Effect of Ulcerative Colitis Duration on the Usefulness of Immunochemical Fecal Occult Blood Test Result as a Disease Activity Biomarker

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Natsuki Ishida[a], Takahiro Miyazu[a], Tomoharu Matsuura[a], Ryosuke Takano[a], Satoshi Tamura[a], Takuma Kagami[a], Shinya Tani[b], Mihoko Yamade[a], Yasushi Hamaya[a], Moriya Iwaizumi[c], Satoshi Osawa[b], Takahisa Furuta[d], and Ken Sugimoto[a]

[a]First Department of Medicine, Hamamatsu University School of Medicine, Shizuoka, Japan

[b]Department of Endoscopic and Photodynamic Medicine, Hamamatsu University School of Medicine, Shizuoka, Japan

[c]Department of Laboratory Medicine, Hamamatsu University School of Medicine, Shizuoka, Japan

[d]Center for Clinical Research, Hamamatsu University School of Medicine, Shizuoka, Japan

Short title: Effect of UC Duration on FIT

Correspondence:

Ken Sugimoto, MD, PhD

The First Department of Medicine, Hamamatsu University School of Medicine, Shizuoka, Japan

1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192 (Japan)

Tel: 81 534352261; Fax: 81 534349447; E-mail: sugimken@hama-med.ac.jp

Declarations

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval

This study was reviewed and approved by the Institutional Review Board of Hamamatsu University School of Medicine.

Consent to participate

Written informed consent to participate was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contributions

Natsuki Ishida and Ken Sugimoto designed the study. Takahiro Miyazu, Tomoharu Matsuura, Ryosuke Takano, Satoshi Tamura, and Takuma Kagami collected the data. Shinya Tani, Mihoko Yamade, Moriya Iwaizumi, and Yasushi Hamaya analyzed the data. Natsuki Ishida and Ken Sugimoto wrote the paper. Satoshi Osawa and Takahisa Furuta provided critical insight regarding paper preparation.

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Abstract

Purpose: The effects of ulcerative colitis (UC) duration on biomarker accuracy are unknown. We investigated the effects of UC duration on the predictive accuracy of biomarkers including immunochemical fecal occult blood test (FOBT, also known as FIT), prostaglandin E-major urinary metabolite (PGE-MUM), and C-reactive protein (CRP).

Methods: We divided 133 samples into groups based on disease duration. Clinical and endoscopic remission was defined as Lichtiger's clinical activity index (CAI) of ≤4, Mayo endoscopic subscore (MES) of 0, and UC endoscopic index of severity (UCEIS) of ≤1.

Results: FIT results were significantly correlated with all activity scores when the disease duration was <4 years. When the disease duration was ≥4 years, FIT results were significantly correlated with the CAI and MES but not with UCEIS. When the disease duration was ≥5 years, FIT and CAI were significantly correlated, whereas FIT and MES or FIT and UCEIS did not show any correlation. When the duration was ≥4 years, PGE-MUM and CRP showed a significant correlation with CAI, MES, and UCEIS. Receiver operating characteristic curve analysis of biomarker data for predicting endoscopic remission showed that the accuracy of FIT was superior to that of PGE-MUM and CRP in the <4 year group.

Conclusions: FIT is an accurate biomarker reflecting the endoscopic score until 4 years in patients with UC. However, owing to the increased number of false negatives, the usefulness of FIT may decline after 4 years. Hence, evaluation of UC in combination with other biomarkers is recommended.

Keywords: ulcerative colitis, disease duration, fecal immunochemical test, prostaglandin E-major urinary metabolite, C-reactive protein.

1. Introduction

Ulcerative colitis (UC) refers to idiopathic intestinal chronic inflammation, and is characterized by diarrhea, bloody stools, abdominal pain, fever, anemia, and weight loss [1]. Previously, clinical symptom scoring and blood examination were mainly used for evaluating UC disease status, which was then used for choosing the right treatment. Mucosal healing has been reported to improve remission maintenance rates, shorten hospital stays, and decrease the possibility of colectomy in patients with UC [2]. In recent years, mucosal healing has become the target of all UC treatments. This makes endoscopic evaluations very important; however, endoscopic examinations are invasive and expensive. Biomarkers can be used to evaluate mucosal healing without endoscopic examinations. For example, acute phase reactants in the blood, such as C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) are commonly used for this purpose. Moreover, the use of fecal biomarkers, such as immunochemical fecal occult blood test (FOBT or FIT) and fecal calprotectin (FC), to evaluate mucosal healing status has also been recently reported [3-9]. Furthermore, the urine level of prostaglandin E-major urinary metabolite (PGE-MUM) is considered a biomarker of UC [10, 11].

FIT is relatively inexpensive and rapid, and can be easily performed at any facility. FIT results indicate disease activity and can be used as a biomarker in patients with UC. Results of FIT reflect bleeding associated with inflammation of the intestinal tract. UC is characterized by relapse and remission, therefore, inflammation and repair are repeated in the intestinal mucosa [12]. This often leads to intestinal fibrosis,

especially when the disease is prolonged.

As fibrosis and scarring of the intestinal mucosa may affect the amount of bleeding from the intestinal tract, we speculated that the accuracy of FIT as a biomarker might be affected in patients with chronic UC. In contrast, blood and urine biomarkers are not affected by intestinal fibrosis. However, whether the accuracy of these biomarkers changes with disease duration has not been reported. In this study, we investigated the effect of disease duration on several biomarkers, including FIT, in patients with UC.

2. Materials and Methods

2.1. Patients

Patients with UC treated at Hamamatsu Medical University Hospital between August 2016 and April 2019 were eligible for enrollment in this study. In this time period, colonoscopy was performed 113 times in 70 patients; 113 samples of urine, feces, and blood were collected. The diagnosis of UC was based on clinical characteristics and endoscopic and histological evaluations according to the current guidelines. Written informed consent was obtained from all patients before enrollment in this study. Patients with Crohn's disease (CD), Behcet's disease, and other irritable bowel diseases (IBD), such as indeterminate colitis and inflammatory bowel disease unclassified, and malignant tumors, such as colorectal cancer, were excluded. Smokers were also excluded, because smoking was reported to increase the PGE-MUM level [13]. Chronic fibrosing interstitial pneumonia increases the PGE-MUM level, therefore, patients with interstitial

pneumonia were also excluded [14].

