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ORIGINAL ARTICLE

Optimal cut-off value of fecal calprotectin for the evaluation of ulcerative colitis: An unsolved issue?

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fecal calprotectin, inflammatory bowel disease, irritable bowel disease, ulcerative colitis.

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Abstract

Introduction: There is variability in the fecal calprotectin (FCP) cut-off level for the prediction of ulcerative colitis (UC) disease activity and differentiation from irritable bowel disease (IBS-D). The FCP cut-off levels vary from country to country.
Aims: We aimed to assess FCP as a marker of disease activity in patients with UC. We determined the optimal FCP cut-off value for differentiating UC and IBS-D.
Methods: In a prospective study, we enrolled 76 UC and 30 IBS-D patients. We studied the correlation of FCP with disease activity/extent as well as its role in differentiating UC from IBS-D. We also reviewed literature regarding the optimal FCP cut-off level for the prediction of disease activity and differentiation from IBS-D patients.
Results: Sensitivity, specificity, positive predictive value, and negative predictive value of FCP (cut-off level, 158 μg/g) for the prediction of complete mucosal healing (using Mayo endoscopic subscore) were 90, 85, 94.7, and 73.3%, respectively. Sensitivity, specificity, positive predictive value, and negative predictive value of FCP (cut-off level, 425 μg/g) for the prediction of inactive disease (Mayo Score ≤ 2) were 94.3, 88.7, 86.2, and 95.4%, respectively. We also found a FCP cut-off value of 188 μg/g for the differentiation of UC from IBS-D.
Conclusions: The study reveals the large quantitative differences in FCP cut-off levels in different study populations. This study demonstrates a wide variation in FCP cut-off levels in the initial diagnosis of UC as well as in follow-up post-treatment. Therefore, this test requires validation of the available test kits and finding of appropriate cut-off levels for different study populations.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon, characterized by alternating periods of activity and remission. The measurement of disease activity is very important for the management of UC patients. Disease activity is measured by clinical, laboratory, and endoscopic features grouped together as disease activity indices.[1]

Colonoscopy and biopsy is the single best test for the diagnosis of UC, but it is invasive and carries a risk of fatal complications. To avoid unnecessary colonoscopy, various biomarkers have been described to assess disease activity in UC. Unfortunately, each biomarker has notable limitations. Widely used laboratory parameters of inflammation, erythrocyte sedimentation rate, and C-reactive protein are not sufficiently specific or sensitive.[2–4] Other commonly used laboratory markers, including leukocyte count, platelets, and albumin, are inefficient in the prediction of disease activity.

As compared to blood or serum biomarkers, stool markers have the advantage of increased specificity for inflammatory processes localized to the bowel. Many neutrophil-derived proteins, such as lactoferrin, lysozyme, elastase, myeloperoxidase, and calprotectin, have been studied in stool samples for the diagnosis and prediction of disease severity in patients of UC. In recent years, fecal calprotectin (FCP) and lactoferrin are the most extensively studied biomarkers. Calprotectin is a 36 kDa calcium- and zinc-binding protein that represents 60% of cytosolic proteins in granulocyte.[5] Calprotectin is found in both plasma and stool and may be significantly raised in inflammatory bowel disease (IBD), necrotizing enterocolitis, and sepsis. FCP appears to be distributed homogeneously in feces. It is resistant to bacterial proteases in feces; hence, it is stable at room temperature for several days, allowing for transport of the sample to the laboratory.

Several studies have compared FCP with disease activity indexes and/or endoscopic/histological evaluation to verify intestinal inflammation in IBD patients.[6] These studies have

demonstrated the usefulness of FCP in detecting inflammation, predicting recurrence, and differentiating IBD from other diseases. Studies have showed a significant correlation between FCP levels and endoscopic disease activity in IBD.

Although manufacturers of the test kit recommend a "cut-off" FCP level for positive test results, various studies showed different cut-off levels for the optimal interpretation of test results. There is a lack of agreement on the best cut-off levels of FCP for differentiating IBD from IBS, and for predicting endoscopic activity, remission, and relapse. The FCP cut-off levels vary from country to country.

