Faecal calprotectin as a novel biomarker for differentiating between inflammatory bowel disease and irritable bowel syndrome

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Abstract. The present study aimed to investigate faecal calprotectin as a diagnostic marker to differentiate between patients with inflammatory bowel disease (IBD) and those with irritable bowel syndrome (IBS). A total of 20 healthy control subjects, 26 patients with IBS and 58 patients with IBD, including 22 with ulcerative colitis (UC) and 36 with Crohn's disease (CD), were recruited for the present study. Calprotectin was analysed in stool samples, and C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) were assessed in blood samples. CRP and calprotectin levels, and the ESR were observed to be significantly higher in patients with CD and UC compared with those of the healthy control subjects (P<0.0001). Furthermore, in patients with IBD and IBS, significant increases in faecal calprotectin and CRP levels were observed (694.8±685.0 μg/g in IBD vs. 85.8±136.1 μg/g in IBS and 0.851±1.200 mg/dl in IBD vs. 0.16±0.23 mg/dl in IBS, respectively; P<0.0001). Area under the receiver operating characteristic curve analysis revealed that, in patients with IBD, the levels of faecal calprotectin [0.931±0.029; 95% confidence interval (CI), 0.874-0.987] were significantly higher than that of CRP (0.865±0.041; 95% CI, 0.785-0.946) and the ESR (0.869±0.042; 95% CI, 0.786-0.952). These findings indicate that faecal calprotectin may represent a novel biomarker for diagnosing IBD and may be effective in distinguishing between IBD and IBS.

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Introduction

Calprotectin is a calcium-binding protein with a molecular weight of 36 kDa, which primarily originates from neutrophils and has a regulatory role in inflammatory processes. Calprotectin is stable and resistant to bacterial degradation in faeces (1,2). Upon inflammatory damage in the intestinal mucosa, calprotectin protein levels increase and calprotectin is released into the intestinal lumen. Therefore, calprotectin may have potential as a noninvasive biomarker of intestinal inflammation. Moreover, faecal calprotectin may represent a beneficial marker for the diagnosis and follow-up of inflammatory bowel disease (IBD) (3,4). Previous studies have shown that the concentration of faecal calprotectin in patients with IBD, including ulcerative colitis (UC) and Crohn's disease (CD), is significantly higher than that in patients with irritable bowel syndrome (IBS) (3,4). Faecal calprotectin levels have been correlated with histological disease activity in colonic biopsies from patients with UC and CD. Therefore, calprotectin is considered to be a marker of intestinal inflammation (2,5,6). Normalisation of faecal calprotectin levels has been reported to be a predictive marker of mucosal healing in patients with IBD; therefore, faecal calprotectin is an important measure when treating IBD. Although endoscopy is the gold standard for diagnosing intestinal inflammation, the procedure is invasive and unsuitable for frequent use. Faecal calprotectin concentration represents an increase in neutrophils and

Table I. Clinical and biochemical data of patients with CD and UC, and the control subjects.

	CD (n=36)		UC (n=22)		Control (n=20)	
Parameter	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
CRP (mg/dl)	1.045±1.333[a]	0.017-4.900	0.536±0.882[a]	0.014-3.330	0.064±0.062	0.007-0.191
ESR (mm/h)	21.94±25.32[a]	4-104	11.9±9.05[a]	2.0-42.0	4.45±2.16	1-8
Faecal calprotectin (μg/g)	815.4±720.7[a]	30-1,800	497.4±584.8[a]	35-1,810	36.85±6.80	30-51

CD, Crohn's disease; UC, ulcerative colitis; SD, standard deviation; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate. [a]P<0.0001 compared with the control subjects; Mann-Whitney U test.

correlates strongly with disease activity (7,8). The present study investigated whether faecal calprotectin may be used to differentiate between patients with IBD and those with IBS when monitoring disease activity and performing follow-ups in patients with IBD.

