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A New Cutoff Value for Fecal Calprotectin Level in Differentiating Functional from Organic Causes of Chronic Diarrhea

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ABSTRACT

Background: The gold standard to establish inflammatory bowel disease (IBD) diagnosis remains in the hands of endoscopists and pathologists. A challenge is thus to distinguish symptoms of IBD from irritable bowel syndrome (IBS). ***Aim of this work:*** was to evaluate the clinical usefulness of fecal calprotectin level as noninvasive marker to distinguish patients with diarrhea in need of intensified follow up from those that do not need further workup. ***Patients and Methods:*** From total 150 patients presented with chronic diarrhea with or without bleeding per-rectum in outpatient clinic of Specialized Medical Hospital Mansoura university, only 60 patients involved in this study. Stool analysis and culture were done. Measurement of fecal calprotectin was performed by ELISA kit. Inflammatory biomarkers as ESR, CRP, and P-ANCA, were tested. Full colonoscopy with histopathological examination was performed. ***Results:*** The frequency of diseases based on diagnostic colonoscopy and pathological examination were as follow; 19 patients with irritable bowel syndrome (31.67%) as non organic gastrointestinal disease (GIT) versus organic GIT diseases 41 patients (68.33%): 32 patients with ulcerative colitis (53.33%), 2 patients with crohn's disease (3.33%), 2 patients with diverticulitis (3.33%), 2 patients with eosinophilic gastroenteritis (3.33%) and 2 patients with cancer colon (3.33%). There was remarkable difference between organic GIT diseases versus non organic groups as regards fecal level of calprotectin ($p<0.001$). Fecal calprotectin value of ≥350 ug /g was diagnostic of ulcerative colitis with sensitivity of 81.82% , specificity of 85.19% ,PPV of 86.32%, NPV of 80.39%.***Conclusion:*** Fecal marker, Calprotectin is helpful as an adjunctive tool in overall evaluation of patients with nonspecific symptoms and as a diagnostic tool in those with inflammatory disease. It is less invasive than colonoscopy and can help guide management in a more cost-effective manner.

KEYWORDS: Inflammatory bowel disease, irritable bowel syndrome, fecal calprotectin, gastrointestinal disease, Positive predictive value (PPV), Negative predictive value (NPP).

INTRODUCTION

Inflammatory bowel disease (IBD) is a life-long disorder that includes two major forms of chronic intestinal inflammation: Ulcerative colitis (UC) and Crohn's disease (CD) The etiology of IBD is not yet fully understood, but the disorder seems to arise from interactions between genetic and environmental factors.(1)IBD is a chronic condition characterized by recurrent episodes of inflammation in the gastrointestinal tract . Patients with IBD experience diarrhea, abdominal pain and cramps, disrupted digestion, rectal bleeding, weight loss and a substantial personal burden.(2) In addition, in IBD many symptoms are similar to the functional non inflammatory disorder like irritable bowel syndrome (IBS). A challenge is thus to distinguish symptoms of IBD from IBS (3), due to the chronicity of these conditions and the early onset of symptoms in the majority of cases. (4) The gold standard to establish IBD diagnosis remains in the hands of endoscopists and pathologists. Precise activity assessment of IBD is essential to determine extent and severity of disease for optimized therapy. (5) Recent studies have identified mucosal healing on endoscopy as a key prognostic parameter in the management of IBD. (6)

Disease activity questionnaires and laboratory inflammatory markers although widely used, show an unreliable correlation with endoscopy and histology, So new markers are needed for detecting and quantifying bowel inflammation and much efforts have been made to identify noninvasive biomarkers able to select patients requiring invasive procedures and avoid unnecessary examinations.(7)

Calprotectin is a 36 kilodalton calcium and zinc binding protein, it is present in neutrophils and monocytes and is released by activation of these cells in plasma, urine, stools and other media as a consequence of disease activity.(8) It represents 60% of cytosolic protein in neutrophils, a major player in gut intestinal

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inflammation.[9] Calprotectin released from neutrophils after cell death or rupture induces apoptosis in other cells as a results, levels of calprotectin from apoptotic neutrophiles rise during cell activation and turn over under inflammatory conditions.[10] In normal individuals most circulating neutrophils migrate through the mucosal membrane of the gut wall and lysis within the gut lumen, this account for the median fecal level of 2.0 mg/L seen in healthy individual.[11] As it is resistant to colonic bacterial degradation and drugs, and stable outside the body for days, fecal calprotectin is a marker of inflammation in IBD. [12]

Aim of this work: was to evaluate the clinical usefulness of fecal calprotectin level as noninvasive marker to distinguish patients in need of intensified follow up from those that do not need further workup and to estimate the possible economic effects of a sequential testing strategy with fecal calprotectin to minimize colonoscopies.

