

Evaluation of Blood and Stool Biomarkers Across Three Fecal Calprotectin Platforms and Combination Testing for Predicting Endoscopic and Histologic Outcomes in Inflammatory Bowel Disease

Thanaboon Chaemsupaphan

Mahidol University

Marianee Salaemae

Mahidol University

Onuma Sattayalertyanyong

Mahidol University

Julajak Limsrivilai

`alimsrivilai@gmail.com`

Mahidol University

Research Article

Keywords: Fecal calprotectin, Fecal immunochemical test, Biomarkers, Inflammatory bowel disease

Posted Date: September 23rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10722887/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Evaluation of Blood and Stool Biomarkers Across Three Fecal Calprotectin Platforms and Combination Testing

for Predicting Endoscopic and Histologic Outcomes in Inflammatory Bowel Disease

Thanaboon Chaemsupaphan, MD¹, Mariane Salaemae, BSc¹, Onuma Sattayalertyanyong, MD¹, Julajak

Limsrivilai, MD, MSc^{*1}

¹Division of Gastroenterology, Department of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

***Corresponding author:**

Julajak Limsrivilai MD, MSc

Associate Professor of Medicine, Division of Gastroenterology, Department of Medicine,

Siriraj Hospital, Mahidol University

2 Wanglang Road, Siriraj, Bangkoknoi, Bangkok Thailand, 10700

Telephone: +66 2419 7281 Email: alimsrivilai@gmail.com

Abstract

Background: Non-invasive biomarkers are increasingly used to monitor inflammatory bowel disease (IBD), but differences among fecal calprotectin assays and the incremental value of combining fecal biomarkers remain uncertain. We aimed to compare the correlation and diagnostic performance of fecal calprotectin (FC) measured using three analytical platforms, fecal immunochemical test (FIT), and serum C-reactive protein (CRP) for predicting endoscopic and histologic outcomes in IBD.

Methods: We conducted a single-center observational study of adults with IBD who underwent ileocolonoscopy at Siriraj Hospital, Thailand. FC was measured using latex agglutination, fluoroenzyme immunoassay, and chemiluminescent immunoassay platforms. Quantitative FIT and serum CRP were also assessed. Endoscopic activity was evaluated using the Mayo Endoscopic Score or Simple Endoscopic Score for Crohn's Disease, and histologic activity using the Nancy Histological Index. Correlations were assessed using Spearman correlation coefficients, and diagnostic performance using receiver operating characteristic analysis and predefined cutoffs. A linear mixed model compared fecal calprotectin levels across platforms.

Results: Among 133 patients, 74 (55.6%) had ulcerative colitis (UC) and 59 (44.4%) had Crohn's disease (CD); 123 (92.5%) were in clinical remission. FC showed excellent correlation across the three platforms (Spearman $r = 0.95\text{--}0.96$, all $P < 0.001$), despite significantly different absolute concentrations. For predicting mucosal healing, FC demonstrated good and comparable discrimination across platforms (AUROC 0.82–0.83), outperforming CRP (0.62). FIT showed high sensitivity (90.7%) but limited specificity (43.1%) at the predefined cutoff, whereas FC provided a more balanced diagnostic profile. In clinically quiescent UC combining FIT with FC increased specificity but did not improve sensitivity. In CD, combination testing provided no additional benefit over FC alone.

Conclusions: FC demonstrated the most consistent overall diagnostic performance for assessing objective disease activity in IBD, although absolute values differed substantially between assay platforms. FIT may be useful as a sensitive screening biomarker, whereas FC is more suitable for confirming mucosal healing. Combining the two tests provides limited additional value in clinically quiescent IBD.

Trial registration: ClinicalTrials.gov, NCT06623383, registered on September 30, 2024

Keywords: Fecal calprotectin, Fecal immunochemical test, Biomarkers, Inflammatory bowel disease

Background

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disorder of the gastrointestinal tract characterized by relapsing and remitting intestinal inflammation. Patients may develop mucosal ulceration, bowel wall inflammation, strictures, or fistula formation involving different segments of the gastrointestinal tract¹⁻³. Over the past decade, the burden of IBD has increased substantially across the Asia-Pacific region, including Thailand⁴, with a growing number of patients and increasing disease heterogeneity accompanied by more complex therapeutic strategies^{5,6}. Management of IBD often requires long-term medical therapy, including biologic agents, and some patients ultimately require intestinal surgery due to disease-related complications. Therefore, regular monitoring of disease activity is essential for optimal disease management⁷.

According to the STRIDE-II recommendations, treatment goals extend beyond symptom control to include mucosal healing, particularly endoscopic healing⁸. Endoscopic assessment remains the gold standard for evaluating intestinal inflammation. In CD, endoscopic activity is commonly assessed using the Simplified Endoscopic Score for Crohn's Disease (SES-CD)⁹, whereas in UC, it is typically evaluated using the Mayo Endoscopic Score (MES)¹⁰. However, colonoscopy is invasive, costly, and may not be suitable for repeated

monitoring in routine clinical practice, leading to increasing interest in non-invasive biomarkers as surrogate markers of intestinal inflammation¹¹. Among these, fecal calprotectin (FC), a neutrophil-derived protein released during intestinal inflammation, has been widely studied and demonstrates high diagnostic value for detecting endoscopic disease activity^{12,13}. FC can now be measured using several analytical platforms. In addition to conventional enzyme-linked immunosorbent assays (ELISA), other technologies such as automated latex agglutination and chemiluminescent immunoassays have increasingly been adopted in clinical laboratories¹⁴. These assays may offer faster turnaround time, improved laboratory automation, and the ability to process larger sample volumes. However, different techniques may result in variability in FC values, with studies demonstrating up to 2–3-fold differences between assays and lack of standardization, which may influence clinical interpretation and diagnostic performance^{15,16}. Furthermore, Other biomarkers, including fecal immunochemical test (FIT), which detects fecal hemoglobin, and serum C-reactive protein (CRP), which reflects systemic inflammation, have also been evaluated as potential tools for disease monitoring^{17,18}. Although several studies have examined the diagnostic performance of each test, studies evaluating combinations of them remain limited. Therefore, our study aimed to evaluate the correlation and diagnostic performance of non-invasive biomarkers, including serum CRP, FIT, FC measured using different assay platforms, and their combinations, for assessing endoscopic and histologic disease activity in patients with IBD.

