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Optimal positioning of biomarkers according to ulcerative colitis activity

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The diagnostic performance and clinical utility of fecal calprotectin (FC), fecal immunochemical occult blood test (FIT), leucine-rich alpha-2 glycoprotein (LRG), C-reactive protein (CRP), and prostaglandin E-major urinary metabolite (PGE-MUM) as established biomarkers for ulcerative colitis (UC) were evaluated. Significant correlations were observed between the clinical activity index, Mayo endoscopic subscore (MES), and each biomarker. Among MES groups, fecal biomarkers demonstrated significant differences, except between MES 2 and MES 3. CRP and LRG showed significant differences, except between MES 1 and MES 2. PGE-MUM exhibited significant differences across all MES groups. Areas under the curve (AUCs) for receiver operating characteristic (ROC) analysis in predicting MES 0 or 1 were as follows: FC, 0.891; FIT, 0.853; LRG, 0.723; CRP, 0.747; PGE-MUM, 0.795. For predicting MES 0 alone, AUCs were as follows: FC, 0.885; FIT, 0.845; LRG, 0.708; CRP, 0.691; PGE-MUM, 0.732. In distinguishing between each MES group, fecal biomarkers exhibited the highest AUC and accuracy in differentiating MES 0 from MES 1, whereas LRG, CRP, and PGE-MUM were most effective in differentiating MES 2 from MES 3. In summary, in UC, fecal biomarkers effectively detect mucosal healing, whereas LRG, CRP, and PGE-MUM are valuable for assessing mucosal healing and active inflammation.

Keyword Biomarker, Fecal calprotectin, Fecal immunochemical occult blood, Ulcerative colitis

In recent years, treatment options for ulcerative colitis (UC), a chronic and difficult-to-treat inflammatory disease, have expanded significantly. This advancement has enabled the attainment of ambitious treatment goals, shifting the recommended treatment objective for UC from clinical remission to mucosal healing (MH), as per the Selecting Therapeutic Targets in Inflammatory Bowel Disease-II guidelines[1]. Biomarkers serve a crucial role in achieving mucosal healing. While colonoscopy (CS) is the gold standard for assessing mucosal healing in UC, its frequent requirement and high costs cause significant patient burden. Therefore, suitable biomarkers offer a practical alternative to ascertain endoscopic activity and measure UC severity.

The biomarkers for UC can be broadly categorized based on the sample used, such as stool, blood, and urine. For instance, fecal calprotectin (FC), a complex S100A protein found in stool due to intestinal inflammation, reportedly reflects mucosal healing and endoscopic remission. The potential of FC to indicate treatment efficacy has been ascertained in numerous large-scale clinical trials[2–4]. Its reliability as a biomarker is emphasized by its recommendation in the European Crohn's and Colitis Organisation (ECCO) guidelines[5]. Another stool-based biomarker, the fecal immunochemical occult blood test (FIT), quantitatively measures bleeding caused by UC inflammation. Like FC, FIT is useful for determining the achievement of mucosal healing and endoscopic remission[6].

Among blood biomarkers, C-reactive protein (CRP) is a conventional marker for systemic inflammation produced in response to interleukin-6 (IL-6), which can be associated with a variety of conditions, including UC[7]. Another blood biomarker, leucine-rich α2-glycoprotein (LRG), which is produced in response to cytokines other than IL-6, specifically reflects intestinal inflammation. Shinzaki et al. indicated that LRG provides a better reflection of endoscopic and histological activity in UC than CRP[8].

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Furthermore, Arai et al. reported that prostaglandin E-major urinary metabolite (PGE-MUM), a novel biomarker measured using urine samples, reflects UC activity more accurately than CRP[9]. However, the measurement method for PGE-MUM has been changed from radioimmunoassay (RIA) to chemiluminescent enzyme immunoassay (CLEIA). Although studies have explored PGE-MUM measured by CLEIA in pediatric UC cases, no studies have examined its application in adult UC[10].

Despite the growing options for measuring biomarkers in UC, their optimal usage remains unclear. Therefore, in this study, we aimed to evaluate the use of the five biomarkers, including FC and FIT (fecal), LRG and CRP (blood), and PGE-MUM (urinary), in assessing UC activity.

Results

Patient characteristics

In total, 149 patients with UC undergoing CS whose samples underwent the measurement of the five biomarkers were enrolled (Table 1). The median age of the participants and their disease duration were 46 and 10 years, respectively. The cohort included 101 men and 48 women. The median clinical activity index (CAI; Rachmilewitz index) was 1. Among the patients, 62, 49, 26, and 12 had Mayo endoscopic score (MES) readings of 0, 1, 2, and 3, respectively. The median values of the five biomarkers were as follows: FC, 326 mg/kg; FIT, 30 ng/mL; LRG, 12.9 μg/mL; CRP, 0.07 mg/dL; and PGE-MUM, 23.6 μg/g·Cr.

