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ORIGINAL ARTICLE

Blood biomarkers reflect integration of severity and extent of endoscopic inflammation in ulcerative colitis

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Abstract

Background and Aim: Blood markers are not always regarded as satisfactory surrogate biomarkers for predicting endoscopic activity in ulcerative colitis (UC). However, those biomarkers have been evaluated solely based on endoscopic activity at the most severe colorectal location, taking no account of the extent of inflammation. This study aimed to examine whether integrated evaluation of severity and extent of endoscopic activity improves the performance of blood biomarkers for UC.

Methods: We performed a retrospective study of UC patients who underwent colonoscopy and blood tests in our hospital. Blood tests were C-reactive protein (CRP), serum albumin (ALB), and platelet count (PLT). We compared blood markers with two versions of endoscopic activity assessed by Mayo endoscopic subscore (MES): the maximum score of MES in the colorectum (mMES, range: 0–3) and the cumulative score of MES of six colorectal regions (cMES, range: 0–18).

Results: All three blood markers correlated well with both mMES and cMES, and each marker showed better correlation with cMES than mMES (Spearman rank correlation coefficient: PLT: 0.54 *vs* 0.47, ALB: –0.65 *vs* –0.52, and CRP: 0.52 *vs* 0.38, respectively). The predictability, including sensitivity and specificity, of each marker for endoscopic activity was also better for cMES, resulting in higher degrees of area under the curve (mMES *vs* cMES: PLT: 0.75 *vs* 0.83, ALB: 0.77 *vs* 0.90, and CRP: 0.75 *vs* 0.90, respectively).

Conclusion: When incorporating the extent of inflammation, blood markers are better at predicting endoscopic activity of UC than previously considered and could be used as a reliable biomarker in clinical practice.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory condition that causes continuous mucosal inflammation of the colon, usually without granulomas on biopsy. It affects the rectum and to a variable extent the colon in a continuous fashion, and is characterized by a relapsing and remitting course. UC patients present symptoms such as visible blood in stools, diarrhea, and abdominal pain. They may live with a considerable symptom burden and high risk of disability despite medical treatment.[1]

Although evaluation of disease status by colonoscopy is necessary for adequate management of UC patients, colonoscopy is burdensome for both patients and physicians. In this context, surrogate biomarkers predicting endoscopic and/or disease activity have been investigated. Commonly used blood markers include C-reactive protein (CRP),[2–4] erythrocyte sedimentation rate (ESR),[3] serum albumin (ALB),[5] and platelet count (PLT),[6] and fecal markers including fecal calprotectin[7–10] and fecal immunochemical test (FIT) are also used.[11–13]

Blood samples are easily obtained in clinical practice and many studies have examined the correlation of blood biomarkers and endoscopic activity. However, the reported performance of blood biomarkers in inflammatory bowel disease (IBD) is not always satisfactory.[14–17] For example, although CRP, the most common inflammatory marker, has been shown to have the best overall performance, there is remarkable heterogeneity among studies in the predictability for activity of UC. Previous studies reported that the sensitivity and specificity for disease activity were 67–73 and 87–97%, respectively, and those for endoscopic activity were 24–67 and 67–100%, respectively.[2,7,8,17,18]

We hypothesized that the insufficient performance of blood markers in IBD is, at least in part, accountable to the methodologies that evaluated disease activities of IBD. In particular, most previous reports evaluated endoscopic activity only at the most severe colorectal location, without consideration of the extent of inflammation. Accurate evaluation of UC disease activity, which requires observation of both severity and extent of

inflammation throughout the colorectum, may improve the predictability of blood markers.

The aim of this study was to examine whether blood biomarker performance for UC is improved by integrated evaluation of severity and extent of endoscopic activity.

Methods

Patients. All colonoscopy records of UC patients who visited Wakayama Medical University Hospital and underwent colonoscopy for evaluation of disease activity or surveillance between May 2010 and August 2016 were collected retrospectively in this study. Blood sample data from the day of colonoscopy or at the nearest hospital visit prior to colonoscopy (approximately within 1 week) were reviewed, and the values of PLT, ALB, and CRP were compared with the colonoscopy findings. Data of demographics of patients, extent of active inflammation at colonoscopy, and medication were also collected from electronic medical charts. In this study, we defined the extent of disease based on the locations where active inflammation was present.

All patients had been diagnosed with UC using the established endoscopic and histologic criteria assessment, and received medical therapy. Exclusion criteria were failed insertion of colonoscopy into the cecum and changes of symptom between the day of blood test and the day of colonoscopy.

