Serum calgranulin C as a non-invasive predictor of activity among inflammatory bowel disease

Hany S. Rasmy, Noha A. Elnakeeb, Mohamed F. Mohamed, and Hossam S. Elbaz

Department of Internal Medicine, Hepatology & Gastroenterology Unit, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Corresponding author: Hany S. Rasmy, Department of Internal Medicine, Hepatology & Gastroenterology Unit, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Email: hanysamir@med.asu.edu.eg.

Abstract

Inflammatory bowel disease is a chronic immune-mediated disorder with a relapsing and remitting course. It leads to disabling gastrointestinal symptoms, low quality of life, and a significant burden for healthcare utilization and associated costs. Therefore, non-invasive biomarkers are needed for early diagnosis and follow up to avoid the complications of invasive diagnostic procedures. Calgranulin C is a calcium binding protein with proinflammatory properties. The aim of this study was to evaluate the role of serum calgranulin C as a non-invasive biomarker for diagnosis and prediction of activity in comparison to different biomarkers and endoscopic activity scores in inflammatory bowel disease. The study included 80 inflammatory bowel disease patients (50 Ulcerative colitis and 30 Chron's patients) and 20 normal controls. Complete blood picture, C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin and serum calgranulin C were measured. Colonoscopies with histopathological examination were done and different activity scoring systems assessed. Among ulcerative colitis group, serum calgranulin C was statistically significantly higher in comparison to control group [723.640±529.055 ng/ml versus 80.850±24.416 ng/ml]. Depending on the American college of gastroenterology ulcerative colitis activity index, fecal calprotectin and serum calgranulin C were statistically significantly higher among moderate to severe ulcerative colitis than those with mild activity and those in remission (p <0.001, for both). Regarding Crohn's disease group, serum calgranulin C was statistically significantly higher in comparison to control group [759.233±797.963 ng/ml versus 80.850±24.416 ng/mL]. Depending on Crohn's disease activity index, both serum calgranulin C and fecal calprotectin were statistically significantly higher among active disease than those in remission (p <0.001, for both). In conclusion, serum calgranulin C could be used as a non-invasive marker to predict activity and severity and to ensure remission among inflammatory bowel disease patients.

Keywords: Serum calgranulin C, Ulcerative colitis, Crohn's disease, inflammatory bowel disease.

Introduction

Inflammatory bowel disease (IBD) is a relapsing disease that requires ongoing proactive monitoring to determine appropriate treatments and follow-up strategies. To date, gastrointestinal endoscopy with histologic examination and contrast-enhanced imaging are

mandatory techniques for diagnosis and activity assessment.[1]

Ulcerative colitis (UC) is an IBD characterized by mucosal inflammation that begins in the rectum and spreads proximally, affecting the entire colon. Although the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) is a well-recognized scoring system for determining disease severity endoscopically, the Mayo endoscopic score (MES) is commonly utilized in clinical practice due to its ease of use.[2]

Crohn's disease (CD) is an IBD characterized by persistent relapsing and remitting inflammation of the gastrointestinal tract (GI) of uncertain cause. It can affect any portion of the GI tract, from mouth to anus, but it is primarily localized in the terminal ileum. It can affect the entire thickness of the bowel wall and leave unaffected areas between patches of diseased tissue. CD is characterized by discrete episodes of acute exacerbation of clinical symptoms (abdominal pain and diarrhea) and signs (elevated inflammatory markers, endoscopic and radiographic findings) followed by periods of clinical remission.[3]

Several serum inflammatory biomarkers have become common laboratory tests for the diagnosis of IBD. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were studied long enough to be used in IBD diagnosis. While neither test has the specificity or accuracy to be regarded as the gold-standard diagnostic method, CRP has several advantages over ESR. For example, the CRP concentration changes quicker than the ESR value in response to disease activity, CRP has a greater range of aberrant values than ESR, and unlike ESR, CRP does not show age-related fluctuation.[4]

Fecal biomarkers are proteins that are specifically identified in IBD patients' stool samples. To present, the fecal biomarkers for IBD that have been described are mostly fecal leukocyte proteins like calprotectin, calgranulin C, lactoferrin, and lipocalin-2. Calgranulin C (encoded by the S100A12 gene) is a calcium-binding protein that belongs to the S100 family of low-molecular-weight proteins which activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and increase cytokine release during pro-

inflammatory processes.[5] Calgranulin C levels were found to be elevated in various inflammatory disorders such as arthritis.[6]

In this study, we aimed to evaluate the usability of serum calgranulin C in determining the extent and severity of IBD disease in patients and to determine whether it can be used as a candidate marker for non-invasive diagnosis, evaluation, and monitoring of IBD.

Subjects and Methods

This observational study was carried out in the IBD clinics of Ain Shams University Hospital and Mahala Teaching Hospitals during the period January 2020 to March 2021. The study included 80 adult patients with IBD (50 Ulcerative colitis and 30 Chron's patients) which were diagnosed using clinical criteria and colonoscopy with biopsy, and 20 normal controls were included.

Subjects with vasculitis, rheumatoid arthritis, Psoriatic arthritis, respiratory distress syndrome, bronchial asthma and glomerulonephritis, pregnancy, lactation, indeterminate colitis, infectious colitis, concurrent infections, colonic malignancy, and history of colorectal surgery were excluded from the study.

The study population was subjected to a well-designed data sheet covering detailed medical history, physical examination, and baseline laboratory investigations, including CRP and ESR.

Fecal Calprotectin in stool samples using (POC Reader) technique as directed by the manufacturer (Quantum Blue Calprotectin, Bühlmann Laboratories AG, Switzerland). Serum Calgranulin C was measured using a double-antibody sandwich S100A12 enzyme-linked immunosorbent assay (ELISA) Kits (Catalog Number : MBS284909, My BioSource, company. Southern California, San Diego, USA), according to the manufacturer's instructions.

