The Egyptian Journal of Internal Medicine

RESEARCH

Open Access

Evaluation of the diagnostic performance of estimated fecal calprotectin and serum intelectin-1 and C-reactive protein solo or in combination for differentiation between patients with query ulcerative colitis and irritable bowel syndrome

Rizk Sayad R. Sarhan[1*], Yasmin M. Marei[2] and Yomna M. Marei[1]

Abstract

Objectives Evaluation of the ability of estimated levels of fecal calprotectin (FCP), serum intelectin-1 (ITLN1), and C-reactive protein (CRP) to differentiate between patients with ulcerative colitis (UC) and irritable bowel syndrome (IBS).

Patients Three-hundred forty-two patients were evaluated clinically for diagnostic criteria of UC and IBD and underwent colonoscopic examination and grading according to Mayo endoscopic scores (MES). Colorectal biopsies were taken for microscopic examination. Fecal and blood samples were obtained for ELISA estimation of levels of the studied variate. Patients were grouped according to microscopic examination of the obtained biopsies as UC and IBD groups. Study outcome is the ability of the laboratory variate for prediction of the microscopic diagnosis.

Results In UC patients, FCP and serum CRP levels were notably elevated compared to controls and IBS patients. Conversely, UC patients exhibited significantly reduced serum ITLN1 levels in comparison to controls and IBS patients with insignificantly lower levels in samples of IBS patients. Statistical analyses defined high FCP and low serum ITLN1 as the significant predictors for UC diagnosis with high specificity for FCP level > 150 µg/ml and high sensitivity for serum ITLN1 ≤ 30 and ≤ 18 ng/ml to predict colonoscopic and microscopic UC diagnosis, respectively.

Conclusion The combination of high FCP and low serum ITLN1 could accurately predict the colonoscopic and microscopic findings of UC and can differentiate UC from IBS and may spare the need for colonoscopy and biopsy especially for IBS patients.

Keywords Ulcerative colitis, Irritable bowel disease, Fecal calprotectin, Intelectin-1, Noninvasive predictors

Introduction

Gastrointestinal (GI) immune homeostasis is the result of collaborative functions of the intestinal epithelium, the immune system and gut nerve supply, and specifically for the colon, gut microbiome, which dynamically regulates the local immune function [1]. The disrupted intestinal epithelial barrier and leakage of the gut microbiome are

*Correspondence:
Rizk Sayad R. Sarhan
rizk.r.sarhan72023@gmail.com
[1] Department of Internal Medicine, Faculty of Medicine, Benha University, Benha, Egypt
[2] Department of Medical Biochemistry, Faculty of Medicine, Benha University, Benha, Egypt

the key factors in different pathophysiological conditions, especially inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and chronic liver diseases [2].

Inflammatory bowel diseases (IBD), including Crohn's disease, ulcerative colitis (UC), and indeterminate colitis, are distinguished by their idiopathic and chronic inflammation of the digestive tract. Unfortunately, they are incurable and demonstrate a worldwide increasing incidence and prevalence [3]. On contrary, IBS is a functional GI disorder that is characterized by gastrointestinal dysmotility and visceral hyperalgesia [4] and is highly prevalent conditions with bothersome abdominal symptoms in the absence of structural abnormalities [5].

Functional GI disorders are recently attributed to gut-brain interaction that starts with mucosal infiltration with immune cells, which release and/or induce the release of nociceptive mediators by intestinal cells [5]. These mediators can activate the sensitized neurons leading to visceral hypersensitivity [6]. The interaction between immune activation and an impaired barrier function of the gut is most likely bidirectional, and the altered microbiota, psychological stress, and food staffs are components of the pathophysiology [7].

Distinguishing patients with IBS and IBD is still dependent on colonoscopic evaluation; however, recently, the development of IBS diarrhea secondary to minimal inflammation was reported in patients with quiescent CD which increased the dilemma [8].

Objectives

In trial to find a noninvasive differentiating modality between cases with query UC or IBS, this study targets to evaluate the diagnostic performance of estimated levels of fecal calprotectin (FCP), serum intelectin-1 (ITLN1), and C-reactive protein (CRP) solely or in combination for such purpose.

Design

It is a prospective double-blinded comparative study.

Setting

It is the Departments of Internal Medicine and Medical Biochemistry, Faculty of Medicine, Benha University, in conjunction with multiple private GIT centers.

Ethical considerations

The study was started in June 2020 after obtaining the preliminary approval of the Faculty of Medicine, Local Ethical Committee, and after completion of case collection in Dec 2022; the final approval was obtained and registered by no. RC: 2.2.23.

Blindness

One author was responsible for case collection and obtaining clinical data, and another was responsible for colonoscopic examination and biopsy taking, and each author was blinded about the data collected by the other. Fecal samples were obtained and sent for estimation of FCP and blood samples for estimation of serum CRP and ITLN1 levels. The obtained biopsies were preserved in formalin and sent for pathological examination at the Department of Pathology, Faculty of Medicine. At the end of case collection, the clinical, colonoscopic, laboratory, and microscopic data were interpreted.

Patients

All patients attending the outpatient clinic with manifestations of either IBD or IBS were evaluated for exclusion and inclusion criteria. Twenty healthy controls free of inclusion and exclusion criteria were enrolled to give fecal specimens and serum samples as control group for laboratory investigations.

Exclusion criteria

Patients who were previously diagnosed as IBD or IBS and were under treatment or not, patients who had recurrent ulceration after surgical treatment to any levels, and patients who had malignancy elsewhere in the body, autoimmune disorders, maintained on immunosuppressant, or refused to participate in the study were excluded from the study.

Inclusion criteria

Patients free of exclusion criteria and presented by symptoms suggestive of IBD or IBS were enrolled in the study.

Evaluation tools

A) *Evaluation tools for UC diseases*

1. UC diagnosis was relied on the presence of at least three of the following items: abdominal pain, diarrhea, hematochezia, and/or pus in stools, the presence of rectal with or without colonic mucosal ulcerations on colonoscopic examination, microscopic findings consistent with UC in the obtained colorectal biopsy, and the absence of manifestations and colonoscopic diagnostic findings of Crohn's disease. To simplify the evaluation, each item was scored by 0 or 1 for a collective score of 0–8 [9].
2. Clinical disease severity was evaluated using the Simple Clinical Colitis Activity Index (SCCAI),

which evaluates day-bowel frequency (score: 0–3), night-bowel frequency (score: 1 or 2), urgency of defecation (score: 1–3), blood in stool (score: 1–3), general wellbeing (score: 0–4), and the presence of extra-colonic features (score: one for each manifestation with a score range of 0–20); a total score was determined for each patients, and score > 5 indicates clinical activity [10].