Methods

Study design

This was a single-center observational study conducted at the IBD center, Siriraj Hospital, Mahidol University, Bangkok, Thailand. The study aimed to evaluate the correlation and diagnostic performance of non-invasive biomarkers, including serum CRP, FIT, and various FC assays, for assessing endoscopic and histologic disease activity in patients with IBD. The study was approved by the Siriraj Institutional Review Board (approval number Si 723/2023) and was registered at ClinicalTrials.gov (NCT06623383 on September 30, 2024).

Study population

This study was conducted between 1 October 2024 and 31 October 2025, enrolling adult participants (>18 years) with an established diagnosis of IBD (UC and CD) who underwent colonoscopy to assess disease activity. The diagnosis of IBD was established and classified based on a combination of clinical, endoscopic, histologic, and radiologic findings. Clinical data, including demographics, disease characteristics, clinical activity scores, laboratory results, and stool tests were collected at the time of colonoscopy. Participants were excluded if stool samples were unavailable, colonoscopy was incomplete, or biomarker measurements were missing. Clinical disease activity was assessed at enrollment using the partial Mayo score (pMayo) for UC¹⁹ and the Harvey–

Bradshaw Index (HBI) for CD²⁰, with clinical remission defined as pMayo ≤ 1 and HBI ≤ 4 , respectively. All

participants provided written informed consent prior to enrollment.

Endoscopic and histologic outcomes

All patients underwent ileocolonoscopy performed by experienced gastroenterologists. Endoscopic disease

activity in UC was evaluated using the MES (range 0–3), with mucosal healing defined as MES 0–1 and

endoscopic remission defined as MES 0¹⁰, while in CD it was assessed using the SES-CD, with endoscopic

remission defined as SES-CD ≤ 2 ⁹. Histologic assessment was performed in patients with available biopsy

specimens obtained during colonoscopy. Biopsy samples were reviewed by experienced gastrointestinal

pathologists according to standard histopathologic criteria. Histologic activity was evaluated using the Nancy

Histological Index, with histologic healing defined as a Nancy score of 0–1.

Biomarker measurements

FC levels were measured using three assay platforms: latex agglutination assay (LAA; OC-Sensor, EIKEN

Chemical Co., Ltd., Tokyo, Japan; measuring range 20–2,720 $\mu\text{g/g}$), fluoroenzyme immunoassay (FEIA; EliA

Calprotectin 2, Thermo Fisher Scientific, Uppsala, Sweden; measuring range 3.8–6,000 $\mu\text{g/g}$), and

chemiluminescent immunoassay (CLIA; Liaison Calprotectin, DiaSorin S.p.A., Italy; measuring range 5–8,000

$\mu\text{g/g}$). Quantitative FIT was performed using an automated immunochemical assay (OC-SENSOR Ceres, EIKEN

Chemical Co., Ltd., Tokyo, Japan), with a detection range of 12–1,000 ng/mL. Patients were instructed to collect

stool samples prior to colonoscopy. Serum CRP levels were measured using standard laboratory methods on the day of colonoscopy. Cutoff values of 150 µg/g for FC and 5 mg/L for CRP were used to define elevated levels according to STRIDE II⁸. For FIT, we selected the cutoff value of 100 ng/mL, which was the most common cutoff value used in previous studies²¹.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data or median with interquartile range (IQR) for non-normally distributed data, while categorical variables were expressed as frequencies and percentages. The Spearman rank correlation coefficient was used to assess correlations between biomarkers (CRP, FIT, and FC) and disease activity indices. Diagnostic performance was evaluated using receiver operating characteristic (ROC) curve analysis, with the area under the ROC curve (AUROC) calculated to assess the discriminative ability of each biomarker for predicting endoscopic remission. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated using predefined cutoff values, and a two-sided *P* value <0.05 was considered statistically significant. Linear mixed model was used to compare calprotectin levels across three methods in the same patients. All statistical analyses were performed using SAS software (SAS Institute, Cary, NC, USA).

Results

A total of 133 patients with IBD were included in the study, of whom 70 (52.6%) were male with a mean age of 47.0 years (Table 1). Among these, 74 patients (55.6%) had UC and 59 (44.4%) had CD. In UC, left-sided (E2) and extensive colitis (E3) were most common (51% and 40%), whereas CD was predominantly ileal disease (19.6%) with perianal involvement of 28.8%. In UC, clinical remission was present in 67 patients (90.5%). Mucosal healing was observed in 62.2% of patients, while 31.1% achieved endoscopic remission. Endoscopically active disease (MES 2–3) was observed in 37.8% of patients. In CD, clinical remission was observed in 56 (95%) patients, while endoscopic remission was present in 49.2% of patients, with the remainder demonstrating mild (27.1%), moderate (20.3%), or severe endoscopic activity (3.4%). The overall median CRP level was 1.6 mg/L (IQR 0.6–4.9), while the median FIT value was 0 ng/mL (IQR 0–75). Median FC levels were 290 µg/g (IQR 91–1312) using LAA, 227 µg/g (IQR 75–1368) using FEIA, and 113 µg/g (IQR 42.9–481) using CLIA. There was no difference between LAA and FEIA, while both of them were significantly higher than CLIA.

