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Serum Amyloid A as a non-invasive predictive biomarker of mucosal healing in ulcerative colitis patients

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Abstract

Ulcerative colitis is a chronic immune-mediated inflammatory condition of large intestine that is frequently associated with inflammation of the rectum but often extends proximally to involve other areas of the colon. The ultimate target of therapy is complete healing in the form of clinical remission, complete endoscopic and histological healing, and transmural healing for which endoscopy is mandatory. Colonoscopy may not always be applicable due to possible complications in active ulcerative colitis. Therefore, non-invasive biomarkers are needed to avoid the disadvantageous complications of invasive diagnostic procedures. The aim of this study was to evaluate the role of serum Amyloid-A (SAA) as a non-invasive predictive biomarker of mucosal healing in comparison to different laboratory biomarkers, and endoscopic activity scores. The study included 100 ulcerative colitis patients classified into two groups: 50 patients in clinical, and biochemical remission and 50 patients in activity. Complete blood picture, C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and SAA were measured and recorded, colonoscopies with histopathological examination were done for all patients. SAA levels were significantly higher in patients with active ulcerative colitis than in clinical remission patients (p <0.001). In clinical, remission patients without full mucosal healing, SAA was positively correlated with endoscopic disease activity represented with Mayo score, Mayo endoscopic sub-score and Ulcerative Colitis Endoscopic Index of Severity (UCEIS) (p< 0.001). However, there was no significant correlation between SAA and endoscopic scores among the activity patients' group. The cut off value of SAA for determining disease activity was > 5.199 µg/ml with 100 % sensitivity, specificity of 92 %, and accuracy of 99.6%. In conclusion, SAA can be used for prediction of mucosal healing in ulcerative colitis remission patients despite not being superior to fecal calprotectin. However, it was unable to differentiate between the different disease activities or extents.

Keywords: Ulcerative colitis; Serum Amyloid A; Mucosal healing.

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Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease characterized by excessive immune response to environmental factors or resident microbiota among genetically susceptible subjects. Inflammation of the colon mucosa plays an essential role in pathogenesis of UC, which leads to ulcer formation. The intestinal mucosal pathology is mainly localized in the rectum and spreads proximally to the other parts of the colon. The most common clinical symptoms are gastrointestinal disorders such as abdominal pain, diarrhea with mucus and/or blood, nausea, and vomiting. In addition, general symptoms include fever, weight loss and anemia together with extra intestinal involvement as peripheral arthritis, cholangitis, pyoderma gangrenosum, erythema nodosum and arthropathies.[1]

The optimal goal of UC management is a sustained and durable period of steroid-free remission, accompanied by appropriate psychosocial support, normal health-related quality of life, prevention of morbidity including hospitalization and surgery, and prevention of cancer. An emerging goal in UC management is that of mucosal healing. To achieve these goals, understanding of the most effective diagnostic, treatment, and preventive strategies is necessary, also involvement of the patients' preferences forms an important component of care.[2]

Endoscopic evaluation is the most accurate way to assess UC activity and screen for colorectal cancer (CRC). The location, extent and severity can be established with this procedure, but its use is prevented by several drawbacks, as it is invasive, time-consuming, and expensive. So, the identification of novel, non-invasive and reliable serum biomarkers are needed to accurately detect inflammation, monitor disease activity, and improve the diagnostic accuracy for CRC.[3,4]

Serum amyloid A (SAA) is a highly conserved acute-phase protein, released in response to inflammation or infection. Production of acute-phase SAA (A-SAA) is stimulated by proinflammatory cytokines, such as interleukin-6 (IL-6), IL-1, tumor necrosis factor (TNF), interferon-γ, and transforming growth factor (TGF). The concentration of A-SAA increases dramatically during acute inflammation and injury, reaching within 5-6 hours levels that are 1000 folds greater than normal.[5]

The liver is the primary source of circulating A-SAA. However, extrahepatic production of SAA by several tissues and cell types has been described in patients with chronic diseases. These include Alzheimer's disease, cancer, diabetes, obesity, insulin resistance, metabolic syndrome, and atherosclerosis.[6]

SAA performed good in predicting intestinal mucosal healing (MH). It performed better than multiple cytokines, including C-reactive protein (CRP). Also, even though fecal calprotectin (FC) corresponds with disease activity status in inflammatory bowel disease (IBD) patients, low compliance with obtaining stool samples and the challenge of collecting samples from diarrhea can disrupt clinical monitoring.[7]

In patients with active IBD and low CRP levels, measurement of SAA might have a role in clinical care. The association between SAA levels and disease activity has been studied in multiple inflammatory entities showing that SAA was a more sensitive test for active disease than CRP but had a lower specificity.[8] Therefore, the aim of the current study was to evaluate the role of SAA as a non-invasive predictive biomarker for mucosal healing in ulcerative colitis patients in comparison to different laboratory biomarkers, and endoscopic activity scores.

Subjects and Methods

This cross-sectional observational study was conducted in the IBD clinic of Ain Shams University Hospitals, from November 2021 to November 2022. The study included 100 adult patients diagnosed by clinical criteria and colonoscopy with biopsy as UC. They were divided into two groups according to disease activity, Group I included 50 UC patients in clinical and biochemical remission stage. And, Group II, included 50 UC patients in clinical, biochemical, and endoscopic activity. They were

age and sex matched. Pregnancy, lactation, indeterminate colitis, infectious colitis, concurrent infections, autoimmune diseases, colonic malignancy, and history of colorectal surgery were the exclusion criteria of the study.

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Sham University (Reference Number: FMASU MD 51/2021). A written informed consent was obtained from each patient before being included in the study.

Data of the study population were collected using a well-designed data sheet covering detailed medical history, physical examination, and baseline laboratory investigations. These included complete blood count (CBC) using an automated blood cell counter (Sysmex XT-1800i autoanalyzer, Sysmex, Japan), according to the manufacturer's instructions. C-reactive protein (CRP) was performed using the latex agglutination method, and erythrocyte sedimentation rate (ESR) using the conventional Westergren method. Serum albumin, ALT and AST were assessed using blood chemistry analyzer (Roche Integra 400 plus, Roche Diagnostic, USA), according to the manufacturer's instructions.