All patients underwent colonoscopy with multiple biopsies to confirm diagnosis, assess severity, and extent of the disease and were classified into Remission and activity according to different scoring systems.

Regarding UC patients, assessment of disease extent was according to the Montreal classification where E1: Ulcerative proctitis with involvement limited to the rectum; that is the proximal extent of inflammation is distal to the rectosigmoid junction, E2: Left-sided UC (distal UC) with involvement limited to a proportion of the colorectum distal to the splenic flexure, and E3: Extensive UC (pancolitis) with involvement extending proximal to the splenic flexure.[7]

The severity was determined using the Truelove and Witt's severity index, which relied on symptoms and basic clinical and laboratory testing, the Ulcerative Colitis Endoscopic Index of Severity (UCEIS), and the Mayo endoscopic sub score.[7]

The UCEIS was calculated as a simple sum of the following three descriptors: vascular pattern (scored 0-2); bleeding (scored 0-3); and erosions and ulcers (scored 0-3). As a result, the UCEIS score ranges from 0 to 8. Patients were classified into four groups: remission (UCEIS 0-1); mild (UCEIS 2-4); moderate (UCEIS 5-6); and severe (UCEIS 7-8).[7]

Mayo Endoscopic Sub score (MES). MES0: normal or inactive (no friability and granularity and intact vascular pattern). MES1: mild (mild erythema or decreased vascular pattern). MES2: moderate (marked erythema, absent vascular pattern, friability, and erosions). MES3: severe (spontaneous bleeding and ulceration).[7]

The American college of gastroenterology ulcerative colitis activity index (ACG-UCAI) was calculated by the following parameters: number of motions, blood in stool, urgency, hemoglobin level, ESR, CRP, fecal calprotectin, MES and UCEIS. It was subdivided according to the score into remission, mild, moderate to severe and fulminant.[8]

Regarding Crohn's disease, the assessment was done by Crohn's disease activity index (CDAI): including different variables with scoring as abdominal pain, general well-being, diarrhea, use of opiate for diarrhea, extraintestinal disease, abdominal mass, hematocrit value and body weight below standard. Accordingly, patients were subdivided into remission [0-149], mild [150- 220], moderate [221-450], severe [451-1100].[9]

Statistical Analysis

The statistical package for the social sciences (SPSS) V20 was used to perform data analysis. Statistical data are presented as mean, standard deviation, student t- test, Chi-square, Linear Correlation Coefficient and Analysis of variance (ANOVA) tests. The unpaired Student T-test was used to compare between two groups in quantitative data. The diagnostic value of serum calgranulin C was evaluated using receiver-operating characteristic (ROC) curve analysis. A p value of <0.05 was considered statistically significant.

Results

This study included 80 patients diagnosed with IBD and divided into: 50 patients with UC, they were 33 females and 17 males with mean age (34.52±9.346) years. The other 30 patients with CD, they were 17 females and 13 males with mean age (31.133±8.838). In addition, 20 apparently healthy controls, they were 11 females and 9 males with mean age (30.11±7.40).

Among the UC group, both serum calgranulin C and fecal Calprotectin were statistically significant higher in comparison to control group (p<0.001, for both) [Table 1]. BY applying different scoring systems for activity among UC patients, there was no statistically significant difference between left sided colitis (E2) and pancolitis (E3) (p > 0.05) [Table 2].

Table 1. Comparison of fecal calprotectin and serum Calgranulin C between ulcerative colitis patients and the control group.

Studied parameter		Studied groups		p-value
		Ulcerative colitis	Control	
Fecal Calprotectin	Range	15-1320	18-57	<0.001
	Mean ±SD	251.188±248.326	34.300±12.566	
Serum calgranulin C	Range	63-2000	52-135	<0.001
	Mean ±SD	723.640±529.055	80.850±24.416	

*t-Test, $p \leq 0.05$ is significant.

Table 2. Comparison of different ulcerative colitis activity scoring systems between subgroups of ulcerative colitis defined by extent (Montreal classification).

Ulcerative colitis		Montreal classification				p-value
		E2		E3		
		N	%	N	%	
Ulcerative Colitis Endoscopic Index of Severity	Remission	11	45.83	6	23.08	NS
	Mild activity	8	33.33	11	42.31	
	Moderate-severe activity	5	20.83	9	34.62	
Vascular pattern	Normal	12	50.00	8	30.77	NS
	Patchy obliteration	4	16.67	4	15.38	
	Obliterated	8	33.33	14	53.85	
Bleeding	Normal	21	87.50	18	69.23	NS
	Luminal mild	2	8.33	4	15.38	
	Luminal moderate or severe	1	4.17	4	15.38	
Erosions and ulcers	Normal	11	45.83	6	23.08	NS
	Erosions	1	4.17	1	3.85	
	Superficial ulcer	12	50.00	19	73.08	
ACG- UCAI	Remission	10	41.67	7	26.92	NS
	Mild activity	7	29.17	5	19.23	
	Moderate to severe activity	7	29.17	14	53.85	
Endoscopy (Mayo sub-score)	Mild disease	12	50.00	7	26.92	NS
	Moderate disease	10	41.67	10	38.46	
	Severe disease	2	8.33	9	34.62	

Chi-Square $p > 0.05$ is not significant (NS).

Depending on ACG-UCAI, the inflammatory markers (ESR, CRP) were statistically significantly higher among UC patients in activity than those in remission (p 0.005, 0.033, respectively) [Table 3]. Also, both fecal Calprotectin and serum calgranulin C were statistically significantly higher among moderate to severe UC than those with mild activity and those in remission (p <0.001, for both) [Table 3].