Correlation between biomarkers, endoscopic score, and the CAI

The CAI showed a significant correlation with all biomarkers. Particularly, fecal biomarkers showed a positive correlation with the CAI, while correlations with LRG, CRP, and PGE-MUM were weaker (Table 2). The MES also exhibited a significant correlation with all biomarkers, with fecal biomarkers showing particularly strong correlations. All biomarkers significantly correlated with each other. In particular, the FIT and FC findings demonstrated a strong correlation.

Achieving MH and endoscopic healing (EH) with biomarkers

The significant differences in each biomarker were evaluated for each of the four groups from MES 0 to 3 (Fig. 1). FC and FIT showed significant differences between all groups except MES 2 and MES 3 (Fig. 1A,B). LRG and CRP showed significant differences between all groups except MES 1 and MES 2 (Fig. 1C,D). PGE-MUM showed significant differences between all groups (Fig. 1E). The significant differences in biomarkers associated

Characteristics	All N = 149
Age (year), median [IQR]	46 [31, 60]
Male/Female, n (%)	101 (67.8)/48 (32.2)
Disease duration (year), median [IQR]	10 [14, 15]
Disease extent, n (%)	
Extensive colitis	108 (72.5)
Left-sided colitis	31 (20.8)
Proctitis	10 (6.7)
CAI (Rachmilewitz index), median [IQR]	1 [0, 3]
MES, n (%)	
MES 0	62 (41.6)
MES 1	49 (32.9)
MES 2	26 (17.4)
MES 3	12 (8.1)
FC (mg/kg), median [IQR]	326 [77, 1,770]
FIT (ng/mL), median [IQR]	30 [30, 579]
LRG (μg/mL), median [IQR]	12.9 [10.4, 17.0]
CRP (mg/dL), median [IQR]	0.07 [0.03, 0.20]
PGE-MUM (μg/g·Cr), median [IQR]	23.6 [16.9, 37.5]
Medication at study, n (%)	
Oral 5-ASA	102 (68.5)
Suppository steroids	9 (6.0)
Systemic steroids	17 (11.4)
Immunomodulators	44 (29.5)
Biologics	67 (45.0)

Table 1. Baseline patient characteristics. 5-ASA, 5-aminosalicylic acid; CAI, clinical activity index; CRP, C-reactive protein; ED, elemental diet; FC, fecal calprotectin; FIT, fecal immunochemical occult blood test; IQR, interquartile range; LRG, leucine-rich alpha 2 glycoprotein; MES, Mayo endoscopic subscore; PGE-MUM, prostaglandin E-major urinary metabolite.

	FC		FIT		LRG		CRP		PGE-MUM	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
CAI	0.520	< 0.001	0.610	< 0.001	0.391	< 0.001	0.394	< 0.001	0.298	< 0.001
MES	0.724	< 0.001	0.715	< 0.001	0.425	< 0.001	0.414	< 0.001	0.496	< 0.001
PGE-MUM	0.243	0.003	0.185	0.024	0.322	< 0.001	0.164	0.046		
CRP	0.245	0.003	0.218	0.008	0.658	< 0.001				
LRG	0.283	< 0.001	0.196	0.017						
FIT	0.707	< 0.001								

Table 2. Correlation between the clinical activity index, endoscopic score, and biomarkers. CAI, clinical activity index; CRP, C-reactive protein; FC, fecal calprotectin; FIT, fecal immunochemical occult blood test; LRG, leucine-rich alpha 2 glycoprotein; MES, Mayo endoscopic subscore; PGE-MUM, prostaglandin E-major urinary metabolite; r, correlation coefficient.