Serum levels of SAA were measured by human serum Amyloid-A Enzyme-linked Immunosorbent Assay (ELISA) kits (Cat. No E1225Hu. BT LAB, Bioassay Technology Laboratory, Sun Red Biotechnology company, Zhejiang, China), according to the manufacturer's instructions. Fecal Calprotectin in stool samples was determined using a fully automated colorectal point of care Reader (Quantum Blue® Calprotectin, Bühlmann Laboratories AG, Switzerland), according to the manufacturer's instructions. Different medications received by patients whether 5-aminosalicylic acid (5-ASA), immunomodulators as Azathioprine or biological therapy were tabulated.

All patients underwent colonoscopy with multiple biopsies to confirm diagnosis, assess severity, extent of the disease and to determine their long-term maintenance therapy. Assessment of disease extent was performed according to the Montreal classification where E1: Ulcerative proctitis with involvement limited to the rectum; that 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.[9]

Assessment of disease activity was performed according to the Truelove and Witt's severity index relying on symptoms, basic clinical and laboratory tests, Ulcerative Colitis Endoscopic Index of Severity (UCEIS), Mayo score and Mayo endoscopic sub score.[9] 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 ranged from 0 to 8. Patients were classified according to UCEIS into four severity groups: remission (UCEIS 0-1); mild (UCEIS 2-4); moderate (UCEIS 5-6); and severe (UCEIS 7-8).[9]

The Mayo Endoscopic Sub score (MES) was arranged in three levels. MES-0: normal or inactive (no friability and granularity and intact vascular pattern). MES-1: mild (mild erythema or decreased vascular pattern). MES-2: moderate (marked erythema, absent vascular pattern, friability, and erosions). MES-3: severe (spontaneous bleeding and ulceration).[9] Finally, Mayo score, included four components which are rectal bleeding, stool frequency, endoscopic picture, physician's global assessment compromised 12 points, where ≤ 2 clinical remission, 3-5 mild activity, 6-10 moderate activity, 11-12 severe activity.[9]

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) Version 20. Data are presented as mean, standard deviation (SD). The student t-test, Chi-square, Linear Correlation Coefficient and Analysis of variance (ANOVA) tests were performed. The unpaired Student T-test was used to compare between two groups in quantitative data. The diagnostic value of serum amyloid A was evaluated using the receiver-

operating characteristic (ROC) curve analysis. A *p* value of <0.05 was considered statistically significant.

Results

This study included 100 UC patients divided into two groups. Group I included 50 patients in clinical and laboratory remission stage. They were 23 females and 27 males with age ranging between 16 and 49 years (mean ±SD: 29.140±8.652). Group II included 50 patients with clinical and endoscopic activity. They were 33 females and 17 males with age ranging between 16 and 68 years (30.460±10.322).

The current study revealed a statistically significantly higher total leucocytic count (TLC), platelet count, CRP, ESR, Fecal calprotectin, and SAA among activity patients in relation to those in remission. However, hemoglobin and albumin levels were statistically significantly lower in activity patients in comparison to remission patients (Table 1).

Table 1. Comparison of different laboratory parameters between Remission and Activity groups of patients.

		Group		*p*-value (T-Test)
		Remission (n=50)	Activity (n=50)	
TLC	Range	4-12.3	3.4-16.1	<0.001
	Mean ±SD	6.260±1.775	9.468±3.395	
Hemoglobin	Range	11-14.5	7-14.4	<0.001
	Mean ±SD	12.562±0.765	10.472±1.567	
PLT	Range	163-640	154-654	0.001
	Mean ±SD	266.060±87.448	341.820±121.072	
CRP	Range	0.2-9	1.4-162	<0.001
	Mean ±SD	1.796±2.310	35.342±33.917	
Fecal calprotectin (mg/kg)	Range	25-193	50-1500	<0.001
	Mean ±SD	90.660±32.633	563.294±354.961	
Albumin	Range	3.2-4.8	1.8-4.5	<0.001
	Mean ±SD	4.222±0.397	3.264±0.681	
AST	Range	8-34	6-71	NS
	Mean ±SD	17.060±5.978	17.040±9.555	
ALT	Range	6-31	5-92	NS
	Mean ±SD	16.920±6.137	14.480±12.795	
ESR	Range	3-14	5-128	<0.001
	Mean ±SD	6.560±2.697	37.980±31.201	
Serum Amyloid A (μg/ml)	Range	1.604-7.796	5.722-40	<0.001
	Mean ±SD	3.703±1.327	24.621±12.782	

p > 0.05 is not significant (NS).

Patients were stratified according to their disease extent according to Montreal classification. In the remission patient group, there was no significant difference between laboratory parameters in patients with different disease extents. However, in activity patients' hemoglobin levels were statistically significantly lower in patients with ulcerative proctitis than those with pancolitis and distal colitis (*p*= 0.017). Also, according to Truelove and Witts criteria, there were 31 patients having severe disease. Of these, 13 patients had extensive colitis (E3), 11 patients had proctitis (E1), and only 7 patients had distal colitis (E2) (Table 2).

Table 2. Comparison between different laboratory parameters of patients in activity group according to Montreal classification.