Methods

This prospective study was a single-center study conducted at a tertiary care teaching hospital in Eastern India. All patients provided informed consent before enrolment. The study was approved by the institute's ethical review committee. All consecutive patients with UC attending the Department of Gastroenterology were included in the study from January 2015 to October 2016. This includes newly diagnosed patients of UC as well as old patients of UC who were under our regular follow up. Patients were excluded from the study if they met the following criteria: use of NSAIDs prior to the endoscopy, infectious enterocolitis, concomitant colorectal cancer in UC, Crohn's disease, indeterminate colitis, and history of colorectal surgery.

A detailed history was taken regarding the clinical features, risk factors, and complications of UC. All patients underwent routine blood tests, including complete blood count, serum albumin, serum electrolytes, and acute-phase reactants like C-reactive protein and erythrocyte sedimentation rate. Stool routine microscopy and stool culture were performed in all patients. Stool samples were delivered to the laboratory from 1 to 3 days before (in previously diagnosed cases of UC) or after (in newly diagnosed cases of UC) sigmoidoscopy/colonoscopy for FCP measurement. FCP was measured using the commercially available kit Phadia 100 Calprotectin (Thermo Fisher Scientific, Uppsala, Sweden) reagents through the enzyme-linked immunoassay (ELiA) method (range 15 to ≥3000 mg/kg). The manufacturer recommends a cut-off level of >50 mg/kg for positive results.

Patients with active disease at admission were evaluated during active disease and in remission. A full colonoscopy is associated with the risk of perforation in acute severe colitis. Therefore, proctosigmoidoscopy was performed, and the colon was assessed up to the splenic flexure and distal transverse colon. The severity and the extent of UC during active disease were assessed as per Montreal classification. However, patients in remission were assessed by full colonoscopy. Disease activity was assessed as per the Mayo score and classified as remission (Mayo Score ≤ 2) or active disease (Mayo Score ≥ 3).[7] The severity of disease was categorized into mild,[3–5] moderate,[6–10] and severe.[11,12] Mayo endoscopic subscores (MES) of 0 and 1 were given for mucosal healing (normal mucosa or inactive UC) and mild inflammation (mild disease), respectively.

Patients presenting with pain abdomen and altered bowel habit were thoroughly evaluated and were diagnosed as having irritable bowel syndrome-diarrhea predominant (IBS-D) using Rome III criteria. Stool samples were analyzed for FCP measurement, and 30 patients with IBS-D were enrolled as controls.

Outcome measures. The primary objective was to assess FCP as a marker of disease activity in patients with UC as compared with the Mayo score. The secondary objective was to determine the optimal FCP cut-off value to differentiate UC and IBS.

Statistical analysis. All analyses were performed using SPSS V17. Results of parametric numerical data were presented as mean ± standard deviation (SD). Median and interquartile range (IQR) were used for nonparametric data. The Kruskal–Wallis test and Wilcoxon matched pairs test were used to assess differences in the laboratory parameters between the groups, and Spearman's correlation was used to analyze the correlation between the parameters. A Shapiro–Wilk test was used to evaluate whether FCP values followed a normal (Gaussian) distribution or not. A P value <0.05 was considered significant.

Results

Of the total, 81 patients with UC were enrolled in the study. Patients[5] were excluded from the study if they met the following criteria: acute gastroenteritis,[2] history of NSAIDS intake,[2] and prior hemicolectomy.[1] We included 76 UC patients and 30 age-matched IBS-D patients in study. Of 76 patients, 71 patients had active disease, and 5 patients were in clinical remission.

The median age in UC patients and control groups were 35 years (14–60) and 30 (21–60), respectively (P = 0.06). The male:female ratio of UC patients and IBS-D patients (controls) were 2:1 and 4:1, respectively. The median age of UC patients at initial presentation was 33.7 years (9–61); 32 (42%) patients were newly diagnosed, and 44 (58%) patients were previously diagnosed cases of UC.