The study protocol was approved by the institutional review board of Wakayama Medical University.

Colonoscopy. Bowel preparation was performed by polyethylene glycol-based or magnesium citrate-based electrolyte solution by oral administration. After colonic lavage fluid was cleared, patients underwent colonoscopy. Colonoscopy was performed by skilled endoscopists, and 10 or more still images were taken at each portion of the colorectum (cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum). Stored images were assessed by two of the authors (M.U. and J.K.) using the Mayo endoscopic subscore (MES) (0, normal or inactive disease; 1, mild disease with erythema, decreased vascular pattern, mild friability; 2, moderate disease with marked erythema, absent vascular pattern, friability, erosions; and 3, severe disease with spontaneous bleeding, ulceration).[19]

Mucosal healing was defined as MES 0 or 1. For the evaluation of endoscopic activity at the point with the most severe inflammation in the colorectum, the maximum score of MES in the colorectum (mMES, range: 0–3) was used. Meanwhile, the cumulative score of MES (cMES) of each of the six portions for the integrated evaluation of endoscopic severity and extent of inflammation (cMES, range: 0–18) was used for analysis.

Statistical analysis. Statistical analysis was conducted using the JMP program (version 12, SAS Institute, Cary, NC, USA). Spearman rank correlation was performed to determine the association between blood markers and mMES or cMES. Categorical variables were compared using the χ^2 test. To obtain optimal cutoff values of blood markers, receiver operating characteristic (ROC) curve analysis was performed and the area under the curve (AUC) was calculated. Based on the obtained optimal cutoff values of blood markers, sensitivity, specificity, positive predictive value, and negative predictive value with 95% confidence intervals (CIs) were also calculated. All *P*-values were two sided and considered statistically significant when <0.05.

Results

Clinical characteristics of the patients. A total of 207 colonoscopies were performed on 68 UC patients between May 2010 and August 2016. Patient characteristics at colonoscopy are shown in Table 1. The median age was 50 years (range: 19–79 years) and median disease duration at colonoscopy was 8 years (range: 0.07–55 years).

Colonoscopy findings and the results of blood markers are shown in Table 1. The mMES 0 in the colorectum was observed in 52 (25%), mMES 1 in 61 (29%), mMES 2 in 55 (27%), and mMES 3 in 39 (19%) patients. Cumulative endoscopic activity throughout the colorectum (cMES) demonstrated three peaks of distribution (Table 1 and Fig. 1b). The first peak was at cMES

Table 1 Characteristics of the study patients

Gender	
Male	33 (49%)
Female	35 (51%)
Median (range) disease duration at colonoscopy, years	8 (0.07–55)
Median (range) age at colonoscopy	50 (19–79)
Medications at colonoscopy	
Aminosalicylate	179 (86%)
Corticosteroids	44 (21%)
Azathioprine/mercaptopurine	72 (35%)
Tacrolimus	4 (2%)
Biologics	16 (8%)
Maximum score of MES (mMES)	
0	52 (25%)
1	61 (29%)
2	55 (27%)
3	39 (19%)
Cumulative score of MES (cMES)	
0	52 (25%)
1	29 (14%)
2	25 (12%)
3	13 (6%)
4	12 (6%)
5	15 (7%)
6	16 (8%)
7	7 (3%)
8	7 (3%)
9	12 (6%)
≥10	19 (9%)
Extent of active inflammation	
Rectum only	15 (7%)
Over the rectum within the splenic flexure	27 (13%)
Beyond the splenic flexure	52 (25%)
Blood markers, median (range)	
PLT ($\times 10^4/\mu L$)	25.1 (9.5–64.5)
ALB (g/dL)	4.3 (2.2–5.3)
CRP (mg/dL)	0.13 (0.02–10.2)

ALB, albumin; CRP, C-reactive protein; MES, Mayo endoscopic subscore; PLT, platelet count.

