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CLINICAL RESEARCH

Clinical value of fecal calprotectin in determining disease activity of ulcerative colitis

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Abstract

AIM: To investigate possibility and clinical application of fecal calprotectin in determining disease activity of ulcerative colitis (UC).

METHODS: The enzyme-linked immunosorbent assay (ELISA) was used to measure the concentrations of calprotectin in feces obtained from 66 patients with UC and 20 controls. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), acid glycoprotein (AGP) were also measured and were compared with calprotectin in determining disease activity of UC. The disease activity of UC was also determined by the Sutherland criteria.

RESULTS: The fecal calprotectin concentration in the patients with active UC was significantly higher than that in the inactive UC and in the controls (402.16 ± 48.0 μg/g *vs* 35.93 ± 3.39 μg/g, 11.5 ± 3.42 μg/g, $P < 0.01$). The fecal calprotectin concentration in the inactive UC group was significantly higher than that in the control group ($P < 0.05$). A significant difference was also found in the patients with active UC of mild, moderate and severe degrees. The area under the curve of the receiver operating characteristics (AUC^{ROC}) was 0.975, 0.740, 0.692 and 0.737 for fecal calprotectin, CRP, ESR and AGP, respectively. There was a strong correlation between the fecal calprotectin concentration and the endoscopic gradings for UC ($r = 0.866$, $P < 0.001$).

CONCLUSION: Calprotectin in the patient's feces can reflect the disease activity of UC and can be used as a rational fecal marker for intestinal inflammation in clinical practice. This kind of marker is relatively precise, simple and noninvasive when compared with other commonly-used markers such as CRP, ESR and AGP.

Key words: Fecal calprotectin; Disease activity; Ulcerative colitis; Enzyme-linked immunosorbent assay

INTRODUCTION

Ulcerative colitis (UC) is a chronic, idiopathic inflammatory bowel disease characterized by remission of disease activity. The incidence and the prevalence rates of UC are increasing in China[1]. It is important to accurately evaluate intestinal mucosa inflammation in the management of these patients, particularly for the assessment of therapeutic effectiveness. Colonoscopy and biopsy are useful in the assessment of intestinal mucosa inflammation of patients with UC, but these examinations can be a heavy burden to the patient[2,3]. Clinical evaluations including laboratory tests such as C-reactive protein (CRP)[4-6], erythrocyte sedimentation rate (ESR)[7], acid glycoprotein (AGP)[8], and platelet count[9,10], have been used for the determination of disease activity of UC, but none of them are specific for gut inflammation[11]. Therefore, a new marker that will be more sensitive and specific for determination of disease activity of UC is urgently needed in clinical practice.

An alternative approach to the assessment of the presence of intestinal inflammation[12] is to analyze the whole gut lavage fluid or to quantitate the protease resistant neutrophil derived proteins such as lactoferrin[13,14] in the patient's feces, and this approach can be non-invasive for the patient. Calprotectin is one of these proteins. It is a major protein in the neutrophilic granulocytes and the macrophages[15], which accounts for 60% of the total protein in the cytosol fraction in these cells[16,17]. This kind of protein can resist metabolic degradation caused by intestinal bacteria, and the protein is relatively stable in stools for up to one week at room temperature[18]. It can differentiate between patients with organic or non-organic intestinal disease, and can be useful in detecting colorectal cancer and inflammatory disorders, and can also be useful in predicting a relapse of inflammatory bowel disease[19].

Our study was aimed at the measurement of the concentration of calprotectin in the feces of the UC patient and at the comparison of it with commonly-used markers in clinical practice, such as CRP, ESR and AGP.

MATERIALS AND METHODS

Study subjects

Sixty-six patients with UC (age, 38.97 ± 2.39 years) were enrolled in the study, including 15 patients with proctitis, 22 with left-sided colitis, and 29 with pancolitis. Of the patients, 44 were hospitalized. The patients' disease activities were assessed according to the Sutherland criteria[20] in which a score of more than two was considered to indicate the active stage of the disease. The control group consisted of 20 subjects (age, 38.95 ± 3.59 years) with no confirmed abnormality in the upper or lower digestive tract.

Methods of stool collection

The patients were instructed to defecate directly into a polystyrene container. The stool samples were stored at -70℃ until the time of measurement.

