New Fecal Biomarker, α1-acid Glycoprotein, for Evaluation of Inflammatory Bowel Disease: Comparison with Calprotectin and Lactoferrin

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Abstract

Objective: Fecal leukocytes biomarkers such as calprotectin（Cal）and lactoferrin（LF）have been shown to reflect the disease activity of inflammatory bowel disease（IBD）. Here, we evaluated fecal α1-acid glycoprotein（AG）, acute-phase reactant protein, as a new fecal biomarker.

Methods: Thirty six patients with ulcerative colitis（UC）and Crohn's disease（CD）were analyzed. Active or inactive conditions determined to clinical activity index（CAI）, Mayo endoscopic subscore and Matts' histopathological grade in UC, while by Crohn's disease activity index（CDAI）and simple endoscopic score for Crohn's disease in CD. The fecal levels of biomarkers were measured by enzyme-linked immunosorbent assay.

Results: All three fecal biomarkers were significantly higher in the CAI active than in the inactive group, but not in the CDAI active　compared with the inactive group. These biomarkers were significantly elevated in endoscopically active compared to the inactive UC and CD, respectively. These biomarkers were also significantly higher in the histologically active than in the inactive UC.

Conclusions: Fecal biomarkers, AG as well as Cal and LF, could differentiate active from inactive UC and CD. Our results strongly suggest that the fecal AG may be valuable noninvasive diagnostic tools for evaluation of the activity of IBD.

Key words： **Fecal α1-acid glycoprotein, Calprotectin, Lactoferrin, Inflammatory bowel disease**

Introduction

Inflammatory bowel disease（IBD）such as ulcerative colitis（UC）and Crohn's disease（CD）are chronic inflammatory disorders of gastrointestinal tract characterized by relapse and remission. It is important to evaluate the activity of the disease for the treatment of IBD. The evaluations of the activity of IBD have been mainly achieved by clinical, laboratory and endoscopic disease activity indices. C-reactive protein（CRP）, erythrocyte sedimentation rate and white blood cells are widely used as noninvasive parameters for IBD. These laboratory markers do not appropriately reflect the activity of the intestinal tract because of a summation of systemic host responses rather than being specific for intestinal inflammation in IBD patients [1, 2]. Subsequently, they have insufficient sensitivity and specificity for intestinal inflammation [3]. Therefore, more reliable biological markers are required to confirm the disease activity.

Current gold standard for assessing intestinal inflammation has been considered to be endoscopic evaluation. Endoscopy allows visual determination of

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Table 1　Characteristics of subjects

	UC	CD	Controls
Number of subjects	20	16	6
Gender (No. , male / female)	9 / 11	3 / 13	6 / 0
Age　(yr. , mean, range)	45.8 (21-71)	35.3 (16-55)	35.8 (31-42)
Disease duration (yr. , mean, range)	15.7 (0-33)	8.1 (0-31)	—
Smoking (No. , never / former / current)	18 / 2 / 0	12 / 2 / 2	5 / 1 / 0
Family history of IBD (No.)	0	0	0
Prior bowel surgery (No.)	0	6	0
Disease type (No.)			
Extensive / Left-sided / Proctitis	12 / 6 / 2	—	—
Ileitis / Ileocolitis / Colitis	—	2 / 11 / 3	—

disease severity and extent, and then mucosal biopsy could provide further information. However, endoscopic procedure is invasive and requires an uncomfortable preparation. The active gut inflammation is closely associated with the migration of neutrophils into the gut. A number of neutrophil-derived proteins in stools have been studied, including fecal calprotectin (Cal), lactoferrin (LF), lysozyme, elastase, and myeloperoxidase [2]. There has been increasing evidence that fecal biomarkers are valuable tools because of a simple, rapid, sensitive, specific, inexpensive and noninvasive to detect and monitor intestinal inflammation in IBD [4]. Recently, fecal Cal and LF have been shown to be excellent markers of intestinal inflammation, as it reflects the migration of neutrophils through the inflamed bowel wall to the mucosa [3, 5, 6].

