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The Role of Fecal Calprotectin in The Diagnosis and Severity of Ulcerative Colitis

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Abstract

Background: The neutrophil-derived protein fecal calprotectin has emerged over the last decade as a novel non-invasive biomarker for the diagnosis and follow-up of intestinal inflammation. **Objectives** The objective of this study was to assess the diagnostic power and role of severity of fecal calprotectin in patients with ulcerative colitis.

Methods: A case-control study was performed in Al-Najaf City in Iraq at Al-Sadr Medical City, from August 2025 to March 2026. A total of 150 subjects were recruited, comprising 63 patients with clinicopathologically proved ulcerative colitis (UC) and 87 apparently healthy controls. The severity of disease was characterized according to the Mayo scoring system into mild, moderate and severe. All subjects were asked to provide fresh stool samples, and fecal calprotectin concentrations were assessed via a commercially available ELISA. **Results**: Fecal calprotectin concentrations in patients with ulcerative colitis were significantly greater than those of healthy controls (412.68 ± 136.45 µg/g vs.38.74 ± 14.62 µg/g; p <0.002). Similarly, fecal calprotectin concentration showed stepwise elevation according to disease severity: 218.54 ± 64.27µg/g, 431.86 ± 95.48µg/g and 732.45 ± 128.91µg/g in patients with mild, moderate and severe disease respectively (F =58·73, p <0·001). In the diagnosis of ulcerative colitis, fecal calprotectin showed a sensitivity of 90.5% and specificity of 87.4% at a cut-off value of 85.5 µg/g. **Conclusions**: Fecal calprotectin stands as a non-invasive biomarker with high sensibility and specificity in the diagnosis of ulcerative colitis, also correlating highly with severity of illness. The sequential accumulation of high fecal calprotectin concentrations with severe disease activity is a further indication of its useful e ness as an indicator of inflammatory burden, which is directly related to treatment choices.

Keywords: Fecal Calprotectin; Inflammatory Bowel Disease; Ulcerative Colitis

Introduction

Ulcerative colitis (UC) is a form of chronic immune-mediated intestinal inflammation with unknown etiology characterized by recurrent inflammatory response limited to the colonic mucosal membrane, starting in the rectal area and extend to the nearby regions in a continuous distribution. It is characterized clinically by rectal bleeding, diarrhea, and abdominal pain urgency and weight loss that greatly affect patients' quality of life and apply a considerable strain on healthcare systems globally (Ungaro et al., 2017). While there is an increasing global incidence and prevalence of UC, especially in economically transitioning countries (Ng et al., 2018), the need for diagnostic and prognostic tools that help with early intervention and appropriate management of disease has never been more pressing.

Traditionally, before the advent of serologic testing, diagnosis and assessment of UC relied on a combination of endoscopic examination, clinical evaluation, histopathological exam and laboratory investigations. Colonoscopy with biopsy is the current gold standard for diagnosis confirmation and disease progression assessment; however, due to being an invasive, costly procedure that cannot be repeated frequently it ought to be replaced as a monitoring tool. Additionally, symptoms by themselves frequently do not correspond with the activity of mucosal inflammation and this has resulted in a heightened interest in non-invasive markers that can proxy disease activity or guide therapeutic decision making (Ma et al., 2021).

An example of a fecal biomarker is fecal calprotectin (FC) which has become one of the important and widely studied markers for assessing intestinal inflammation. Calprotectin is a protein containing calcium and zinc of the S100 family, mainly expressed in neutrophilic cells, then to a lesser extent in macrophages and monocytes. In the presence of active gut inflammation, activated neutrophilic cells migrate into the gastrointestinal lumen and secrete calprotectin which leads to high stool concentrations of this protein. FC is the only inflammatory marker, and due to its excellent stability in feces for days at room temperature, it serves as practical and reproducible markers for regular clinical use (Roseth et al., 1997).