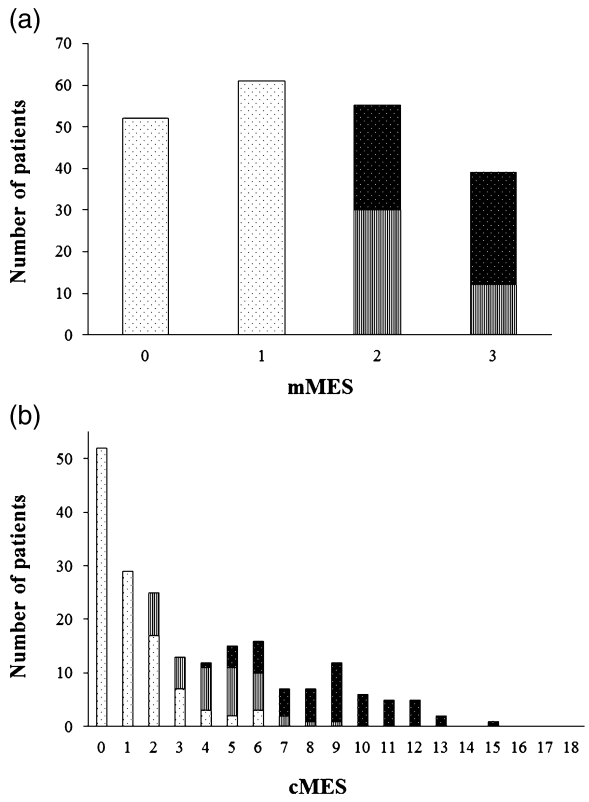

Figure 1 The distributions of (a) maximum and (b) cumulative scores of Mayo endoscopic subscore (mMES and cMES) and the extent of inflammation. The area of endoscopic inflammation extended with increase in severity (■, beyond the splenic flexure; ▥, within the splenic flexure; ⊡, mucosal healing).

0 in 52 (25%) patients. The second and third peaks were seen at cMES 6 in 16 (8%) and cMES 9 in 12 (6%) patients, respectively. cMES ≥ 10 was observed in 19 (9%) patients. As we expected, the area of endoscopic inflammation extended with increase in severity (Fig. 1). Approximately 55% of patients with mMES 2 had extent of inflammation within the splenic flexure. In contrast, the extent of inflammation beyond the splenic flexure was observed in 69% of patients with mMES 3 (Fig. 1a). As for the cumulative endoscopic activity, the extent of inflammation in patients with cMES 6 or lower were likely to be confined within the splenic flexure, while most patients with cMES 7 or higher had inflammation beyond the splenic flexure (Fig. 1b).

Correlation between blood markers and endoscopic activity. Correlation between blood markers (PLT, ALB, and CRP) and endoscopic activity (mMES and cMES) is shown in Figures 2 and 3. All three blood markers correlated well with both mMES and cMES. More importantly, each marker showed better correlation with cMES than mMES (Spearman rank correlation coefficient: PLT: 0.54 *vs* 0.47, ALB: –0.65 *vs* –0.52, and CRP: 0.52 *vs* 0.38). In addition, the changes of blood markers (ΔPLT, ΔALB, and ΔCRP) observed in the intervals between colonoscopies in patients who underwent two or more colonoscopies (139 intervals in 68 patients) also

