Both Fecal Calprotectin and Fecal Immunochemical Tests are Useful in Children with Inflammatory Bowel Disease

Short title: FCP/FIT use in pediatric IBD

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Abstract

Background: Noninvasive biomarkers of intestinal inflammation can reduce the number of endoscopies in children with inflammatory bowel disease (IBD). This study aimed to prospectively investigate the usefulness of fecal calprotectin (FCP) and fecal immunochemical test (FIT) in pediatric IBD.

Methods: Patients aged 6–17 years who underwent ileocolonoscopy for established or suspected IBD were eligible for this study. Fecal samples for FCP and FIT were collected before colonoscopy.

Results: A total of 251 samples were analyzed: 88 from ulcerative colitis (UC), 74 from Crohn's disease (CD), 75 from healthy controls (HC), and 14 from children with functional gastrointestinal disorders and normal colonoscopy (NC). At IBD diagnosis, both FCP and FIT were significantly higher in the newly diagnosed UC/CD group than in the HC/NC group (P<0.001). The optimal cutoffs of FCP and FIT to predict IBD diagnosis were 217 mg/kg and 87 ng/mL, respectively. Patients without mucosal healing (MH) showed higher FCP and FIT than those with MH in both UC and CD (P<0.001). The FCP increased exponentially as the endoscopic activity score increased. The optimal cutoff values of FCP and FIT for predicting MH were 161 mg/kg and 106 ng/mL for UC and 367 mg/kg and 57 ng/mL for CD, respectively. FCP showed better specificity than the FIT. Patients with CD and normal ileocolonoscopy had elevated FCP during active small intestinal inflammation.

Conclusions: Both FCP and FIT correlate well with endoscopic activity in

pediatric patients with IBD. The FCP is a superior marker for predicting MH.

Keywords:

Fecal calprotectin

Fecal immunochemical test

Inflammatory bowel disease

Ulcerative colitis

Crohn's disease

Introduction

Calprotectin is a 36.5 kD calcium-binding protein in the S100 protein family, found primarily in neutrophils, monocytes, and macrophages. It accounts for approximately 60% of the total cytosolic protein content in these cells. Moreover, fecal calprotectin (FCP) level is stable at room temperature for a few days. Thus, FCP reflects the migration of these inflammatory cells into the intestinal epithelium [1].

The gold standard for diagnosing and evaluating pediatric-onset IBD is the combination of esophagogastroduodenoscopy (EGD) and ileocolonoscopy with biopsy [2]. With the advancement of endoscopic devices, endoscopy has become a relatively safe procedure for pediatric populations [3]. However, fasting, bowel preparation, sedation, or general anesthesia, and the risks associated with endoscopic procedures remain a concern. Therefore, FCP is expected to be a useful, noninvasive surrogate marker for intestinal inflammation.

There have been many studies on FCP in adults. Tibble et al. [4] reported the effectiveness of FCP in distinguishing organic intestinal diseases from non-organic diseases. Moreover, its correlation with endoscopic activity in ulcerative colitis (UC) [5, 6] and Crohn's disease (CD) [7-9] have been described. In addition, the usefulness of the fecal immunochemical test for hemoglobin (FIT) has also been demonstrated in adult patients with IBD [10, 11].

Henderson et al. [12] conducted a systematic review and meta-analysis of FCP in a pediatric population. The pooled sensitivity and specificity for the diagnostic utility of FCP were 0.978 (95% confidence interval [CI], 0.947–0.996) and 0.682 (95% CI, 0.502–0.863), respectively. However, no study has compared the usefulness of FCP and FIT in pediatric populations.

Of note, FCP results varied according to the assay used. EliA-Calprotectin demonstrated higher mean FCP values (765.6 μg/g) compared to Bühlmann Calprotecitn (222.5 μg/g) and PhiCal Calprotectin (247.2 μg/g) despite the excellent correlation among the three assays (r >0.9) by Passing-Bablok regression analysis [13]. Moreover, a comparison of six available FCP assays showed good qualitative correlations with a poor quantitative agreement [14]. The importance of evaluating each assay for the intended patient population should not be ignored.

Thus, this study aimed to investigate the diagnostic accuracy and correlation to the endoscopic activity of FCP measured by EliA-Calprotectin 2 and FIT in pediatric patients with IBD.

Methods

Patients

Three tertiary care pediatric institutions participated in this study: the National Center for Child Health and Development, Saitama Children's Medical Center, and Juntendo University. Patients aged 6–17 years who

underwent ileocolonoscopy for established or suspected IBD were eligible for this study. The diagnosis of IBD was based on the diagnostic criteria developed by the Pediatric IBD Porto Group of ESPGHAN [15]. Patients undergoing apheresis therapy, who used non-steroidal anti-inflammatory drugs more than twice a week within 3 months before endoscopy, failed to complete colonoscopy with terminal ileum intubation, had positive stool culture for pathogenic bacteria, or were in a menstrual period were excluded. For the healthy control (HC) group, children who had never been diagnosed with chronic gastrointestinal diseases and had no gastrointestinal symptoms such as diarrhea or abdominal pain were enrolled. Participants suspected of having IBD but demonstrated no abnormal findings were categorized into the normal colonoscopy (NC) group. All participants completed a questionnaire to collect information for fecal sampling (stool consistency, usual bowel habits, and gross bleeding in feces).

Evaluations of Endoscopic and Clinical Disease Activity in Patients with UC

In patients with UC, endoscopic activity was evaluated by experienced endoscopists using the Mayo endoscopic score (MES; range 0–3) [16]. The total colon was divided into five segments (cecum and ascending colon, transverse colon, descending colon, sigmoid colon, and rectum), and MES was assessed in each segment. The sum of these five segments was calculated as the modified score (MS; range 0–15) [17]. We regarded the MS

of 0 as mucosal healing (MH). Clinical activity was scored according to the pediatric ulcerative colitis index (PUCAI; range, 0–85) [18]. These scores were interpreted independently without knowledge of the FCP/FIT results.

Evaluations of Endoscopic and Clinical Disease Activity in Patients with CD

For patients with CD, the endoscopic disease activity of the colon and terminal ileum was assessed by experienced endoscopists using the simple endoscopic score for Crohn's disease (SES-CD; range 0–60) [19]. The intestine was divided into five segments (the ileum, right colon, transverse colon, left colon, and rectum), and the endoscopic activity in each segment was evaluated using four parameters: the presence and size of ulcers (score 0–3), the extent of the ulcerated surface (score 0–3), area of the affected surface (score 0–3), and presence and level of narrowing (score 0–3). Then, the SES-CD was calculated as the sum of the scores of the five segments. The SES-CD scores of 0–2, 3–6, 7–15, and <15 indicated remission, mild, moderate, and severe endoscopic activity, respectively.

In patients who underwent small bowel capsule endoscopy (SBCE) within 4 weeks of colonoscopy, the Lewis score [20], which was validated for isolated small-bowel CD [21], was calculated. The Lewis score classifies small bowel inflammatory activity into three grades based on the characteristics and distribution of villous edema, ulceration, and the existence of stenosis: normal or clinically insignificant mucosal inflammatory change (score <135), mild disease (score ≥135–<790), and moderate-to-severe disease (score

158 ≥790).

159 Clinical disease activity was measured using the weighted Pediatric
160 Crohn's Disease Activity Index (wPCDAI; range, 0–125) [22]. These scores
161 were interpreted independently without knowledge of the FCP/FIT results.