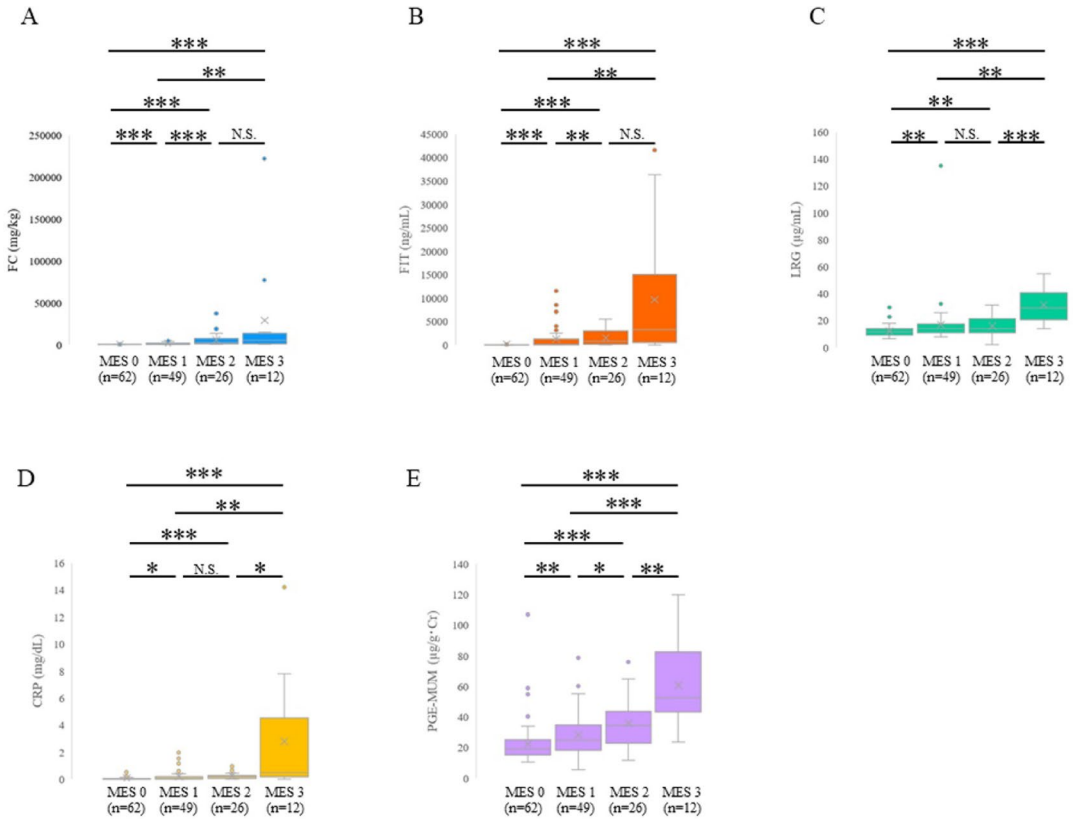

Fig. 1. Differences in five biomarkers across the Mayo endoscopic subscore (MES) groups. The panels show differences in fecal calprotectin (FC) (**A**), fecal immunochemical occult blood test (FIT) (**B**), leucine-rich alpha 2 glycoprotein (LRG) (**C**), C-reactive protein (CRP) (**D**), and prostaglandin E-major urinary metabolite (PGE-MUM) (**E**) between the MES groups. Statistical significance is indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; N.S., not significant.

with achieving MH and EH were evaluated (Fig. 2). The values of all five biomarkers were significantly higher in the MES 2 and 3 than those in the MES 0 and 1 groups, and in the MES 1, 2, and 3 groups than those in the MES 0 group.

Receiver operating characteristic (ROC) analyses were performed to predict MH and EH (Table 3). The cutoff values for predicting MH were 416 mg/kg for FC, 38 ng/mL for FIT, 17.8 μg/mL for LRG, 0.10 mg/dL for CRP, and 32.0 μg/g Cr for PGE-MUM. The area under the curve (AUC) for predicting MH was approximately 0.8 for FC and FIT and 0.7 for LRG, CRP, and PGE-MUM. For predicting EH, the cutoff values were 261 mg/kg for FC, 31 ng/mL for FIT, 14.9 μg/mL for LRG, 0.10 mg/dL for CRP, and 29.5 μg/g · Cr for PGE-MUM. The AUC for predicting EH was approximately 0.8 for FC and FIT, 0.7 for LRG and PGE-MUM, and 0.6 for CRP.

