



Combination of leucine-rich alpha-2 glycoprotein and fecal markers detect Crohn's disease activity confirmed by balloon-assisted enteroscopy

Ami Kawamoto^{1,2*}, Kento Takenaka^{1*}, Shuji Hibiya^{1,2}, Yoshio Kitazume³, Hiromichi Shimizu¹, Toshimitsu Fujii¹, Eiko Saito¹, Kazuo Ohtsuka^{1,2}, Ryuichi Okamoto¹

¹Department of Gastroenterology and Hepatology, ²Endoscopic Unit, and ³Department of Radiology, Tokyo Medical and Dental University, Tokyo, Japan

Background/Aims: Endoscopic activity confirmed by enteroscopy is associated with poor clinical outcome in Crohn's disease (CD). We investigated which of the existing biomarkers best reflects endoscopic activity in CD patients including the small bowel, and whether their combined use can improve accuracy. **Methods:** One hundred and four consecutive patients with ileal and ileocolonic type CD who underwent balloon-assisted enteroscopy (BAE) from October 2021 to August 2022 were enrolled, with clinical and laboratory data prospectively collected and analyzed. **Results:** Hemoglobin, platelet count, C-reactive protein, leucine-rich alpha-2 glycoprotein (LRG), fecal calprotectin, and fecal hemoglobin all showed significant difference in those with ulcers found on BAE. LRG and fecal calprotectin showed the highest areas under the curve (0.841 and 0.853) for detecting ulcers. LRG showed a sensitivity of 78% and specificity of 80% at a cutoff value of 13 µg/mL, whereas fecal calprotectin showed a sensitivity of 91% and specificity of 67% at a cutoff value of 151 µg/g. Dual positivity for LRG and fecal calprotectin, as well as LRG and fecal hemoglobin, both predicted ulcers with an improved specificity of 92% and 100%. A positive LRG or fecal calprotectin/hemoglobin showed an improved sensitivity of 96% and 91%. Positivity for LRG and either of the fecal biomarkers was associated with increased risk of hospitalization, surgery, and relapse. **Conclusions:** The biomarkers LRG, fecal calprotectin, and fecal hemoglobin can serve as noninvasive and accurate tools for assessing activity in CD patients confirmed by BAE, especially when used in combination. (*Intest Res* 2024;22:65-74)

Key Words: Crohn disease; Leucine-rich alpha2-glycoprotein, human; Leukocyte L1 antigen complex; Biomarkers; Inflammatory bowel diseases

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disease characterized by ulcers of the small and large bowels, progressing to the development of complications including strictures and fistulae. The treat-to-target approach is the treatment strategy that

is currently recommended in treating inflammatory bowel disease (IBD) patients, where not only clinical remission but also endoscopic remission is set as a formal target.¹ Endoscopic remission, in this context, is defined as that which is confirmed by ileocolonoscopy, the preferred method worldwide for assessing mucosal healing in patients with CD. However, patients can often manifest with more proximal lesions that cannot be reached by ileocolonoscopy. We have previously shown that evaluation of small bowel mucosal healing more proximal to the terminal ileum using balloon-assisted enteroscopy (BAE) is important, as active (ulcerative) lesions in this area are associated with a higher rate of clinical relapse, hospitalization, and

Received August 1, 2023. **Revised** September 13, 2023.

Accepted October 5, 2023.

Correspondence to Kento Takenaka, Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. Tel: +81-3-5803-5877, Fax: +81-3-5803-0268, E-mail: ktakenaka.gast@tmd.ac.jp

*These authors contributed equally to this study as first authors.

surgery.² Nonetheless, BAE is an invasive procedure with potential risks, high cost, and limited accessibility, as are other available imaging techniques, such as capsule endoscopy, magnetic resonance enteroscopy (MRE), and bowel ultrasound.

Compared to these imaging modalities, biomarkers are non-invasive and less expensive, and can be performed in non-specialized centers. C-reactive protein (CRP) and fecal calprotectin are 2 such biomarkers that have been well established for use in IBD patients. Recently, the CALM trial has shown that tighter disease control based on these biomarkers irrespective of symptoms led to improved prognosis and endoscopic remission.³ They have been defined as formal targets in the aforementioned treat-to-target approach. However, studies focusing on the use of fecal calprotectin with regards to small bowel activity have been limited, with mixed results, suggesting room for improvement.⁴⁻⁷ Fecal hemoglobin has also been reported to be useful in CD, with some reports suggesting similar accuracy as fecal calprotectin,⁸ while others reporting a lower specificity in terms of small bowel activity.⁹ Leucine-rich alpha-2 glycoprotein (LRG) is a novel biomarker that has recently been approved for use in the diagnosis and monitoring of IBD patients.¹⁰ Unlike CRP which is induced mainly by interleukin (IL)-6, LRG expression is also induced by tumor necrosis factor- α , IL-1 β , and IL-22, enabling a more sensitive reflection of a wider range of inflammation. It has been reported to be a sensitive marker for detecting small intestinal activity and transmural healing in CD patients.¹¹ Our previous study involving CD patients undergoing BAE showed that LRG was significantly more accurate in detecting endoscopic activity compared to CRP.¹² Several other studies have also shown accuracy of LRG in assessing for small bowel lesions using BAE,¹³ and capsule endoscopy.¹⁴ Shinzaki et al.¹⁵ showed that LRG correlates better with endoscopic activity than fecal calprotectin or CRP, in CD patients evaluated using colonoscopy. However, no previous study has directly compared these existing biomarkers in terms of monitoring for endoscopic activity including the small bowel.

Several studies have combined biomarkers for IBD in the past. Combining fecal calprotectin, CRP, and clinical activity scores have been reported to be useful for monitoring endoscopic activity in both CD and ulcerative colitis,^{16,17} however, no reports have evaluated their combined use for detecting endoscopic activity in CD evaluated by enteroscopy.

We investigated which of the existing biomarkers best reflects endoscopic activity in CD patients evaluated using BAE, and whether combining these biomarkers could improve their accuracy.