162

163 **Fecal sampling**

164 Three fecal samples were collected simultaneously within three weeks of
165 bowel preparation. First, samples for FCP were collected using a standard
166 sterile stool container and stored at -20°C at each hospital before being
167 transferred to a central laboratory (Thermo Fisher Scientific, Tokyo, Japan)
168 and analyzed using a fluoroscence enzyme immunoassay (FEIA) (Thermo
169 Fisher EliA Calprotectin 2: Thermo Fisher Scientific, Tokyo, Japan)
170 according to the manufacturer's instructions. Second, samples for FIT were
171 collected using a dedicated plastic serrated tip sampling probe and stored at
172 4°C until measurement using a colloidal gold agglutination assay
173 (Nescauto® Hemo Plus: Alfresa Pharma Corp., Osaka, Japan) on a high-
174 throughput discrete clinical chemistry analyzer (Hemo Techt NS-Plus C,
175 Alfresa Pharma Corp., Osaka, Japan) in a central laboratory (SRL, Inc.
176 Tokyo, Japan). The measurement range was between 3.8 mg/kg and 6,000
177 mg/kg for FCP and between 20 ng/mL and 1,200 ng/mL for FIT. Samples
178 with FCP values above 6,000 mg/kg were diluted further and measured
179 again to obtain quantitative values. The samples were analyzed
180 independently without considering the colonoscopy results. In addition, to

exclude participants with bacterial gastroenteritis, stool bacterial culture tests were performed on all samples.

Laboratory data (complete blood count [CBC], C-reactive protein [CRP], erythrocyte sedimentation rate [ESR], total protein, and albumin) within 3 weeks before ileocolonoscopy were also collected, if available.

Statistical Analyses

Continuous variables were compared using the Mann-Whitney U test for two independent groups and the Kruskal-Wallis test for three or more independent groups. When multiple pairwise comparisons were performed, a Bonferroni P-value correction was applied. Spearman's rank correlation test was used to assess the correlation between FCP/FIT and the endoscopic activity score. Receiver operating characteristic (ROC) curves were constructed to analyze the optimal FCP/FIT value for predicting MH. A 2-sided P value <0.05 was considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [23].

Ethical Considerations

The study was approved by the institutional review board (No.1911). Regarding participants under 16 years of age, a written consent form was obtained from the parents or guardians of participants, and signed

permission was also obtained from the patient, where appropriate. A written consent form was obtained from all participants aged 16 years and over.

Results

The characteristics of participants

A total of 257 fecal samples were collected. Among them, six samples were excluded from the study due to the diagnosis of atypical CD (no colonic inflammation) in two, *Yersinia* enterocolitis in one, nonspecific chronic inflammation that could not be diagnosed with IBD in two, and substitution of colonoscopy results at the referring hospital in one.

Therefore, 88 samples from UC (21 newly diagnosed UC and 67 established UC), 74 samples from CD (20 newly diagnosed CD and 54 established CD), 14 samples from NC, and 75 samples from HC were analyzed (Figure 1). Among 162 patients with UC and CD, the duration between stool sampling and colonoscopy was within 1 day for 125 patients (77.2%), 2–3 days for 26 patients (16.0%), 4–7 days for 4 patients (2.5%), and 8–17 days for 7 patients (4.3%). As a result, fecal samples were collected within 1 week of colonoscopy in more than 95% of study participants. Likewise, the durations between stool sampling and submission to each hospital were within 1 day for 149 samples (91.9%), 2 days for 9 samples (5.6%), and 3 days for 4 samples (2.5%), respectively.

The baseline characteristics of the patients with UC and CD are shown in

Table 1.

Concerning HC, 75 samples were collected from children aged 12.1 ± 3.5 years (mean ± SD). There was no relationship observed between age and FCP/FIT. However, five samples showed a high FCP (> 100 mg/kg). Among them, three participants agreed with the re-examination, and FCP decreased to under 100 mg/kg within 6 months in all of the participants (Supplementary Table 1).

Comparison of FCP/FIT in newly diagnosed patients with IBD to controls

First, the diagnostic performance of FCP/FIT in distinguishing patients with IBD from controls was evaluated. Thus, FCP/FIT levels were compared among the newly diagnosed UC, newly diagnosed CD, NC, and HC groups (Figure 2). Kruskal-Wallis analysis indicated that at least one group stochastically dominated another group (P<0.001 for both FCP and FIT). The Mann-Whitney U test showed that both FCP and FIT were significantly higher in the newly diagnosed UC/CD group than in the HC/NC group (P <0.001 for both FCP and FIT). The optimal cutoff values of FCP and FIT for the diagnosis of IBD were 217 mg/kg and 87 ng/mL, respectively. The sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and the area under the receiver operating curve (AUROC) are shown in Table 2A.

Comparison of FCP/FIT in patients with IBD stratified by endoscopic

activity score

Regarding patients with UC, the median FCP/FIT was compared between UC with MH, UC without MH, and HC. Kruskal-Wallis analysis indicated that at least one group stochastically dominated another group (P<0.001 for both FCP and FIT). Pairwise comparisons using the Mann-Whitney U test showed that UC without MH (FCP: 1411 mg/kg [interquartile range (IQR), 407–4010], and FIT 247 ng/mL [IQR, 20–1200]) were significantly higher than UC with MH (FCP: 46 mg/kg [IQR, 17–131], P<0.001; FIT: 20 ng/mL [IQR, 20–24.5], P<0.05) and HC (FCP: 20.2 mg/kg [IQR, 13.3–36.0], P <0.001; FIT: 20 ng/mL [IQR, 20–20], P<0.001) (Figures 3A and 3C).

Similarly; for CD, Kruskal-Wallis analysis indicated stochastical domination of at least one group (P<0.001 for both FCP and FIT), and the Mann-Whitney U test showed that median FCP/FIT in patients without MH (FCP: 1639 mg/kg [IQR, 560–5236], and FIT: 381 ng/mL [IQR, 27–1200]) were higher than in patients with CD with MH (FCP: 107 mg/kg [IQR, 35–335] P<0.001, and FIT: 20 ng/ml [IQR, 20–33], P<0.001) and HC (FCP: 20.2 mg/kg [IQR, 13.3–36.0], P<0.001; FIT: 20 ng/mL [IQR, 20–20], P<0.001) (Figures 3B and 3D).

Comparison of FCP/FIT in patients with non-MH stratified by disease extent or disease location

Figure 3 also shows the comparison of FCP/FIT in patients with non-MH stratified by disease extent or disease location. In UC, both FCP and FIT

were independent of disease extent (P = 0.481 and P = 0.153, respectively; Kruskal-Wallis analysis) (Figures 3A and 3C). For CD, although both FCP and FIT tended to be high in L2 patients, they did not reach statistical significance (P = 0.07 and P = 0.09, respectively; Kruskal-Wallis analysis) (Figures 3B and 3D).

FCP and FIT levels compared by endoscopic severity

Figure 4 shows the box plot of FCP/FIT stratified by endoscopic severity. In UC, both FCP and FIT tended to increase with endoscopic severity. In particular, FCP had a wider measurement range than FIT and increased exponentially with MS (Figure 4A). However, FIT was negative in half of the patients with mild inflammation, corresponding to an MS of 1–2 (Figure 4C).

For CD, FCP tended to rise exponentially with SES-CD (Figure 4B), while FIT remained low in patients with mild endoscopic activity (Figure 4D). However, seven patients showed FCP >300 mg/kg even with SES-CD ≤2. In the sub-analysis, they all had L4 disease (upper intestinal disease), which SES-CD did not consider. In four patients who showed an FCP of >1,000 mg/kg, significant small bowel inflammation was confirmed by SBCE, which showed a Lewis score exceeding 600. However, the FIT was <100 ng/mL in most of these patients (Supplementary Table 2). It is noteworthy that, a 10-year-old boy showed completely normal laboratory or clinical findings except FCP of 1025 mg/kg but had severe small bowel inflammation with a Lewis

score of 1200.

Correlation analysis for endoscopic inflammation

In UC, the correlations between MS and the following parameters were analyzed: FCP, FIT, CRP, ESR, and PUCAI (Table 3A). Overall, both FCP and FIT had a good correlation with MS (Spearman's rank correlation coefficient: 0.67, P<0.0001 vs. 0.65, P< 0.0001, respectively), which were higher than that of ESR and CRP. FCP showed a slightly higher correlation coefficient than FIT, although the difference was not statistically significant (P= 0.154). Among these markers, PUCAI showed the strongest correlation with MS.

