Diagnostic Value of Fecal Calprotectin in Patient with Ulcerative Colitis

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Received: 02 Feb. 2013
Accepted: 28 Mar. 2013

ABSTRACT

BACKGROUND

Ulcerative colitis (UC) is characterized by recurrent episodes of inflammation limited to the mucosal layer of the colon. Calprotectin is a zinc and calcium binding protein derived from neutrophils and monocytes. It is easily detectable in tissue samples, body fluids, and stools, which makes it a potentially valuable marker of inflammation. The aim of the current study is to evaluate the value of fecal calprotectin (FC) as a marker of disease activity in patients with UC.

METHODS

Seventy three eligible subjects underwent ileocolonoscopy and multiple biopsies were obtained from different parts of the colon and terminal ileum. All patients underwent blood and stool sampling as well as an interview to assess the disease severity utilizing ulcerative colitis activity index (UCAI), subjectively. The diagnostic value of the FC in comparison with Mayo disease activity index as the gold standard technique, was then evaluated.

RESULTS

Mean FC level increased linearly according to Mayo disease activity index ($r=0.44$, $p<0.001$) and was significantly different between levels of Mayo disease activity index ($p=0.003$). In multivariate analysis, Mayo disease activity index, positive CRP and ESR were associated with FC level. FC level > 21.4 ng/ml was able to discriminate between active and inactive phases of UC according to Mayo disease activity index>2 with 72.3% sensitivity and 73.1% specificity. The combination of FC > 21.4 ng/ml and UCAI score of 7 had a 46.8% sensitivity and 88% specificity to diagnose Mayo disease activity index >2. Furthermore, FC level <21.4 ng/ml in combination with UCAI score of <3 showed a highly considerable specificity of 98% to discriminate the remission phase of UC (Mayo disease activity index <2), although with a low sensitivity (31%).

CONCLUSION

FC appears to be a non-invasive biomarker with moderate accuracy to discriminate the active phase of inflammatory bowel disease (IBD). The value of FC especially in combination with UCAI is highly considerable to rule out the Mayo disease activity index >2.

KEYWORDS

Colitis, ulcerative; Leukocyte L1 Antigen Complex; Calprotectin; Colonoscopy

Please cite this paper as:
Zamani H, Barzin G, Yousefinia M, Mohammadkhani A, Ostovaneh MR, Sharifi AH, Tayebi S, Malekzadeh R, Ansari R. Prognostic Value of Fecal Calprotectin in Patient with Ulcerative Colitis. *Middle East J Dig Dis* 2013;5:76-80.

INTRODUCTION

Ulcerative colitis (UC) is a chronic, idiopathic inflammatory condition of the large bowel with a typically relapsing and remitting course.[1-3] UC exacerbations are characterized by symptoms of diarrhea, urgency of defecation, rectal bleeding and abdominal pain.[4,5] Currently, colonoscopy followed by mucosal biopsy is the standard of choice to diagnose inflammatory bowel disease (IBD) and assess its severity. However, these techniques are costly and invasive in nature. Accordingly, simple, inexpensive, and objective tools for the assessment of mucosal inflammation are therefore desirable.[6-9]

Fecal calprotectin (FC) has been introduced as a sensitive and specific marker to diagnose IBD.[10,11] Neutrophil infiltration is a prominent feature of mucosal histology in patients with active IBD.[12,13] Calprotectin is a major protein in neutrophils, accounting for about 60% of the cytosol. Elevated FC levels correlate with intestinal inflammation.[14,15] FC levels remain stable in stool. Stool samples are easy to collect. Despite some evidences suggesting FC as a surrogate marker to assess disease severity in IBD, the value of FC, however, is not completely understood.[16,17] The aim of this study is to compare the value of FC with clinical and endoscopic markers in the discrimination between inactive and active forms of UC.

MATERIALS AND METHODS

Patients with UC, referred for colonoscopy to Shariati Hospital, Tehran, Iran were consecutively recruited between May 2010 and May 2011. Exclusion criteria were: Crohn's disease, pregnancy, infectious colitis, history of colorectal cancer, alcohol consumption, and treatment with NSAIDs, antibiotic and cytotoxic medications. Subjects who met the eligibility criteria enrolled in the study after informed consent was obtained. The Ethics Committee of the Digestive Disease Research Center (DDRC) approved the study protocol which conformed to the guidelines of the 1975 Declaration of Helsinki.

We subjectively interviewed patients to obtain baseline characteristics and assess disease severity, utilizing the ulcerative colitis activity index (UCAI) scoring system.[18] Blood samples were taken for all subjects after tourniquet application to evaluate the blood cell counts and erythrocyte sedimentation rate (ESR).