Table 1 Baseline characteristics of patients included in the study

	N (%)
Male	70 (52.6)
Age (years) (mean ± SD)	47.0 ± 17.1

Clinical remission	123 (92.5)
Endoscopic remission	52 (39.1)
Histologic remission (N=121)	24 (19.8)
Ulcerative colitis	74 (55.6)
L1/L2/L3	7 (9.5)/ 35 (47.3)/ 32 (43.2)
Full Mayo score	1 (0-2)
Clinical remission (pMayo 0-1)	67 (90.5%)
Endoscopic activity	
Endoscopic remission (MES 0)	23 (31.1)
MES 1	23 (31.1)
MES 2	18 (24.3)
MES 3	10 (13.5)
Histologic healing	20 (27.0)
Crohn's disease	59 (44.4)
L1/L2/L3/L4	26 (19.6)/ 14 (10.5)/ 19 (14.3)/ 8 (6.0)
Stricturing phenotype (B2)	32 (54.2)
Fistulizing phenotype (B3)	10 (17.0)
Perianal involvement	17 (28.8)
HBI	0 (0-1)

Clinical remission (HBI 0-4)	56 (95.0)
Endoscopic activity	
Endoscopic remission (SES-CD 0-2)	29 (49.2)
Mild (SES-CD 3-6)	16 (27.1)
Moderate (SES-CD 7-15)	12 (20.3)
Severe (SES-CD >16)	2 (3.4)
Hb (g/dL) (mean $\pm$ SD)	13.3 $\pm$ 2.3
CRP (median, IQR)	1.6 (0.6-4.9)
Albumin (g/dL)	4.3 $\pm$ 0.4
FIT quantitative (ng/mL)	0 (0-75)
Fecal calprotectin (LAA) (μ g/g)	290 (91-1312)
Fecal calprotectin (FEIA) (μ g/g)	227 (75-1368)
Fecal calprotectin (CLIA) (μ g/g)	113 (42.9-481)

Abbreviations: UC, ulcerative colitis; CD, Crohn's disease; pMayo, partial Mayo score; HBI, Harvey-Bradshaw

Index; MES, Mayo Endoscopic Score; SES-CD, Simple Endoscopic Score for Crohn's Disease; L1-L4, Montreal

classification for disease location in CD; Hb, hemoglobin; CRP, C-reactive protein; FIT, fecal immunochemical

test; FC, fecal calprotectin; LAA, latex agglutination assay; FEIA, fluoroenzyme immunoassay; CLIA,

chemiluminescent immunoassay; IQR, interquartile range; SD, standard deviation.

Correlation between non-invasive biomarkers and endoscopic activity

The correlations between non-invasive biomarkers and endoscopic activity were analyzed. All three FC assays

were highly correlated with each other, with Spearman coefficients ranging from 0.95 to 0.96 (all $P < 0.001$). FIT

had significant moderate to strong correlation with FC, with Spearman coefficients ranging from 0.58 to 0.62 (all P

< 0.001), while CRP was not significantly correlated with FC, with Spearman coefficients of 0.14 to 0.16 ($P > 0.05$)

(Supplementary Table S1).

The distribution of FC, FIT and CRP levels stratified by endoscopic severity is presented in Table 2. In UC, FC

levels measured using all three assays increased with worsening endoscopic activity, with significant moderate

correlations with MES, with Spearman coefficients of 0.49 for LAA, 0.54 for FEIA, and 0.48 for CLIA (all $P < 0.01$).

Notably, the differences in absolute values were observed between platforms. The CLIA levels appeared to be

lower than the others. FIT levels increased progressively with higher MES and demonstrated a significant

moderate correlation ($r = 0.55$, $P < 0.01$). CRP levels showed minimal variation across MES categories, with

overlapping values despite increasing endoscopic severity. It was not significantly correlated with MES ($r = 0.16$,

$P = 0.18$). The same trends were observed in CD. FC levels measured using all three assays also increased with

worsening endoscopic activity, with significant strong correlations with SES-CD ($r = 0.67$ for LAA, 0.67 for FEIA,

and 0.65 for CLIA, all $P < 0.01$). Furthermore, differences in absolute values were also observed between

platforms. Unlike UC, FIT and CRP showed significant but weak correlation to SES-CD severity ($r = 0.29$, $P = 0.03$

for CRP, $r = 0.38$, $P < 0.01$ for FIT).

Table 2 Comparison of serum C-reactive protein, fecal immunochemical test, and fecal calprotectin

measurements using three techniques stratified by endoscopic severity

UC (MES)	LAA ($\mu\text{g/g}$)	FEIA ($\mu\text{g/g}$)	CLIA ($\mu\text{g/g}$)	FIT (ng/mL)	CRP (mg/L)	CD (SES- CD)	LAA ($\mu\text{g/g}$)	FEIA ($\mu\text{g/g}$)	CLIA ($\mu\text{g/g}$)	FIT (ng/mL)	CRP (mg/L)
0	116 (36-189)	71 (41-138)	54.5 (15.5-73.6)	0 (0-0)	1.2 (0.6-3.8)	0-2	132 (52-256)	146 (66-222)	62.1 (23.9- 85.6)	0 (0-6)	1.2 (0.6-6.6)
1	228 (63-1015)	232 (72-868)	102 (17.6-360)	0 (0-20)	0.8 (0.6-3.8)	3-6	651.5 (348.5- 1617)	746 (200.5- 1802.5)	267.5 (143.5- 564)	11 (0-91)	2.3 (0.6-9.8)
2	1655.5 (291- 2636)	1769 (280- 3253)	572 (124-867)	285.5 (1-1083)	1.3 (0.8-2.0)	7-15	1598.5 (535.5- 3374.5)	1615.5 (787.5- 2429.5)	580.5 (251.5- 940)	8.5 (0-191.5)	4.6 (1.6-18.8)

3	2258.5 (53-5798)	2904.5 (53-5345)	679.5 (31.6- 2060)	178 (16-9055)	3.1 (1.4-8.9)	>15	866 (105- 1627)	1101 (284- 1918)	403 (54.1-752)	1054.5 (8 -2101)	40.9 (2.8-79.1)
---	---------------------	---------------------	--------------------------	------------------	------------------	-----	-----------------------	------------------------	-------------------	---------------------	--------------------

Abbreviations: UC, ulcerative colitis; CD, Crohn's disease; MES, Mayo Endoscopic Score; SES-CD, Simple

Endoscopic Score for Crohn's Disease; CRP, C-reactive protein; FIT, fecal immunochemical test; LAA, latex

agglutination assay; FEIA, fluorescence enzyme immunoassay; CLIA, chemiluminescent immunoassay

Diagnostic performance of non-invasive biomarkers for predicting mucosal healing

The diagnostic performance of biomarkers for predicting mucosal healing, assessed using AUROC, is shown in

Figure 1. In the overall cohort, FC demonstrated good and comparable accuracy across all assays, with AUROC

values of 0.83 for the LAA, 0.82 for FEIA, and 0.82 for CLIA. These were significantly higher than the AUROC of

CRP (AUROC 0.62, $P < 0.05$), and higher but not significantly than the AUROC of FIT (AUROC 0.75, all $P = 0.06$).

