

## **Evaluation of mucosal healing in ulcerative colitis by fecal calprotectin versus fecal immunochemical test**

### **Short Title: Fecal calprotectin vs. FIT for mucosal healing**

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**Conflict of Interest**

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**Abbreviations:** UC, ulcerative colitis; IBD, inflammatory bowel disease; FIT, fecal immunochemical test; MES, Mayo endoscopic subscore; CRC, colorectal cancer; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; ROC, receiver operating characteristic; AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay.

**Word count:** 3801 words.

## Abstract

**OBJECTIVES:** We previously showed that a quantitative fecal immunochemical test (FIT) can predict mucosal healing (MH) in ulcerative colitis (UC). Fecal calprotectin (Fcal) has also been reported as an important biomarker of UC activity. The aim of this study was to compare the predictive ability of these two fecal markers for MH in UC.

**METHODS:** FIT and Fcal were examined in stool samples from consecutive UC patients who underwent colonoscopy. Mucosal status was assessed via the Mayo endoscopic subscore (MES).

**RESULTS:** In total, 105 colonoscopies in 92 UC patients were evaluated in conjunction with the FIT and Fcal results. Both FIT and Fcal results were significantly correlated with MES (Spearman rank correlation coefficient: 0.61 and 0.58, respectively). The sensitivity and specificity of the FIT values (< 100 ng/mL) for predicting MH (MES 0 alone) were 0.95 and 0.62, respectively, while those of Fcal (< 250 µg/g) were 0.82 and 0.62, respectively. The sensitivities became similar when MH was defined as MES 0 or 1 (0.86 vs. 0.86). Although the predictability of MH evaluated by the area under the receiver operating characteristics curve was similar for the two fecal markers (FIT 0.83 vs. Fcal 0.82 for MES 0 alone), the FIT results were relatively robust regardless of the cutoff value selected.

**CONCLUSIONS:** Both FIT and Fcal can efficiently predict MH in UC, but FIT appears to be more sensitive than Fcal for predicting MES 0 alone.

## **STUDY HIGHLIGHTS**

### **1. WHAT IS CURRENT KNOWLEDGE**

- Mucosal healing (MH) in ulcerative colitis (UC) is associated with sustained clinical remission and is considered to be a treatment goal.
- A quantitative fecal immunochemical test (FIT) can predict MH in UC.
- Fecal calprotectin (Fcal) is an effective biomarker of UC that is used worldwide.
- The ability of FIT versus Fcal to predict MH has not previously been evaluated.

### **2. WHAT IS NEW HERE**

- The FIT and Fcal values both effectively reflected the mucosal status of UC.
- Both markers predicted MH with sufficient sensitivity and specificity.
- FIT appears to be more sensitive than Fcal for predicting MES 0 alone.

## INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that causes diffuse inflammatory mucosal injury, including erosions and ulcers in the colon and rectum. Patients suffer from uncomfortable symptoms such as diarrhea, rectal bleeding, and abdominal pain, unless appropriate treatment is provided. Although immunological disorders have been shown to be involved in the occurrence of UC, its cause remains largely unknown and a complete cure is rare with the medical resources that are currently available. Therefore, the treatment strategy for UC consists of remission induction therapy in the active stage, followed by maintenance of remission after successful remission induction.

In the past, UC patients were treated to achieve and sustain clinical remission, as indicated by the disappearance of clinical symptoms. Recently, however, not only clinical remission but also endoscopic mucosal healing is being pursued as the treatment goal for UC. Patients with UC who achieve mucosal healing have been shown to have a lower rate of relapse and a reduced risk of hospitalization and colectomy (1-3). Although evaluation of mucosal healing absolutely requires colonoscopic observation, colonoscopy is an invasive and costly procedure, and cannot be performed frequently. Therefore, noninvasive methods to evaluate mucosal status without performing colonoscopy are desirable. Among promising candidates that might reflect mucosal status, surrogate markers present in stool samples have been evaluated (4-12).

Fecal calprotectin, a major protein found in the cytosol of inflammatory cells, is the most widely used marker of mucosal inflammation. Initially, fecal calprotectin was used to predict the presence of inflammation in IBD patients without performing colonoscopy, and was shown to predict the presence of active inflammation in UC patients

with sensitivity ranging from 71% to 93% and specificity ranging from 71% to 100% (5-7). Subsequently, the ability of this marker to predict mucosal healing was evaluated. Although fewer studies have demonstrated the predictability of fecal calprotectin for mucosal healing than for the presence of active inflammation in UC patients, its sensitivity for mucosal healing reportedly ranges from 65% to 100%, and its specificity from 53% to 90% (8-11).

