Utility of fecal calprotectin as a discriminative biomarker between ulcerative colitis and irritable bowel syndrome and its ability to be used for the assessment of the remission stage of ulcerative colitis

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Background and aim
Fecal calprotectin (FC) has been proposed in recent studies as a sensitive, specific biomarker for the diagnosis of ulcerative colitis (UC). Hence, the present study sought to investigate the efficacy of FC for the diagnosis and monitoring of UC, as well as to assess the correlation of FC with other disease activity indexes.

Research design and methods
The present study included 96 consecutive patients who presented with lower gastrointestinal complaints. Patients were classified into two groups: group I (which included patients with UC) and group II (which included patients with irritable bowel syndrome); then, according to the disease activity, group I was subdivided into the following: group Ia (which included patients with active UC) and group Ib (which included only those patients of group Ia who were in the remission stage of UC). For all patients, erythrocyte sedimentation rate and C-reactive protein were determined; moreover, all patients underwent quantitative determination of calprotectin in stool samples, abdominal ultrasonography, and complete colonoscopy with biopsies for the histopathological examination to assess the disease severity by using the UC activity index according to the Mayo endoscopic and Geboes histological scores. The diagnostic validity of FC levels in correlation with Mayo Disease Activity Index (MDAI) was then investigated.

Results
FC levels showed highly significant increase in patients with active UC compared with inactive UC and irritable bowel syndrome (524.17 ± 48.0 vs. 184.48 ± 3.33 and 47.17 ± 5.32 mg/kg, respectively, $P < 0.001$). FC level has 100% accuracy, sensitivity, specificity, positive predictive value, and negative predictive value in distinguishing UC patients from the control group at a cutoff value of 140 mg/kg, but at a cutoff value of 223 mg/kg FC level shows 93.4% accuracy, 89.8% sensitivity, 97% specificity, 97.4% positive predictive value, and 55% negative predictive value to distinguish the active phase from the remission phase of UC. In addition, there was a statistically significant proportional correlation between FC and the MDAI, but the correlation between FC and histological inflammatory activity statistically was more significant than with MDAI ($r = 0.75$, $P < 0.001$).

Conclusion
FC level is an accurate, noninvasive biomarker in clinical practice with high specificity and sensitivity for the diagnosis and monitoring of UC, as well as good marker for the evaluation of disease activity. Therefore, it can be used as a monitoring test to assess medical response and to predict clinical relapse of the disease.

Keywords:
biological markers, disease activity index, fecal calprotectin, ulcerative colitis

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Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease affecting gastrointestinal tract and is characterized by relapse and remission. Disease activity index in UC has been extensively evaluated using various tools incorporating clinical, laboratory, and endoscopic indexes. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and total leukocytic count (TLC) are widely used as noninvasive parameters for inflammatory bowel disease (IBD). These laboratory markers do not appropriately reflect the activity of the intestinal tract because they have insufficient sensitivity and specificity for intestinal inflammation. Therefore, more reliable biological markers are required to confirm the disease activity of UC [1,2].

Current gold standard for assessing intestinal inflammation of UC is endoscopic evaluation. Endoscopy allows visual assessment of disease severity and extent, and then mucosal biopsy is used to provide further information. The most commonly used scoring system for endoscopic disease activity is the Mayo score (Table 1) [3].

However, endoscopic procedure is invasive and requires an uncomfortable preparation for a long time. The active gut inflammation is closely associated with the migration of neutrophils into the gut. Therefore, some neutrophilic products in stool can be considered as valuable tools because of simple, rapid, sensitive, specific, inexpensive, and noninvasive biomarkers to detect and monitor intestinal inflammation in UC [4–6].

Calprotectin is an abundant neutrophil protein found in both plasma and stool, and is markedly elevated in infectious and inflammatory conditions, including UC. Accumulation of neutrophils at the site of inflamed mucosa in the gastrointestinal tract results in the release of calprotectin into feces where it is stable and resistant to bacterial degradation [7].

Fecal calprotectin (FC) has been proposed in recent studies as a sensitive and specific marker for the diagnosis of UC [8–11]. Hence, the present study sought to evaluate and confirm the efficacy of FC as a sensitive and specific biochemical for the diagnosis and monitoring of UC, with correlation with other disease activity indexes including clinical, laboratory, endoscopic, and histological measures.