Measurement of fecal calprotectin by ELISA

The stool samples were thawed, and 50-100 mg of the sample was suspended with 2500-5000 μL of the fecal extraction buffer, and was homogenized; then, the suspernatant was diluted to 1:50, and the calprotectin was analyzed by the enzyme-linked immunosorbent assay (ELISA) using the Nycotest Phical ELISA kit (Nycomed, Norway). Microcapture and immunoaffinity-purified rabbit anticalprotectin conjugated with alkaline phosphatase was used for the development. The ELISA read the absorbance at 405 nm for 96-well plates. The results of the sample tests were evaluated from the standard curves. CRP, ESR and AGP were measured in the clinical laboratory, West China Hospital, based on the instructions provided by the reagent manufacturer.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS 11.5. The data were expressed as means ± SD. The Mann-Whitney test was used to assess differences in the laboratory parameters between the groups, and Spearmans' correlation was used to analyze the correlation between the parameters. All the P values were two tailed; P values < 0.05 were considered statistically significant. The receiver operating characteristics (ROC) (sensitivity and specificity) were assessed by the curve analysis as described by Henderson[21].

RESULTS

Concentrations of fecal calprotectin, CRP, ESR and AGP in patients with UC and in controls

There was a significant difference in the fecal calprotectin concentration between the patients with active UC and the patients with inactive UC ($P < 0.01$) (Table 1, Figure 1). The calprotectin concentration was significantly greater in the patients with inactive UC than in the controls ($P < 0.05$). The patients with active UC had higher levels of CRP, ESR and AGP than the patients with inactive UC and the controls ($P < 0.05$), but there was no significant difference between the patients with inactive UC and the controls.

Relationship between the concentrations of fecal calprotectin, CRP, ESR and AGP and the disease activity index (DAI) in UC

As shown in Figure 2, the concentrations of fecal calprotectin, CRP, ESR and AGP in UC had a good correlation with DAI. The correlation coefficients between DAI and the concentrations of fecal calprotectin, CRP, ESR and AGP were 0.866, 0.492, 0.433 and 0.533, respectively. This association was strongest for fecal calprotectin and weakest for ESR.

Specificity and sensitivity

The correspondence between the DAI-based classification of the active or the inactive disease status and the classification based on the parameter cut-offs was analyzed for each parameter (Table 2) and was expressed as the percentage of the samples that were correspondingly identified (specificity and sensitivity). Specificity was highest for fecal calprotectin, and lowest for AGP. The specificity rates for fecal calprotectin, CRP, ESR and AGP were 79.4%, 69.0%, 68.9% and 65.5%, respectively. The sensitivity for fecal calprotectin was relatively high, but was relatively low for CRP. The sensitivity rates for fecal calprotectin, CRP, ESR and AGP were 91.9%, 62.2%, 64.9%, and 67.6%, respectively. The ROC curves showed the trade-off between specificity and sensitivity for fecal calprotectin (the area under the curve, AUC, 0.975 ± 0.015; $P < 0.001$), for CRP (AUC, 0.740 ± 0.061; $P < 0.001$), for ESR (AUC, 0.692 ± 0.064; $P < 0.01$), and for AGP (AUC, 0.737 ± 0.062; $P < 0.001$) (Figure 3). The AUC^{ROC} of fecal calprotectin was greater than that of CRP, ESR or AGP ($P < 0.01$).

DISCUSSION

Chronic relapsing and remitting inflammation of the gastrointestinal tract is the hallmark of UC. One of the most prominent histological features observed in UC is infiltration of the neutrophils into the inflamed mucosa at an early stage of inflammation. The neutrophils are major sources of inflammatory cytokines, chemokines proteases, active lipids, and reactive oxygen derivatives, as well as a full complement of factors needed to exacerbate mucosal inflammation and tissue injury[22-25] The fecal calprotectin excretion of indium-labeled autologous granulocytes has for a long time been suggested as the gold standard test in assessing bowel inflammation in inflammatory bowel disease. However, as it involves an exposure to radiation and prolonged fecal collection, which is unpopular with patients and laboratory staff, it is only used as a research tool. In patients with inflammatory bowel disease, a three day excretion of indium-labeled granulocytes correlated well with a daily excretion and a one-off fecal calprotectin level[26].

