivity of 70% and specificity of 81% for the detection of UCEIS=0 (prediction of mucosal healing) (AUC 0.76). Lactoferrin (cut-off 78.3 ng/ml) had a sensitivity of 57% and specificity of 88% for the detection of UCEIS=0 (AUC 0.71). Hemoglobin (cut-off 31 ng/ml) had a sensitivity of 64% and specificity of 88% for the detection of UCEIS=0 (AUC 0.74). FC: fecal calprotectin; Lf: lactoferrin; UCEIS: Ulcerative Colitis Endoscopic Index of Severity

Figure 2b. Receiver operating curve analysis for the fecal markers (for differentiating between ME=A and $\geq B$)

Calprotectin, at a cut-off of 280 $\mu\text{g/ml}$, had a sensitivity of 68% and specificity of 86% for the detection of ME=A (prediction of mucosal healing) (AUC 0.78). Lactoferrin (cut-off 43.6 ng/ml) had a sensitivity of 71% and specificity of 76% for the detection of ME=A (AUC 0.77). Hemoglobin (cut-off of 34 ng/ml) had a sensitivity of 71% and specificity of 86% for the detection of ME=A (AUC 0.78). FC: fecal calprotectin; Lf: lactoferrin; ME: magnifying endoscopic stratification

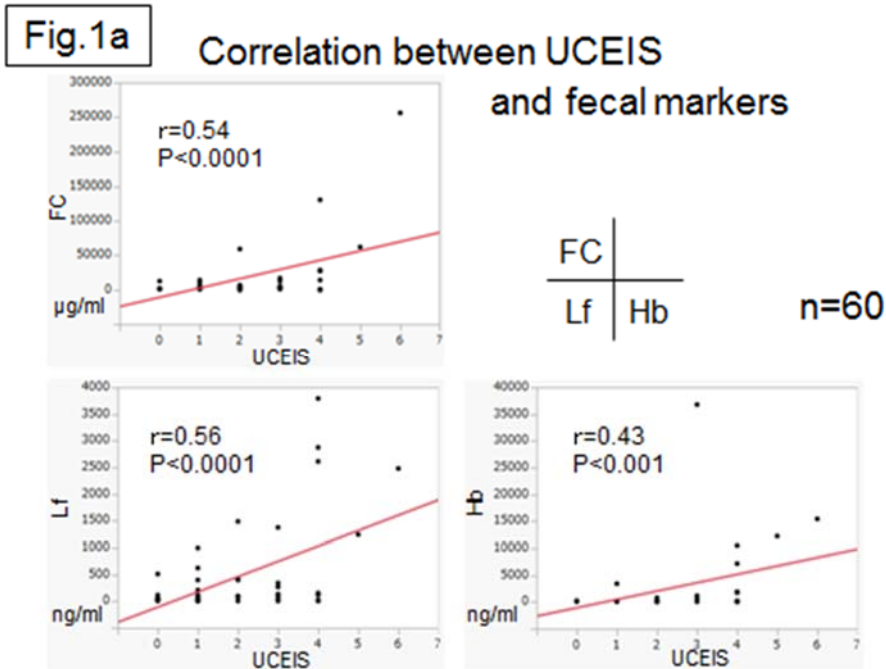


Figure 1a. Correlation between UCEIS and fecal markers

The relationships between calprotectin, lactoferrin, hemoglobin, and UCEIS were analyzed. A significant correlation was observed between UCEIS and all fecal markers (Spearman's rank correlation coefficient for calprotectin $r = 0.54$, $P < 0.0001$; lactoferrin $r = 0.56$, $P < 0.0001$; and hemoglobin $r = 0.43$, $P < 0.001$). FC: fecal calprotectin; Lf: lactoferrin; UCEIS: Ulcerative Colitis Endoscopic Index of Severity