α1-acid glycoprotein (AG) (orosomucoid) is an acute phase protein synthesized predominantly by hepatocytes in response to tissue injury, inflammation or infection [7]. During an acute phase condition, the concentration rises several times, making it one of the predominant proteins in serum [8]. Serologically, serum AG has been proved to have a stronger correlation with CDEIS, endoscopic disease score, compared with CRP [9]. The serum level of AG has been assessed as a standard disease activity index, although a long half life limits its usefulness [7]. Recently, urinary AG has been proposed as a potential biomarker in CD [10]. However, there has been no report

evaluating a disease of IBD using fecal AG. The aim of this study was to investigate the clinical utility of fecal AG as the evaluation of IBD activity. Here we identified validity of fecal biomarkers in the evaluation of IBD activity compared with various indices.

Subjects and Methods

Subjects

Thirty-six IBD patients (UC, 20; CD, 16) were included at the Fukuoka University Hospital, Fukuoka, Japan. Control group consisted of six healthy subjects who showed no evidence of abnormality under ileocolonoscopy. Their demographic characteristics are shown in Table 1. Six patients with CD had undergone prior ileocecal resection. Informed consent was obtained from all patients and control subjects. Study protocol was approved by the Ethics Committee of the Fukuoka University Hospital (09-5-09).

Evaluation of clinical, endoscopic and pathological disease activity in UC

As the clinical parameter, clinical activity index (CAI) was used [11]. Endoscopic activity indices were based on Mayo endoscopic subscore [12]. Pathological evaluation of biopsy specimens was performed after stained with hematoxylin and eosin. A single experienced gastrointestinal pathologist (S.N.), who was completely

blinded to the endoscopic findings, scored the pathological findings according to the scoring system of Matts' histopathological grade [13]. The endoscopic and pathological evaluations were performed regarding the most severely diseased area.

Evaluation of clinical and endoscopic disease activity in CD

Crohn's disease activity index（CDAI）was used as a clinical index [14]. Endoscopic activity indices were based on simple endoscopic score for Crohn's disease（SES-CD）[15].

Assessment of activity in UC and CD

As shown in Table 2, CAI, Mayo endoscopic subscore, Matts' histopathological grade, CDAI and SES-CD were divided into active and inactive conditions [11, 14, 16, 17]. Endoscopically active conditions in UC were defined as subscore of 2-3, while inactive conditions were defined as subscore of 0-1, which was previously considered as mucosal healing（MH）[17].

Collection of fecal samples and fecal analysis

Fecal samples were taken within one week before or after endoscopic examination in the absence of the change of treatments, and then stocked at -30℃ within 5 hours after sampling until analysis. Cal was measured by enzyme linked immunosorbent assay（ELISA）method（Calprotectin ELISA Kit, Immundiagnostik AG, Bensheim, Germany）. Moreover, LF and AG were similarly assayed using ELISA method at the Kyoto

Institute of Medical Science（Kyoto Medical Science Laboratory, Kyoto, Japan）.

Statistical analysis

The data were analyzed using the Mann-Whitney U-test and the Spearman rank correlation coefficient. For all analyses, P–values less than 0.05 were considered statistically significant.

Results

Comparison of fecal biomarkers (AG, Cal and LF) among UC, CD and control groups

Fecal Cal, LF and AG levels were significantly higher in both UC and CD groups than in the control group（Cal, UC $P<0.05$, CD $P<0.001$; LF, UC $P<0.001$, CD $P<0.001$; AG, UC $P<0.05$, CD $P<0.005$）（Figure 1）.

Correlation of fecal biomarkers (Cal, LF and AG) with CAI in UC

All fecal biomarkers were significantly correlated with CAI（Cal, $r=0.789$, $P<0.001$; LF, $r=0.647$, $P<0.01$; AG, $r=0.777$, $P<0.001$）. Fecal Cal, LF and AG levels were significantly higher in the active phase than in the inactive phase（Cal, $P<0.001$; LF, $P<0.001$; AG, $P<0.001$）（Figure 2）.