In UC subgroup, FC assays also showed similar diagnostic performance, with AUROC values of 0.78 for the LAA,

0.79 for FEIA, and 0.77 for CLIA. FIT demonstrated comparable accuracy in this subgroup (AUROC 0.80),

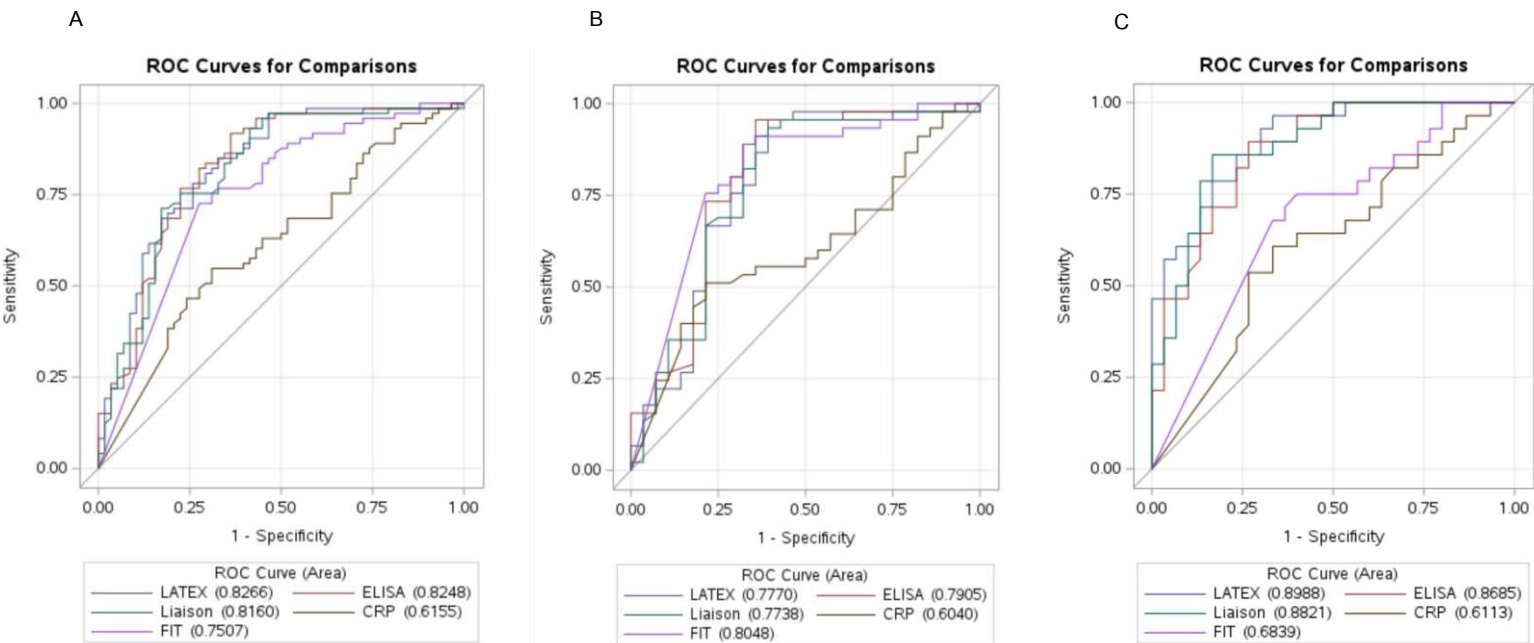
whereas CRP showed limited discriminative ability (AUROC 0.60, $P < 0.05$). In CD, FC achieved the highest

predictive performance, with AUROC values of 0.90 for the LAA assay, 0.87 for FEIA, and 0.88 for CLIA. FIT and

CRP demonstrated significantly lower accuracy, with AUROC values of 0.68 and 0.61, respectively. AUROC for

predicting endoscopic remission is provided in Supplementary Figure S1. Overall, the performance was comparable but slightly lower than that observed for mucosal healing.

Figure 1: Area under Receiver operating characteristic (AUROC) curves demonstrating the performance of non-invasive biomarkers for predicting mucosal healing in (A) overall IBD, (B) ulcerative colitis, and (C) Crohn’s disease



Using a cutoff value of <150 µg/g to predict mucosal healing, sensitivities of FC assays in the overall cohort ranged from 54.7% to 76.0% and specificities from 70.7% to 87.9%. Among the assays, CLIA showed higher sensitivity (76.0%) with lower specificity (70.7%), whereas LAA and FEIA demonstrated higher specificities (87.9% and 84.5%, respectively) but lower sensitivities. FIT (cutoff value <100 ng/mL) showed the higher sensitivity (90.7%) but with relatively low specificity (43.1%). The diagnostic performance of the biomarkers across the overall cohort, UC, and CD subgroups, is detailed in Table 3. In UC, all FC assays showed comparable sensitivities (52.2–69.6%) and specificities (71.4–78.6%). FIT maintained high sensitivity (91.3%) with moderate specificity (64.3%), outperforming FC in sensitivity. In CD, the LAA exhibited the highest specificity (96.7%) with moderate sensitivity (58.6%), whereas CLIA showed higher sensitivity (86.2%) but lower specificity (70.0%). FIT again demonstrated high sensitivity (89.7%) but poor specificity (23.3%). CRP demonstrated consistently suboptimal diagnostic performance across the study.