Patients and methods

Patient samples. In the present study, stool samples were obtained from 104 patients, including 20 healthy participants, 26 patients with IBS and 58 patients with IBD, of which 22 were diagnosed with UC and 36 with CD. Written informed consent was obtained from the patients or their famililes. Faecal samples (5 g) were collected from all of the patients over 12 months. Diagnoses of CD and UC were based on standard criteria (9,10). In accordance with their medical history, the healthy participants did not have any type of bowel disease and were required to undergo endoscopy and routine laboratory blood tests to exclude IBS. Following the exclusion of organic pathology by performing routine blood tests and thyroid function tests, stool examination for bacteria and parasites, ultrasound examination, colonoscopy and intestinal radiology, IBS was diagnosed according to the Rome III Diagnostic Criteria (11). Patient stool samples were used for measuring the calprotectin levels and blood samples were used for measuring the levels of C-reactive protein (CRP) as well as the erythrocyte sedimentation rate (ESR). The study was approved by the Ethics Committee of the Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan (DMR98-013-IRB-251).

Inclusion criteria. All of the patients with IBD were diagnosed based on standard endoscopic, radiological and histological criteria, which included; disease duration of less than three months, complete ileocolonoscopy including biopsies, an age range between 20 and 70 years, and the delivery of faecal samples between one and three days prior to bowel preparation.

Exclusion criteria. The exclusion criteria for the present study were as follows: Patients with gastric cancer, incomplete ileocolonoscopy (ileum not intubated), infectious enterocolitis or acute infection diseases, pregnant females, individuals with a history of extensive bowel resection, alcohol abuse, symptoms associated with perianal penetration, and individuals who regularly consumed aspirin, antibiotics, cytotoxic drugs and non-steroidal anti-inflammatory drugs (more than two tablets per week).

Faecal calprotectin. Stool samples were collected and placed in clean tubes within 72 h and stored at 2-8˚C. Faecal calprotectin was measured using a commercial qualitative point-of-care testing assay and a Bühlmann Quantum® Blue kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland). In brief, faecal samples were placed in an extraction tube and diluted to 1:16 (w/v) with an extraction buffer (lower range Quantum Blue® LF-CAL) or 1:150 (w/v) with chase buffer (high range Quantum Blue® LF-CHR) to provide quantitative results from 30 to 300 μg/g or from 100 to 1,800 μg/g faecal calprotectin, respectively. For quantitative measurements, unknown samples reading >300 μg/g can be re-tested in the Bühlmann Quantum Blue® High Range calprotectin assay (LF-CHR25). The mixture was vortexed for 1 min and centrifuged at 3000 x g for 5 min. Following a predetermined dilution, large particles were allowed to settle and the supernatant was assayed for 12 min. The high faecal calprotectin concentration was assayed for 15 min using the calibrated Bühlmann Quantum Blue Reader® (Bühlmann Laboratories AG). The cut-off level of faecal calprotectin was 50 μg/g.

Statistical analysis. Statistical analyses were performed using the SPSS software for Windows 16.0 (SPSS, Inc., Chicago, IL, USA). Biochemical parametric data were presented as the mean ± standard deviation. Calprotectin values were presented as medians, ranges and 95% confidence intervals (CIs). Mann-Whitney U tests were performed to compare faecal calprotectin and CRP levels, and the ESR between the CD and UC, healthy control, IBS and IBD groups. The cut-off values for the ESR (normal range, >9 mm/h) and CRP (upper limit of normal, <0.4 mg/dl) were determined as routine laboratory values. For the clinical section of the present study, the cut-off point was 50 μg/g, which was recommended as positive for gastrointestinal inflammation. Kruskal-Wallis tests were used to analyse faecal calprotectin levels within the groups, whereas Dunn's multiple comparison tests were used to compare differences in faecal calprotectin levels between the groups. Receiver operating characteristic (ROC) curves were used to assess faecal calprotectin as previously described by Henderson (12). All significant values were two-sided and P<0.05 was considered to indicate a statistically significant difference.

Table II. Clinical and biochemical data of the IBD and IBS patients.