In an alternative approach, we previously reported the predictability of mucosal healing in UC by a fecal immunochemical test (FIT) (12). Quantitative FIT measures fecal hemoglobin concentrations using an antibody specific for human hemoglobin, and has been used to screen for colorectal neoplasia (13). The FIT that we used predicted mucosal healing in UC, defined as a Mayo endoscopic subscore of 0 (MES 0), with 92% sensitivity and 71% specificity (12). Moreover, FITs have the advantage that the amount of blood can be rapidly measured in many fecal samples in one batch with automated equipment, because the original purpose of FITs was rapid screening for colorectal cancer (CRC) among a large population (13). However, it is unknown whether fecal calprotectin or FIT predicts mucosal healing more accurately in UC patients.

In this study, therefore, we prospectively examined fecal calprotectin and conducted a FIT simultaneously using stool samples from UC patients who underwent colonoscopy. The ability to predict mucosal healing in UC was then compared between the two modalities.

## METHODS

### *Patients*

Since 2006, UC patients who regularly attend Okayama University Hospital have been requested to routinely prepare and bring fecal samples at each visit, including the day of colonoscopy, in order to evaluate the amount of fecal occult blood via FIT. In addition, all consecutive UC patients who underwent scheduled colonoscopy between October 2012 and February 2014 were requested to bring another fecal sample for the examination of fecal calprotectin. Both fecal samples were collected on the day or a few days before colonoscopy, and stored in a refrigerator until the day of colonoscopy. All of the patients had an established diagnosis of UC according to endoscopic and histologic assessments and had received adequate medical therapy. Mucosal status assessed via colonoscopy was compared with the results of FIT versus fecal calprotectin.

Clinical disease activity was evaluated by using the Mayo score (Scoring Systems for Assessment of UC), consisting of the following four subscores: stool frequency (0, normal number of stools for this patient; 1, 1–2 stools more than normal; 2, 3–4 stools more than normal; and 3, 5 or more stools more than normal), rectal bleeding (0, no blood seen; 1, streaks of blood with stool less than half the time; 2, obvious blood with stool most of the time; and 3, blood alone passed), endoscopic findings (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), and physician's global assessment (0, normal; 1, mild disease; 2, moderate disease; and 3, severe disease) (14). Clinical remission was defined as a Mayo stool frequency subscore of 0 or 1 and a Mayo rectal bleeding subscore of 0 (15). Patients who failed to fulfill the definition of clinical

remission were considered to have clinically active disease.

### ***FIT analysis***

Details of the method used for FIT have been described previously (12, 16). In brief, patients collected fecal samples using an OC-Hemodia sampling probe (Eiken Chemical, Tokyo, Japan). Submitted stool samples were immediately processed and examined using OC-SENSOR neo (Eiken Chemical), which can accurately measure fecal hemoglobin concentrations from 50 ng/mL to 1000 ng/mL. Fecal specimens with a hemoglobin concentration of more than 1000 ng/mL were measured by further dilution, whereas those with a hemoglobin concentration of less than 50 ng/mL were categorized as one (0–50 ng/mL) because FIT results are inaccurate when the hemoglobin concentration is lower than 50 ng/mL.

### ***Fecal calprotectin analysis***

Fecal samples collected by the patients for calprotectin analysis were stored at -70°C until shipment to the laboratory. The samples were sent to the Institute of Applied Technology for Innate Immunity (Kagawa, Japan), where calprotectin in stools is measured by a Phical<sup>®</sup> Calprotectin ELISA kit (Immundiagnostik AG, Germany). The quantitative range of calprotectin was between 0.65 µg/g and 84,000 µg/g after the appropriate dilution of fecal samples ranging from 1:50 to 1:100,000.

### ***Colonoscopy***

Bowel preparation was performed with a polyethylene glycol-based or magnesium citrate-based electrolyte solution according to the standard protocol in our hospital. After the colonic lavage fluid was cleared, patients underwent colonoscopy. Patients were

excluded from the study if the colonoscopic examination was incomplete because of problems with the bowel preparation or if the colonoscope could not be inserted into the cecum.