2.2. Disease assessment

Lichtiger's clinical activity index (CAI) was used to evaluate the clinical disease activity based on the following criteria: diarrhea (number of daily stools), nocturnal diarrhea, visible blood in stools (percentage of movements), fecal incontinence, abdominal pain or cramping, general well-being, abdominal tenderness, and need for anti-diarrheal drugs [15]. The score was evaluated on the same day as the endoscopic examination. Clinical remission was defined as the CAI of ≤4.

Bowel preparation was performed with a polyethylene glycol-based electrolyte solution or glycerin enema. The mucosal status of UC was assessed using the Mayo endoscopic subscore (MES) classification system and ulcerative colitis endoscopic index of severity (UCEIS) [16-18]. The MES was assessed 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. The UCEIS score was calculated as the simple sum of three descriptors: vascular pattern (scores 0–2), bleeding (scores 0–3), and erosions and ulcers (scores 0–3). These were evaluated in the most active lesions of the colon. Endoscopic remission and mucosal healing were defined by the MES of 0 and UCEIS score of 0 or 1.

2.3. FIT analysis

To avoid the effect of the endoscopic examination, fecal samples were obtained 2 days prior to colonoscopy. The patients prepared fecal samples from their stools using the collection kit (Eiken Chemical, Tokyo, Japan). The submitted samples were immediately processed and examined using OC Sensor IO (Eiken Chemical, Tokyo, Japan).

2.4. PGE-MUM analysis

Urine samples were obtained on the morning of the colonoscopic examination in our hospital and sent to SRL Hachioji Laboratory (Tokyo, Japan). The samples were frozen and stored at -20°C until the assay. Briefly, each spot of urine sample was analyzed using a γ-counter (Hitachi) and the Bicyclic PGE-MUM Radioimmunoassay (RIA) kit (Fuji Rebio, Tokyo, Japan). The measured PGE-MUM values were corrected using urine creatinine levels.

2.5. Study design

This was a prospective 3-year observational study. The purpose of this study was to determine whether differences in the duration of UC affected the correlation between the FIT result and endoscopic scores (MES and UCEIS). The primary endpoint was the correlation between the FIT result and endoscopic score (MES or UCEIS) at various periods of the disease. Disease duration was assessed from less than 2 years to

more than 8 years. Secondary endpoints included correlation between PGE-MUM or CRP and endoscopic score (MES or UCEIS). Cut-off values for the FIT result, PGE-MUM, and CRP were defined when the endoscopic activity (MES or UCEIS) was performed during the disease period, with a significant correlation between the FIT result and endoscopic activity.

2.6. Ethical consideration

This study was approved by the Ethics Committee of Hamamatsu University School of Medicine (Registration number 18-228). Complete verbal and written explanations of this study were provided to the patients and written consents were obtained from them.

2.7. Statistical analysis

Statistical analysis was performed using SPSS statistical software (SPSS for Windows, Version 16.0, Ekuseru-Toukei 2010; Social Survey Research Information Co., Ltd., Tokyo, Japan) and R program (http://cran.r-project.org). The results are expressed as mean ± standard deviation. Correlations between the biomarkers (FIT, PGE-MUM, and CRP) and activity index values were analyzed using a logistic regression analysis. Intergroup differences were compared using Student's *t* test. The accuracy of each biomarker was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. The results with a P-value of >0.05 were considered statistically significant.

3. Results

3.1. Patients' characteristics

The baseline characteristics of the patients are shown in Table 1. Urine, feces, and blood specimens (113 specimens each) were collected from 70 patients with UC. The mean age of patients was 48.3 years (Table 1) and the mean disease duration was 7.95 years (range, 0.1–34). Seventy-two (63.7%) patients had extensive colitis, 27 (23.9%) had left colitis, and 14 (12.4%) had proctitis. The mean of FIT result, PGE-MUM, and CRP levels was 2787 ng/mL, 30.3 μg/g·Cr, and 0.32 mg/dL, respectively.

During sampling, 72 (63.7%) patients were taking oral 5-aminosalicylic acid (5-ASA), 22 (19.4%) were taking 5-ASA suppositories, 17 (15.0%) were taking systemic steroids, 42 (37.2%) were taking immunomodulators, 10 (10.9%) were taking tacrolimus, and 30 (26.6%) were taking biologics.

3.2. Correlation between FIT results and clinical and endoscopic scores among patients with disease durations of <4 vs. ≥4 years

We analyzed the correlation between FIT result and clinical or endoscopic score by dividing the samples into two groups according to the disease duration: <4 years (46 samples) and ≥4 years (67 samples) (Figure 1). FIT results significantly correlated with the CAI and MES in both groups (<4 years: $r = 0.767$, $P < 0.001$ and $r = 0.534$, $P < 0.001$, respectively; ≥4 years: $r = 0.565$, $P < 0.001$ and $r = 0.356$, $P = 0.003$, respectively). Although FIT result significantly correlated with the UCEIS score in the group with disease duration of <4

years, it did not significantly correlate with the UCEIS score in the group with disease duration of ≥4 years (<4 years: r = 0.603, P < 0.001; ≥4 years: r = 0.235, P = 0.056).

3.3. Correlation between FIT results and clinical and endoscopic scores among patients with disease durations of 2, 3, 5, 6, 7, and 8 years

Regression analysis was performed for patients grouped by disease duration i.e. 2, 3, 5, 6, 7, and 8 years of UC (Table 2). In groups with disease durations of <2 and ≥ 2 years, FIT result showed a significant correlation with the CAI, MES, and UCEIS score; these results were similar to those observed for patients with disease duration of 3 years (<2 years: r = 0.821, P < 0.001, r = 0.495, P = 0.016, and r = 0.678, P < 0.001, respectively; ≥2 years: r = 0.516, P < 0.001, r = 0.367, P = 0.001, and r = 0.241, P = 0.022, respectively). Similarly, FIT result showed a significant correlation with the CAI, MES, and UCEIS scores among patients with disease durations of less than 5, 6, 7, and 8 years (<5 years: r = 0.661, P < 0.001, r = 0.566, P < 0.001, and r = 0.524, P < 0.001, respectively; <6 years: r = 0.673, P < 0.001, r = 0.582, P < 0.001, and r = 0.547, P < 0.001, respectively; <7 years: r = 0.672, P < 0.001, r = 0.570, P < 0.001, and r = 0.538, P < 0.001, respectively; <8 years: r = 0.675, P < 0.001, r = 0.531, P < 0.001, and r = 0.518, P < 0.001, respectively). Notably, FIT result correlated significantly with CAI, but not with MES and UCEIS scores in groups with disease durations of 5, 6, 7, and 8 years or more (≥5 years: r = 0.579, P < 0.001, r = 0.249,

P = 0.065, and r = 0.176, P = 0.195, respectively; ≥6 years: r = 0.557, P < 0.001, r = 0.222, P = 0.121, and r = 0.135, P = 0.351, respectively; ≥7 years: r = 0.568, P < 0.001, r = 0.216, P = 0.141, and r = 0.124, P = 0.406, respectively; ≥8 years: r = 0.569, P < 0.001, r = 0.243, P = 0.112, and r = 0.130, P = 0.399, respectively).