Table 1 Components of the Mayo scoring system [1]

Stool frequency
0 = normal
1 = 1–2 stools/day more than normal
2 = 3–4 stools/day more than normal
3>4 stools/day more than normal
Rectal bleeding
0 = none
1 = visible blood with stool less than half the time
2 = visible blood with stool half of the time or more
3 = passing blood alone
Mucosal appearance at endoscopy
0 = normal or inactive disease
1 = mild disease (erythema, decreased vascular pattern, mild friability
2 = moderate disease (marked erythema, absent vascular pattern, friability, erosions)
3 = severe disease (spontaneous bleeding, ulceration)
Physician rating of disease activity
0 = normal
1 = mild
2 = moderate
3 = severe

Patients and methods

Patients

This study was carried out in the Department of Internal Medicine in Ibn Sina National Colleague Hospitals, Jeddah, Kingdom Saudi Arabia, from March 2013 to September 2015. A total of 96 patients with lower gastrointestinal complaints were recruited for the present study and were selected randomly from the Outpatient Gastroenterology Clinic and Endoscopy Unit of Ibn Sina National Colleague Hospitals.

This study was approved by the research ethics committee of the Ibn Sina National Colleague Hospitals. All patients were invited to participate and were provided with all the information necessary to dispel their doubts regarding the study. The individuals who agreed to participate signed an informed consent before participation. All participants met the following inclusion criteria: age older than 18 years; willing and able to participate in all the procedures of the study, such as collection of blood and stool samples and undergoing a colonoscopy; and being able to come to the clinic where the study was taking place, whenever necessary. Moreover, there are nine exclusion criteria:

(1) Individuals with active or latent tuberculosis;
(2) Patients with a previous history of colon cancer;
(3) History of infectious diarrhea during the previous 6 months;
(4) Positive for HIV or hepatitis B or C;
(5) Infection with intestinal parasites;
(6) Colostomy or ileostomy up to 1 month before the study;
(7) Pregnancy;
(8) Concurrent menstruation or sever epistaxis in the previous 48 h; and
(9) Long-term use of NSAIDs, anticoagulant, and proton pump inhibitors to avoid false results for FC.

Patients were classified into two groups: group I (which included patients with UC) and group II (which included patient with irritable bowel syndrome (IBS) and acted as control group); then, according to the disease activity, group I was subdivided into the following: group Ia (patients with active UC) and group Ib (the same patients of group Ia but on the remission stage of UC). Group Ia included patients who had clinical, laboratory, endoscopic, and histopathological manifestations of UC; the patients received aminosalicylates, steroids, immunosuppressive, and biological therapy according to the severity of disease and received iron therapy and blood transfusion only in severe anemia (hemoglobin% <8 g/dl). Group Ib comprised only those patients of group Ia who were in the period of remission stage of UC. Remission in UC should mean complete cessation of rectal bleeding,

urgency, and increased stool frequency, which is best confirmed by endoscopic mucosal healing [12]. Group II (the control group) included patients of IBS, who had normal colonoscopy and not had any clinical manifestation of UC. Patients were diagnosed as IBS according to Rome III criteria [13].

Methods

The following methods were carried out [once for controls (group II) and many times for the patients (group II)], once at the active phase and another time at the remission phase of UC:

(1) Full history taking, by using carefully designed questionnaire about the manifestations of UC.
(2) Careful clinical examination, stressing on abdominal examination.
(3) Laboratory measurements, including complete stool analysis, complete blood count, ESR and CRP titer, serum albumin, aspartate aminotransferase, alanine aminotransferase, serum bilirubin total and direct prothrombin time, partial thromboplastin time and international normalized ratio, fasting and postprandial blood glucose, serum creatinine, blood urea nitrogen, and quantitative determination of calprotectin in stool samples. Other investigations were required for the exclusion of some diseases such as tuberculosis.
(4) Abdominal ultrasonography and abdominal plain radiography to exclude other causes of abdominal pain.
(5) Complete colonoscopy, which was carried out after the patient was made to drink clear liquids only for 24 h before exam, no solid foods for 2 days before exam, and special oral laxatives such as polyethylene glycol and enema for cleansing, and under cautious sedation using propofol and midazolam. Histopathological examination of multiple biopsies and the measurement of UC activity index according to the Mayo endoscopic index and Geboes histological scores were carried out, and then FC was correlated well with clinical, Mayo endoscopic and Geboes histological indexes, and its sensitivity, specificity, and accuracy was determined. Colonoscopy with biopsy was repeated for patients of UC to confirm the remission phase of UC [14,15].