Table 3: Diagnostic performance of serum and stool assays for predicting mucosal healing in IBD

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Overall cohort				
FC LAA	54.7 (42.7-66.2)	87.9 (76.7-95.0)	85.4 (72.2-93.9)	60.0 (48.8-70.5)

FC FEIA	57.4 (45.4-68.7)	84.5 (72.6-92.7)	82.7 (69.7-91.8)	60.5 (49.0-71.2)
FC CLIA	76.0 (64.7-85.1)	70.7 (57.3-81.9)	77.0 (65.8-86.0)	69.5 (56.1-80.8)
FIT	90.7 (81.7-96.2)	43.1 (30.2-56.8)	67.3 (57.3-76.3)	78.1 (60.0-90.7)
CRP	77.3 (66.2-86.2)	29.3 (18.1-42.7)	58.6 (48.2-68.4)	50.0 (32.4-67.6)
Ulcerative colitis				
FC LAA	52.2 (36.9-67.1)	78.6 (59.0-91.7)	80.0 (61.4-92.3)	50.0 (34.6-65.4)
FC FEIA	60.9 (45.4-74.9)	78.6 (59.0-91.7)	82.4 (65.5-93.2)	55.0 (38.5-70.7)
FC CLIA	69.6 (54.2-82.3)	71.4 (51.3-86.8)	80.0 (64.4-90.9)	58.8 (40.7-75.4)
FIT	91.3 (79.2-97.6)	64.3 (44.1-81.4)	80.8 (67.5-90.4)	81.8 (59.7-94.8)
CRP	82.6 (68.6-92.2)	21.4 (8.3-41.0)	63.3 (49.9-75.4)	42.9 (17.7-71.1)
Crohn's disease				
FC LAA	58.6 (38.9-76.5)	96.7 (82.8-99.9)	94.4 (72.7-99.9)	70.7 (54.5-83.9)
FC FEIA	51.7 (32.5-70.6)	90.0 (73.5-97.9)	83.3 (58.6-96.4)	65.9 (49.4-79.9)
FC CLIA	86.2 (68.3-96.1)	70.0 (50.6-85.3)	73.5 (55.6-87.1)	84.0 (63.9-95.5)
FIT	89.7 (72.6-97.8)	23.3 (9.9-42.3)	53.1 (38.3-67.5)	70.0 (34.8-93.3)
CRP	69.0 (49.2-84.7)	36.7 (19.9-56.1)	51.3 (34.8-67.6)	55.0 (31.5-76.9)

The diagnostic performance was evaluated using a cutoff value of <150 µg/g for fecal calprotectin, 100 ng/mL for fecal immunochemical test, and <5 mg/L for C-reactive protein.

Abbreviations: FC, fecal calprotectin; LAA, latex agglutination assay; FEIA, fluoroenzyme immunoassay; CLIA, chemiluminescent immunoassay; CRP, C-reactive protein; FIT, fecal immunochemical test; Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value

Diagnostic performance of combining FC and FIT for predicting endoscopic remission and histologic healing in clinically quiescent IBD patients

Of 74 patients with UC, 67 were in clinical remission. FC measured using the LAA methods demonstrated higher specificity than FIT (73.9% vs 56.5%) but lower sensitivity (52.3% vs 93.2%) (Table 4). FIT showed high sensitivity and negative predictive value, whereas FC provided better specificity and positive predictive value. Combination testing improved specificity and positive predictive value but did not improve sensitivity. For endoscopic and histologic remission, FIT consistently showed high sensitivity but poor specificity, while FC demonstrated similar performance. The combination assay provided slightly better specificity.

Among 59 patients with CD, 56 were in clinical remission. FC showed high specificity (96.3%) but only moderate sensitivity (58.6%), whereas FIT showed high sensitivity (89.7%) but very low specificity (18.5%). Combination testing did not provide additional benefit beyond FC alone.

Table 4: Diagnostic performance of fecal immunochemical test and fecal calprotectin at specific cutoffs and

combination testing, for predicting endoscopic and histologic outcomes in clinically quiescent IBD patients

Outcomes	Tests	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Mucosal healing	FC	52.3 (36.7-67.5)	73.9 (51.6-89.8)	79.3 (60.3-92.0)	44.7 (28.6-61.7)
	FIT	93.2 (81.3-98.6)	56.5 (34.5-76.8)	80.4 (66.9-90.2)	81.2 (54.4-96.0)
	Combination FIT + FC	52.3 (36.7-67.5)	82.6 (61.2-95.0)	85.2 (66.3-95.8)	47.5 (31.5-63.9)
Endoscopic remission	FC	56.5 (34.5-76.8)	63.6 (47.8-77.6)	44.8 (26.4-64.3)	73.7 (56.9-86.6)
	FIT	100.0 (85.2-100.0)	36.4 (22.4-52.2)	45.1 (31.1-59.7)	100.0 (79.4-100.0)
	Combination FIT + FC	56.5 (34.5-76.8)	68.2 (52.4-81.4)	48.1 (28.7-68.1)	75.0 (58.8-87.3)
Histologic remission	FC	70.0 (45.7-88.1)	68.1 (52.9-80.9)	48.3 (29.4-67.5)	84.2 (68.7-94.0)
	FIT	100.0 (83.2-100.0)	34.0 (20.9-49.3)	39.2 (25.8-53.9)	100.0 (79.4-100.0)
	Combination FIT + FC	70.0 (45.7-88.1)	72.3 (57.4-84.4)	51.9 (31.9-71.3)	85.0 (70.2-94.3)

Endoscopic remission	FC	58.6 (38.9-76.5)	96.3 (81.0-99.9)	94.4 (72.7-99.9)	68.4 (51.3-82.5)
	FIT	89.7 (72.6-97.8)	18.5 (6.3-38.1)	54.2 (39.2-68.6)	62.5 (24.5-91.5)
	Combination FIT + FC	58.6 (38.9-76.5)	96.3 (81.0-99.9)	94.4 (72.7-99.9)	68.4 (51.3-82.5)