	IBD (n=58)		IBS (n=26)		
Parameter	Mean ± SD	Range	Mean ± SD	Range	P-value
CRP (mg/dl)	0.851±1.200	0.014-4.900	0.16±0.23	0.010-1.040	<0.0001
ESR (mm/h)	18.14±21.16	2-104	9.11±4.02	5-22	0.220
Faecal calprotectin (μg/g)	694.8±685.0	30-1810	85.8±136.1	30-622	<0.0001

IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SD, standard deviation.

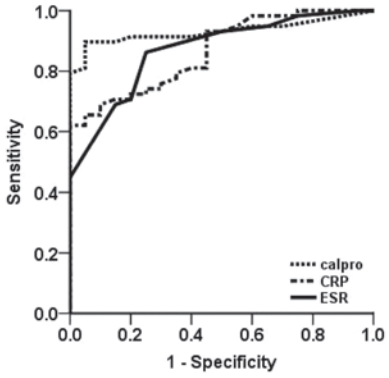

Figure 1. ROC curves of faecal calprotectin, CRP and the ESR in all patients with IBD, including those with Crohn's disease and ulcerative colitis. The ROC curve indicates that a faecal calprotectin level of 48.5 μg/g is the optimal cut-off value. The AUC of faecal calprotectin in the patients with IBD (AUC, 0.931±0.029; 95% CI, 0.874-0.987) was significantly higher than that of CRP (AUC, 0.865±0.041; 95% CI, 0.785-0.946) and the ESR (AUC, 0.869±0.042; 95% CI, 0.786-0.952). ROC, receiver operator characteristic; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; calpro, calpronectin; IBD, inflammatory bowel disease; AUC, area under the ROC curve.

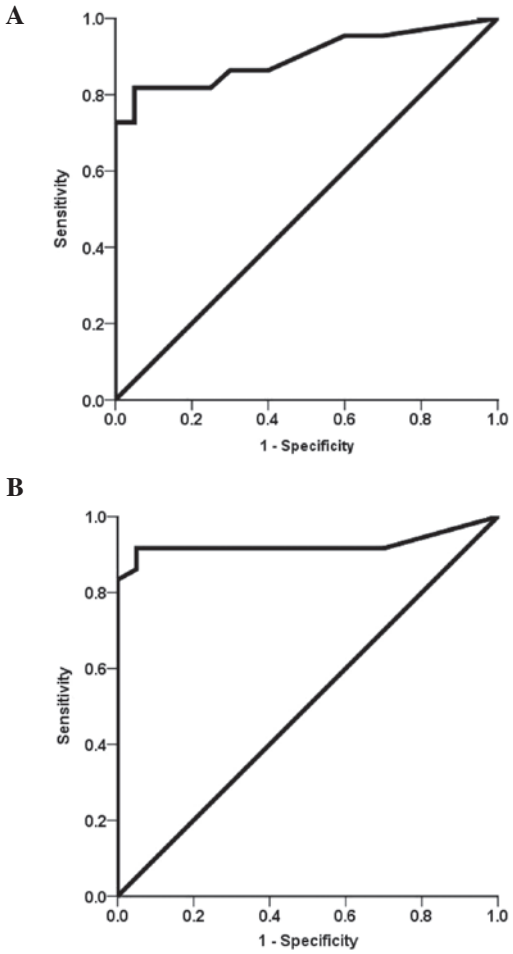

Figure 2. Receiver operator characteristic (ROC) curve of faecal calprotectin in patients with (A) Ulcerative colitis and (B) Crohn's disease. (A) Area under the ROC curve (AUC) , 0.939; 95% CI, 0.869-1.01. (B) AUC, 0.925; 95% CI, 0.84-1.00. (A and B) P<0.0001 indicated asymptotic significance.