Fecal calprotectin test

The assessment of the FC is a quantitative test carried out using the PhiCal Calprotectin ELISA Kit (Immundiagnostik AG, Bensheim, Germany) for in-vitro determination of calprotectin in stool.

The samples of stool were stored at −20°C for joint analysis. On the day of processing, the samples were thawed at room temperature according to the manufacturers' instructions. To measure calprotectin, 1–5 g of feces was taken from each sample; subsequently, a 100-mg aliquot was separated using a precision scale, placed in a test tube, mixed in a vortex for 30 s, and placed on a horizontal agitator (speed of 1000 rpm) for 35 min. Approximately, 1–2 ml of the supernatant was transferred to an Eppendorf tube and centrifuged at 10 000*g* for 20 min. The extract was diluted, and the ELISA test was carried out in duplicate. An appropriate reader with a 450-nm filter was used. The optical density of all of the standards (included in the kit) was calculated, and a standard curve was obtained. The values corresponding to each sample were located on that curve, and the concentrations were calculated as ng/ml, which were multiplied by 2.5 to obtain the equivalents expressed as mg/kg. In this test, values up to 50 mg/kg of FC were considered to be normal [16].

Statistical analysis

Data were presented as mean ± SD or *n* (%). Statistical difference was tested using the nonparametric Kruskal–Wallis *H* test. Spearman's coefficient was used to assess the correlation between the variables. We performed linear regression modeling to assess the independent effect of the variables on square root transformed FC level. Variables with a *P* value of less than 0.2 in univariate regression were included in the multivariate model. The assumptions of the linear regression were assessed through testing normality and homogeneity of variance of residuals and the linear relation between outcome variable and predictors. Variance inflation factor and correlation coefficients were calculated to assess multicollinearity. We used receiver–operating characteristics curve analysis to evaluate the accuracy of FC, ulcerative colitis activity index (UCAI), and their combination with the intent to discriminate between active and inactive phases of UC. Statistical analyses were performed with SPSS software (version 23.0; SPSS Inc., Chicago, Illinois, USA), and a *P* value of less than 0.05 was considered statistically significant.

Results

A total of 96 patients were included in the study, with mean age of 49 years (range: 18–59 years). After clinical, laboratorial, endoscopic, and histological evaluation and considering the final diagnosis, patients were allocated to the following groups: group Ia (the UC group), which included 48 patients (15 women and 33 men); group II (the IBDs group), which included 48 patients (28 women and 20 men; and group Ib, which included those patients from group Ia who were in the remission stage of UC, as shown in Table 1. In

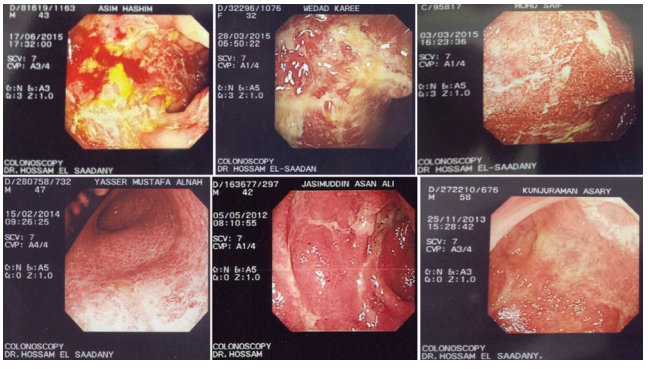
addition, there are some endoscopic pictures of UC in different grades, as shown in Fig. 1.

No significant difference in the FC levels was observed on comparing sex or age in the studied patients. There was a significant increase in the mean value of ESR, TLC, platelets count, and CRP, with a highly significant decrease in the mean value of hemoglobin in GIa (active UC) in comparison with other groups. Moreover, the mean levels of calprotectin in UC were highly significantly increased in patients with active UC than that in the inactive UC and in controls (524.17 ± 48.0, 184.48 ± 3.33, 47.17 ± 5.32 mg/kg), respectively, *P* value less than 0.001 as shown in Table 2).

Statistical analysis of the receiver–operating characteristic curve showed that at a cutoff value of 140 mg/kg FC level has 100% accuracy, sensitivity, specificity, positive predictive value, and negative predictive value in distinguishing UC patients from the control group, but at a cutoff value of 223 mg/kg, FC level reaches an accuracy, sensitivity, specificity, and positive and negative predictive values of 93.4%, 89.8% [95% confidence interval (CI): 89–97], 97% (95% CI: 79.7–99.8), 97.4%, and 55%, respectively, to distinguish the active phase from the remission phase of UC, as shown in Figs 2 and 3.