3. IBD Disease Activity Index (DAI) was assessed depending on the combination of SCCAI score and estimated levels of FCP or serum CRP, and SCCAI is > 5 with *FCP* ≥ 150 μg/ml, or serum CRP is > 5 mg/L which indicates active UC disease; otherwise, patient was considered to be either in remission or had inactive UC disease [11].

B) *Evaluation tools for IBS*

1. Diagnosis of IBS was dependent on fulfillment of at least two of the documented Rome IV criteria which include recurrent abdominal pain on at least 1 day/week during the last 3 months in patients complaining since 6 months, and this recurrent abdominal pain is related to defecation and/or associated with change in stool frequency or form according to Bristol Stool Scale (BSS) that consists of seven types for description of forms and consistency of stool. IBS was categorized as IBS with predominant constipation (IBS-C), or diarrhea (IBS-D), mixed type (IBS-M), or unclassified (IBS-U) [12, 13].
2. The IBS disease activity index (IBS-DAI) evaluated six items each was scored using 4-point score (0–3) indicating no, mild, moderate, or severe symptom, and a total score of ≥ 10 indicates IBS activity; these items include nausea, bloating, abdominal pain, diarrhea, constipation, and anorexia [14].

C) *Colonoscopic evaluation*

– All patients underwent colonoscopic examination for macroscopic appearance of the mucosa and was graded according to Mayo endoscopic score (MES) as MES-0 if no friability and granularity were detected with intact vascular pattern, thus indicating normal colonoscopy or inactive disease in patient previously diagnosed as UC; MES-1 if there was mild erythema or decreased vascular pattern with mild friability, thus indicating mild inflammatory disease activity; MES-2 if there is marked erythema, lack of vascular pattern, friability, and/or erosions, thus indicating moderate disease activity; and MES-3 if there is spontaneous bleeding and/or large ulcerations, so indicates severe disease activity [15].

Laboratory investigations

Fecal sampling and processing

Fresh fecal samples (about 30 ml) were obtained and stored at − 20 °C. The stored feces samples were thaw at room temperature; about 100 mg of feces was taken by the inoculation loop, placed into a screw-cap tube, and weighted to obtained net feces weight. Then, the pre-diluted extraction buffer was added to tube contents in 1:50 Wt.:vol., the tube was closed and shake vigorously for 30 s, and contents were homogenized for about 25 min on a shaker. One milliliter of the homogenate was put in a tube, centrifuged for 20 min at 10,000 g, and 0.5 ml of the clear extract supernatant was pipetted and put in Eppendorf tube and stored at − 80 °C until being assayed.

Blood sampling and processing

At time of obtaining the fecal sample, blood samples were obtained from the antecubital vein, allowed to clot, and centrifuged at 1500 rpm for 15 min, and serum was collected and put in dry Eppendorf tube at − 80 °C until being assayed.

Investigations

a. Fecal calprotectin (FCP) level was estimated using ELISA kit Abcam (Abcam Inc., Cambridge, USA; catalog no. ab267628), [16] and levels were interpreted as follows: FCP level < 50 μg/ml indicates negative/normal result, FCP level ≥ 150 μg/ml assured positive and indicated UC disease, and concentrations ≥ 50– < 150 μg/ml indicate moderate positivity [17].
b. Serum human intelectin-1 (ITLN1; omentin-1) and C-reactive protein (CRP) levels were ELISA estimated using Abcam kit (Abcam Inc., Cambridge, USA; catalog nos. ab269554 & ab260058, respectively). Estimated serum CRP level of < 0.3 mg/dl indicates normal level [18], and serum ITLN1 level in range of 24.7–33.2 ng/ml was documented to be the normal range estimated in samples of healthy subjects [19].

Patients' grouping

Considering the gold standard for UC diagnosis is the microscopic detection of mucosal changes consistent with UC, patients with positive microscopic examination

for UC were categorized as UC group and patients with negative findings consistent with UC diagnosis as IBS group.

Study outcomes

1. *Primary outcome*: Is the predictability of the estimated biomarkers for the results of the microscopic examination of the obtained mucosal biopsies
2. *Secondary outcomes*

   – The diagnostic performance of the studied biomarkers solely or in combination for blinded differentiation between patients with UC and IBS
   – The relation of estimated biomarkers to disease activity scores

Statistical analysis

Results were analyzed using one-way ANOVA and chi-square test (X^2 test), and correlation analysis was performed using Pearson's correlation analysis. Regression analysis of correlated variate was conducted by the use of the stepwise method. Receiver operating characteristic (ROC) curve analysis was performed to compare the area under curve (AUC) for each variate versus the area under the standard line ($AUC = 0.5$), and performance of each variate was judged according to the AUC as sensitive ($AUC < 0.5$) or specific ($AUC > 0.5$). Paired analysis of difference of AUC was performed for each two variates to determine the best predictor. Kaplan-Meyer analysis was used to determine the cutoff point for the studied variate that might predict the cumulative hazard for UC diagnosis. Performance characters of lab variate were evaluated versus MES and microscopic diagnosis of UC using the determined cutoff points. IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) which was for Windows statistical package was the applied system. Significance was considered at the cutoff point for *P*-value at < 0.05.

Results

During the study duration, 373 patients presented by manifestations suggestive of UC or IBS; 13 patients were excluded because 9 patients were under treatment for UC, and 4 patients had recurrent ulceration after previous surgical resection, and 360 patients were enrolled in the study. Eighteen patients refused to undergo colonoscopic examination and were excluded from the study, and data of 342 patients were analyzed. According to the microscopic examination of the obtained mucosal biopsies, 51 biopsies showed changes consistent with diagnosis of UC (UC group), while 291 biopsies were free of these changes (IBS group) as shown in Fig. 1.