Activity		Montreal classification			*p*-value (ANOVA)
		E1 (n= 13)	E2 (n= 17)	E3 (n= 20)	
TLC	Range	4.9-15	3.4-16.1	3.6-15	NS
	Mean ±SD	10.431±3.602	9.529±3.342	8.790±3.318	
Hemoglobin	Range	7-12	9-13.8	8.1-14.4	0.017
	Mean ±SD	9.492±1.259	11.082±1.392	10.590±1.641	
PLT	Range	195-529	178-504	154-654	NS
	Mean ±SD	374.462±129.676	308.294±88.787	349.100±136.944	
CRP	Range	6-74.5	4.5-102	1.4-162	NS
	Mean ±SD	31.515±22.116	32.982±25.346	39.835±45.612	
Fecal calprotectin (mg/kg)	Range	185-1305	110-1400	50-1500	NS
	Mean ±SD	670.362±348.599	520.235±356.278	530.300±361.456	
Albumin	Range	1.8-4.2	2.7-4.5	1.8-4.5	NS
	Mean ±SD	2.923±0.652	3.500±0.521	3.285±0.753	
AST	Range	6-25	10-22	8-71	NS
	Mean ±SD	18.385±5.679	14.176±3.729	18.600±13.866	
ALT	Range	6-28	5-23	6-92	NS
	Mean ±SD	13.308±6.356	11.294±4.767	17.950±18.839	
ESR	Range	5-128	11-119	10-90	NS
	Mean ±SD	40.154±36.131	41.294±35.603	33.750±24.225	
Serum Amyloid A	Range	15.47-40	5.722-40	6.599-40	NS
	Mean ±SD	31.337±10.586	21.961±12.435	22.516±13.359	
Chi-Square		N %	N %	N %	*p*-value
*Truelove and Witts	Moderate	2 15.38	10 58.82	7 35.00	0.049
	Severe	11 84.62	7 41.18	13 65.00	

*Data is represented in number (N) and percent (%), $p > 0.05$ is not significant (NS).

In the group of patients with disease activity, according to Mayo score, four patients experienced mild disease and 46 patients had moderate disease. On comparing them, the number of bloody motions was statistically significantly higher in patients with moderate Mayo score than in those with mild score (Tables 3 and 4).

Table 3. Comparison between Laboratory parameters and symptomatology of activity patients' group in relation to Mayo score.

Activity		Mayo score		*p*-value (T-Test)
		Mild (n= 4)	Moderate (n= 46)	
TLC	Range	5.4-11.4	3.4-16.1	NS
	Mean ±SD	9.025±2.580	9.507±3.477	
Hemoglobin	Range	9.8-12	7-14.4	NS
	Mean ±SD	10.800±1.117	10.443±1.606	
PLT	Range	195-517	154-654	NS
	Mean ±SD	307.000±144.342	344.848±120.230	
CRP	Range	3.3-35	1.4-162	NS
	Mean ±SD	17.325±15.234	36.909±34.724	
Fecal calprotectin (mg/kg)	Range	50-918	110-1500	NS
	Mean ±SD	338.250±392.342	582.863±349.345	
Albumin	Range	1.8-4.5	1.8-4.5	NS
	Mean ±SD	3.425±1.218	3.250±0.635	
AST	Range	6-22	8-71	NS
	Mean ±SD	16.500±7.188	17.087±9.795	
ALT	Range	8-23	5-92	NS
	Mean ±SD	15.000±6.782	14.435±13.236	
ESR	Range	7-20	5-128	NS
	Mean ±SD	14.250±5.377	40.043±31.682	
No. of bowel motions	Range	4-10	4-10	NS
	Mean ±SD	7.250±3.202	6.957±1.534	
No. of bloody motions	Range	1-4	1-7	0.045
	Mean ±SD	2.500±1.291	4.109±1.509	
Temperature	Range	36.8-37.1	36.8-38	NS
	Mean ±SD	36.975±0.126	37.233±0.374	
Heart rate	Range	88-98	77-110	NS
	Mean ±SD	92.750±4.573	92.304±8.899	
Serum Amyloid A	Range	15.47-40	5.722-40	NS
	Mean ±SD	29.833±12.230	24.167±12.857	
Chi-Square		N	%	N	%	*p*-value
*Truelove and Witts	Moderate	2	50.00	17	36.96	NS
	Severe	2	50.00	29	63.04	

*Data is represented in number (N) and percent (%), $p > 0.05$ is not significant (NS).

Table 4. Comparison between different disease severity scores among patients in activity group in relation to their Mayo Score.

Activity		Mayo score				*p*-value (T-Test)
		Mild (n=4)		Moderate (n=46)		
Age	Range	28-42		16-68		NS
	Mean ±SD	33.500±6.807		30.196±10.584		
Chi-Square		N	%	N	%	*p*-value
Gender	Male	2	50.00	15	32.61	NS
	Female	2	50.00	31	67.39	
Mayo endoscopic sub score	Moderate	3	75.00	18	39.13	NS
	Severe	1	25.00	28	60.87	

Table 4. Continued.

Activity		Mayo score				*p*-value (T-Test)
		Mild (n=4)		Moderate (n=46)		
UCEIS	Mild	3	75.00	38	82.61	NS
	Moderate	1	25.00	8	17.39	
Medications	Non-Biological	3	75.00	38	82.61	NS
	Biological	1	25.00	8	17.39	

$p > 0.05$ is not significant (NS).

Moreover, on classifying patients with disease activity according to UCEIS, there were 41 patients with mild disease and 9 patients with moderate disease. However, there was no significant difference in gender, laboratory data, and other endoscopic severity scores between them (Tables 5 and 6).

Table 5. Comparison between Laboratory parameters and symptomatology of activity patients' group in relation to UCEIS.

Activity		UCEIS				*p*-value (T-Test)
		Mild (n=41)		Moderate (n=9)		
TLC	Range	3.4-16.1		3.6-15		NS
	Mean ±SD	9.554±3.307		9.078±3.964		
Hemoglobin	Range	8.1-14.4		7-12		NS
	Mean ±SD	10.641±1.553		9.700±1.467		
PLT	Range	154-654		195-529		NS
	Mean ±SD	341.878±119.889		341.556±133.849		
CRP	Range	1.4-162		6-97		NS
	Mean ±SD	36.195±34.506		31.456±32.738		
Fecal calprotectin (mg/kg)	Range	50-1500		300-1305		NS
	Mean ±SD	529.944±360.245		715.222±301.966		
Albumin	Range	1.8-4.5		2.5-4.2		NS
	Mean ±SD	3.266±0.701		3.256±0.615		
AST	Range	6-71		11-28		NS
	Mean ±SD	16.561±10.137		19.222±6.241		
ALT	Range	5-92		9-32		NS
	Mean ±SD	13.585±13.576		18.556±7.650		
ESR	Range	10-128		5-78		NS
	Mean ±SD	39.878±32.359		29.333±24.995		
No. of bowel motions	Range	4-10		4-10		NS
	Mean ±SD	7.073±1.649		6.556±1.810		
No. of bloody motions	Range	1-7		3-7		NS
	Mean ±SD	3.902±1.578		4.333±1.414		
Temperature	Range	36.8-38		36.8-38		NS
	Mean ±SD	37.202±0.352		37.256±0.445		
Heart rate	Range	77-110		83-110		NS
	Mean ±SD	92.220±8.656		92.889±8.852		
Serum Amyloid A	Range	5.722-40		11.56-40		NS
	Mean ±SD	24.409±12.967		25.587±12.596		
Chi-Square		N	%	N	%	*p*-value
*Truelove and Witts	Moderate	16	39.02	3	33.33	NS
	Severe	25	60.98	6	66.67	