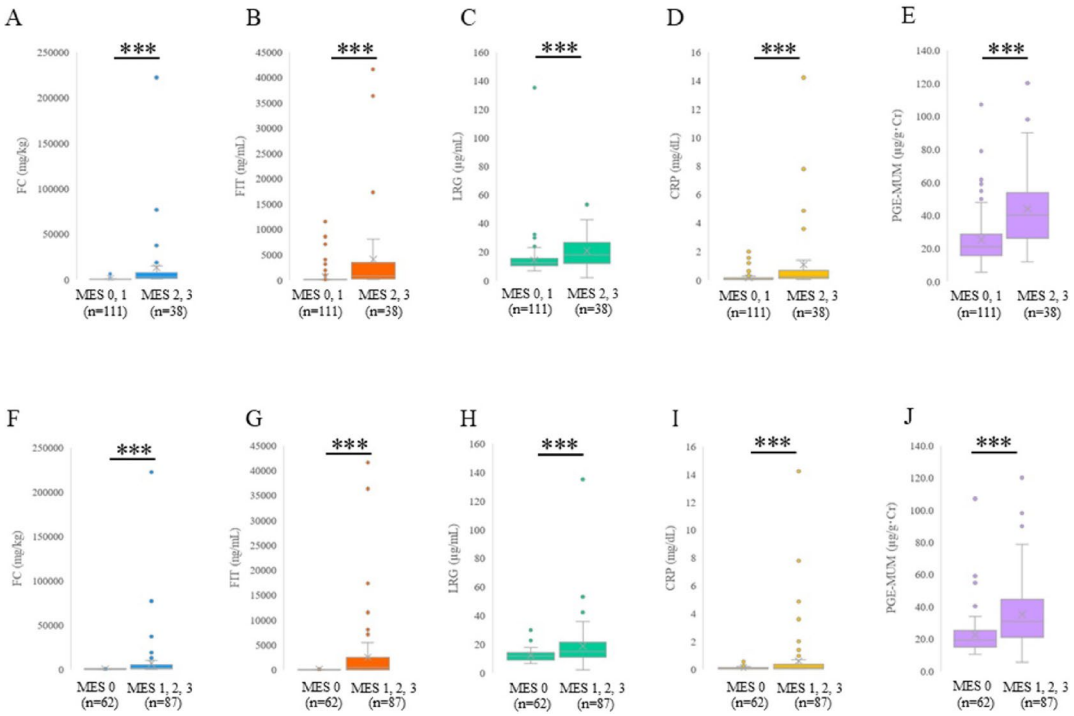

Fig. 2. Differences in biomarkers between the Mayo Endoscopic Subscore (MES) groups. The panels show differences in fecal calprotectin (FC) (**A**), fecal immunochemical occult blood test (FIT) (**B**), leucine-rich alpha 2 glycoprotein (LRG) (**C**), C-reactive protein (CRP) (**D**), and prostaglandin E-major urinary metabolite (PGE-MUM) (**E**) between the MES 0, 1 versus MES 2, 3 groups. Panels (**F**) through (**J**) display differences in FC (**F**), FIT (**G**), LRG (**H**), CRP (**I**), and PGE-MUM (**J**) between MES 0 versus MES 1, 2, and 3 groups.

	Biomarker	Cutoff value	AUC [95% CI]	PPV	NPV	Sensitivity	Specificity	Accuracy
MES 0, 1	FC	416 mg/kg	0.891 [0.839–0.944]	0.976	0.545	0.730	0.947	0.785
	FIT	38 ng/mL	0.853 [0.789–0.917]	0.976	0.554	0.739	0.947	0.792
	LRG	17.8 μg/mL	0.723 [0.623–0.823]	0.845	0.606	0.883	0.526	0.792
	CRP	0.10 mg/dL	0.747 [0.650–0.844]	0.894	0.453	0.685	0.763	0.705
	PGE-MUM	32.0 μg/g·Cr	0.795 [0.713–0.878]	0.883	0.231	0.820	0.333	0.752
MES 0	FC	261 mg/kg	0.885 [0.833–0.937]	0.761	0.866	0.823	0.816	0.819
	FIT	31 ng/mL	0.845 [0.792–0.897]	0.707	0.940	0.935	0.724	0.812
	LRG	14.9 μg/mL	0.708 [0.625–0.790]	0.553	0.818	0.839	0.517	0.651
	CRP	0.10 mg/dL	0.691 [0.608–0.775]	0.565	0.781	0.774	0.575	0.658
	PGE-MUM	29.5 μg/g·Cr	0.732 [0.651–0.814]	0.573	0.868	0.887	0.529	0.678

Table 3. Receiver operating characteristic curve results for predicting Mayo endoscopic subscore (MES) 0, 1 and MES 0. AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; FC, fecal calprotectin; FIT, fecal immunochemical occult blood test; LRG, leucine-rich alpha 2 glycoprotein; NPV, negative predictive value; PPV, positive predictive value; PGE-MUM, prostaglandin E-major urinary metabolite.

Usefulness of biomarkers for UC activity

To assess the utility of biomarkers in relation to disease activity, we first evaluated the correlation coefficients within the MES 0–2 group (remission to moderate disease) and the MES 1–3 group (mild to severe disease) (Table 4). Ideally, participants would be divided into MES 0–1 and MES 2–3 groups for separate analysis; however, we proceeded with the broader groupings as described due to limitations in correlation analysis with such division. To account for limitations in correlation analysis, patients were grouped as MES 0–2 and MES 1–3, rather than MES 0–1 and MES 2–3. Despite this broader grouping, significant correlations were observed in both groups, with higher correlation coefficients for FC and FIT in the MES 0–2 group than those in the MES 1–3 group. Conversely, the correlation coefficients for LRG, CRP, and PGE-MUM were higher in the MES 1–3 group than those in the MES 0–2 group.