For CD, the correlations between SES-CD and the following markers were also evaluated: FCP, FIT, CRP, ESR, and wPCDAI (Table 3B). Overall, both FCP and FIT had a good correlation with SES-CD (Spearman's rank correlation coefficient: 0.70, P<0.0001 vs. 0.72, P<0.0001, respectively), which were higher than the correlation of ESR, CRP, and wPCDAI. FIT showed a slightly higher correlation coefficient than FCP, although the difference was not statistically significant (P= 0.143).

Predicting Mucosal Healing in UC

For the performance of FCP/FIT in predicting MH, the AUROC for each parameter is shown in Table 3. The best cutoff values of FCP and FIT for predicting MH in UC (MS = 0) were 161 mg/kg and 106 ng/mL, respectively.

In this analysis, PUCAI showed the strongest correlation with MS. However, the AUROC for PUCAI in predicting MH was only 0.675. To examine the significance of adding fecal markers to clinical symptoms in predicting MH, we compared the AUROC of PUCAI alone and those of PUCAI with FCP or FIT (Supplementary Figure 1). We found that the AUROC significantly increased to 0.889 by adding FCP to PUCAI (P = 0.01) and that this was the most significant increase in AUROC observed. Hence, no additional diagnostic accuracy was observed when FIT was added to PUCAI and FCP.

Predicting Mucosal Healing in CD

Regarding CD, the best cutoff values of FCP and FIT for predicting MH (SES-CD ≤2) were 367 mg/kg and 57 ng/mL, respectively. Although FCP showed relatively high AUROC in both UC and CD, the differences were not statistically significant (P>0.05).

Discussion

In this study, we investigated the usefulness of FCP and FIT in children with IBD. First, for the diagnostic accuracy in distinguishing patients with IBD from healthy children, both FCP and FIT had excellent AUROC. When these markers are used as screening tools for IBD, high sensitivity should be weighted more than specificity. If we set the cutoff value as FCP of 50

mg/kg and FIT of 100 ng/mL, the sensitivity and specificity were 0.976 and 0.831 for FCP, and 0.927 and 0.966 for FIT, respectively. The sensitivity and specificity of FCP were consistent with those of a meta-analysis by Henderson et al. [12] To the best of our knowledge, there are no data on the effectiveness of FIT in detecting pediatric IBD. Our results suggest that both FCP and FIT are valuable tools to consider which patients should undergo colonoscopy.

Second, in UC, the median FCP increased exponentially as MS increased. MS is the sum of the MESs from five segments of the colon. Summarily, FCP is considered to reflect both the severity and extent of inflammation. Conversely, FCP can be used as a marker of MH in patients with UC. In addition, many studies have reported the usefulness of FCP as a surrogate marker for MH in both adults [6, 11, 24] and pediatric [25] patients.

Hiraoka et al. [26] compared the correlation of fecal markers with endoscopic findings between a pair of colonoscopies in adults. They reported that FIT is useful in confirming and predicting MH, while FCP correlates well with endoscopic activities during the active phase of UC.

Dai et al. [27] conducted a meta-analysis to assess the utility of the FIT for predicting MH in adults with UC. They reported that the pooled sensitivity and specificity were 0.77 (95% CI, 0.72–0.81) and 0.81 (95% CI, 0.76–0.85), respectively. Our lower specificity compared to their meta-analysis may be caused by the strict definition of MH and the cutoff value. Their meta-analysis included studies whose definition of MH was an MES of 0–1, while

363 the present study used an MES of 0 alone.

364 However, Ryu et al. [24] defined MH as MES of 0 and set the cutoff as FIT
365 ≤100 ng/mL. They reported that the sensitivity and specificity of FIT for
366 predicting MH were 0.980 and 0.374, respectively, and the PPV was as low
367 as 0.394. For FCP, the sensitivity and specificity were 0.784 and 0.748,
368 respectively, and the PPV was 0.563 when the cutoff was set to FCP ≤170
369 mg/kg. These results were consistent with those of the present study. Under
370 low specificity and PPV, negative FIT does not necessarily mean the
371 achievement of MH. In addition, more than half of our patients with MS of
372 1–2 showed negative FIT results.

373 Summarily, FCP has higher specificity and PPV and can be considered as a
374 suitable marker for predicting MH. Conventionally, MH has been predicted
375 in daily practice using clinical symptoms and laboratory data. This study
376 has shown the significance of adding FCP to PUCAI to improve the
377 diagnostic accuracy. FCP can, therefore, improve the differential diagnosis
378 of MH and has the added benefit of being a non-invasive biomarker, which is
379 important, especially in children. In Japan, the reference value of FCP for
380 predicting MH in UC was set to ≤300 mg/kg based on the performance
381 testing results. However, our results showed that the cutoff of FCP 300
382 mg/kg produces a PPV as low as 40%. An FCP of approximately 160 mg/kg
383 might be the better cutoff for predicting MH in pediatric UC, although
384 further studies are needed.

385 Regarding the correlation of fecal markers and the extent of UC,

Naganuma et al. [28] reported that median FCP was lower in proctitis (E1) than in left-sided colitis and pancolitis (E2–E4), while the FIT was independent of the disease extent [29]. In the present study, both FCP and FIT showed no association with the disease extent. It has been shown that proctitis accounts for only 5–7% of pediatric patients with UC in Japan [30] and Europe [31]. Hence, the scarcity of pediatric patients with proctitis (E1) might have affected our results due to selection bias.

For CD, the median FCP also increased exponentially as the SES-CD increased. The SES-CD reflects the entire endoscopic activity in the terminal ileum and the four parts of the colon. Hence, FCP may be used as a marker for MH in CD. In addition, the ECCO-ESPGHAN guidelines for pediatric CD recommend applying FCP as a treatment response marker and relapse predictor [30].

Previous studies have reported variable correlations between FCP and SES-CD (Spearman's rank correlation coefficient, $r = 0.45–0.75$) [31-33]. Our results showed a relatively strong correlation between FCP/FIT and SES-CD (correlation coefficient of approximately 0.7). Although FIT showed a high sensitivity for predicting MH, its specificity was low. On the other hand, the FCP cutoff of 367 mg/kg produced a sensitivity of 86% and a PPV of 70%. In pediatric patients with CD, FCP seemed to be superior to FIT in predicting MH.

Notably, CD could have transmural inflammation. In such cases, FCP may not correlate with the endoscopic activity score, which assesses mucosal

409 inflammation. Indeed, Weinstein-Nakar et al. [34] analyzed data from the
410 ImageKids study to determine associations among mucosal, transmural
411 healing, and FCP levels in children with CD. They reported that the median
412 FCP level was lowest in children who achieved both mucosal and
413 transmural healing (defined as deep healing) and highest in patients with
414 mucosal and transmural inflammation. Thus, FCP may reflect transmural
415 inflammation in patients with CD. They reported that an FCP cutoff value
416 of 100 mg/kg identified patients with deep healing with 71% sensitivity and
417 92% specificity. Interestingly, they also reported that FCP of 300 mg/kg
418 identified patients with MH with 80% sensitivity and 81% specificity, and
419 these results are consistent with our results.
420 Moreover, another aspect of the CD should be considered. CD affects all
421 areas of the gastrointestinal tract and is characterized by skip lesions. SES-
422 CD does not cover the upper intestinal lesions (L4 disease in the Paris
423 classification). Arai et al. [35] reported that FCP was correlated with small
424 bowel inflammation. In the present study, seven patients achieved MH by
425 SES-CD but showed a high FCP of >300 mg/kg. Significant small bowel
426 inflammation corresponding to a Lewis score of >600 was confirmed in four
427 patients with an FCP of >1,000 mg/kg. These results might imply that we
428 should search for small bowel inflammation when no inflammation was
429 detected by ileocolonoscopy despite a high FCP. Interestingly, in these 4
430 patients, the levels of FIT, CRP, and ESR were not necessarily elevated. In
431 this regard, FCP could be the “cue” for searching L4 disease.