Patients of UC group were significantly older than patients who had IBS, while other demographic data showed nonsignificant differences between patients of both groups. Recurrent abdominal pain was the main complaint of 330 patients (96.5%) including all IBS patients and 39 (76.5%) of UC patients with significantly higher incidence among IBS patients. One-hundred

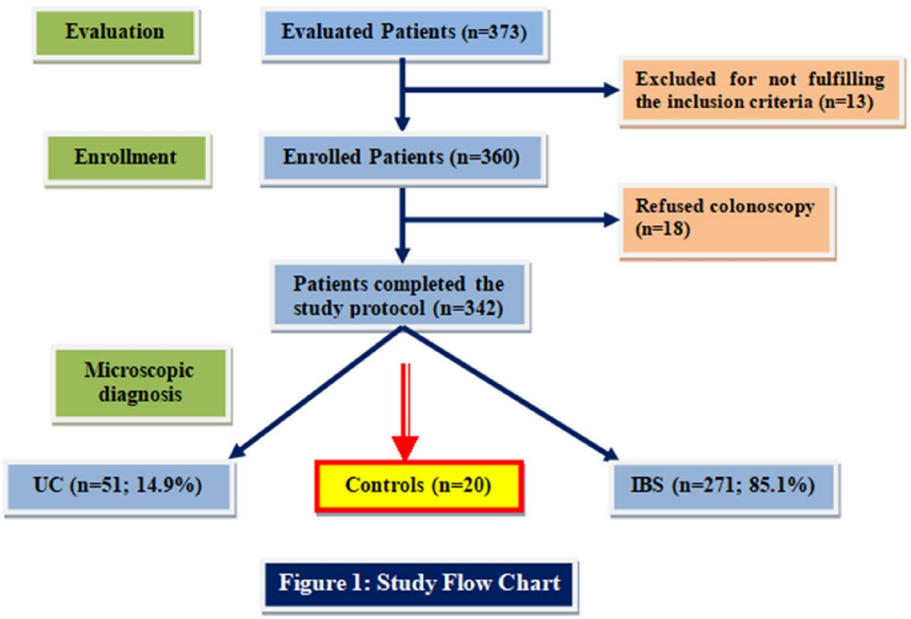

Fig. 1 Study flow chart

and five patients complained of diarrhea with significantly higher frequency among UC patients. Among IBS patients, constipation was the main bowel habit compliant of 42 patients, while 49 patients had bowel habit alternating between diarrhea and constipation, and 137 could not classify their bowel habits. Hematochezia and pus in stool were reported by 51 and 59 patients, respectively, with significantly higher incidence among UC patients (Table 1).

Colonoscopic examination of patients of UC group detected 75 ulcers: 46 in the rectum and 29 were distributed through the colon: 17 in the sigmoid, 8 in the left, and 4 ulcers in the right colon. Twenty-four patients (47.1%) had both rectal and colonic ulcerations, while 22 patients (43.1%) had only rectal ulcerations, and 5 patients (9.8%) had only colonic ulcer. Colonoscopic examination of patients of IBS group detected no ulceration, but 72 patients (24.7%) showed only mild erythema with normal vascular pattern and no friability. In grading of colonoscopic findings according to MES, defined 219 patients (64%) were of MES-0, 79 patients (23.1%) of MES-1, 34 patients (9.9%) of MES-2, and 10 patients (3%) of MES-3 (Table 2).

Mean FCP levels estimated in samples of UC patients were significantly ($P<0.001$) higher than levels estimated in samples of controls and IBS patients with significantly ($P=0.0015$) higher levels in samples of IBS patients than control samples. Twenty-eight patients had FCP level ≥ 150 µg/ml, 152 patients had levels ranging between > 50 and < 150 µg/ml, and 162 patients had levels ≤ 50 µg/ml with significantly ($P<0.001$) higher frequency of patients who had $FCP \geq 150$ µg/ml among UC patients than IBS patients. Mean serum CRP levels estimated in samples of UC and IBS patients were significantly ($P<0.001$) higher than control levels with significantly ($P<0.001$) higher levels in samples of UC than samples of IBS patients. In relation to 5 mg/L as cutoff point for serum CRP, 55 patients had serum CRP levels < 5, while 287 patients had serum CRP level ≥ 5 mg/L with nonsignificant ($P=0.893$) difference between UC and IBS patients. Mean serum ITLN1 levels estimated in samples of UC patients were significantly ($P<0.001$) lower than levels estimated in control and IBS patients' samples with nonsignificantly ($P=0.366$) lower levels in samples of IBS patients than in control samples (Table 3).

Diagnosis of UC according to the collected clinical, colonoscopic, and microscopic data detected 18 patients (35.3%) had three positive items, 14 patients (27.5%) had four positive items, 13 patients (25.5%) had five positive items, and 6 patients (11.7%) had six positive items. According to SCCAI for clinical severity of UC disease, 15 patients (29.4%) had score of < 5,

Table 1 Demographic data and clinical presentations of the enrolled UC and IBS patients

Data			UC ($n=51$)	IBS ($n=291$)	*p*-value
Age (years)	Age strata	< 2 5	2 (3.9%)	11 (3.8%)	**0.005**
		25–30	10 (19.6%)	88 (30.2%)	
		31–40	14 (27.5%)	117 (40.2%)	
		41–50	23 (45.1%)	55 (18.9%)	
		> 50	2 (3.9%)	20 (6.9%)	
	Average (± SD)		39 (± 8.7)	35.5 (± 8)	**0.0018**
Gender	Males		16 (31.4%)	85 (29.2%)	**0.755**
	Females		35 (68.6%)	206 (70.8%)	
BMI (kg/m^2)	Strata	< 25	0	4 (1.4%)	**0.265**
		25–30	9 (17.6%)	77 (26.5%)	
		> 30–35	42 (82.4%)	210 (72.1%)	
	Average (± SD)		31.5 (± 1.88)	31.2 (± 2.8)	**0.312**
Smoking	Smoker		13 (25.5%)	69 (23.7%)	**0.648**
	Ex-smoker		9 (17.6%)	39 (13.4%)	
	Un-smoker		29 (56.9%)	183 (62.9%)	
Clinical presentation	Recurrent abdominal pain		39 (76.5%)	291 (100%)	**< 0.001**
	Bowel habit	Diarrhea	43 (84.3%)	63 (14.4%)	**< 0.001**
		Constipation	0	42 (14.4%)	
		Alternating	0	49 (16.8%)	
		Uncategorized	0	137 (47.1%)	
	Hematochezia		32 (62.7%)	20 (6.9%)	**< 0.001**
	Pus in stool		**26 (51%)**	**33 (11.3%)**	< 0.001

26 patients (51%) had score ranging between 5 and 10, and 10 patients (19.6%) had score of > 10 with a median SCCAI of 6 (*IQR*: 4–9). Evaluated disease activity using UC-DAI detected 30 patients with active UC, and 21 patients were in remission (Table 4).