Mean hemoglobin, mean total leukocyte count, mean platelet count, mean albumin, and median erythrocyte sedimentation rate (mm/h) were 11 ± 2.56 gm/dL, 9522 ± 3778/mm^3, 318 535 ± 113 853/mm^3, 3.67 gm/dL, and 40 (8–117), respectively. Median (range) FCP (μg/g) was 3000 (186–3000).

Of 71 patients, mild, moderate, and severe disease were noted in 11 (15.5%), 46 (64.8%), and 14 (19.7%), respectively. Five (6.6%) patients had inactive disease (remission). Proctitis, left-sided colitis, and pancolitis were seen in 10 (14%), 39 (55%), and 22 (31%) patients, respectively.

At the time of enrolment, the percentages of treatment-naïve and treated patients were 42 and 58%, respectively. All patients (76) were treated with mesalazine. Thirty-four (45.33%) patients required steroids treatment. Seven (9.3%) patients were steroid dependent and responded to azathioprine therapy. Of 71 patients, 48 (67.6%) went into remission during the study period. One patient was diagnosed with pyoderma gangrenosum. One patient died due to cerebrovascular stroke.

The characteristics of UC patients are summarized in Table 1.

FCP and disease severity/extent. In 71 patients with endoscopic evidence of inflammation, FCP (>188 μg/g), erythrocyte sedimentation rate (>20 mm/h), and positive C-reactive protein (>6 mg/L) were seen in 98.6, 71.8, and 42.3%, respectively.

FCP levels increased with increasing severity of endoscopic inflammation, as evaluated by the Mayo score. Median

Table 1 Characteristics of ulcerative colitis patients

Patient characteristics	Ulcerative colitis (N = 76)
Age (years) median (range)	35 (14–60)
Gender (male: female)	51:25
Median (range) age (years) at initial presentation	33.7 (9–61)
Hemoglobin (gm/dL) (mean ± SD)	11.0 ± 2.56
Serum albumin (mg/dL) (mean ± SD)	3.67 ± 0.94
Disease extent	
Inactive	5 (6.6%)
E1 (proctitis)	10 (14.1%)
E2 (left sided colitis)	39 (54.9%)
E3 (pancolitis)	22 (31.0%)
Disease severity as per Mayo score	
Mild[3–5]	11 (15.5%)
Moderate[6–10]	46 (64.8%)
Severe[11,12]	14 (19.7%)
Treatment history	
Naïve	32 (42.1%)
Experienced	44 (57.9%)
Therapy	
Mesalazine	76 (100%)
Mesalazine + steroid	34 (45.33%)
Mesalazine + steroid + azathiprine	7 (9.3%)

(IQR) values of FCP during active disease and during remission were 3000 μg/g (1342–3000) and 88 μg/g (58–167), respectively ($P < 0.0001$). The correlation coefficients between FCP and Mayo score during active disease and remission were $r = 0.527$ ($P < 0.0001$) and $r = 0.663$ ($P < 0.0001$), respectively. There was a significant difference in FCP levels between mild and moderate (450 *vs* 3000 μg/g; $P < 0.0001$) and mild and severe disease (450 *vs* 3000 μg/g; $P < 0.0001$). However, we did not find a significant difference between moderate and severe disease (3000 *vs* 3000 μg/g; $P = 0.80$) (Fig. 1). During remission, patients with MES 0 ($n = 40$) and MES 1 ($n = 13$) had a median FCP (IQR) of 77.5 μg/g (39–109) and 190 μg/g (163–557) ($P < 0.0001$), respectively. The correlation coefficients between FCP and MES during active disease and remission were $r = 0.599$ ($P < 0.0001$) and $r = 0.662$ ($P < 0.0001$), respectively.

FCP increased with increasing disease extent ($r = 0.503$; $P < 0.0001$). Patients with proctitis, left-sided colitis, and pancolitis had median FCPs (IQR) of 712 μg/g (372–1509), 3000 μg/g (1685–3000), and 3000 μg/g (2917–3000), respectively. There was a significant difference ($P < 0.0001$) in the FCP levels between proctitis (712 μg/g) and left-sided colitis (3000 μg/g) or pancolitis (3000 μg/g). However, no difference was noted between FCP levels of patients with left-sided colitis and pancolitis (3000 *vs* 3000 μg/g; $P = 0.056$) (Fig. 1).