The mucosal status of UC patients was assessed via the MES classification. Evaluation was performed at each portion of the colorectum (cecum and ascending colon combined, transverse colon, descending colon, sigmoid colon, and rectum), and the maximum score in the colorectum of each patient was used for analysis. The total inflammation score was defined as the sum of MES in the five colonic portions, and ranged from 0 (no inflammation) to 15 (severe and extensive inflammation). Mucosal healing was defined as an MES of 0, or 0 or 1 throughout the colorectum. Some patients underwent biopsy from the portion with maximum endoscopic inflammation for pathological examinations. All colonoscopic examinations were performed by experienced colonoscopists who were blind to the results of the FIT and fecal calprotectin results. In addition, the MES and the fecal results of each patient were determined independently by investigators who did not know patient's status including symptoms.

### ***Pathologic Findings***

Histologic studies were evaluated using Geboes scores (17) by a gastrointestinal pathologists. Geboes scores for assessment of UC histologic disease activity and mucosal healing are classified into 6 grades from grade 0 to grade 5: grade 0 (subgrades 0.0 to 0.3), structural (architectural changes); grade 1 (subgrades 1.0 to 1.3), chronic inflammatory infiltrate; grade 2, lamina propria neutrophils and eosinophils (subgrades 2A.0 to 2A.3 for eosinophils, subgrades 2B.0 to 2B.3 for neutrophils); grade 3 (subgrades 3.0 to 3.3), neutrophils in epithelium; grade 4 (subgrades 4.0 to 4.3), crypt destruction; grade 5 (subgrades 5.0 to 5.4), erosion or ulceration. When one or more biopsy specimens were

evaluated on each patient, the highest score was used for analysis.

### *Statistical analysis*

Patient characteristics were analyzed by the JMP program (version 11.0 for Windows, SAS Institute, USA). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with 95% confidence intervals (CIs) for detecting mucosal status were determined based on the FIT and calprotectin results. To estimate appropriate cutoff values for FIT and calprotectin, receiver operating characteristic (ROC) curve analysis was performed, and the area under the curve (AUC) was calculated. Spearman's rank correlation test was performed to determine the correlation coefficient between the FIT values or fecal calprotectin levels and the Mayo endoscopic scores. All *p* values were two-sided and considered statistically significant when less than 0.05.

### *Ethical considerations*

This study was approved by the institutional review board of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences. Informed consent was obtained from each patient before bringing fecal samples.

## RESULTS

### *Clinical characteristics of patients*

The study included 105 colonoscopies that were accompanied by corresponding FIT and calprotectin results and were performed in 92 UC patients (53 men, 39 women; median age at UC onset, 35.5 years) (Table 1). Among the 92 UC patients, 63 (69%) had pancolitis, 23 (25%) had left-side colitis, and 6 (6%) had proctitis.

Of the 105 colonoscopy cases, 77 (73%) were performed in patients in clinical remission, while the other 28 (27%) were performed in patients with clinically active disease. Colonoscopy findings revealed that the maximum MES for the colorectum was MES 0 in 44 (42%) cases, MES 1 in 20 (19%) cases, MES 2 in 35 (33%) cases, and MES 3 in 6 (6%) cases. The distribution of the FIT results showed 56 (53%) cases with a hemoglobin concentration of 50 ng/mL or lower, 9 (9%) with 51–100 ng/mL, and 40 (38%) with 101 ng/mL or higher. On the other hand, the results of fecal calprotectin indicated 51 (49%) cases with a concentration of 200 µg/g or lower, 25 (24%) with 201–400 µg/g, and 29 (27%) with 401 µg/g or higher.

Of the total 105 cases, 93 underwent biopsy for pathological examinations. Of these, 4 were excluded because of suspected neoplastic change, and the remaining 89 were evaluated with Geboes score (Table 2).

### *Correlations between FIT, fecal calprotectin, and colonoscopic findings*

The correlations between FIT, fecal calprotectin, and colonoscopic findings (maximum MES in the colorectum and the total inflammation score) were analyzed. Both the FIT results and fecal calprotectin levels were significantly correlated with both the maximum MES (Spearman rank correlation coefficient: 0.61,  $p < 0.0001$  vs. 0.58,  $p <$

0.0001), and the total inflammation score (Spearman rank correlation coefficient: 0.64,  $p < 0.0001$  vs. 0.60,  $p < 0.0001$ ) (Figures 1 and 2). Thus, the correlation coefficients of the FIT values to endoscopic scores were slightly higher than those of fecal calprotectin values to endoscopic scores. A significant correlation was also observed between the FIT values and fecal calprotectin levels (Spearman rank correlation coefficient: 0.64,  $p < 0.0001$ ) (Figure 3).