METHODS

1. IRB Approval

The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Tokyo Medical and Dental University (IRB No. M2019-11). Written informed consent was obtained from all patients.

2. Study Population

We performed BAE (SIF-H190; Olympus Medical Systems, Tokyo, Japan) in 104 consecutive patients with ileal and ileocolonic type CD from October 2021 to August 2022 at our hospital (Tokyo Medical and Dental University, Japan), a tertiary referral hospital, for assessment of known or suspected small bowel lesions. Clinical and endoscopic data were prospectively collected. MRE results were also collected if performed within 8 weeks of undergoing BAE (n = 32). Hemoglobin, platelet count, CRP, and LRG measurements were made together with other routine blood at outpatient clinic within 8 weeks of performing BAE, before any changes were made to treatment from time of endoscopy. Both CRP and LRG were measured using commercially available latex agglutination kits (N-Assay LA CRP-T kit, Nitto Medical, Tokyo, Japan; Nanopia LRG, Sekisui Medical, Tokyo, Japan) within the hospital. Patients were asked to bring a fecal sample on the day of BAE, which was collected at home before initiating bowel preparation. Fecal samples were analyzed for hemoglobin and calprotectin within the hospital (EIKEN OC-sensor; Eiken Chemical Co., Ltd., Tokyo, Japan). All patients filled the diagnostic criteria outlined by the Japanese Ministry of Health, Labour and Welfare.¹⁸ Exclusion criteria included patients with colostomy/ileostomy, those with abdominal abscesses, those who were intolerant or contraindicated to BAE, those on nonsteroidal anti-inflammatories, and those with confirmed intestinal infections or malignancies. Patients were prospectively followed for at least 6 months for evaluation of clinical outcome (hospitalization, surgery, and relapse related to CD). Relapse was defined as clinical or endoscopic activity which required change of treatment by the physician in charge.

3. Imaging Modalities

The protocol for BAE at our institution has been described previously.¹⁹ Retrograde insertion was performed for all cases. Scope insertion was terminated when large ulcers were detected in order to avoid complications. Endoscopic evaluations were performed using the modified Simple Endoscopic Score for

CD (modified SES-CD) as described previously.²⁰ In short, this excluded “stenosis” from the original SES-CD which includes 4 endoscopic variables (size of ulcers, proportion of ulcerated surface, proportion of affected surface, and stenosis), as we aimed to focus on active disease. The small bowel was divided into 3 segments; terminal ileum, proximal ileum, and jejunum. Each variable was scored 0 to 3 for each segment, and the modified SES-CD was calculated as the sum. Anastomotic ulcers were also included. Endoscopists blinded to the results of the laboratory tests evaluated the score.

We performed and evaluated MRE as outlined previously.¹¹ A 3.0-T scanner (FUJIFILM Healthcare Corporation [formerly HITACHI], Tokyo, Japan) was used with oral and intravenous contrast. All imaging procedures covered the entire small bowel. A radiologist, who was blinded to the laboratory test results, determined the simplified magnetic resonance index of activity.²¹ Transmural activity was defined as a maximum segmental simplified magnetic resonance index of activity ≥ 4 , in line with our previous study.¹¹

4. Study Design

Primary outcome was set as the relationship between each biomarker and the presence of intestinal ulcers identified at BAE. “Presence of ulcer” was defined as presence of a mucosal defect ≥ 0.5 cm, as defined in SES-CD. The effect of combining biomarkers was also evaluated. Subanalyses were performed for patients with absence of colonic ulcers, extraintestinal manifestations, active anal disease, and patients who also underwent MRE.

5. Statistical Analysis

Between-group differences in continuous variables were assessed by the Mann-Whitney test. The Fisher exact test was used to analyze between-group differences for categorical variables using 2×2 contingency tables. The relationship between the presence of ulcers and each biomarker was examined using receiver operating characteristic (ROC) curves and the area under the curve (AUC). The optimal threshold value for each biomarker was calculated using the Youden index. Logistic regression analysis was used to determine the relationship between different biomarkers and the presence of ulcers. The presence of ulcers was the outcome variable (or dependent variable) (i.e., a binomial variable taking the value 1 if presence of ulcer ≥ 0.5 cm and the value 0 if < 0.5 cm). Each biomarker with their calculated optimal cutoffs (CRP, LRG, hemoglobin, platelet, fecal hemoglobin, and fecal calprotectin) were em-

ployed as explanatory variables (or independent variables). Univariable analysis was used to identify candidate predictors. Then, a multivariable model was fitted. All variables with $P < 0.05$ were retained in the model. Confidence intervals were calculated using the profile likelihood method. The Spearman correlation coefficient was used to analyze the strength of correlation between various biomarkers and modified SES-CD. To evaluate patient prognosis, the Kaplan-Meier method and the log-rank test were used to calculate cumulative end point-free rates, whereas Cox proportional hazards model was used to evaluate the risks for prognosis. The level of statistical significance was set at a P -value of < 0.05 . Box plot figures present median (line), interquartile range (box), range (whiskers), outliers (open circles), and extreme outliers (stars). GraphPad Prism 9.5.1 (GraphPad Software, San Diego, CA, USA) and R version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria) were used.

RESULTS

1. Patient Characteristics and Endoscopic Findings

The main characteristics of the 104 patients included in the study are shown in Table 1. Their disease classed by the Montreal classification included 26 patients with inflammatory disease (B1), 55 patients with stricturing disease (B2), and 23 patients with penetrating disease (B3). The majority of the patients (87%) were treated with biologics including infliximab, adalimumab, ustekinumab, and vedolizumab. Most of the patients were in clinical remission, with a median Crohn’s Disease Activity Index of 57. The small bowel was intubated in all 104 patients, and the proximal ileum was reached in almost all of the cases (96%). Median length of small bowel visualized from the ileocecal valve (or anastomotic site in the case of post-surgical patients) was 50 cm. The extent of intubation was often limited by the presence of a stricture or difficulty in manipulation. There were 55 patients in whom ulcers were identified at BAE; of whom 52 patients had small bowel ulcers, with 19 patients having both small bowel and colonic ulcers.