In this study, we focused on the evaluation of any relationship that might exist between the mucosal neutrophil infiltration represented by calprotectin, CRP, ESR, AGP and the UC disease activity represented by the Sutherland criteria. The DAI score of UC is the sum score of the following four parameters (each scoring

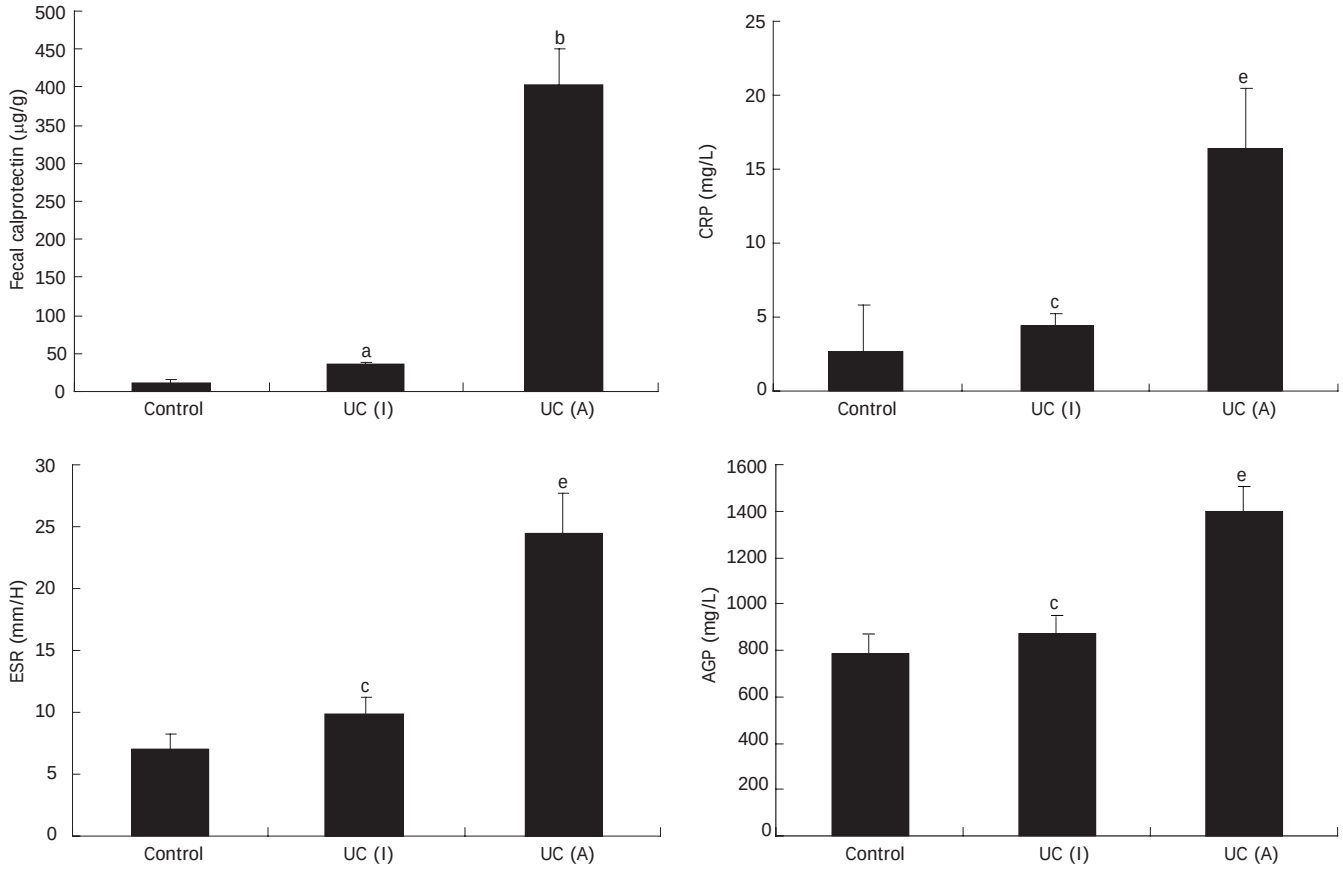

Figure 1 Concentrations of fecal calprotectin, CRP, ESR and AGP in the UC patients and the controls. UC (A): Ulcerative colitis (active phase); UC (I): Ulcerative colitis (inactive phase). [a]$P < 0.05$ *vs* the control, [b]$P < 0.01$ *vs* UC (I) and the control, [c]$P > 0.05$ *vs* the control, [e]$P < 0.05$ *vs* UC (I) and the control.

