Neutrophil and Eosinophil Granule Proteins as Potential Biomarkers of Assessing Disease Activity and Severity in Patients With Ulcerative Colitis

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Background: Colonoscopy can assess disease activity and severity of ulcerative colitis (UC) accurately, but it is invasive and costly. Role of noninvasive biomarkers of intestinal inflammation in evaluation of patients with UC is not well understood. In this study, we assessed fecal eosinophil cationic protein (FECp), fecal myeloperoxidase (FMPO), and fecal calprotectin (FC) as surrogate markers of disease activity and severity in patients with UC, and then evaluated effect of the combination of these markers. **Methods:** Sixty-three UC patients and 59 cases of age-matched controls were investigated. All patients underwent clinical, endoscopic, and histological assessment for disease activity and severity. Fecal samples were analyzed for FECp, FC, and FMPO. **Results:** All three fecal biomarkers were elevated in patients compared with controls ($P = 0.000$). Significant differences were found between inactive UC

and controls ($P = 0.000$). Cases with severe UC had significantly higher FECp levels than those with mild UC ($p < 0.05$), but there were no significant differences in FC and FMPO levels among disease severity groups. All three biomarkers showed positive correlation with Ulcerative Colitis Activity Index (UCAI). The areas under the ROC curve of FECp, FC, and FMPO were 0.939, 0.783, and 0.785, respectively. Sensitivity and specificity of fecal biomarkers in assessing disease activity were FECp—88.46%, 89.47%; FC—80.77%, 68.42%; and FMPO—84.62%, 63.16%. **Conclusions:** All three fecal biomarkers could be used as surrogate markers for assessing disease activity of UC, and FECp provided superior discrimination than FMPO and FC. Moreover, FECp could distinguish between mild disease and severe disease group. J. Clin. Lab. Anal. 30:776–778, 2016. © 2016 Wiley Periodicals, Inc.

Key words: fecal calprotectin; fecal eosinophil cationic protein; fecal myeloperoxidase; severity; activity

INTRODUCTION

Ulcerative colitis (UC) is a chronic relapsing disease of unknown etiology involving the rectum and, to a various extent, the colon. After a definite diagnosis of UC, assessment of disease activity and severity is essential for tailoring therapy to individual. Endoscopy has been considered to be the gold standard for assessing of disease severity in UC. However, the limitations of colonoscopy, such as invasiveness, poor patient acceptability, high costs, and overburden to the healthcare system, are well known (1). Ulcerative Colitis Activity Index (UCAI) (2) is widely used in clinical to reflect the disease activity, but it is a complex index as it is based on stool frequency, rectal bleeding, sigmoidoscopic appearance, and the physician's assessments.

Recently, there have been several studies on fecal biomarkers, such as fecal eosinophil cationic protein (FECp), fecal myeloperoxidase (FMPO), and fecal calprotectin (FC), in UC and their relationship to disease activity and severity, but there is no consensus (3,4). The aim of this study was to assess FECp, FMPO, and FC as surrogate markers of disease activity and severity in

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patients with UC, and then evaluate effect of the combination of these markers.

MATERIALS AND METHODS

Consecutive patients with UC and controls from February 2012 to October 2014 were prospectively studied. All protocols were approved by local Scientific Ethical Committees and written consents were obtained from all participants. Diagnosis of UC was based on World Gastroenterology Organization Practice Guidelines for the Diagnosis and Management of inflammatory bowel diseases (IBD) (5) and disease activity of UC was further assessed by UCAI.

Feces samples were collected and extracted (6). Fecal biomarkers in the extraction were measured. ECP was determined by ImmunoCAP250 System (Thermo Fisher Scientific, America), FC (BÜhlmann company, Sweden), and FMPO (RB company, America) were measured by ELISA. In order to eliminate the effects of feces character on the results, the measured concentrations of fecal biomarkers ($\mu\text{g/L}$ or U/L) were converted to semisolid mass ($\mu\text{g/g}$ or U/g). Blood samples were collected from the antecubital vein. CRP was detected by rate nephelometry (IMMAGE 800, Beckman Coulter, America), and ESR was tested by Westergren method (Greiner bio-one, Germany). Data were analyzed using SPSS software (v. 13.0). $P < 0.05$ was considered statistically significant.