Table 3. Comparison of laboratory parameters, serum calgranulin C, calprotectin and Mayo sub-score between subgroups of ulcerative colitis according to ACG-UCAI.

Ulcerative colitis		ACG- UCAI						*p-value
		Remission		Mild activity		Moderate-severe activity		
		N	%	N	%	N	%	
Endoscopy (Mayo sub-score)	Mild disease	17	100.00	2	16.67	0	0.00	<0.001
	Moderate disease	0	0.00	10	83.33	10	47.62	
	Severe disease	0	0.00	0	0.00	11	52.38	

Table 3. Continued.

Ulcerative colitis		ACG- UCAI			#*p*-value
		Remission	Mild activity	Moderate-severe activity	
Hemoglobin (g/dl)	Range	8.4-14.9	8.8-14.9	7.9-15.9	NS
	Mean ±SD	12.376±1.689	11.300±1.924	11.662±2.176	
Platelet (X 10^9cells/L)	Range	162-386	200-485	176-609	NS
	Mean ±SD	292.529±70.513	342.000±87.472	331.190±110.249	
Total leucocytic count (X 10^9 cells/L)	Range	3.6-10.2	5.3-13.2	3.9-11	NS
	Mean ±SD	6.900±2.007	7.792±2.097	8.434±2.036	
ESR	Range	12-90	25-150	20-170	0.005
	Mean ±SD	35.294±22.025	66.583±32.701	71.667±41.002	
CRP	Range	0.3-22	2-96	3-48	0.033
	Mean ±SD	5.918±7.683	20.342±26.146	16.619±11.536	
Creatinine mg/dl	Range	0.6-1.2	0.5-1.1	0.56-1.2	NS
	Mean ±SD	0.888±0.154	0.789±0.174	0.928±0.214	
Urea mg/dl	Range	20-32	21-30	18-34	NS
	Mean ±SD	26.176±3.844	24.917±3.315	25.429±4.057	
ALT (U/L)	Range	10-61	12-24	12-45	NS
	Mean ±SD	18.882±12.257	16.667±3.822	20.095±7.395	
AST (U/L)	Range	12-40	14-32	14-70	NS
	Mean ±SD	20.000±7.697	19.500±5.745	23.429±11.531	
Albumin (g/dl)	Range	3.4-5	3.7-4.7	3.9-5	NS
	Mean ±SD	4.229±0.444	4.233±0.380	4.414±0.403	
Total Bilirubin mg/dl	Range	0.4-1.9	0.3-0.9	0.5-4.5	NS
	Mean ±SD	0.847±0.337	0.708±0.178	0.918±0.834	
Direct Bilirubin mg/dl	Range	0.1-0.8	0.1-0.31	0.1-0.7	NS
	Mean ±SD	0.259±0.169	0.186±0.057	0.230±0.134	
Fecal Calprotectin	Range	15-180	67-426	53-1320	<0.001
	Mean ±SD	74.765±49.422	254.917±109.011	391.876±308.778	
Serum calgranulin C (ng/ml)	Range	63-435	438-1812	505-2000	<0.001
	Mean ±SD	226.706±101.888	765.50±365.172	1102.00±492.76	

*Chi-Square, #ANOVA *p* > 0.05 is not significant (NS).

Regarding clinical symptoms, UC patients with diarrhea, blood in stool and urgency have statistically significantly higher serum calgranulin C than those without these symptoms (p <0.001, for all) [Table 4].

Regarding the different endoscopic activity scoring systems, patients with severe disease on mayo endoscopic sub-score, obliterated vascular pattern, presence of ulcers and luminal bleeding have statistically significantly higher serum calgranulin C than those with mild disease on different endoscopic parameters (p<0.001, for all) [Table 4].

Table 4. Serum calgranulin C in relation to socio-clinical and laboratory parameters in the ulcerative colitis group.

Ulcerative colitis		Serum calgranulin c		p-value
		N	Mean±SD	
Sex	Male	17	534.059±462.442	NS
	Female	33	821.303±541.054	
Smoking	No	41	736.634±501.478	NS
	Yes	9	664.444±672.439	
Abdominal pain	No	35	722.886±520.883	NS
	Yes	15	725.400±566.325	
Diarrhea	No	15	251.267±128.239	<0.001
	Yes	35	926.086±505.945	
Blood in stools	No	35	531.029±400.618	<0.001
	Yes	15	1173.067±529.824	
Urgency	No	24	402.083±372.209	<0.001
	Yes	26	1020.462±479.849	
Tenesmus	No	39	771.795±547.589	NS
	Yes	11	552.909±436.985	
Montreal classification	E2	24	611.083±483.438	NS
	E3	26	827.538±556.954	
Endoscopy (Mayo sub-score)	Mild disease	19	292.947±220.935	<0.001
	Moderate disease	20	848.150±444.884	
	Severe disease	11	1241.182±479.071	
Vascular pattern	Normal	20	471.950±442.967	<0.001
	Patchy obliteration	8	488.250±322.682	
	Obliterated	22	1038.045±505.827	
Bleeding	Normal	39	566.795±417.121	<0.001
	Luminal mild	6	1006.000±401.147	
	Luminal moderate or severe	5	1608.200±487.036	
Erosions and ulcers	Normal	17	296.647±230.389	<0.001
	Erosions	2	261.500±164.756	
	Superficial ulcer	31	987.613±488.258	
CRP	Normal	22	377.273±376.394	<0.001
	Elevated	28	995.786±472.457	
ESR	<30 mm/hr	10	454.900±527.911	NS
	>30 mm/hr	40	790.825±514.011	
Fecal calprotectin	<150 mg/kg	19	327.842±379.913	<0.001
	150-200 mg/kg	31	966.226±458.850	

t-Test or ANOVA, $p > 0.05$ is not significant (NS).