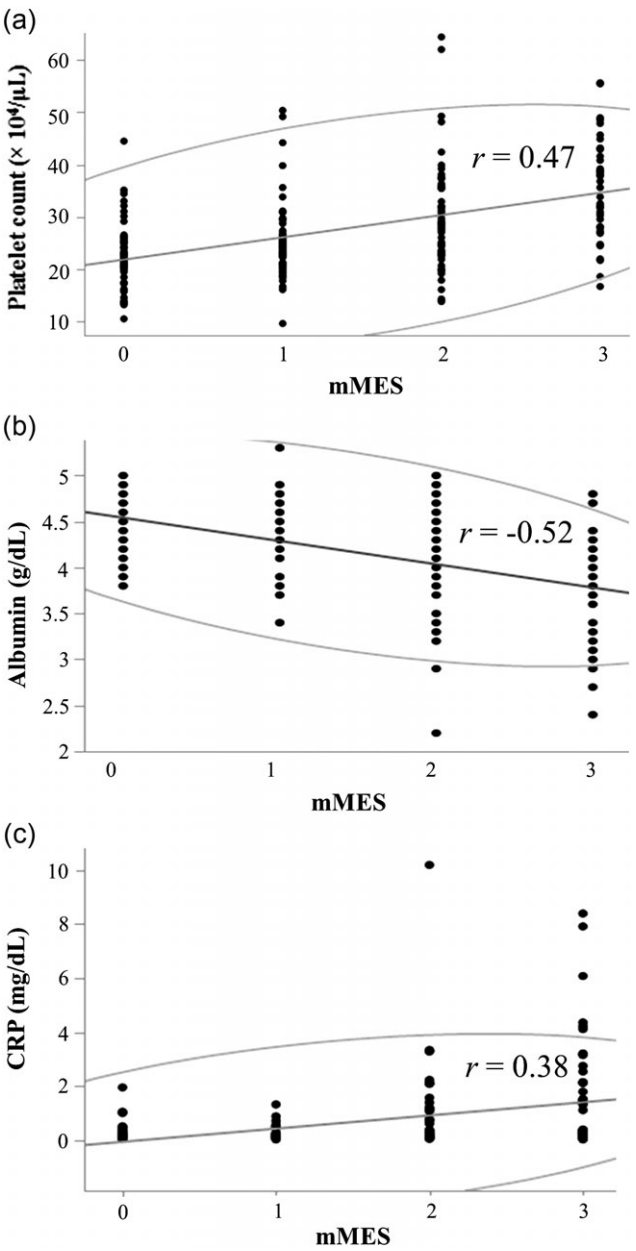

Figure 2 The correlations between blood markers (platelet count [PLT], albumin [ALB], and C-reactive protein [CRP]) and maximum score of Mayo endoscopic subscore (mMES). All three blood markers correlated well with mMES.

correlated well with the changes of cMES (ΔcMES) (Spearman rank correlation coefficient: ΔPLT: 0.57, ΔALB: –0.49, and CRP: 0.54) (Fig. S1, Supporting information).

Predictability of blood markers for mMES. The predictability of blood markers for maximum endoscopic severity in the colorectum (mMES) is shown in Table 2. The cutoff value of each parameter for endoscopic active disease (mMES ≥ 2) was determined by ROC curve analysis, and PLT ≥ 26.7 × 10^4/μL, ALB ≤ 4.2 g/dL, and CRP ≥ 0.23 mg/dL could discriminate

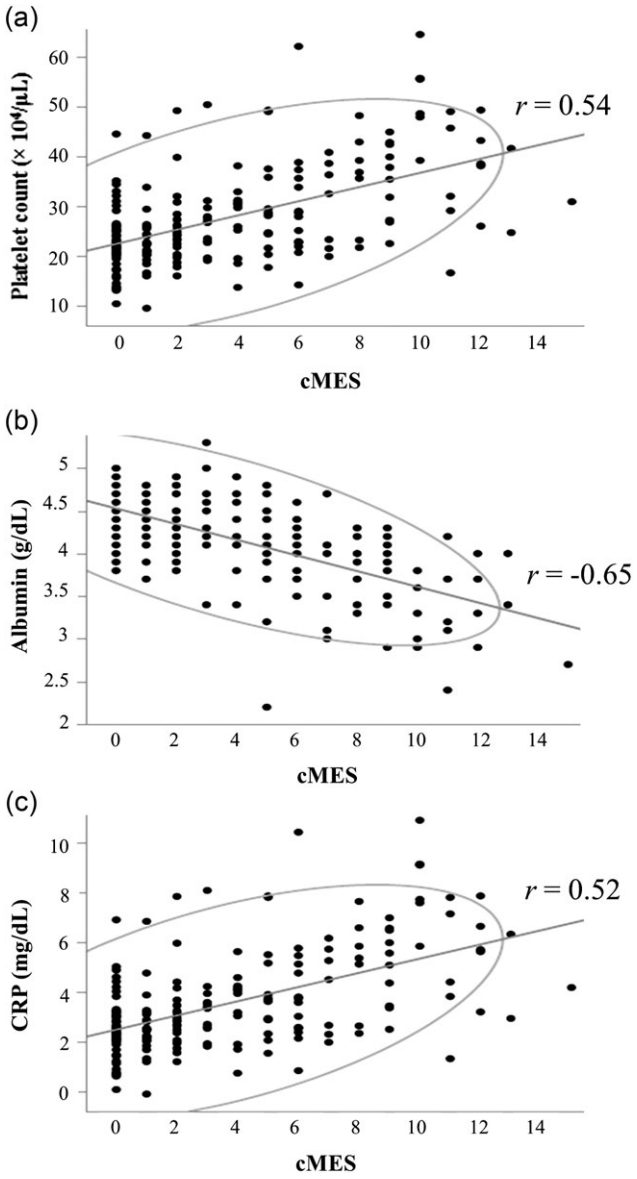

Figure 3 The correlations between blood markers (platelet count [PLT], albumin [ALB], and C-reactive protein [CRP]) and cumulative score of Mayo endoscopic subscore (cMES). Each marker showed better correlation with cMES than maximum score of Mayo endoscopic subscore (mMES).

patients with active disease with the highest AUC values (0.75, 0.77, and 0.75, respectively). With these cutoff values, the sensitivity of each parameter was 0.68, 0.73, and 0.57, respectively, and the specificity was 0.79, 0.73, and 0.81, respectively.