Furthermore, to evaluate the discriminatory ability for each severity, ROC analysis was performed to discriminate between the two MES groups (Fig. 3). For example, in the analysis of MES 0 vs. 1, ROC analysis

	All (N = 149)		MES 0–2 (n = 137)		MES 1–3 (n = 87)	
	r	*P*	r	*P*	r	*P*
FC	0.724	< 0.001	0.690	< 0.001	0.496	< 0.001
FIT	0.715	< 0.001	0.689	< 0.001	0.398	< 0.001
LRG	0.425	< 0.001	0.290	< 0.001	0.360	< 0.001
CRP	0.414	< 0.001	0.317	< 0.001	0.370	< 0.001
PGE-MUM	0.496	< 0.001	0.390	< 0.001	0.459	< 0.001

Table 4. Correlation between the Mayo endoscopic subscores (MES) and biomarkers in the groups of MES 0–2 and MES 1–3. CRP, C-reactive protein; FC, fecal calprotectin; FIT, fecal immunochemical occult blood test; LRG, leucine-rich alpha 2 glycoprotein; PGE-MUM, prostaglandin E-major urinary metabolite; r, correlation coefficient.

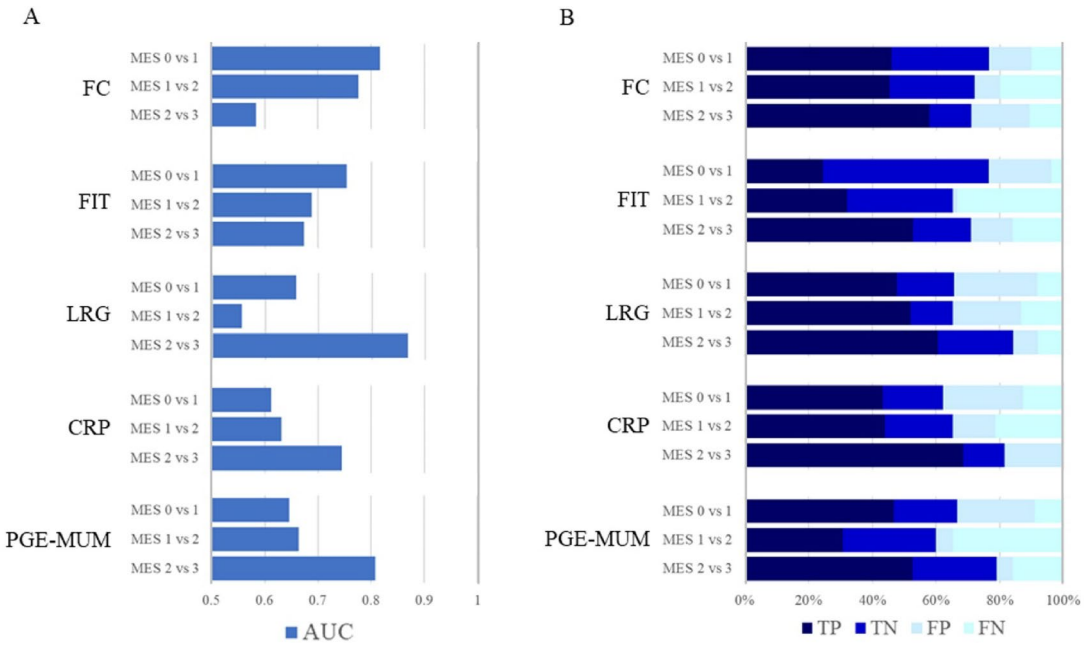

Fig. 3. Receiver operating characteristic (ROC) analysis for predicting the division of two Mayo endoscopic subscore (MES) groups. The area under the curve (AUC) bar graphs of fecal calprotectin (FC), fecal immunochemical occult blood test (FIT), leucine-rich alpha 2 glycoprotein (LRG), C-reactive protein (CRP), and prostaglandin E-major urinary metabolite (PGE-MUM) in ROC analysis for predicting MES group divisions (**A**). Panel (**B**) shows the accuracy of each biomarker as derived from ROC analysis, with TP indicating true positive, TN indicating true negative, FP indicating false positive, and FN indicating false negative.