Regarding the remaining 291 patients, according to Rome IV criteria, in addition to recurrent abdominal pain, 177 patients (60.8%) had change in stool frequency, and 152 patients (52.2%) had change in stool form. Collectively, there were 38 patients (13.1%) fulfilling > 2 of Rome IV criteria, while 253 patients (86.9%) fulfilled two

Table 2 Colonoscopic findings of the enrolled patients

Data			Number (%)
Distribution of ulcers through the colorectal tract	Rectum		46 (61.3%)
	Sigmoid colon		17 (22.7%)
	Left colon		8 (10.7%)
	Right colon		4 (5.3%)
	Total		75 (100%)
Site of ulcerations among UC patients	Colorectal		24 (47.1%)
	Rectum		22 (43.1%)
	Colon		5 (9.8%)
Mayo endoscopic score (MES)	UC	MES-1	7 (13.7%)
		MES-2	34 (66.7%)
		MES-3	10 (19.6%)
	IBS	MES-0	219 (75.3%)
		MES-1	72 (24.7%)

Table 3 Estimated laboratory parameters estimated in samples of the enrolled patients compared to samples of control subjects

Laboratory parameters	Group Data		Control ($n=20$)	UC ($n=51$)	IBS ($n=291$)
Fecal calprotectin (µg/ml)	Average (± SD)		31.86 ± 10.8	146.9 ± 54.4	**66.4 ± 47**
	Significance vs. control			< 0.001	**0.0015**
	Significance vs. UC				**< 0.001**
	Patients' distribution according to the probable diagnostic FCP levels	≤ 50	20 (100%)	0	**162 (55.7%)**
		> 50– < 150	0	30 (58.8%)	**152 (41.9%)**
		≥ 150	0	21 (41.2%)	**28 (2.4%)**
Serum CRP (mg/l)	< 5		20 (100%)	11 (21.6%)	**44 (15.1%)**
	≥ 5		0	40 (78.4%)	**152 (84.9%)**
	Average (± SD)		2.08 ± 0.4	11.74 ± 8.5	**8.81 ± 3.5**
	Significance vs. control			< 0.001	**< 0.001**
	Significance vs. UC				**< 0.001**
Serum ITLN1 (ng/ml)	Average (± SD)		29.36 ± 2.95	15.58 ± 3.3	**27.69 ± 8.23**
	Significance vs. control			< 0.001	**0.366**
	Significance vs. UC				**< 0.001**

Table 4 Diagnostic scorings of UC

UC diagnosis scoring		SCCAI		UC-DAI		
Score = 3	18 (35.3%)	< 5	15 (29.4%)	Active	SCCAI > 5 + FCP ≥ 150 + CRP > 5	15 (29.4%)
Score = 4	14 (27.5%)	5–10	26 (51%)		SCCAI > 5 + FCP ≥ 150	10 (19.6%)
Score = 5	13 (25.5%)	> 10	10 (19.6%)		SCCAI > 5 + CRP > 5	5 (9.8%)
Score = 6	6 (11.7%)			Remission		21 (41.2%)

of Rome IV criteria. According to Bristol Stool Scale, 42 patients had IBS-C (14.4%), 63 patients had IBS-D (21.6%), 49 patients had IBS-M (16.8%), and 137 patients (47.2%) had uncategorized stool form and considered as unclassified IBS (IBS-U). According to IBS disease activity index, there were 44 patients (15.1%) who had active IBS (≥10) with median value of IBS-DAI of 11 (*IQR*: 10.75–12) points, while 237 patients (84.9%) showed IBS-DAI < 10 (Table 5). The median value of IBS-DAI score for these 291 IBS patients was 6 (*IQR*: 4–7) points.

Diagnosis of UC as judged by MES and by microscopic diagnosis was positively correlated with patients' age, FCP, and serum CRP levels while was negatively correlated with serum ITNL1 levels. Diagnosis of UC was nonsignificantly correlated with male gender and BMI. Regression analysis defined high FCP and low serum ITLN1 as the significant predictors for colonoscopic diagnosis for UC. Regarding prediction of the microscopic findings suggestive of UC, regression analysis defined old age, high FCP, and low serum ITLN1 as the significant predictors while excluded other correlated variables (Table 6). Furthermore, estimated serum ITLN1 levels showed negative significant correlation with BMI ($r = -0.122$, $P = 0.025$), FCP ($r = -0.132$, $P = 0.012$), and serum CRP ($r = -0.265$, $P < 0.001$).

The ROC curve showed high sensitivity for low serum ITLN1 ($AUC = 0.307$, $SE = 0.032$, $P < 0.001$; 95% *CI*: 0.245–0.370) and high specificity for high FCP ($AUC = 0.643$, $SE = 0.032$, $P < 0.001$; 95% *CI*: 0.581–0.705) to predict colonoscopic findings (Fig. 2).

Table 5 Diagnostic scoring of IBS

Data			Number (%)			
Rome IV	**Recurrent abdominal pain**		291 (100%)			
	Change in frequency		177 (60.8%)			
	Change in form		152 (52.2%)			
	Number of criteria	**2**	253 (86.9%)			
		> 2	38 (13.1%)			
Bristol Stool Score	**Constipation**		42 (14.4%)			
	Diarrhea		63 (21.6%)			
	Mixed		49 (16.8%)			
	Unclassified		137 (47.2%)			
IBS disease activity index	**Items**		**Score = 0**	**Score = 1**	**Score = 2**	**Score = 3**
	Abdominal pain		0	85 (29.2%)	117 (40.2%)	89 (30.6%)
	Diarrhea		228 (78.4%)	27 (9.3%)	19 (6.5%)	17 (5.8%)
	Constipation		249 (85.6%)	17 (5.8%)	13 (4.5%)	12 (4.1%)
	Nausea		144 (49.5%)	49 (16.8%)	57 (19.6%)	41 (14.1%)
	Bloating		103 (35.4%)	73 (25.1%)	41 (14.1%)	74 (25.4%)
	Anorexia		105 (36.1%)	81 (27.8%)	56 (19.2%)	49 (16.8%)
	Patients' distribution according to total score	**< 10**	247 (84.9%)			
		≥ 10	44 (15.1%)			

Table 6 Correlation and regression analyses for patients' data and laboratory findings as predictors for colonoscopic and microscopic diagnosis of UC