The ratios of fulfillment of each cutoff in patients with active inflammation (mMES ≥ 2) were examined based on the extent of inflammation (Table 3). Patients with extent of inflammation beyond the splenic flexure were more likely to fulfill the cutoff values than those with inflammation confined within the splenic flexure in all of three blood markers (50% *vs* 81%, $P = 0.0016$, 43% *vs* 94%, $P < 0.0001$, and 26% *vs* 83%, $P < 0.0001$, respectively). These results suggest that only the confrontation with pinpoint endoscopic severity could not evaluate the performance of blood markers sufficiently in UC, and that the extent of inflammation should be incorporated in evaluation.

Table 2 Predictive values of PLT, serum ALB, and CRP for mMES ≥ 2

	PLT ≥26.7 (×10⁴/μL)	ALB ≤4.2 (g/dL)	CRP ≥0.23 (mg/dL)
AUC	0.75	0.77	0.75
Sensitivity	0.68 (0.60–0.73)	0.73 (0.65–0.79)	0.57 (0.51–0.63)
Specificity	0.79 (0.74–0.85)	0.73 (0.67–0.78)	0.81 (0.76–0.86)
PPV	0.74 (0.66–0.80)	0.70 (0.62–0.75)	0.73 (0.63–0.80)
NPV	0.74 (0.69–0.79)	0.75 (0.70–0.81)	0.68 (0.65–0.74)

Values in parenthesis indicate 95% confidence interval.
ALB, albumin; AUC, area under the curve; CRP, C-reactive protein; mMES, maximum score of Mayo endoscopic subscore; NPV, negative predictive value; PLT, platelet count; PPV, positive predictive value.

Table 3 Ratios of fulfillment of each cutoff value in patients with active inflammation (mMES ≥ 2) according to the extent of inflammation

	Within the splenic flexure (n = 42)	Beyond the splenic flexure (n = 52)	P-value
PLT ≥ 26.7 (×10⁴/μL)	21 (50%)	42 (81%)	0.0016
ALB ≤ 4.2 (g/dL)	18 (43%)	49 (94%)	<0.0001
CRP ≥ 0.23 (mg/dL)	11 (26%)	43 (83%)	<0.0001

ALB, albumin; CRP, C-reactive protein; mMES, maximum score of Mayo endoscopic subscore; PLT, platelet count.

Predictability of blood markers for cMES. To further examine the performance of blood markers in association with disease severity and extent, we performed comparison with the values of cMES (cumulative inflammation throughout the colorectum) (Table 4). The cutoff values calculated by ROC curve analysis, PLT ≥ 30.9×10^4/μL, ALB ≤ 4.0 g/dL, and CRP ≥ 0.34 mg/dL, for discrimination of patients with cMES ≥ 9 showed better AUC values (0.83, 0.90, and 0.90, respectively) than the cutoff values for mMES ≥ 2. The sensitivity and specificity for cMES ≥ 9 were also higher than those for mMES ≥ 2 (sensitivity: 0.76, 0.88, and 0.85, specificity: 0.79, 0.79, and 0.81 respectively). These results confirm that blood markers reflect integration of severity and extent of inflammation more specifically than focal endoscopic severity in UC patients.

Discussion

The results of the present study indicate that blood markers (PLT, ALB, and CRP) correlate with both focal endoscopic severity and cumulative severity of endoscopic activity throughout the colorectum. More importantly, all three markers showed better predictability for cumulative activity than for focal activity. Thus, blood markers reflect integration of severity and extent of inflammation more specifically than focal endoscopic severity in UC patients.

Table 4 Predictive values of PLT serum ALB, and CRP for cMES ≥ 9

	PLT ≥30.9 ($\times 10^4$/μL)	ALB ≤4.0 (g/dL)	CRP ≥0.34 (mg/dL)
AUC	0.83	0.90	0.90
Sensitivity	0.76 (0.62–0.88)	0.88 (0.73–0.95)	0.85 (0.69–0.93)
Specificity	0.79 (0.77–0.82)	0.79 (0.76–0.80)	0.81 (0.78–0.82)
PPV	0.40 (0.33–0.46)	0.43 (0.35–0.45)	0.45 (0.36–0.48)
NPV	0.95 (0.92–0.98)	0.97 (0.94–0.99)	0.97 (0.94–0.99)

Values in parenthesis indicate 95% confidence interval.
ALB, albumin; AUC, area under the curve; cMES, cumulative score of Mayo endoscopic subscore; CRP, C-reactive protein; NPV, negative predictive value; PLT, platelet count; PPV, positive predictive value.