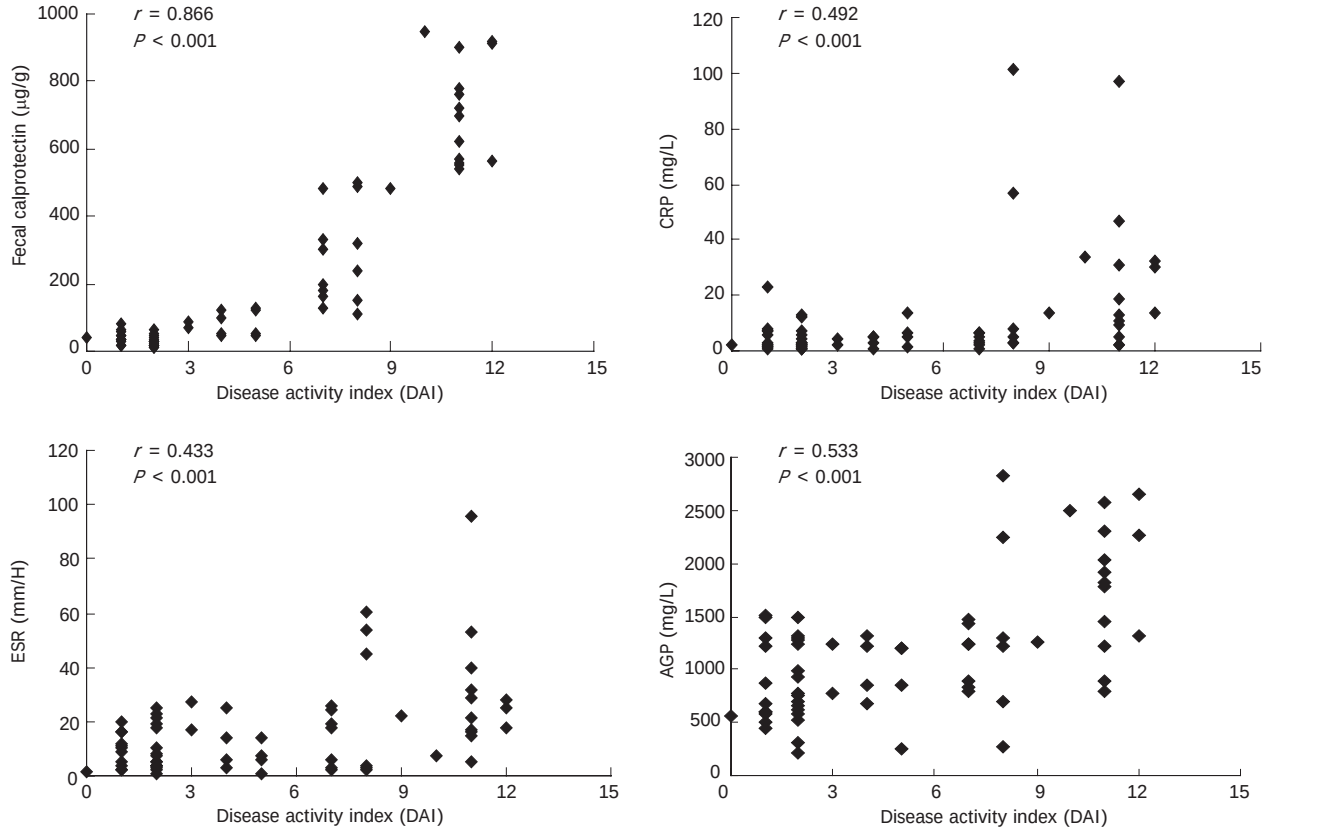

Figure 2 Concentrations of fecal calprotectin, CRP, ESR and AGP in UC and the DAI score of UC.

Table 1 Concentrations of fecal calprotectin, CRP, ESR and AGP in the UC patients and the controls (mean ± SD)

Group	Calprotectin (μg/g)	CRP (mg/L)	ESR (mm/H)	AGP (mg/L)
Control	11.5 ± 3.42	2.66 ± 3.2	7.05 ± 1.2	786.65 ± 77.65
Inactive UC	35.93 ± 3.39	4.39 ± 0.89	9.84 ± 1.36	870.14 ± 71.04
Active UC	402.16 ± 48.0	16.45 ± 3.98	21.44 ± 3.29	1394.9 ± 109.3

UC: Ulcerative colitis; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; AGP: Acid glycoprotein.

Table 2 Specificity and sensitivity for fecal calprotectin, CRP, ESR and AGP

Marker	Cut-off	Specificity (%)	Sensitivity (%)
Fecal calprotectin	50.0 μg/g	79.4	91.9
CRP	5.0 mg/L	69.0	62.2
ESR	15.0 mm/h	68.9	64.9
AGP	1200 mg/L	65.5	67.6