The diagnostic performance was evaluated using a cutoff value of <150 µg/g for fecal calprotectin and <100

ng/mL for fecal immunochemical test

Abbreviations: FIT, fecal immunochemical test; FC, fecal calprotectin; Sn, sensitivity; Sp, specificity; PPV, positive

predictive value; NPV, negative predictive value

Discussion

In this study, we comprehensively evaluated the performance of non-invasive biomarkers, including CRP, FIT, and

FC across multiple analytical platforms for predicting endoscopic and histologic activity in IBD patients. FC

demonstrated the strongest correlation with endoscopic activity and superior diagnostic performance for mucosal

healing compared with FIT and CRP. Despite variability in absolute values, FC assays showed excellent inter-

assay correlation, supporting the robustness. At a certain cutoff, FIT exhibited high sensitivity but low specificity to

predict mucosal healing, whereas FC assays provided a more balanced profile. In clinically quiescence disease,

combining FIT and FC did not provide meaningful incremental benefit beyond FC alone. Further, CRP showed limited utility as a surrogate marker of disease activity.

Our findings are consistent with prior studies demonstrating that FC is a reliable biomarker for endoscopic activity in IBD. Multiple studies have reported moderate to strong correlations between FC and endoscopic indices, generally ranging from 0.5 to 0.7^{17,22-27}. For instance, Takashima et al. reported a correlation of 0.58 between FC and MES in UC²³, while Inokuchi et al. demonstrated a correlation of 0.67 with SES-CD in CD¹⁸. Similarly, Jain et al. reported a correlation of 0.827 for SES-CD in CD which is stronger than of 0.793 for UCEIS in UC²⁶. In our study, FC demonstrated good diagnostic performance across all IBD subtypes. A recent study on UC from Mayo Clinic also reported an excellent AUROC of 0.915 for predicting mucosal healing²⁸. A higher AUROC was observed in CD compared with UC. However, prior meta-analysis suggested superior performance in UC compared with CD (AUROC 0.88 in UC vs 0.79 in CD), along with higher sensitivity (0.87 vs 0.67)²⁹. This discrepancy may be explained by differences in disease distribution. In the meta-analysis, many CD cohorts included patients with isolated ileal disease, where FC is known to have reduced sensitivity. In contrast, our cohort had a higher proportion of colonic and ileocolonic involvement, which might have enhanced the FC performance.

Another key aspect of this study is the comparison of FC across different analytical platforms. Despite substantial differences in absolute FC values between assays, all platforms demonstrated excellent correlation. This finding is consistent with previous study comparing ELISA and CLIA platforms, demonstrating significant variability in absolute values ($P = 0.039$), but strong correlation³⁰. CLIA, as a newer immunoassay platform, offers advantages including higher analytical sensitivity, faster turnaround time, and automated processing compared with traditional ELISA-based methods^{31,32}. In our study, slight differences in diagnostic performance were observed across platforms in our study, with CLIA showing higher sensitivity but lower specificity, while LAA and FEIA demonstrated slightly higher specificity. These differences likely reflect variations in assay calibration and analytical range. Therefore, this finding reinforces that FC assays are not interchangeable.

FIT has also been increasingly studied as an alternative biomarker. Previous studies have consistently demonstrated high sensitivity but variable specificity. Takashima et al. and Nakarai et al. reported sensitivity above 90% in UC, while Inokuchi et al. demonstrated similarly high sensitivity in CD but with substantially lower specificity^{18,23,33}. Our findings are consistent with these results, showing high sensitivity but limited specificity, particularly in CD. This supports the role of FIT as a rule-out test rather than a confirmatory marker of mucosal healing. Notably, FIT detects mucosal bleeding rather than inflammation directly, and therefore its performance

may vary depending on disease severity, particularly in the presence or absence of ulceration. This mechanistic difference likely explains its reduced specificity compared with FC.

When directly comparing FC and FIT, prior studies have demonstrated complementary diagnostic characteristics.

In UC, Takashima et al. reported that FIT achieved higher sensitivity for endoscopic remission compared to FC

(95% vs 82%), albeit with only moderate specificity (62%)²³. Similarly, in CD, Inokuchi et al. showed that FIT

maintained very high sensitivity (96%) but had substantially lower specificity (48%), particularly in patients with

isolated small bowel disease, whereas FC provided better specificity (71%) and remained reliable across disease

locations¹⁸. Our study also confirmed that FC has superior specificity with moderate sensitivity, appropriate for

confirming mucosal healing. In contrast, FIT is more suited as a screening test for active mucosal inflammation,

particularly in patients in clinical remission where a negative result may reduce the need for endoscopic

reassessment. However, cutoff values for both FIT and FC vary considerably across studies, highlighting the need

for cautious interpretation.

Previous studies evaluating combination testing to predict endoscopically active disease have shown mixed

results, with some suggesting combining FIT and FC improved specificity but reduced sensitivity³⁴. More recent

data showed potential synergy with improved overall diagnostic performance, although this benefit varies by

disease locations and endpoints³⁵. In contrast, our study, which specifically evaluated clinically quiescent

population, combining FIT with FC did not provide additional benefit beyond FC alone. In a setting of low inflammatory burden, the high sensitivity of FIT may lead to persistent false positives even when mucosal healing has been achieved, thereby limiting its additive value when combined with FC.

CRP showed weak or non-significant correlations with endoscopic and histologic activity and inferior diagnostic performance compared with stool-based biomarkers. This aligns with previous data demonstrating that CRP reflects systemic rather than mucosal inflammation and has limited sensitivity, particularly in UC³⁶. Even in CD, where CRP is more frequently elevated, its correlation with endoscopic activity remains modest. These findings further support that CRP should not be used as a standalone marker for mucosal healing.

This study has several strengths, including its prospective design, inclusion of both UC and CD populations, evaluation of multiple FC platforms, and assessment of relevant objective outcomes. Biomarker measurements were performed in close temporal proximity to colonoscopy, minimizing bias. However, several limitations should be acknowledged. First, the majority of patients were in clinical remission, resulting in a limit number of patients with moderate-to-severe endoscopic activity, which may affect diagnostic performance estimates across the full disease spectrum. Second, the total number of participants was not sufficient to allow for meaningful subgroup analyses according to disease location, extent, or phenotype. Lastly, we used predefined cutoff values based on

prior studies. However, optimal cutoff values may vary depending on disease subtype, assay platform, and clinical context. This may have influenced the observed diagnostic performance.