3.4. Correlation of PGE-MUM and CRP with the clinical and endoscopic scores among patients with disease duration of <4 years vs. ≥4 years

No significant correlation was observed between FIT results and UCEIS scores in groups with disease duration of 4 years or more. Therefore, we investigated the correlation of PGE-MUM and CRP with clinical and endoscopic scores among patients with disease durations of <4 and ≥4 years. PGE-MUM showed a significant correlation with the CAI, MES, and UCEIS score in both groups i.e. with disease durations of <4 and ≥4 years (<4 years: r = 0.517, P < 0.001, r = 0.406, P = 0.005, and r = 0.442, P = 0.002, respectively; ≥4 years: r = 0.331, P = 0.008, r = 0.438, P < 0.001, and r = 0.479, P < 0.001, respectively) (Figure 2).

CRP correlated significantly with the CAI and UCEIS score in the group with disease duration of <4 years (r = 0.569, P < 0.001, and r = 0.418, P = 0.004, respectively) (Figure 3). Although CRP tended to show an association with the MES, statistically significant correlation was not observed (r = 0.289, P = 0.051). CRP showed a significant correlation with the CAI, MES, and UCEIS score in the group with a disease duration of ≥4 years (r = 0.383, P = 0.001, r = 0.454, P < 0.001, and r = 0.489, P < 0.001,

respectively).

We also performed a regression analysis of the endoscope scores (MES and UCEIS scores) for PGE-MUM and CRP. PGE-MUM significantly correlated with endoscopic scores (MES and UCEIS score) in both the groups with short and long disease duration i.e. <4 and >4 years (from 2 to up to 8 years; Table 3). Although CRP did not show a significant correlation with MES in the groups with disease durations of <2 years and <4 years, CRP and MES or UCEIS score showed a significant correlation in other groups (Table 4).

3.5. Accuracy of biomarkers in the group with a disease duration of <4 years

We analyzed the accuracy of each biomarker in assessing the endoscopic activity in groups with a disease duration of <4 years. We defined the MES score of 0 and UCEIS score of 0 or 1 as endoscopic remission. The MES of ≥1 and UCEIS score of ≥2 were considered to indicate endoscopic activity. Based on this definition, ROC curves of the biomarkers were analyzed for samples in group with a disease duration of <4 years. The optimal cut-off values of each biomarker for predicting endoscopic remission by MES were as follows: FIT = 31.0 ng/mL, PGE-MUM = 22.1 μg/g･Cr, and CRP = 0.05 mg/mL (Figure 4a). The area under the curve (AUC) of the ROC curve of FIT result for predicting endoscopic remission using the MES was 0.911 (95% CI: 0.829–0.992), whereas that of PGE-MUM and CRP was 0.681 (95% CI: 0.509–0.853) and 0.747 (95% CI: 0.581–0.912), respectively. When a similar analysis was performed using the UCEIS

score, the optimal cut-off value for FIT result was 31.0 ng/mL, for PGE-MUM was 24.0 μg/g･Cr, and for CRP was 0.11 mg/mL (Figure 4b). The AUC of the ROC curve for UCEIS was 0.843 (95% CI: 0.726–0.961), 0.642 (95%CI: 0.472–0.811), and 0.742 (95%: 0.590–0.894), respectively. The results of ROC curve analysis in the group with a disease duration of <4 years showed that the AUC of the FIT results for both endoscopic scores were larger than those of other biomarkers and that FIT result was the best among tested biomarkers in terms of sensitivity and specificity.

4. Discussion

In this study, we investigated how the duration of UC affects the results of different biomarkers, including FIT. We divided the subjects based on disease duration by 1 year increments, and evaluated the correlation between the FIT result and clinical score or endoscopic score in the groups with short and long disease durations. We found that FIT result reflected the endoscopic score until a disease duration of <4 years, but its reliability may decrease when disease duration is >4 years. FIT can be performed with a small amount of specimen; moreover, it can be easily performed in most facilities. Although FIT is a useful biomarker of UC [7, 9, 19, 20], it should be cautiously used, as we found that the FIT results for UC patients with a disease duration of ≥4 years were less reliable.

We speculated that intestinal fibrosis was responsible for the effect of disease duration on FIT in patients with UC. Intestinal fibrosis develops with an increase in disease duration due to persistent chronic

inflammation, and relapse and remission of UC. Although reports have shown that fibrosis and extent of bleeding affect the results of FIT, clinical evidence has shown that scarring tissue is less likely to cause bleeding. In this study, we analyzed bleeding based on UCEIS scoring to evaluate the degree of hemorrhage from the intestinal mucosa. The rate of bleeding score greater than 1 in the group with short disease duration was significantly higher than that in the long disease duration group at any point from 2 to 8 years (2 years: P = 0.006, 3 years: P =0.012, 4 years: P =0.016, 5 years: P =0.017, 6 years: P =0.047, 7 years: P =0.029, and 8 years: P =0.015; data not shown). This finding indicated that the group with a short disease duration tended to have a higher frequency of bleeding. Thus, the usefulness of FIT in UC may decrease over time. Besides FIT, we also measured PGE-MUM and CRP levels as biomarkers for UC. PGE-MUM is a biomarker for evaluation of the UC disease activity; Arai et al. reported that it reflects the degree of mucosal inflammation in UC [10, 21]. PGE-MUM levels may also increase due to smoking and lung diseases [22, 23]. PGE-MUM measurement has been reported to be useful in colorectal, lung, breast, and pancreatic cancers [24-28]. Because of these reasons, those with smoking habits, lung diseases, or malignant tumors were excluded from this study.

