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Research Article

Diagnostic Performance of Faecal Calprotectin in Asian Patients

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Abstract

Background and Aim: Faecal calprotectin (FC) is useful for detecting inflammation of the intestinal mucosa which can help to diagnose organic bowel disorders such as inflammatory bowel disease (IBD). The manufacturer's recommended cut-off level is 50 µg/g to diagnose pathology, but there is lack of data in Asian patients. We correlated the FC levels with the colonoscopy findings in Singaporean patients..

Methods: Consecutive patients scheduled for colonoscopy were invited for the study between July 2015 to November 2016. The FC levels were determined from the stool specimens collected around the time of the colonoscopy. The colonoscopy findings, histology results and relevant clinical data were collected from the medical records. Subjects were categorized into organic bowel disease (OBD) and non-organic bowel disease (NOBD). The FC levels were correlated with colonic findings and analysis of data was carried out using SPSS21.

Results: A total of 292 patients were included in the final analysis with 104/292 adjudicated to have OBD. In multivariate analysis, colorectal carcinoma (CRC), colitis and adenomas ≥ 10mm were independent predictors for FC ($r = 0.592$, $p = 0.049$). For the prediction of OBD, FC has an AUROC = 0.843 (95% CI 0.795-0.890, $p < 0.001$). The optimal FC cut-off was 104 µg/g and corresponded to a sensitivity of 80.8% and specificity of 75.5%.

Conclusion: The optimal FC cut-off level was found to be higher in our study compared to the manufacturer's recommendation. FC is a reasonably accurate test for OBD. Further research into its clinical utility is required.

Keywords: *Endoscopy; Gastrointestinal; Irritable Bowel Syndrome; Inflammatory Bowel Disease; Calprotectin; Faecal; Organic Bowel Disease*

Introduction and Aims

Gastrointestinal complaints are amongst the most common reasons for seeking medical attention. Determining the underlying cause can be challenging and often requires costly and invasive investigations such as a colonoscopy. Simple non-invasive tests may reduce this need.

Faecal calprotectin (FC) is one such measure. Calprotectin is a 24 kDa dimer of the calcium binding proteins S100A8 and S100A9, and accounts for up to 60% of the soluble protein content in the neutrophil cytosol [1-4]. Faecal calprotectin results from influx of neutrophils into the intestinal lumen and thus is a marker of inflammation of the lower intestinal tract [5,6].

Faecal calprotectin is useful in distinguishing between irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Several systematic reviews and meta-analysis have demonstrated that a FC cut-off of 50 µg/g has high sensitivity and specificity for differentiating IBD from IBS [7-11]. The United Kingdom National Institute for Health Care and Excellence (NICE) recommends the use of FC by primary care physicians to help determine whether further specialist assessment for IBD is required in adults patients with gastrointestinal symptoms [12].

The prevalence of IBD in Asia has been increasing in recent decades [13]. Prevalence rates in Japan and Korea has shown a 3 to 10-fold increase in the incidence of IBD [14,15]. The costs associated with expensive investigations to diagnose IBD versus other organic pathology for patients is imposing huge economic burdens on local healthcare systems in Asia [16,17]. Most of the available literature for the diagnostic cut-offs for FC is based on data in Western patients. It is uncertain whether this can be extrapolated to Asian subjects.

Therefore, we conducted a study to describe the correlation of FC with colonoscopy findings in an Asian population. The diagnostic performance of FC in predicting organic vs non-organic bowel disease was determined. The optimal cut-off was calculated and evaluated against the manufacturer recommendation of a cut-off level of 50 µg/g to diagnose pathology [7].

Methods

Patient selection

The study was performed at Alexandra Health, Khoo Teck Puat Hospital Yishun Singapore between July 2015 and November 2016. Approval was granted by the local ethics committee. Consecutive patients who were scheduled for colonoscopy from either outpatient clinics or from hospital wards were screened for eligibility. Exclusion criteria included age below 21 or greater than 95 years, current pregnancy, or unable to give informed consent. Those with active infection for infectious gastroenteritis, ingestion of non-steroidal anti-inflammatory medications or proton pump inhibitors in the past 2 weeks were also excluded from recruitment. Patients were selectively recruited into the study depending on the pathological findings after colonoscopy. This was necessary to ensure there were adequate numbers of patients with less common colonic findings (e.g. colorectal carcinoma vs normal colonoscopy) so that statistical power could be achieved. Relevant clinical and demographic data of recruited subjects was obtained through medical records.