### ***Correlation between FIT, fecal calprotectin, colonoscopic findings and histological findings***

The correlation between FIT, fecal calprotectin, colonoscopic findings and histological findings in the 89 cases were analyzed. The maximum Geboes score was significantly correlated with the maximum MES and the sum of MES (Spearman rank correlation coefficient: 0.70,  $p < 0.0001$ , and 0.67,  $p < 0.0001$ , respectively). In addition, the histological score was also correlated with both FIT and fecal calprotectin to a similar extent (Spearman rank correlation coefficient: 0.43,  $p < 0.0001$ , and 0.43,  $p < 0.0001$ , respectively).

### ***Sensitivity, specificity and predictive value of FIT versus fecal calprotectin for mucosal healing***

The sensitivity, specificity, PPV, NPV and accuracy of FIT versus fecal calprotectin data in relation to mucosal healing were calculated for the 105 colonoscopy cases. The calculation was performed for two definitions of mucosal healing: MES 0 alone (Tables 3 and 4), and MES 0 or 1 (Tables 5 and 6). In addition, two types of cutoff value were set for each fecal marker: the cutoff commonly used for CRC screening by FIT ( $< 100$  ng/mL) or for mucosal healing in IBD by fecal calprotectin ( $< 250$   $\mu$ g/g) (standard

cutoffs); and the cutoffs optimized by ROC analysis (optimal cutoffs).

When mucosal healing was defined as MES 0 alone, the sensitivity of FIT was more than 10 points higher than that of fecal calprotectin based on either the standard or the optimal (< 75 ng/mL for FIT and < 200 µg/g for fecal calprotectin) cutoffs (standard cutoffs, 0.95 (95%CI 0.89-1.02) vs. 0.82 (95%CI 0.70-0.93); optimal cutoffs, 0.93 (95%CI 0.86-1.01) vs. 0.77 (95%CI 0.65-0.90)). The specificity did not differ largely when either cutoff was used (standard cutoffs, 0.62 (95%CI 0.50-0.74) vs. 0.62 (95%CI 0.50-0.74); optimal cutoffs, 0.67 (95%CI 0.55-0.79) vs. 0.72 (95%CI 0.61-0.83)). Similar AUCs were obtained by ROC analysis for the FIT and fecal calprotectin results (0.83 vs. 0.82).

When mucosal healing was defined as MES 0 or 1, the sensitivity of the FIT data was more than 10 points higher than that of the fecal calprotectin data based on the standard cutoffs (0.81 (95%CI 0.72-0.91) vs. 0.70 (95%CI 0.59-0.82)); however, similar sensitivity was observed based on the optimal (< 280 ng/mL for FIT and < 369 µg/g for fecal calprotectin) cutoffs (0.86 (95%CI 0.77-0.94) vs. 0.86 (95%CI 0.77-0.94)). The specificity was also similar regardless of which cutoff was used (standard cutoffs, 0.68 (95%CI 0.54-0.83) vs. 0.66 (95%CI 0.51-0.80); optimal cutoffs, 0.66 (95%CI 0.51-0.80) vs. 0.63 (95%CI 0.49-0.78)). Also in this case, similar AUCs were obtained by ROC analysis between the FIT and fecal calprotectin (0.79 vs. 0.80).

We also performed subanalysis using the data of only the first colonoscopy case per patient (Supplemental tables 1-4), and subanalysis for patients with pancolitis and for those with left-side colitis, separately (Supplemental tables 5-8). These results did not differ largely from those of the results using all colonoscopy data. Moreover, we confirmed that fulfillment of both FIT < 100 ng/mL and fecal calprotectin < 250 µg/g could raise the specificity to MES 0 alone up to 0.77 (Supplemental table 9). All thorough the analyses, the presence of inflammatory polyps did not affect the results of FIT and

fecal calprotectin (data not shown).

These results suggest that both FIT and fecal calprotectin can predict mucosal healing in UC to a similar extent. However, FIT appeared to have an advantage over fecal calprotectin with higher sensitivity to predict complete mucosal healing (MES 0 alone), although the superiority was not proved because of overlapped confidence intervals. The robust predictability regardless of the cutoff value used for prediction seemed to be another merit of FIT.

