SYMPTOMS OF IRRITABLE BOWEL SYNDROME IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

James William Berrill MB BCh MRCP

A thesis submitted to Cardiff University
for the degree of Doctor of Medicine

June 2014

Contents

Chapter 2: Methods

Chapter 3: IBS-type symptoms in patients with IBD: the role of sub-clinical inflammation and the impact on clinical assessment of disease activity

Chapter 4: Albumin catalysed coelenterazine chemiluminescence as a biomarker of IBS

Thesis Summary

Irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) are both chronic relapsing intestinal disorders. Their symptom profiles overlap in terms of abdominal discomfort and altered bowel habit. Meta-analysis of patients with IBD demonstrates that 25-46% of those in clinical remission have symptoms compatible with IBS. These patients report lower quality of life scores compared to their asymptomatic counterparts. There is uncertainty as to the cause of these symptoms, and concern for the influence they may exert on clinical management.

The work described in this thesis investigated the nature of IBS-type symptoms occurring in patients with IBD, examined potential diagnostic tools to distinguish between the respective conditions, and conducted a therapeutic trial for the management of functional symptoms in this setting.

IBS-type symptoms were observed to occur more commonly in female IBD patients, were associated with high anxiety levels, and occurred in patients with no active inflammation as confirmed by a normal faecal calprotectin level. These findings are characteristic of irritable bowel syndrome, and suggest that this disorder may cause persistent symptoms during IBD remission.

Two potential biomarkers of IBS were investigated. The first explored a hypothesis that IBS may be a systemic condition caused by the absorption of toxic metabolites produced by the bacterial fermentation of dietary carbohydrates. This mechanism would potentially explain both the gastrointestinal and the systemic symptoms that are observed in patients with IBS. It was proposed that toxic metabolites may covalently modify albumin in patients with IBS, however on investigation of this theory there was no significant difference observed between the plasma samples of IBS patients, IBD patients and healthy controls. The presence of systemic symptoms in patients with IBS and IBD was associated with higher anxiety levels.

Cognitive function was also assessed as a potential biomarker of IBS following anecdotal reports that IBS patients experience impaired concentration. However no significant difference between IBS patients, IBD patients, and healthy controls was identified. Concurrent mood disorders, in particular depression, were associated with impaired performance of specific tasks in patients with IBD.

A randomised-controlled trial of a mindfulness-based psychological intervention was performed in IBD patients with IBS-type symptoms or high perceived stress levels. Sub-group analysis demonstrated a significant improvement in quality of life in the intervention group in those patients who were experiencing IBS-type symptoms.

Overall, these findings support the theory that IBS can cause persistent symptoms in IBD patients who are in remission. However, until the molecular mechanisms underlying IBS are identified and reliable biomarkers are developed, a systematic diagnostic approach is required to evaluate these patients. IBS-type symptoms in IBD patients represent a therapeutic target to improve quality of life and further trials of psychological intervention, medication and dietary modification are required.

Acknowledgements

First and foremost I would like to offer my sincere thanks to Dr John Green and Professor Anthony Campbell who have supervised my research. Their support and guidance during the last four years has been invaluable. I am also grateful to Professor Kerry Hood who has been extremely generous in offering advice and encouragement on many aspects of the work. I have greatly enjoyed working with all of them throughout this period.

I have collaborated with several departments within Cardiff University during this research. I would like to thank Professor Kenneth Wann, Mr Charles Cox, and Mr Steffan Jones for their assistance with the laboratory work at the school of pharmacy and pharmaceutical sciences. I would also like to thank Professor Andy Smith in the school of psychology, and Professor John Gallacher and Mr Clive Mitchell in the school of medicine for their help in investigating cognitive function, and for developing the cognitive testing website.

I am grateful to all of the patients and volunteers that participated in the studies described in this thesis. I am extremely grateful to Sister Helen Ludlow for her assistance in recruiting patients with inflammatory bowel disease.