was performed to predict only MES 0 for a total of 111 patients, including 62 patients with MES 0 and 49 patients with MES 1. Figure 3A shows a bar graph of the AUC values calculated from the ROC analysis. The AUC values for FC and FIT decreased in the order of MES 0 vs. 1, MES 1 vs. 2, and MES 2 vs. 3, whereas those for CRP and PGE-MUM increased. For LRG, the AUC value was the highest for the MES 2 vs. 3 comparison. Figure 3B shows a bar graph of the true positive (TP), true negative (TN), false positive (FP), and false negative (FN) values based on the cutoff value obtained from ROC analysis, providing an evaluation of test accuracy. The accuracy of the test is represented by the sum of TP and TN. FC and FIT demonstrated high accuracy, particularly in distinguishing MES 0 from MES 1. Conversely, LRG, CRP, and PGE-MUM have higher accuracy in differentiating between MES 2 and MES 3.

Biomarkers and histological evaluation

A retrospective study was conducted on 116 cases, in which biomarker measurements were paired with histological evaluation using the Geboes histological score (GHS) (Table 5). ROC analysis for predicting histological healing (HH) identified the following cutoff values: 416 mg/kg for FC and 31 ng/mL for FIT, with high AUCs of 0.802 and 0.769, respectively. For the same biomarkers, the cutoff values were 15.5 µg/mL for LRG, 0.09 mg/dL for CRP, and 30.7 µg/g · Cr for PGE-MUM. The AUCs for these biomarkers ranged around 0.6, which was slightly lower than those for the fecal biomarkers.

Biomarker	Cutoff value	AUC [95% CI]	PPV	NPV	Sensitivity	Specificity	Accuracy
FC	416 mg/kg	0.802 [0.722–0.882]	0.667	0.894	0.902	0.646	0.759
FIT	31 ng/mL	0.769 [0.695–0.842]	0.657	0.857	0.863	0.646	0.741
LRG	15.5 μg/mL	0.607 [0.503–0.710]	0.506	0.714	0.804	0.385	0.569
CRP	0.09 mg/dL	0.649 [0.549–0.749]	0.554	0.706	0.706	0.554	0.621
PGE-MUM	30.7 μg/g·Cr	0.624 [0.520–0.728]	0.554	0.762	0.804	0.492	0.629

Table 5. Receiver operating characteristic for predicting histological healing. AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; FC, fecal calprotectin; FIT, fecal immunochemical occult blood test; LRG, leucine-rich alpha 2 glycoprotein; NPV, negative predictive value; PPV, positive predictive value; PGE-MUM, prostaglandin E-major urinary metabolite.

Discussion

The utility of various biomarkers in UC has been well-documented in previous studies, yet their optimal application remains uncertain. This study compared five biomarkers: fecal biomarkers, FC and FIT; blood biomarkers, LRG and CRP; and a novel urinary biomarker, PGE-MUM. All five biomarkers demonstrated significant correlations with clinical and endoscopic scores. The ROC analyses indicated that fecal biomarkers are particularly effective for determining MH and EH. However, the ROC analysis for MES 0 vs. 1, MES 1 vs. 2, and MES 2 vs. 3 suggested that LRG, CRP, and PGE-MUM could also be valuable for assessing various levels of endoscopic activity.

FC is a widely used biomarker for evaluating disease activity in large-scale clinical trials, with its use being endorsed by the ECCO[11]. FIT, which uses the same stool sample as FC, is similarly employed in clinical practice. CRP has long been utilized as a blood biomarker, whereas the novel blood biomarker, LRG, promises superior performance to that of CRP and is increasingly implemented in practice[8]. PGE-MUM, a urinary biomarker, reportedly reflects endoscopic activity in adult UC cases, although previous studies relied on the RIA method[9,12,13]. Hagiwara et al. reported that PGE-MUM was measured by CLEIA and RIA in the same patient with pediatric UC and showed a correlation[10]. Currently, PGE-MUM is measured using the CLEIA method; however, reports on its application in adult UC are scarce. Additionally, no studies have directly compared this biomarker with the other four.

Although no significant difference was observed for FC and FIT between the MES 2 and MES 3 groups in the significance tests, their AUCs were ≥ 0.8 in ROC analyses for predicting MH (MES 0, 1) and EH (MES 0). These AUC values were higher than those of other biomarkers. Additionally, the ROC analysis for discriminating between MES 0 and 1 showed higher AUCs and accuracy for FC and FIT, suggesting that fecal biomarkers are particularly useful for assessing remission status.