Differentiation from IBS-D. FCP values were higher in the UC patients as compared to the controls (IBS-D). Median (IQR) values of FCP in active UC patients, inactive UC patients, and controls were 3000 μg/g (1342–3000), 88 μg/g (58–167), and 21.5 μg/g (15–65.7), respectively ($P < 0.0001$).

Receiver operating characteristic (ROC) curve analysis. ROC curve analysis revealed a FCP cut-off level of 425 μg/g for diagnosing active disease (Mayo score ≥ 3) from inactive disease (score ≤ 2) [sensitivity 94.3%, specificity 88.7%; positive predictive value 86.2%, negative predictive value 95.4%; AUC 0.985 (CI: 0.967–1.00)]. The study also revealed a FCP cut-off level of 158 μg/g [sensitivity 90%, specificity 85%; positive predictive value 94.7%, negative predictive value 73.3%; AUC 0.944 (CI: 0.883–1.00)] for predicting complete mucosal healing by MES 0 from MES 1. A FCP cut-off level of 188 μg/g was found to be a predictor for differentiating IBS-D from UC [sensitivity 98.5%, specificity 96.6%; positive predictive value 98.5%, negative predictive value 96.6%; AUC 0.999 (CI: 0.00–1.00)] (Table 2, Fig. 2).

Comparison of biomarkers. FCP did not correlate with total leukocyte count ($r = 0.166$; $P = 0.165$), platelet count ($r = 0.053$; $P = 0.663$), erythrocyte sedimentation rate ($r = 0.178$; $P = 0.137$), and C-reactive protein ($r = 0.10$; $P = 0.405$). MES significantly correlated with C-reactive protein ($r = 0.391$, $P < 0.001$) and erythrocyte sedimentation rate ($r = 0.330$; $P < 0.005$) (Table 3). MES also correlated with total leukocyte count ($r = 0.282$, $P = 0.017$) but did not correlate with platelet count ($r = 0.214$; $P = 0.074$).

Discussion

FCP has recently emerged as a simple, noninvasive test for the assessment of disease activity in patients of UC. Studies regarding the utility of FCP in the management of UC are limited in this part of the world. Studies from different parts of the world showed wide variability in the FCP cut-off level for prediction of disease activity and differentiation from IBS-D patients. In this study, we have prospectively analyzed the data regarding the usefulness of FCP in the management of UC patients. In this study, the concentration of FCP was significantly related to the disease activity that was evaluated by the Mayo Score and MES. We determined the optimal FCP cut-off value for differentiating UC and IBS in our study cohort. We also reviewed literature regarding the optimal FCP cut-off levels for the prediction of disease activity and differentiation from IBS-D patients.

The mean age of our patient cohort was 35 years (range 14–60), with a male to female ratio of 2:1. These findings are nearly similar to those of other studies from India where patients with UC usually presented in their fourth decade of life with a slight male preponderance.[8,9] There is an overall female preponderance for IBD in the Western literature. Asian data showed an equal or slight male preponderance for UC.[9] The major clinical parameters of UC patients in our study were nearly consistent with that of other studies from this region.

In this cohort, FCP, C-reactive protein, and erythrocyte sedimentation rate correlated with endoscopic inflammation. However, FCP was elevated more frequently than C-reactive protein and erythrocyte sedimentation rate in patients with endoscopic active disease. Our findings support previous observations that the FCP is better than other biomarkers for the prediction of endoscopic inflammation.[10–13]

In our study cohort, two-third of patients went into remission during the study period. There was a significant difference