2. Biomarkers Detect and Correlate with Endoscopic Activity in CD Patients

There was a significant difference in all blood and fecal biomarker values between CD patients in whom ulcers were observed by BAE compared to those without (Fig. 1). CRP, LRG, fecal hemoglobin, fecal calprotectin, and platelet count were significantly increased in those with ulcers, whereas hemoglo-

Table 1. Patient Characteristics and Endoscopic Findings

Variable	Value (n = 104)
Male sex	76 (73.1)
Age (yr)	37 (19–80)
Disease duration (yr)	9 (1–48)
Location	
L1: Ileal	40 (38.5)
L3: Ileocolonic	64 (61.5)
History of upper gastrointestinal involvement	12 (11.5)
Behavior	
B1: Inflammatory	26 (25.0)
B2: Stricturing	55 (52.9)
B3: Penetrating	23 (22.1)
Active anal disease	13 (12.5)
Active extraintestinal manifestation	2 (1.9)
Crohn's Disease Activity Index	57 (0–338)
Hemoglobin (g/dL)	13.8 (9.8–17.9)
Platelet count ($\times 10^4/\mu\text{L}$)	27.1 (10.0–51.3)
C-reactive protein (mg/L)	0.8 (0.3–37.1)
Leucine-rich alpha-2 glycoprotein ($\mu\text{g}/\text{mL}$)	13.5 (6.4–49.1)
Fecal hemoglobin (ng/mL)	15 (0–15,000)
Fecal calprotectin ($\mu\text{g}/\text{g}$)	341 (0–10,100)
Concomitant treatment	
Steroids	3 (2.9)
Immunomodulator	41 (39.4)
Biologics	90 (86.5)
Intubation into the proximal ileum	100 (96.2)
Length intubated (cm)	50 (5–500)
Presence of intestinal ulcers, ≥ 0.5 cm	55 (52.9)
Ulcers present in both small bowel and colon	19 (18.3)
Small bowel ulcers only	33 (31.7)
Colonic ulcers only	3 (2.9)

Values are presented as number (%) or median (range).

bin was significantly decreased in those with ulcers. We then evaluated the correlation of endoscopic activity detected at BAE with each of the biomarkers. All biomarkers significantly correlated with modified SES-CD, a score for endoscopic activity which evaluates the small bowel in detail as well as the colon (Fig. 2). Out of the 6 biomarkers evaluated, fecal calprotectin showed the strongest correlation with endoscopic activity ($r=0.64$, $P<0.001$), while CRP, LRG, fecal hemoglobin, hemoglobin, and platelet count all showed moderate correlation ($r=0.49$, $r=0.55$, $r=0.42$, $r=-0.55$, $r=0.49$ respectively, all $P<0.001$).

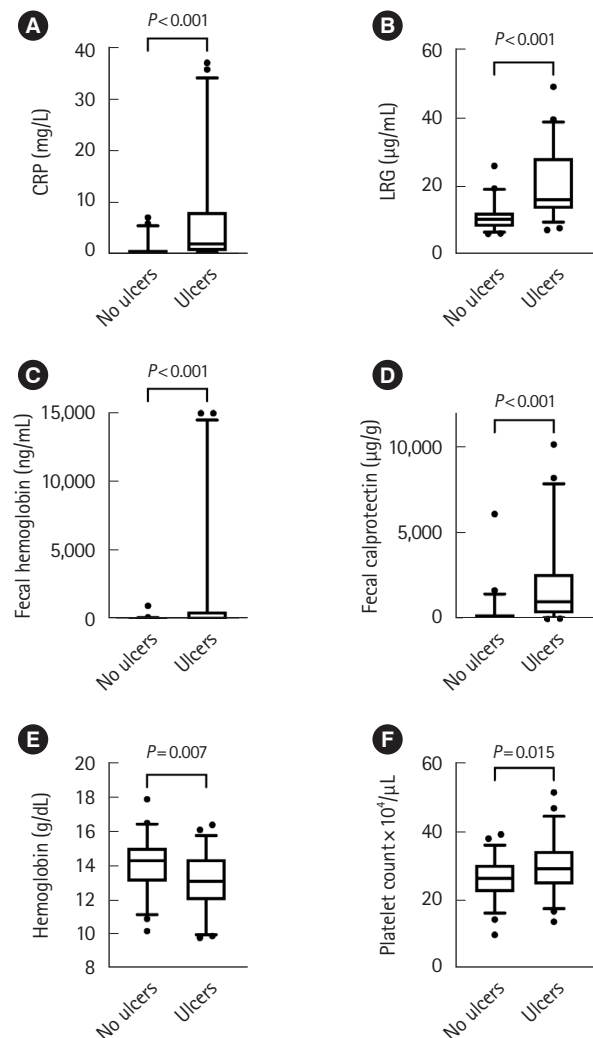


Fig. 1. Blood and fecal biomarkers detect Crohn's disease patients with intestinal ulcers. Box and whisker plots showing levels of serum CRP (A), serum LRG (B), fecal hemoglobin (C), fecal calprotectin (D), hemoglobin (E), and platelet count (F) determined in patients in whom no ulcers were observed with enteroscopy ($n=49$) and in patients with confirmed ulcers ($n=55$). CRP, C-reactive protein; LRG, leucine-rich alpha-2 glycoprotein.