Variate	Pearson's correlation analysis				Regression analysis			
	MES		Microscopic diagnosis		MES		Microscopic diagnosis	
	"r"	*P*	"r"	*P*	*β*	*P*	*β*	*P*
Age	0.147	0.006	0.152	0.005	0.094	0.062	0.086	0.006
Male gender	0.065	0.230	0.017	0.756	0.004	0.932	−0.061	0.064
BMI	0.055	0.312	0.078	0.149	−0.006	0.876	0.002	0.953
FCP	0.406	< 0.001	0.514	< 0.001	0.537	< 0.001	0.677	< 0.001
Serum CRP	0.222	< 0.001	0.218	< 0.001	0.070	0.103	0.024	0.454
Serum ITLN1	−0.394	< 0.001	−0.488	< 0.001	−0.528	< 0.001	−0.656	< 0.001

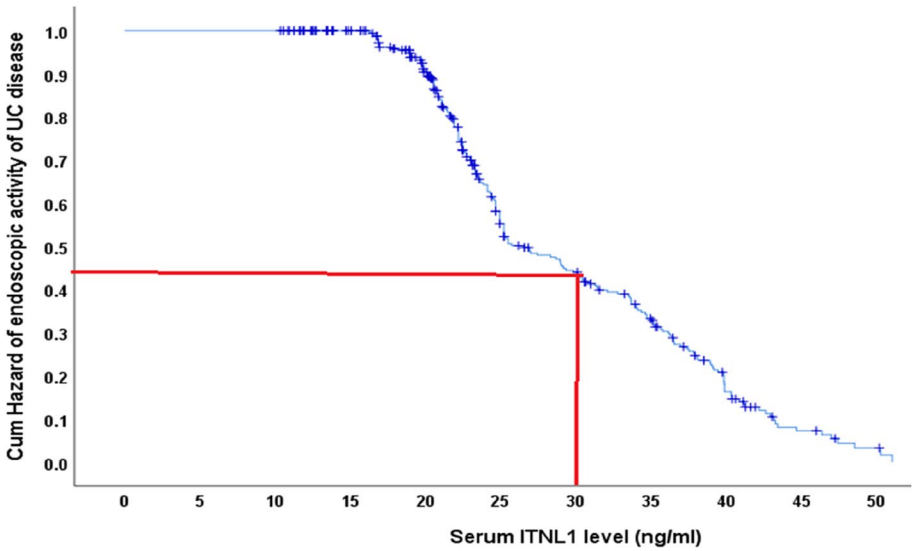

Fig. 2 Kaplan-Meyer curve for estimated serum ITLN1 as predictor for the cumulative hazard of UC endoscopic diagnosis of activity

For prediction of microscopic findings, ROC curve analysis showed high sensitivity for low serum ITNL1 levels (*AUC* = 0.038, *SE* = 0.010, *P* < 0.001; 95% *CI*: 0.019–0.058), while older age (*AUC* = 0.611, *SE* = 0.044, *P* = 0.012; 95% *CI*: 0.524–0.698) and high FCP (*AUC* = 0.871, *SE* = 0.026, *P* < 0.001; 95% *CI*: 0.821–0.921) showed high specificity for prediction of microscopic findings diagnostic for UC (Fig. 3). Paired analysis for AUC difference for age and FCP detected significantly high AUC difference (AUC difference = − 0.260, SE = 0.266, *P* < 0.001; 95% *CI*: [− 0.364]–[− 0.156]) in favor of FCP as specific predictor for microscopic diagnosis of UC.

Kaplan-Meyer regression analysis of serum ITLN1 levels defined serum levels at ≥ 30 ng/ml as the cutoff point to predict reduced cumulative risk of positive colonoscopic examination for UC down to 45% (Fig. 2) while at cutoff point of ≥ 18 ng/ml as the appropriate cutoff point for defining a cumulative risk of 90% for having UC on microscopic examination of colorectal biopsy (Fig. 3).

Evaluation of the diagnostic performance characteristics of the studied lab variate for colonoscopic and

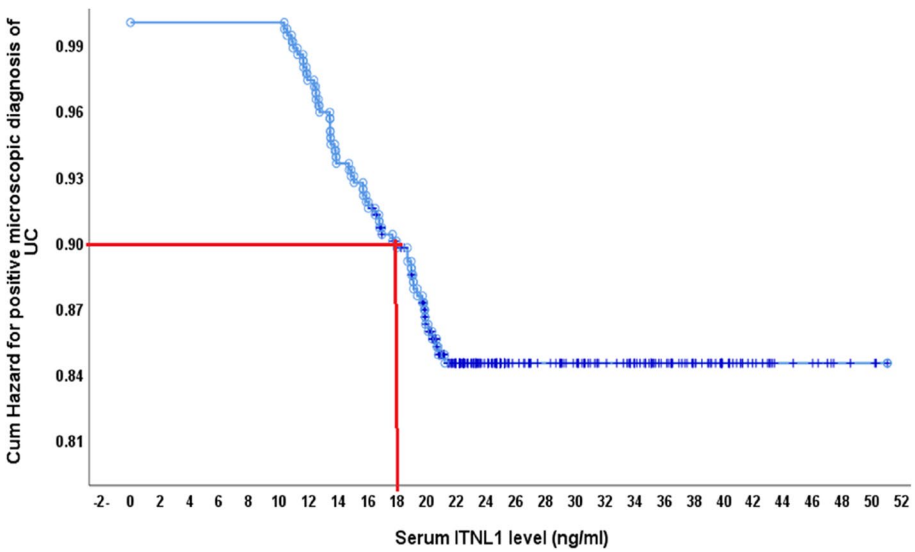

Fig. 3 Kaplan-Meyer curve for estimated serum ITLN1 as predictor for the cumulative hazard of microscopic diagnosis of UC

microscopic diagnosis of UC showed high specificity for FCP level > 150 µg/ml and high sensitivity for serum ITLN1 ≤ 30 and ≤ 18 ng/ml to predict colonoscopic and microscopic UC diagnosis, respectively. Both high FCP and low serum ITLN1 showed accuracy for diagnosis of 76% and > 90%, respectively, for colonoscopic and microscopic diagnosis of UC (Table 7).

Discussion

Decreased serum levels of intelectin-1 (ITLN1) could differentiate patients with UC from healthy controls and IBS patients due to the significantly lower levels in samples of UC patients than controls and IBS samples and might differentiate between IBS patients and healthy controls because of the nonsignificant difference between samples of IBS patients and controls.