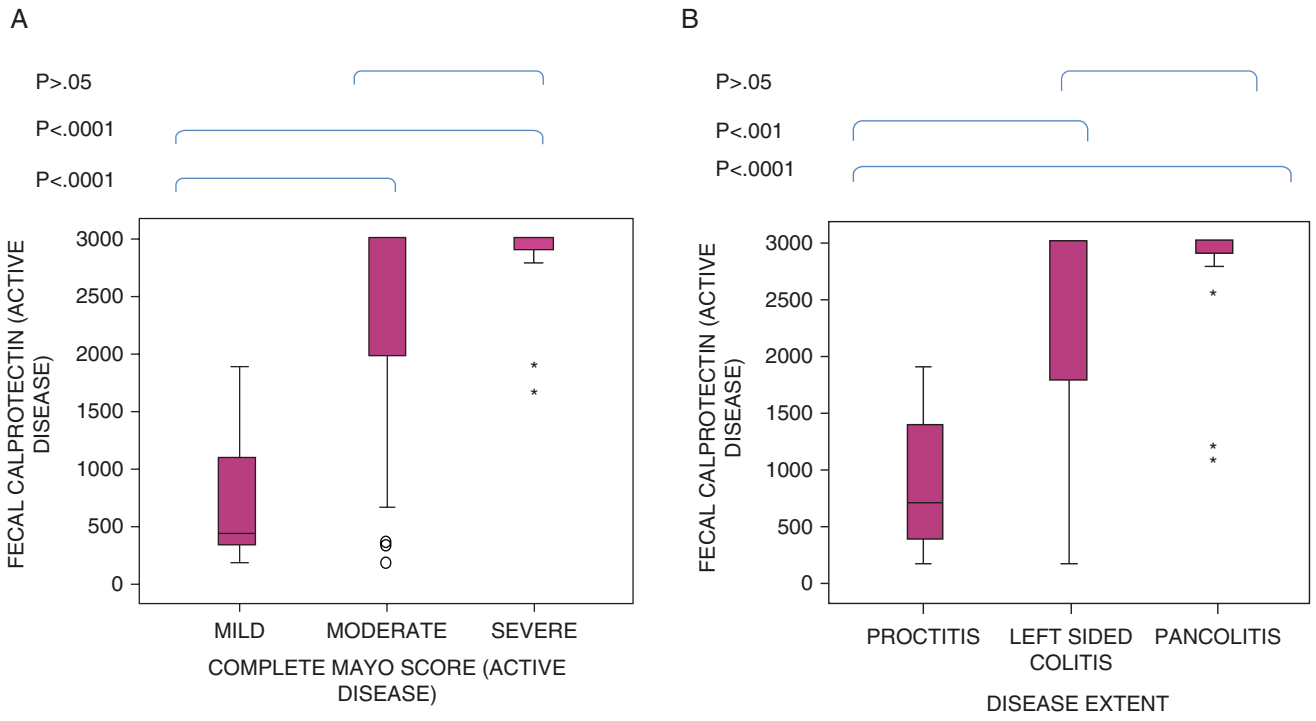

Figure 1 Boxplot to illustrate the concentration of fecal calprotectin (FCP) in relation to (a) disease severity and (b) disease extent.

in the FCP concentration between patients with active UC and inactive UC (3000 vs. 88 μg/g; $P < 0.0001$). Yamamoto *et al.* showed significant decline of median FCP levels in patients of UC with clinical and endoscopic remission.[14] In our case cohort, the FCP level was significantly higher in the patients with inactive UC than in the controls (IBS-D) (88 *vs* 21.5; $P < 0.0001$). A meta-analysis by Von Roon *et al.* shows that the FCP can discriminate between patients with IBD and controls. FCP was higher (by 219 μg/g) in IBD patients than in controls.[15]

We found a significant difference in the FCP level between mild versus moderate and severe disease but not between moderate and severe disease. During remission, a significant difference was observed in FCP level between patients with an MES score of 0 and 1 [median (IQR) 77.5 μg/g (39–109) and 190 μg/g (163–557), respectively ($P < 0.0001$)]. The association between FCP and endoscopic disease activity has been evaluated in several studies. In a study by Schoepfer *et al.*, FCP had a strong correlation with endoscopic disease evaluated with both Rachmilewitz and modified Baron Score; FCP was the only marker that could significantly discriminate between different endoscopic grades (as per Mayo score).[16] Ricaneket *et al.* showed a significant correlation between FCP levels and endoscopic grades (Mayo score) and MES for discriminating between mild versus moderate and severe disease ($P < 0.05$). However, FCP was unable to discriminate between moderate and severe inflammation ($P > 0.05$).[17] In a study by Theede *et al.* using the MES, patients of UC with a score of 0 and 1 had a significant difference in FCP level (mean [IQR], 250 μg/g [30–203] *vs* 1103 μg/g [207–1645]; $P < 0.0001$).[18]