The present study has some limitations. First, the pathological findings were not assessed in this study. However, MH is considered a therapeutic target in clinical practice. Second, the endoscopic score was not independently assessed by central reviewers. However, it was evaluated by pediatric endoscopists who specialize in pediatric IBD without knowing the results of FCP/FIT. Third, this study defined MH in CD as SES-CD $\leq$2. As mentioned above, SES-CD-based assessment might overlook upper gastrointestinal inflammation and transmural inflammation. These types of inflammation might affect the results of fecal markers and, therefore, further studies assessing these forms of inflammation are required. Despite these limitations, this is a multicenter, prospective study that includes more than 250 samples, which is a relatively large sample size for a pediatric study. Furthermore, to the best of our knowledge, no pediatric study has adopted the EliA Calprotectin 2 for FCP measurement and compared it to FIT in pediatric populations with endoscopic evaluation.

In conclusion, the present study revealed that both FCP and FIT correlate well with endoscopic activities in pediatric patients with IBD. FCP seemed to be a superior marker for predicting MH with better specificity.

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Figure Legends

Fig. 1 Patients flow chart

UC, ulcerative colitis; CD, Crohn's disease; CS, colonoscopy; HC, healthy control. Paris classification UC extent [E1, ulcerative proctitis; E2, left-sided UC (distal to splenic flexure); E3, extensive (hepatic flexure distally); E4, Pancolitis (proximal to the hepatic flexure)]; CD location [L1, terminal ileal ± limited cecal disease; L2, colonic; L3, ileocolonic; L4a, upper disease proximal to the ligament of Treitz; L4b, upper disease distal to the ligament of Treitz and proximal to distal 1/3 ileum].

Fig. 2 Comparisons of fecal biomarker levels in patients with newly diagnosed ulcerative colitis and newly diagnosed Crohn's disease against controls

Median (a) FCP and (b) FIT levels in newly diagnosed UC and newly diagnosed patients with CD are significantly higher than in controls (P<0.001, respectively; Kruskal-Wallis analysis).

In the pairwise comparisons using the Mann-Whitney U test, P-values are adjusted using the Bonferroni method (** P<0.001).

FCP, fecal calprotectin; FIT, fecal immunochemical test for hemoglobin; HC, healthy control; NC, normal colonoscopy; UC, ulcerative colitis; CD, Crohn's disease; IBD, inflammatory bowel disease.

Fig. 3 Comparisons of fecal biomarker levels by disease extent or location

Median (a) FCP and (c) FIT levels in patients with ulcerative colitis and median (b) FCP and (d) FIT levels in patients with CD. Among patients with UC with MH, UC without MH, and HC, UC without MH shows higher FCP and FIT than others (P <0.001, respectively; Kruskal-Wallis analysis). Likewise, among CD with MH, CD without MH, and HC, CD without MH shows higher FCP and FIT than others (P <0.001, respectively; Kruskal-Wallis analysis). For UC without MH, FCP (a) and FIT (c) was independent of the disease extent (FCP, P= 0.48; FIT, P= 0.15, respectively; Kruskal-Wallis analysis). For CD without MH, FCP (b) and FIT (d) tend to be high in L2 patients, but are not statistically significant (P= 0.07 and P= 0.09, respectively; Kruskal-Wallis analysis). In the pairwise comparisons using the Mann-Whitney U test, P-values are adjusted using the Bonferroni method (* P<0.05, ** P<0.001).

FCP, fecal calprotectin; FIT, fecal immunochemical test for hemoglobin; UC, ulcerative colitis; CD, Crohn's disease; HC, healthy control; MH, mucosal healing; Paris

605 classification UC extent [E1, ulcerative proctitis; E2, left-sided UC (distal to splenic
606 flexure); E3, extensive (hepatic flexure distally); E4, Pancolitis (proximal to the hepatic
607 flexure)]; CD location [L1, terminal ileal ±limited cecal disease; L2, colonic; L3,
608 ileocolonic].
609
610 **Fig. 4 Comparisons of fecal biomarker levels by endoscopic activity score**
611 Median (a) FCP and (c) FIT levels in patients with various MS and median (b) FCP and
612 (d) FIT levels in patients with various SES-CD. FCP levels increase exponentially as
613 MS or SES-CD increases (P<0.001, respectively; Kruskal-Wallis analysis). In the
614 pairwise comparisons using the Mann-Whitney U test, Pvalues are adjusted using the
615 Bonferroni method (* P<0.05, ** P<0.001).
616 FCP, fecal calprotectin; FIT, fecal immunochemical test for hemoglobin; HC, healthy
617 control; UC, ulcerative colitis; CD, Crohn's disease; MH, mucosal healing; MS, modified
618 Mayo endoscopic score; SES-CD, simple endoscopic score for Crohn's disease.
619

Table 1: Characteristics of patients with ulcerative colitis and Crohn's disease

UC	n = 88	CD	n = 74
Age, y (mean ± SD)	13.9 ± 2.3	Age, y (mean ± SD)	13.6 ± 2.5
Males, n (%)	43 (48.8%)	Males, n (%)	46 (62.1%)
Paris classification, n (%)		Paris classification, n (%)	
E1 (proctitis)	4 (4.5%)	L1 (TI & Cecum)	17 (23.0%)
E2 (left-sided)	8 (9.0%)	L2 (colonic)	6 (8.1%)
E3 (extensive)	10 (11.4%)	L3 (ileocolonic)	51 (68.9%)
E4 (pancolitis)	66 (75.0%)	L4a and/or L4b	62 (83.8%)
FCP, n (%)		FCP, n (%)	
<100 mg/kg	11 (12.5%)	<100 mg/kg	15 (20.3%)
<300 mg/kg	25 (28.4%)	<300 mg/kg	26 (35.1%)
FIT, n (%)		FIT, n (%)	
<50 ng/mL	36 (40.9%)	<50 ng/mL	34 (45.9%)
<100 ng/mL	40 (45.5%)	<100 ng/mL	38 (51.4%)
<300 ng/mL	50 (56.8%)	<300 ng/mL	44 (59.5%)
CRP, n (%)		CRP, n (%)	
<3 mg/L	72 (81.8%)	<3 mg/L	47 (63.5%)
<5 mg/L	81 (92.0%)	<5 mg/L	51 (68.9%)
ESR, n (%)		ESR, n (%)	
<10 mm/h	36 (40.9%)	<10 mm/h	25 (33.8%)
<20 mm/h	65 (73.9%)	<20 mm/h	43 (58.1%)
Modified score (MS), n (%)		SES-CD, n (%)	
0, mucosal healing	10 (11.4%)	0–2, mucosal healing	22 (29.7%)
1-2, mild disease	26 (29.5%)	mild disease	17 (23.0%)
3–5, moderate disease	23 (26.1%)	moderate disease	17 (23.0%)
6–15, severe disease	29 (33.0%)	severe disease	18 (24.3%)
PUCAI, n (%)		wPCDAI, n (%)	
<10, remission	46 (52.3%)	<12.5, remission	36 (48.6%)
10–30, mild	19 (21.6%)	12.5–22.5, mild	5 (6.8%)
35–60, moderate	18 (20.5%)	25–57.5, moderate	22 (29.7%)
65–85, severe	5 (5.7%)	60–125, severe	11 (14.9%)
	Treatment (current user / past user), n		
5-ASA	52/15	5-ASA	41/9
Immunomodulator	29/10	Immunomodulator	24/5
Corticosteroids	11/33	Corticosteroids	6/11
Infliximab	7/4	Infliximab	9/7
Adalimumab	2/2	Adalimumab	18/5
Golimumab	9/1	Ustekinumab	8/2
Vedolizumab	2/0	Vedolizumab	1/2

UC, ulcerative colitis; MH, mucosal healing; CD, Crohn's disease; SD, standard deviation; FCP, fecal calprotectin; FIT, fecal immunochemical test; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PUCAI, pediatric ulcerative colitis activity index; 5-ASA, 5-aminosalicylic acid.