Patients and methods:

This prospective study comprised one hundred and fifty patients who presented with chronic diarrhoea with or without bleeding per-rectum at gastroenterology clinic of the Specialized Medical Hospital, Mansoura University. All outpatients referred to our tertiary hospital for colonoscopy from June 2011 to December 2012. Written informed consents were obtained from all subjects before enrolment. The Ethical Scientific Committee of the Faculty of Medicine, Mansoura University approved this study**.** Only 60 patients out of 150 completed the study, the remaining were diagnosed as infectious colitis and respond well to antibiotics guided stool culture. All patients were subjected to:

Thorough historical evaluation with stress on frequency and consistency of diarrhea per day. Analysis of any associated symptoms as weight loss, fever, bleeding per-rectum.

Thorough physical examination with stressing on pulse and temperature.

Laboratory investigations:

1- A 6 ml venous blood was withdrawn from each subject ,3 ml on EDTA for CBC and ESR, 3 ml blood collected into a plain tube, prompt separation of serum was done for assay of serum albumin and CRP. Part of serum was stored at – 20˚C until assay of CMV antibody and P-ANCA (in ulcerative colitis cases) . Complete blood count was performed using coulter B66, Miami, Florida, USA, serum albumin was done on Synchron CX9 autoanalyzer (Brea, California, USA), and Quantitative CRP was done using nephelometry.
2- Stool analysis and culture to exclude the presence of infection.
3- CMV antibody was done using ELISA assay (Inova Diagnostics, San Diego,CA), while atypical P-ANCA detection was done by Indirect immunoflourescense (Inova Diagnostics, San Diego, CA/EuroImmun, Germany).
4- Measurement of fecal calprotectin: stool sample was taken into dry, well capped container and stored at -20ºC until analysis.

Detection of fecal calprotectin in stool using immune diagnostic AG ELISA kit, (MRP 8/14) Stubenwald-Allee 8a–D-64625 Bensheim as follow:

100 mg of stool was suspended in 5 ml extraction buffer (diluted 1:2.5) using vortex, the suspension was centrifuged and diluted 1:50 (20µl supernatant + 980 µl wash buffer (diluted 1:10).Then 100 µl of this diluted supernatant and reconstituted standards and controls were added into respective wells, covered and incubated for 1 hour at 37 ˚C on a horizontal mixer. After incubation, wells were washed 5 times, dried and 100 µl of conjugated (diluted 1:100) were added to each well, then the plate was incubated for 1 hour at 37 ˚C on a horizontal mixer. After incubation, wells were washed 5 times, dried and 100 µl of substrate were added, the plate was incubated for 10-20 minutes at room temperature, then the reaction was stopped using 50 µl stop solution. The absorbance was determined with ELISA reader at 450 nm and the results were calculated by bloating standard curve using 4-parameter- algorithm.

Radiological investigations: abdominal ultrasonography, and barium enema in selected cases.

Full colonscopy and endoscopic findings were collected by the endoscopists by a standardised questionnaire. According to this questionnaire, the following endoscopic diagnoses were considered: normal endscopic signs, active ulcerative colitis, active crohn´s disease, diverticula with peridiverticular inflammation, ischemic colitis, colorectal cancer or polyps and miscellaneous diagnoses.

Endoscopic activity of ulcerative colitis and Crohn´s Disease were evaluated by Truelove & Witt's scoring system and Crohn´s Disease endoscopic Index of Severity (CDEIS) respectively.

Patients with one or more of the following criteria were excluded from the study:

1. Diabetes mellitus.
2. Thyroid disorders.
3. Immnuo-compromised patients or on chemotherapy.
4. Patients diagnosed as diarrhoea type-IBS, already on treatment without flag alarming symptoms.

Statistical Analysis:

The statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA, version 17.0 for Windows). All quantitative variables were expressed as the mean ± standard deviation.