3. Fecal Calprotectin and LRG Detect Endoscopic and Transmural Activity

We evaluated the diagnostic ability of the biomarkers in detecting the presence of intestinal ulcers detected on BAE, by analyzing the AUC of ROC curves (Table 2). The AUCs for fecal calprotectin and LRG were 0.853 and 0.841 respectively, and were higher than the AUCs for CRP, hemoglobin, platelet count, or fecal hemoglobin (0.813, 0.653, 0.638, 0.761, respectively). The optimal threshold value for each marker was calculated from the respective ROC curves using the Youden in-

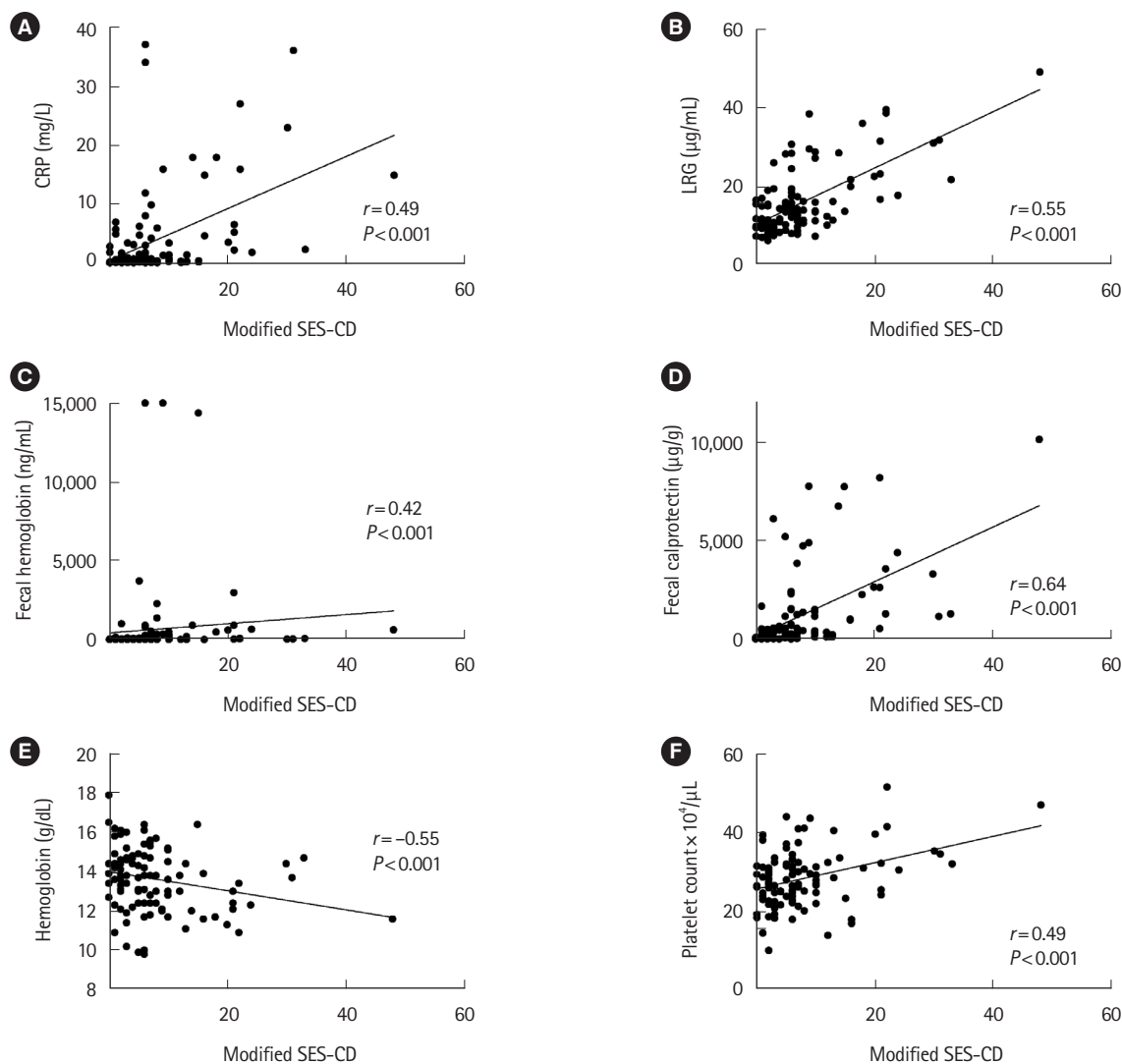


Fig. 2. Fecal calprotectin and LRG show a stronger correlation with endoscopic severity compared to the other biomarkers. Scatterplots showing the correlation of serum CRP (A), serum LRG (B), fecal hemoglobin (C), fecal calprotectin (D), hemoglobin (E), and platelet count (F) with modified SES-CD (r =Spearman rank correlation coefficient). LRG, leucine-rich alpha-2 glycoprotein; CRP, C-reactive protein; SES-CD, Simple Endoscopic Score for Crohn's Disease.

dex. Fecal calprotectin showed a high sensitivity of 91%, specificity of 67%, positive predictive value (PPV) of 76%, and negative predictive value (NPV) of 87% at a cutoff value of 151 µg/g. LRG also showed a high sensitivity of 78%, specificity of 80%, PPV of 80%, and NPV of 76% at a cutoff value of 13 µg/mL. Because our ulcer population consisted of patients with both small bowel and colonic ulcers, in addition to patients with active extraintestinal manifestations and active anal disease, we performed subanalysis excluding 22 patients with colonic ulcers, 13 patients with active anal disease, and 2 patients with active extraintestinal manifestations (n=75). LRG and fecal calprotectin still showed high accuracy for detecting solely

small bowel ulcers compared to those without, with an AUC of 0.805 and 0.784 respectively, with CRP also yielding a comparable result with an AUC of 0.785 (Table 3).

