INTRODUCTION

Clinicians are frequently challenged to interpret gastrointestinal symptoms in patients with inflammatory bowel disease (IBD) who, on the basis of conventional tests, appear to be in remission. In two separate studies, irritable bowel syndrome (IBS)-type symptoms were described in 33% of patients with ulcerative colitis (UC) and in 42–57% with Crohn’s disease (CD), who were in remission (1,2). How does the clinician interpret such symptoms? Do they reflect the coincident occurrence of IBS or the presence of persistent, but clinically undetected, low-grade inflammation? This management dilemma is particularly problematic in CD and, especially in those with disease confined to the small bowel, where frequent disease monitoring is logistically difficult.

There is a statistically definable likelihood of coincidence of IBD and IBS, as both syndromes are common with prevalence rates in the developed world for IBD ranging between 0.1 and 0.2% (3) and for IBS from 9 to 12% (4,5) and both may significantly impact on quality of life (QOL) (6–10). However, in the absence of an identifiable cause, or a specific biomarker for either condition, both IBS and IBD are diagnosed clinically as being mutually exclusive. In both cases, diagnosis requires not only the presence of compatible clinical features with chronicity, but clinically undetected, low-grade inflammation. This management dilemma is particularly problematic in CD and, especially in those with disease confined to the small bowel, where frequent disease monitoring is logistically difficult.

OBJECTIVES: Do gastrointestinal symptoms in patients with inflammatory bowel disease (IBD) in apparent remission reflect the coexistence of irritable bowel syndrome (IBS) or subclinical inflammation? The aims of this study were as follows: (i) to prospectively determine the prevalence of IBS symptoms in IBD patients in remission; and (ii) to determine whether IBS symptoms correlate with levels of fecal calprotectin.

METHODS: Remission was defined by physician assessment: Crohn’s disease (CD) activity index ≤150 and ulcerative colitis disease activity index ≤3, and serum C-reactive protein < 10, while off corticosteroids or biologics. Quality of life (QOL) (by inflammatory bowel disease questionnaire), the hospital anxiety and depression scale (HAD), and fecal calprotectin were measured.

RESULTS: Rome II criteria for IBS were fulfilled in 37/62 (59.7%) of CD patients and by 17/44 (38.6%) of those with ulcerative colitis (UC). However, fecal calprotectin was significantly elevated above the upper limit of normal in both IBD patient groups, indicating the presence of occult inflammation. Furthermore, calprotectin levels were significantly higher in CD and UC patients with criteria for IBS than in those without IBS-type symptoms. QOL scores were lower and HAD scores higher among UC patients with IBS symptoms in comparison to those who did not have IBS symptoms.

CONCLUSIONS: IBS-like symptoms are common in patients with IBD who are thought to be in clinical remission, but abnormal calprotectin levels suggest that the mechanism in most cases is likely to be occult inflammation rather than coexistent IBS.

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patients in whom symptoms cannot be attributed to readily identifiable inflammatory disease activity. Conventional clinical activity indices and traditional laboratory markers of inflammation, such as C-reactive protein, frequently lack sufficient sensitivity to detect low-grade inflammation and to help clinicians distinguish subclinical IBD from coexisting IBS.

To help address this, we selected patients with both CD and UC, who were considered to be in clinical remission, on the basis of predefined criteria and determined their prevalence of IBS-type symptoms and the relationship of such symptoms to fecal calprotectin, which was used as a surrogate marker of subclinical inflammation. The results confirm the common occurrence of IBS-type symptoms in patients with both forms of IBD, but suggest that subclinical inflammation is a more likely explanation than coexisting true IBS.

**METHODS**

This study was approved by Cork University Hospitals ethics committee, and patient recruitment was between 1 July 2006 and 30 June 2007. Informed consent was obtained from all research participants. A single gastroenterologist was responsible for assessment of the IBD patients (FS) and a separate gastroenterologist for the IBS study patients (EQ).

**Study subjects**

All patients attending a specialized IBD clinic over an 18-month period were prospectively reviewed. Consecutive patients in clinical remission were recruited for inclusion in the study. Remission was defined as follows:

(i) Physicians global assessment (based on history and physical examination),
(ii) C-reactive protein < 10 mg/l,
(iii) Absence of use of corticosteroids or biological agents over the prior 6 months, and
(iv) CD activity index ≤ 150 (11) or an UC activity index ≤ 3 (12).