In the retrospective analysis, fecal biomarkers demonstrated larger AUCs and higher accuracy in the ROC analysis for histological evaluation than the other biomarkers. Takashima et al. reported that the sensitivity and specificity of FIT values (< 100 ng/mL) for MES 0 were 0.95 and 0.62, respectively, whereas FC values (< 250 μg/g) exhibited sensitivities of 0.82 and 0.62, respectively[6]. Furthermore, when MES was defined as 0 and 1, the sensitivity for FC and FIT was nearly identical (0.86 vs. 0.86), indicating that both biomarkers are highly accurate for determining MH and EH. Additionally, the International Delphi Consensus, which examined FC and CRP, recommended prioritizing FC levels over CRP levels for assessing remission with 100% agreement[14]. Recent studies have increasingly focused on comparing various biomarkers directly with respect to their association with endoscopic activity.

Yasutomi et al. conducted a ROC analysis to predict MES 0 using LRG, CRP, FC, and FIT. The AUCs for FC and FIT were 0.72 and 0.75, respectively, whereas those for LRG and CRP were 0.61 and 0.59, respectively[15]. Similarly, Shimoyama et al. reported AUCs of 0.91, 0.80, and 0.72 for FC, LRG, and CRP, respectively, in predicting MES 1 or higher[16]. Consistent with these findings, the present study also supports the utility of fecal biomarkers in determining EH at MES 0, reinforcing their value for assessing remission.

Conversely, CRP, PGE-MUM, and LRG may be less accurate in evaluating remission but could be useful for assessing the extent of UC activity. There is limited research on the optimal biomarkers for assessing endoscopic activity during active disease phases. Our previous study evaluated the relationship between endoscopic scores for the entire colon and various biomarkers[17]. In that study, FC and FIT were relatively well correlated with descriptors of the Ulcerative Colitis Colonoscopic Index of Severity (UCCIS) for UC in remission (MES 0 and 1). In contrast, only CRP showed a correlation with UCCIS descriptors in UC with active disease (MES 2 and 3), suggesting that CRP may be particularly useful for assessing endoscopic activity.

Furthermore, fecal biomarkers, such as FC and FIT, which measure protein leakage and bleeding, respectively, can detect more subtle intestinal pathology and reflect activity more precisely. Conversely, blood and urinary biomarkers, which are derived from systemic circulation, may be less sensitive to subtle changes but can indicate a broader range of disease activity. Although clinicians working with inflammatory bowel disease (IBD) biomarkers may intuitively understand this distinction, the current study provides further statistical validation for these observations.

Although previous studies have evaluated multiple biomarkers, most have focused on determining remission states, and few aimed to investigate the active phase of the disease. A key strength of this study includes its comprehensive evaluation across the full spectrum of disease activity using biomarkers that are widely applied in clinical practice. Specifically, our findings suggest that fecal biomarkers, such as FC and FIT, are useful for

evaluating remission. In contrast, LRG, CRP, and PGE-MUM may be more effective for assessing active disease. However, the present study has certain limitations. First, this was a single-center study, which may limit the generalizability of the findings. Second, although the study included an assessment of active disease, the sample size for active patients was small and not balanced by the level of disease activity. Third, the small number of MES 2 and 3 cases may have reduced the statistical power of subgroup analyses, potentially leading to instability in estimates. Finally, histological data were retrospectively collected, limiting the ability to evaluate all registered patients, which may have introduced selection bias due to incomplete pathology evaluations.

In conclusion, fecal biomarkers, such as FC and FIT, proved effective for assessing endoscopic activity in states closer to remission but were less useful for evaluating active disease. Conversely, blood biomarkers, such as LRG and CRP, and the urinary biomarker, PGE-MUM, were less effective than fecal biomarkers for assessing endoscopic activity near remission but may be valuable for evaluating a broader range of disease activity. These findings underscore the clinical utility of these biomarkers and support their continued use as newer biomarkers emerge.

Methods

Ethics approval

The study was conducted in accordance with Good Clinical Practice principles in adherence to the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of Hamamatsu University School of Medicine (number 20-178). The authors are accountable for all aspects of the work and ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Study design

The purpose of this prospective single-center study was to evaluate the usefulness of biomarkers used in UC and to determine their relevance in assessing disease activity. The primary outcome was to assess MH and EH using each biomarker, which was achieved by analyzing the correlation between biomarkers and conducting ROC analysis to predict MH and EH. The secondary outcome was the ability of each biomarker to assess activity across all severity levels. This involved determining whether each biomarker could distinguish between MES 0, 1, 2, and 3 activity levels.