When using the Pearson multivariate correlation, a significant positive correlation was observed between serum calgranulin C and bowel motion, platelets, ESR, UCEIS, fecal Calprotectin (p <0.001, p= 0.010, p=0.016, p<0.001, and p=0.011, respectively) [Table 5]. However, there was a significant negative correlation with hemoglobin (p=0.042) [Table 5].

Table 5. Correlation between serum calgranulin C with different parameters among ulcerative colitis patients.

Ulcerative colitis	Serum calgranulin c	
	r	*p*-value
Age	-0.076	NS
Bowel motion	0.716	<0.001
Hemoglobin (g/dl)	-0.288	0.042
Platelet (X 10^9cells/L)	0.362	0.010
Total leucocytic count (X 10^9 cells/L)	0.210	NS
ESR	0.339	0.016
CRP	0.271	NS
Ulcerative Colitis Endoscopic Index of Severity	0.747	<0.001
Fecal Calprotectin	0.357	0.011

p > 0.05 is not significant (NS).

The ROC curve analysis showed that serum calgranulin C, at cut off >135 ng/ml, had 92.00% sensitivity, 100% specificity, 100% PPV, 83.3% NPV and 97.7% accuracy in prediction of UC. [Figure 1]. Depending on UCEIS, the ROC curve analysis was used to reveal the accuracy of calgranulin C and fecal calprotectin levels in prediction of activity among UC patients [Table 6]. Calgranulin C, at cut off >625, had 100.0 % sensitivity, specificity 66.67 %, PPV 45.8 %, NPV 100.0 % and accuracy 88.2% in prediction of luminal bleeding in endoscopy among UC patients [Figure 2].
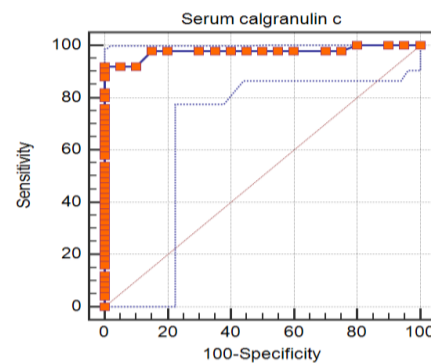
Figure 1. Receiver-operating characteristic (ROC) curve analysis of serum calgranulin c level in prediction of UC.

Table 6. Receiver-operating characteristic (ROC) curve for serum calgranulin C and fecal Calprotectin in prediction of activity in ulcerative colitis patients depending on UCEIS.

ROC curve between Remission and Active Ulcerative Colitis						
	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum calgranulin C	>435	96.97	94.12	97.0	94.1	97.1%
Fecal Calprotectin	>219	69.70	94.12	95.8	61.5	89.4%
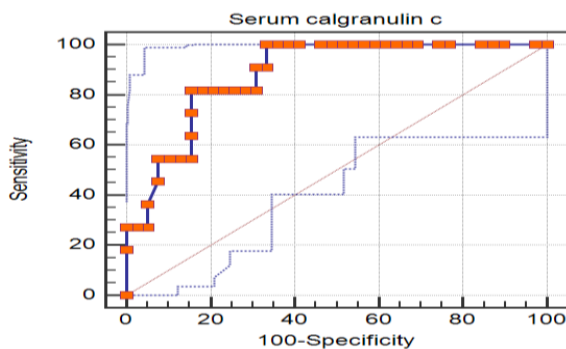
Figure 2. Receiver-operating characteristic (ROC) curve analysis for serum calgranulin c in prediction of luminal bleeding in endoscopy in UC

Regarding the CD group, both serum calgranulin C and fecal Calprotectin were statistically significantly higher in comparison to the control group (*p*=0.002 and, *p*=0.001, respectively) [Table 7].

Depending on CDAI, both serum calgranulin C and fecal calprotectin were statistically significantly higher among active disease than those in remission (*p* <0.001 for both). Also, the inflammatory markers (ESR, CRP) were statistically significantly higher among active disease than those in remission (*p*=0.001 and, *p*=0.004, respectively) [Table 8].

When using Pearson multivariate correlation, a significant positive correlation was observed between serum calgranulin C and bowel motion, ESR, CRP, fecal Calprotectin and CDAI [Table 9].

Table 7. Comparison of Calgranulin C and fecal calprotectin between Crohn's disease and the control group.

Studied parameter		Diagnosis		*p*-value
		Crohn's disease	Control	
Fecal Calprotectin	Range	23-917	18-57	0.002
	Mean ±SD	196.100±223.449	34.300±12.566	
Serum calgranulin C	Range	110-2000	52-135	<0.001
	Mean ±SD	759.233±797.963	80.850±24.416	

*t-Test, *p* ≤ 0.05 is significant.

Table 8. Comparison of all laboratory parameters, calgranulin C and fecal calprotectin between subgroups of Crohn's disease according to CDAI.

Crohn's disease		CDAI		*p*-value
		Remission	Active CD	
Hemoglobin (g/dl)	Range	10.2-15.7	10.2-13.8	0.257
	Mean ±SD	12.695±1.485	12.080±1.092	
Platelet (X 10^9cells/L)	Range	202-468	147-696	NS
	Mean ±SD	314.950±78.923	356.900±169.185	
Total leucocytic count (X 10^9 cells/L)	Range	4.2-9.2	4.2-12	NS
	Mean ±SD	6.730±1.577	6.640±2.882	
ESR	Range	18-63	22-114	0.001
	Mean ±SD	34.750±14.668	68.800±35.727	
CRP	Range	0.3-18	2-140	0.004
	Mean ±SD	5.613±5.710	33.680±39.413	
Creatinine mg/dl	Range	0.5-1.03	0.7-1	NS
	Mean ±SD	0.788±0.181	0.850±0.118	
Urea mg/dl	Range	0.3-32	21-27	NS
	Mean ±SD	24.515±6.983	24.000±2.357	
ALT (U/L)	Range	11-34	13-75	NS
	Mean ±SD	18.400±5.576	22.300±18.709	
AST (U/L)	Range	13-36	14-110	NS
	Mean ±SD	20.800±6.212	29.400±28.849	
Albumin (g/dl)	Range	3.8-4.8	4-4.9	NS
	Mean ±SD	4.195±0.352	4.410±0.345	

Table 8. Continued.