Comparison of fecal biomarkers (Cal, LF and AG) with endoscopic activity indices in UC

Endoscopically active UC patients showed significantly higher fecal Cal, LF and AG levels than inactive UC

Table 2　Assessment of activity in UC and CD

	Inactive	Active
UC		
CAI	≦ 4	5 ≦
Mayo endoscopic subscore	0 – 1	2 – 3
Matts' histopathological grade	1 – 2	3 – 5
CD		
CDAI	≦ 150	151 ≦
SES-CD	0 – 3	4 ≦

UC, ulcerative colitis; CD, Crohn's disease; CAI, clinical activity index; CDAI, Crohn's disease activity index; SES-CD, simple endoscopic score for Crohn's disease.
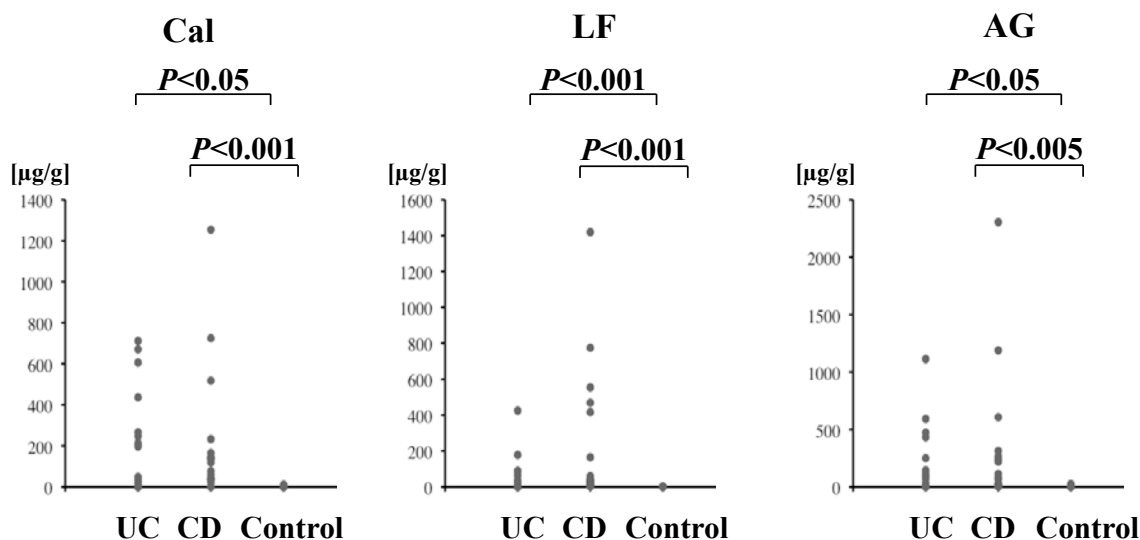
Cal