FCP levels increased with increasing disease extent ($r = 0.503$, $P < 0.0001$). Studies have found variable results regarding the correlation between FCP and extent of disease. There are some studies that showed a significant correlation between FCP levels and extent of disease in UC patients.[17] However, other studies showed no relation between FCP concentration and extent of disease.[18]

In our study, FCP with a cut-off level of 188 μg/g (AUC 0.99 [CI: 0.00–1.00]) differentiated patients of UC from IBS-D

Table 2 Receiver operator characteristic (ROC) curves and predictive value of fecal calprotectin (FCP)

	Active disease (Mayo score ≥ 3)	Mucosal healing (MES = 0)	UC *vs* IBS-D
Cut-off value (μg/g)	>425	<158	>188
Sensitivity (95% CI)	0.94 (0.84–0.98)	0.90 (0.76–0.97)	0.98 (0.92–0.99)
Specificity (95% CI)	0.88 (0.78–0.95)	0.85 (0.54–0.98)	0.96 (0.82–0.99)
AUC (95% CI)	0.985 (0.967–1.00)	0.944 (0.883–1.00)	0.999 (0.00–1.00)
Positive predictive value (95% CI)	0.86 (0.74–0.93)	0.94 (0.82–0.99)	0.98 (0.92–0.99)
Negative predictive value (95% CI)	0.95 (0.87–0.99)	0.73 (0.44–0.92)	0.96 (0.82–0.99)

IBS-D, irritable bowel syndrome-diarrhea; MES, Mayo endoscopic sub score; UC, ulcerative colitis.