RESULTS

A total of 63 patients with UC and 59 cases of controls were studied. The median age was 35 (range 13–81 years) in patient group and 33 (range 15–82 years) in control group. The median duration of illness was 5.6 years (range 0.2–23 years). According to UCAI, 44.4% (28/63) of patients were in remission (UCAI scored 1 to 2) and 55.6% (35/63) were in active period (UCAI scored more than 3). Among active patients, 20.0% (7/35) had mild disease (UCAI scored 3 to 5), 34.3% (12/35) had moderate disease (UCAI scored 6 to 10), and 45.7% (16/35) had severe disease (UCAI scored 11 to 12).

The concentrations of three fecal biomarkers in active group were significantly higher than that of inactive group ($P < 0.001$). Compared with control groups, fecal biomarkers concentrations of inactive group were significantly higher ($P < 0.001$). The median concentration of serum CRP showed significant differences between inactive group and controls ($P < 0.01$), while ESR in active group were higher than inactive group ($P < 0.05$). The differences in FECP concentrations between mild active group and severe active group were statistically significant ($P = 0.002$). However, there were no significant differences between mild active group and moderate active group

Fig. 1. The receiver operator curve for all biomarkers.

($P = 0.086$), and between moderate active group and severe active group ($P = 0.382$). No significant differences were found in FC, FMPO, CRP, and ESR among disease severity groups.

To evaluate the performance of markers in differentiating active group from inactive group, ROC curves were generated using UCAI as gold standard. As shown in Figure 1, FECP produced an ROC curve with an area under the curve (AUC) of 0.939, which was significant larger than the AUC of other biomarkers ($P < 0.05$). The AUC of FC and FMPO was very close (AUC 0.783 and 0.785, respectively), which were higher than the AUC of CRP and ESR (AUC 0.578 and 0.721, respectively). Of all three fecal biomarkers, the efficacy of FECP (sensitivity 88.46%, specificity 89.47%) in evaluating disease activity was better than FC (80.77%, 68.42%) and FMPO (84.62%, 63.16%). The combination of three fecal markers was more efficient than any of them used separately (sensitivity 91.43%, specificity 83.14%).

DISCUSSION

Several studies on fecal biomarkers in assessment of severity of UC have been reported. However, most studies (7, 8) have had a small sample size. More studies with a larger sample size are needed to validate the role of fecal biomarkers. Thus, we conducted this study to evaluate the utility of FECP, FMPO, and FC in assessing disease activity and severity in patients with UC, and then evaluate effect of the combination of these markers.

Accumulation and activation of eosinophils indicates involvement in the inflammatory process (9). In this study, we showed that concentrations of FECP in active UC were significantly higher than inactive group and controls.

Moreover, FECP could discriminate mild active from severe active group. However, there was no significant difference between mild active and moderate active group, and between moderate active and severe active group. The reason was likely due to the relatively small sample size of the study. ROC curves also showed that FECP had higher diagnostic efficiency in assessing disease activity of UC, compared with FMPO, FC, and routine parameters. Therefore, FECP was an effective biomarker in assessing disease activity in patients with UC.

The observation of this study demonstrated that both FC and FMPO could distinguish active from inactive group and controls, which confirmed previous findings that neutrophil involved in active UC (10, 11). We also found that concentrations of FC and FMPO in inactive group were significantly higher than those of controls. This might confirm the hypothesis that low-grade intestinal inflammation was a constant feature even in inactive UC patients. Although the AUC was lower than that of FECP, FC and FMPO showed superior diagnostic efficiency in assessing disease activity of UC compared with routine parameters CRP and ESR. Thus, FC and FMPO were also useful biomarkers in assessing disease activity in patients with UC. Combination of FECP, FC, and FMPO can be more efficient in assessing disease activity (sensitivity 91.43%, specificity 83.14%). Nevertheless, for both FC and FMPO, we found no significant differences among disease severity groups, which indicated that these biomarkers played little roles in evaluating the severity of disease.

Our observations confirm that FECP, FC, and FMPO showed superior efficiency in assessing disease activity of UC when compared with routine parameters, CRP and ESR. Of these three fecal biomarkers, FECP had the best correlation with clinical inflammatory activity, especially referring to the specificity. We conclude that FECP, FC, and FMPO could be used as surrogate markers for assessing disease activity of UC, and FECP provided superior discrimination than FMPO and

FC. Moreover, FECP could distinguish between mild disease and severe disease group. Of course, more studies with a larger sample size are needed to validate the role of FECP, FC, and FMPO in the management of UC.

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