Finally, I would like to thank my family for all of the support they have given me. My parents Bill and Jane, and brother David, who have encouraged me throughout my education and career. My wife Rhiannon whose patience and understanding have enabled this thesis to be possible. And my beautiful daughter Lowri whose smile has given everything in life a new perspective.

Publications and Presentations

Publications:

Mindfulness-based therapy for inflammatory bowel disease patients with functional abdominal symptoms or high perceived stress levels.
Berrill JW, Sadlier M, Hood K, Green JT
Journal of Crohn's and Colitis. 2014; doi: 10.1016/j.crohns.2014.01.018.

An observational study of cognitive function in patients with irritable bowel syndrome and inflammatory bowel disease
Berrill JW, Gallacher J, Hood K, Green JT, Matthews SB, Campbell AK, Smith A
Neurogastroenterology and Motility. 2013;25(11):918-925

Symptoms of irritable bowel syndrome in patients with inflammatory bowel disease: examining the role of sub-clinical inflammation and the impact on clinical assessment of disease activity
Berrill JW, Green JT, Hood K, Campbell AK
Alimentary Pharmacology and Therapeutics. 2013; 38(1):44-51

Oral Presentations:

Psychological stress in patients with inflammatory bowel disease
Welsh Association for Gastroenterology and Endoscopy
Bridgend, March 2012

Functional abdominal symptoms in inflammatory bowel disease
Welsh Association for Gastroenterology and Endoscopy
Llandrindod Wells, October 2011

Poster Presentation:

Functional abdominal symptoms in inflammatory bowel disease
Congress of the European Crohn's and Colitis Organisation
Barcelona, February 2012

Abbreviations

5-HT	Serotonin
ACTH	Adrenocorticotrophic Hormone
ANCOVA	Analysis Of Covariance
ANOVA	Analysis Of Variance
ANS	Autonomic Nervous System
Anti-TNF	Anti-Tumour Necrosis Factor
AVP	Arginine Vasopressin
BAM	Bile Acid Malabsorption
BFI	Big Five Inventory
BSA	Bovine Serum Albumin
CAI	Clinical Activity Index
CBT	Cognitive Behavioural Therapy
CD	Crohn's Disease
CI	Confidence Intervals
CNS	Central Nervous System
CRH	Corticotrophin-Releasing Hormone
CRP	C-Reactive Protein
ELISA	Enzyme Linked Immunosorbent Assay
ENS	Enteric Nervous System
FC	Faecal Calprotectin
FGID	Functional Gastrointestinal Disorders
FODMAP	Fermentable Oligosaccharides Disaccharides Monosaccharides and Polyols

GI	Gastrointestinal
GWAS	Genome-Wide Association Studies
HAD	Hospital Anxiety and Depression Scale
HBI	Harvey-Bradshaw Index
HPA	Hypothalamo-Pituitary-Adrenal
HSA	Human Serum Albumin
IBD	Inflammatory Bowel Disease
IBDQ	Inflammatory Bowel Disease Questionnaire
IBS	Irritable Bowel Syndrome
IBS-C	Irritable Bowel Syndrome with Constipation
IBS-D	Irritable Bowel Syndrome with Diarrhoea
IBS-M	Mixed Irritable Bowel Syndrome
IBS-U	Unsubtyped Irritable Bowel Syndrome
IBS-SSS	Irritable Bowel Syndrome Symptom Severity Scale
ISEL	Interpersonal Support Evaluation List
ITT	Intention-To-Treat
IQ	Intelligence Quotient
MCT	Multi-Convergent Therapy
MM	Mindfulness Meditation
NART	National Adult Reading Test
NNT	Number Needed to Treat
PI-IBS	Post-Infectious Irritable Bowel Syndrome
PSQ	Perceived Stress Questionnaire
RDHS	Revised Daily Hassles Scale

SBBO	Small Bowel Bacterial Overgrowth
SCCAI	Simple Clinical Colitis Activity Index
SCFAs	Short Chain Fatty Acids
SSRIs	Selective Serotonin Reuptake Inhibitors
TCAs	Tricyclic Antidepressants
TRPV1	Transient Receptor Potential Vanilloid 1
UC	Ulcerative Colitis
UK	United Kingdom
WCC	Ways of Coping Checklist