P<0.05

P<0.001

LF

P<0.001

P<0.001

AG

P<0.05

P<0.005

Figure 1　Comparison of fecal biomarkers（Cal, LF and AG）among UC, CD and control groups.
In both UC and CD groups, Cal, LF and AG levels are significantly higher compared with the control group, respectively（Cal, *P*<0.05, *P*<0.001; LF, *P*<0.001, *P*<0.001; AG, *P*<0.001, *P*<0.001）.
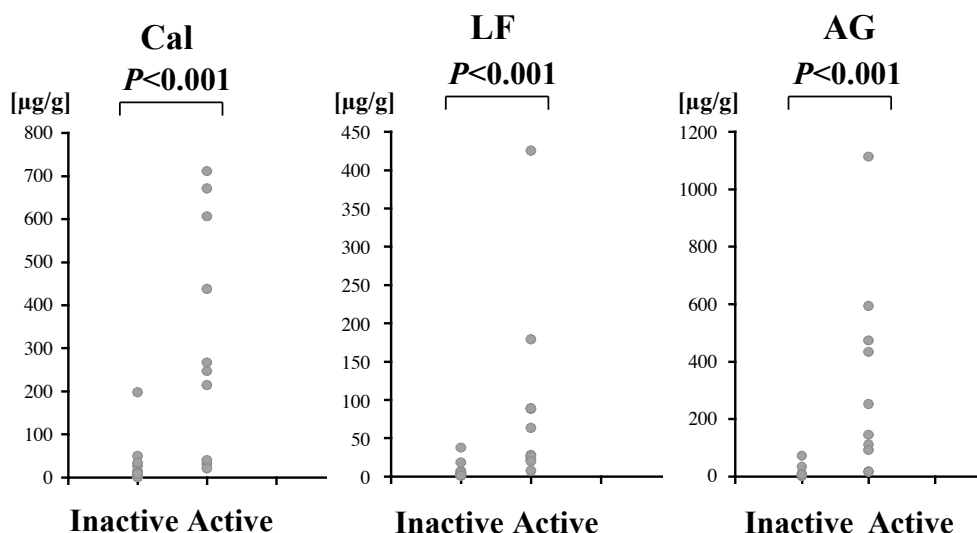
Cal

P<0.001

LF

P<0.001

AG

P<0.001

Figure 2　Comparison of fecal biomarkers（Cal, LF and AG）with CAI in UC.
The fecal Cal, LF and AG levels were significantly higher in the active phase than the inactive phase groups（Cal, *P*<0.001; LF, *P*<0.001; AG, *P*<0.001）.

patients by Mayo endoscopic subscore（Cal, *P*<0.001; LF, *P*<0.05; AG, *P*<0.001）（Figure 3）.

Comparison of fecal biomarkers (Cal, LF and AG) with histopathological grade in UC

Fecal Cal, LF and AG levels were significantly higher in the active conditions than in the inactive conditions by Matts' histopathological grade（Cal, *P*<0.005; LF, *P*<0.05; AG, *P*<0.001）（Figure 4）.

Correlation of fecal biomarkers (Cal, LF and AG) with CDAI in CD

Fecal Cal and AG were poorly correlated with CDAI （Cal, *r*=0.603, *P*<0.05; AG, *r*=0.503, *P*<0.05）, but LF was not（LF, *r*=0.298, *P*=0.262,）. There were no significant differences in fecal Cal, LF and AG levels between inactive and active CDAI（Figure 5）.

Comparison of fecal biomarkers (Cal, LF and AG) with SES-CD in CD

Endoscopically active CD patients showed significantly

Cal
P<0.001

[µg/g]

Inactive　Active

LF
P<0.05

[µg/g]