There have been several reports referring to the performance of blood markers on endoscopic activity in UC. However, most reports evaluated endoscopic activity using only the most severe inflammation in the colorectum. Our study provided clear evidence that evaluation by comparison with focal endoscopic activity underestimates the performance of blood markers and that the markers show better association with integration of severity and extent of inflammation. Therefore, these markers are more reliable than previously considered.

Blood markers are considered to substantially reflect inflammatory status occurring in the entire body. Therefore, our hypothesis and obtained results with regard to incorporation of extent of inflammation appear to be reasonable. The difference in the cutoff values obtained from ROC analysis in discrimination of endoscopic activity (CRP and PLT: higher for cMES, ALB: lower for cMES) in this study also confirms the concept of the reflection of extensive inflammation of blood markers. In this context, the strength of this study was that all the endoscopic data were based on colonoscopy findings from the rectum to the cecum. We previously showed that in evaluation of endoscopic activity of UC, sigmoidoscopy alone was not sufficient, particularly in patients with severe disease activity.[20] Uneven prevalence of inflammation in the colorectum of UC appears to be one of the reasons for previous underestimation of blood markers.

CRP, the most common serum inflammatory marker, has been examined as the biomarker for endoscopic activity of UC. A previous study indicated that CRP is not so useful in UC as it is in Crohn's disease (CD) for the assessment of disease activity, except in acute severe colitis.[21] A systematic review with meta-analysis demonstrated that the pooled sensitivity, specificity, and AUC estimates of CRP for endoscopic active disease were 0.49, 0.92, and 0.72, respectively.[4] Our results (sensitivity: 0.57, specificity: 0.81, and AUC: 0.72) for mMES ≥ 2 were in line with the meta-analysis results because all the papers included in the meta-analysis evaluated endoscopic activity of UC with focal maximum severity. In the present study, the sensitivity and AUC of CRP clearly increased when the target was changed from mMES to cMES (for cMES ≥ 9: sensitivity 0.85 and AUC 0.90). Thus, CRP, a sensitive marker for systemic inflammation, showed sufficiently high predictability even in UC when the extent of active inflammation was incorporated. In this context, the reported higher performance of CRP in CD may be attributable to the method of evaluation of endoscopic activity for CD, because endoscopic index for CD usually include the parameters of extent of disease.

Seo *et al.*[22] showed that disease severity in patients with UC is significantly influenced by five factors: bloody stool, bowel movements, erythrocyte sedimentation rate, hemoglobin, and ALB (Seo index). Thus, ALB has been regarded as a biomarker for disease activity in IBD, although the correlation with endoscopic activity in UC has rarely been reported. ALB is a negative acute phase reactant, and decreased levels can be found during inflammation. During the active phase of IBD, inflammation causes decrease in ALB with two scenarios: leak from intestinal mucosa due to mucosal injury caused by damage of vascular endothelium and enhanced permeability, and suppression of synthesis in the liver. Therefore, hypoalbuminemia is an inevitable accompaniment to the inflammatory process. Moreover, malnutrition and malabsorption due to IBD also cause low ALB levels. Thus, hypoalbuminemia is a consequence of the combined effects of inflammation and inadequate protein and caloric intake.[23] In the present study, the performance of ALB was equivalent to that of CRP. In addition, it should be noted that the cutoff for cMES (4.0 g/dL) was commonly used as the cutoff of normal range, suggesting that ALB levels below the normal limit may be a sensitive indicator of inflammation of UC.

Mean PLT was higher in UC patients with active disease than in inactive UC or healthy controls.[24,25] The relevance of PLT dysfunction to IBD pathogenesis is still unclear, but in addition to the role in hemostasis, PLTs can also function as potent proinflammatory cells.[26] Activated PLTs by inflammation express CD40 ligand and can interact with a large number of CD40-bearing immune and non-immune cells.[27] This correlation may contribute to the increase in PLT in UC patients with active disease.[28–30] Schoepfer *et al.*[31] showed the correlation of PLT with modified Baron index ($r = 0.49$). In addition, Lobatón *et al.*[32] showed the correlation with MES ($r = 0.38$). Both reports evaluated focal severity of inflammation, and the results of those reports were consistent with our result for mMES ($r = 0.47$). Similar to the other two blood markers, PLT also better reflects inflammation incorporating disease extent (for cMES [$r = 0.54$]). Although PLT levels are likely to show variation among individuals, physicians should note that increase in PLT to more than 30×10^4/μL indicates expansive mucosal inflammation.