In addition, there was a statistically significant proportional correlation between FC and Mayo Disease Activity Index; moreover, the correlation between FC and histological inflammatory activity was statistically more significant ($P < 0.001$) (Fig. 4). Furthermore, multivariate analysis indicated a positive correlation between FC and other inflammatory markers (ESR, CRP, TLC, and platelets count) of disease activity ($r = 0.75$, $P < 0.001$), as shown in Tables 3–6.

Figure 1



Endoscopic pictures of ulcerative colitis in different grades: (a, b) Sever form; (c, d) Moderate form; and (e, f) Mild form of ulcerative colitis.

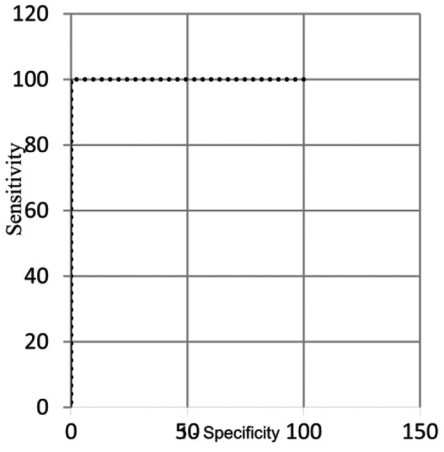
Discussion

Intestinal inflammation plays a major role in the pathogenesis of different lower gastrointestinal disorders such as UC. Intestinal inflammation is characterized by an increased activity of immune cells, with the migration of polymorphonuclear neutrophils from the circulation to the inflamed intestinal mucosa. Because of leukocyte shedding in the intestinal lumen, proinflammatory proteins such as calprotectin can be detected and measured in the stool [17]. When intestinal inflammation occurs, FC rises rapidly and correlates with endoscopic and histological alterations

Table 2 Basic demographic and main clinical characteristics of the patients

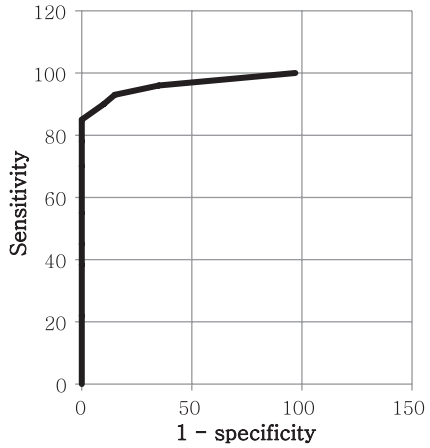
Characteristics	*N* (%)	
	Group I (*N* = 48)	Group II (*N* = 48)
Age (mean ± SD) (years)	49 ± 10	47+11
Sex (male/female)	33 (68.75)/15 (31.2)	28 (58.33)/20 (41.66)
Race		
Black	33 (68.6)	35 (72)
White	15 (31.4)	13 (28)
Diarrhea	22 (47)	18 (39)
Abdominal pain	48 (100)	48 (100)
abdominal tenderness	19 (40)	–
Rectal bleeding	45 (95)	–
Tenesmus	16 (35)	–
Fever	4 (10)	–
Pallor	31 (66)	–
Tachycardia	33 (70)	–
Weight loss	14 (31)	–
Extraintestinal features	Aphthous ulcer in two patients and oligoarthritis in one patient	–

Figure 2



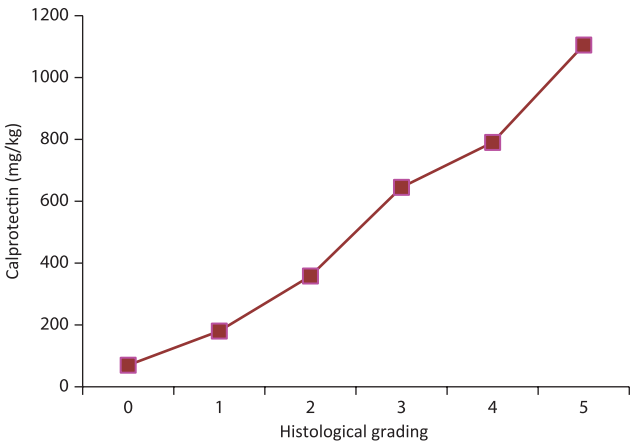
ROC curve to analyze the best cutoff point of fecal calprotectin in distinguishing active ulcerative colitis (group Ia) from inactive ulcerative colitis (group Ib), demonstrated area under the curve of 0.922 ± 0.025 ($P < 0.001$). ROC, receiver–operating characteristic.