Many studies have either shown or suggested that FC is useful for the differentiation of differentiate inflammatory bowel disease from physiological gastrointestinal disorders such as irritable bowel disease. determination of fecal calprotectin has good specificity and sensitivity for differentiating between IBD and other non-inflammatory conditions, resulting in more targeted gastrointestinal imaging with fewer patients directed to numerous endoscopic procedures. Similarly, Turvill et al. When used for screening and diagnosis in patients with lower gastrointestinal symptoms, FC has been shown to have very high accuracy, as evidenced by the work of Etzioni et al (2016).

In addition to its diagnostic usefulness, fecal calprotectin has attracted particular interest as a predictive biomarker of mucosal healing and disease activity in UC. FC levels have a powerful correlation between endoscopy and histology inflammation and are also used as predictive biomarkers for therapy response and disease activity. Park et al. (9) showed that endoscopic severity is closely associated with FC concentrations and low FC levels may predict relapse in clients with ulcerative colitis (UC), even for those well under clinical remission. Likewise, D'Haens et al. (2012) reported FC levels closely correlate with mucosal lesions and add value to information on disease activity, hence support its use as a less invasive alternative providing valuable information without the tedious follow up by repeat colonoscopic evaluations.

Studies in ulcerative colitis continue to highlight the prognostic value of FC. Mao et al. (2021) performed a meta-analysis and systematic review study and demonstrated that FC had a relatively high sensitivity of 0.88 (95% CI: 0.85-0.91) when detecting endoscopic activity in UC with the specificity equal to 0.81 (95% CI: 0.76-0-86). Furthermore, Khan et al. (2022) reported that increased FC concentration is associated with the risk of clinical relapse in subsequent 6–12 months, proving its role in long-term monitoring of the disease. Fecal calprotectin was not only a biomarker of the current inflammatory status but also an indicator of disease outcomes.

The investigation of the relationship between FC levels and disease severity has also been done so extensively. Fecal calprotectin levels are basically greatly increased in patients with moderate-to-severe UC, whereas lower concentrations are reported in patients with mild disease activity (Mosli et al., 2015). In the study conducted by Lee et al. (2015), FC values greater than 500–600 μg/g were associated with severe endoscopic inflammation and impaired outcomes. In addition, the specialized international institutions had approved new standards perceiving FC as a vital marker for treatment reaction observing and mucosal recuperating evaluation in patients with IBD (Turner et al., 2021).

Fecal calprotectin levels might be affected by a variety of factors, including gastrointestinal infections, colorectal neoplasia as well as the usage of non-steroidal anti-inflammatory drugs (NSAIDS), resulting in false positives (Fagerberg et al., 2017). The heterogeneity of assay methods and contenders across studies also highlights the need to standardize for improved clinical interpretation. Nonetheless, FC is still one of the most reproducible non-invasive biomarkers available today for

intestinal inflammatory evaluation. The present study was performed to evaluate the significance of fecal calprotectin in diagnosing (UC) and assessing disease severity with respect to its application as a non-invasive biomarker for follow up of this disorder as well.

Patients and Methods

Study Design and Setting

This is a case-control study performed at Al-Sadr Medical City, Al-Najaf, Iraq during the implementation of 2025 to march 2026. To assess the diagnostic values of fecal calprotectin (FC) and its relationship with disease severity in UC. Such a study was recommended by the Institutional Ethical Committee of Al-Sadr Medical City and was also later permitted by the relating health authorities. All procedures were based on the ethical criteria of the Declaration of Helsinki. All patients formally agreed to participate through written informed consent before enrollment.

Study Population

This study enrolled a total of 150 participants, comprising 63 patients with ulcerative colitis and 87 apparently healthy controls. The participants had an age distribution from 18 to 70 years of age. Ulcerative colitis was diagnosed by gastroenterologists using clinical presentations, laboratory tests, colonosopic examinations and histopathological results of colonic biopsy specimens in accordance with the established criteria. Disease activity and severity were rated according to the Mayo scoring system and categorized as remission, mild, moderate, or severe. Patients with indeterminate colitis, Crohn's disease, infectious colitis without pouchitis or CVP, ulcerative colitis; any prior open abdominal surgery or bowel surgery including colectomy; non-specific in the last 30 days attributable to ulcerative colitis (eg dysentery or gastrointestinal bleeding of any etiology); severe hepatic dysfunction (Child-Pugh class C), borderline liver function tests where active inflammation or thrombocytopenia exists; renal failure requiring dialysis and/or inflammatory bowel diseases correlated with autoimmune illnesses such as ankylosing spondylitis, rheumatoid arthritis, and sarcoidosis Pregnancy within the previous month of study enrolment Recent use of anti-inflammatory drugs (information obtained by an interview questionnaire having good inter-rater reliability) was excluded from this trial after screening the results and discarded. We also excluded subjects who previously received antibiotics, corticosteroids or biological therapy within 2 weeks before sample collection in order to reduce the confounding impact on fecal calprotectin.

