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Diagnostic accuracy of faecal calprotectin estimation in prediction of abnormal small bowel radiology

S. DOLWANI*, M. METZNER*, J. J. WASSELL†, A. YONG‡ & A. B. HAWTHORNE*
*Department of Gastroenterology, †Department of Clinical Biochemistry, ‡Department of Radiology, University Hospital of Wales, Cardiff, UK

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SUMMARY

Backgound: Patients being investigated for symptoms of abdominal pain, diarrhoea and or weight loss often undergo small bowel radiology as part of their diagnostic workup mainly to exclude inflammatory bowel disease.

Aim: To assess and compare the utility of a single faecal calprotectin estimation to barium follow through as well as conventional inflammatory markers such as erythrocyte sedimentation rate and C-reactive protein in exclusion of intestinal inflammation.

Methods: Seventy-three consecutive cases undergoing barium follow through for investigation of symptoms of diarrhoea and or abdominal pain with or without weight loss were studied. The control group comprised 25 cases with known active Crohn's disease (positive controls), 26 normal healthy volunteers (negative controls) and 25 cases of irritable bowel syndrome diagnosed by Rome II criteria. Symptoms, erythrocyte sedimentation rate and C-reactive protein were recorded at recruitment and a single stool sample assayed for calprotectin within 7 days prior to or after barium follow through.

Results: The median calprotectin value in the active Crohn's group, irritable bowel syndrome group and normal volunteers was 227 μg/g of stool, 19 and 10 μg/g respectively ($P < 0.0001$). A faecal calprotectin above a cut-off value of 60 μg/g was able to predict all nine cases with an abnormal barium follow through as well as all six cases with a normal barium follow through but with organic intestinal disease. The negative predictive value of a single calprotectin result below 60 μg/g of stool was 100% compared with 91% each for erythrocyte sedimentation rate > 10 mm and C-reactive protein > 6 mg/L and 84% for a combination of erythrocyte sedimentation rate and C-reactive protein in predicting absence of organic intestinal disease.

Conclusion: A single stool calprotectin value < 60 μg/g of stool obviates the need for further barium radiology of the small bowel, is more accurate than measurement of erythrocyte sedimentation rate or C-reactive protein and effectively excludes Crohn's disease or non-functional gastrointestinal disease.

INTRODUCTION

Faecal calprotectin estimation has been studied as a surrogate marker in intestinal inflammatory and neoplastic conditions.[1, 2] The clinical situations in adults where it has been proposed to be useful are, in screening for colorectal cancer,[3] diagnosis of 'organic' gastrointestinal disease in patients with persisting gastrointestinal symptoms,[4] diagnosis of non-steroidal anti-inflammatory medication (NSAID) induced enteropathy,[5] in monitoring disease activity in inflammatory bowel disease (IBD)[6] and in a specific syndrome of disordered zinc metabolism.[7] The characteristics of this test that

Correspondence to: Dr A. B. Hawthorne, Department of Radiology, University Hospital of Wales, Cardiff, UK.
E-mail: barney.hawthorne@cardiffandvale.wales.nhs.uk

have been assessed when considering its clinical applicability have been its high sensitivity and relatively low specificity in these settings along with its correlation with endoscopic and histologic inflammation[8] as well as with Indium labelled neutrophil excretion[9] in IBD. The main focus of most studies has been the differentiation of functional [irritable bowel syndrome (IBS)] from non-functional gastrointestinal disease. Small bowel radiology is often one of the investigations planned in the work up of patients with diarrhoea or abdominal pain in order to exclude small bowel Crohn's disease and given the above properties of this test we felt it would be appropriate to investigate whether faecal calprotectin estimation would be an accurate surrogate test instead of barium follow through (BaFT) in this group of patients and reduce the need for barium meal and follow-through examinations performed for this indication.