Inactive　Active
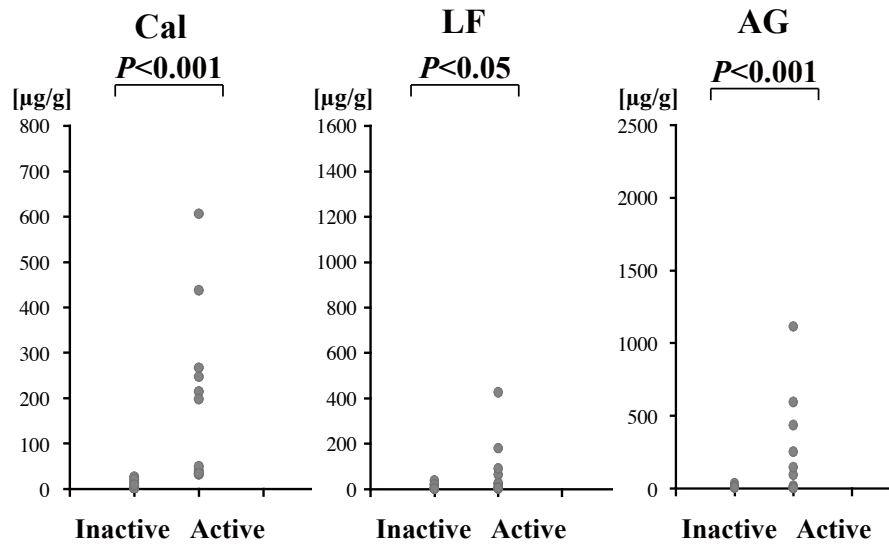
AG
P<0.001

[µg/g]

Inactive　Active

Figure 3　Comparison of fecal biomarkers（Cal, LF and AG）with endoscopic activity indices in UC.
A significant difference was observed in fecal Cal, LF and AG when dividing into inactive and active conditions（Cal, *P*<0.001; LF, *P*<0.05; AG, *P*<0.001）.

Cal
P<0.005

[µg/g]

Inactive　Active

LF
P<0.05

[µg/g]

Inactive　Active

AG
P<0.001
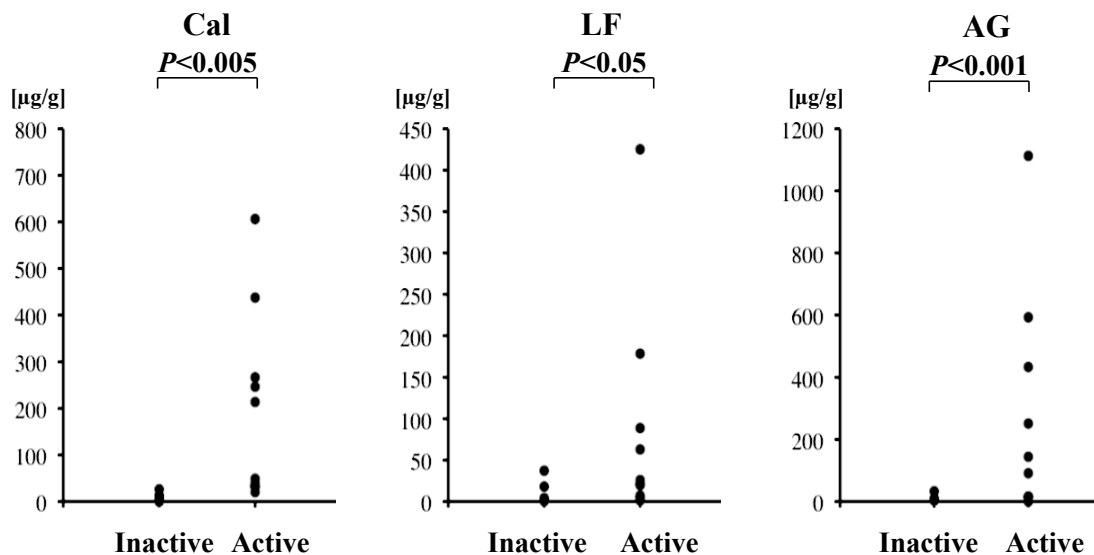
[µg/g]

Inactive　Active

Figure 4　Comparison of fecal biomarkers（Cal, LF and AG）with histopathological grade in UC.
A significant difference was observed in fecal Cal and LF when dividing into inactive and active conditions（Cal, *P*<0.005; LF, *P*<0.05; AG, *P*<0.001）.

higher fecal Cal, LF and AG levels than inactive CD patients by SES-CD（Cal, *P*<0.001; LF, *P*<0.001; AG, *P*<0.001）（Figure 6）.

Discussion

　The present study first demonstrated that fecal AG in similar to fecal Cal and LF was determined to be significantly higher in both UC and CD groups than in the control group, and to associate with endoscopic disease activity scores in UC and CD. In UC, all three fecal

biomarkers were significantly higher in the CAI active group than in the inactive group, but not in the CDAI active group compared with the inactive group in CD. These fecal biomarkers were also significantly higher in the histologically active group than in the inactive group in UC.