Although CRP is a blood biomarker and its levels sometimes do not increase despite active inflammation in UC, it is commonly evaluated in clinical practice because it can be easily measured in most facilities [29].

In this study, both biomarkers (CRP and PGE-MUM) were analyzed in a way similar to that used

for FIT in groups with disease durations of <4 and ≥4 years. The results showed that PGE-MUM and CRP were useful as biomarkers even when the disease duration of ≥4 years. In the regression analysis of results for samples divided in groups based on disease duration (2 to 8 years), PGE-MUM showed a significant correlation with endoscopic scores over the entire period, indicating that PGE-MUM was a useful biomarker. In contrast, CRP did not show significant correlation with endoscopic scores in groups with disease duration of < 2 and < 4 years, indicating that CRP was not useful for the UC patients with short disease duration.

Next, we assessed the utility of FIT as a biomarker in UC. FIT can acts as a marker that sharply reflects the inflammatory state of the mucosa. Takashima et al. reported that the sensitivity of FIT for MES = 0 was more than 10 points higher than that of FC [8]. We investigated the accuracy of FIT in groups with a disease duration of <4 years and compared it with other biomarkers. The analysis was performed using the ROC curves for each biomarker to predict mucosal healing. Currently, the definition of mucosal healing has not been clearly established; it has been defined as MES of 0 or 1 [2], although several recent studies defined only MES = 0 as a reflection of mucosal healing [7, 8, 19] Therefore, to enable more accurate evaluation of mucosal healing by measuring biomarkers, we defined only MES = 0 as mucosal healing in this study. When MES was used to predict musical healing, AUC of FIT was larger than that of PGE-MUM or CRP, and the FIT result was superior to PGE-MUM and CRP in both sensitivity and specificity. Similarly,

when the UCEIS score was used, FIT was the best biomarker to reflect mucosal healing. From these results, we concluded that FIT was a more accurate biomarker as compared to PGE-MUM and CRP when disease duration was <4 years. Therefore, in patients with UC, FIT can be used as a biomarker for disease duration of up to 4 years and PGE-MUM and CRP in combination with FIT should be used after 4 years of disease duration.

Till date, only a few studies have examined the effect of UC disease duration on biomarker accuracy. Piotr et al. reported that disease duration and time-dependent change in Crohn's disease phenotype do not affect the diagnostic utility of FC measurement [30]. They reported that FC is a biomarker of inflammation similar to PGE-MUM and CRP, however, it was based on a mechanism different from that of FIT, which is evaluated from quantitative intestinal bleeding. They concluded that disease duration only negligibly affected FC. However, they did not report any findings on UC and FIT. Similar to Crohn's disease, fibrosis of the intestinal tract in UC develops with an increase in disease duration; thus, inflammation of the intestinal tract is less likely to correlate with the amount of mucosal bleeding. However, we did not analyze the correlation between the biomarkers and disease duration in CD, and this will be investigated in the future.

This study had some limitations. First, prognostic prediction was not analyzed in this study. Biomarkers should be able to predict endoscopic activity and relapse in quiescent patients with UC, as prediction of relapse is essential for treatment. Second, analysis of other fecal biomarkers, including that of

FC, was not performed in this study. Currently, FC is the most commonly used fecal biomarker for assessing the activity of UC. Moreover, FC has been reported to be useful as a biomarker to predict relapse in clinically quiescent UC patients [31-34]. In addition, it is one of the most widely used fecal biomarkers in clinical trials for evaluating medical condition. In the future, we plan to investigate the effect of disease duration on FC. Finally, this study was performed in a single center.

In conclusion, the duration of UC affects the efficacy of FIT as a biomarker. FIT is a highly accurate biomarker and reflects endoscopic score until disease duration of <4 years in patient with UC. However, the utility of FIT as a biomarker decreases after a duration of 4 years, and therefore, a comprehensive evaluation in combination with other biomarkers such as PGE-MUM and CRP should be considered.

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Figure legends

Fig. 1 Correlation between immunochemical fecal occult blood test (FOBT or FIT) and the clinical activity index (CAI), Mayo endoscopic subscore (MES), and ulcerative colitis endoscopic index of severity (UCEIS) score in patients with ulcerative colitis (UC) among patients with disease durations of <4 and >4 years. Scatter plots of FIT results vs. CAI in groups with disease durations of <4 (a) and >4 years (b). Scatter plots of FIT results vs. MES in groups with disease durations of <4 (c) and >4 years (d). Scatter plots of FIT results vs. UCEIS score in groups with disease durations of <4 (e) and >4 years (f)

Fig. 2 Correlation of the prostaglandin E-major urinary metabolite (PGE-MUM) levels with clinical activity index (CAI), Mayo endoscopic subscore (MES), and ulcerative colitis endoscopic index of severity (UCEIS) score in patients with ulcerative colitis (UC) among patients with disease durations of <4 and >4 years. Scatter plots of PGE-MUM vs. CAI in groups with disease durations of <4 (a) and >4 years (b). Scatter plots of PGE-MUM vs. MES in groups with disease durations of <4 (c) and >4 years (d). Scatter plots of PGE-MUM vs. UCEIS score in groups with disease durations of <4 (e) and >4 years (f)

Fig. 3 Correlation of the C-reactive protein (CRP) level with clinical activity index (CAI), Mayo endoscopic subscore (MES), and ulcerative colitis endoscopic index of severity (UCEIS) score in patients with ulcerative colitis (UC) among patients with disease durations of <4 and >4 years. Scatter plots of CRP vs.