Results

CRP and faecal calprotectin levels, and ESR in patients with CD and UC. Table I shows clinical and biochemical data of the patients with CD (n=36) and UC (n=22) and the healthy control subjects (n=20). The mean CRP (1.045±1.333mg/dl; range, 0.017-4.900), ESR (21.94±25.32 mm/h; range, 4-104) and faecal calprotectin (815.4±720.7 μg/g; range, 30-1,800) values were observed to be significantly higher in the patients with CD compared with the healthy control subjects (all P<0.0001). Similar to the patients with CD, the mean CRP (0.536±0.882 mg/dl; range, 0.014-3.330), ESR (11.9±9.05 mm/h; range, 2-42) and faecal calprotectin (497.4±584.8 μg/g; range, 35-1810) values in the patients with UC were found to be significantly higher compared with the healthy control subjects (all P<0.0001).

CRP, faecal calprotectin and ESR in patients with IBD and IBS. Table II shows clinical and biochemical data of the patients with IBD (n=58) and IBS (n=26). The mean CRP (0.851±1.200 mg/dl; range, 0.014-4.900) and faecal calprotectin (694.8±685.0 μg/g; range, 30-622) values were observed to be significantly higher in the patients with IBD compared with the patients with IBS (CRP: 0.162±0.229 mg/dl; range, 0.010-1.040; faecal calprotectin, 85.77±136.1 μg/g; range, 30-622; P<0.0001). The difference in the mean ESR between the patients with IBD (18.14±21.16 mm/h; range, 2-104) and those with IBS (9.11±4.02 mm/h; range, 5-22) was not identified to be significant (P=0.220).

ROC curve analysis of faecal calprotectin, CRP and ESR in patients with IBD. The area under the ROC curve (AUC) of faecal calprotectin (AUC, 0.931±0.029; 95% CI, 0.874-0.987)

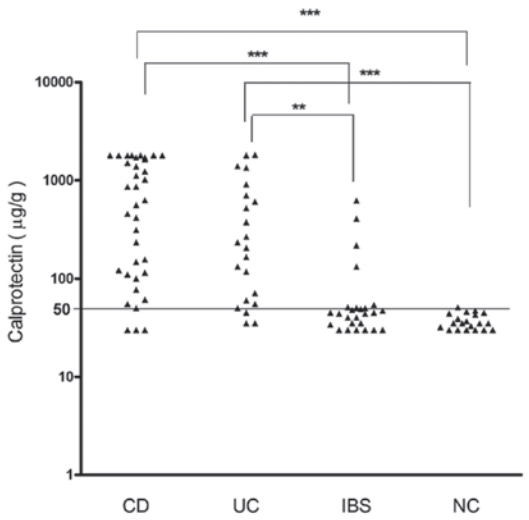

Figure 3. Faecal calprotectin levels (log scale) in the examined groups. The cut-off value was 50 μg/g. Median faecal calprotectin values were increased in the CD and UC groups compared with the IBS or NC groups. **P<0.001; ***P<0.0001. CD, Crohn's disease; UC, ulcerative colitis; IBS, irritable bowel syndrome; NC, normal control.

was significantly higher than that of CRP (AUC, 0.865±0.041; 95% CI, 0.785-0.946) and the ESR (AUC, 0.869±0.042; 95% CI, 0.786-0.952) in patients with IBD (Fig. 1). The ROC curve indicated that a faecal calprotectin level of 48.5 μg/g was the optimal cut-off value in the group of patients with IBD, with a sensitivity of 90%, a specificity of 95%, a positive (P) predictive value (PV) of 94% and a negative (N) PV of 89%. In the patients with IBD, the sensitivity, specificity, PPV, and NPV were 62, 95, 92 and 71% for CRP, and 86, 75, 77 and 84% for ESR, respectively.

ROC curve analysis of faecal calprotectin in patients with CD and UC. The median calprotectin concentration was 595 μg/g (95% CI, 571-1059; range, 30-1,800 μg/g) in the patients with CD and 219 μg/g (95% CI, 238-756; range, 35-1810 μg/g) in the patients with UC. The AUC was significantly higher in the patients with UC (0.939; 95% CI, 0.869-1.01; Fig. 2A) than that in the patients with CD (0.925; 95% CI, 0.84-1.00; Fig. 2B). The sensitivity, specificity, PPV and NPV were 86, 95, 94 and 87% in the patients with UC, and 91, 95, 94 and 91% in those with CD, respectively.