Crohn's disease		CDAI		*p*-value
		Remission	Active CD	
Total Bilirubin mg/dl	Range	0.5-1	0.4-0.9	NS
	Mean ±SD	0.820±0.140	0.757±0.148	
Direct Bilirubin mg/dl	Range	0.1-0.4	0.1-0.4	NS
	Mean ±SD	0.255±0.065	0.229±0.101	
Fecal Calprotectin	Range	23-187	121-917	<0.001
	Mean ±SD	83.750±46.243	420.800±268.737	
Serum calgranulin C(ng/ml)	Range	110-437	1125-2000	<0.001
	Mean ±SD	218.600±101.160	1840.500±285.603	

t-Test, *p* > 0.05 is not significant (NS).

Table 9. Correlation between calgranulin C among Crohn's disease and different laboratory parameters, age and CDAI.

Crohn's disease	Serum calgranulin c	
	r	*p*-value
Age	-0.106	NS
Bowel motion	0.357	0.05
Hemoglobin (g/dl)	-0.250	NS
Platelet (X 10^9cells/L)	0.182	NS
Total leucocytic count (X 10^9 cells/L)	-0.023	NS
ESR	0.621	<0.001
CRP	0.533	0.002
Fecal Calprotectin	0.736	<0.001
CDAI	0.821	<0.001

p > 0.05 is not significant (NS).

The ROC curve analysis showed that serum calgranulin C, at cut off >110 ng/ml, had 96.67% sensitivity, 85.00% specificity and 97.8% accuracy in prediction of CD. [Figure 3].

Depending on CDAI, the ROC curve analysis was used to reveal the accuracy of calgranulin C and fecal calprotectin levels in prediction of activity among CD patients [Table 10].
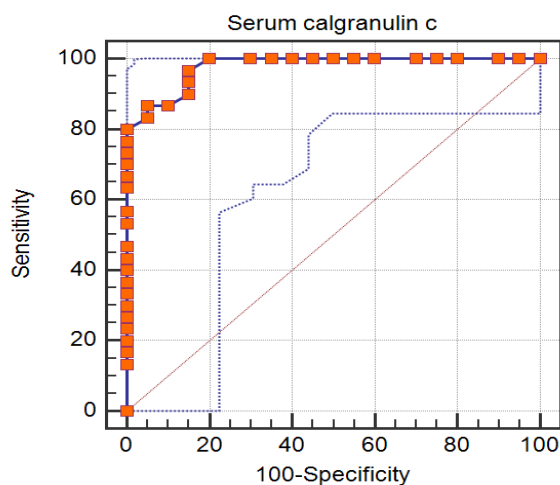

Figure 3. Receiver-operating characteristic (ROC) curve analysis of serum calgranulin c level in prediction of CD.

Table 10. Receiver-operating characteristic (ROC) curve for serum calgranulin c and fecal Calprotectin in prediction of activity of Crohn's disease according to CDAI.

ROC curve between Remission and Active CD						
CD	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum calgranulin C	>437	100.0	100.0	100.0	100.0	100%
Fecal Calprotectin	>231	80.0	100.0	100.0	90.9	97.7%

Discussion

IBD refers to two chronic idiopathic inflammatory diseases: ulcerative colitis (UC) and Crohn's disease (CD). Clinical, endoscopic, histologic, and radiologic characteristics are used to distinguish one from the other. Both disorders can have an effect on many aspects of a patient's life, including education, job, social, and family life. A patient-centered approach with strong multidisciplinary care can result in improved quality of life for patients of all ages.[10]

Biomarkers play critical roles in IBD. These include identifying inflammatory changes in those with undifferentiated symptoms (which leads to a clear diagnosis), determining response to an intervention, and monitoring disease course including identifying those at risk of recurrence in the next months and those with unfavorable disease course. These biomarkers included serum and fecal markers of inflammation, and serological markers reflecting immune responses.[11]

The S100 calcium-binding protein A12 (S100A12), also known as calgranulin C, is a member of the S100 protein family which, in humans, consists of twenty-five EF-hand (a helix-loop-a helix), calcium-binding proteins, of which the vast majority is in a homodimer, heterodimer i.e. S100A8/A9, or more complex form.[12]

S100A12, like S100A8/A9 (calprotectin), is phagocyte 2 specific, has proinflammatory features, and has already been associated with a variety of inflammatory disorders, including IBD.[13] Several studies using the determination of S100A12 in feces, revealed a significant association between fecal S100A12 levels and IBD, and especially the active disease.[14] The aim in this study was to evaluate the role of serum calgranulin C as a diagnostic and prognostic marker in IBD patients and to correlate its level with different biochemical, endoscopic and clinical parameters.