In no case did the physicians global assessment disagree with the other three criteria of remission.

The Rome II criteria for IBS were used for the definition of IBS symptoms (13). Participants were, accordingly, divided into four groups: CD with and without IBS symptoms and UC patients with/without IBS symptoms. QOL was assessed using the inflammatory bowel disease questionnaire (IBDQ) (14) and the hospital anxiety and depression (HAD) scale (15).

To provide reference ranges for controls and IBS subjects, two further groups were studied: 34 healthy female controls (average age 24 years) without gastrointestinal symptoms and 41 female Rome II IBS patients (average age 41 years) who were recruited consecutively at a specialty clinic for this condition.

**Fecal calprotectin**

A stool sample was obtained from each patient and all samples were frozen at −80°C for fecal calprotectin assay (16). Fecal calprotectin was measured by quantitative enzyme linked immuno-}

sorbent assay using the Phical test (CALPRO AS, Oslo, Norway); the procedure followed the manufacturer's instructions. This is a well validated and Food and Drug Administration-approved assay for measuring fecal calprotectin and has been used in discriminating IBS from IBD (17), assessing disease activity in IBD (18) and as a predictor of IBD relapse (19). This involves extracting the protein from as little as 100 mg of faeces and testing the supernatant using an enzyme immuno assay specific for calprotectin. The mean optical densities of all samples tested in duplicate were matched to a standard curve with actual calprotectin concentrations of standards (mean $r^2 = 0.984$) and corresponding mean optical density values on a xy linear system. All concentrations were obtained from absorbance readings within the linear range (min = 0.06, max = 1.2). No calprotectin measurements were obtained from extrapolated data. To ensure accuracy, samples were assayed using at least two starting dilutions. Where samples fell outside the linear range of the standard curve, they were diluted appropriately to achieve a measurement within the linear range of the standard curve.

**Statistical analysis**

Descriptive statistics are expressed as percentages for categorical data and compared using $\chi^2$-test. Parametric data were compared using an unpaired t-test and nonparametric data with Mann–Whitney U-test. A P value < 0.05 was considered to be significant. All calculations were performed by Graphpad Prism v4 and InStat V3 (Graphpad, San Diego, CA, USA).

**RESULTS**

A total of 106 patients were recruited: 62 CD and 44 UC patients. Despite being considered to be in clinical remission by the predefined criteria, 59.7% ($n = 37$) of CD patients and 38.6% ($n = 17$) of UC patients fulfilled Rome II criteria for IBS.

**Clinical features of patients with and without IBS-like symptoms**

Tables 1 and 2 compare the baseline demographics of IBD patients with and without IBS symptoms in the CD and UC populations, respectively.

Among the CD patients, two factors, smoking status and CD activity index score, differentiated those with IBS symptoms; these patients were more likely to be smokers ($P < 0.0008$) and to have higher CD activity index scores (53.4±31.3 vs. 35.6±35.6, $P = 0.044$), although within the range considered as being in remission. Among the UC patients, the UC activity index score was numerically higher in those with IBS symptoms but did not reach statistical significance. Importantly, all other demographic features were similar for those with and without IBS symptoms in both CD and UC patient groups.

When classified according to disease location and phenotype using the Montreal system (20), no significant difference was evident for those with and without IBS-type symptoms, but subset numbers became too small for robust comparative statistics. Thus, of the patients with CD and IBS-type symptoms, 40.5% (15) had ileocolonic disease, 21.6% (8) had colonic only, and 37.9% (14)
ileal only. Of those with CD without IBS-type symptoms, 33.3% (8) had ileocolonic disease, 25% (6) colonic only, and 41.7% (10) ileal only. With regard to phenotype, of those with CD with IBS-type symptoms, 48.6% (18) had stricturing disease, 40.5% (15) non-stricturing, non-penetrating, and 10.8% (4) penetrating disease. Similarly, of those with CD without IBS, 33.3% (8) had stricturing disease, 58.3% (14) had non-stricturing, non-penetrating, and 8.3% (2) penetrating disease.

With regard to the distribution of UC; of those with IBS-type symptoms, 29.4% (5) had proctitis, 35.3% (6) had left-sided or distal colitis, and 35.3% (6) had pancolitis. Similarly, of those without IBS-type symptoms, 29.6% (8) had proctitis, 33.3% (11) had distal colitis, and 29.6% (8) had pancolitis.