Eligible subjects underwent ileocolonoscopy with multiple mucosal biopsy sampling by a single expert gastroenterologist. Colonoscopic disease activity was evaluated using the Mayo Disease Activity Index (0: normal mucosa, 1: erythema, 2: erosion, 3: ulcer).[19] Subjects with Mayo disease activity index levels ≥2 were considered to have active UC.

Stool samples were also collected in screw-capped plastic containers on the day of the colonoscopy and kept at -70°C. FC was measured using calprotectin ELISA kits (Demeditec Diagnostics GmbH, Germany). Stool extracts were prepared and analyzed for FC according to the manufacturer's instructions. FC level was expressed as ng/ml. The diagnostic value of the FC in comparison with Mayo disease activity index as the gold standard technique, was then evaluated.

Statistical analysis

STATA version 11 (StataCorp, College Station, TX, USA) was used for statistical analysis. Data were presented as mean±SD or number (%) when appropriate. Statistical difference was tested using the non-parametric Kruskal Wallis H test. Spearman's coefficient was used to assess the correlation between the variables. We performed linear regression modeling to assess the independent effect of the variables on square root transformed FC level. Variables with $p<0.2$ in univariate regression were included in the multivariate model. The assumptions of the linear regression were assessed through testing normality and homogeneity of variance of residuals and the linear relation between outcome variable and predictors. Variance inflation factor and correlation coefficients were calculated to assess multi-collinearity. We used receiver operating characteristics (ROC) curve analysis to evaluate the

accuracy of FC, UCAI and their combination with the intent to discriminate between active and inactive phases of UC. $p<0.05$ was considered statistically significant.

RESULTS

Of 86 subjects assessed for eligibility, we excluded 13 subjects as follows: Crohn's disease (3); pregnant (1); NSAIDs, antibiotics, and cytotoxic medication use (5); alcohol use (1); infectious enteritis (2); and colon cancer (1). Included in the study were 73 patients whose mean age was 34.57±11.4 years. Of these, there were 35 (47.9%) females. Table 1 outlines baseline characteristics of the patients.

As shown in Table 2 and Figure 1, the mean FC level increased linearly according to Mayo disease activity index (r=0.44, $p<0.001$) and was significantly different between different levels of Mayo disease activity index ($p=0.003$). The UCAI score differed significantly between different levels of Mayo disease activity index with a positive correlation (r=0.37, $p=0.001$, Table 2).

According to univariate regression analysis, patient age, Mayo disease activity index, disease duration, positive CRP, ESR and WBC count correlated with square root transformed FC with a p-value <0.2 and were included in the multivariate model. In multivariate analysis, only the Mayo disease activity index, positive CRP and ESR had independent associations with FC. The strength of association between FC and Mayo disease activity index was higher for a Mayo score of 3 compared to lower Mayo scores (Table 3).

The area under the ROC curve (AUROC) for FC to discriminate active phase of UC (defined as Mayo disease activity index > 2) was 74% (95% CI: 62%-86%) with a 72.3% sensitivity and 73.1% specificity for a FC cutoff value of 21.4 ng/ml. The UCAI score distinguished a Mayo disease activity index >2 with AUROC of 72.5% (95% CI: 60%-85%). The UCAI cutoff score of 7 had a 57.4% sensitivity and 84.7% specificity in this regard.

The combined FC value of 21.4 ng/ml and UCAI

Table 1: Patients' baseline characteristics.

Age, mean (SD)		34.57 (11.4)
Female gender, n (%)		35 (47.9)
Fecal calprotectin (FC), median (IQR)		27 (6-98.4)
Erythrocyte sedimentation rate (ESR), mean (SD)		13 (8-30)
Positive CRP, n (%)		24 (32.9)
WBC, mean (SD)		6750 (5750-8000)
Disease duration in months, mean (SD)		92 (30-121)
UCAI score, median (IQR)		6 (4-8)
UCAI >3, n (%)		70 (95.9)
UCAI >7, n (%)		31 (42.5)
Mayo score	**0**	12 (16.44)
	1	14 (19.18)
	2	15 (20.55)
	3	32 (43.84)

SD: standard deviation; IQR: interquartile range; CRP: C reactive protein; WBC: white blood cell; UCAI: ulcerative colitis activity index

Table 2: Median fecal calprotectin (FC) and median UCAI score according to Mayo score.