In terms of IBD symptomatology, the current study showed that serum calgranulin C levels were statistically significantly higher in UC patients with diarrhea, blood in stool, and urgency than in those without these symptoms. Also, a significant positive correlation between serum calgranulin C and bowel motion was noted. This result agreed with a previous study who found that serum calgranulin C was substantially linked with clinical disease activity in both CD and UC.[15] This is because most patients with active illness experience regular passage of loose or watery stools and may experience nocturnal diarrhea.[14]

Also, our findings agreed with those of a previous study which included a group of patients presented with diarrhea ± abdominal pain, IBD patients, diagnosed after a full work-up (endoscopies, histopathology, cultures etc.), and concluded that serum Calgranulin C in IBD patients with diarrhea and abdominal pain were higher than control.[16]

Moreover, another study was conducted on a total number of 337 children and teenagers with chronic abdominal pain and diarrhea.[17] Eventually a total of 93 patients (27.6%) were diagnosed with IBD. They showed that calgranulin C had better specificity for IBD than calprotectin in patients diagnosed with IBD with abdominal pain and diarrhea.

The present study showed that serum calgranulin C was statistically significantly higher among the UC group in comparison to the control group (723.640±529.055 ng/ml versus 80.850±24.416 ng/ml). This was in accordance with a previous study, showed significant elevation in serum calgranulin C levels in patients with IBD but not with functional bowel disorder, thus allowing the distinction between the two entities.[16]

In addition, an earlier study evaluated serum calgranulin C in 300 adults with IBD (150 CD and 150 UC), 100 non-IBD inflammatory controls (including diverticulitis, infectious enterocolitis, and ischemic colitis) and 143 healthy controls.[18] Significantly elevated serum calgranulin C concentrations were seen in both IBD groups and non-IBD inflammatory controls compared with healthy individuals.

These findings were supported and explained by a former study which proposed that human calgranulin C expressed and secreted by neutrophil granulocytes and, therefore, has been assigned to the S100 protein subfamily of calgranulins or myeloid-related proteins.[19] Human calgranulin C is markedly overexpressed in inflammatory compartments, and elevated serum levels of calgranulin C are found in patients suffering from various inflammatory, neurodegenerative, metabolic, and neoplastic disorders.

In the current study, the UC group showed significant positive correlation between serum calgranulin C and fecal calprotectin, ESR and platelets ($p<0.001$, $p=0.016$ and, $p<0.001$, respectively) and a significant negative correlation with hemoglobin ($p= 0.042$). These were consistent with the findings of a previous study, found that in IBD patients, calgranulin C levels were correlated with the histological inflammatory score, ESR, CRP, thrombocytes, white blood cell count, hemoglobin, and hematocrit.[20] In addition, both ESR and calgranulin C correlated with disease activity.[20]

These findings were explained by evidence that platelets play a role in the initiation and maintenance of inflammatory mechanisms by releasing many proinflammatory mediators (P-selectin, platelet-derived microparticles, serotonin, CD40 ligands, and chemokines known as regulated on activation, normal T cell expressed and secreted) when activated, as in IBD.[21] It has also been proved that altered platelets function in IBD is related with several morphological changes, including a decrease in mean platelet volume, increased granular content and density, and an increase in absolute number (thrombocytosis).[22]

According to the Montreal classification of UC, our present study showed no statistically significant difference in serum calgranulin C level between E2 (LT sided colitis) and E3 (pancolitis) ($p>0.05$). The same result was mentioned by a previous study, stating that the variations in the median of serum S100A12 did not show a significant association with extent in UC ($p = 0.590$).[16] According to their results, determination of serum S100A12 could not be used to predict disease extent in UC patients, since the S100A12 serum values were not significantly different in patients with proctitis, left-sided colitis or pancolitis.

In the current study, regarding the various endoscopic scoring systems, we found that patients with severe disease on the Mayo score, obliterated vascular pattern, presence of ulcers, and luminal bleeding had statistically significantly higher serum calgranulin C than those with mild disease on all endoscopic parameters, ($p = 0.001$). This finding was consistent with a previous study, found that serum calgranulin C closely linked with clinical disease activity in both CD and UC.[23] There was a strong correlation between serum calgranulin C and both endoscopic (r=0.72; $p<0.01$) and histological (r=0.83; $p<0.001$) scores.

Depending on ACG-UCAI, our study showed that serum calgranulin C was statistically significantly higher among patients with moderate to severe activity than those in mild activity and patients in remission (1102.00±492.76 ng/ml versus 765.50±365.172 ng/ml versus 226.706±101.888 ng/ml) ($p <0.001$). Also, fecal calprotectin was statistically significantly higher in patients with disease activity than those in remission ($p= 0.002$). In addition, the present study showed significant positive correlation between serum calgranulin C and both fecal calprotectin and UCEIS ($p<0.001$, and $p= 0.011$, respectively). This was supported by the finding of an earlier study, demonstrated that immunohistochemistry labelling of tissue sections verified S100A12 (calgranulin C) expression in the gut of patients with active IBD more than inactive illness.[24] This is comparable to a previous study, reported elevated S100A12 levels in a group of 74 adult IBD patients, of whom 34 had UC and 40 had CD.[19] S100A12 levels were greater in active CD (470–125 ng/mL) and active UC (401–20 ng/mL)

than in healthy control individuals (75-25 ng/mL).

Our study showed that Serum calgranulin C, at cut off >135 ng/ml, had 92.00% sensitivity, 100% specificity, 100% PPV, 83.3% NPV and 97.7% accuracy in prediction of UC. These findings agreed with those of a previous study, included 83 consecutive patients with an established diagnosis of IBD or symptoms suggesting gastrointestinal inflammation.[25] Calgranulin C at a cutoff point of 0.8 mg/kg was used to distinguish active IBD from healthy controls and irritable bowel syndrome.

The present study revealed that there was a statistically significantly higher level of fecal calprotectin in UC (in exacerbation was 391.876±308.778 ng/ml) compared to UC (in remission was 74.765± 49.422 ng/ml.). The diagnostic performance analysis revealed that at cutoff > 219 ng/ml, the sensitivity and specificity were 69.70% and 94.12%, respectively for predicting the UC activity.