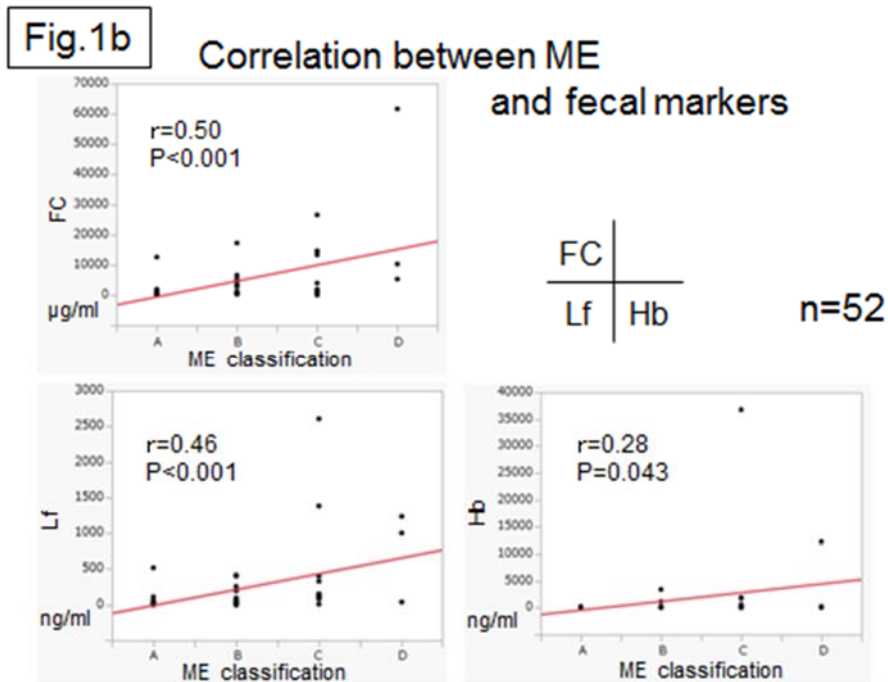


Figure 1b. Correlation between ME and fecal markers

The relationship between calprotectin, lactoferrin, hemoglobin, and ME were analyzed. ME correlated significantly with calprotectin and lactoferrin levels but slightly with hemoglobin levels (calprotectin $r = 0.50$, $P < 0.001$; lactoferrin $r = 0.46$, $P < 0.001$; and hemoglobin $r = 0.28$, $P = 0.043$). FC: fecal calprotectin; Lf: lactoferrin; ME: magnifying endoscopic stratification

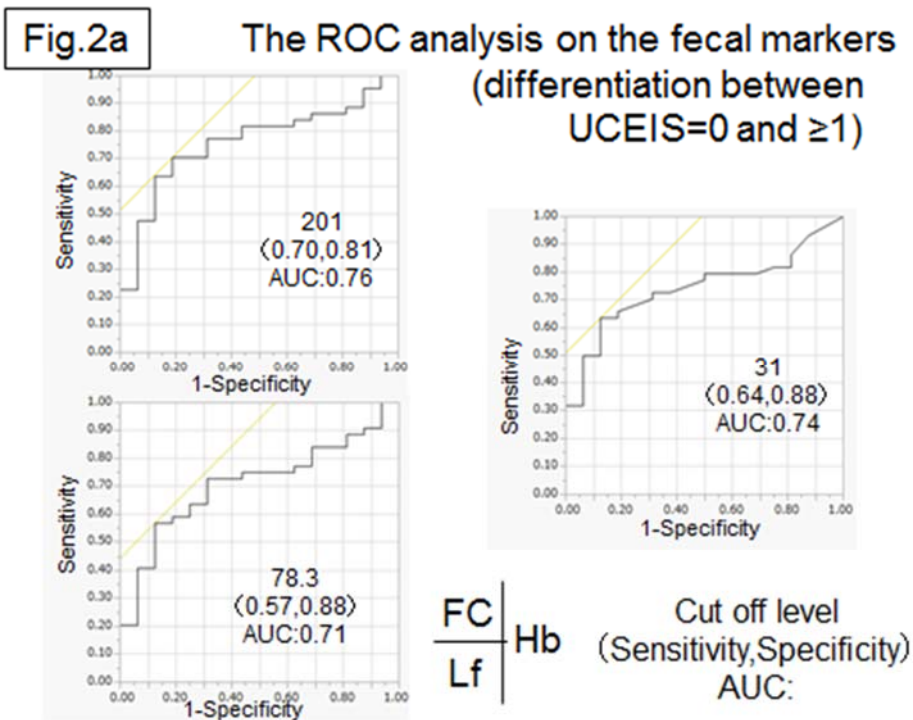


Figure 2a. Receiver operating curve analysis for the fecal markers (for differentiating between UCEIS=0 and ≥ 1)