*Data is represented in number (N) and percent (%), $p > 0.05$ is not significant (NS).

Table 6. Comparison between different disease severity scores among patients in activity group in relation to their UCEIS.

Activity		UCEIS				*p*-value
		Mild (n=41)		Moderate (n=9)		T-Test
Age	Range	16-68		17-56		NS
	Mean ±SD	30.927±9.885		28.333±12.560		
Chi-Square		N	%	N	%	*p*-value
Gender	Male	12	29.27	5	55.56	NS
	Female	29	70.73	4	44.44	
Mayo endoscopic Sub score	Moderate	19	46.34	2	22.22	NS
	Severe	22	53.66	7	77.78	
Medications	Non-Biological	33	80.49	8	88.89	NS
	Biological	8	19.51	1	11.11	

Data is represented in number (N) and percent (%), $p > 0.05$ is not significant (NS).

The current study also found no significant relation between SAA and gender, different laboratory parameters and endoscopic severity scores among the group of patients with disease activity. While in the remission stage group, some patients with clinical remission did not have full endoscopic remission. In addition, SAA was statistically significantly higher in mild cases in comparison to remission cases according to Mayo score and UCEIS. Also, SAA was statistically significantly higher in severe and moderate cases than in mild cases according to Mayo endoscopic sub score (Tables 7 and 8). Nevertheless, among the group with disease activity, SAA showed a statistically significant positive correlation with the TLC and a significant negative correlation with hemoglobin level (Table 9).

Table 7. Relation between serum Amyloid A and different parameters in Activity patients' group.

Activity		Serum Amyloid A		*p*-value
		N	Mean±SD	(T-Test)
Gender	Male	17	22.807±13.772	NS
	Female	33	25.555±12.356	
True love and Witts	Moderate	19	20.264±11.221	NS
	Severe	31	27.291±13.111	
Mayo endoscopic sub score	Moderate	21	25.831±12.758	NS
	Severe	29	23.744±12.951	
Mayo score	Mild	4	29.833±12.230	NS
	Moderate	46	24.167±12.857	
UCEIS	Mild	41	24.409±12.967	NS
	Moderate	9	25.587±12.596	
Medications	Non-Biological	41	25.109±12.775	NS
	Biological	9	22.397±13.335	
CRP	Normal	6	24.115±12.623	NS
	Elevated	44	24.690±12.946	
Fecal calprotectin (mg/kg)	<150 mg/kg	5	24.126±12.522	NS
	>150 mg/kg	45	24.676±12.948	
ESR	<30	30	22.086±13.220	NS
	>30	20	28.423±11.367	

$p > 0.05$ is not significant (NS). N: number.

Table 8. Relation between serum Amyloid A and different parameters in the remission patients' group.

Remission		Serum Amyloid A		*p*-value (T-Test)
		N	Mean±SD	
Gender	Male	27	3.865±1.305	NS
	Female	23	3.512±1.357	
Mayo score	Remission	34	3.239±0.977	<0.001
	Mild	16	4.689±1.459	
UCEIS	Remission	41	3.366±1.032	<0.001
	Mild	9	5.236±1.497	
Medications	Non-Biological	28	3.589±1.236	NS
	Biological	22	3.848±1.452	
CRP	Normal	44	3.730±1.377	NS
	Elevated	6	3.501±0.951	
Fecal calprotectin (mg/kg)	<150 mg/kg	48	3.706±1.334	NS
	>150 mg/kg	2	3.613±1.622	
ESR	<30	50	3.703±1.327	-
	>30	0	0.000±0.000	
				p-value (ANOVA)
Mayo endoscopic sub score	Mild	34	3.239±0.977	<0.001
	Moderate	14	4.555±1.425	
	Severe	2	5.629±1.877	

$p > 0.05$ is not significant (NS). N: number

Table 9. Correlation between serum Amyloid A and different parameters among both remission and activity groups of patients.

	Serum Amyloid A			
	Remission		Activity	
	r	*p*-value	r	*p*-value
Age	-0.037	NS	0.118	NS
TLC	-0.019	NS	0.330	0.019
Hemoglobin	0.059	NS	-0.286	0.044
PLT	0.201	NS	0.235	NS
CRP	0.115	NS	-0.050	NS
Fecal calprotectin (mg/kg)	-0.032	NS	0.151	NS
Albumin	0.046	NS	-0.136	NS
AST	-0.138	NS	-0.115	NS
ALT	-0.128	NS	-0.230	NS
ESR	0.053	NS	0.160	NS
No. of bowel motions	-0.156	NS	-0.055	NS
No. of bloody motions	-	-	0.056	NS
Temperature	0.000	NS	0.081	NS
Heart rate	0.122	NS	-0.029	NS

$p > 0.05$ is not significant (NS).

The ROC curve analysis was used to determine the diagnostic value of SAA to differentiate UC disease activities. The best cutoff value was > 5.199 µg/ml, with 100 % sensitivity, specificity of 92 %, positive predictive value (PPV) of 92.6%, 100% negative predictive value (NPV)

and overall accuracy of 99.6% (Figure 1). While at a cutoff value of ≤15.24 µg/ml SAA can discriminate between mild and moderate disease activity, according to Mayo score, with sensitivity of 39.13 %, 100 % specificity, 100% PPV, NPV of 12.5% and overall accuracy of 65.8 % (Figure 2).