Comparisons between the groups were performed using the Student's t-test whenever applicable. The qualitative or categorical variables were described as proportions. Proportions were compared using the chi-squared test or Fisher's exact test whenever applicable. Correlations between fecal calprotectin and other variables were established by using Pearson or Spearman's rank correlation. Mann-Whitney U-test was used for the continuous ordinal data between two qualitative variables. Variables that achieved statistical significance with the univariate analysis were included in multiple regression analysis to evaluate the independent factors associated with high fecal calprotectin. P values < 0.05 were considered statistically significant.

RESULTS

From total 150 patients presented with diarrhea with or without bleeding per-rectum in outpatient clinic, only 60 patients involved in this study whom were not respond to medical treatment, underwent specific laboratory investigations and diagnostic colonoscopy. The basic patient's characteristics of non-organic GIT diseases versus organic GIT diseases were mentioned in table (1). IBS was more in female gender, there was statistically significant difference between both groups as regards decreased hemoglobin level, elevated white blood cells count although its value within normal ranges elevated ESR & CRP as markers of inflammation, occurrence of bleeding per rectum in organic group. There was remarkable difference between organic GIT diseases versus non organic groups as regards fecal level of calprotectin.

Table 1: Patient's characteristics of non-organic GIT diseases versus organic GIT diseases.

	Non organic GIT disease no=19	Organic GIT disease no=41	p value
Age (years)	37.21±9.94	34.54±9.49	0.322
Sex			
Male	5	24	0.02
Female	14	17	
HB (g/dl)	11.60±0.58	9.47±2.28	<0.001
Platelets (10^9/L)	287.89±9.177	298.49±90.374	0.187
WBC (10^9/L)	4.90±0.90	7.01±2.74	<0.001
ESR (ml/h)	12.11±2.18	39.2±3.39	<0.001
CRP(mg/dl)	6	27.19±15.617	<0.001
S. albumin (g/dl)	3.72±0.11	3.29±0.81	0.147
Pulse (/minute)	71.84±4.9	77.22±10.19	0.03
Temperature (ºC)	37.11±0.05	37.21±0.36	0.36
Frequency of diarrhea			
Mild (<4/day)	5	9	0.442
Moderate (4-6/day)	8	12	
Severe(>6/day)	6	20	
Bleeding per rectum	0	19(31.66)	0.005
Fecal calprotectin (ug/gm)	12.5	989.134±728.18	<0.001

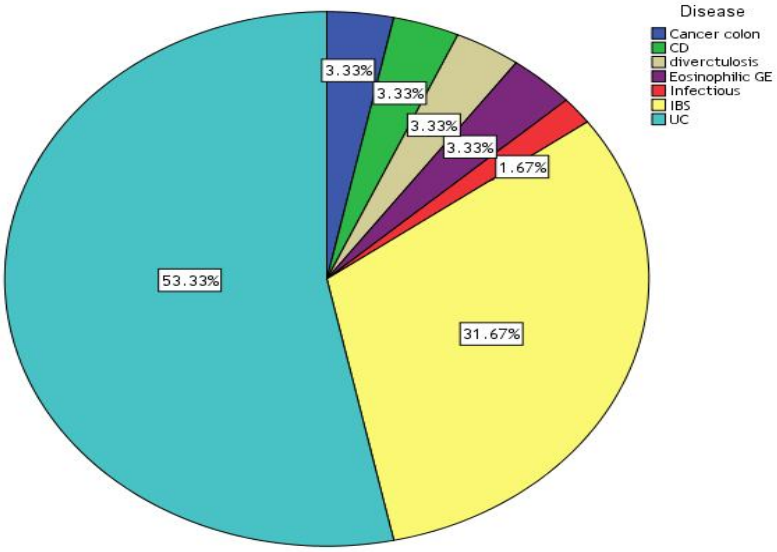

Figure 1: Distribution of all diseases in the studied population :CD**(**crohn's disease) ,GE(gastroenteritis**),** UC**(**ulcerative colitis**)** IBS**(**irritable bowel syndrome**).**

From total 60 cases, 32 cases were diagnosed as ulcerative colitis (53.33%), the remaining cases diagnosed as different entities; IBS 19 patients (31.67%), 2 cases as crohn's disease (3.33%), their values for fecal calprotectin were 1325 and 875 ug/g , 2 cases were diverticulitis (3.33%), with fecal calprotectin levels 150, 125 ug /g respectively, 2 cases with Eosinophilic gastroenteritis represented (3.33%) with fecal calprotectin levels 237.5, 1315 ug /g and 2 cases with cancer colon (3.33%), with values 525, 350 ug/g (figure 1).