Because BAE cannot assess the whole of the small bowel, nor detect for transmural inflammation, subanalysis was performed for the 32 cases that also underwent MRE as well as BAE (Supplementary Table 1). LRG and fecal calprotectin were accurate as markers to detect cases where activity was seen on either BAE or MRE, compared to those without any activity on either modality, with an AUC of 0.836 and 0.806 respectively. Although not statistically significant, biomarker levels tended to be lower in cases of endoscopic remission where MRE

Table 2. AUC, Sensitivity, Specificity, PPV, and NPV of Blood and Fecal Biomarkers, Alone and in Combination, for Predicting Intestinal Ulcers Seen on Balloon-Assisted Enteroscopy

	AUC (95% CI)	Optimal cutoff	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	PPV (%; 95% CI)	NPV (%; 95% CI)
Biomarker						
CRP (mg/L)	0.813 (0.732–0.895)	1	69 (61–76)	82 (72–89)	81 (71–88)	70 (62–76)
LRG ($\mu\text{g/mL}$)	0.841 (0.765–0.916)	13	78 (70–85)	80 (71–86)	80 (71–86)	76 (67–83)
Hemoglobin (g/dL)	0.653 (0.548–0.758)	13.1	49 (36–62)	78 (64–87)	71 (55–83)	58 (46–69)
Platelet ($\times 10^4/\mu\text{L}$)	0.638 (0.532–0.743)	31.1	42 (30–55)	84 (71–92)	74 (57–86)	56 (45–67)
Fecal hemoglobin (ng/mL)	0.761 (0.668–0.855)	41	64 (55–70)	86 (77–92)	83 (73–91)	68 (60–73)
Fecal calprotectin ($\mu\text{g/g}$)	0.853 (0.779–0.927)	151	91 (83–96)	67 (59–73)	76 (69–80)	87 (76–94)
Combination of biomarkers						
LRG (+) and fecal calprotectin (+)			73 (65–77)	92 (84–97)	91 (82–96)	75 (68–79)
LRG (+) or fecal calprotectin (+)			96 (90–99)	53 (46–56)	70 (65–72)	93 (80–98)
LRG (+) and fecal hemoglobin (+)			51 (46–51)	100 (94–100)	100 (89–100)	65 (61–65)
LRG (+) or fecal hemoglobin (+)			91 (83–96)	63 (55–69)	74 (67–77)	86 (74–94)
LRG (+) and fecal hemoglobin (+) and fecal calprotectin (+)			51 (46–51)	100 (94–100)	100 (89–100)	65 (61–65)
LRG (+) or fecal hemoglobin (+) or fecal calprotectin (+)			96 (90–99)	54 (46–57)	71 (66–73)	93 (80–98)

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; CRP, C-reactive protein; LRG, leucine-rich alpha-2 glycoprotein.

Table 3. AUC, Sensitivity, Specificity, PPV, and NPV of Serum and Fecal Biomarkers for Predicting Solely Small Intestinal Ulcers

Biomarker	AUC (95% CI)	Cutoff value	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	PPV (%; 95% CI)	NPV (%; 95% CI)
CRP (mg/L)	0.785 (0.679–0.891)	1	65 (52–74)	84 (75–91)	74 (60–85)	77 (69–83)
LRG ($\mu\text{g/mL}$)	0.805 (0.703–0.907)	13	71 (58–81)	80 (71–87)	71 (58–81)	80 (71–87)
Fecal hemoglobin (ng/mL)	0.703 (0.575–0.832)	41	53 (41–63)	86 (78–93)	73 (56–86)	73 (66–79)
Fecal calprotectin ($\mu\text{g/g}$)	0.784 (0.678–0.890)	151	84 (71–92)	66 (57–72)	63 (54–70)	85 (74–93)

Enteroscopy, excluding 22 patients with colonic ulcers, 13 patients with active anal disease, and 2 patients with active extraintestinal disease, with overlapping patients (n = 75).

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; CRP, C-reactive protein; LRG, leucine-rich alpha-2 glycoprotein.

also confirmed remission (radiological remission), compared to cases in endoscopic remission where MRE showed activity (Supplementary Fig. 1).

4. LRG and Fecal Biomarkers Independently Predict Ulcers

In order to determine the relationship between the biomarkers, we analyzed the correlation between each of the biomarkers (Supplementary Table 2). LRG showed the strongest correlation with CRP ($r=0.78$, $P<0.001$), another blood biomarker, whereas fecal calprotectin showed the strongest correlation with fecal hemoglobin ($r=0.64$, $P<0.001$), a fecal biomarker.

There was a tendency for the correlation to be weaker between blood and fecal biomarkers. Although LRG and fecal calprotectin were both accurate in detecting ulcers, these results suggested that blood and fecal biomarkers may detect slightly different inflammatory profiles. We therefore determined the relationship between each of the biomarkers with ulcers found on BAE using logistic regression. Univariable analysis showed statistical significance for all biomarkers, however only LRG ≥ 13 $\mu\text{g/mL}$ (odds ratio, 4.946; $P=0.033$), fecal hemoglobin ≥ 41 ng/mL (OR, 5.824; $P=0.016$), and fecal calprotectin ≥ 151 $\mu\text{g/g}$ (odds ratio, 6.082; $P=0.012$), were independent predictors for the presence of ulcers on BAE (Table 4).

Table 4. Relationship between Biomarkers and Ulcers Found on Enteroscopy

Biomarker	Univariable analysis		Multivariable analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
CRP ≥ 1 mg/L	9.935 (3.952–24.977)	<0.001	3.544 (0.786–17.000)	0.101
LRG ≥ 13 µg/mL	12.379 (4.898–31.288)	<0.001	4.946 (1.175–23.370)	0.033
Fecal hemoglobin ≥ 41 ng/mL	10.500 (3.978–27.715)	<0.001	5.824 (1.463–27.210)	0.016
Fecal calprotectin ≥ 151 µg/g	20.625 (6.892–61.725)	<0.001	6.082 (1.555–27.210)	0.012
Hemoglobin < 13.1 g/dL	3.331 (1.447–8.071)	0.006	1.622 (0.478–5.688)	0.437
Platelet count ≥ 31.1 × 10 ⁴ /µL	3.684 (1.504–9.796)	0.006	2.092 (0.554–8.645)	0.285

OR, odds ratio; CI, confidence interval; CRP, C-reactive protein; LRG, leucine-rich alpha-2 glycoprotein.