CAI in groups with disease durations of <4 (a) and >4 years (b). Scatter plots of CRP vs. MES in groups with disease durations of <4 (c) and >4 years (d). Scatter plots of CRP vs. UCEIS score in groups with disease durations of <4 (e) and >4 years (f)

Fig. 4 Receiver-operating characteristic (ROC) curves for each biomarker for predicting mucosal healing of ulcerative colitis (UC) patients with a disease duration of <4 years. (a) ROC curve for immunochemical fecal occult blood test (FOBT or FIT), prostaglandin E-major urinary metabolite (PGE-MUM), and C-reactive protein (CRP) evaluated with Mayo endoscopic subscore (MES). (b) ROC curve for FIT, PGE-MUM, and CRP evaluated with ulcerative colitis endoscopic index of severity (UCEIS) score

1 **Tables**

2 Table 1 Characteristics of patients with ulcerative colitis included in this study

Characteristics	N = 113
Age (year), mean (range) ± SD	48.3 (14–83) ±15.9
Male/Female, n (%)	94/34 (83.2/16.9)
Disease duration (year), mean (range) ± SD	7.95 (0.1–34) ± 8.16
disease duration < 1, n (%)	13 (11.5)
1 ≤ disease duration < 2, n (%)	10 (8.8)
2 ≤ disease duration < 3, n (%)	14 (12.4)
3 ≤ disease duration < 4, n (%)	9 (8.0)
4 ≤ disease duration < 5, n (%)	11 (9.7)
5 ≤ disease duration < 6, n (%)	6 (5.3)
6 ≤ disease duration < 7, n (%)	2 (1.8)
7 ≤ disease duration < 8, n (%)	4 (3.5)
8 ≤ disease duration, n (%)	44 (38.9)
Disease extent, n (%)	
Extensive colitis	72 (63.7)
Left-sided colitis	27 (23.9)
Proctitis	14 (12.4)
CAI (Lichtiger's score) mean (range) ± SD	2.08 ± 2.72
MES mean (range) ± SD	1.01 (0–3) ± 0.97
MES 0, n (%)	45 (39.8)
MES 1, n (%)	29 (25.7)
MES 2, n (%)	32 (28.3)
MES 3, n (%)	7 (6.2)
UCEIS mean (range) ± SD	2.07 (0–7) ± 1.97
FIT (ng/mL) mean (range) ±SD	2787 (0–45900) ± 6533
PGE-MUM (μg/g･Cr) mean (range) ± SD	30.3 (5.1–107) ± 20.6

CRP (mg/dL) mean (range) ± SD		0.32 (0.01–5.44) ± 0.70
Medication at study, n (%)		
	Oral 5-ASA	72 (63.7)
	Suppository 5-ASA	22 (19.4)
	Systemic steroids	17 (15.0)
	Immunomodulators	42 (37.2)
	Biologics	30 (26.6)

CAI, clinical activity index; MES, Mayo endoscopic subscore; UCEIS, ulcerative colitis endoscopic index of severity; FIT, immunochemical fecal occult blood test; PGE-MUM, prostaglandin E-major urinary metabolite; CRP, C-reactive protein; 5-ASA, 5-aminosalicylic acid.

17

18 Table 2 Regression analysis of FIT results and CAI, MES, and UCEIS in groups with long-term and short-term disease duration

19

Dependent variable	Independent variable	α	β	P	r
FIT (<2 years)	CAI	-938.419	1625.019	<0.001	0.821
n = 23	MES	1.052	0.116	0.016	0.495
	UCEIS score	-2964.422	2530.475	<0.001	0.678
FIT (≥2 years)	CAI	-150.072	1412.370	<0.001	0.516
n = 90	MES	84.309	2384.515	<0.001	0.367
	UCEIS score	772.696	800.040	0.022	0.241
FIT (<3 years)	CAI	-650.751	1396.688	0.002	0.775
n = 37	MES	-549.578	3397.200	0.001	0.538
	UCEIS score	-1220.566	1863.585	<0.001	0.639
FIT (≥3 years)	CAI	-307.636	1685.918	<0.001	0.551
n = 76	MES	87.304	2521.238	0.001	0.360
	UCEIS score	841.448	826.726	0.048	0.228
FIT (<4 years)	CAI	2136.675	2597.211	0.001	0.462
n = 46	MES	-502.665	3198.720	<0.001	0.534
	UCEIS score	-1049.037	1689.602	<0.001	0.603
FIT (≥4 years)	CAI	-288.382	1814.066	<0.001	0.565
n = 67	MES	136.094	2592.656	0.003	0.356
	UCEIS score	891.988	889.755	0.056	0.235
FIT (<5 years)	CAI	1.576	1332.526	<0.001	0.661
n = 57	MES	-792.390	3903.940	<0.001	0.566
	UCEIS score	-534.950	1748.100	<0.001	0.524
FIT (≥5 years)	CAI	-920.469	2052.590	<0.001	0.579
n= 56	MES	417.153	1646.614	0.065	0.249
	UCEIS score	837.132	590.309	0.195	0.176

FIT (<6 years)	CAI	3.774	1336.947	<0.001	0.673
n = 63	MES	-728.816	3865.934	<0.001	0.582
	UCEIS score	-484.964	1733.701	<0.001	0.547
FIT (≥6 years)	CAI	-1012.984	2067.245	<0.001	0.557
n = 50	MES	515.335	1517.584	0.121	0.222
	UCEIS score	1036.740	474.395	0.351	0.135
FIT (<7 years)	CAI	62.476	1330.260	<0.001	0.672
n = 65	MES	-570.421	3638.352	<0.001	0.570
	UCEIS score	-366.659	1647.131	<0.001	0.538
FIT (≥7 years)	CAI	-1120.200	2038.733	<0.001	0.568
n = 48	MES	512.635	1557.179	0.141	0.216
	UCEIS score	1081.691	458.878	0.406	0.123
FIT (<8 years)	CAI	34.245	1332.565	<0.001	0.675
n = 69	MES	-335.021	3265.134	<0.001	0.531
	UCEIS score	-348.926	1558,780	<0.001	0.518
FIT (≥8 years)	CAI	-1124.280	2107.785	<0.001	0.569
n = 44	MES	337.231	1902.495	0.112	0.243
	UCEIS score	1071.901	516.634	0.399	0.130

α, intercept; ß, regression coefficient; r, Personal correlation coefficient; FIT, fecal immunochemical occult blood test; CAI, clinical activity index; MES, Mayo endoscopic subscore; UCEIS, ulcerative colitis endoscopic index of severity

Table 3 Regression analysis of the PGE-MUM and MES or UCEIS in groups with long-term and short-term disease duration