Clinical Assessment

Data on demographic and clinical information were obtained using interviews and medical record review. Recorded variables included age, sex, place of residence, smoking status, disease duration, family history of inflammatory bowel disorder, body mass index (BMI), number of diarrhea/day and also rectal bleeding as well as abdominal pain and medication history. The specialist gastroenterologists at Al-Sadr Medical City documented colonoscopic findings and Mayo disease activity scores.

Stool Sample Collection

All participants provided fresh stool samples in sterile, wide-mouthed plastic containers. Samples were carried immediately to laboratory and maintained at −20°C until analysis. Fecal calprotectin determination was performed on between 50–100 mg of each fecal sample based on the manufacturer's instructions. All samples have been handled in a centralized laboratory under strict controlled conditions to ensure reproducibility of results.

Measurement of Fecal Calprotectin

Fecal calprotectin concentrations have been estimated using a the kit of enzyme-linked immunosorbent assay (ELISA) kit (Calprotectin ELISA Kit, Sunlong Biotech Co., Ltd., China), in accordance with the manufacturer instructions. In short, stool extracts were added with the extraction buffer provided by the kit and the absorbance was read out at 450 nm in an automated microplate reader. Calprotectin was measured and presented in terms of micrograms per gram (μg/g) of stool. All samples were run in duplications, and internal quality-control procedures implemented throughout the analytical procedure ensured accuracy and reproducibility. As described by the manufacturer, values obtained from fecal calprotectin analysis <50 μg/g were considered normal in this study, while increased values indicated that intestinal inflammation was present.

Statistical Analysis

Data were analysis to de descriptive and inferential statistics by using the Statistical Package for Social Sciences (SPSS) version 26.0 (IBM Corp., USA). Quantitative data have been described as mean ± standard deviation (SD), and qualitative variables have been expressed as percentages and frequency. Assessments of differences in fecal calprotectin concentrations between clients with UC and healthy controls were performed accordingly with the independent samples t-test. One-way analysis of variance (ANOVA) with LSD post hoc test was employed for comparing between the different groups of disease severity (Al-fahham, 2018). "Receiver operating characteristic (ROC) curve analysis" was performed to obtain the diagnostic performance of fecal calprotectin and all outcomes were evaluated according to cut-off

value, sensitivity, specificity area under the ROC curves (AUC). Statistical significance was set at $p<0.05$.

Results

Demographic information of patients with Ulcerative colitis and healthy controls are presented in table 1. Most of respondents in both groups were between 35 and 44 age, average category by years was from 45–54. The patient group comprised 54.0% males and 49.4% females, while the control group included 46.0% males and 50.6% females, respectively (p=1). As for residence, the urban places were relatively high, 61.9% for the patients and 66.7% for the controls. There were no significant differences regarding age distribution (χ^2 = 1.95, P = 0.59), gender (χ^2 = 0.42, P = 0.52) or residence between patients and controls by statistical analysis.

Table 1. General information of subjects with UC in comparison with healthy control group

Indicators		`Patients (No. = 63)		Control (No. = 87)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	25-34	16	25.4	24	27.6	1.95	0.59 (NS)
	35-44	19	30.2	28	32.2		
	45-54	17	27	21	24.1		
	≥ 55	11	17.4	14	16.1		
Gender	Male	34	54	43	49.4	0.42	0.52 (NS)
	Female	29	46	44	50.6		
Residence	Rural	24	38.1	29	33.3	0.77	0.38 (NS)
	Urban	39	61.9	58	66.7		

NS: Non-significant at P>0.05

Figure 1 exhibited the distribution of subjects with UC according to severity of the disease, it shows that the prevalence of moderate level of ulcerative colitis (n=31, 49.2%) was the most common among the studied patients, followed by mild level (n=21; 33.33%); and finally, severe of ulcerative colitis (n=11; 17.47%).