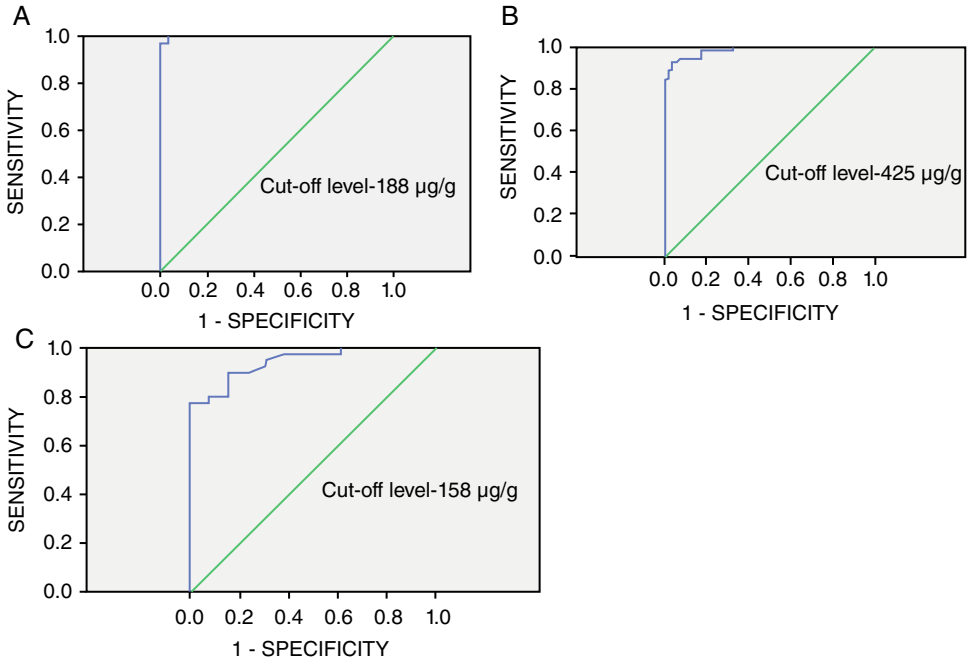

Figure 2 Receiver operator characteristic (ROC) curves to illustrate the ability of fecal calprotectin (FCP) to differentiate (a) inflammatory bowel disease (IBD) from irritable bowel syndrome-diarrhea (IBS-D) [AUC 0.999 (CI 0.00–1.00)], (b) active (Mayo score ≥ 3) from inactive diseases (Mayo score ≤ 2) [AUC 0.985 (0.967–1.00)], and (c) complete mucosal healing (MES 0) from partial healing (MES 1) [AUC 0.944 (0.883–1.00)].

with a sensitivity of 98.5% and specificity of 96.6%. Sensitivity and specificity of FCP in differentiating patients of UC from IBS or nonorganic disease ranged from 70 to 100% and 75 to 100%, respectively (Table 4). FCP cut-off values in these studies were markedly variable (ranged from 40 to 217 µg/g). Menees *et al*. performed a meta-analysis to evaluate the utility of biomarkers to distinguish between patients with IBD and IBS and healthy controls. Patients with C-reactive protein ≤ 0.5 mg/dL or FCP ≤ 40 µg/g were found to have ≤1% probability of having IBD.[19]

In this study, ROC analysis revealed a FCP cut-off level of 158 µg/g (AUC 0.944; CI: 0.883–1.000) for predicting complete mucosal healing (MES 0) with 90% sensitivity and 85% specificity. ROC analysis also revealed a FCP cut-off level of 425 µg/g (AUC, 0.985; 95% CI, 0.967–1.00) for predicting inactive UC (Mayo Score ≤ 2) with sensitivity and specificity of 94.3 and 88.7%, respectively. Sensitivity and specificity of FCP for predicting inactive UC or complete mucosal healing range from 70 to 96% and 70 to 100%, respectively. FCP cut-off values in these studies were markedly variable (ranges from 50 to 800 µg/g). Theede *et al*. revealed a FCP cut-off level of 192 µg/g (AUC 0.888; CI: 0.825–0.950) for predicting mucosal healing (MES 0).[18] Lobation *et al*. showed a FCP cut-off level of 250 µg/g to predict the remission (MES ≤ 1) (AUC 0.924) in UC patients (N = 123).[25] In a study by D'Haens *et al*., sensitivity of 71.0% and a specificity of 100.0% (positive predictive value 100.0%, negative predictive value 47.1%) were noted with a FCP cut-off level of 250 µg/g for active mucosal disease (Mayo score > 0).[24] In another study, the FCP cut-off level to identify active UC (Mayo score ≥ 2) was 800 µg/g (sensitivity 96%, specificity 71%; AUC 0.80 [CI: 0.58–1.00]).[12]

Optimal cut-off levels of FCP. There is a paucity of data regarding the correlation of FCP with disease activity in patients with IBD from underdeveloped Asian countries. Manufacturers of test kits recommend a "cut-off" FCP level of 50 mg/g, above which all results are positive. However, various studies showed different cut-off levels for the optimal interpretation of test results. Different threshold concentrations ranging from 50 to 800 mg/g have been proposed in the literature (Table 4). There is still a lack of agreement on the best cut-off levels of FCP for differentiating IBD from IBS, and for predicting endoscopic activity, remission, and relapse.

Variability in the cut-off level is due to the use of different test kits and different study populations.[32] FCP levels may also be elevated because of a few unspecific reasons. Chronic subclinical intestinal inflammation may explain the higher cut-off FCP levels in

Table 3 Biomarkers in active ulcerative colitis (UC) patients and correlation with endoscopic activity (Mayo endoscopic sub score [MES])

Characteristics	Active UC (N = 71)	Correlation coefficients	P value
Erythrocyte sedimentation rate (ESR) median (range)	40 (8–117) (mm/h)	0.330	<0.005
Positive C-reactive protein (CRP) (>6 mg/L)	30 (42.3%)	0.391	<0.001
Fecal calprotectin (FCP) median (range)	3000 (186–3000) (µg/gm)	0.599	<0.0001