Calprotectin, at a cut-off of 201 $\mu\text{g/ml}$, had a sensitivity of 70% and specificity of 81% for the detection of UCEIS=0 (prediction of mucosal healing) (AUC 0.76). Lactoferrin (cut-off 78.3 ng/ml) had a sensitivity of 57% and specificity of 88% for the detection of UCEIS=0 (AUC 0.71). Hemoglobin (cut-off 31 ng/ml) had a sensitivity of 64% and specificity of 88% for the detection of UCEIS=0 (AUC 0.74). FC: fecal calprotectin; Lf: lactoferrin; UCEIS: Ulcerative Colitis Endoscopic Index of Severity

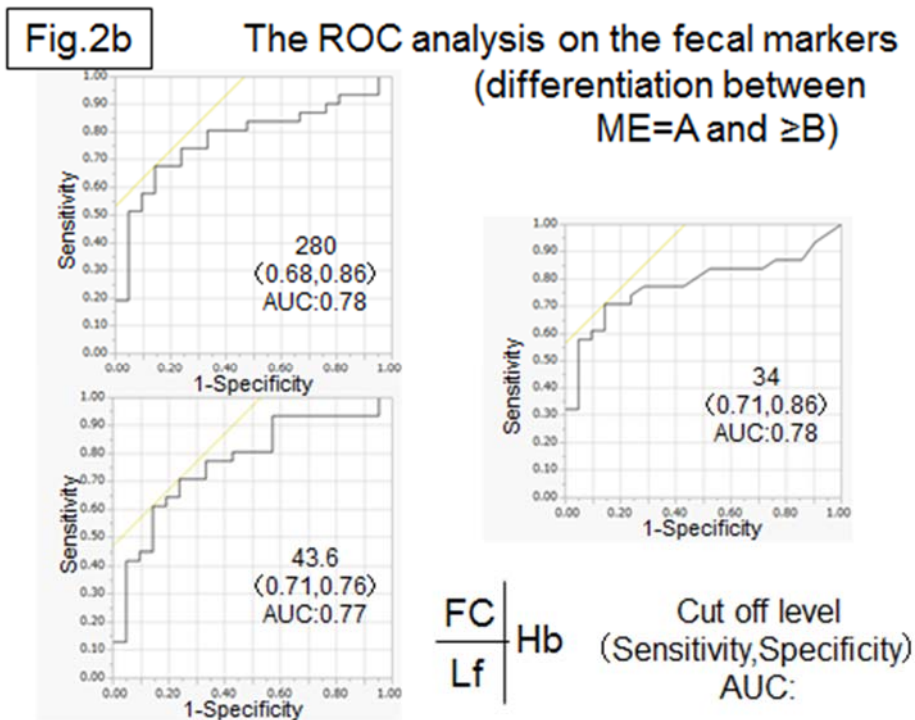


Figure 2b. Receiver operating curve analysis for the fecal markers (for differentiating between ME=A and \geq B)

Calprotectin, at a cut-off of 280 μ g/ml, had a sensitivity of 68% and specificity of 86% for the detection of ME=A (prediction of mucosal healing) (AUC 0.78). Lactoferrin (cut-off 43.6 ng/ml) had a sensitivity of 71% and specificity of 76% for the detection of ME=A (AUC 0.77). Hemoglobin (cut-off of 34 ng/ml) had a sensitivity of 71% and specificity of 86% for the detection of ME=A (AUC 0.78). FC: fecal calprotectin; Lf: lactoferrin; ME: magnifying endoscopic stratification

Table 1. Characteristics of patients

Patients	
Total	60
Age [Median (range)] years	51 (20-79)
Gender	
Male	35 (58%)
Female	25 (42%)
Extent of inflammation	
Extensive colitis	40 (67%)
Left-sided colitis	13 (22%)
Proctitis	7 (12%)
Concomitant medications	
None	6
Salazosulfapyridine	5
Mesalamine	43
Azathioprine / Mercaptopurine	9 / 4
Infliximab / Adalimumab	3 / 2

Table 2. Summary of the relationship between magnifying endoscopic (ME) classifications and Ulcerative Colitis Endoscopic Index of Severity (UCEIS)

ME-classification	0 (n=15)	1 (n=18)	2 (n=6)	3 (n=8)	4 (n=4)	5< (n=1)
ME-A (n=21)	15	6				
ME-B (n=17)		9	4	3	1	
ME-C (n=12)		3	2	4	3	
ME-D (n=2)				1		1

A significant correlation was observed between ME and UCEIS ($r = 0.74$, $P < 0.0001$).