Also, there was a statistically significantly higher serum calgranulin C level in UC in exacerbation was 1102.00 ± 492.786 ng/ml compared to UC in remission was 226.706 ± 101.88 ng/ml. The diagnostic performance analysis revealed that at cut-off > 435 ng/ml, the sensitivity and specificity were 96.97% and 94.12%, respectively for predicting the UC activity.

Such data are consistent with findings of a previous study, showed that fecal calprotectin at cutoff value of 164 μg/g, the sensitivity was 85.42%, specificity 73.68%, in predicting clinical active disease.[26] They concluded that fecal calprotectin is a clinically relevant biomarker for both clinically active disease and mucosal healing in patients with UC. However, the cutoff value still needs large and multicenter studies for confirmation.

Moreover, our findings agreed with those of a previous study, found that both fecal calprotectin and fecal-S100A12 correlated with markers of inflammation among UC and CD patients.[27] They found differences between patients in clinical relapse and remission (fecal calprotectin: mean 1027 ± 818 mcg/ml vs 580 ± 695 mcg/ml, respectively, p = 0.028); (fecal S100A12: mean 66.4 ± 48.2 mcg/ml vs 42.7 ± 40

mcg/ml, respectively p = 0.02). Moreover, they reported a significant difference in fecal calprotectin between children with endoscopic inflammation and remission (mean 825 ± 779 mcg/ml vs 473.3 ± 492 mcg/ml, respectively p = 0.048), as well as for fecal S100A12 (53 ± 43 mcg/ml vs mean 31 ± 33 mcg/ml vs, respectively p = 0.019). Similar findings were also reported by another study.[28]

Regarding laboratory markers in CD patients, our study showed that fecal calprotectin, ESR and CRP were statistically significantly higher among active CD than those in remission (p <0.001, p=0.001, and p=0.004, respectively. These were in accordance with those reported by an earlier study which included 273 CD patients and found that fecal calprotectin level was significantly positively correlated with the CDAI and simple endoscopic score-CD, with correlation coefficients of 0.666 and 0.674, respectively.[29] The median fecal calprotectin levels in patients with clinical remission and mildly active and moderately–severely active disease was 41.01 μg/g, 164.20 μg/g, and 444.45 μg/g, respectively. These values were 26.94 μg/g, 66.77 μg/g, and 327.22 μg/g during endoscopic remission and mildly and moderately–severely active stages respectively. They concluded that compared with CRP, the ESR, and other biomarker parameters, fecal calprotectin was better at predicting disease activity for CD patients.

Our study showed that serum calgranulin C was statistically significantly higher among CD patients than in controls (759.233±797.963 ng/ml versus 80.850±24.416 ng/ml). Also, it was statistically significantly higher among active CD than those in remission (1840.500±285.603 ng/ml versus 218.600±101.160 ng/ml) (p <0.001).

The current study showed a significant positive correlation between serum calgranulin C and CDAI (p< 0.001). This result agreed with that published by another study which concluded that fecal S100A12 and fecal calprotectin are both useful non-invasive biomarkers in the management of pediatric IBD in follow up and in monitoring endoscopic and clinical relapse.[27]

Our study showed that serum calgranulin C at a cutoff >110 ng/ml, had 96.67% sensitivity, 85.00% specificity and 97.8% accuracy in prediction of CD. Moreover, serum calgranulin C, at cutoff >437 ng/ml, had 100 % sensitivity, 100%, specificity and 100% accuracy in prediction of activity in CD depend on CDAI.

This agreed with previous studies, demonstrated that serum levels of calgranulin C strongly correlated with clinical disease activity in CD.[14, 15] Also, a previous study showed a significant correlation between calgranulin C serum levels and disease activity.[25] Both UC and CD patients with active disease seemed to have higher values of calgranulin C compared to IBD subjects with inactive disease. On the contrary another study, found that fecal calgranulin correlated with fecal calprotectin (r= 0.689), ESR (r= 0.524), CRP (r= 0.499), and albumin (r= -0.446), but not with CDAI (r=0.045).[30]

Finally, some data also demonstrated that fecal calgranulin C fall down following anti-inflammatory therapy, indicating that this marker could become a further easy way to assess response to therapy. Additional potential roles of S100A12 may be as indication of mucosal healing and in the prediction of potential relapse. Furthermore, intense scientific research on S100 proteins has revealed a wide range of possibilities for novel S100- oriented therapeutic interventions.[31]

In conclusion, our study findings indicated that serum calgranulin C could be used as a non-invasive diagnostic marker to predict activity, severity and to ensure remission among inflammatory bowel disease patients.

Acknowledgements

Author Contributions

In addition to being the corresponding author, HSR proposed the research idea and authoring. NAE & HSE made significant contributions to the authoring and critical revision. MFM aided by data collection and revising the laboratory analysis and tabulating the data. The submitted manuscript was reviewed and approved by all authors.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

Ethical approval

The study protocol was reviewed and approved by the Research Ethics Review Committee of the Faculty of Medicine, Ain Sham University (Reference Number: FMASU MS 57/2019).

Informed consent

A written informed consent was obtained from each study participant.

References

1. Dragoni G, Innocenti T, Galli A. (2021). Biomarkers of inflammation in inflammatory bowel disease: how long before abandoning single-marker approaches? *Dig Dis*; 39(3):190–203.

2. Albayrak B, Sebin E. (2023). A novel inflammatory marker for extensive ulcerative colitis; *Endocan. BMC Gastroenterol* 23: 118.

3. Porter AC, Aubrecht J, Birch C, et al. (2020): Biomarkers of Crohn's Disease to Support the Development of New Therapeutic Interventions. *Inflamm Bowel Dis*; 26(10): 1498-1508.