PATIENTS AND METHODS

Subjects

Ethical approval was obtained from the Bro Taf Local Research Ethics Committee. The study group comprised 73 consecutive patients undergoing small bowel BaFT examination as part of their clinical workup after presenting with abdominal pain and or diarrhoea to out-patient gastroenterology or general surgical clinics as well as patients undergoing in-patient investigations for the same symptoms at the University Hospital of Wales. The majority of cases were recruited from a gastroenterology out-patient clinic with a special interest in IBD. Of this group of 73, 10 cases already had known Crohn's disease and were therefore excluded from the final analysis. All patients underwent rigid sigmoidoscopy and stool cultures as part of their workup and those with abnormal rigid sigmoidoscopy or positive stool cultures were excluded. The control group comprised 25 patients with known clinically active Crohn's disease and 26 healthy volunteers acting as positive and negative controls respectively. A further group of 24 patients with IBS as diagnosed by Rome II criteria were studied for comparison of the discriminant value of calprotectin in arriving at a diagnosis of non-functional bowel disease particularly IBD. All patients provided informed consent prior to sample collection.

Methods

Patients with known malignancy, those on NSAIDs, known coeliac disease, on steroids for any indication, and those with severe cardiopulmonary, renal or hepatic impairment, significant psychiatric disease or alcohol and drug dependency were excluded from the study. After informed consent was obtained patients were asked to supply a single stool sample within 7 days before, or (where this was not possible) 7–10 days after the BaFT examination by posting it directly to the laboratory. At the time of recruitment a symptom questionnaire was administered, drug history noted and a blood sample taken for estimation of full blood count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). The patients in the IBS group all had a clearly established diagnosis of IBS on the basis of the Rome II criteria, and exclusion of other organic pathology where clinically indicated. Blood was taken for ESR and CRP. Normal healthy volunteers who were not on any medication with no symptoms of bowel disease and no previous abdominal surgery were recruited in the negative control group. Patients in the Crohn's disease group had symptoms of active disease, blood was taken for ESR and CRP, and Crohn's disease activity index (CDAI) was calculated.

Biochemical analysis

The biochemist performing the assay was blinded to all clinical details. On arrival in the laboratory, a portion of stool was frozen at −20 °C. Samples were then assayed in batches. Sample extraction and assay was performed as previously described.[10] An ELISA-based method (Calprotech Ltd, London, UK) was used; all reagents were supplied with the kit.

Calprotectin concentration was calculated using Biolise software (version 1.65, Life Sciences International, Cergy-Pontoise, France, 1994) and expressed as micrograms of calprotectin per gram (μg/g) of stool.

The detection limit was determined from multiple determinations of the zero level standard. The performance of the assay was also assessed with respect to its postal stability by incubating six stool samples at room temperature or 4 °C for up to 7 days. On days 0, 1, 3 and 7 a sample of stool was taken and frozen for later analysis. The distribution of calprotectin in stools was determined by sampling from 10 different sites within two separate stool samples and results compared. Within and between

batch precision were determined by analysing the same sample 10 times in a single assay (within batch) and 10 times in 10 separate assays (between batch).

Radiological reporting

All BaFT examinations were carried out and reported by the same two gastrointestinal radiologists who were blinded to any biochemical data and who reported on specific parameters on the radiographs, which had been agreed prior to commencement of the study. These included radiological early 'inflammatory' changes (nodular lymphoid hyperplasia, thickened valvulae, wall thickening, aphthous ulceration, featureless mucosa, skip lesions, rose thorn ulceration and cobblestoning), advanced features (pseudosacculations, strictures, proximal bowel dilatation, intramural sinuses, fistulous tracts and abcess) and disease distribution was clearly specified with a global assessment at the end as normal/abnormal.

Statistics

Normal values for ESR were defined as < 10 mm/h, and for CRP < 6 mg/L. Although somewhat arbitrary in a specific clinical setting, these are quoted as normal values in our laboratory. Bayesian analysis with calculation of sensitivity, specificity, positive and negative predictive value of the calprotectin test was performed initially using the active Crohn's disease group, and comparing with the IBS and healthy control groups, and receiver operating curve (ROC) curves were constructed. Similar calculations were performed for ESR and CRP values. Cut-off values were determined using this and co-ordinates of the curve.

RESULTS

Assay characteristics

Calprotectin was stable in stool samples after up to 3 days at room temperature ($P < 0.01$, $n = 6$). There was no significant difference in calprotectin concentration ($P < 0.01$, $n = 10$) when samples were taken from different sites within the same stool. The within batch precision of the assay was 2.8% (1.9 μg/g of stool) and 6.9% (60.5 μg/g of stool). The between batch precision was 19% (24.2 μg/g of stool) and 14% (61.7 μg/g of stool). The detection limit was 4.4 μg/g of stool.