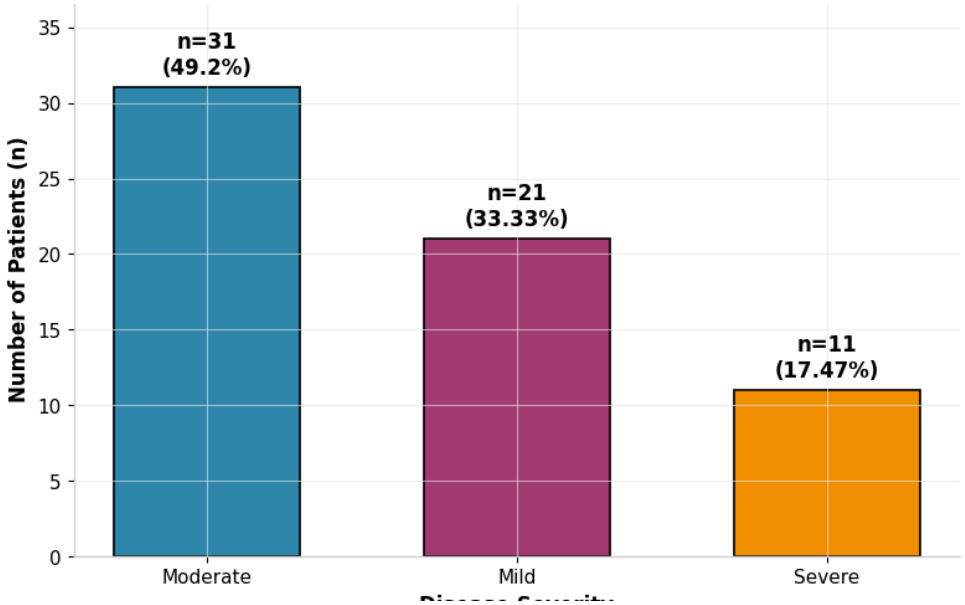

Figure 1. Distribution of patients with according to severity of ulcerative colitis

Comparison of serum fecal calprotectin in patients with UC and healthy individuals. In their result, serum fecal calprotectin concentrations were reported to be statistically greater in patients with ulcerative colitis (412.68 ± 136.45 µg/g) than the control group (38.74 ± 14.62 µg/g).

Table 2. Comparison of fecal calprotectin between subjects with UC and control subjects

Groups	Patients Mean ± SD	Control Mean ± SD	T Test (P Value)
Fecal Calprotectin (µg/g)	412.68 ± 136.45	38.74 ± 14.62	t = 20.84 p < 0.002 (HS)

HS: High significant at P<0.01

The distribution of fecal calprotectin among subjects with UC stratified by ulcerative colitis disease severity is illustrated in Table 3. The mean concentration of fecal calprotectin in patients with mild disease was 218.54 ± 64.27 µg/g, while moderate disease showed a statistically significant greater level (431.86 ± 95.48 µg/g). Mean concentrations were highest in subjects with severe ulcerative colitis, at 732.45 ± 128.91 µg/g (analysis of variance: F = 58.73; p < 0.001 among the three severity groups).

Table 3. Measurement of fecal calprotectin levels in patients' subgroups classified according to disease severity

Groups	No.	Fecal Calprotectin (µg/g)	F Test (P Value)
Mild	21	218.54 ± 64.27	F = 58.73 p < 0.000 (HS)
Moderate	31	431.86 ± 95.48	
Severe	11	732.45 ± 128.91	

HS: High significant at P<0.001

Table 4 reveals the analysis using a receiver operating characteristic (ROC) curve, fecal calprotectin displayed very good diagnostic accuracy for discriminating subjects with UC from healthy controls. The area under curve (AUC) was 0.931, reflecting excellent discriminative ability. In addition, the diagnostic performance was statistically very significant (p < 0.001). Fecal calprotectin reached a sensitivity of 90.5% and specificity of 87.4% at an optimal cut-off value of 85.5 µg/g.