These findings supported the earlier studies detected significantly lower serum ITLN1 in patients with active Crohn's disease (CD) than in patients in remission and patients with IBS and healthy controls [20]. Another study also found serum ITLN1 is significantly decreased in CD and UC patients than healthy controls and with active than inactive diseases [21]. These results showed an inverse relation between the presence and severity of inflammation and serum levels of ITLN1; in support of this assumption, the applied correlation analysis showed negative significant correlation between serum ITLN1 levels and Mayo's endoscopic score (MES), which is recently documented as the most common endoscopic index recommended in guidelines and widely used in clinical trials and practice [22] and with the microscopic diagnosis of UC. Intelectin-1 (omentin-1) is one of the anti-inflammatory adipocytokines including chemerin, vaspin, omentin, and visfatin [23], and recently, the relation between various adipocytokines and IBD presence and severity was reported, wherein Sochal et al. [24] reported a relation between serum chemerin and IBD severity of inflammation and Saadoun et al. [25] documented the use of estimated serum visfatin levels to detect UC activity and as predictor for disease extension.

In support of the relation between serum ITLN1 and inflammation, Kukla et al. [26] detected significantly decreased serum levels of ITLN1 and chemerin in samples of COVID-19 patients compared to healthy volunteers, and Gültekin et al. [27] found serum ITLN1 levels were significantly higher in non-sepsis than sepsis and septic shock patients and concluded that ITLN1 may have a role in development of inflammatory and metabolic complications in intensive care patients and is associated with poor outcomes and mortality.

In trial to explore the mechanisms underlying the relation between serum ITLN1 and UC disease, using animal model of induced UC inflammation, Ma et al. [28] detected decreased expression levels of ITLN1 in colonic tissues and found overexpression of ITLN1 inhibited endoplasmic reticulum stress-related proteins, colonic damage, inflammation, barrier damage, and cell apoptosis. Clinically, Nasir et al. [29] found an inverse significant relation between serum ITLN1 levels and levels of cholesterol and consumption of monounsaturated fatty acid and total fat intakes and established a relation between obesity and low serum ITLN1 levels. In support of this, the current study detected a negative relation between serum ITLN1 and BMI of the studied patients. Also, Li et al. [30] detected a similar negative relation between total body fat mass and serum ITLN1 levels.

On contrary, estimated serum CRP levels were significantly higher in UC and IBS patients than controls' levels, despite the significantly higher levels in UC patients than IBS patients. Similarly, Xu et al. [31]

Table 7 Diagnostic performance characters of the estimated levels of the studied lab variate for UC diagnosis

Variate		MES			Microscopic diagnosis		
		FCP	CRP	ITLN1	FCP	CRP	ITLN1
Cutoff point		> 150 µg/ml	> 5 mg/L	≤ 30 ng/ml	> 150 µg/ml	> 5 mg/L	≤ 18 ng/ml
Sensitivity	Rate (%)	27.9	78.7	81.7	60.8	72.6	95.7
	95% CI	19.8–37.2	70.4–85.6	75.5–86.9	46.1–74.2	58.3–84.1	92.7–97.7
Specificity	Rate (%)	99.1	14.1	68.9	97.9	14.8	61
	95% CI	96.9–99.9	9.8–19.4	60.8–76.2	95.6–99.2	10.9–19.4	44.5–75.8
PPV	Rate (%)	93.9	33.7	76.85	83.8	13	94.7
	95% CI	79–98.5	31.3–36.1	72.2–80.9	69.4–92.2	11.1–15.1	92.5–96.4
NPV	Rate (%)	74.1	54.4	74.8	93.4	75.4	65.8
	95% CI	71.8–76.3	42.7–65.7	68.4–80.3	91–95.3	64.5–83.9	51.7–77.5
Accuracy	Rate (%)	76	37.1	76	92.4	23.4	91.2
	95% CI	71.1–80.5	32–42.5	71.1–80.5	89.1–95	19–28.2	88–94.3

reported increased serum CRP levels with decreased serum total leucocytic count, and Sakemi et al. [32] detected significantly higher levels of inflammatory markers including CRP in UC and CD patients than in controls with significant difference between UC and CD. Further, the current study showed a negative significant relation between serum levels of CRP and ITLN1; such relation assured the anti-inflammatory action of ITLN1. The action of ITLN1, which was consumed or its synthesis, was suppressed by the inflammatory cascade. Similarly, inverse relations were detected between serum CRP and lymphocyte:monocyte ratio [31] and interleukin-22, which have an essential role in mucosal repair in IBD [32].

Estimated levels of FCP were significantly higher in patients than in controls and in UC than IBS patients with significantly higher frequency of patients who had FCP level > 150 μg/ml among UC than IBS patients. Further, there was positive relation between FCP and endoscopic and microscopic UC diagnostic findings and serum CRP, while the relation was negative with serum ITLN1 levels. These findings go in hand with recent studies that applied FCP for different purposes to diagnose or monitor UC patients [33, 34].

Statistical analyses found high FCP and serum ITLN1 levels were highly predictive for the UC disease, but with different performance where high FCP was specific and low serum ITLN1 was sensitive biomarker for the presence of UC, and were correlated with disease activity as judged by colonoscopic and microscopic findings for UC. Further, estimated levels of FCP and serum ITLN1 might predict the colonoscopic and microscopic findings with accuracy rate of about 76% and 90%, respectively. These complimentary roles of both markers allow using this combination for differentiation between UC and IBS and sparing colonoscopy with its financial and psychological impacts.

In line with this assumption for using complimentary investigation to replace colonoscopy goes in hand with Goodsall et al. [35] who found the composite use of FCP and intestinal ultrasonography to reduce the need for colonoscopy in routine care for UC patients. Also, Singh et al. [36] suggested the applicability a biomarker combination based on FCP and symptom-based monitoring strategy over a symptom-based monitoring strategy and to reduce endoscopic use for assessment of disease activity to guide treatment decisions. Further, Magalhaes et al. [37] in a systemic review found blood neutrophil-expressed biomarkers as adjuvants to basic diagnostics for IBD can help to modify treatment decision-making.