Table 2: Diagnostic accuracy of fecal calprotectin and fecal immunochemical test

A: IBD diagnosis		Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	Accuracy
FCP	cutoff 50	0.976 (0.871–0.999)	0.831 (0.737–0.902)	0.987 (0.928–1.000)	0.727 (0.590–0.839)	0.877 (0.808–0.928)
	cutoff 100	0.951 (0.835–0.994)	0.921 (0.845–0.968)	0.976 (0.917–0.997)	0.848 (0.711–0.937)	0.931 (0.873–0.968)
	cutoff 217*	0.927 (0.801–0.985)	0.989 (0.939–1.000)	0.967 (0.907–0.993)	0.974 (0.865–0.999)	0.969 (0.923–0.992)
	cutoff 300	0.878 (0.738–0.959)	0.989 (0.939–1.000)	0.946 (0.879–0.982)	0.973 (0.858–0.999)	0.954 (0.902–0.983)
FIT	cutoff 50	0.951 (0.835–0.994)	0.966 (0.905–0.993)	0.977 (0.920–0.997)	0.929 (0.805–0.985)	0.962 (0.913–0.987)
	cutoff 87*	0.951 (0.835–0.994)	0.966 (0.905–0.993)	0.977 (0.920–0.997)	0.929 (0.805–0.985)	0.962 (0.913–0.987)
	cutoff 100	0.927 (0.801–0.985)	0.966 (0.905–0.993)	0.966 (0.905–0.993)	0.927 (0.801–0.985)	0.954 (0.902–0.983)
	cutoff 300	0.854 (0.708–0.944)	0.989 (0.939–1.000)	0.936 (0.866–0.976)	0.972 (0.855–0.999)	0.946 (0.892–0.978)
B: predicting MH in UC		Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	Accuracy
FCP	cutoff 50	0.500 (0.187–0.813)	0.930 (0.830–0.981)	0.914 (0.810–0.971)	0.556 (0.212–0.863)	0.866 (0.760–0.937)
	cutoff 100	0.600 (0.262–0.878)	0.930 (0.830–0.981)	0.930 (0.830–0.981)	0.600 (0.262–0.878)	0.881 (0.778–0.947)
	cutoff 161*	0.900 (0.555–0.997)	0.860 (0.742–0.937)	0.980 (0.894–0.999)	0.529 (0.278–0.770)	0.866 (0.760–0.937)
	cutoff 300	0.900 (0.555–0.997)	0.754 (0.622–0.859)	0.977 (0.880–0.999)	0.391 (0.197–0.615)	0.776 (0.658–0.869)
FIT	cutoff 50	0.800 (0.444–0.975)	0.526 (0.390–0.660)	0.938 (0.792–0.992)	0.229 (0.104–0.401)	0.567 (0.440–0.688)
	cutoff 100	0.900 (0.555–0.997)	0.474 (0.340–0.610)	0.964 (0.817–0.999)	0.231 (0.111–0.393)	0.537 (0.411–0.660)
	cutoff 106*	1.000 (0.587–1.000)	0.474 (0.340–0.610)	1.000 (0.817–1.000)	0.250 (0.127–0.412)	0.552 (0.426–0.674)
	cutoff 300	1.000 (0.587–1.000)	0.351 (0.229–0.489)	1.000 (0.762–1.000)	0.213 (0.107–0.357)	0.448 (0.326–0.574)
C: predicting MH in CD		Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	Accuracy
FCP	cutoff 50	0.381 (0.181–0.616)	0.939 (0.798–0.993)	0.705 (0.548–0.832)	0.800 (0.444–0.975)	0.722 (0.584–0.835)
	cutoff 80	0.429 (0.218–0.660)	0.939 (0.798–0.993)	0.721 (0.563–0.847)	0.818 (0.482–0.977)	0.741 (0.603–0.850)
	cutoff 100	0.524 (0.298–0.743)	0.909 (0.757–0.981)	0.750 (0.588–0.873)	0.786 (0.492–0.953)	0.759 (0.624–0.865)
	cutoff 367*	0.857 (0.637–0.970)	0.758 (0.577–0.889)	0.893 (0.718–0.977)	0.692 (0.482–0.857)	0.796 (0.665–0.894)
FIT	cutoff 50	0.857 (0.637–0.970)	0.545 (0.364–0.719)	0.857 (0.637–0.970)	0.545 (0.364–0.719)	0.667 (0.525–0.789)
	cutoff 57*	0.905 (0.696–0.988)	0.545 (0.364–0.719)	0.900 (0.683–0.988)	0.559 (0.379–0.728)	0.685 (0.544–0.805)
	cutoff 100	0.905 (0.696–0.988)	0.485 (0.308–0.665)	0.889 (0.653–0.986)	0.528 (0.355–0.696)	0.648 (0.506–0.773)
	cutoff 300	0.952 (0.762–0.999)	0.364 (0.204–0.549)	0.923 (0.640–0.998)	0.488 (0.329–0.649)	0.593 (0.450–0.724)

A: Diagnostic accuracy for the diagnosis of inflammatory bowel disease, B: Diagnostic accuracy for predicting mucosal healing in patients with ulcerative colitis, C: Diagnostic accuracy for predicting mucosal healing in patients with Crohn's disease. *optimal cutoff point. Mucosal healing is defined as a modified score of 0 for UC and an SES-CD ≤2 for CD. IBD, inflammatory bowel disease; CI, confidence interval; NPV,

negative predictive value; PPV, positive predictive value; FCP, fecal calprotectin; FIT, fecal immunochemical test for hemoglobin; UC, ulcerative colitis; CD, Crohn's disease.

Table 3: Correlations between laboratory and clinical markers and endoscopic disease activities and the area under the receiver operating characteristic curve for predicting mucosal healing in patients with established ulcerative colitis and Crohn's disease

A: ulcerative colitis

	Correlation coefficient	Predicting MH in established UC				
	r	Cutoff	Sensitivity	Specificity	AUROC	95%CI
FCP	0.669**	161 mg/kg	0.900	0.860	0.874	0.724–1.000
FIT	0.645**	106 ng/mL	1.000	0.474	0.732	0.616–0.849
CRP	0.478**	0.02 mg/dl	0.700	0.649	0.641	0.456–0.826
ESR	0.390*	3 mm/h	0.400	0.772	0.520	0.297–0.744
PUCAI	0.752**	10	1.000	0.333	0.675	0.555–0.794