Control groups

In the active Crohn's disease group, the median calprotectin level was 226 μg/g stool, significantly higher than levels in IBS and healthy volunteers groups (Table 1). Other characteristics are shown in Table 1. Two patients with clinically active Crohn's disease had CDAI scores < 150, (but interestingly, both had raised calprotectin), and calprotectin did not correlate with level of CDAI ($R^2 = 0.004$). ROC analysis gave a calprotectin value of 60 μg/g as the best discriminator between active Crohn's and IBS, with a sensitivity of 84%, specificity of 96%, positive predictive value of 95% and negative predictive value of 85%. In contrast, an ESR ≥ 10 mm/h had much lower sensitivity (67%), with specificity of 95%, and a raised CRP ≥ 6 mg/L gave higher sensitivity (92%), but much lower specificity (43%). If a raised ESR and CRP were taken together, this gave a specificity of 100%, but much lower sensitivity of 67%.

Table 1. Control group results

	Healthy volunteers	Irritable bowel syndrome	Active Crohn's disease
Number	26	24	25
Age median (range)	34 (27–57)	38 (17–67)	36 (16–71)
Sex female/male	18/8	20/4	18/7
Calprotectin (μg/g) median (range)	10 (2–43)	19 (2.7–87)**	227 (3–11966)*
ESR (mm/hr) median (range)	–	3 (1–11)	14 (2–147)***
CRP (mg/L) median (range)	–	6 (2–19)	22 (2–54)***
CDAI median (range)	–	–	260 (60–460)

* $P < 0.0001$ vs. healthy volunteer and IBS group.
** $P = 0.04$ vs. healthy volunteer.
*** $P < 0.0001$ vs. IBS group.
ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CDAI, Crohn's disease activity index; IBS, irritable bowel syndrome.

Cases undergoing BaFT examination

Seventy-three patients were recruited in this group, but 10 were excluded as they had had previously diagnosed Crohn's disease. Of the remaining 63 patients, 20 were male, the median age was 47 (range 17–86), and 86% had symptoms of pain, 67% diarrhoea, and 44% weight loss. Nine patients had abnormal BaFT results, six with small intestinal Crohn's, (median calprotectin 211 μg/g). one a jejunal lipoma (calprotectin 80 μg/g), one a Roux loop with ulceration (calprotectin 140 μg/g), thought to be due to previous NSAID use, and one a caecal carcinoma (calprotectin 896 μg/g). A further six patients had normal BaFT, but were found subsequently to have organic bowel disease (for the purposes of this study defined as non-functional gastrointestinal disease) namely, Crohn's colitis in five patients (median calprotectin 250 μg/g)., and coeliac disease in the sixth (median calprotectin 180 μg/g). The remaining 48 patients had normal BaFT. Results are presented for these three groups in Figure 1 and Table 2. Calprotectin was significantly higher in both groups with organic disease, compared with the normal group, indeed none of these patients had calprotectin below 60 μg/g. In the normal group, however, 10 patients (21%) had a raised calprotectin. Of these 10 patients, six had a final diagnosis of IBS (median calprotectin 117 μg/g); one had a questionable increase in lymphocytes on rectal biopsy, not thought to be sufficient to diagnose microscopic colitis (calprotectin 134 μg/g); one had a strong family history of Crohn's disease (affecting mother and sister), but all investigations were negative including upper gastrointestinal endoscopy, colonoscopy, BaMFT and technetium labelled leucocyte scan (calprotectin 114 μg/g); and two patients had other abnormalities but no clear diagnosis [a 55-year-old male with an abnormal distal duodenal biopsy, but not typical of coeliac disease, with negative tissue transglutaminase antibodies, and a lesion in the lung on CT scan (calprotectin 69 μg/g); a 20-year-old male with hypertension, gastro-oesophageal reflux disease, and an unexplained rise in CRP (16 mg/L) and ESR (37 mm/h) with calprotectin of 102 μg/g]. Of the other 38 in this group with normal calprotectin, all had a final diagnosis of IBS or non-specific abdominal pain.