Conclusion

The combined estimation of serum ITLN1 and FCP could be used as an array for screening patients presented by manifestations suggestive of UC. Elevated FCP and decreased serum ITLN1 could accurately predict the colonoscopic and microscopic diagnostic findings of UC and thus can differentiate UC from IBS. Serum ITLN1 near normal levels suggest IBS especially with *FCP* < 50 or in the gray zone of > 50– < 150 μg/ml. The suggested combination may spare the need for colonoscopy and biopsy taking for UC diagnosis.

Limitation

Estimation of serum ITLN1 in UC patients in remission was a limitation of the current study.

Recommendations

Wider-scale comparative studies were mandatory to establish the suggested cutoff points for ITLN1 as predictor for MES grade and microscopic findings. Also, comparative studies for serum ITLN1 levels in UC patients who were in remission versus IBS patients and controls are required to assure its distinguishing ability between various colorectal disorders.

Abbreviations

ANOVA	One-way analysis of variance
AUC	Area under the curve
BSS	Bristol Stool Scale
CD	Crohn's disease
CRP	C-reactive protein
FCP	Fecal calprotectin
GI	Gastrointestinal
IBD	Inflammatory bowel diseases
IBS	Irritable bowel syndrome
IBS-DAI	IBS disease activity index
ITLN1	Intelectin-1
MES	Mayo endoscopic scores
ROC	Receiver operating characteristic
SCCAI	Simple Clinical Colitis Activity Index
UC	Ulcerative colitis
UC-DAI	UC Disease Activity Index

Authors' contributions

RSR underwent colonoscopic examination and biopsy taking and at the end of case collection interpreted the clinical, lab, colonoscopic, and microscopic data. YMM collected the clinical data and performed the clinical diagnosis and after interpretation of the studies evaluated the diagnostic performance of each variate in conjunction with the statistician. YM analyzed the obtained samples for estimation of the levels of the studied parameters. All authors read and approved the final manuscript.

Funding

No fund.

Availability of data and materials

Data is available when required.

Declarations

Competing interests

The authors declare that they have no competing interests.

Received: 15 May 2023 Accepted: 31 October 2023
Published online: 10 November 2023