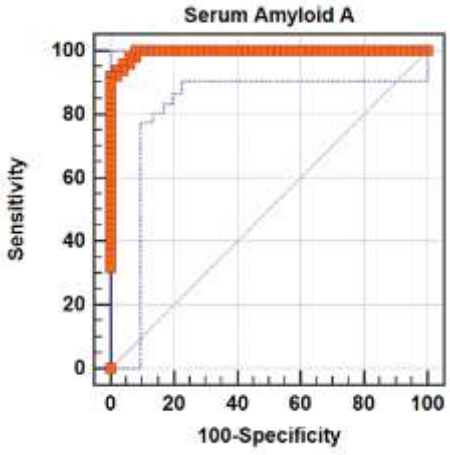

Figure 1. Receiver-operating characteristic (ROC) curve of serum Amyloid A for differentiation of ulcerative colitis disease activity.

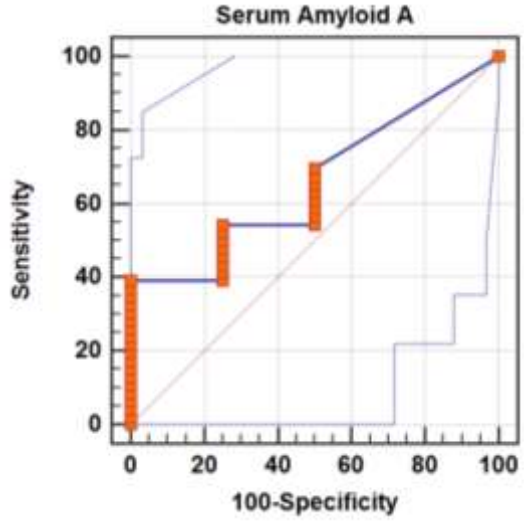

Figure 2. Receiver-operating characteristic (ROC) curve of serum Amyloid A for differentiation of ulcerative colitis disease severity according to Mayo score.

Also, at a cutoff value of ≤16.29 µg/ml, SAA can discriminate between mild and moderate disease activity, according to Mayo endoscopic Sub score, with a sensitivity of 48.28 %, specificity of 71.43 %, PPV of 70%, NPV of 50% and overall accuracy of 56.3 % (Figure 3). Finally, the best cutoff value of SAA in differentiation between extensive colitis (E3) from (E1 and E2) based on Montreal classification, was ≤14.01 µg/ml with sensitivity of 50 %, specificity of 80 %, PPV of 62.5%, NPV of 70.6% and overall accuracy of 59.7 % (Figure 4).

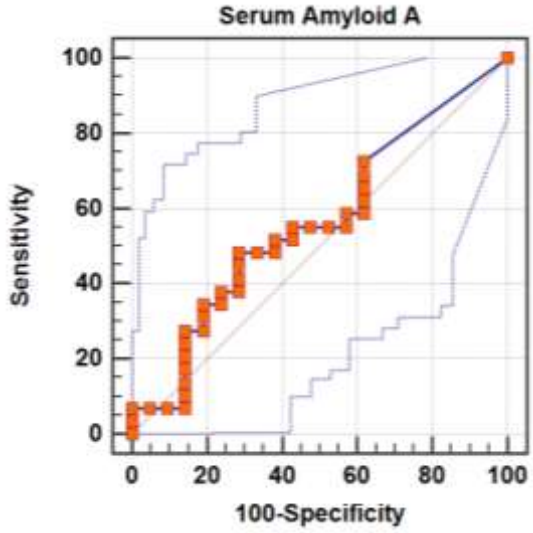

Figure 3. Receiver-operating characteristic (ROC) curve of Amyloid A for differentiation of ulcerative colitis disease severity according to Mayo endoscopic sub score.

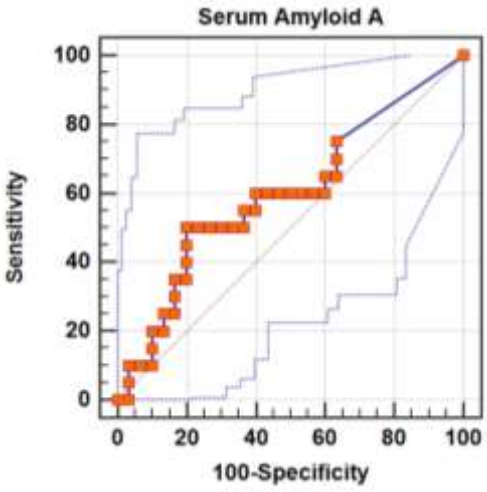

Figure 4. Receiver-operating characteristic (ROC) curve of serum Amyloid A for differentiation of ulcerative colitis Extensive colitis (E3) according to Montreal classification.

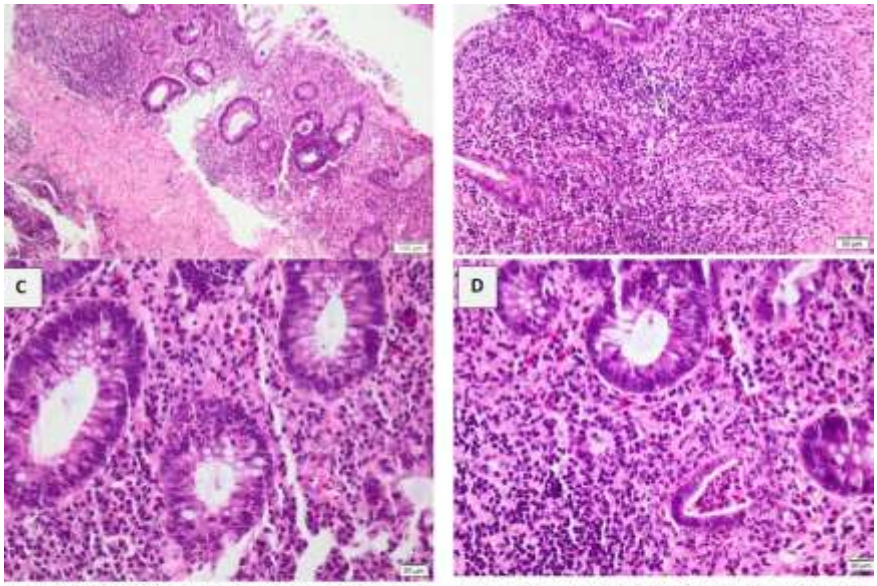

Figure (A): Rectosigmoid biopsy revealed focally ulcerated surface mucosa and glandular distortion (H&E x100). **Figure (B):** Section revealed increased basal lymphoplasmacytic infiltrate (H&E X200). **Figure (C):** Section revealed moderate cryptitis (H&E X400). **Figure (D):** Section revealed cryptitis and crypt abscess formation (H&E x400)

Figure 5. Different histopathological findings of Ulerative colitis.