Table 4. Diagnostic power parameters of fecal calprotectin for the diagnosis of ulcerative colitis

Biomarker	(AUC)	Sig. p-value	Cut-off Point	Sensitivity (%)	Specificity (%)
Fecal Calprotectin	0.931	< 0.001	85.5	90.5	87.4

AUC: Area Under the curve

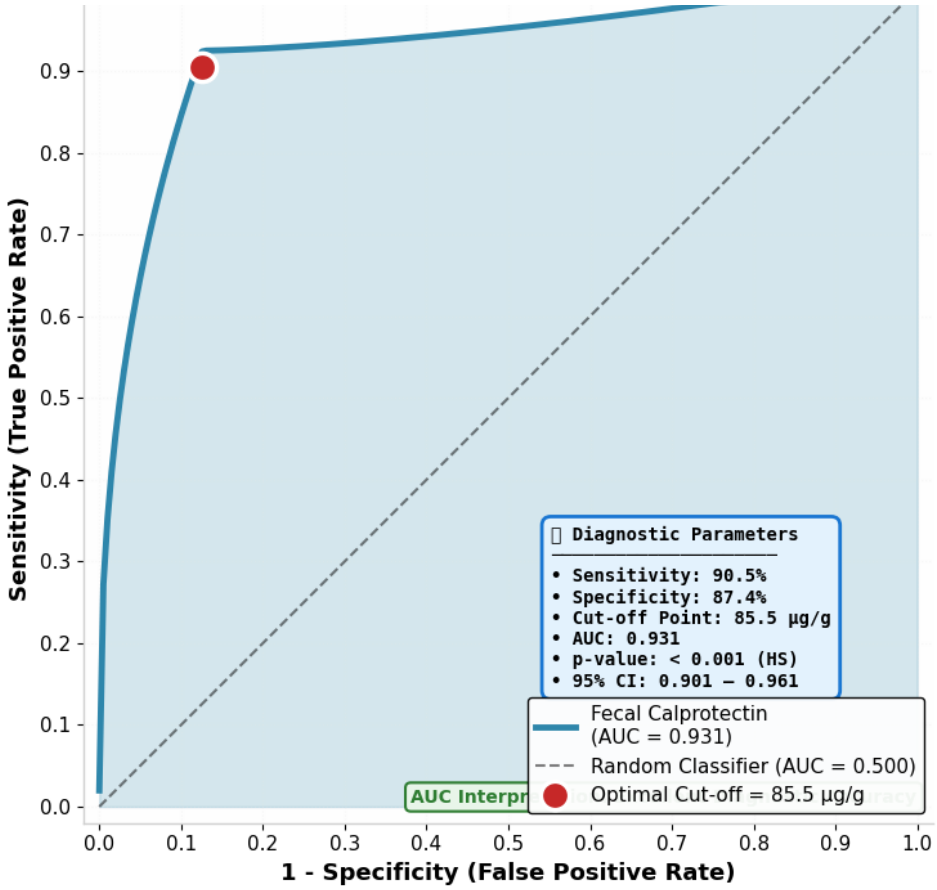

Figure 2. ROC Curve of the fecal calprotectin for the diagnosis of ulcerative colitis

Discussion

The demographic characteristics of the study population showed no significant association when comparing patients to controls across age, residence and gender (Table 1) These results demonstrate that the groups were sufficiently well matched, reducing demographic confounding of the measured biomarker. Maaser et al. (2019) have observed similar phenomenon as well, who argued that age and sex have only minor influences on fecal calprotectin concentrations in adults and that increased levels are mainly correlated to inflammation, not demographic data.