Stool collection and measurement of calprotectin

Stool specimens were collected within a mean +/- 2 days (SD 7.0) of colonoscopy. Subjects were requested to send their stool specimens to the laboratory within 24 hours of the bowel motion. After submission to the laboratory, specimens were kept in a freezer (minus 20°C) until analysis. The BUHLMANN CALEX® Elisa test kit was used to determine the calprotectin with the methodology as follows: Using an inoculation loop, 50 - 100 mg of the stool sample was placed in an empty polypropylene tube with extraction buffer that is 49x the weight of the stool added. The sample was then homogenized on a vortexer after which the homogenate is further centrifuged. The supernatant is decanted and 100 µl is placed onto a microtiter plate that is pre-coated with a monoclonal capture antibody highly specific to the Calprotectin heterodimeric and polymeric complexes. After incubation for 30 minutes at room temperature and a washing step, a detection antibody conjugated to horseradish peroxidase detects the calprotectin molecules bound to the monoclonal antibody coated onto the plate. There is further incubation and washing step, after which tetramethylbenzidine is added followed by a stopping reagent.

The absorption is measured at 450 nm. The measuring range of the test was 10 - 600 µg calprotectin/g faeces with an intra- and inter-assay coefficient of 4.7% and < 15%, respectively. The quantification of calprotectin values > 600 µg/g was performed with additional dilution series of the extracts.

Determination of the final diagnosis and defining organic bowel disease

The colonoscopy and histology results (whenever biopsies were taken) and relevant clinical data for each recruited subject was reviewed independently by 2 gastroenterologists (R.K and T.A) blinded to the FC results. The final diagnosis of each case was determined by consensus. Patients were categorised as having "Organic Bowel Disease" (OBD) when there were any of the following findings: colorectal carcinoma (CRC), large adenomatous polyps, and any form of inflammation (colitis, ileitis or proctitis) or ulceration. Patients were categorised as Non-organic bowel disease (NOBD) when there was a normal colonoscopy or clinically insignificant benign abnormalities, which included uncomplicated diverticulosis, haemorrhoids, miscellaneous benign pathology (e.g. colonic lipoma, leiomyoma, healed solitary rectal ulcer). With regards to polyps, adenomas greater/equal 10 mm were categorised as OBD, while adenomas less than < 10mm or hyperplastic polyps were categorised as NOBD. The rationale for this is based the findings from the CEDAR study, which showed that FC was able to have reasonable diagnostic accuracy when only adenomas ≥ 10 mm was considered as organic disease, but not when all polyps were included [18].

Analysis of results was carried out using SPSS 21. The relationship between FC and other independent variables was examined in univariate analysis which included calculating the median, interquartile range, minimum/maximum values and Pearson's correlation coefficient. The comparison of the distribution of FC between different colonic findings was performed with the Mann-Whitney U test. Multivariate analysis was performed using multiple linear regression. The diagnostic performance of FC for OBD was determined by calculating the area under the receiver operator characteristics curve (AUROC). The coordinates of the curve were used to determine the FC cut-off values for the corresponding sensitivity, specificity and diagnostic odds ratios. In all instances, a significant p-value was taken to be < 0.05. The optimal cut-off was determined with the Youden Index (YI).

Results

Patient characteristics

A total of 292 patients were recruited and included in the final analysis. The mean age was 56.2 years (yrs) standard deviation (SD) 13.2 yrs. The proportion of male subjects was 57.5% (168/292). The ethnic distribution of the study population was as follows: Chinese 78% (225/292), Malay 13% (39/292), Indian 7% (21/292) and others 2% (7/292). The most common indication for performing colonoscopy was rectal bleeding (40.4%) followed by altered bowel habit (20.9%). Table 1 summarises the patient characteristics.