In the clinical practice of UC, fecal markers, particularly fecal calprotectin, have been frequently used as well as blood markers. The predictability of fecal calprotectin for endoscopic activity in UC was reported in a systematic review and meta-analysis[4] with 0.88 sensitivity, 0.73 specificity, and 0.89 AUC. According to the predictive values, fecal calprotectin has been considered to be superior to blood markers. Interestingly, however, those values are quite similar to the values of blood markers for cMES in the current study. Therefore, in incorporating the severity and extent of activity, the predictive value of blood markers appears to be no lower than that of fecal calprotectin. In this regard, however, fecal calprotectin should also be evaluated in comparison to endoscopic activity incorporating extent of inflammation.

There are several limitations to this study. It was conducted with a retrospective design in a single hospital. However, as it was a cross-sectional observation study of clinical practices

without intervention, the study design would not have caused great bias. Also, blood markers and endoscopic findings could not be compared with fecal markers and histological findings, because they were not routinely examined at the time. Most previous reports examined the correlation of fecal markers or histological findings with endoscopic activity using only focal endoscopic activity, so comparison with endoscopic activity incorporating extent of inflammation would reveal more clinical significance of those factors. The strength of the current study was incorporation of extent of inflammation into evaluation of blood makers. For this purpose, however, the optimal method for evaluation may have been continuous integration of endoscopic activity from the rectum to the cecum. Such methodology might show more reliable data for the performance of blood markers in the future.

In conclusion, our study revealed that blood markers (PLT, ALB, and CRP) reflect integration of severity and extent of inflammation more specifically than focal endoscopic severity in UC patients. Blood markers are more reliable than previously considered for disease evaluation of UC. They can be a surrogate instrument for colonoscopy, resulting in being helpful in the determination of treatment strategy. Physicians should pay more attention to the changes of blood markers in clinical practice of UC.

References

1 Magro F, Gionchetti P, Eliakim R *et al*. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J. Crohns Colitis*. 2017; **11**: 649–70. https://doi.org/10.1093/ecco-jcc/jjx008.

2 Karoui S, Laz S, Serghini M *et al*. Correlation of C-reactive protein with clinical and endoscopic activity in patients with ulcerative colitis. *Dig. Dis. Sci.* 2011; **56**: 1801–5.

3 Yoon JY, Park SJ, Hong SP *et al*. Correlations of C-reactive protein levels and erythrocyte sedimentation rates with endoscopic activity indices in patients with ulcerative colitis. *Dig. Dis. Sci.* 2014; **59**: 829–37.

4 Mosli MH, Zou G, Garg SK *et al*. C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am. J. Gastroenterol.* 2015; **110**: 802–19.

5 Jensen KB, Jarnum S, Koudahl G, Kristensen M. Serum orosomucoid in ulcerative colitis: its relation to clinical activity, protein loss, and turnover of albumin and IgG. *Scand. J. Gastroenterol.* 1975; **11**: 177–83.

6 Nakarai A, Kato J, Hiraoka S *et al*. Prognosis of ulcerative colitis differs between patients with complete and partial mucosal healing, which can be predicted from the platelet count. *World J. Gastroenterol.* 2014; **20**: 18367–74.

7 Onal IK, Beyazit Y, Sener B *et al*. The value of fecal calprotectin as a marker of intestinal inflammation in patients with ulcerative colitis. *Turk. J. Gastroenterol.* 2012; **23**: 509–14.

8 Schoepfer AM, Beglinger C, Straumann A *et al*. Ulcerative colitis: correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. *Inflamm. Bowel Dis.* 2009; **15**: 1851–8.

9 Boon GJ, Day AS, Mulder CJ, Gearry RB. Are faecal markers good indicators of mucosal healing in inflammatory bowel disease? *World J. Gastroenterol.* 2015; **21**: 11469–80.

10 Mooiweer E, Severs M, Schipper ME *et al*. Low fecal calprotectin predicts sustained clinical remission in inflammatory bowel disease patients: a plea for deep remission. *J. Crohns Colitis*. 2015; **9**: 50–5.

11 Kuriyama M, Kato J, Takemoto K, Hiraoka S, Okada H, Yamamoto K. Prediction of flare-ups of ulcerative colitis using quantitative immunochemical fecal occult blood test. *World J. Gastroenterol.* 2010; **16**: 1110–14.