Patients

In total, 149 patients with UC who were treated at the Hamamatsu University School of Medicine between July 2021 and May 2024 were enrolled. These patients presented typical clinical symptoms, endoscopic findings, and histological findings and were diagnosed with UC based on established criteria[11]. Patients with IBD other than UC, such as Crohn's disease (CD), IBD unclassified, or Behçet's disease, were excluded. Moreover, those using non-steroidal anti-inflammatory drugs, as well as those with infections and malignancies, which affect the FC and PGE-MUM levels, were excluded to maintain the specificity of biomarker characteristics[5,18–20]. Additionally, patients with lung disease and smoking habits were also excluded due to the reported elevation of PGE-MUM in these conditions[21,22].

Disease assessment

The clinical activity of UC was evaluated using the CAI, according to the method described by Rachmilewitz[23]. Endoscopic evaluation was performed using the MES, classified as follows: 0, normal or inactive disease; 1, mild disease with erythema, decreased vascular pattern, and mild friability; 2, moderate disease with marked erythema, absence of vascular patterns, friability, and erosions; and 3, severe disease with spontaneous bleeding and ulceration[24]. In this study, MES 0 and 1 were defined as MH, and MES 0 as EH.

Biomarker measurement

As colonoscopic preparation could influence the results of the fecal biomarkers, samples for FC and FIT measurements were collected a few days ahead of colonoscopic preparation. Fecal samples for FC measurement were collected in plastic tubes and stored at − 20 °C until shipment to the laboratory (SRL Inc., Tokyo, Japan). FC measurement was performed using a Phadia 250 immunoanalyzer (Hitachi Ltd., Tokyo, Japan) and the Elia A Calprotectin 2 reagent (Phadia GmbH, Freiburg, Germany) based on fluorescence enzyme immunoassay principles. Fecal samples for FIT measurements were collected using a dedicated kit (Eiken Chemical, Tokyo, Japan). The samples were immediately processed and examined by OC Sensor IO (Eiken Chemical) at our facility.

Blood and urine samples were collected on the day of endoscopy or several days before. Blood samples for LRG measurement were centrifuged at 1,500 rpm for 15 min and frozen at − 80 °C until use. The samples were sent to a laboratory (SRL Inc.) where LRG was measured using a JCA-BM8000 analyzer (JEOL Ltd., Tokyo, Japan) and the Nanopia LRG (Sekisui Medical Company Limited, Tokyo, Japan) employing the latex agglutination immunoassay method. The CRP levels were measured in the same blood samples used for measuring LRG at our facility as part of routine clinical testing.

Spot urinary samples collected for PGE-MUM measurements were frozen at − 20 °C and delivered to the SRL Hachioji Laboratory (Tokyo, Japan). PGE-MUM measurements were performed using the LUMIPULSE System (Fujirebio Inc., Tokyo, Japan). This study included only PGE-MUM measured by the CLEIA method, excluding measurements by the RIA method. The presented PGE-MUM values were corrected for urinary creatinine levels to avoid the influence of urine concentration.

Pathological assessment

Histological evaluations were performed at our institution, with some patients undergoing pathological evaluation during CS. In 116 patients, histological evaluation was performed using the GHS; however, obtaining evaluations for all patients was not feasible, as the data were retrospectively collected during routine clinical practices[25]. Patients without pathology evaluation included cases where tissue sampling was not performed at the discretion of the CS physician or where the pathologist did not assess all Gebose scores. Cases lacking histological data were excluded from the analysis and were not imputed. Biopsies were endoscopically conducted from the site of the most severe disease. GHS was classified on a scale from 0 to 5, and in this study, a GHS < 3 was defined as HH.

Statistical analysis

Correlations between endoscopic scores and biomarkers were assessed using Spearman's correlation coefficient. Differences between the two groups were evaluated with the Mann–Whitney U test. For comparisons among three or more groups, the Kruskal–Wallis test was used to test for significance. ROC analysis was performed to predict the achievement of MH, EH, and each endoscopic score group. Because this study was an exploratory analysis, no adjustments were made for multiple comparisons. The level of significance was set at $P < 0.05$. Statistical analyses were performed with IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, NY, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) software[26].

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Received: 20 March 2025; Accepted: 29 May 2025
Published online: 06 June 2025

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Acknowledgements
We thank the enrolled patients with IBD who provided samples for this study. We would like to express our sincere gratitude to the staff of the gastroenterology ward, outpatient clinic, endoscopic center, and laboratory at our institution. No funding was received for conducting this study.

Author contributions
NI contributed to this work. NI and KS designed the study. NI, TT, KT, and YA collected the data. NI analyzed the data. NI and KS wrote the paper. TM, MY, MI, YH, TY, and SO provided critical insight regarding paper preparation.

Declarations

Ethics approval and consent to participate
This research was approved by the Ethics Committee of Hamamatsu University School of Medicine (number 20-178). Written informed consent was obtained from all participants.

Competing interests
The authors declare no competing interests.

Additional information
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