Figure 3



ROC curve to analyze the best cutoff point of fecal calprotectin in distinguishing active ulcerative colitis (group Ia) from irritable bowel disease (group II). ROC, receiver–operating characteristic.

Figure 4



Correlation between fecal calprotectin and the Geboes histological score in patients of ulcerative colitis. $P = 0.000$ (Spearman's rank correlation). Grade 0, indicative structural change only; grade 1, chronic inflammation; grade 2, lamina propria neutrophils; grade 3, neutrophils in epithelium; grade 4, crypt destruction; and grade 5, erosions or ulcers.

Table 3 Endoscopic characteristics and scoring of disease activity in group Ia

Mayo score		Geboes histology score	
Grade	*N* (%)	Grade	*N* (%)
0	0	0	0
1	9 (4.3)	1	3 (1.4)
2	21 (10)	2	6 (2.8)
3	18 (8.6)	3	10 (4.8)
		4	14 (6.7)
		5	15 (7.2)
Endoscopic characteristics of patient with active ulcerative colitis group Ia [*N* (%)]			
Extension		Severity	
Pancolitis	6 (12.4)	Mild	10 (20.8)
Rectal	8 (16.6)	Moderate	20 (41.6)
Rectal+sigmoid	12 (24.9)	Sever	18 (37.4)
Sigmoid	11 (22.8)		

in patients with IBD, supporting the idea that it is a sensitive and specific means to identity inflammatory activity in these patients. Thus, FC has emerged in recent studies as a new diagnostic tool to detect and monitor intestinal inflammation in patients with UC [18].

The present study was aimed to investigate the efficacy of FC as an accurate and noninvasive biomarker for the diagnosis and monitoring of UC, as well as to assess the correlation of FC with other disease activity indexes. Our results were consistent with one of the most recently published study, which concluded that the level of FC identifies patients with UC who have endoscopic and histological features of mucosal healing and correlates with endoscopic and histological inflammatory activity [19].

Our results are in agreement with that of another study [20], which showed that FC in the patient's feces can reflect the disease activity of UC and can be used as a rational fecal marker for intestinal inflammation in clinical practice. This kind of marker is relatively precise, simple, and noninvasive when compared with other commonly used markers such as CRP and ESR. Furthermore, our findings were in agreement with that of a recent study by Kotze *et al.* [21], who showed that the determination of FC assists in differentiating between active and inactive IBDs and between IBDs and IBS.

Moreover, our findings are in agreement with that of a recent study by Nouh *et al.* [22], who showed that FC is a valuable, simple, easily performed, and cost-effective noninvasive marker for the evaluation of patients with UC. It differentiated UC and other diseases causing colonic symptoms (cutoff value of 131 μg/g) and between active and inactive UC (cutoff value of 235 μg/g) with high accuracy, sensitivity, and specificity. It also correlated well with other markers for UC activity (UCAI, ESR, CRP, TLC, and platelets count) and could be a reliable surrogate marker for the severity of UC. Similarly, our results showed that at a cutoff value of 140 mg/kg, FC level has 100% accuracy, sensitivity, specificity, positive predictive value, and negative predictive value in discriminating UC patients from the control group, but at a cutoff value of 223 g/kg, FC level has an accuracy, sensitivity, specificity and positive predictive value, and negative predictive value of 93.4%, 89.8% (95% CI: 89–97), 97% (95% CI: 79.7–99.8), 97.4%, and 55%, respectively, to discriminate the active phase from the remission phase of UC.

Table 4 Basic laboratory characteristics of the studied groups

Characteristics	Mean ± SD			Paired *t*-test (*P* value for GIa and GIb)	Paired *t*-test (*P* value for GIa and GII)	Paired *t*-test (*P* value for GIb and GII)
	Group Ia (*N* = 48)	Group Ib (*N* = 48)	Group II (*N* = 48)			
Fecal calprotectin (mg/kg)	524.17 ± 48	184.48 ± 3.3	47.17 ± 5.3	7.551 (<0.001)	7.971 (<0.001)	5.951 (<0.015)
Hb (g/dl)	8.81 ± 2.1	13.15 ± 19	13 ± 19	11.95 (<0.001)	11.99 (<0.001)	–0.177 (0.87)
TLC (10^3/dl)	14.8 ± 2	8.11 ± 1.12	6.11 ± 0.22	15.95 (<0.001)	16.85 (<0.001)	3.88 (0.003)
CRP (mg/l)	22.1 ± 0.12	5.1 ± 3.03	4.65 ± 1.89	17.15 (<0.001)	17.35 (<0.001)	2.121 (0.082)
ESR (mm/h)	54.1 ± 12.1	25 ± 10.6	18.99 ± 8.9	16.45 (<0.001)	17.12 (<0.001)	2.15 (0.115)
Platelets	370.1 ± 52.1	295.3 ± 6.21	284.2 ± 52.1	4.15 (<0.001)	4.55 (<0.001)	0.45 (0.54)