Preface

Inflammatory bowel disease is a chronic relapsing disorder that is associated with significant morbidity. Active disease is characterised by the presence of intestinal inflammation and typically causes symptoms of abdominal pain, diarrhoea, and weight loss. During remission a proportion of patients continue to experience abdominal symptoms, that are compatible with a diagnosis of irritable bowel syndrome, despite there being no clinically apparent active inflammation. These persistent symptoms can be difficult to manage and frequently do not respond to conventional inflammatory bowel disease therapies. Further characterisation of this group of patients may provide an insight into the aetiology of these persistent symptoms and assist in the development of therapeutic strategies to alleviate them.

Chapter 1

Introduction

1.1 Inflammatory Bowel Disease

1.1.1 Introduction

It is one hundred years since Thomas Dalziel provided the first report of a condition that he referred to as chronic interstitial enteritis, making a memorable comparison of the affected intestine to "*An eel in a state of rigor mortis*" (1). Twenty years later, in 1932, Burrill Crohn published a case series of 'regional enteritis' that subsequently dictated the eponym 'Crohn's disease' by which the disease is now known (2).

Ulcerative colitis was first described by the physician Sir Samuel Wilks in 1859, although documented cases of non-infectious diarrhoea date back as far as Roman literature (3). Together these two conditions, Crohn's disease (CD) and ulcerative colitis (UC), form the chronic relapsing disorder termed inflammatory bowel disease (IBD).

1.1.2 Epidemiology

Historically IBD has been considered a disease of western society, with the highest incidence and prevalence rates in northern Europe, the United Kingdom (UK), and North America (Figures 1.1 and 1.2). However more recently there have been reports of an increasing burden in other parts of the world including Asia and Africa that appear to correlate with the industrialisation of these areas (4, 5).

Figure 1.1 Worldwide Crohn's disease incidence and / or prevalence for countries reporting data after 1980. Taken with permission from Molodecky, Gastroenterology, 2012 (6).