Faecal calprotectin concentration in patients with CD, UC and IBS and the healthy control subjects. Fig. 3 displays the faecal calprotectin levels in the examined groups. The median calprotectin levels were observed to be significantly increased in the patients with CD (595 μg/g; 95% CI, 571-1,059 μg/g) and UC (219 μg/g; 95% CI, 238-756 μg/g) compared with the healthy control subjects (35 μg/g; 95% CI, 33-40 μg/g; all P<0.0001). Furthermore, significant variations were observed among the IBS, CD and UC groups (P<0.0001 and P<0.001). Normal calprotectin levels were found in the patients with IBS (44.50 μg/g; 95% CI, 32.6-141.9 μg/g), in addition, no statistical difference was identified between the IBS patients and the healthy control subjects. These findings demonstrate that the group of patients with CD and UC exhibited higher faecal calprotectin levels than those in the group of patients with IBS. These data indicate that faecal calprotectin levels may be used to differentiate between patients with IBD and IBS.

Discussion

In the present study, CRP and faecal calprotectin levels, and the ESR were found to be significantly higher in patients with CD and UC compared with those of the healthy control subjects. Furthermore, it was identified that faecal calprotectin may be a potential marker of intestinal inflammation to enable differentiation between IBD and IBS in patients. A significant difference was observed in CRP and faecal calprotectin levels between patients with IBS and those with IBD; however, no significant difference was identified in the ESR between these patients. In the patients with IBD, the AUC of faecal calprotectin was significantly higher than that of CRP and ESR; however, the sensitivity for CRP and ESR was low. It was identified that faecal calprotectin levels may be used to distinguish between IBD and IBS according to its high sensitivity, specificity, PPV and high NPV enabling exclusion of IBD in undiagnosed patients with abdominal pain or diarrhoea. Furthermore, it was observed that the AUC of faecal calprotectin concentration was significantly higher in patients with UC than those with CD. It has previously been reported that a high concentration of faecal calprotectin is associated with a two-fold relapse risk in patients with CD and a 14-fold relapse risk in patients with UC, indicating that a high concentration of faecal calprotectin may be a more accurate predictive marker of relapse in UC than in CD. Therefore, detecting faecal calprotectin may facilitate the identification of patients with UC and CD who possess a high risk of clinical disease relapse (13,14). Furthermore, a previous study demonstrated that faecal calprotectin may be used to distinguish between active and inactive UC (6), and faecal calprotectin and CRP have also been shown to be more adequate than ESR in detecting leukocytosis in patients with active UC (15). Faecal calprotectin levels have been found to be beneficial in predicting clinical relapse in patients with IBD, including those with UC and CD, in a large long-term follow-up study and faecal calprotectin determination may be useful in predicting impending clinical relapse, particularly during the subsequent three months (16). The effectiveness of faecal calprotectin measurements in screening for spontaneous bacterial peritonitis and hepatic encephalopathy (HE) in cirrhosis has also been reported to facilitate the grading of HE-severity (17). In addition, a previous study reported that measuring faecal calprotectin levels was a useful screening tool for identifying patients who were likely to require an endoscopy for suspected IBD. The sensitivity and specificity of faecal calprotectin testing was found to be greater in adults than in children (sensitivity, 0.93 vs. 0.92 and specificity, 0.96 vs. 0.76). Thus, measuring faecal calprotectin levels may provide the capacity to distinguish between IBD and IBS (18) and faecal calprotectin has been reported to be an accurate marker of IBD in children and adults (19).

In conclusion, faecal calprotectin was observed to be a more direct and useful biomarker than CRP or ESR for diagnosing intestinal inflammation in patients with IBD. Faecal calprotectin may facilitate the diagnosis of patients with UC and CD who are at a high risk of clinical relapse. Furthermore,

faecal calprotectin may potentially be used to distinguish between IBD and IBS.

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