Table 2: Patient's characteristics of ulcerative colitis group.

Parameter	Mean ±SD or no (%) total no= 32
Age(year)	33.13±7.79
Sex	
Male	19(59.4%)
Female	13(40.6%)
HB (g/dl)	9.25±2.33
Platelets (10^9/L)	303.44±89.23
WBC (10^9/L)	6.81±2.74
ESR (ml/h)	43.16±23.71
CRP (mg/L)	30.41±14.68
S. albumin (g/dl)	3.25±0.84
Pulse (/minute)	77.19±10.91
Temperature (ºC)	37.20±0.349
Frequency of diarrhea	
Mild (<4/day)	9(28.1%)
Moderate (4-6/day)	4(12.5%)
Severe(>6/day)	19(59.4%)
Bleeding per rectum	19(59.4%)
Fecal calprotectin (ug/gm)	1110.22±743.20
Disease location	
Rectal	5(15.6%)
Lt side colon	6(18.8%)
All colon	11(34.4%)
Ileocolonic	10(31.2%)
Pseudopolyps	4(12.5%)
Truelove & Witt's scoring system	
Mild	8(25%)
Moderate	7(21.9%)
Severe	17(53.1%)
P-ANCA (IU/L)	7(21.87%)
CMV-IgM Ab (IU/L)	3(9.37%)

Patient's characteristics of ulcerative colitis were demonstrated in table (2).

There was a positive correlation between fecal calprotectin level and chronicity of the disease ($p<0.01$), male gender ($p<0.007$), increased inflammatory markers as ESR ($p<0.001$) and CRP ($p<0.001$), elevated white blood cells count ($p<0.01$), increased pulse rate ($p<0.02$), increased frequency of diarrhea ($p<0.001$), occurrence of bleeding per-rectum ($p<0.009$), more involvement of colon ($p<0.001$) and finally with severity of ulcerative colitis ($p<0.001$). On the other hand, there was a negative correlation between fecal calprotectin level and age of the patients ($p<0.03$), decreased hemoglobin level ($p<0.001$), and serum albumin ($p<0.002$) (table 3).

Table 3: Correlation between fecal calprotectin and other parameters in ulcerative colitis group.

Parameter	Correlation Co-efficient	p value	95% Confidence Interval	
			Upper bound	Lower bound
Age(year)	-.277	0.03	-2.68	8.028
Sex	.465	0.007		
Duration (days)	.303	0.01	-2.07	0.21
HB (g/dl)	-.511	0.001	1.059	3.194
Platelets (10^9/L)	.172	0.18	-52.38	31.19
WBC(10^9/L)	.311	0.01	-3.40	-0.81
ESR (ml/h)	.765	0.001	-37.83	-16.20
CRP (mg/L)	.790	0.001	-28.40	-13.99
S. albumin (g/dl)	-.392	0.002	0.054	0.808
Pulse (/minute)	.299	0.02	-10.319	-0.436
Temperature (ºC)	.101	0.442	-.207	0.109
Increased frequency of diarrhea	.561	0.001		
Bleeding per rectum	.453	0.009		
Involvement of colon	.618	0.001		
Truelove & Witt's scoring system	.892	0.001		

Table (4) demonstrates the linear regression analysis of fecal calprotectin to other parameters. High level of fecal calprotectin could predict presence of high WBCs (p=0.01), low level of hemoglobin (p<0.001), high ESR (p<0.001) and CRP(p<0.001), low serum albumin(p=0.002), high pulse rate (p=0.02), increased frequency of diarrhea (p<0.001), occurrence of bleeding per-rectum (p<0.001), more involvement of colon(p<0.001), and more active disease(p<0.001).

Table 4: Regression analysis between fecal calprotectin and other parameters in ulcerative colitis group

Parameter	B	p value
Age(year)	37.791	0.03
Sex	1.496	0.725
Duration(days)	2.225	0.01
HB (g/dl)	11.137	<0.001
WBC(10^9/L)	5.633	0.01
ESR(ml/h)	14.645	<0.001
CRP(mg/L)	8.916	<0.001
S. albumin(g/dl)	3.677	0.002
Pulse(/minute)	37.04	0.02
Increased frequency of diarrhea	5.183	<0.001
Bleeding per rectum	.037	<0.001
Involvement of colon	1.343	<0.001
Truelove & Witt's scoring system	.370	<0.001

Fecal calprotectin value of ≥350 ug /g with ROC 0.931 (95% CI, 0.864 to 0.971) was diagnostic of ulcerative colitis with sensitivity of 81.82% (95% CI, 64.5 to 93), specificity of 85.19% (95% CI, 66.3 to 95.8), PPV of 86.32%, and NPV of 80.39% (Table 5, and figure 2).