Discussion

Ulcerative colitis (UC) is a chronic, progressive inflammatory bowel disorder characterized by frequent flares followed by periods of remission.[10] diagnosis depends on clinical manifestations together with radiological investigations, endoscopic and histopathological examination.[11] Simple non-invasive biomarkers are needed to avoid the disadvantageous complications of invasive diagnostic procedures. Previously studied biomarkers for predicting activity in UC, are either serum markers like CRP and ESR or serological and antibody markers or fecal markers as calprotectin and lactoferrin.[12] This study aimed to evaluate the role of SAA as an evolving possible biomarker in predicting mucosal healing in ulcerative colitis patients in comparison to other laboratory biomarkers, endoscopic severity scores and histopathology.

Patients in the current study were 44% males and 56% females with age range 16-49 years in the remission stage group and 16-68 years in UC disease activity group. There was no difference in age and gender between both groups. This observation agreed with that of a study by Zhang et al., 2020[13] who stated that age and gender did not significantly differ between patients with mild-to-moderate UC and those with severe UC. Also, the study by Ahmed et al., 2017[14] reported no significant difference in the age between patients with mild, moderate, or severe activity.

In the current study hemoglobin and albumin levels were statistically significantly lower in disease activity patients in comparison to remission patients. This is in accordance with that observed by Zhang et al., 2020[13] who reported that patients with severe UC had lower levels of hemoglobin, albumin, and total protein than normal control people. Also, we observed that CRP, ESR, TLC and platelets counts were statistically significantly higher in patients in disease activity in comparison to those in remission. This observation is consistent with that reported by Elnagdy et al., 2022[15] who found that active UC patients showed significantly greater TLC, absolute neutrophilic count, absolute monocytic count, CRP, and ESR than inactive UC patients and controls.

In the present study, FC was higher in the disease activity group with mean values of (563.294±354.961 mg/kg) in comparison to remission group (90.660±32.633 mg/kg) ($p<$ 0.001). This goes in agreement with that reported by Theede et al., 2015[16] who stated that FC level identified patients with UC who have endoscopic and histologic features of

mucosal healing and correlates with endoscopic and histologic inflammatory activity. These results also agreed with recommendations of the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE), stating that the cutoff value of FC is dependent on the desired outcome. Where lower thresholds (<100 μg/g) were proposed for reflecting deep healing (both endoscopic and transmural healing) whereas higher values (<250 μg/g) reflect less stringent outcomes.[17]

In the present study, SAA levels were statistically significantly higher among UC disease activity group in comparison to remission stage group (24.621±12.782 vs 3.703±1.327, *p* <0.001). This finding is consistent with that of Elkholy et al., 2023,[18] stated that SAA was significantly higher in the moderate and severe UC activity groups than in the inactive group (*p*=0.002). This also agreed with findings of Bourgonje, 2023,[19] who demonstrated that UC patients with high endoscopic disease activity (either moderate or severe) had significantly elevated serum concentrations of Eotaxin-1, SAA, TNF-α, IL-6, IL-8 and IL-17A as compared to patients with low endoscopic disease activity, either in remission or mild disease.

Within the same context, the study by Yarur et al., 2017,[8] concluded that high circulating SAA levels can correlate with lack of intestinal mucosal healing (MH) and may be a surrogate marker for disease activity, even in patients whose CRP levels do not correlate with disease activity in Crohn's disease. The same observation was reported by Ishihara et al., 2018,[20] results indicating that SAA level was significantly higher in endoscopic active phases as compared to inactive phase.

In the present study, FC levels did not correlate with disease extent as it was higher in patients with active ulcerative proctitis than in those with much extensive disease. However, this difference did not reach statistical significance. Such finding contradicts those observed by Theede et al., 2015,[16] who stated that FC levels increased significantly with disease extent advancement.

According to findings of the present study, SAA levels did not differ between ulcerative proctitis (E1) patients of both activity and remission groups and in patients with much extensive diseases. This observation goes in agreement with Shin et al., 2020,[21] who found no statistically significant difference in disease extent according to the endoscopic remission status. However, this observation disagreed with that reported by Wakai et al., 2020,[22] who found that the usefulness of SAA may be more enhanced in widespread inflammation such as total colitis than in less extensive disease.

In the present study, there was no statistically significant difference in SAA levels with different treatment modalities in both study groups. This was also reported by Shin et al., 2020,[21] who found no statistical difference in SAA levels related to offered medications regardless of the endoscopic remission status. This may be attributed to fact that most of ulcerative proctitis patients enrolled in the present study (11 out of 13) had severe disease according to Truelove and Witts criteria[9.] Also, this may explain the statistically significantly lower hemoglobin levels in patients with ulcerative proctitis in comparison to other disease extents.

In the current study, FC did not differ in patients with moderate disease and mild disease activity according to Mayo score (582.863±349.345 vs 338.250±392.342). FC was higher in patients with moderate than mild UCEIS (715.222±301.966 vs 529.944±360.245). These results are in accordance with those of Mak et al., 2018,[23] who stated that the level of FC increased with MES advancement, and with those of Mańkowska-Wierzbicka et al., 2015,[24] who declared that FC was closely correlated with the MES and can be used to evaluate severity of UC.