Ulcerative colitis patients had the most important rise from all the groups with a very high statistical significance in fecal calprotectin level compared with healthy subjects which was another main point of our study. This is biologically plausible since the major source of calprotectin is activated neutrophils associated with gut inflammation migration into the lumen. As a result, neutrophil infiltration increase will be followed by more calprotectin release in feces, having extremely high concentrations. Roseth et al. were one of the earliest investigators to show that fecal calprotectin precisely reflects underlying intestinal inflammation and can be a useful biomarker for inflammatory bowel diseases. Similarly, D'Haens et al. (2012) indicated that fecal calprotectin levels associate strongly with endoscopic lesions and may serve as a predictor marker for mucosal inflammation in subjects with intestinal bowel disease.

The large difference seen between patients and controls in this study is consistent with the results of Menees et al. (2015). Likewise, Turvill et al. (2016) revealed that the fecal calprotectin is a valid biomarker for identifying patients that required additional endoscopic evaluation. This substantially higher level found in patients from the present study supports the use of fecal calprotectin as a non-invasive diagnostic biomarker for ulcerative colitis.

Another interesting finding was the increase of fecal calprotectin concentrations according to the stage of disease severity. In clients with UC, the prevalence of SCFA concentrations was greater in individuals with severe disease compared to those who had mild disease. The findings suggest a strong association of fecal calprotectin with the severity of mucosal inflammation. Greater amounts of calprotectin are released into the intestinal lumen given that extensive mucosal ulceration and increased neutrophilic infiltration characterize severe disease (Mosli et al.,

2015). According to Lee et al (2015), the levels of fecal calprotectin are significantly associated with endoscopic disease activity and concentrations > 500 µg/g are frequently associated with moderate-to-severe endoscopic inflammation. Moreover, Park et al. (2017) used fecal calprotectin levels to predict both disease recurrence and endoscopic severity in a group of patients with UC.

The major differences seen between the mild, moderate and severe disease categories in this study were also agreed with the results of Mao et al. (2021), whose systematic review and meta-analysis has shown that fecal calprotectin is a good diagnostic test to detect active mucosal inflammation. Likewise, Turner et al. (2021) explained this concept and emphasized in their recent STRIDE-II recommendations which consider fecal calprotectin normalization an important therapeutic target in inflammatory intestinal disease due to its close association with mucosal healing.

Receiver operating characteristic (ROC) analysis conducted in the present study demonstrated a statistically significant area under the curve (AUC) of 0.931, suggesting that fecal calprotectin has a very good ability to discriminate ulcerative colitis from healthy controls. The fact that high sensitivity and specificity values were achieved also supports its diagnostic utility. The current results are consistent with those documented by Mao et al. (2021) demonstrated pooled specificity and sensitivity of >80% for the detection of the presence of endoscopic activity in UC. Similarly, Menees et al. (2015) found that fecal calprotectin is among the most accurate non-invasive biomarkers available for diagnosing inflammatory bowel disease.

High sensitivity in the current study suggests that fecal calprotectin can detect a large proportion of those people with active disease, while high specificity prevents a false-positive diagnosis in healthy controls. Thus, determination of fecal calprotectin may avoid unnecessary colonoscopies and can optimize patient management. These observations correlate with the European Crohn's and Colitis Organisation guidelines that endorse fecal calprotectin for evaluating inflammatory intestinal diseases (IBDs) and monitoring (Maaser et al., 2019).

Besides its role as a diagnostic biomarker, fecal calprotectin has been identified as prognostic marker for predicting disease relapse and treatment response. Khan et al. A recent study on 400 clients with biological therapy for IBD) reported that higher fecal calprotectin concentrations were independently associated with relapse during follow-up. Hence, serial fecal calprotectin measurements may allow timely prescription of therapy and markedly enhance long-term outcomes. In the current study, we noted a close association between disease severity and calprotectin levels, confirming its role as a surrogate marker for inflammatory burden and clinical progression (Kostas et al., 2017).