4. Alghoul Z, Yang C, Merlin D. (2022). The Current Status of Molecular Biomarkers for Inflammatory Bowel Disease. *Biomedicines*; 10(7):1492. doi: 10.3390/biomedicines10071492.

5. Mendoza J.L., Abreu M.T. (2009). Biological markers in inflammatory bowel disease: Practical consideration for clinicians. *Gastroentérologie Clin.* Biol; 33: 158–S173.

6. Perera C., McNeil H.P., Geczy C.L. (2010). S100 Calgranulins in inflammatory arthritis. *Immunol. Cell Biol*; 88:41–49.

7. Pabla BS & Schwartz DA. (2020). Assessing severity of disease in patients with Ulcerative colitis. *Gastroenterol Clin N Am*; 49:671-688.

8. Rubin DT, Ananthakrishnan AN, Siegel CA, et al. (2019). ACG clinical guideline: Ulcerative colitis in adults. *American Journal of Gastroenterology*; 114(3): 384-413.

9. Feldman M, Friedman LS & Brandt LJ (2020). Sleisenger and Fordtran's gastrointestinal and liver disease E-*book: pathophysiology, diagnosis, management, 11th Edition-Elsevier-* eBook ISBN: 9780323710947

10. Sairenji T, Collins KL & Evans DV (2017). An update on inflammatory bowel disease. *Primary Care: Clinics in Office Practice*, 44: 673-692.

11. Day AS, Leach ST & Lemberg DA (2017). An update on diagnostic and prognostic biomarkers in inflammatory bowel disease. *Expert Review of Molecular Diagnostics,* 17: 835-843.

12. Zackular JP, Chazin WJ & Skaar EP (2015). Nutritional immunity: S100 proteins at the host-pathogen interface. *Journal of Biological Chemistry*, 290: 18991-18998.

13. Pruenster M, Vogl T, Roth J, et al. (2016). S100A8/A9: from basic science to clinical application. *Pharmacology & therapeutics,* 167: 120-131.

14. Roh JS & Sohn DH (2018). Damage-Associated Molecular Patterns in Inflammatory Diseases. *Immune Netw*.13;18(4): e27.

15. Däbritz J, Langhorst J, Lügering A, et al. (2013). Improving relapse prediction in inflammatory bowel disease by neutrophil derived S100A12. *Inflammatory bowel diseases*, 19: 1130-1138.

16. Manolakis AC, Kapsoritakis AN, Georgoulias P, et al. (2010). Moderate performance of serum S100A12, in distinguishing inflammatory bowel disease from irritable bowel syndrome. *BMC gastroenterology*, 10: 1-7.

17. Heida A, Van De Vijver E, Van Ravenzwaaij D, et al. (2018). Predicting inflammatory bowel disease in children with abdominal pain and diarrhoea: calgranulin-C versus calprotectin stool tests *Archives of Disease in Childhood* 103:565-571.

18. Brinar, M., Cleynen, I., Coopmans, T., et al. (2010). Serum S100A12 as a new marker for inflammatory bowel disease and its relationship with disease activity. *Gut*, 59, 1728-1729.

19. Meijer B, Gearry RB & Day AS (2012). the role of S100A12 as a systemic marker of inflammation. *Int J Inflam*. 907078.

20. Harbord M, Eliakim R, Bettenworth D, et al. (2017). Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 2: current management. *Journal of Crohn's and Colitis*, 11: 769-784.

21. Voudoukis E, Karmiris K, Oustamanolakis P, et al. (2013). Association between thrombocytosis and iron deficiency anemia in inflammatory bowel disease. *European journal of gastroenterology & hepatology*, 25: 1212-1216.

22. Lee SL, Ng CY, Sidhu J, et al. (2021). Mean Platelet Volume as a Complementary Non-Invasive Biomarker for Disease Activity in Inflammatory Bowel Disease: A Single Centre Study. *Asian Journal of Research and Reports in Gastroenterology*, 1-9.

23. Foell D, Seeliger S, Vogl T, et al (2003). Expression of S100A12 (EN-RAGE) in cystic fibrosis. *Thorax,* 58: 613-617.

24. Kaiser T, Langhorst J, Wittkowski H et al. (2007). Faecal S100A12 as non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome. *Gut,* 56(12): 1706–1713.

25. Däbritz J, Musci J & Foell D (2014). Diagnostic utility of faecal biomarkers in patients with irritable bowel syndrome. *World journal of gastroenterology: WJG*, 20: 363.

26. Chen F, Hu Y, Fan Y-H et al. (2021). Clinical Value of Fecal Calprotectin in Predicting Mucosal Healing in Patients with Ulcerative Colitis. *Front. Med.* 8:679264.

27. Cenni S, Casertano M, Trani M, et al. (2023). The use of calgranulin-C (S100A12) and fecal zonulin as possible non-invasive markers in children with inflammatory bowel disease: a clinical study. *Eur J Pediatr*. Mar;182(3):1299-1308.

28. De Jong N, Leach S, Day A (2006). Fecal S100A12: a novel non-invasive marker in children with Crohn's disease. *Inflamm Bowel Dis.,* 12: 566–572

29. Li J, Xu M, Qian W, et al. (2023). Clinical value of fecal calprotectin for evaluating disease activity in patients with Crohn's disease. *Front Physiol*. Jun 1; 14:1186665.

30. Sun-Ho Lee, Sung Wook Hwang, Sang Hyoung Park, et al. (2022). Fecal S100A12 is associated with future hospitalization and step-up of medical treatment in patients with Crohn's disease in clinical remission: a pilot Study. *Intestinal Research* 20(2):203-212.

31. Fengming Yi, Long Feng and Jianbing Wu (2017). Evaluation of fecal protein S100A12 in patients with inflammatory bowel disease. *Medica lExpress (São Paulo, online).* 2017. Vol. 4(3).