Distribution of colonic abnormalities in the study population, and classification into organic versus non-organic bowel disease

There was a total of 104 out of 292 subjects adjudicated to have OBD. These subjects were diagnosed to have CRC (47/104 cases); any form of colitis or ulceration (47/104 cases); or adenomas ≥ 10 mm (23/104 cases). There were 13 subjects with more than 1 form of OBD. In the 47 subjects diagnosed with colitis, the most common aetiology was ulcerative colitis (15/47 subjects) followed by Crohns (9/47).

There were 188/292 subjects adjudicated to have NOBD. These subjects had a normal colonoscopy (49/188 cases), or were diagnosed to have the following abnormalities: polyps that were adenomas < 10mm or hyperplastic polyps (59/188); asymptomatic diverticulosis (35/188); haemorrhoids (94/188), other benign pathology (7/188). There were 56 subjects who had more than one abnormality classified as NOBD. Table 2a and 2b provide a summary of each number and type of diagnoses classified into OBD and 13 patients had more than one form of OBD. Hence the total for OBD is the sum of the number of cases of CRC, colitis and adenomas ≥ 10mm minus 13.

Patient Characteristics (n = 292)	
	N (%)
Mean Age years, SD	56.2 (13.2)
Male	168 (57.5)
Ethnicity	
Chinese	225 (78)
Malay	39 (13)
Indian	21 (7)
Others	7 (2)
Main Indication for Colonoscopy	
PR bleeding/FOBT Positive	118 (40.4)
Altered bowel habit (Constipation, diarrhea or both)	61 (20.9)
Abdominal Pain/Bloating	22 (7.6)
Anaemia	21 (7.2)
Asymptomatic Bowel Cancer Screening	20 (6.8)
Polyp/Post-operative CRC Surveillance	20 (6.8)
Monitoring of known IBD	12 (4.1)
PR mass	5 (1.7)
Perianal Fistula	4 (1.4)
Weight Loss	4 (1.4)
Others	5 (1.7)

Table 1: *Patient characteristics.*

Organic Bowel Disease			**N = 104/292**
	N (% within group)		**N (% within group)**
Colorectal carcinoma	47		
		Colorectal Carcinoma Only	36
Colitis	47		
Ulcerative Colitis	15	Colorectal Carcinoma + Adenoma ≥10 mm	10
Crohns	9		
IBD indeterminate	4	Colorectal Carcinoma + Colitis	1
Ischaemic	2		
Infective	6	Colitis Only	44
Solitary Rectal Ulcer syndrome +/- proctitis	6		
NSAID colitis	1	Colitis + Adenoma ≥ 10 mm	2
Idiopathic colitis	4		
		Adenoma ≥ 10 mm only	11
Adenoma ≥ 10 mm	23		
Non-Organic Bowel Disease			**N = 188/292**
Adenoma < 10 mm or Hyperplastic Polyps	59		
		Adenoma < 10 mm or Hyperplastic Polyps only	25
		Adenoma < 10 mm or Hyperplastic Polyps + Haemorrhoids	18
		Adenoma < 10 mm or Hyperplastic Polyps + Diverticulosis	9
		Adenoma < 10mm or Hyperplastic Polyps + Diverticulosis + Haemorrhoids	5
		Adenoma < 10 mm or Hyperplastic Polyps + Other Benign pathology	1
		Adenoma < 10 mm or Hyperplastic Polyps + Haemorrhoids + Other Benign Pathology	1
Diverticulosis	35	Diverticulosis Only	7
		Diverticulosis + Haemorrhoids	13
		Diverticulosis + Haemorrhoids + Other Benign Pathology	1
Haemorrhoids	94	Haemorrhoids Only	55
		Haemorrhoids + Other Benign Pathology	1
Other Benign Pathology	7	Other Benign Pathology Only	3
No structural abnormality	49		49

Table 2a: *Distribution of colonic abnormalities.*

Organic Bowel Disease			
Colorectal carcinoma		47	
Colitis		47	
	Ulcerative Colitis		15
	Crohns		9
	IBD indeterminate		4
	Ischaemic		2
	Infective		6
	Solitary Rectal Ulcer syndrome +/- proctitis		6
	NSAID colitis		1
	Idiopathic colitis		4
Adenoma ≥ 10 mm		23	
	Total	104/292†	
Non-Organic Bowel Disease			
Adenoma < 10 mm or Hyperplastic Polyps		59	
Diverticulosis		35	
Haemorrhoids		94	
Other Benign Pathology		7	
No structural abnormality		49	
	Total	188/292‡	

Table 2b: *Distribution of colonic abnormalities.*

† 13 patients had more than one form of OBD. Hence the total for OBD is the sum of the number of cases of CRC, colitis and adenomas ≥ 1 0mm minus 13.