　The previous studies have indicated that fecal Cal and LF assay are useful markers for identifying IBD from irritable bowel syndrome or healthy controls, and that these markers provide better correlations with endoscopic disease activities [3, 16, 18, 19]. Our results are in accordance
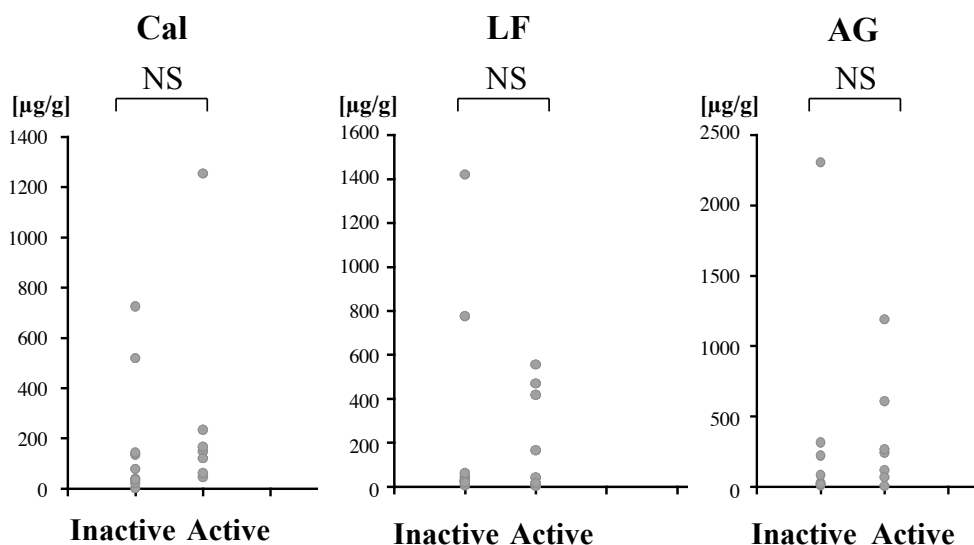
Cal
LF
AG

NS

NS

NS

[µg/g]

[µg/g]

[µg/g]

Inactive Active

Inactive Active

Inactive Active

Figure 5 Comparison of fecal biomarkers (Cal, LF and AG) with CDAI in CD.
There were no significant differences in fecal Cal, LF and AG levels between inactive and active CDAI.
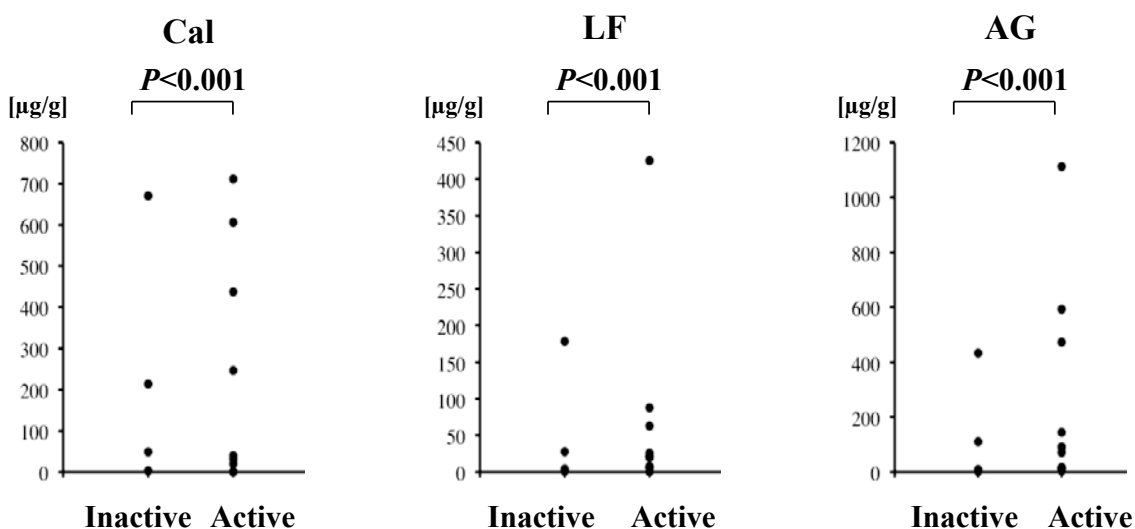
Cal
LF
AG