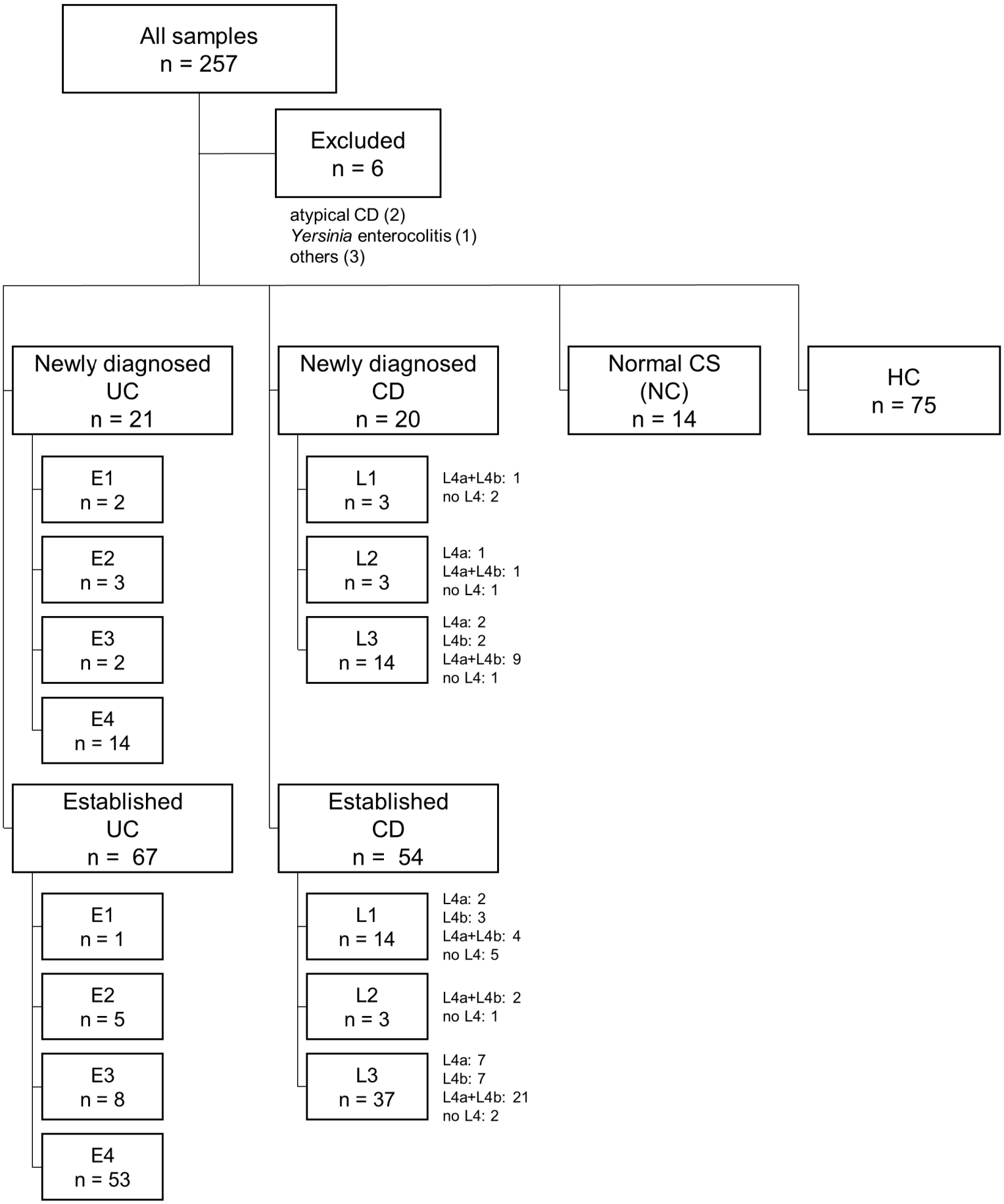
B: Crohn's disease

	Correlation coefficient	Predicting MH in established UC				
	r	Cutoff	Sensitivity	Specificity	AUROC	95%CI
FCP	0.698**	367 mg/kg	0.857	0.758	0.823	0.704–0.942
FIT	0.720**	57 ng/mL	0.905	0.545	0.716	0.588–0.844
CRP	0.600**	0.07 mg/dl	0.762	0.758	0.740	0.600–0.881
ESR	0.585**	16 mm/h	0.857	0.576	0.765	0.636–0.894
wPCDAI	0.581**	7.5	0.762	0.545	0.657	0.511–0.802

*P<0.001 **P<0.0001

UC, ulcerative colitis; MS, modified Mayo endoscopic score; MH, mucosal healing; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; FCP, fecal calprotectin; FIT, fecal immunochemical test for hemoglobin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PUCAI, pediatric ulcerative colitis activity index; CD, Crohn's disease; SES-CD, simple endoscopic score for Crohn's disease; wPCDAI, weighted pediatric Crohn's disease activity index.

Figure 1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Excluded
n = 6
atypical CD (2)
Yersinia enterocolitis (1)
others (3)
Newly diagnosed
UC
n = 21
E1
n = 2
E2
n = 3
E3
n = 2
E4
n = 14
Newly diagnosed
CD
n = 20
L1
n = 3
L4a+L4b: 1
no L4: 2
L2
n = 3
L4a: 1
L4a+L4b: 1
no L4: 1
L3
n = 14
L4a: 2
L4b: 2
L4a+L4b: 9
no L4: 1
Normal CS
(NC)
n = 14
HC
n = 75
Established
UC
n = 67
E1
n = 1
E2
n = 5
E3
n = 8
E4
n = 53
Established
CD
n = 54
L1
n = 14
L4a: 2
L4b: 3
L4a+L4b: 4
no L4: 5
L2
n = 3
L4a+L4b: 2
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L3
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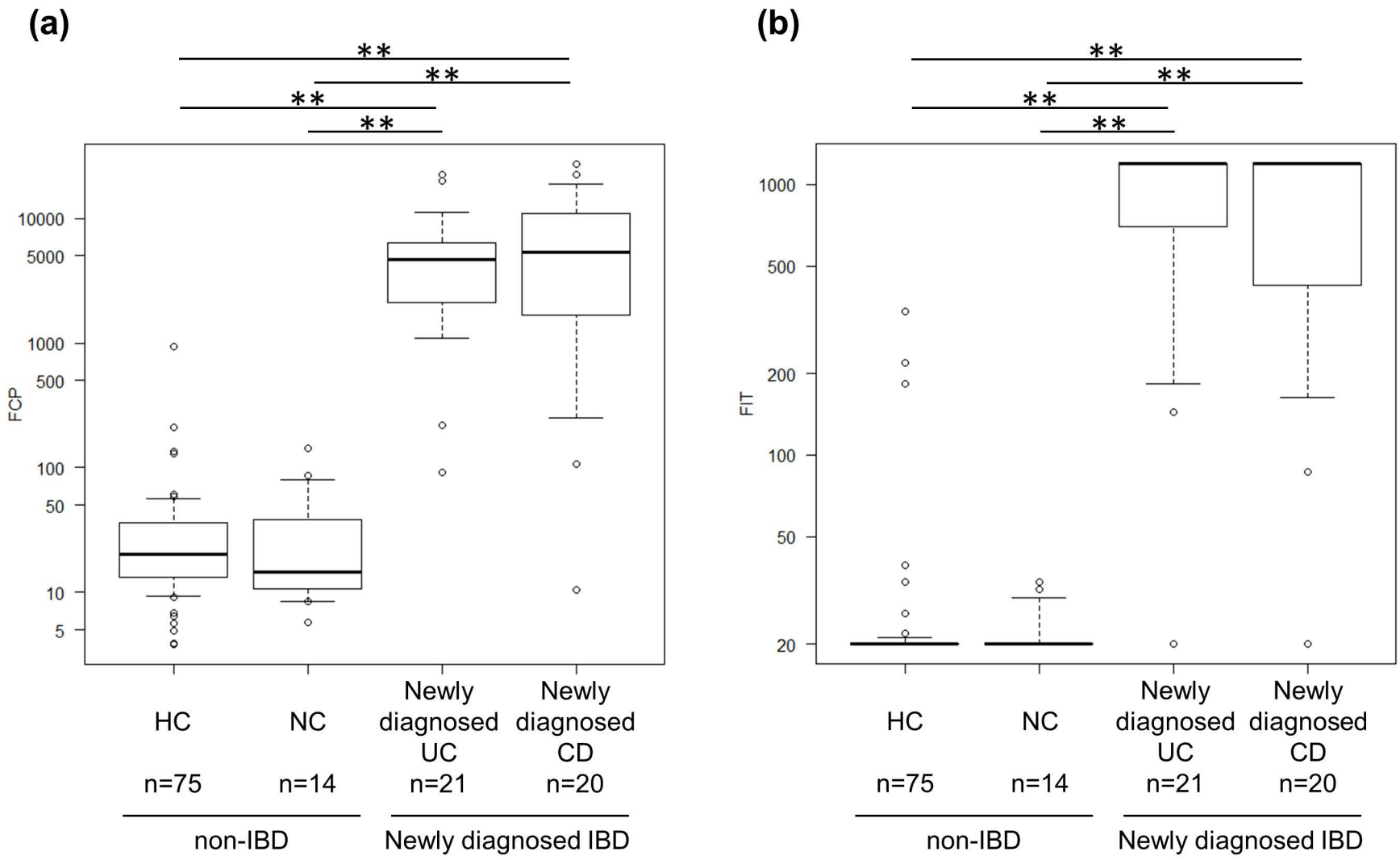
L4b: 7
L4a+L4b: 21
no L4: 2