‡ 56 patients had more than one form of NOBD. Hence the total for NOBD is the sum of the number of cases with normal colonoscopy, adenomas < 10 mm or hyperplastic polyps, diverticulosis, haemorrhoids and other benign pathology minus 56.

Correlation of faecal calprotectin with patient demographics and colonic abnormalities

In univariate analysis, a significant correlation was found between FC and the following: Chinese ethnicity, other ethnicities, colorectal carcinoma, colitis, haemorrhoids, other benign abnormalities, and adenomas that were ≥ 10 mm or had high grade dysplasia. However, in multivariate analysis, the only significant associations were CRC, colitis and adenomas ≥ 10mm. In the multivariate model, the correlation coefficient (r) was = 0.384, $p < 0.001$ when colitis was the sole predictor; $r = 0.585$, $p < 0.001$ when colitis and CRC were the predictors; and $r = 0.592$, $p = 0.049$ when colitis, CRC and adenomas ≥ 10 mm were the predictors. Table 3 provides a summary.

Patient Characteristics	Pearsons Correlations (r)	p-Value(univariate)	R (multivariate model)	P-value (multivariate)
Age (yrs)	0.112	0.028		
Male Gender	-0.058	0.160		
Ethnicity				
Chinese	-0.114	0.026		
Malay	0.052	0.188		
Indian	0.046	0.214		
Others	0.119	0.021		
Colonic abnormalities				
Malignant	0.371	< 0.001*	0.592	0.049
Colitis	0.384	< 0.001*	0.592	0.049
Diverticulum	0.005	0.469		
Haemorrhoids	-0.216	< 0.001		
Normal	-0.205	< 0.001		
Other Benign Abnormalities	-0.073	0.105		
Polyps				
All polyps	0.051	0.192		
≥ 3 polyps	0.006	0.461		
≥ 10 mm Polyp	0.162	0.003*	0.592	0.049
High Grade Dysplasia	0.142	0.008		

Table 3: *Correlation of faecal calprotectin with patient demographics and colonic abnormalities.*

The FC results of colonic abnormalities were compared against the FC results of normal colonoscopies. The FC in those with normal colonoscopy was 37 μg/g (median), 20 - 131 μg/g (IQR). The FC of was significantly greater in colorectal carcinoma (median 697 μg/g, IQR 170 - 927 μg/g, $p < 0.001$), colitis (710 μg/g, 158 - 861 μg/g, $p < 0.001$) and polyps (72 μg/g, 7 - 1083 μg/g, $p = 0.031$). Adenomas ≥ 10 mm did not show a significant difference, but the numbers were small (n = 11/23) because more than half the cases (n = 12/23) were excluded due to the presence of other co-existing colonic abnormalities. No difference was also found between those with diverticulosis and "other benign lesions". Table 4 provides a summary.

	N	Median, Interquartile range (µg/g)	Range	Vs. Normal Colonoscopy P-value
Normal Colonoscopy	49	37 (20 - 131)	10 - 823	n/a
Colorectal Carcinoma	14	697 (170 - 927)	61 - 1119	< 0.001
Colitis	27	710 (158 - 861)	21 - 1268	< 0.001
Polyps	40	72 (32 - 400)	7 - 1083	0.031
Adenoma ≥ 10 mm	11	66 (13 - 477)	10 - 1012	0.410
Adenoma < 10 mm or Hyperplastic Polyp	25	69 (12-243)	7 - 911	0.262
Diverticulosis	7	46 (19 - 511)	2 - 1382	0.734
Haemorrhoids	55	31 (11 - 76)	2 - 1384	0.122
Other Benign Abnormalities	3	47	29 - 75	0.883

Table 4: *FC results of colonic abnormalities VS FC results of normal colonoscopies.*