Conclusion

FC should be prioritized in treat-to-target strategy as it demonstrates the strongest correlation and overall diagnostic performance for assessing endoscopic and histologic activity in IBD. However, clinicians should be aware of assay-specific differences in the measurements and interpret results accordingly. Although FIT provides high sensitivity, the specificity is limited, making it useful only as a screening tool while limiting its role as a confirmatory marker. Combination FIT with FC does not offer meaningful benefit in clinically quiescent patients. Longitudinal studies are needed to determine the predictive value of these biomarkers for treatment response, and to define optimal cutoff values to guide clinical decision-making.

List of abbreviations

AUROC: area under the receiver operating characteristic curve

CD: Crohn's disease

CLIA: chemiluminescent immunoassay

CRP: C-reactive protein

FC: fecal calprotectin

FEIA: fluoroenzyme immunoassay

FIT: fecal immunochemical test

HBI: Harvey–Bradshaw Index

IBD: inflammatory bowel disease

IQR: interquartile range

LAA: latex agglutination assay

MES: Mayo Endoscopic Score

NPV: negative predictive value

pMayo: partial Mayo score

PPV: positive predictive value

ROC: receiver operating characteristic

SD: standard deviation

SES-CD: Simple Endoscopic Score for Crohn's Disease

Sn: sensitivity

Sp: specificity

UC: ulcerative colitis

Declarations

Ethics approval and consent to participate

The study was approved by the Siriraj Institutional Review Board (approval number Si 723/2023) and was registered at ClinicalTrials.gov (NCT06623383 on September 30, 2024). All participants provided written informed consent prior to enrollment.

Consent for publication

Not applicable

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Siriraj IBD research foundation, Siriraj Hospital, Bangkok, Thailand

Authors' contributions

Conceptualization and supervision: JL; Methodology: TC, JL; Data acquisition: TC, MS; Investigation: TC, MS, OS

Formal analysis: TC, JL; Writing original draft: TC, JL; Review and editing: TC, JL

Acknowledgements

Not applicable

References

1. Seyedian SS, Nokhostin F, Malamir MD. A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. *J Med Life* 2019;12(2):113-122. DOI: 10.25122/jml-2018-0075.
2. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007;369(9573):1627-40. DOI: 10.1016/S0140-6736(07)60750-8.

3. Chaemsupaphan T, Arzivian A, Leong RW. Comprehensive care of ulcerative colitis: new treatment strategies. *Expert Rev Gastroenterol Hepatol* 2025;19(10):1063-1079. DOI: 10.1080/17474124.2025.2457451.
4. Ng SC, Kaplan GG, Tang W, et al. Population Density and Risk of Inflammatory Bowel Disease: A Prospective Population-Based Study in 13 Countries or Regions in Asia-Pacific. *Am J Gastroenterol* 2019;114(1):107-115. DOI: 10.1038/s41395-018-0233-2.
5. Aniwan S, Limsrivilai J, Pongprasobchai S, et al. Temporal trend in the natural history of ulcerative colitis in a country with a low incidence of ulcerative colitis from 2000 through 2018. *Intest Res* 2021;19(2):186-193. DOI: 10.5217/ir.2020.00028.
6. Limsrivilai J, Aniwan S, Sudcharoen A, et al. Temporal trend of disease recognition, treatment paradigm, and clinical outcomes of Crohn disease in Thailand from 2000 through 2017: Is early use of thiopurines beneficial? *Medicine (Baltimore)* 2020;99(38):e22216. DOI: 10.1097/MD.00000000000022216.
7. Kucharzik T, Taylor S, Allocca M, et al. ECCO-ESGAR-ESP-IBUS Guideline on Diagnostics and Monitoring of Patients with Inflammatory Bowel Disease: Part 1. *J Crohns Colitis* 2025;19(7). DOI: 10.1093/ecco-jcc/jjaf106.
8. Turner D, Ricciuto A, Lewis A, et al. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD

- (IOIBD): Determining Therapeutic Goals for Treat-to-Target strategies in IBD. *Gastroenterology* 2021;160(5):1570-1583. DOI: 10.1053/j.gastro.2020.12.031.
9. Daperno M, D'Haens G, Van Assche G, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. *Gastrointest Endosc* 2004;60(4):505-12. DOI: 10.1016/s0016-5107(04)01878-4.
 10. Lobaton T, Bessissow T, De Hertogh G, et al. The Modified Mayo Endoscopic Score (MMES): A New Index for the Assessment of Extension and Severity of Endoscopic Activity in Ulcerative Colitis Patients. *J Crohns Colitis* 2015;9(10):846-52. DOI: 10.1093/ecco-jcc/jjv111.
 11. Gonczi L, Bessissow T, Lakatos PL. Disease monitoring strategies in inflammatory bowel diseases: What do we mean by "tight control"? *World J Gastroenterol* 2019;25(41):6172-6189. DOI: 10.3748/wjg.v25.i41.6172.
 12. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis* 2012;18(12):2218-24. DOI: 10.1002/ibd.22917.
 13. Zhulina Y, Cao Y, Amcoff K, Carlson M, Tysk C, Halfvarson J. The prognostic significance of faecal calprotectin in patients with inactive inflammatory bowel disease. *Aliment Pharmacol Ther* 2016;44(5):495-504. DOI: 10.1111/apt.13731.