**Fecal calprotectin in IBD patients with and without IBS-type symptoms**

Fecal calprotectin levels were significantly higher in CD patients with IBS compared with those without (414.7 ± 80.3 vs. 174.9 ± 49.1 mg/kg, \( P = 0.0087 \) (Figure 1). The difference remained statistically significant when smokers were excluded (CD + IBS, 425.8 ± 101.7 vs. CD-IBS, 185.6 ± 55.1 mg/kg; \( P = 0.037 \)). Likewise, UC patients with IBS had higher calprotectin levels compared with those without IBS symptoms (591.1 ± 172.5 vs. 229.8 ± 83.4 mg/kg, \( P = 0.0041 \) (Figure 2). As with CD, when smokers were excluded, the difference in calprotectin remained significantly different for those with IBS-type symptoms (655.8 ± 189.7) vs. those UC patients without IBS (253.6 ± 92.8; \( P = 0.0076 \). For both CD and UC, the numbers of smokers were too small to analyze the smoking subsets alone. It is noteworthy that calprotectin levels for all CD and UC patients greatly exceeded those of control subjects.

**Table 1. Summary of CD patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>CD + IBS (n=37)</th>
<th>CD – IBS (n=25)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean ± s.d., years (range)</td>
<td>42±11.6 (21–65)</td>
<td>38.3±9.8 (20–67)</td>
<td>0.3</td>
</tr>
<tr>
<td>Females/males</td>
<td>22/15</td>
<td>10/14</td>
<td>0.25</td>
</tr>
<tr>
<td>Duration of disease, years, mean ± s.d. (range)</td>
<td>11.2±7.7 (2–30)</td>
<td>11±8.7 (1–30)</td>
<td>0.97</td>
</tr>
<tr>
<td>Surgery (Crohn’s-related)</td>
<td>67% (n=26)</td>
<td>56% (n=14)</td>
<td>0.15</td>
</tr>
<tr>
<td>Smokers</td>
<td>27% (n=9)</td>
<td>8% (n=2)</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

**Table 2. Summary of UC patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>UC + IBS (n=17)</th>
<th>UC – IBS (n=27)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± s.d., years (range)</td>
<td>42±11.5 (18–56)</td>
<td>44±13.6 (23–67)</td>
<td>0.84</td>
</tr>
<tr>
<td>Females/males</td>
<td>12/5</td>
<td>14/13</td>
<td>0.36</td>
</tr>
<tr>
<td>Mean duration of disease ± s.d. (range)</td>
<td>12.1±9.3 (1–29)</td>
<td>10.3±6.3 (1–29)</td>
<td>0.49</td>
</tr>
<tr>
<td>Surgery (IBD-related)</td>
<td>None</td>
<td>None</td>
<td>NS</td>
</tr>
<tr>
<td>Smoker</td>
<td>11.7% (n=2)</td>
<td>11.1% (n=3)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

**Medications**

<table>
<thead>
<tr>
<th></th>
<th>CD + IBS (n=37)</th>
<th>CD – IBS (n=25)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-ASA</td>
<td>62.2% (n=23)</td>
<td>52% (n=13)</td>
<td>0.2</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>0%</td>
<td>4% (n=1)</td>
<td>0.13</td>
</tr>
<tr>
<td>Steroids</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>AZA/6MP</td>
<td>32% (n=14)</td>
<td>37.8% (n=7)</td>
<td>0.55</td>
</tr>
<tr>
<td>No meds</td>
<td>13.5% (n=6)</td>
<td>20% (n=4)</td>
<td>0.35</td>
</tr>
<tr>
<td>CDAI (mean ± s.d.)</td>
<td>53.4±31.3</td>
<td>35.6±35.6</td>
<td>0.04</td>
</tr>
</tbody>
</table>

5-ASA, 5-aminosalicylate; AZA, azathioprine; CD, Crohn’s disease; CDAI, CD activity index; IBS, irritable bowel syndrome; 6MP, 6-mercaptopurine; NS, not significant.

**Figure 1.** Fecal calprotectin levels in patients with Crohn’s disease, with and without inflammatory bowel disease (IBS)-like symptoms.