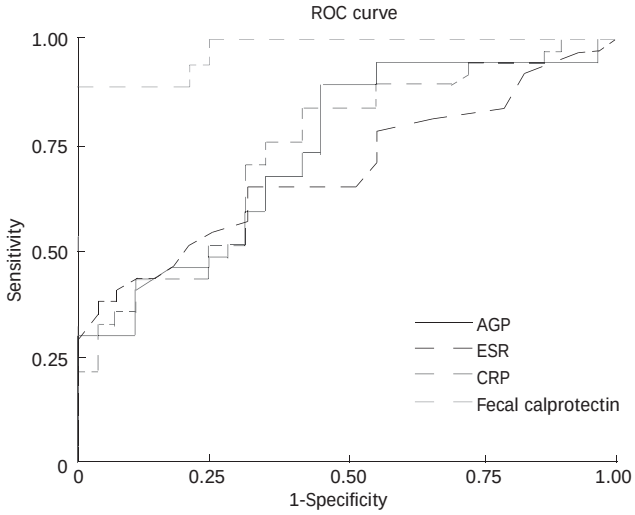

Figure 3 The ROC curve analysis on the abilities of calprotectin, CRP, ESR and AGP to make a difference between active UC and inactive UC.

between nought and three, making 12 the worst score): stool frequency, rectal bleeding, mucosa appearance, and physician's global assessment[27]. It reflects the clinical representation of UC and is the most extensive and simplest DAI. It is adopted by the guidelines for the management of inflammatory bowel disease in the fourth Asia Pacific Digestive Week[28]. Our results showed that fecal calprotectin concentrations were significantly higher in the patients with active UC than in the patients with inactive UC and in the controls. In addition, our results showed that fecal calprotectin concentration was higher in the patients with inactive UC than in the controls. In the patients with UC, the fecal calprotectin concentration had a better correlation with DAI than the CRP, ESR or AGP concentration.

The ROC curve is adopted not only to ensure the right cut-off point, but also to compare the diagnostic values of two or more diagnostic tests. The ROC analysis on the fecal calprotectin concentrations under these circumstances, showed that a cut-off point of 50.0 μg/g for calprotectin had a 91.9% sensitivity and a 79.4% specificity for making a differentiation between active UC and inactive UC. These were significantly better than those obtained with CRP, ESR and AGP.

In this study, ELISA was used to determine the fecal calprotectin concentrations in the patients with UC and in the controls. In comparison to endoscopy this method is simple, noninvasive and inexpensive. However, fecal calprotectin can only reflect the excretion of neutrophils. Many infective diseases can cause a large number of neutrophils to infiltrate, so that fecal calprotectin is elevated in a number of organic gastroenterological disorders[29-31]. Therefore, fecal calprotectin is not desirable as a method that is required to differentiate efficiently between UC and infective colitis, so it cannot take the place of an endoscope in diagnosing UC. Regardless of how sensitive the calprotectin technique may be in the detection of disease activity in patients with previously diagnosed UC, its greater potential use is in identifying patients with UC and in differentiating between patients with UC and patients with non-inflammatory disorders, still requires further study.

In conclusion, the determination of fecal calprotectin is an objective approach to grading the mucosal disease activity in patients with inflammatory bowel disease. The advantages of fecal calprotectin are simplicity, noninvasiveness, and relatively low cost. Fecal calprotectin can be useful not only in research but also in clinical practice.

COMMENTS

Background

The incidence and the prevalence rates of ulcerative colitis (UC) are increasing in China. It is important to accurately evaluate intestinal mucosa inflammation in the management of these patients, particularly for the assessment of therapeutic effectiveness. No clinical evaluations are specific for gut inflammation. Therefore, a new marker that will be more sensitive and specific for determination of disease activity of UC is urgently needed in clinical practice.

Research frontiers

Calprotectin can resist metabolic degradation caused by intestinal bacteria, and the protein is relatively stable in stools for up to one week at room temperature. Some foreign research has shown that fecal calprotectin concentrations were significantly higher in patients with active UC than in patients with inactive UC and in the controls.

Innovations and breakthroughs

Our study improved the originality of the manuscript assessing the relationship between calprotectin level and extension of colitis. This question was not described enough in the medical literature.

Applications

The aim of our study was to investigate the possibility and clinical application of fecal calprotectin in determining disease activity of UC. Our findings suggest that calprotectin in the patient's feces can reflect disease activity of UC and can be used as a rational fecal marker for intestinal inflammation in clinical practice.

Terminology

Receiver operating characteristics (ROC) curve is adopted not only to ensure the right cut-off point but also to compare the diagnostic values of two or more diagnostic tests. The accuracy of diagnostic test is characterized by the sensitivity and specificity. A ROC curve displays the sensitivity of a diagnostic test over all possible false-positive rates.

Peer review

This manuscript provided compelling evidence that fecal calprotectin was a better marker than C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) or acid glycoprotein (AGP) and improved the originality of the manuscript assessing the relationship between calprotectin level and extension of colitis. It deserves to be published.

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