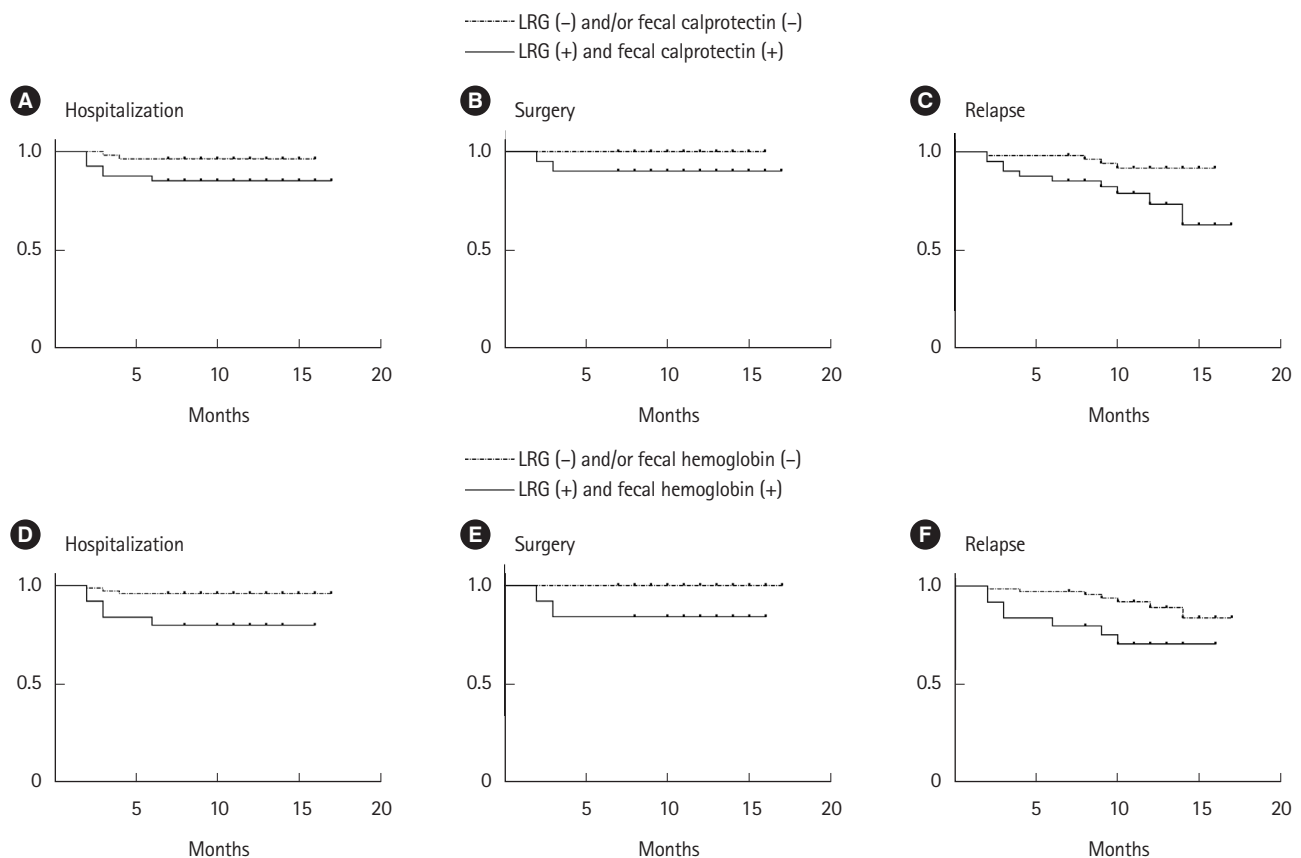


Fig. 3. Kaplan-Meier curves demonstrating poorer outcome-free survival for patients with high leucine-rich alpha-2 glycoprotein (LRG) and fecal calprotectin levels compared with patients who have low LRG and/or fecal calprotectin levels (A-C), and those with high LRG and fecal hemoglobin levels compared with patients who have low LRG and/or fecal hemoglobin levels (D-F) ($P < 0.05$ for all).

5. Combination of LRG and Fecal Biomarkers Accurately Detect Ulcers on BAE

We therefore hypothesized that combining the different types of biomarkers could improve their accuracy. The ability to detect ulcers on BAE using various combinations of LRG, fecal hemoglobin, and fecal calprotectin, with their pre-determined optimal cutoff values, were determined (Table 2). As predict-

ed, combining LRG, a serum biomarker, together with each of the fecal biomarkers improved the accuracy. Dual-positivity for LRG and fecal calprotectin predicted the presence of ulcers with an improved specificity of 92% and PPV of 91%, whereas having either a positive LRG or fecal calprotectin showed an improved sensitivity of 96% and NPV of 93%. The combination of LRG and fecal hemoglobin showed a similar improve-

ment in accuracy; dual-positivity for LRG and fecal hemoglobin predicted ulcers with an improved specificity of 100% and PPV of 100%, whereas having a positive LRG or fecal hemoglobin showed an improved sensitivity of 91% and NPV of 86%. There was no marked added improvement when all 3 of the biomarkers were combined.

6. Combination of LRG and Fecal Biomarkers Predict Patient Prognosis

Finally, we sought to determine the association of biomarkers with patient prognosis. Out of the 104 patients followed after a median of 11 months (range, 0–17 months), 8 patients were hospitalized, 4 patients underwent surgery, and 14 patients relapsed. Relapse was defined as those requiring a change of treatment. Kaplan-Meier analysis showed that patients with dual-positivity for LRG and fecal calprotectin demonstrated a poorer outcome-free rate in terms of hospitalization, surgery, and relapse, compared to those with either low LRG and/or low fecal calprotectin (Fig. 3A-C). Cox proportional hazard regression showed a significant hazard ratio of 3.846 (95% confidence interval, 1.285–14.03; $P < 0.05$) for relapse in the dual-positive group. Similar results were obtained with LRG and fecal hemoglobin, where those who were dual-positive demonstrated a poorer outcome-free rate compared to those with either low LRG and/or low fecal hemoglobin (Fig. 3D-F). Cox proportional hazard regression showed a significant hazard ratio of 3.325 (95% confidence interval, 1.134–9.755, $P < 0.05$) for relapse in the dual-positive group.