**Figure 2.** Fecal calprotectin in patients with ulcerative colitis, with and without inflammatory bowel disease (IBS)-like symptoms.
Figure 3 illustrates the range of calprotectin levels for our healthy control and IBS subjects; calprotectin levels were similar in both groups (calprotectin (mg/kg), mean ± s.e., control vs. IBS = 23.7 ± 3.9 vs. 24.7 ± 4.7, not significant) with the mean level in both groups well below 50 mg/kg.

Quality of life
The impact of the presence of IBS-type symptoms on QOL among IBD patients was assessed using the inflammatory bowel disease questionnaire (IBDQ) and the HAD (Figure 4). The IBDQ has a range of 32–224, a low score corresponding to a lower QOL. Among CD patients, the IBDQ did not differ significantly between those with or without IBS symptoms: 175.0 ± 4.7 vs. 186.7 ± 5.1, P = 0.09. However, among UC patients, the IBDQ score was a significantly lower for those with IBS symptoms compared with those without: 179.2 ± 6.1 vs. 205.9 ± 2.2, P = 0.0001.

The HAD score consists of 14 questions, 7 for anxiety and 7 for depression. It has a range of 0–42 and was designed for hospital general medical outpatients. A score of 0–7 for either subscale is considered normal. Although the HAD score was elevated in both CD groups, there was no significant difference between those with or without IBS symptoms: 10.6 ± 1.2 vs. 10.2 ± 1.5, P = 0.835 (Figure 5). In contrast, among the UC patients, the HAD score was significantly higher for those with IBS symptoms compared with those without: 8.6 ± 1.2 vs. 5.2 ± 0.6, P = 0.016 (Figure 5).

DISCUSSION
While confirming what others have previously shown, namely, that IBS symptoms are common among IBD patients in apparent remission (1,2), this study provides convincing evidence that such symptoms reflect ongoing IBD activity. This conclusion is supported, first, by the presence of substantially elevated levels of the fecal marker calprotectin among IBD patients with IBS-type symptoms, levels higher than those without IBS symptoms and substantially higher than those of IBS patients and control subjects, second, by higher IBD activity indices in the IBD–IBS groups and, finally, by the observation that CD patients with IBS symptoms were more likely to smoke.

In approaching this complex issue, to minimize confounding variables, we selected patients with both CD and UC who were, by predefined conventional criteria, considered to be in clinical remission, although acknowledging that clinical remission may differ from endoscopic or biochemical evidence of remission. In practice, clinical remission does not necessarily mean the absence of symptoms; it may include symptoms of insufficient severity to be regarded as active disease or to require a change of treatment. A greater dilemma is the presence of symptoms due to coexisting IBS. Although confirmation of disease activity is relatively easy in UC, the diagnostic dilemma may be particularly problematic in CD, particularly if confined to the small bowel. The problem is compounded by insufficient correlation between disease activity indices with endoscopic findings and thereby, as reviewed elsewhere (21–24), reflecting the need for biochemical markers, such as calprotectin.

Until recently, the notion that at least some forms of IBS and IBD could be related and, therefore, coexist would have been dismissed by many clinicians. There is, however, ample evidence for the presence of low-grade inflammation or immune dysfunction...
in IBS (25–27) and it appears that, at least in some instances, IBS may represent an aberrant mucosal response to disturbances in the intestinal microbiota (28–30), thereby, echoing current theories of IBD pathogenesis. Finally, an extensive literature attests to the potential for low-grade inflammation, in IBD, to produce symptoms reminiscent of IBS (31,32). In the past, the clinician attempting to distinguish active IBD from superimposed IBS was hampered by the absence of a sufficiently sensitive marker of inflammatory activity and had to rely on clinical activity indices such as the CD activity index (11). The advent of a well validated and sensitive fecal marker of intestinal inflammation, fecal calprotectin, offers the potential to resolve these issues. Calprotectin is a calcium- and zinc-bound protein that accounts for 60% of the soluble proteins in the cytosol of neutrophil granulocytes. It is a marker of neutrophil turnover and is released into the intestine during colonic inflammation. It is resistant to colonic degradation and can be easily measured in the stool by means of an enzyme linked immunosorbent assay (16).