Figure 2


(a)
**
**
**
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FCP
10000
5000
1000
500
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50
10
5
HC
n=75
NC
n=14
Newly diagnosed UC
n=21
Newly diagnosed CD
n=20
non-IBD
Newly diagnosed IBD
(b)
**
**
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FIT
1000
500
200
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20
HC
n=75
NC
n=14
Newly diagnosed UC
n=21
Newly diagnosed CD
n=20
non-IBD
Newly diagnosed IBD

Figure 3

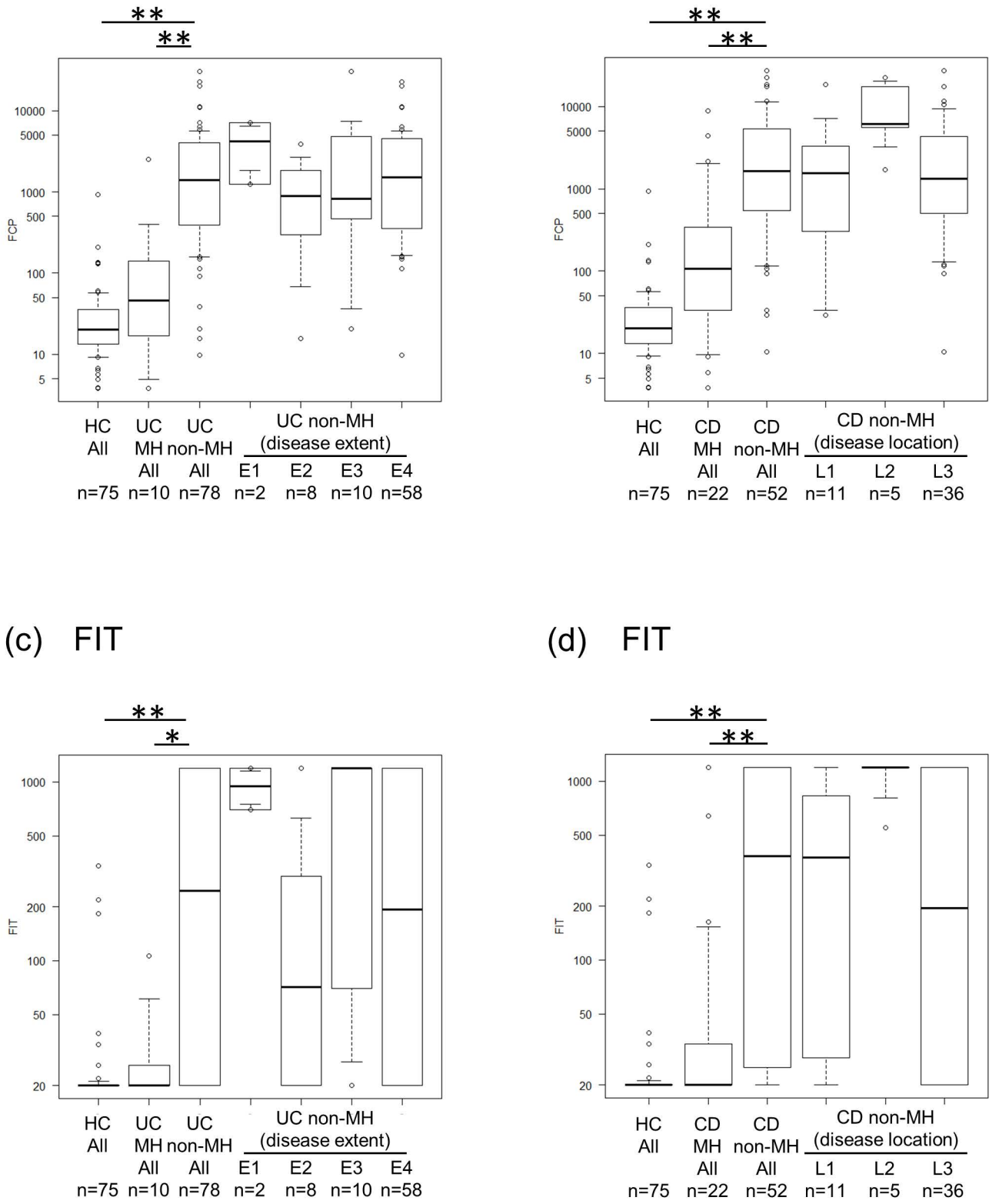
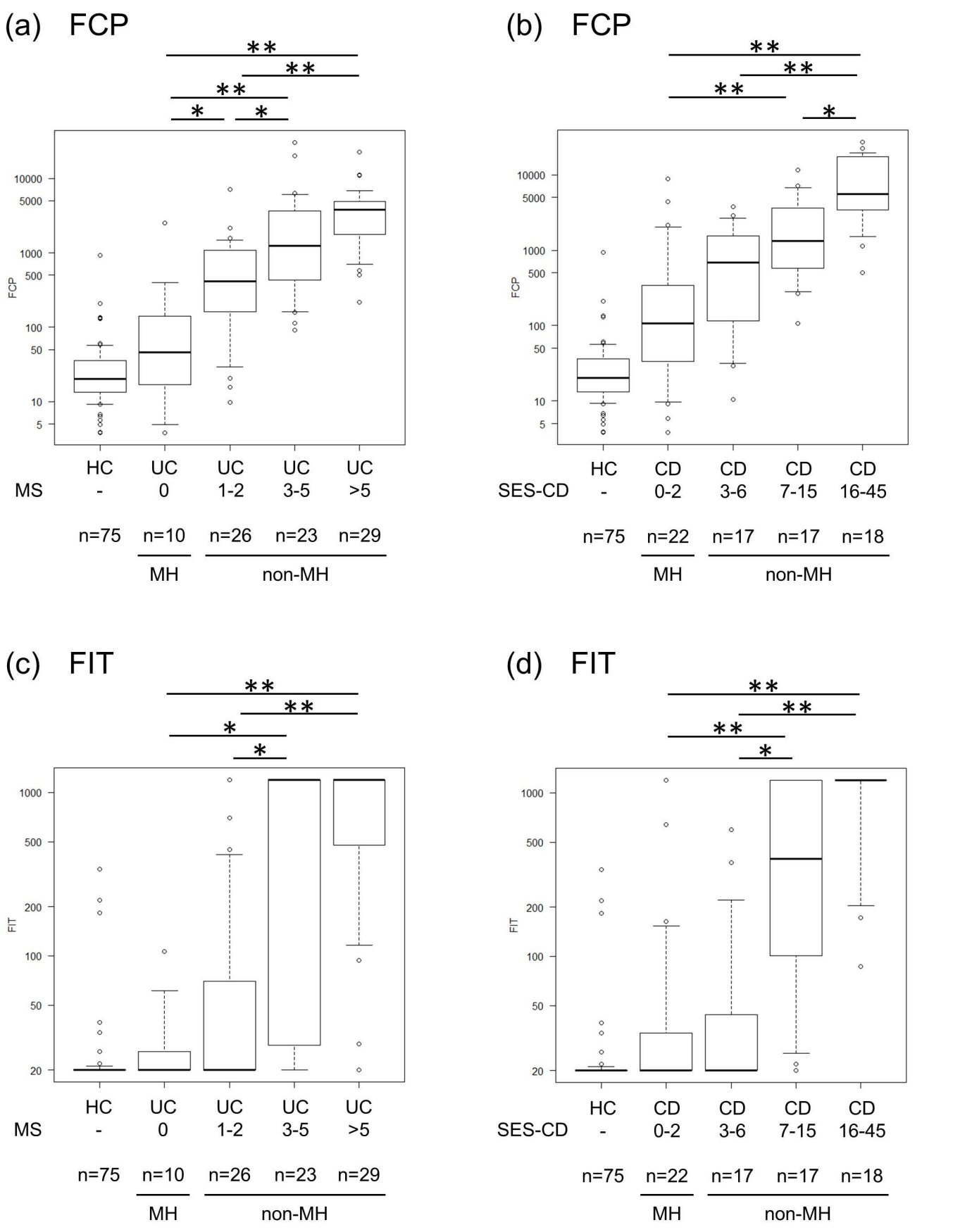