14. Murray J, Kok KB, Ayling RM. Fecal Calprotectin in Gastrointestinal Disease. Clin Chem 2023;69(7):699-710. DOI: 10.1093/clinchem/hvad051.
15. Oyaert M, Boel A, Jacobs J, et al. Analytical performance and diagnostic accuracy of six different faecal calprotectin assays in inflammatory bowel disease. Clin Chem Lab Med 2017;55(10):1564-1573. DOI: 10.1515/cclm-2016-1012.
16. Lin L, Wyness SP, Jensen R, et al. Comparison of Next-Generation Assays for Fecal Calprotectin vs the PhiCal Assay. Am J Clin Pathol 2022;157(2):252-256. DOI: 10.1093/ajcp/aqab114.
17. Mooiweer E, Fidder HH, Siersema PD, Laheij RJ, Oldenburg B. Fecal hemoglobin and calprotectin are equally effective in identifying patients with inflammatory bowel disease with active endoscopic inflammation. Inflamm Bowel Dis 2014;20(2):307-14. DOI: 10.1097/01.MIB.0000438428.30800.a6.
18. Inokuchi T, Kato J, Hiraoka S, et al. Fecal Immunochemical Test Versus Fecal Calprotectin for Prediction of Mucosal Healing in Crohn's Disease. Inflamm Bowel Dis 2016;22(5):1078-85. DOI: 10.1097/MIB.0000000000000728.
19. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med 1987;317(26):1625-9. DOI: 10.1056/NEJM198712243172603.

20. Harvey RF, Bradshaw JM. A simple index of Crohn's-disease activity. *Lancet* 1980;1(8167):514. DOI: 10.1016/s0140-6736(80)92767-1.
21. Dai C, Jiang M, Sun MJ, Cao Q. Fecal immunochemical test for predicting mucosal healing in ulcerative colitis patients: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2018;33(5):990-997. DOI: 10.1111/jgh.14121.
22. Iwamoto F, Matsuoka K, Motobayashi M, et al. Prediction of disease activity of Crohn's disease through fecal calprotectin evaluated by balloon-assisted endoscopy. *J Gastroenterol Hepatol* 2018;33(12):1984-1989. DOI: 10.1111/jgh.14310.
23. Takashima S, Kato J, Hiraoka S, et al. Evaluation of Mucosal Healing in Ulcerative Colitis by Fecal Calprotectin Vs. Fecal Immunochemical Test. *Am J Gastroenterol* 2015;110(6):873-80. DOI: 10.1038/ajg.2015.66.
24. Sakuraba A, Nemoto N, Hibi N, et al. Extent of disease affects the usefulness of fecal biomarkers in ulcerative colitis. *BMC Gastroenterol* 2021;21(1):197. DOI: 10.1186/s12876-021-01788-4.
25. Latorre Ano P, Torrente Sanchez J, Perez Ibanez AA, et al. Fecal immunochemical test for haemoglobin versus fecal calprotectin to monitoring endoscopic activity in inflammatory bowel disease. *Rev Esp Enferm Dig* 2023. DOI: 10.17235/reed.2023.9536/2023.

26. Xu M, Li J, Qian W, et al. The value of fecal calprotectin measured by fluorescent immunochromatography assay in evaluating clinical and endoscopic activity in ulcerative colitis. *BMC Gastroenterol* 2025;25(1):566. DOI: 10.1186/s12876-025-04200-7.
27. Simon EG, Wardle R, Thi AA, Eldridge J, Samuel S, Moran GW. Does fecal calprotectin equally and accurately measure disease activity in small bowel and large bowel Crohn's disease?: a systematic review. *Intest Res* 2019;17(2):160-170. DOI: 10.5217/ir.2018.00114.
28. Suttichaimongkol T, Coelho-Prabhu N, Bruining DH, Tariq R, Snyder MR, Loftus EV, Jr. Diagnostic Performance of a Fecal Calprotectin Assay as a Biomarker for Mayo Endoscopic Subscore in Ulcerative Colitis: Result From a Tertiary Referral Center. *Inflamm Bowel Dis* 2024;30(12):2347-2355. DOI: 10.1093/ibd/izae005.
29. Xiang BJ, Jiang M, Sun MJ, Dai C. Optimal Range of Fecal Calprotectin for Predicting Mucosal Healing in Patients with Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis. *Visc Med* 2021;37(5):338-348. DOI: 10.1159/000514196.
30. Osredkar J, Ekart N, Drobne D. Calprest ELISA vs. Liaison((R)) Chemiluminescence: Evaluating Accuracy, Efficiency, and Clinical Utility in Fecal Calprotectin Testing. *Biomedicines* 2026;14(1). DOI: 10.3390/biomedicines14010143.

31. Khan M, Shah SH, Salman M, Abdullah M, Hayat F, Akbar S. Enzyme-Linked Immunosorbent Assay versus Chemiluminescent Immunoassay: A General Overview. *Global Journal of Medical, Pharmaceutical, and Biomedical Update*;18:1. DOI: 10.25259/GJMPBU_77_2022.
32. Pelkmans LPJ, de Groot MJM, Curvers J. Analytical Performance and Clinicopathologic Correlation of Four Fecal Calprotectin Methods. *Am J Clin Pathol* 2019;152(3):392-398. DOI: 10.1093/ajcp/aqz051.
33. Nakarai A, Kato J, Hiraoka S, et al. Ulcerative colitis patients in clinical remission demonstrate correlations between fecal immunochemical test results, mucosal healing, and risk of relapse. *World J Gastroenterol* 2016;22(21):5079-87. DOI: 10.3748/wjg.v22.i21.5079.
34. Mak LY, Tong TSM, Cheung KS, et al. Combined Use of Common Fecal and Blood Markers for Detection of Endoscopically Active Inflammatory Bowel Disease. *Clin Transl Gastroenterol* 2020;11(3):e00138. DOI: 10.14309/ctg.00000000000000138.
35. Bohra A, Batt N, Dutt K, et al. The synergy of dual faecal immunochemical and faecal calprotectin testing for accurate assessment of endoscopic and histological activity in inflammatory bowel disease. *Ther Adv Gastroenterol* 2024;17:17562848241237895. DOI: 10.1177/17562848241237895.
36. Chen P, Zhou G, Lin J, et al. Serum Biomarkers for Inflammatory Bowel Disease. *Front Med (Lausanne)* 2020;7:123. DOI: 10.3389/fmed.2020.00123.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial13082026.docx](#)