This study confirms the validity of calprotectin in distinguishing IBD from both IBS and control subjects (17–19,33–35) and again illustrates that calprotectin levels are within the normal range in IBS (17,33,34). As neutrophils are not a feature of the immunoinflammatory changes found in the mucosa of some patients with IBS, an elevated fecal calprotectin would not be expected in such patients. Most importantly, calprotectin levels were considerably higher in the IBD patients with IBS symptoms than in IBS or control subjects, and were comparable with those reported in IBD patients who were about to relapse (19), providing strong support for our contention that IBS symptoms in our IBD patients were, indeed, reflective of ongoing activity of their IBD. In short, low-grade IBD can produce IBS-type symptoms but the development of such symptoms reflects the nonspecificity of gut’s symptomatic repertoire. Regardless of their compatibility with available diagnostic criteria for IBS, their presence, in the context of IBD, does not equate to a diagnosis of IBS and emphasises the caution that needs to be exerted in interpreting such symptoms in the IBD patient. In these challenging situations, which are most likely to occur in the patient with small intestinal CD, as ongoing disease activity can be fairly readily detected in UC by means of colonoscopy or flexible sigmoidoscopy, the clinician has to balance the risks of inappropriate imaging (36) and empiric therapy against the need to correctly identify the source of the patient’s symptoms. We contend that fecal calprotectin and, perhaps, other fecal markers of inflammatory activity, may help to resolve this clinical dilemma. An analogous situation may arise in celiac disease in which IBS symptoms may not only feature prominently at the time of presentation but may also persist following apparent institution of a gluten-free diet and contribute to impaired QOL (37,38); here the availability of highly sensitive and specific antibodies that can be detected in the serum as well as a low threshold for the performance of duodenal biopsy have contributed greatly to diagnostic accuracy.

What do these findings tell us about relationships between IBS and IBD? While the observation that IBS-type symptoms in IBD, though frequent, are reflective of low-grade IBD activity rather than coincident or related IBS, tends to emphasize that these are distinct entities, it also provides a further illustration of the ability of intestinal inflammation, whether occurring in the context of IBD or celiac disease, to lead to “functional” symptoms and underlines the need for caution, by the physician, in the interpretation of such symptoms in these contexts. Finally, these findings also highlight the distinctive nature of the immune-mediated processes that are operative in IBD and IBS (39). Not only is IBS unlikely to lead to IBD (40), but also several features of their mucosal and systemic immunology are quite different. Most obviously, frank, light microscopic inflammation, a hallmark of IBD, is not a feature of IBS and, while a variety of abnormalities in the mucosal immune response have been documented in IBS at a molecular level, their nature appears quite different to IBD. One recent report, for example, documented reduced expression, in IBS, of a number of chemokines and cytokines, which were all quite clearly elevated in IBD (28). Furthermore, the scale of any of the immunological changes seen in IBS is orders of magnitude lower than that reported in IBD.

In acknowledging fundamental differences between IBD and IBS, this study should not be interpreted as support for a conceptual biomedical dichotomy of organic vs. functional disease. The limitation of these and other descriptors has been addressed by us elsewhere (41), and a more unifying or integrated model of IBS and IBD has been presented by others (42).

In summary, although IBS-like symptoms are common among IBD patients in apparent remission and may lead them to qualify for a diagnosis of IBS according to current criteria, it is evident that such symptoms reflect ongoing, although subclinical, activity of IBD and their presence should always be interpreted as such. Bottom line, IBD is IBD unless proven otherwise!

CONFLICT OF INTEREST
Guarantor of the article: Fergus Shanahan, MD, FACG.
Specific author contributions: F.S. and E.M.Q. designed the study and the other authors helped with assays and analysis.
Financial support: The authors are supported, in part, by Science Foundation Ireland in the form of a Centre grant, and by the Higher Education Authority of Ireland.
Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE
✓ Distinguishing occult inflammation from concomitant irritable bowel syndrome (IBS) is an important diagnostic dilemma in patients with inflammatory bowel disease (IBD).
✓ We confirm that IBS-like symptoms are common in both Crohn’s disease and ulcerative colitis.

WHAT IS NEW HERE
✓ For patients judged to be in clinical remission, fecal calprotectin levels are higher in patients with IBS-like symptoms than in those without such symptoms.
✓ Subclinical inflammation should be sought first before assuming a diagnosis of coexisting IBS.
REFERENCES