The diagnostic performance of faecal calprotectin for organic bowel disease

For the prediction of OBD, FC has an AUROC = 0.843 (95% CI 0.795 - 0.890, p < 0.001) (Figure 1). The optimal FC cut-off was 104 µg/g and corresponded to a sensitivity of 80.8% and specificity of 75.5% (YI = 0.56). The diagnostic odds ratio (DOR) for FC ≥ 104 µg/g is 13.0. As the main use of FC is to accurately exclude OBD so that further invasive testing is not required, a high sensitivity is desirable. At the manufacturer's recommended cut-off of > 50 µg/g, the overall accuracy was lower than our optimal cut-off of 104 (YI 0.44 vs 0.56). The sensitivity, specificity and DOR are 90.4%, 53.2% and 10.7 respectively. At a cut-off of > 20 µg/g, there is a higher sensitivity (95.2%) but with a trade off in specificity (30.9%), YI (0.26) and DOR (8.8). The diagnostic performance of FC is superior when it is used to predict any colonic abnormality considered to be OBD, rather than a specific colonic abnormality. Table 5 is a summary of the results.

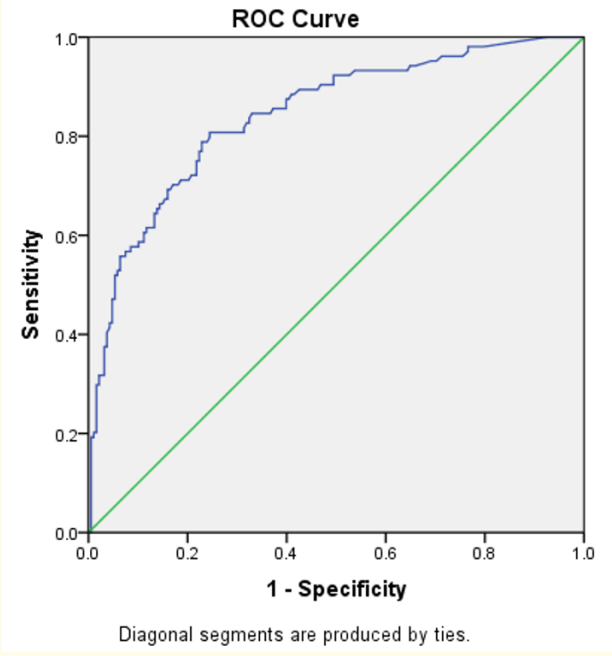

Figure 1: *The diagnostic performance of faecal calprotectin for organic bowel disease. AUROC = 0.843 (95% CI 0.795 - 0.890, p < 0.001).*

						Optimal Sens and Spec		FC Cut Offs ug/g corresponding to ~90, 95 and 100% sensitivity or specificity (Sens, Spec)					
	N	AUROC	95% CI	p-Value	FC cut-off	Sens	Spec	~90%Sens	~95% Sens	~100% Sens	~90% Spec	~95% Spec	~100% Spec
Organic Bowel Disease	104	0.843	0.795-0.890	< 0.001	104	80.8	75.5	51 (90.4, 53.2)	21 (95.2, 30.9)	10 (100, 7)	250 (58.7, 90.0)	440 (51.9, 94.6)	1385 (0, 100)
Malignancy	47	0.795	0.732-0.858	< 0.001	106	85.1	63.7	64 (89.4,49.4)	44 (95.7,41.6)	19 (100,23.7)	711 (40.4,89.8)	868 (29.8,95.1)	1337 (0,99.6)
Colitis	47	0.806	0.748-0.865	< 0.001	116	85.1	65.3	82 (91.5,57.1)	36 (95.7,38.8)	21 (100,25.7)	714 (38.3,90.2)	917 (19.1,95.1)	1385 (0,100)
Polyps	95	0.526	0.455-0.598	0.464	60	63.8	44.5	11 (86.2,13.6)	10 (97.9,6.1)	3 (100,1.0)	837 (13,89.9)	952 (8.5,94.9)	1337 (0,99.5)
Diverticulum	55	0.528	0.444-0.613	0.513	171	54.5	67.5	11 (85.5,1.0)	10 (96.4,0.1)	1 (100,0)	851 (9.1,89.9)	990 (3.6,94.9)	1255 (1.8,99.6)
Haemorrhoids	117	0.367	0.303-0.431	< 0.001	74	46.2	41.1	10 (89.7,9.1)	6 (94.9,4.5)	1 (100,0)	899 (4,90)	1047 (1.7,94)	1337 (0,99)

Table 5: *Diagnostic performance of FC for OBD.*

Discussion

To the best of our knowledge, this is the largest study in an Asian population comparing organic vs non-organic pathology using faecal calprotectin as a marker [17,19-22].