Fecal calprotectin was proved to be an extremely precise non-invasive biomarker for diagnosing ulcerative colitis type (UC); an area under the ROC curve (AUC) of 0.931 was observed at valid cut-off values of 85.5 µg/g, with a sensitivity and specificity estimated at 90.5% and 87.4% correspondingly. Altogether, these results highlight the strong discriminatory value of FC for identifying UC patients from healthy individuals, which agrees with all previous evidence consolidating this biomarker as a pillar in IBD. The significantly higher levels of FC in subjects with UC, as compared to controls, is directly linked to neutrophil-mediated mucosal inflammation fundamental to the disease; calprotectin represents ~60% of the content of cytosolic protein within neutrophilic cells and can be released into the intestinal lumen during active inflammation (Moein et al., 2017)

The diagnostic performance demonstrated in the current study corroborates that seen in previous literature. A systematic review, supported by the "National Institute for Health and Care Excellence (NICE)", found that FC at a cutoff of 50 µg/g achieved an AUC of 0.97 for differentiating IBD from irritable bowel syndrome (IBS); pooled sensitivity was 93%, and pooled specificity was 94% across various ELISA-based studies (Waugh et al., 2013). In accordance with this view, A meta-analysis conducted by Ye et al. FC assessment for histological activity in UC (2021) presented pooled sensitivity and specificity 0.69 (95% CI: 0.52, 0.82) and 0.77 (95% CI: 0.63–0.87), respectively. Although the sensitivity in the present study (90.5%) is higher than the pooled estimate from meta-analysis, it could be explained by differences in population, the cut-off value used as high probability for IBD and distribution of disease activity. Importantly, the cut-off of 85.5 µg/g defined in this work lies within the limits proposed by very recent clinical guidelines which classify FC values as less than 50 µg/g indicating active disease; between 50 and 150 µg/g corresponding to a diagnostic grey zone; while leading to values greater than 150–250–mu g/g determined more possible active disease (D'Amico et al., 2021; Turner et al., 2021). Fecal calprotectin has been shown in several studies to outperform the conventional serological inflammatory biomarkers, including ESR and CRP. Moein et al. (2017) explained that FC was highly associated with endoscopic disease activity indices (r = 0.798 for UC), whereas CRP (r = 0.463) and ESR (r = 0.467) were poorly correlated, and the AUC of FC versus non-IBD was significantly better than that of CRP or ESR in discriminating IBD subjects from non-IBD subjects.

The present study indicates that the most appropriate cut-off valued, 85.5 µg/g, is compatible with other independent studies.

A study by Li et al. (2021) found that an FC cut-off of 164 µg/g reached an AUC of 0.830 for the diagnosis of clinically active UC, with corresponding values for sensitivity and specificity being 85.42% and 73.68%, respectively, while a cut-off of 154.5 µg/g diagnosed mucosal healing with an AUC = 0.839 (73) (Table4). The reduced cut-off point used in this study may be a consequence of including patients at the lower end of disease severity inclusively, as illustrated by the variety of patient distribution seen here. Moreover, in a systematic review it has been demonstrated that FC is most accurate for differentiating IBD from functional gastrointestinal disorders with pooled sensitivity to rule out IBD ranging from 89%–99% along with an NPV for exclusion of IBD of 0.99 at the standard cut off of 50 µg/g, consistent with the high sensitivity found in this study (Annisa, 2025).

These findings have important clinical implications as well. FC testing also has a strong negative predictive value at this cut-off, which means that FC is good for ruling out active intestinal inflammation, and thus may decrease the frequency of unnecessary invasive endoscopic procedures on subjects with suspected UC and normal or mildly elevated FC levels. This is especially important, as colonoscopy is resource-intensive and entails patient burden. Both ECCO consensus and AGA guidelines support FC as a potential biomarker of mucosal healing in UC, with recommendations for serial monitoring every 3–6 months throughout active therapy and every 6–12 months throughout maintenance therapy (D'Amico et al., 2021). However, there are some caveats you need to recognize. The wide variation FC cut-off values between studies from 50 µg/g to >250 µg/g indicates that there is a need for validation of assays and populations before clinical implementation (Ye et al., 2021).

Conclusions

The results demonstrate the medical relevance of fecal calprotectin as an easy-to-measure, non-invasive, and highly accurate biomarker for diagnosing UC and assessing disease severity in patients with UC up to date. Its high level in patients and its gradual rise with more severe disease activity highlights its potential as a marker of severity for regular clinical use. The introduction of fecal calprotectin measurement into ulcerative colitis management could lead to better disease monitoring, less reliance on invasive procedures and more effective therapeutic approaches.

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