In the present study, SAA was insignificantly higher in mild than moderate disease activity according to Mayo score which is in accordance with findings of Mańkowska-Wierzbicka et al., 2015,[24] who stated that there were no significant correlations between the Mayo endoscopic scores and markers investigated for UC, as CRP, IL2, IL10, IFN- alpha. This is also consistent with findings of Bourgonje, 2023,[19] who stated that in UC, using the Mayo endoscopic sub score, serum concentrations of

SAA did not correlate in moderate-to-severe disease activity as compared to remission or mild disease activity. However, this was partially contradicted with what Wakai et al., 2020,[22] stated, that in UC patients, both CRP and SAA were correlated with MES, with much stronger correlation between SAA and mucosal inflammation than that of CRP. Therefore, SAA was found to reflect the state of the mucosa more accurately than CRP.

In another context, in our remission patients' group, there was a subset of patients with reported remission symptoms and even normal laboratory results who weren't found to have full endoscopic remission, with many discrepancies in their disease severity among different endoscopic scores.

These discrepancies may be partially dependent on that Mayo score evaluates subjective symptoms. Also, since erythema, vascular texture, brittleness, erosion, ulcers, and spontaneous bleeding are the foundation of MES, it may not differentiate between ulcers' depths, with all scoring MES3. While during MH, ulcers tend to shrink and become shallower, with Mayo score being unable to detect such subtle alternation.[25] Compared with Mayo score, UCEIS can distinguish the depth of ulcers, precisely depict the actual endoscopic manifestations of patients with severe UC, and more accurately reflect clinical outcomes.[26]

In such patients SAA was statistically significantly higher in mild disease in comparison to endoscopic remission as determined by Mayo score and UCEIS. And, also higher in severe and moderate disease than in mild disease according to MES (p <0.001). This agreed with that of Wakai et al., 2020,[22] who reported that SAA can be an excellent marker in predicting mucosal healing in clinical remission patients.

However, in patients who did not achieve clinical remission. No significant difference was found. When the disease activity of UC increases, CRP level also tends to increase, and the significance of SAA decreases. Thus, SAA can be a better monitoring tool to predict mucosal inflammation than CRP in patients with clinical remission with low disease activity.[22] This explains that in our activity patients, there were no statistically significant relations between SAA and gender, different laboratory parameters or endoscopic severity scores. Such finding led Wakai et al., 2020,[22] to propose that endoscopic examinations should be considered in clinical remission patients with elevated SAA, even if their CRP results are negative.

Consecutively, there were no significant correlations between SAA and age, clinical symptoms, FC, ESR, CRP or platelet count neither in remission nor activity group. These contradict with findings of Bourgonje et al., 2019[27] who detected a significant positive correlation between SAA and FC. And, with Elkholy et al., 2023[18] who found a significant positive correlation between SAA and FC, CRP, and platelet count.

According to UCEIS, SAA was higher in patients with moderate than mild disease (25.587±12.596 µg/ml vs 24.409±12.967 µg/ml), but this did not reach statistical significance. However, based on our best knowledge, there are no previous studies that ever dealt with the correlation between SAA and UCEIS as an indicator of endoscopic disease activity in ulcerative colitis.

Based on current study results, SAA could predict disease activity at a cutoff value of > 5.199 µg/ml with 100% sensitivity, specificity of 92%, PPV of 92.6%, 100% NPV and overall accuracy of 99.6%. Such data indicate more sensitivity and specificity than the results of a study by Wakai et al., 2020,[22] that reported SAA levels < 5.8 µg/ml could discriminate mucosal inflammation from mucosal healing with sensitivity of 0.722, specificity of 0.850, PPV of 0.760, NPV of 0.823, and accuracy of 0.799. Also, our reported data are more sensitive and specific than those indicated by Elkholy et al., 2023,[18] who reported that the cutoff was >3.97 µg/ml, with sensitivity, specificity, PPV, NPV and accuracy were 84.44%, 55.56%, 65.5%, 78.1% and 74.2%, respectively.

The present study has several limitations including the relatively small sample size. SAA was measured in each patient at a single time point during the clinical course. It should be measured at multiple time points in both the active and remission phases of the disease in the same patient for accurate monitoring of its

level changes during disease activity. Since not all patients underwent urine tests, chest X-ray examination, computed tomography, etc., we cannot completely exclude infectious diseases and malignant tumors that may have caused the elevated CRP and SAA levels.

In summary, according to the present study, SAA was found to be higher in patients with clinical and endoscopic activity but with no statistically significant correlation with endoscopic mucosal activity scores representing mucosal inflammation like Mayo score and UCEIS and may be a possible useful marker for predicting endoscopic activity in UC patients in clinical remission. It would be more helpful when used together with serum laboratory inflammatory indices (ESR, CRP and fecal calprotectin) along with clinical and endoscopic activity scores. In conclusion, our data indicated that SAA could be used for prediction of mucosal healing in ulcerative colitis remission patients despite not being superior to fecal calprotectin. However, it was unable to differentiate between the different disease activities or extents. Although the therapeutic goal of UC is mucosal healing, clinical and endoscopic findings do not necessarily match. Therefore, among the clinical remission patients without symptoms, it is important to evaluate intestinal inflammation using biomarkers than through frequent endoscopies. SAA can be used in parallel with other inflammatory markers and endoscopic scores to predict disease activity.

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Author Contributions

AI designed and edited the manuscript. MWK reviewed and modified the work. Data were gathered by SMM. HSE aided in drafting of the manuscript. SAE contributed by manuscript revision and pathological examination. AAA aided with methodology and data revision. The submitted manuscript was reviewed and approved by all authors.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Sham University (Reference Number: FMASU MD 51/2021).

Informed consent

A written informed consent was obtained from each patient before being included in the study.