DISCUSSION

Biomarkers are recommended for monitoring CD activity; however, their accuracy has been debated, especially in detecting small bowel activity.^{4,9} This is the first study comparing the major existing biomarkers for CD in terms of monitoring for endoscopic activity evaluated using BAE, as well as the added value of their use in combination. We showed that LRG and fecal biomarkers, especially fecal calprotectin, are both accurate biomarkers on their own in detecting and correlating with endoscopic activity, but their accuracy is further improved when used in combination. Dual positivity for LRG and either fecal biomarker shows high specificity and PPV, suggesting that when a patient tests positive for both markers, there is a high likelihood that the patient will have endoscopic activity detected with BAE. Conversely, the sensitivity and NPV are high if LRG or either fecal biomarker is positive, indicating that

if a patient tests negative for both of these markers, there is a low likelihood of the patient having endoscopic activity detected with BAE. We also showed that the combined use of LRG and fecal calprotectin or fecal hemoglobin predicts the clinical outcome of CD patients better than each on its own. Decreased hemoglobin and increased platelet count are known as robust inflammatory markers in IBD and were included in the study, however, their abilities to detect endoscopic activity were poor compared to the other markers. Although CRP showed a relatively high AUC, it did not emerge as an independent predictor as a result of multivariate analysis.

In this study, we included patients with both ileal and ileocolonic type CD, and set the primary outcome to include any intestinal ulcers confirmed on enteroscopy, assessing for both the small bowel and the colon. We also decided to include patients with active anal disease and extraintestinal manifestations, to reflect the patient population encountered in actual clinical practice. As reported previously,²² small bowel healing is much more difficult to achieve compared to colonic healing in cases of ileocolonic CD; rarely resulting in solely colonic residual ulcers. This is confirmed in our study where out of the 64 patients with ileocolonic type CD, only 3 patients had residual colonic ulcers alone, compared to 15 patients with ulcers only in the small intestine, and 19 patients with ulcers in both the small bowel and colon. In clinical practice, we do not only deal with ileal type CD but also ileocolonic CD, with varying degrees of small bowel and colonic ulcers, in addition to patients with perianal lesions and extraintestinal manifestations. We, therefore, included these patients in our main analysis as any biomarker will have to perform well even in patients with these commonly seen complications. We hypothesized that including these “extra” inflammatory burdens would result in a lower specificity for the biomarkers, especially the serum markers, however on the contrary, our subanalysis excluding these sets of patients to assess for purely small bowel ulcers resulted in a lower accuracy for detecting endoscopic activity (Table 3). This may be because of the high rate of endoscopically active lesions in this group of patients (24 out of 29 patients had endoscopically confirmed ulcers). We were able to show that these biomarkers will be useful in patients in a real-life clinic setting.

LRG is a serum biomarker which is induced by systemic inflammation, providing an advantage as it can reflect transmural inflammation in CD, as previously reported.¹¹ However, this feature is also a disadvantage as it responds to non-specific inflammation other than IBD.²³ On the other hand, fecal

biomarkers are more specific towards bowel mucosal inflammation. Our subanalysis of a limited number of patients who also underwent MRE showed that CRP and LRG were better markers for detecting transmural activity than fecal hemoglobin or fecal calprotectin. This difference in mechanism between the markers is possibly the reason for their improved accuracy in detecting endoscopic activity when used in combination, complementing their weaknesses. Whilst our study could not identify particular parameters associated with false positivity/negativity of individual biomarkers, future larger-scale studies are warranted to address this question.

Although fecal hemoglobin was less accurate than fecal calprotectin in detecting endoscopic activity compared to fecal calprotectin (AUC: 0.761 vs. 0.853, $P=0.05$), the accuracy of fecal hemoglobin improved when combined with LRG. In clinical settings where fecal calprotectin measurement is not available, or when LRG and fecal calprotectin cannot be simultaneously measured due to insurance restrictions, as is the case in Japan, the combined use of LRG and fecal hemoglobin can be an alternative monitoring method.

We also showed for the first time that combining LRG and fecal biomarkers can better predict the clinical outcome of CD patients in the long term. Patients who are dual-positive for LRG and fecal calprotectin or hemoglobin should be considered for endoscopic evaluation including the small bowel and assessed for change in treatment, as they are at higher risk for hospitalization, relapse, and surgery.

The limitation of our study is that it was conducted at a single tertiary referral hospital in Tokyo. The length of small intestine intubated varied in each individual, with possibility that the modified SES-CD scores do not accurately reflect the endoscopic activity of the whole intestine. Our study also did not consider the areas not intubated by enteroscopy (i.e., the proximal small intestine, stomach, and duodenum). We, therefore, performed subanalysis on patients who also underwent MRE, however, the number of cases was limited. The patient population consisted of mostly biologic-treated patients in clinical remission, therefore may not be representative of the whole patient population. Blood and fecal biomarkers were not necessarily taken on the same day as each other, and not simultaneously with BAE or MRE, though tests were all conducted in a limited timespan before change of treatment. Because patients enrolled were mostly in remission with stable disease undergoing routine elective endoscopy, we predicted that disease activity would not change markedly within the 8-week time period.

In conclusion, the combined use of LRG and fecal biomarkers detects with high accuracy the presence of endoscopic activity evaluated by BAE in CD patients. The use of these biomarkers in clinical practice may enable earlier intervention, leading to better prognosis.

ADDITIONAL INFORMATION

Funding Source

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Data Availability Statement

The data that support the findings of this study are available upon reasonable request from the corresponding author.

Author Contributions

Conceptualization: Kawamoto A, Takenaka K. Data curation: Kawamoto A. Formal analysis: Kawamoto A, Takenaka K. Investigation: Kawamoto A, Takenaka K, Hibiya S, Saito E, Ohtsuka K, Kitazume Y. Methodology: Kawamoto A, Takenaka K. Project administration: Kawamoto A, Takenaka K. Resources: Kawamoto A, Takenaka K. Supervision: Hibiya S, Fujii T, Shimizu H, Ohtsuka K, Okamoto R. Visualization: Kawamoto A, Takenaka K. Writing – original draft: Kawamoto A. Writing – review & editing: all authors. Approval of final manuscript: all authors.