## DISCUSSION

To date, the goal of treatment in UC has been considered to be mucosal healing because it reduces the risk of relapse and colectomy (1). Although mucosal healing may be a good marker in the treatment of UC, a major disadvantage of using mucosal healing in clinical practice is its absolute requirement for colonoscopy, which is an invasive and costly procedure. Therefore, surrogate markers of mucosal status have been sought and evaluated. Among several candidates, fecal calprotectin has become a front-runner, particularly in Western countries. Meanwhile, we have reported the utility of FIT in predicting mucosal healing in UC (12); furthermore, this marker is promising due to its growing availability worldwide because it has been replacing the guaiac-based test in the field of CRC screening. Against this background, in this study we compared the ability of fecal calprotectin and FIT to predict mucosal healing. Our prospective analysis indicated that fecal calprotectin and FIT were equivalent in their ability to predict mucosal healing in UC patients.

Several reports have indicated that fecal calprotectin can predict mucosal healing in UC with 65%–100% sensitivity and with 53%–90% specificity (8-11). In this study, the sensitivity and specificity of fecal calprotectin using optimal cutoffs were 77% and 72%, respectively, for MES 0 alone as mucosal healing. In addition, the values were 86% and 63%, respectively, for MES 0 or 1 as mucosal healing. Thus, the present study data for fecal calprotectin are in line with those in previous studies.

On the other hand, there are discrepancies in the sensitivity, specificity, and optimal cutoff value of the FIT between this and our previous study (12). In particular, the sensitivity for predicting MES 0 or 1 was higher in the present study than in the previous report (86% vs. 58%). The discrepancy may be attributable to the difference in study

design (prospective vs. retrospective) and/or to characteristics of the patients (patients with MES 0, 42% vs. 15%). By contrast, the sensitivity for predicting MES 0 is similarly high (93% vs. 94%) between the two studies. Thus, the FIT results of this study are reasonable.

A previous study compared the predictability of mucosal inflammation between fecal calprotectin and fecal hemoglobin (18). In contrast to the present study, the report examined fecal hemoglobin concentrations by using an enzyme-linked immunosorbent assay (ELISA) system. The study revealed that the sensitivity and specificity of fecal hemoglobin toward the presence of mucosal inflammation were equivalent to those of fecal calprotectin. In clinical practice, however, the prediction of mucosal healing is more relevant than the prediction of mucosal inflammation, because UC patients with mucosal inflammation are likely to have symptoms, but asymptomatic UC patients do not always show mucosal healing. Moreover, mucosal healing has been considered to be the treatment goal because it reduces the risk of relapse. To our knowledge, this is the first report to compare the predictability of mucosal healing by fecal calprotectin and FIT. Our results indicate that FIT can replace fecal calprotectin with regard to the prediction of mucosal healing, particularly the prediction of MES 0 alone. As the automated FIT analyzer grows in availability owing to its use in CRC screening, FIT may become more common in IBD practice.

As compared with fecal calprotectin, FIT was found to be a better predictor of more strictly defined mucosal healing (MES 0 alone) in the present study. Until now, the definition of mucosal healing has not been established. Older reports were likely to define mucosal healing as MES 0 or 1 (15, 19-21), whereas more recent studies have defined mucosal healing as MES 0 alone (2, 22, 23). Mucosal healing should be determined in correlation with prognosis including risk of relapse and colectomy. In this context, some studies reported that the prognosis of patients with MES 0 did not differ from that of

patients with MES 1 (15, 20, 21), while others showed a significant difference in prognosis between patients with MES 0 and those with MES 1 (2). We also observed a significant difference in risk of relapse between patients with each definition in a previous study (22). Although further studies are required to confirm the difference in prognosis between the definitions of mucosal healing, the higher sensitivity of FIT as compared with fecal calprotectin toward MES 0 alone suggests that FIT might be a more useful marker than fecal calprotectin in predicting a reduced risk of relapse.

Cost-saving is another merit of using FIT in place of fecal calprotectin in monitoring UC. The cost of one-time FIT is approximately \$22, while that of fecal calprotectin is \$180. Because monitoring with fecal samples has to be performed repeatedly during years of each patient's disease course, the cost-saving effect would be extremely large. More importantly, of 28 cases with clinically active disease, 23 (82%) presented positive FIT ( $> 100$  ng/mL) with active mucosal features (MES 1 or more). Therefore, these patients could be regarded as those who require additional treatment without performing colonoscopy, and only the remaining 5 cases with a negative FIT result would have to receive colonoscopy to confirm the activity of mucosal inflammation. Thus, the potential of reduction of the cost of colonoscopy by using FIT would also be large.