Dependent variable	Independent variable	Short-disease duration		Long-disease duration	
		P	r	P	r
PGE-MUM (2 years division)	MES	0.027	0.461	<0.001	0.440
	UCEIS score	<0.001	0.679	<0.001	0.449
PGE-MUM (3 years division)	MES	0.010	0.416	<0.001	0.426
	UCEIS score	0.001	0.515	<0.001	0.436
PGE-MUM (4 years division)	MES	0.005	0.406	<0.001	0.438
	UCEIS score	0.002	0.442	<0.001	0.479
PGE-MUM (5 years division)	MES	0.012	0.332	<0.001	0.485
	UCEIS score	0.003	0.388	<0.001	0.499
PGE-MUM (6 years division)	MES	0.006	0.344	<0.001	0.497
	UCEIS score	0.001	0.393	<0.001	0.515
PGE-MUM (7 years division)	MES	0.006	0.335	<0.001	0.515
	UCEIS score	0.002	0.384	<0.001	0.535
PGE-MUM (8 years division)	MES	0.001	0.386	0.002	0.454
	UCEIS score	0.001	0.375	<0.001	0.543

r, Personal correlation coefficient; FIT, fecal immunochemical test; PGE-MUM, prostaglandin E-major urinary metabolite; MES, Mayo endoscopic subscore; UCEIS, ulcerative colitis endoscopic index of severity

30 Table 4 Regression analysis of the CRP and MES or UCEIS in long-disease duration group and short-disease duration group

31

Dependent variable	Independent variable	Short-disease duration		Long-disease duration	
		P	r	P	r
CRP	MES	0.158	0.304	0.003	0.314
(2 years division)	UCEIS score	0.001	0.625	0.001	0.337
CRP	MES	0.045	0.332	<0.001	0.375
(3 years division)	UCEIS score	0.003	0.472	<0.001	0.404
CRP	MES	0.051	0.289	<0.001	0.454
(4 years division)	UCEIS score	0.004	0.418	<0.001	0.489
CRP	MES	0.015	0.320	0.002	0.400
(5 years division)	UCEIS score	<0.001	0.450	0.001	0.423
CRP	MES	0.007	0.338	0.009	0.363
(6 years division)	UCEIS score	<0.001	0.462	0.008	0.374
CRP	MES	0.006	0.338	0.020	0.335
(7 years division)	UCEIS score	<0.001	0.458	0.016	0.346
CRP	MES	0.009	0.313	0.007	0.398
(8 years division)	UCEIS score	<0.001	0.438	0.007	0.402

32 r, Personal correlation coefficient; CRP, C-reactive protein; MES, Mayo endoscopic subscore; UCEIS, ulcerative colitis endoscopic index of severity