Table 4 Fecal calprotectin (FCP) cut-off levels in ulcerative colitis patients

Reference	Country	*N*	Subject/controls	Cut-off (μg/g)	Sn/Sp	Inference
Garcia Sanchez *et al.*[20]	Spain	25	IBD/Healthy	217	85/–	Diagnosis of organic disease
von Roon *et al.*[15]	United Kingdom	5983	IBD/Healthy	100	95/91	Diagnosis
Dhaliwal *et al.*[21]	United Kingdom	88	IBD/IBS	100	97/76	Distinguish IBD from IBS
D'Incà *et al.*[22]	Italy	46	UC	80	78/70	Active disease
Xiang *et al.*[23]	China	66	UC/Healthy	50	79/92	Active disease
D'Haens*et al.*[24]	The Netherlands	126	UC/IBS	250	71/100	Active disease
Schoepfer *et al.*[16]	Switzerland	228	UC/Healthy	57	91/90	Active disease
Samant *et al.*[12]	India	32	UC	800	96/71	Active disease
Lobatón *et al.*[25]	Spain	123	UC	250	74/90	Remission
Lin *et al.*[26]	Taiwan	52	UC	191	88/75	Remission
Dhaliwal *et al.*[21]	United Kingdom	88	IBD/IBS	250	90/76	Remission
Costa *et al.*[27]	Italy	41	UC	150	89/82	Relapse
Tibble *et al.*[28]	England	80	IBD	50	90/83	Relapse
D'Inca et al.[29]	Italy	97	UC	130	70/70	Relapse
Gisbert *et al.*[30]	Spain	74	UC	164	75/69	Relapse
Garcia-Sanchez *et al.*[31]	Spain	69	UC	120	81/63	Relapse
Yamamoto *et al.*[14]	Japan	160	UC	55	88/80	Relapse

Sn, sensitivity; Sp, specificity.

underdeveloped populations. Studies from China showed higher FCP levels in infants in rural areas than urban areas. Due to relatively poor socioeconomic status and unhygienic/unsanitary environment, rural or suburban populations are more prone to frequent gastrointestinal infections, resulting in impaired intestinal mucosal function and chronic subclinical gastrointestinal inflammation.[33,34] Raised FCP levels have been found to be associated with chronic giardia infection and microscopic duodenal inflammation.[35] Studies have demonstrated high levels of FCP in patients with intestinal tuberculosis.[36]

There is scarcity of data regarding the usefulness of FCP in patients with UC from South Asian countries. To the best of our knowledge, our study is the second study conducted in this part of the world. The current results are consistent with the previous study, which showed higher FCP cut-off values. However, the current study had a larger sample size ($N = 72$) than that of the previous study ($N = 32$).[12]

This study has some limitations. Nonsignificant differences between moderate and severe disease could be because of the maximum limit of assay, 3000 μg/g, and a similar maximum value of FCP in both moderate and severe disease or because of a small number of patients in each group. Our study was also limited by a relatively small sample size and noninclusion of healthy controls. Studies have showed comparable FCP levels between IBS patients and healthy control subjects. In one study, FCP levels in IBS patients and healthy control subjects were 44.50 and 35 μg/g, respectively. No statistical difference was identified between the IBS patients and the healthy control subjects.[37] Bonnín Tomàs *et al*. found similar FCP levels in healthy children (20 μg/g [16–25 μg/g]) and children with functional gastrointestinal disease (25 μg/g [19.2–32.5 μg/g]) ($P = 0.264$).[38] In a study by von Roon *et al*., a quantitative meta-analysis was performed on 30 prospective studies. There was no significant difference in FCP levels when comparing patients with IBS with healthy controls (weighted mean difference −4.01, 95% CI −21.63 to 13.61, $P = 0.66$).[15]

Conclusions

This study reveals the large quantitative differences in FCP cut-off levels in different study populations and demonstrates a wide variation in FCP cut-off levels in the initial diagnosis of UC as well as in follow up post-treatment. Therefore, this test requires validation of the available test kits and the finding of appropriate cut-off levels for different study populations.

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