12 Nakarai A, Kato J, Hiraoka S *et al*. Evaluation of mucosal healing of ulcerative colitis by a quantitative fecal immunochemical test. *Am. J. Gastroenterol.* 2013; **108**: 83–9.

13 Takashima S, Kato J, Hiraoka S *et al*. Evaluation of mucosal healing in ulcerative colitis by fecal calprotectin vs fecal immunochemical test. *Am. J. Gastroenterol.* 2015; **110**: 873–80.

14 Sands BE. Biomarkers of inflammation in inflammatory bowel disease. *Gastroenterology*. 2015; **149**: 1275–85.

15 Cioffi M, Rosa D, Serao R, Picone I, Vietri MT. Laboratory markers in ulcerative colitis: current insights and future advances. *World J. Gastrointest. Pathophysiol.* 2015; **6**: 13–22.

16 Gomes P, du Boulay C, Smith CL, Holdstock G. Relationship between disease activity indices and colonoscopic findings in patients with colonic inflammatory bowel disease. *Gut*. 1986; **27**: 92–5.

17 Miranda-García P, Chaparro M, Gisbert JP. Correlation between serological biomarkers and endoscopic activity in patients with inflammatory bowel disease. *Gastroenterol. Hepatol.* 2016; **39**: 508–15.

18 Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am. J. Gastroenterol.* 2008; **103**: 162–9.

19 Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N. Engl. J. Med.* 1987; **317**: 1625–9.

20 Kato J, Kuriyama M, Hiraoka S, Yamamoto K. Is sigmoidoscopy sufficient for evaluating inflammatory status of ulcerative colitis patients? *J. Gastroenterol. Hepatol.* 2011; **26**: 683–7.

21 Solem CA, Loftus EV, Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. *Inflamm. Bowel Dis.* 2005; **11**: 707–12.

22 Seo M, Okada M, Yao T, Ueki M, Arima S, Okumura M. An index of disease activity in patients with ulcerative colitis. *Am. J. Gastroenterol.* 1992; **87**: 971–6.

23 Don BR, Kaysen G. Serum albumin: relationship to inflammation and nutrition. *Semin. Dial.* 2004; **17**: 432–7.

24 Kapsoritakis AN, Koukourakis MI, Sfiridaki A *et al*. Mean platelet volume: a useful marker of inflammatory bowel disease activity. *Am. J. Gastroenterol.* 2001; **96**: 776–81.

25 Kayahan H, Akarsu M, Ozcan MA *et al*. Reticulated platelet levels in patients with ulcerative colitis. *Int. J. Colorectal Dis.* 2007; **22**: 1429–35.

26 Danese S, De La Motte C, Fiocchi C. Platelets in inflammatory bowel disease: clinical, pathogenic, and therapeutic implications. *Am. J. Gastroenterol.* 2004; **99**: 938–45.

27 Henn V, Slupsky JR, Gräfe M *et al*. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature*. 1998; **391**: 591–4.

28 Garlichs CD, Eskafi S, Raaz D *et al*. Patients with acute coronary syndromes express enhanced CD40 ligand/CD154 on platelets. *Heart*. 2001; **86**: 649–55.

29 Khan SY, Kelher MR, Heal JM *et al*. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40,

and is a potential cofactor in the development of transfusion-related acute lung injury. *Blood*. 2006; **108**: 2455–62.
30 Phipps RP, Kaufman J, Blumberg N. Platelet derived CD154 (CD40 ligand) and febrile responses to transfusion. *Lancet*. 2001; **357**: 2023–4.
31 Schoepfer AM, Beglinger C, Straumann A *et al*. Fecal calprotectin more accurately reflects endoscopic activity of ulcerative colitis than the Lichtiger Index, C-reactive protein, platelets, hemoglobin, and blood leukocytes. *Inflamm. Bowel Dis.* 2013; **19**: 332–41.
32 Lobatón T, Rodríguez-Moranta F, Lopez A, Sánchez E, Rodríguez-Alonso L, Guardiola J. A new rapid quantitative test for fecal calprotectin predicts endoscopic activity in ulcerative colitis. *Inflamm. Bowel Dis.* 2013; **19**: 1034–42.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1 The correlations between the changes of blood markers (Δ platelet [ΔPLT], Δ albumin [ΔALB], and Δ C-reactive protein [ΔCRP]) and the changes of cumulative score of Mayo endoscopic subscore (ΔcMES) in patients who underwent two or more colonoscopies. The changes of all three blood markers were correlated well with the changes of cMES.