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34

Figure

Figure 1

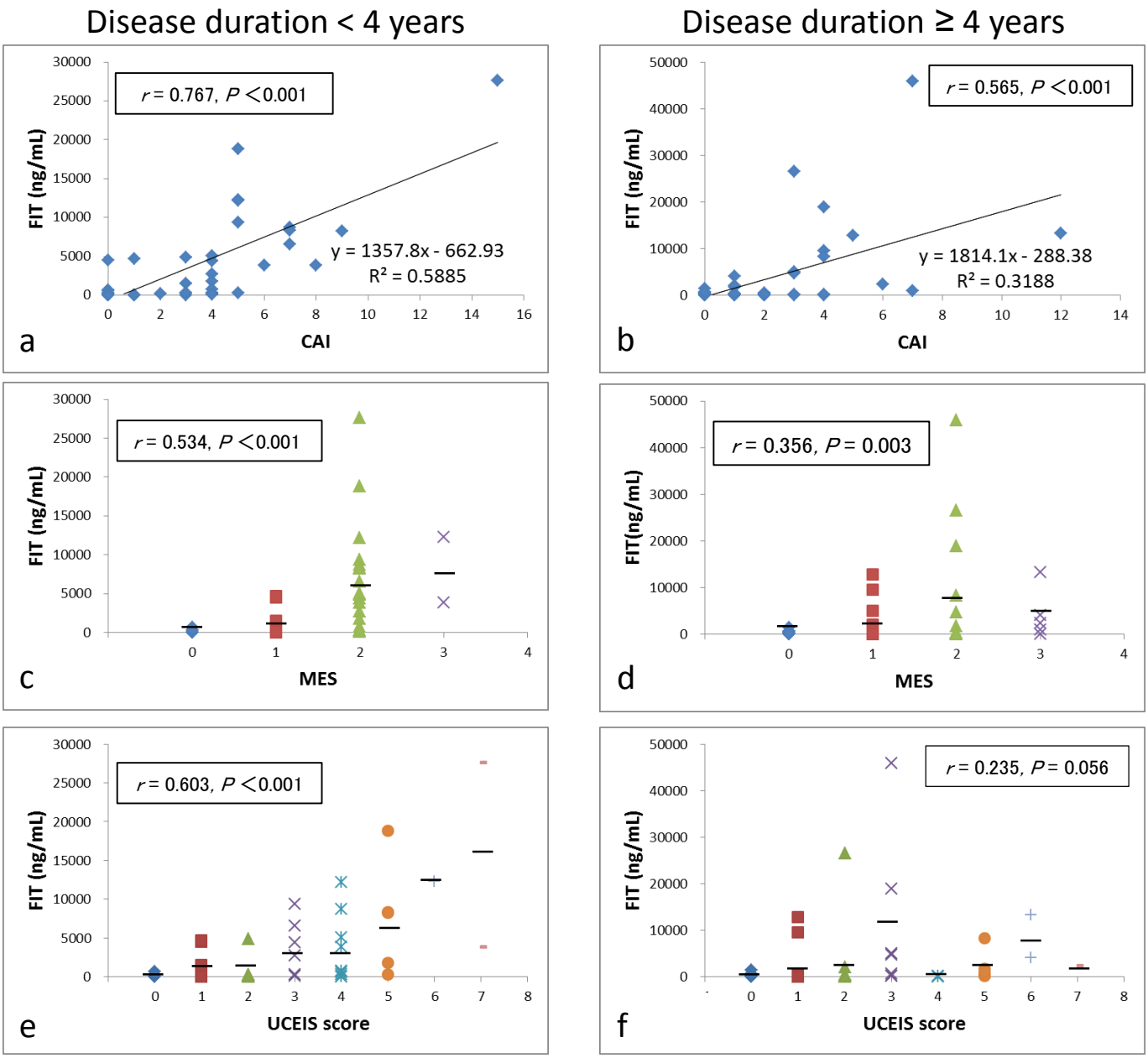


Figure 2

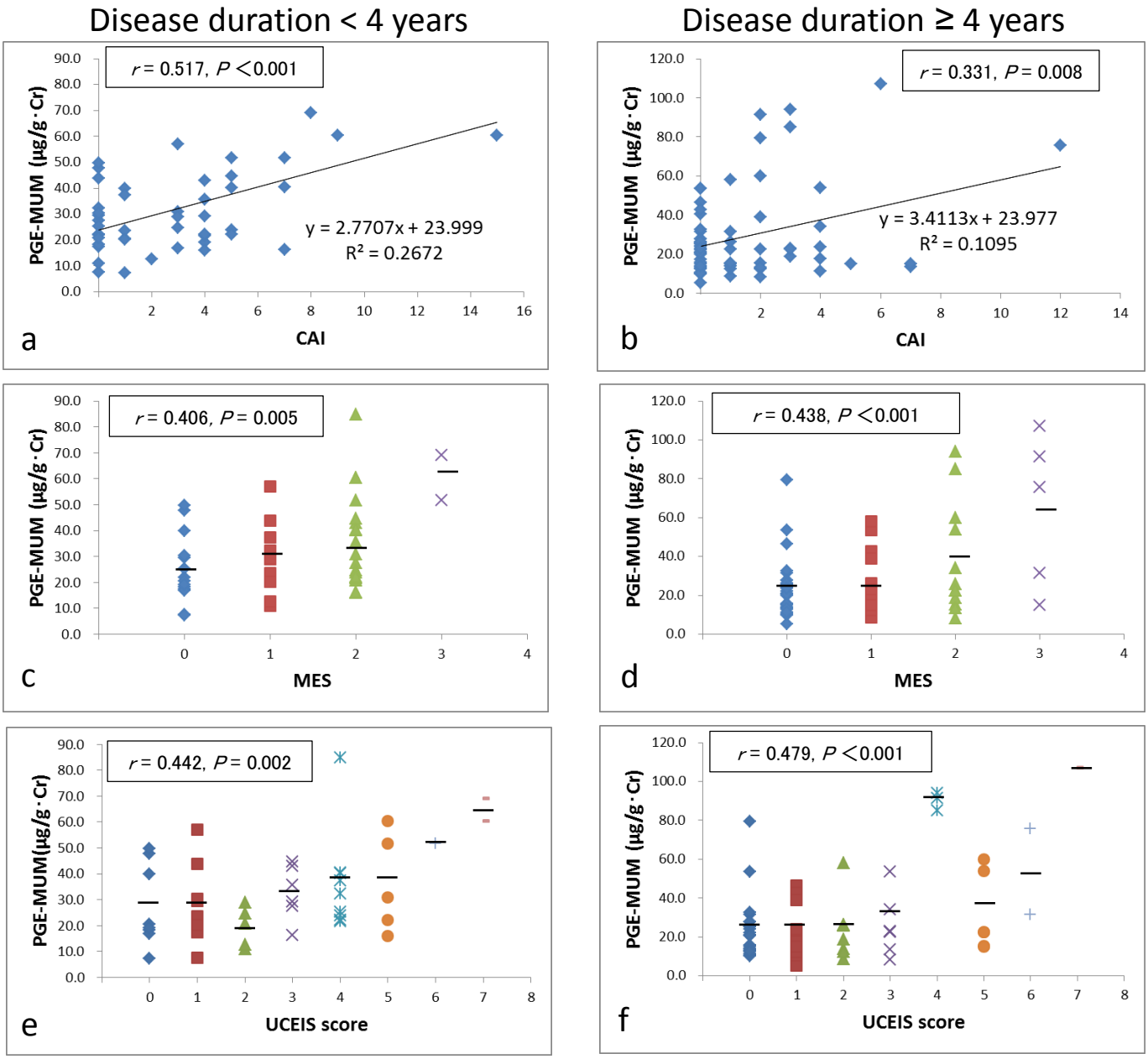

Disease duration < 4 years
Disease duration ≥ 4 years
r = 0.517, P < 0.001
y = 2.7707x + 23.999
R² = 0.2672
PGE-MUM (μg/g·Cr)
CAI
a
r = 0.331, P = 0.008
y = 3.4113x + 23.977
R² = 0.1095
PGE-MUM (μg/g·Cr)
CAI
b
r = 0.406, P = 0.005
PGE-MUM (μg/g·Cr)
MES
c
r = 0.438, P < 0.001
PGE-MUM (μg/g·Cr)
MES
d
r = 0.442, P = 0.002
PGE-MUM(μg/g·Cr)
UCEIS score
e
r = 0.479, P < 0.001
PGE-MUM (μg/g·Cr)
UCEIS score
f