P<0.001

P<0.001

P<0.001

[µg/g]

[µg/g]

[µg/g]

Inactive Active

Inactive Active

Inactive Active

Figure 6 Comparison of fecal biomarkers (Cal, LF and AG) with SES-CD in CD.
Fecal Cal, LF and AG assay were significantly higher in the active group compared with the inactive group by SES-CD (Cal, *P*<0.001; LF, *P*<0.001; AG, *P*<0.001).

with their findings regarding the good performance of fecal Cal and LF to discriminate between different inflammatory activities. In this study, fecal AG levels also have good diagnostic accuracy in distinguishing IBD from healthy controls. Furthermore, here we identified that fecal AG levels were well correlated with the endoscopic activity in UC and CD. It is considered that these results showed fecal AG, in addition to Cal and LF, reflect the activity of intestinal mucosa in IBD.

Serum AG is an acute phase protein which has been determined to be correlated well with disease activity [7].

The leakage of elevated serum AG reflects both of the increase in serum AG and the mucosal defect. In addition to fecal excretion of AG, several reports have shown that urinary excretion of AG significantly increases in lupus nephritis patients with active renal disease compared with inactive disease [20], and that urinary AG is associated with diabetic nephropathy, in which urinary AG is an independent risk factor [21]. Thus AG is appeared to be a promising fecal and urinary biomarker.

From our study, fecal biomarkers (Cal, AF and AG) were associated with the discrimination between active

and inactive clinical indices in UC, but not in CD. The similar discrepancy has also been documented for the correlation between symptoms and endoscopic appearance [22, 23]. In CD, evaluation of remissions and relapses are usually based on the CDAI value, which represents a subjective and indirect assessment of gut inflammatory activity because it includes certain variables and symptoms not directly correlated with active inflammation and expression of severity rather than activity of disease [24]. Thus the fecal biomarkers might be correlated with endoscopic activities rather than clinical indices, suggesting that fecal biomarkers are more reliable for assessing the disease activity in IBD.

Recently, the treatment in IBD has aimed at MH on endoscopy since MH after 1 year of treatment is predictive of reduced subsequent disease activity and decreased need for active treatment such as colectomy [25]. Furthermore, endoscopic monitoring after treatment has now become of significance [26]. However, endoscopic examinations have the disadvantage of being invasive, time-consuming, expensive, and sometimes uncomfortable for patients [16]. The definition of MH is ill-defined in the small intestine, compared with the large intestine. Therefore, there is a growing importance of fecal biomarkers, which is closely correlated with endoscopic disease activity and MH.

Our study has some limitations. Although serum AG has been shown to be well correlated with disease activity, its longer half life, compared with CRP, have been considered to make its limit in clinical practice [2, 7]. However, for the evaluation of fecal excretion, the longer half life may be advantage. Second, the sensitivity and specificity of fecal AG were not obtained because of the small number of patients. Further studies are required to evaluate of fecal AG assay in a large number of IBD patients.

In conclusion, the present study first demonstrated the clinical usefulness of fecal AG, in addition to Cal and LF, in IBD. The close associations are demonstrated between fecal biomarkers and CAI, endoscopic activity and histological activity in UC, and between those and endoscopic activity in CD. These results suggest that fecal AG reflect the degree of intestinal inflammation, and could be a promising noninvasive diagnostic tool for evaluation of the activity in patients with IBD.

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