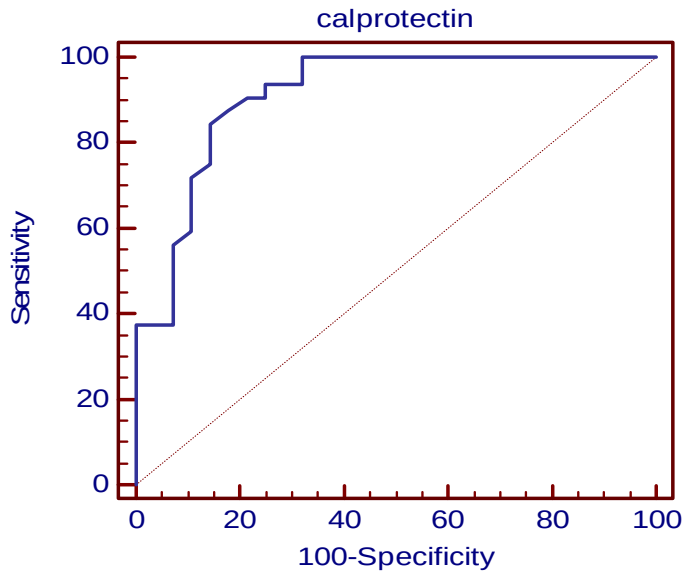

Figure 2: Receiver Operating Characteristics (ROC) curve for fecal calprotectin results in ulcerative colitis patients.

Table 5 : Accuracy of fecal calprotectin measurement in diagnosis of ulcerative colitis.

Value± 95% confidence interval	
Cutoff value (ug/gm)	**≥350**
AUROC	**0.931 (0.864 to 0.971)**
Sensitivity (%)	**81.82 (64.5 – 93)**
Specificity (%)	**85.19 (66.3 - 95.8)**
Positive predictive value (%)	**86.32(68.861 to 96.062)**
Negative predictive value(%)	**80.39(61.502 to 92.7)**
Positive likehood ratio	**5.52**
Negative likehood ratio	**0.21**

DISCUSSION

inflammatory bowel diseases are very common, most of them are functional not organic in nature but clinicians suffer a lot in differentiating between the two categories especially the management is completely different.(13) Many methods of assessing bowel inflammation have been proposed, including laboratory indices and clinical scores that generally are considered nonspecific and many cases the definitive diagnosis needs invasive procedures including lower colonoscopy and biopsy for histopathological documentation .(14) In many studies, fecal calprotectin has been claimed to be a specific, sensitive, non-invasive, cheap and accessible marker for gut inflammation.(15)

In our study, fecal calprotectin can significantly differentiate organic gastrointestinal diseases including UC from non organic disorders including IBS patients ($p<0.001$). In the IBD, many symptoms are similar to the functional disorder IBS. A challenge is thus to distinguish symptoms of IBD from IBS. ***Jelsness-Jørgensen*** et al., concluded that calprotectin levels are elevated in subgroups of IBD patients that are in remission and have coexisting IBS-like symptoms. (3) Fecal calprotectin can still differentiate inflammatory bowel diseases from functional bowel disorders. Comparison studies have found an overall diagnostic accuracy in IBD of 80% to 100% for calprotectin. (16) CRP is one of the many acute phase proteins that increase in the serum of patients with acute phase IBD. It is more sensitive in cases of CD than UC. So, use of this marker alone to identify patients with symptoms compatible with IBD that undergo further evaluation would delay diagnosis for many cases. (17)

In this study, we found that there is a female predominance in patients with IBS, gender differences in IBS are well established. In one of the pivotal studies in this area, ***Drossman*** et al (18) noted that the ratio of men:women with IBS was as high as 1:2, also ***Ambe***r, 2013 found that IBS is twice more common in female than male and he explained this high incidence due to several physical, chemical, social and emotional differences between male and female gender.(19) patients with organic gastrointestinal diseases were significantly presented by high pulse rates although within normal value, high ESR and CRP, high WBC count, bleeding per rectum and high fecal calprotectin level. Similarly ***Xiang et al,.*** 2008 found that the patients with active UC had higher levels of CRP and ESR than patients with inactive UC and control group.(20)This could be explained by the fact that these parameters are increased in inflammatory conditions as acute phase reactant.