(a) FCP
**
**
FCP
10000
5000
1000
500
100
50
10
5
HC All n=75
UC MH All n=10
UC non-MH All n=78
UC non-MH (disease extent)
E1 n=2
E2 n=8
E3 n=10
E4 n=58
(b) FCP
**
**
FCP
10000
5000
1000
500
100
50
10
5
HC All n=75
CD MH All n=22
CD non-MH All n=52
CD non-MH (disease location)
L1 n=11
L2 n=5
L3 n=36
(c) FIT
**
*
FIT
1000
500
200
100
50
20
HC All n=75
UC MH All n=10
UC non-MH All n=78
UC non-MH (disease extent)
E1 n=2
E2 n=8
E3 n=10
E4 n=58
(d) FIT
**
**
FIT
1000
500
200
100
50
20
HC All n=75
CD MH All n=22
CD non-MH All n=52
CD non-MH (disease location)
L1 n=11
L2 n=5
L3 n=36

Figure 4


(a) FCP
**
**
**
*
*
FCP
10000
5000
1000
500
100
50
10
5
HC
UC
UC
UC
UC
MS
-
0
1-2
3-5
>5
n=75
n=10
n=26
n=23
n=29
MH
non-MH
(b) FCP
**
**
**
*
FCP
10000
5000
1000
500
100
50
10
5
HC
CD
CD
CD
CD
SES-CD
-
0-2
3-6
7-15
16-45
n=75
n=22
n=17
n=17
n=18
MH
non-MH
(c) FIT
**
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FIT
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HC
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1-2
3-5
>5
n=75
n=10
n=26
n=23
n=29
MH
non-MH
(d) FIT
**
**
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*
FIT
1000
500
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100
50
20
HC
CD
CD
CD
CD
SES-CD
-
0-2
3-6
7-15
16-45
n=75
n=22
n=17
n=17
n=18
MH
non-MH

Supplementary Table 1: Results of re-examination in healthy controls who showed high FCP

Age [years]	Sex	1st FCP [mg/kg]	2nd FCP [mg/kg]	1st FIT [ng/mL]	2nd FIT [ng/mL]	Interval [months]
9	Male	933	67	340	40	5
12	Female	210	28	20	20	6
13	Female	134	71	20	20	3
16	Female	136	NA	20	NA	NA
17	Male	136	NA	20	NA	NA
12	Female	129	NA	20	NA	NA

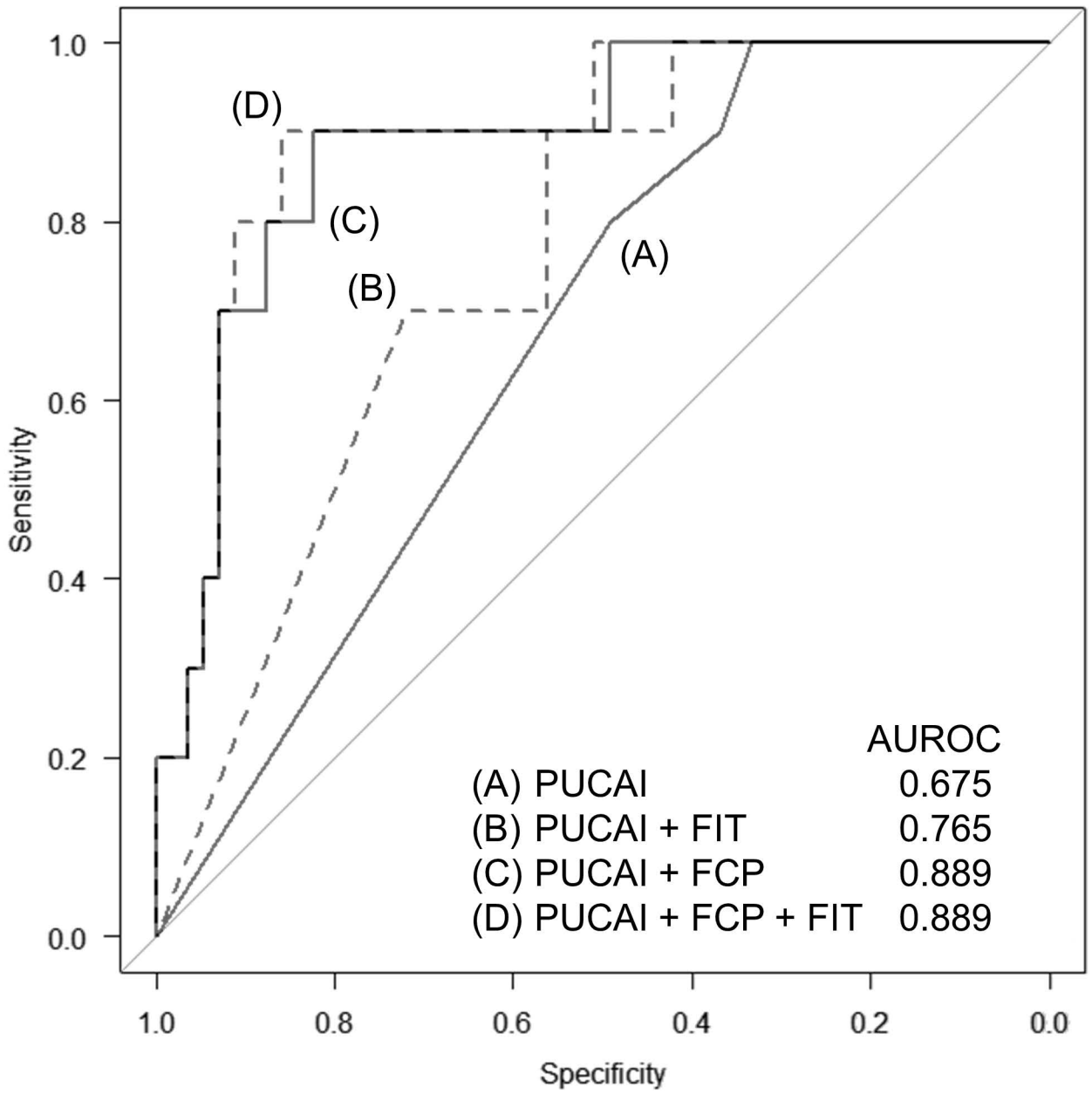
FCP, fecal calprotectin; FIT, fecal immunochemical test for hemoglobin; NA, not available.

Supplementary Table 2: Characteristics of patients with high FCP despite SES-CD ≤2

Age, sex	Location	Behavior	CRP	ESR	FCP	FIT	wPCDAI	SES-CD	Lewis score
13 y, F	L3, L4a, L4b	B1	0.02	7	313	34	0	2	0-0-0
15 y, M	L3, L4a, L4b	B1	0.01	1	342	20	7.5	0	0-0-0
10 y, F	L1, L4b	B1	0.01	4	367	28	47.5	2	0-0-900
10 y, M	L3, L4b	B1	0.02	8	1025	57	0	0	450-450-1200
12 y, M	L1, L4a, L4b	B1	0.55	16	2145	29	42.5	2	451-601-135
14 y, M	L1, L4a, L4b	B2p	7.07	41	4441	31	70	2	900-225-225
14 y, M	L3, L4a, L4b	B1	3.42	28	8893	>1200	80	2	908-908-0

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; FCP: fecal calprotectin; FIT: fecal immunochemical test for hemoglobin; wPCDAI: weighted pediatric Crohn's disease activity index, SES-CD: simple endoscopic score for Crohn's disease, location (L1: terminal ileal disease, L3: ileocolonic disease, L4a: upper intestinal disease proximal to the ligament of Treitz, L4b: upper intestinal disease distal to the ligament of Treitz), behavior (B1: inflammatory, B2 stenotic, p: perianal disease)

S. Figure 1


Comparison of ROC curves
1.0
0.8
0.6
0.4
0.2
0.0
Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
Specificity
(D)
(C)
(B)
(A)
AUROC
(A) PUCAI 0.675
(B) PUCAI + FIT 0.765
(C) PUCAI + FCP 0.889
(D) PUCAI + FCP + FIT 0.889