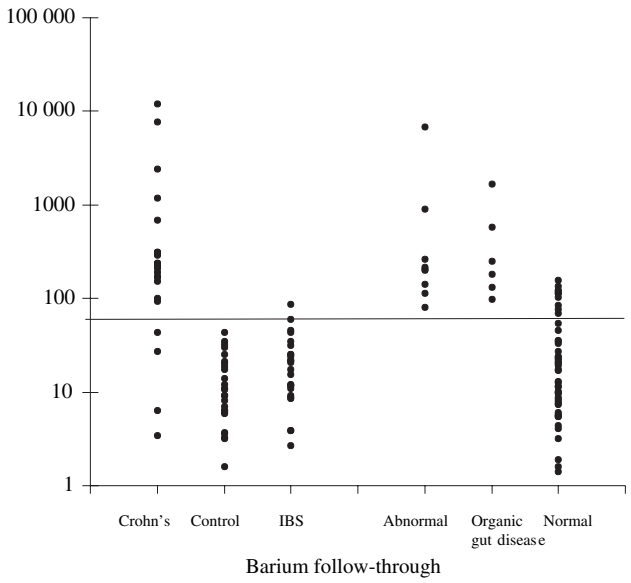

Figure 1. Scatterplot of faecal calprotectin values (μg/g stool) in three control groups: active Crohn's disease ($n = 25$), irritable bowel syndrome (IBS) ($n = 24$), and healthy volunteers ($n = 26$); also results from patients undergoing barium follow-through, divided into three groups: abnormal barium result ($n = 9$), normal barium result but with organic gut disease ($n = 6$), and normal barium result without organic gut disease ($n = 48$). Horizontal line indicates cut-off value of 60 μg/g.

The value of calprotectin used as a screening test for organic bowel disease, compared with CRP and ESR is shown in Table 3. Calprotectin gave the best results, with a negative predictive value of 100%, compared with 91% for the other tests.

DISCUSSION

A significant proportion of the referrals to specialist hospital based gastroenterology clinics consist of patients with diarrhoea and or abdominal pain. A significant proportion of these may not have a clear diagnosis on clinical grounds alone and may require further investigations. Irritable bowel syndrome accounts for up to 12% of primary care consultations and 28% of referrals to specialist GI practice.[11, 12] The currently used standard criteria for this condition are the Rome II criteria. These are successful in identifying the majority of patients with IBS. However they suffer from an inherent limitation in that while they are extremely useful in research studies they are cumbersome for routine use. Along with this there is a lack of a 'gold standard test' for IBS and it is argued that this group may actually comprise several heterogeneous subgroups.[13] In some patients, the diagnosis is not obvious, and further investigation in the form of endoscopic or radiologic procedures is needed to exclude

Table 2. Results in barium follow-through (BaFT) patients divided into three groups: abnormal BaFT; normal BaFT but organic disease (non-functional gastrointestinal disease); normal BaFT without organic disease (non-functional gastrointestinal disease) confirmed

	Abnormal BaFT	Normal BaFT with organic disease	Normal BaFT without organic disease
Number	9	6	48
Calprotectin (μg/g) median (range)	205 (80–6804)*	215 (99–1678)*	17 (1.4–156)
Calprotectin ≥60 μg/g no./total (%)	9/9 (100%)	6/6 (100%)	10/48 (21%)
ESR (mm/h) median (range)	17 (3–47)**	16 (11–18)***	w 5 (1–45)
ESR ≥ 10 mm/hr No./total (%)	5/8 (63%)	6/6 (100%)	15/45 (33%)
CRP (mg/L) median (range)	7 (1–100)	8 (3–26)**	3 (1–47)
CRP ≥ 6 mg/L no./total (%)	6/8 (75%)	4/5 (80%)	14/46 (30%)

* $P < 0.0001$ vs. 'normal BaFT with no organic disease' group.
** $P < 0.05$ vs. 'normal BaFT with no organic disease' group.
*** $P = 0.005$ vs. 'normal BaFT with no organic disease' group.
ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 3. Value of faecal calprotectin, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in detecting organic bowel disease