Anemia is a frequent and serious complication in patients with IBD affecting about one third of the patients. Although in many cases anemia correlates the clinical activity of the disease, many patients in remission have anemia, due to iron, vitamin B12 and/or folic acid deficiency which have important consequences in the clinical status and quality of life of the patients. (21)

In this study, the mean of fecal calprotectin levels were 1325 µg/g in UC, 875µg/g in CD, and 525µg/g in cancer colon. Using ROC statistics, a cutoff value of 350µg/g indicated the presence of ulcerative colitis with a sensitivity of 81.82% and a specificity of 85.179.5% (positive predictive value (PPV) 86.3%, negative predictive value (NPV) 80.39%). In a recent study by ***Nancey et al.***, fecal calprotectin concentrations correlated closer with endoscopic scores in UC ($r =0.75$; $p < 0.001$). This study was in accordance with our result ($r=0.892$; $p<0.001$). Using cutoff of 250 µg/g for fecal calprotectin, in addition, fecal markers had similar overall accuracies to predict endoscopic activity in patients with UC (88%), whereas accuracies of C-reactive protein were lower. (22)

In a study by ***Dranga et al.,*** 50 patients with UC the calprotectin values were evaluated during the active disease. The correlation between the calprotectin values and lesions localization was analyzed, without finding any statistical differences. The data have shown a very strong correlation between the severity of the active disease, assessed through the UCDAI (Mayo) score, and the calprotectin value. (23)

Our study revealed that, fecal calprotectin level could be predictor of increased white blood cell count, high ESR and CRP, frequency of diarrhea, occurrence of bleeding per-rectum, more involvement of colon, and activity scoring system in ulcerative colitis patients. On the same side ***Hesham et al***.,2011 revealed that faecal calprotectin correlated significantly with the TLC, PLT count, ESR, CRP and UC activity index, however non-significantly with CD activity index.(24)

Another study evaluated the diagnostic accuracy of fecal calprotectin against complete colonic and small bowel endoscopy in 83 adult patients referred for suspected IBD. In total, 40 patients were diagnosed with the optimal cutoff of fecal calprotectin was 150 µg/g, providing 85% sensitivity and 81% specificity, with AUROC for fecal calprotectin significantly superior to that of CRP.(25) ***Tibble et al***., 2002 found that faecal calprotectin at cut off value of 10 mg/L had maximal sensitivity and specificity of 89% and 79% respectively with a PPV of 76% and a

NPV of 89% in differentiating patients with organic and non organic intestinal diseases.[26] ***Carroccio et al.***, 2003 found that the calprotectin value with the highest diagnostic accuracy was 170 µg/g, it was 100% sensitive and 95% specific in differentiating CD from IBS adult patients.[27] Also, ROC curve analysis showed a sensitivity of 95%, specificity of 91%, and an area under the curve (AUC) of 0.95 for the diagnosis of IBD .[28]

A recent retrospective cost–minimization analysis further stressed the potential use of fecal calprotectin as a screening tool, because it resulted in a 50% reduction of the estimated demand of colonoscopies when the 50 µg/g cutoff was used and 67% reduction when the cutoff was doubled to 100 µg/g, with a cost avoidance of €1.57 million and €2.13 million, respectively.[29,30]

The wide ranges of different cutoffs and different numbers of these studies make it difficult to have a universe optimal cutoff point for fecal calprotectin with different sensitivities. In comparing results from different studies with our study, it is important to define the test operating characteristic to compare cutoff points. Beside, because positive and negative predictive values based on the prevalence of disease in different population and countries so it changes with wide change in the study population.

CONCLUSION

Fecal marker, Calprotectin is helpful as an adjunctive tool in overall evaluation of patients with nonspecific symptoms and as a diagnostic tool in those with inflammatory bowel diseases. They are less invasive than colonoscopy and can help guide management in a more cost-effective manner.

Acknowledgment

The authors declare that they have no conflicts of interest in this research.

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