Notably, measurement of fecal calprotectin is performed by ELISA systems, for which there are many assay kits including Phical Calprotectin ELISA kit (used in this study), Calprest (10), Calprotectine Buhlmann ELISA (8, 9) and quantitative point-of-care test (8). Previous studies have used different assay kits; therefore, the sensitivity, specificity, and predictive values as well as cutoff values used for prediction have varied among studies. Moreover, even when the same kit has been used in studies, the cutoff values for prediction have sometimes differed. Thus, fecal calprotectin has a major

drawback in terms of the lack of a standard assay method and standard cutoff values.

In contrast, the FIT system used in this study (OC-sensor, Eiken Chemical) is the system that is most widely used worldwide. The FIT cutoff value of 100 ng/mL is the standard value used for CRC screening (3, 13). In this study, we analyzed the sensitivity, specificity and predictive values of FIT using two cutoff values—the fixed value of 100 ng/mL and the optimal cutoff calculated by ROC analysis—and large differences were not observed between values using either cutoff. In this regard, moreover, the relatively high optimal cut-off to detect MES 0 or 1 of FIT (280 ng/mL) may be attributable to this highly sensitive nature of FIT to slight mucosal inflammation. Thus, the availability of a standard method and the robust cutoff value are strengths of the FIT. Moreover, the fact that FIT can be measured within a short time (approximately 7 minutes) with automated equipment is also a strong advantage.

There are several limitations to this study. First, although the study was designed in a prospective manner, the sample size was relatively small, and the number of patients with MES 0 was large. The practical utility of FIT vs. fecal calprotectin needs to be verified in large studies in the future. Second, the difference in the manner of sampling of stools between FIT and fecal calprotectin might have affected the results. On the one hand, the sampling method for FIT was standardized with a specific sampling kit including a probe and a tube; thus, the variation in results due to sampling is expected to be relatively small. On the other hand, there are no standardized collection kits for fecal calprotectin. The lack of the standardized kits may have yielded the difference in the way of collecting stools among patients and affected the results of fecal calprotectin. In this regard, the potentially small variation caused by sampling may be considered to be a further advantage of FIT. Third, the results of FIT may have been affected by the presence of hemorrhoid. In this regard, however, previous reports regarding CRC screening indicated

that the presence of hemorrhoid did not significantly affect the results of FIT (24, 25).

In conclusion, our study revealed that both FIT and fecal calprotectin effectively predicted mucosal healing in UC patients. The results also indicated that FIT is more sensitive toward MES 0 than fecal calprotectin. The difference in clinical utility, including predictability for risk of relapse or colectomy, between the two fecal tests should be further investigated.

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## Figure Legends

### **Figure 1. Correlation between FIT or fecal calprotectin and colonoscopic findings (maximum MES in the colorectum).**

A: FIT results were significantly correlated with the maximum MES (Spearman rank correlation coefficient: 0.61,  $p < 0.0001$ ). The proportions of patients with a hemoglobin concentration of  $<100$  ng/mL were 95% with MES 0, 50% with MES 1, 31% with MES 2 and 33% with MES 3.

B: Fecal calprotectin levels were significantly correlated with the maximum MES (Spearman rank correlation coefficient: 0.58,  $p < 0.0001$ ). The proportions of patients with a calprotectin concentration of  $<250$   $\mu\text{g/g}$  were 82% with MES 0, 45% with MES 1, 37% with MES 2 and 17% with MES 3.

### **Figure 2. Correlation between FIT or fecal calprotectin and colonoscopic findings (the sum of MES of five colorectal portions: the total inflammation score).**

A: FIT results were significantly correlated with the total inflammation score (Spearman rank correlation coefficient: 0.64,  $p < 0.0001$ ).

B: Fecal calprotectin levels were significantly correlated with the total inflammation score (Spearman rank correlation coefficient: 0.60,  $p < 0.0001$ ).

### **Figure 3. Correlation between FIT values and fecal calprotectin levels**

The FIT and fecal calprotectin values in each patient were significantly correlated. (Spearman rank correlation coefficient: 0.64,  $p < 0.0001$ ).