Figure 3

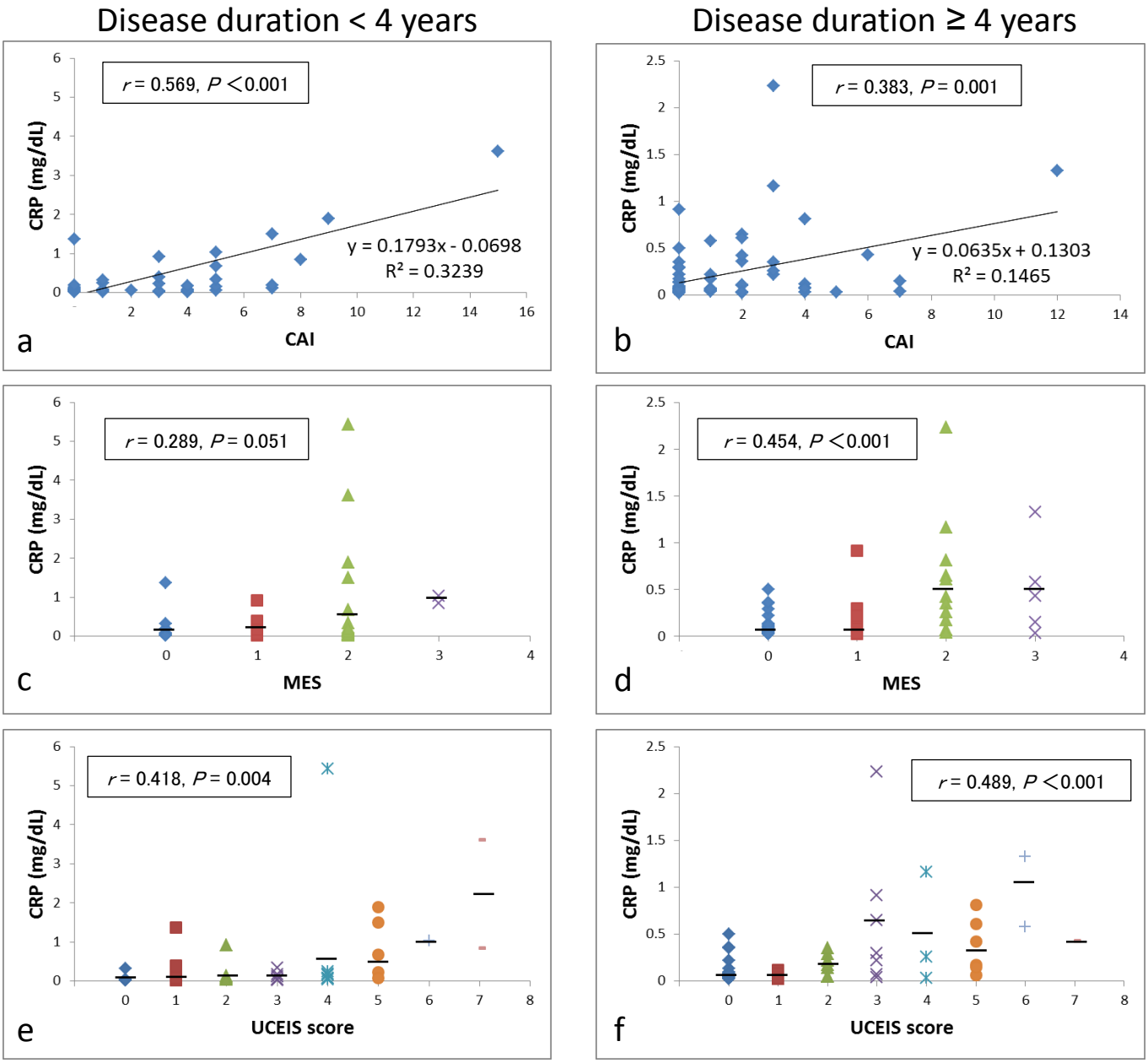
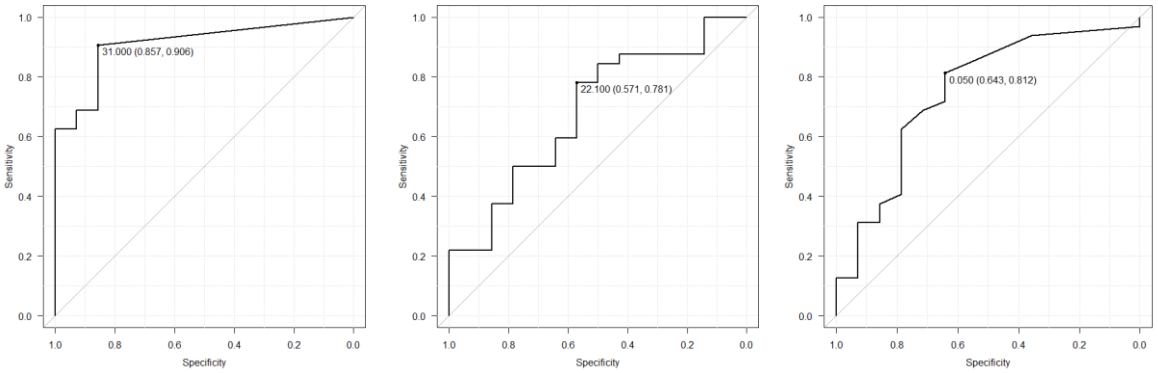

Disease duration < 4 years
Disease duration ≥ 4 years
r = 0.569, P <0.001
y = 0.1793x - 0.0698
R² = 0.3239
CRP (mg/dL)
CAI
a
r = 0.383, P = 0.001
y = 0.0635x + 0.1303
R² = 0.1465
CRP (mg/dL)
CAI
b
r = 0.289, P = 0.051
CRP (mg/dL)
MES
c
r = 0.454, P <0.001
CRP (mg/dL)
MES
d
r = 0.418, P = 0.004
CRP (mg/dL)
UCEIS score
e
r = 0.489, P <0.001
CRP (mg/dL)
UCEIS score
f

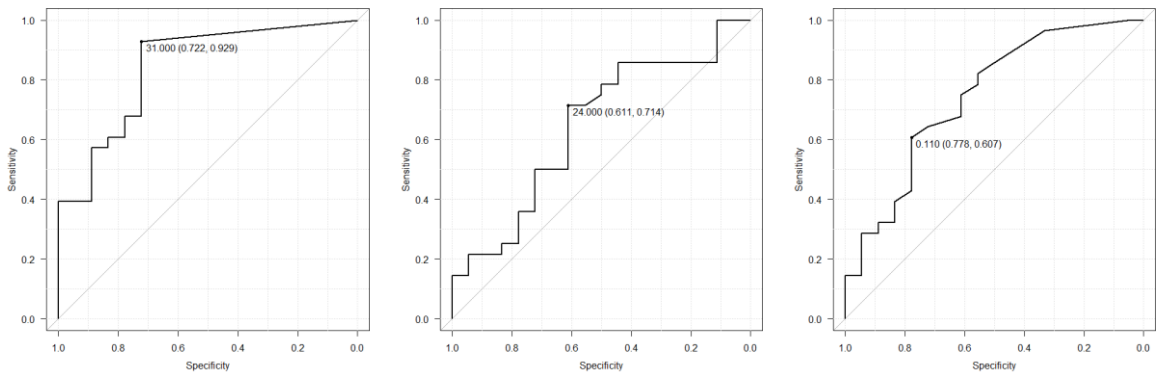
Figure 4

a



	FIT	PGE-MUM	CRP
Cut-off value	31.0 ng/mL	22.1 μg/g･Cr	0.05 mg/mL
AUC	0.911	0.681	0.747
95% CI	0.829 - 0.992	0.509 - 0.853	0.581 -0. 912
Sensitivity	85.7 %	57.1 %	64.3 %
Specificity	90.6 %	78.1 %	81.2 %

b



	FIT	PGE-MUM	CRP
Cut-off value	31.0 ng/mL	24.0 μg/g･Cr	0.11 mg/mL
AUC	0.843	0.642	0.742
95% CI	0.726 - 0.961	0.472 - 0.811	0.590 -0. 894
Sensitivity	72.2 %	61.1 %	77.8 %
Specificity	92.9%	71.4 %	60.7 %