Mayo score	Median FC (IQR)	Median UCAI (IQR)
0	6.4 (2.6-17.5)	4 (3-6)
1	11.8 (8-46.7)	5 (3-6)
2	26.8 (5.4-122.2)	6 (4-9)
3	65.9 (24.8-118.5)	7 (5-9)

UCAI: ulcerative colitis activity index; IQR: interquartile range.

Table 3: Results of linear regression analysis to determine predictors of square root transformed fecal calprotectin (FC).

		Standardized beta (p-value)	
		Univariate analysis	**Multivariate analysis**
Age		-0.16(0.168)	0.05 (0.68)
Female gender		0.09 (0.46)	-
Mayo score (0 as reference layer)	**1**	0.1 (0.47)	0.01 (0.92)
	2	0.25 (0.09)	0.16 (0.29)
	3	0.5 (0.002)	0.33 (0.04)
Disease duration		-0.19 (0.1)	-0.13 (0.32)
Positive CRP		0.36 (0.002)	0.24 (0.04)
Erythrocyte sedimentation rate (ESR)		0.36 (0.002)	0.24 (0.04)
WBC		0.18 (0.13)	0.1 (0.3)

CRP: C reactive protein; WBC: white blood cell

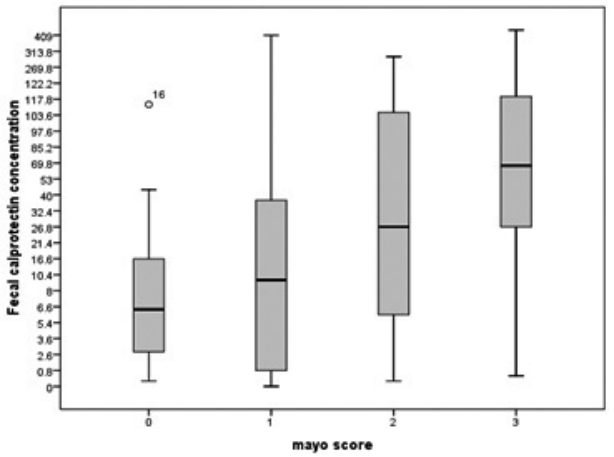

Fig. 1: Fecal calprotectin concentration for different levels of Mayo disease activity index.

score of 7 had a 46.8% sensitivity and 88% specificity for the diagnosis of a Mayo disease activity index >2 (AUROC: 78%; 95% CI: 68-89). On the other hand an FC level <21.4 ng/ml when combined with a UCAI score of <3 showed a highly considerable specificity of 98% to discriminate the remission phase of UC (Mayo score <2), however the sensitivity was low (31%).

DISCUSSION

All of our patients were known cases of UC with at least two separate colonoscopies and pathologic confirmation. Since other concomitant inflammatory and malignant disorders also increase fecal concentrations of calprotectin we only included patients in this study who had diagnoses of UC but no other concomitant diseases such as Crohn's disease, infections or malignancies.

All patients were on medications but had symptoms indicative of inflammatory flare up or IBS. We planned this study to clearly answer the question of whether the patient experienced a flare or not.

Several groups have shown that FC can differentiate active from inactive UC as well as from IBS.[12,20-23] There is good evidence from some studies showing that relapse of UC can be predicted by measuring elevations in FC.[24,25] However, studies assessing the correlation between endoscopic disease activity and FC are scarce, particularly amongst adult UC patients. As shown in Table 2 there is a positive correlation between median FC and Mayo score (p<0.05). There is a similar correlation between UCAI and Mayo score (p<0.05). Therefore use of FC and UCAI are predictive of UC mucosal activity. These findings are in accordance with that of Costa et al. who have found a strong association of UCAI and FC with UC relapse.[26] As shown in Table 3 with univariate regression analysis and the multivariate models CRP, ESR and Mayo score correlated with FC. This correlation was more significant with Mayo score 3.

ROC curve analysis showed that a FC cut off value of 21.4 ng/L offered the best diagnostic accuracy; (sensitivity: 72.3%, specificity: 73.1%) for discriminating active phase of UC with a Mayo score ≥2 (95% CI: 60%-85%). AUROC was performed for UCAI which showed 72.5% under the curve for a Mayo score >2 (95% CI: 60%-85%) with a 57.4% sensitivity and 84.7% specificity for UCAI score >7.

In this study, we showed that concomitant use of FC and UCAI were predictive of UC activity. An FC level >21.4 ng/ml and UCAI score >7 might be useful for distinguishing endoscopic active UC with Mayo scores of 2 and 3 from endoscopic quiescent disease (Mayo scores 1 and 2), For subjects with FC <21.4 ng/ml and UCAI score <3, we were able to rule out the active phase of UC (Mayo score <2) with a high confidence.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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