ORCID

Kawamoto A	https://orcid.org/0000-0002-8549-4999
Takenaka K	https://orcid.org/0000-0002-7861-8383
Hibiya S	https://orcid.org/0000-0001-8823-7755
Kitazume Y	https://orcid.org/0000-0002-6411-8660
Shimizu H	https://orcid.org/0000-0003-3268-0613
Fujii T	https://orcid.org/0000-0003-3048-7270
Saito E	https://orcid.org/0000-0002-4806-130X
Ohtsuka K	https://orcid.org/0000-0002-2409-9709
Okamoto R	https://orcid.org/0000-0002-7047-571X

Supplementary Material

Supplementary materials are available at the Intestinal Research website (<https://www.irjournal.org>).

REFERENCES

1. Turner D, Ricciuto A, Lewis A, et al. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. *Gastroenterology* 2021;160:1570-1583.
2. Takenaka K, Ohtsuka K, Kitazume Y, et al. Utility of magnetic resonance enterography for small bowel endoscopic healing in patients with Crohn's disease. *Am J Gastroenterol* 2018;113:283-294.
3. Colombel JF, Panaccione R, Bossuyt P, et al. Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial. *Lancet* 2017;390:2779-2789.
4. Simon EG, Wardle R, Thi AA, Eldridge J, Samuel S, Moran GW. Does fecal calprotectin equally and accurately measure disease activity in small bowel and large bowel Crohn's disease? A systematic review. *Intest Res* 2019;17:160-170.
5. Iwamoto F, Matsuoka K, Motobayashi M, et al. Prediction of disease activity of Crohn's disease through fecal calprotectin evaluated by balloon-assisted endoscopy. *J Gastroenterol Hepatol* 2018;33:1984-1989.
6. Sipponen T, Savilahti E, Kolho KL, Nuutinen H, Turunen U, Färkkilä M. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's Disease Activity Index and endoscopic findings. *Inflamm Bowel Dis* 2008;14:40-46.
7. Zittan E, Kelly OB, Gralnek IM, Silverberg MS, Hillary Steinhart A. Fecal calprotectin correlates with active colonic inflammatory bowel disease but not with small intestinal Crohn's disease activity. *JGH Open* 2018;2:201-206.
8. Mooiweer E, Fidder HH, Siersema PD, Laheij RJ, Oldenburg B. Fecal hemoglobin and calprotectin are equally effective in identifying patients with inflammatory bowel disease with active endoscopic inflammation. *Inflamm Bowel Dis* 2014;20:307-314.
9. Inokuchi T, Kato J, Hiraoka S, et al. Fecal immunochemical test versus fecal calprotectin for prediction of mucosal healing in Crohn's disease. *Inflamm Bowel Dis* 2016;22:1078-1085.
10. Serada S, Fujimoto M, Terabe F, et al. Serum leucine-rich alpha-2 glycoprotein is a disease activity biomarker in ulcerative colitis. *Inflamm Bowel Dis* 2012;18:2169-2179.
11. Takenaka K, Kitazume Y, Kawamoto A, et al. Serum leucine-rich α 2 glycoprotein: a novel biomarker for transmural inflammation in Crohn's disease. *Am J Gastroenterol* 2023;118:1028-1035.
12. Kawamoto A, Takenaka K, Hibiya S, Ohtsuka K, Okamoto R, Watanabe M. Serum leucine-rich α 2 glycoprotein: a novel biomarker for small bowel mucosal activity in Crohn's disease. *Clin Gastroenterol Hepatol* 2022;20:e1196-e1200.
13. Kawamura T, Yamamura T, Nakamura M, et al. Accuracy of serum leucine-rich alpha-2 glycoprotein in evaluating endoscopic disease activity in Crohn's disease. *Inflamm Bowel Dis* 2023;29:245-253.
14. Omori T, Sasaki Y, Koroku M, et al. Serum leucine-rich alpha-2 glycoprotein in quiescent Crohn's disease as a potential surrogate marker for small-bowel ulceration detected by capsule endoscopy. *J Clin Med* 2022;11:2494.
15. Shinzaki S, Matsuoka K, Tanaka H, et al. Leucine-rich alpha-2 glycoprotein is a potential biomarker to monitor disease activity in inflammatory bowel disease receiving adalimumab: PLANET study. *J Gastroenterol* 2021;56:560-569.
16. Zubin G, Peter L. Predicting endoscopic Crohn's disease activity before and after induction therapy in children: a comprehensive assessment of PCDAI, CRP, and fecal calprotectin. *Inflamm Bowel Dis* 2015;21:1386-1391.
17. Bodelier AG, Jonkers D, van den Heuvel T, et al. High percentage of IBD patients with indefinite fecal calprotectin levels: additional value of a combination score. *Dig Dis Sci* 2017;62:465-472.
18. Ueno F, Matsui T, Matsumoto T, et al. Evidence-based clinical practice guidelines for Crohn's disease, integrated with formal consensus of experts in Japan. *J Gastroenterol* 2013;48:31-72.
19. Takenaka K, Ohtsuka K, Kitazume Y, et al. Comparison of magnetic resonance and balloon enteroscopic examination of the small intestine in patients with Crohn's disease. *Gastroenterology* 2014;147:334-342.
20. Takenaka K, Ohtsuka K, Kitazume Y, et al. Correlation of the endoscopic and magnetic resonance scoring systems in the deep small intestine in Crohn's disease. *Inflamm Bowel Dis* 2015;21:1832-1838.
21. Ordás I, Rimola J, Alfaro I, et al. Development and validation of a simplified magnetic resonance index of activity for Crohn's disease. *Gastroenterology* 2019;157:432-439.
22. Takenaka K, Fujii T, Suzuki K, et al. Small bowel healing detected by endoscopy in patients with Crohn's disease after treatment with antibodies against tumor necrosis factor. *Clin Gastroenterol Hepatol* 2020;18:1545-1552.
23. Naka T, Fujimoto M. LRG is a novel inflammatory marker clinically useful for the evaluation of disease activity in rheumatoid arthritis and inflammatory bowel disease. *Immunol Med* 2018;41:62-67.