References

1. Feng J, Xie Z, Hu H (2023) Ion channel regulation of gut immunity. J Gen Physiol 155(2):e202113042
2. Inczefi O, Bacsur P, Resál T, Keresztes C, Molnár T (2022) The influence of nutrition on intestinal permeability and the microbiome in health and disease. Front Nutr 9:718710. https://doi.org/10.3389/fnut.2022.718710
3. Wellens J, Vissers E, Matthys C, Vermeire S, Sabino J (2023) Personalized dietary regimens for inflammatory bowel disease: current knowledge and future perspectives. Pharmgenomics Pers Med 16:15–27. https://doi.org/10.2147/PGPM.S359365
4. Li J, Liu H, Guo F, Guo R, Zhang H, He X, Ming X, Ma X, Shang X, Ji P, Song L, Gao S (2023) Increased GABAergic projections in the paraventricular nucleus regulate colonic hypersensitivity via oxytocin in a rat model of irritable bowel syndrome. NeuroReport 34(2):108–115. https://doi.org/10.1097/WNR.0000000000001867
5. Vanuytsel T, Bercik P, Boeckxstaens G (2023): Understanding neuroimmune interactions in disorders of gut-brain interaction: from functional to immune-mediated disorders. Gut. gutjnl-2020–320633. https://doi.org/10.1136/gutjnl-2020-320633.
6. Tome J, Amrit K, Kamboj AK, Loftus CG CG (2023) Approach to disorders of gut-brain interaction. Mayo Clin Proc 98(3):458–467. https://doi.org/10.1016/j.mayocp.2022.11.001
7. Mayer EA, Ryu BRR (2023) The neurobiology of irritable bowel syndrome. Mol Psychiatry. https://doi.org/10.1038/s41380-023-01972-w
8. Tomita T, Fukui H, Morishita D, Maeda A, Makizaki Y, Tanaka Y, Ohno H, Oshima T, Miwa H (2023) Diarrhea-predominant irritable bowel syndrome-like symptoms in patients with quiescent Crohn's disease: comprehensive analysis of clinical features and intestinal environment including the gut microbiome, organic acids, and intestinal permeability. J Neurogastroenterol Motil 29(1):102–112. https://doi.org/10.5056/jnm22027
9. Moon JR, Lee CK, Hong SN, Im J, Ye B, Cha J, Jung S, Lee K, Park D, Jeen Y, Park Y, Cheon J, Kim H, Seo B, Kim Y, Kim H, MOSAIK study group of the Korean Association for Study of Intestinal Diseases (2020) unmet psychosocial needs of patients with newly diagnosed ulcerative colitis: results from the nationwide prospective cohort study in Korea. Gut Liver 14:459–467. https://doi.org/10.5009/gnl19107
10. Walmsley R, Ayres R, Pounder R, Allan R (1998) A simple clinical colitis activity index. Gut 43(1):29–32. https://doi.org/10.1136/gut.43.1.29
11. Aluzaite K, Al-Mandhari R, Osborne H, Ho C, Williams M, Sullivan M, Hobbs SM (2019) Detailed multi-dimensional assessment of fatigue in inflammatory bowel disease. Inflamm Intest Dis 3:192–201
12. Lacy BE, Mearin F, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R (2016) Bowel disorders. Gastroenterology 150:1393–1407. https://doi.org/10.1053/j.gastro.2016.02.031
13. Lacy BE, Patel NK (2017) Rome criteria and a diagnostic approach to irritable bowel syndrome. J Clin Med 6(11):99. https://doi.org/10.3390/jcm6110099
14. Mathias JR, Clench MH, Reeves-Darby VG (1995) Effect of leuprolide acetate in patients with moderate to severe functional bowel disease: double-blind, placebo-controlled study. Dig Dis Sci 39:1155–1162
15. Theede K, Holck S, Ibsen P, Ladelund S, Nordgaard-Lassen I, Nielsen AM (2015) Level of fecal calprotectin correlates with endoscopic and histologic inflammation and identifies patients with mucosal healing in ulcerative colitis. Clin Gastroenterol Hepatol 13(11):1929–36.e1. https://doi.org/10.1016/j.cgh.2015.05.038
16. Tøn H, Brandsnes Ø, Dale S, Holtlund J, Skuibina E, Schjønsby H, Johne B (2000) Improved assay for fecal calprotectin. Clin Chim Acta 292:41–54
17. Bjarnason I (2017) The use of fecal calprotectin in inflammatory bowel disease. Gastroenterol Hepatol (N Y) 13(1):53–56
18. Eschborn S, Weitkamp JH (2019) Procalcitonin versus C-reactive protein: review of kinetics and performance for diagnosis of neonatal sepsis. J Perinatol 39(7):893–903
19. Yin J, Hou P, Wu Z, Nie Y (2015) Decreased levels of serum omentin-1 in patients with inflammatory bowel disease. Med Sci Monit 21:118–122. https://doi.org/10.12659/MSM.892081
20. Lu Y, Zhou L, Liu L, Feng Y, Lu L, Ren X, Dong X, Sang W (2014) Serum omentin-1 as a disease activity marker for Crohn's disease. Dis Markers 2014:162517. https://doi.org/10.1155/2014/162517
21. Yin J, Hou P, Wu Z, Nie Y (2015) Decreased levels of serum omentin-1 in patients with inflammatory bowel disease. Med Sci Monit 10(21):118–122. https://doi.org/10.12659/MSM.892081
22. Sharara AI, Malaeb M, Lenfant M, Ferrante M (2022) Assessment of endoscopic disease activity in ulcerative colitis: is simplicity the ultimate sophistication? Inflamm Intest Dis 7:7–12
23. Nosrati-Oskouie M, Asghari G, Yuzbashian E, Aghili-Moghaddam N, Zarkesh M, Safarian M, Mirmiran P (2021) Does dietary intake impact omentin gene expression and plasma concentration? A systematic review. Lifestyle Genom 14(2):49–61. https://doi.org/10.1159/000513885
24. Sochal M, Fichna J, Gabryelska A, Talar-Wojnarowska R, Białasiewicz P, Małecka-Wojciesko E (2021) Serum levels of chemerin in patients with inflammatory bowel disease as an indicator of anti-TNF treatment efficacy. J Clin Med 10(19):4615. https://doi.org/10.3390/jcm10194615
25. Saadoun M, Nosair N, Abdel-Azeez H, Sharaf S, Ahmed M (2021) Serum visfatin as a diagnostic marker of active inflammatory bowel disease. J Gastrointestin Liver Dis 30(3):339–345. https://doi.org/10.15403/jgld-3504
26. Kukla M, Menżyk T, Dembiński M, Winiarski M, Garlicki A, Bociąga-Jasik M, Skonieczna M, Hudy D, Maziarz B, Kusnierz-Cabala B, Skladany L, Grgurevic I, Wójcik-Bugajska M, Grodzicki T, Stygar D, Rogula T (2021) Anti-inflammatory adipokines: chemerin, vaspin, omentin concentrations and SARS-CoV-2 outcomes. Sci Rep 11(1):21514. https://doi.org/10.1038/s41598-021-00928-w
27. Gültekin Y, Biri I, Gojayev A, Işıkhan S, Akçin O, Kılıç Y (2021) Can omentin-1 be a prognostic marker in surgical intensive care patients? Turk J Med Sci 51(5):2485–2493. https://doi.org/10.3906/sag-2009-158
28. Ma L, Zhang X, Zhang C, Zhou Y, Zhang H (2022) Omentin-1 attenuates inflammation and barrier damage in DSS-induced ulcerative colitis in mice by inhibiting endoplasmic reticulum stress. Gen Physiol Biophys 41(3):221–230. https://doi.org/10.4149/gpb_2022012
29. Nasir Y, Farzollahpour F, Mirzababaei S, Maghbooli Z, Mirzaei K (2021) Associations of dietary fats intake and adipokines levels in obese women. Clin Nutr ESPEN 43:390–396. https://doi.org/10.1016/j.clnesp.2021.03.018
30. Li S, Xue J, Hong P (2021) Relationships between serum omentin-1 concentration, body composition and physical activity levels in older women. Medicine (Baltimore) 100(10):e25020. https://doi.org/10.1097/MD.0000000000025020
31. Xu M, Cen M, Chen X, Chen H, Liu X, CaO Q (2019) Correlation between serological biomarkers and disease activity in patients with inflammatory bowel disease. Biomed Res Int 2019:6517549. https://doi.org/10.1155/2019/6517549
32. Sakemi R, Mitsuyama K, Morita M, Yoshioka S, Kuwaki K, Tokuyasu H, Fukunaga S, Mori A, Araki T, Yoshimura T, Yamasaki H, Tsuruta K, Morita T, Yamasaki S, Mizoguchi A, Sou S, Torimura T (2020) Altered serum profile of the interleukin-22 system in inflammatory bowel disease. Cytokine 136:155264. https://doi.org/10.1016/j.cyto.2020.155264
33. Sauk JS, Ryu HJ, Labus JS, Khandadash A, Ahdoot AI, Lagishetty V, Katzka W, Wang H, Naliboff B, Jacobs JP, Mayer EA (2023) High perceived stress is associated with increased risk of ulcerative colitis clinical flares. Clin Gastroenterol Hepatol 21(3):741-749.e3. https://doi.org/10.1016/j.cgh.2022.07.025
34. Angelo Cortesi P, Fiorino G, Peyrin-Biroulet L, Mantovani LG, Jairath V, Paridaens K, Andersson FL, Danese S (2023) Non-invasive monitoring and treat-to-target approach are cost-effective in patients with mild-moderate ulcerative colitis. Aliment Pharmacol Ther 57(5):486–495. https://doi.org/10.1111/apt.17261
35. Goodsall T, Day A, Andrews J, Ruszkiewicz A, Ma C, Bryant R (2023): Composite assessment using intestinal ultrasound and calprotectin is accurate in predicting histological activity in ulcerative colitis: a cohort study. Inflamm Bowel Dis. izad043. https://doi.org/10.1093/ibd/izad043.

36. Singh S, Ananthakrishnan A, Nguyen N, Cohen B, Velayos F, Weiss J, Sultan S, Siddique S, Adler J, Chachu K (2023) AGA clinical practice guideline on the role of biomarkers for the management of ulcerative colitis. Gastroenterology 164(3):344–372. https://doi.org/10.1053/j.gastro.2022.12.007
37. Magalhaes D, Peyrin-Biroulet L, Estevinho M, Danese S, Magro F (2023) Pursuing neutrophils: systematic scoping review on blood-based biomarkers as predictors of treatment outcomes in inflammatory bowel disease. Therap Adv Gastroenterol 16:17562848231155988. https://doi.org/10.1177/17562848231155987

Publisher's Note