References

1. Kałużna, A., Olczyk, P., & Komosińska-Vassev, K. (2022). The Role of Innate and Adaptive Immune Cells in the Pathogenesis and Development of the Inflammatory Response in Ulcerative Colitis. *J clin Med*, 11(2), 400.
2. Bressler, B., Marshall, J. K., Bernstein, C. N., et al (2015). Clinical practice guidelines for the medical management of non-hospitalized ulcerative colitis: the Toronto consensus. *Gastroenterology*, 148(5), 1035–1058.e3.
3. Nikolaus, S., & Schreiber, S. (2007). Diagnostics of inflammatory bowel disease. *Gastroenterology*, *133*(5), 1670–1689.
4. Fefferman, D. S., & Farrell, R. J. (2005). Endoscopy in inflammatory bowel disease: indications, surveillance, and use in clinical practice. *Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association*, *3*(1), 11–24.
5. Targońska-Stępniak, B., & Majdan, M. (2014). Serum amyloid A as a marker of persistent inflammation and an indicator of cardiovascular and renal involvement in patients with rheumatoid arthritis. *Mediators of inflammation*, 793628.
6. Wang, Z., & Nakayama, T. (2010). Inflammation, a link between obesity and cardiovascular disease. *Mediators of inflammation*, 535918.

7. Bathe, A. L., Mavropoulou, E., Mechie, N. C., et al. (2019). Impact of faecal calprotectin measurement on clinical decision-making in patients with Crohn's disease and ulcerative colitis. *PloS one*, *14*(10), e0223893
8. Yarur, A. J., Quintero, M. A., Jain, A., et al.(2017). Serum Amyloid A as a Surrogate Marker for Mucosal and Histologic Inflammation in Patients with Crohn's Disease. *Inflammatory bowel diseases*, 23(1), 158–164.
9. Pabla, B. S., & Schwartz, D. A. (2020). Assessing Severity of Disease in Patients with Ulcerative Colitis. *Gastroenterology clinics of North America*, *49*(4), 671–688.
10. Ungaro, R., Mehandru, S., Allen, P. B., et al. (2017). Ulcerative colitis. *Lancet (London, England)*, *389*(10080), 1756–1770.
11. Makkar, R. & Bo, S. (2013): Colonoscopic perforation in inflammatory bowel disease. *Gastroenterol Hepatol*; 9: 573-583.
12. Okba, A. M., Amin, M. M., Abdelmoaty, A. S., et al. (2019). Neutrophil/lymphocyte ratio and lymphocyte/monocyte ratio in ulcerative colitis as non-invasive biomarkers of disease activity and severity. *Auto- immunity highlights*, *10*(1), 4.
13. Zhang, M. H., Wang, H. G., Shi, Y. T., et al., (2020). Efficacy of Serum Total Bilirubin in Predicting the Severity of Ulcerative Colitis: A Cross-Sectional Study. *Annals of clinical and laboratory science*, *50*(2), 228–232.
14. Ahmed, R., El-Atrebi, K. A., Hassan, A., et al. (2017): Fecal calprotectin and CRP as biochemical markers in predicting inflammatory bowel disease activity in patients with ulcerative colitis. *J the Med Res Instit*; 38(1):10-15.
15. Elnagdy, M., Mansour, R., Abd El Aziz, S., et al. (2022): Evaluation of Serum Amyloid (A) Level in Ulcerative Colitis as a new Predictor of Disease Activity. *Al-Azhar Inter Med. J*; 3(5): 35-39.
16. Theede, K., Holck, S., Ibsen, P., et al., (2015). Level of Fecal Calprotectin Correlates With Endoscopic and Histologic Inflammation and Identifies Patients With Mucosal Healing in Ulcerative Colitis. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*, *13*(11), 1929–36.e1.
17. Turner, D., Ricciuto, A., Lewis, A., et al. (2021): STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): Determining Therapeutic Goals for Treat-to-Target strategies in IBD. *Gastroenterology*; 160(5):1570-1583.
18. Elkholy, H., Biomy, H., Ahmad, E., et al. (2023): Serum amyloid A as a biomarker of ulcerative colitis disease activity. *Benha Med. J*; 40: 87-102.
19. Bourgonje, A. R. (2023). Biomarker signatures in pathophysiology and therapeutic interventions in inflammatory bowel diseases: a multimodal approach. [Groningen]: University of Groningen, 1108 p
20. Ishihara, S., Tada, Y., Kawashima, K., et al., (2018). Serum amyloid A level correlated with endoscopic findings in patients with Crohn's disease-Possible biomarker for evaluating mucosal healing. *Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver*, *50*(6), 553–558.
21. Shin, J., Kong, S. M., Seong, G., & Kim, Y. H. (2020). What is the appropriate cut-off value of CRP to predict endoscopic remission in patients with ulcerative colitis in clinical remission?. *Inter J colorectal disease*, 35(12), 2249–2255.
22. Wakai, M., Hayashi, R., Tanaka, S., et al. (2020). Serum amyloid A is a better predictive biomarker of mucosal healing than C-reactive protein in ulcerative colitis in clinical remission. *BMC gastroenterology*, 20(1), 85.
23. Mak, W. Y., Buisson, A., Andersen, M. J., et al. (2018). Fecal Calprotectin in Assessing Endoscopic and Histological Remission in Patients with Ulcerative Colitis. *Digestive diseases and sciences*, *63*(5), 1294–1301.
24. Mańkowska-Wierzbicka, D., Swora-Cwynar, E., Poniedziałek, B., et al. (2015). Usefulness of selected laboratory markers in ulcerative colitis. *European cytokine network*, 26(2), 26–37.
25. Ikeya, K., Hanai, H., Sugimoto, K., et al. (2016). The Ulcerative Colitis Endoscopic Index of Severity More Accurately Reflects Clinical Outcomes and Long-term Prognosis than the Mayo Endoscopic Score. *J Crohn's & colitis*, *10*(3), 286–295.
26. Li, X., Tang, Z., Liu, Y., et al. (2023). Risk prediction model based on blood biomarkers for predicting moderate to severe endoscopic activity in patients with ulcerative colitis. *Frontiers in Medicine*, *10*, 1101237.
27. Bourgonje, A. R., von Martels, J. Z. H., Gabriëls, R. Y., et al. (2019). A Combined Set of Four Serum Inflammatory Biomarkers Reliably Predicts Endoscopic Disease Activity in Inflammatory Bowel Disease. *Frontiers in medicine*, *6*, 251.