We determined from our data that a cut-off of 104 μg/g was found to be more accurate in determining OBD compared to the manufacturer's recommendation of 50 μg/g. However, higher overall accuracy was at the cost of lower sensitivity (sens 80.8% vs 90.4%). The best use of FC may be as a screening tool, and so a lower cut-off to optimise sensitivity but at the cost of specificity may be desirable. On the other hand, lower cut-offs will lead to greater false positives which may generate further costly investigations. The selection of the appropriate cut-offs should consider local economic and resource factors.

In our study, Faecal Calprotectin was found to have an inferior accuracy to pooled sensitivity and specificity analysis that was reported in meta-analysis (Sn 80.8%, Sp 75.5%, YI 0.56 vs Sn 93% Sp 96% YI 0.89 respectively) [9]. The main reason for FC to be less accurate was because we examined its performance to diagnose the broad spectrum of pathology that is considered OBD. Whereas in the meta-analysis, high diagnostic accuracy was observed because FC was studied in the rather specific situation of IBS vs IBD only. In these studies, patients are carefully selected such that they either have IBS or IBD, and non-IBD causes of elevated FC are excluded [8,23]. On the other hand, OBD consists of a mix of pathology including cancers and polyps, any type of inflammation and not just IBD. Hence the performance of FC will always be less accurate when it is used to determine OBD vs NOBD compared to the very specific situation of IBS vs IBD.

In spite of this, the manufacturer's recommended cut-offs of 50 μg/g is commonly used for general clinical application. We feel this would be a mis-interpretation of FC and that clinicians need to be aware of this issue.

Carroccio., *et al*. also examined OBD vs NOBD and found less reliable results compared to IBD VS IBS in adults. In this study, the YI was 0.44 (Sn 64%, Sp 80%) when a cut-off of 50 was used [6,24]. However, this study included patients with celiac disease in which the FC was shown to be less sensitive. This led to a decrease in accuracy in determining OBD (celiac disease was included) vs NOBD. This study finding is also limited by a smaller sample.

Tibble., *et al*. also examined FC levels in organic pathology vs IBS [6]. The study reported an optimal cut-off of 10 mg/L which corresponded to Sn 89%, Sp 79% YI 0.68 DOR 27.8. The difference in results can be attributed to a different type of population that was examined. This study specifically examined IBS patients (as fulfilled by the Rome 1 Criteria) vs organic pathology. We assessed all patients with non-organic pathology regardless of whether there was a diagnosis of IBS. The higher diagnostic accuracy of FC in this study may therefore be attributed to a different selection criterion.

When compared to similar studies, our data shows fairly consistent results. The highest DOR (13.0) is achieved at a cut-off 104 μg/g, which is within the range of confidence intervals reported by meta-analysis (DOR at the cut-off 100 μg/g = 33, 95%CI 3.5 - 1472.1) [11]. Furthermore, in contrast to IBS vs IBD studies, our study and meta-analysis of OBD vs NOBD did not find the optimal cut-off to occur at 50 μg/g, but rather at higher levels.

Our data in Asian patients have produced similar results to studies based on non-Asian populations for OBD. We did not find any differences in multivariate analysis between the ethnic groups and it would not appear that FC performance is any different, but further studies are needed.

Cost is an important issue as many Asian countries are of lower income and may not have as much financial capability for expensive procedures. Burden to the cost of healthcare should be weighed when referrals to colonoscopy are being made.

Conclusion

In the largest study Asian to date, the optimal FC cut-off level of 104 μg/g was found to be more accurate in our study compared to the manufacturer's recommendation of 50 μg/g. FC is a reasonably accurate test for OBD. Further research into its clinical utility.

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