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; TLC, total leukocytic count.

Table 5 Diagnostic validity of fecal calprotectin in comparison with other inflammatory markers for the activity of ulcerative colitis

Index	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ESR	61.1	58	65	82	52
TLC	68	65	72	89	44
CRP	66	60	71	86	49
MDAI	92.3	88.2	96.5	96	59
Fecal calprotectin	93.4	89.8	97	97.6	55

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MDAI, Mayo Disease Activity Index; NPV, negative protective value; PPP, positive protective value; TLC, total leukocytic count.

Table 6 Correlation between fecal calprotectin and disease activity indexes

Index	Fecal calprotectin (mg/kg)	
	r value	*P* value
ESR	0.62	<0.001
CRP	0.59	<0.001
MDAI	0.75	<0.001
TLC	0.52	<0.001
Platelet count	0.44	<0.001

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MDAI, Mayo Disease Activity Index; TLC, total leukocytic count.

Another study by Vieira *et al*. [23] evaluated the efficacy of FC as an indicator of inflammatory activity, and concluded that, first, FC is highly sensitive and specific marker for the detection of intestinal inflammation in IBD patients; and, second, that FC levels are directly proportional to the degree of inflammation in the intestinal mucosa. In addition, another meta-analysis by van Rheenen *et al*. [24] reported FC to be a useful screening tool for identifying patients most likely in need of endoscopy for suspected IBD where the pooled sensitivity and pooled specificity of calprotectin was 0.93 (95% CI: 0.85–0.97) and 0.96 (95% CI: 0.79–0.99). Similarly, our results showed high sensitivity and specificity of 89.8% (95% CI: 89–97) and 97% (9% CI: 79.7–99.8), respectively, for FC in differentiating UC from IBS, and there was statistically significant proportional correlation between FC and disease activity index. In contrast, Kalantari *et al*. [25] reported FC as a noninvasive method that can be used to identify patients with UC from IBS patients has low sensitivity and specificity, where at cutoff value of more than 164 μg/g with sensitivity and specify of 57% (95% CI: 41–71.6), and 75% (95% CI: 59.7–56.8), respectively, was the best for discrimination between patients with UC and those with IBS. Low sensitivity and specificity in the previous study in comparison with our results can be attributed to the dissimilarities in the study design and the studied population.

Several studies have established that calprotectin levels correlate well with endoscopic and histological UC activity. The correlation could be demonstrated for both UC [26–28].

The present study results are in agreement with that of Schroder *et al*. [29], who concluded that although all fecal biomarkers studied provide a reliable and simple noninvasive means in the differentiation of IBD and IBS, calprotectin appears to represent the most accurate marker to discriminate between these two common causes of chronic diarrhea.

Two major limitations of the present study were observed: (a) lack of a control group of healthy adults, as the comparison of the level of FC in patients with UC with the level in healthy adults is preferred; (b) the FC is useful for discriminating patients with UC from those with IBS, but it is a nonspecific disease biomarker for discriminating patients with UC from those with Crohn's disease. The fact that FC cannot be used for the localization of UC is another limitation of the present study.

Finally, large-sized prospective studies are recommended to investigate the best cutoff values in clinical practice in patients with UC for monitoring intestinal inflammation. Other studies are recommended to investigate the utility of FC for predicting the relapse of IBD and to assess the efficacy of medical therapy.

Conclusion

FC can be considered as accurate noninvasive biomarker in clinical practice with high specificity and

sensitivity for the diagnosis of UC, as well as good marker for the evaluation of disease activity. Therefore, these findings indicate that FC should be used in distinguishing IBD from functional bowel disease (IBS), and thus avoiding the need of invasive tests such as colonoscopy. In addition, measuring FC can be used as a monitoring test to assess the medical response and to predict clinical relapse of the disease.

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Conflicts of interest
There are no conflicts of interest.

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