	Calprotectin ≥ 60 μg/g	ESR ≥ 10 mm/h	CRP ≥ 6 mg/L	Both ESR ≥ 10 mm/h and CRP ≥ 6 mg/L
Sensitivity	100%	79%	77%	50%
Specificity	79%	67%	70%	84%
Positive predictive value	60%	42%	42%	50%
Negative predictive value	100%	91%	91%	84%

significant pathology (particularly IBD). Although enteroclysis has been shown to be sensitive as well as specific in the situation of excluding Crohn's disease this involves radiation exposure.[14] In this setting, our study has demonstrated that faecal calprotectin is very useful, with extremely high sensitivity, and high negative predictive value for abnormal small bowel radiology and for organic intestinal pathology. The implication of these findings is that a normal faecal calprotectin result removes the need for small bowel barium radiology, or colonoscopy, in this clinical setting (i.e. patients who have undergone clinical assessment for diarrhoea or abdominal pain in a medical gastroenterology outpatient clinic, and where rigid sigmoidoscopy and stool culture are negative. As demonstrated in Table 3, the test is superior to both ESR, CRP, and a combination of the two. The specificity of the test (79%) is somewhat lower, but it is worth emphasizing that several of the patients with apparent false positive raised calprotectin, had unexplained abnormalities. Two cases seemed to have some evidence of a multisystem disorder rather than inflammation limited to the gastrointestinal tract. This is consistent with previous findings demonstrating that plasma concentrations of calprotectin are elevated in other inflammatory conditions such as rheumatoid arthritis, lupus and sepsis.[15] One patient had a strong family history of Crohn's disease along with suggestive symptoms, but a diagnosis could not be made on conventional tests. This is entirely consistent with observations from studies involving family members of cases with Crohn's disease[16] and may either represent an inherited abnormality or a forme fruste of the disease or possibly a stage in its evolution. However the core false positive group were the six patients where the diagnosis was IBS and this is similar to the findings in the control IBS group, where calprotectin levels were raised in some patients. It supports the argument regarding the heterogeneity of this condition.[17] It would thus be useful to characterize this group further by follow-up of these patients over time.

Calprotectin was clearly able to discriminate between active Crohn's disease (Median CDAI 260) and normal healthy volunteers. The cut off values taken in this study were based on the co-ordinates of the ROC curve which suggest a sensitivity of 0.84 and false positivity (1 – specificity) of 0.059 at a calprotectin level of 50 and

sensitivity of 0.84 and false positivity of 0.039 at a calprotectin level of 57 respectively. Previous studies have used a cut off of 50 μg/g of stool. However as has been noted previously the cut-off value may vary according to the situation in which it is being used, a value above 30 mg/mL having up to 100% sensitivity for diagnosing Crohn's disease[18] whereas a value of above 50 mg/mL has 90% sensitivity to predict clinical relapse for Crohn's disease and ulcerative colitis in remission.[6] The cut-off value also differs according to the specific assay used and direct comparisons would only be valid if the same assay was being used.[19]

It is also of note that of the 15 cases in the abnormal follow through group one had an initial diagnosis of abnormal terminal ileum appearance consistent with Crohn's disease but a subsequent colonoscopy confirmed a final diagnosis of T3N1MX carcinoma of the ascending colon. This patient had a calprotectin level of 896 μg/g of stool. This is consistent with the observations in previous studies using it as a surrogate marker of colonic neoplasia. A further case illustrating the clinical usefulness of faecal calprotectin was a 24-year-old woman with a family history of ulcerative colitis. Her initial barium follow-through was reported as normal, but symptoms worsened and a further BaFT was performed which showed typical ileal Crohn's disease. The original BaFT was then reviewed and subtle but definite abnormalities were noted. Her calprotectin (taken at the time of initial BaFT but reported only at the end of the study when blinding was removed) was recorded as 6804 μg/g.

In conclusion, clinical application of faecal calprotectin estimation in the setting of investigation of patients with abdominal pain and or diarrhoea could result in reduction in the number of unnecessary radiation exposures (because of BaFT examinations) and a more rapid exclusion of significant intestinal pathology in these patients. We would suggest that a single stool calprotectin value < 60 μg/g of stool obviates the need for further barium radiology of the small bowel and effectively excludes Crohn's disease or organic gut disease. The test is more accurate than measurement of ESR or CRP, and makes an extremely useful addition to the diagnostic armamentarium.

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