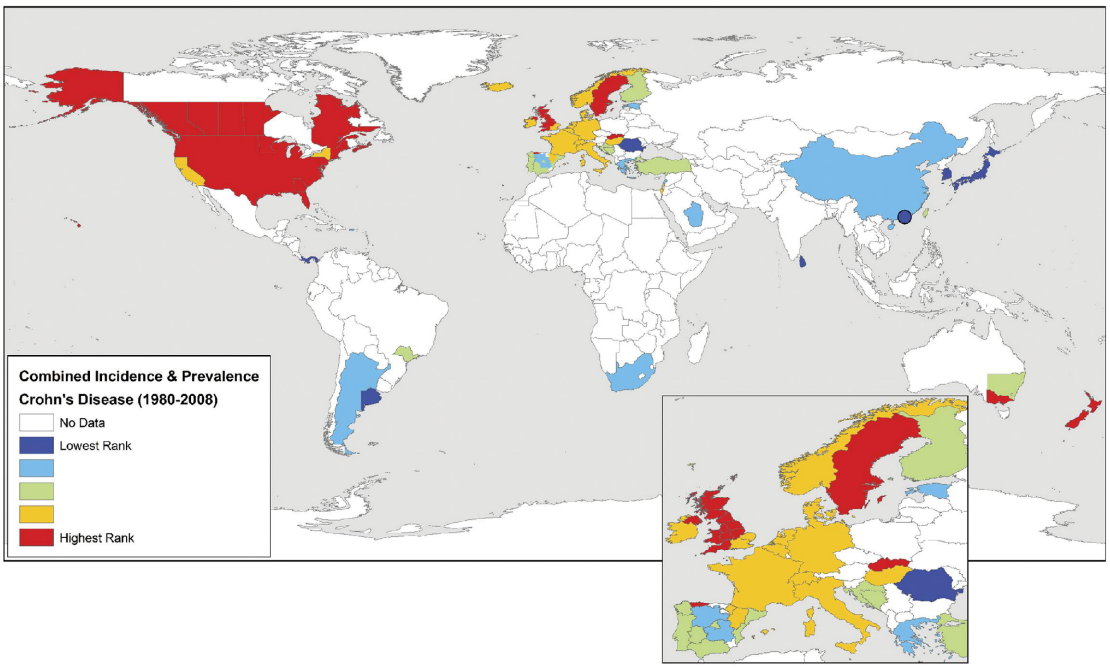
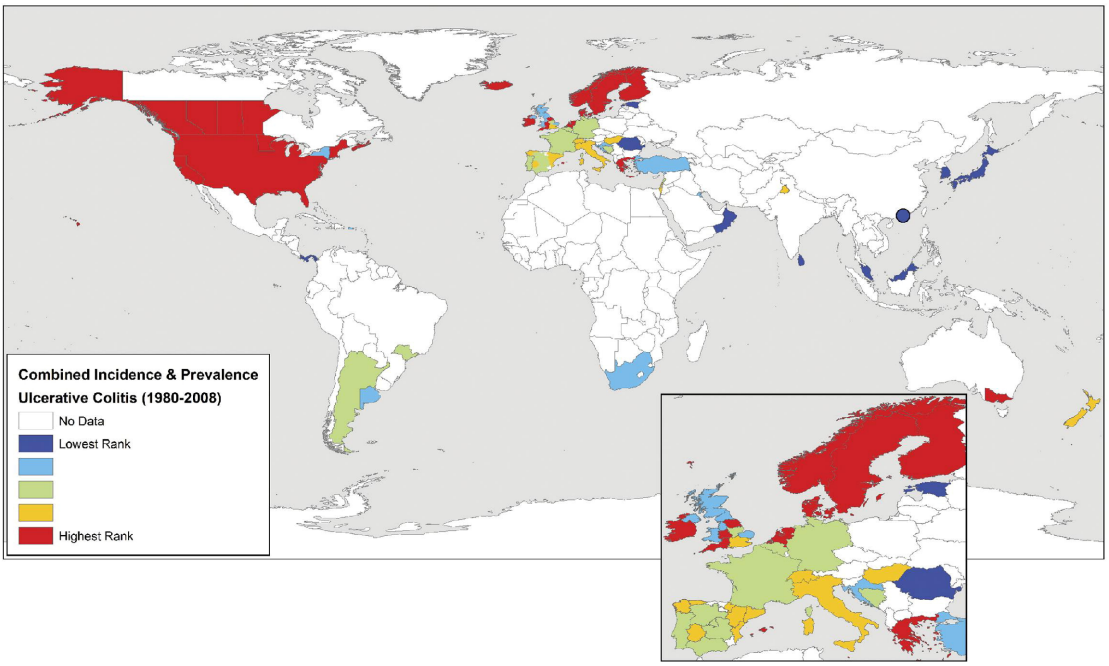


Figure 1.2 Worldwide ulcerative colitis incidence and / or prevalence for countries reporting data after 1980. Taken with permission from Molodecky, Gastroenterology, 2012 (6).



In general, those high-incidence areas experienced a marked increase in the number of cases between the 1950s and 1980s but since then it appears to have stabilised (Figures 1.3 and 1.4). A systematic review of more than 200 reports on the incidence of IBD in locations throughout the world demonstrated that since 1980, 56% of CD and 29% of UC studies have identified a statistically significant increasing incidence, whereas a significant decrease in incidence was reported in only six percent of UC studies and in no CD studies (6).

Considering that mortality in IBD is low and that diagnosis is frequently made at a young age, the global prevalence of IBD is expected to increase substantially. In the UK, the prevalence of IBD is approximately 400 per 100,000 (CD = 145 per 100,000, and UC = 243 per 100,000), with an equal distribution between males and females (7). The onset may occur at any age, but it is most commonly diagnosed in late adolescence and early adulthood.

Studies of migrating populations imply that environmental factors associated with geographic location are an important factor in the development of IBD. Emigrants from countries with a low prevalence of IBD who move to areas with a high prevalence have been observed to have similar incidence rates of IBD to that of their new local population (8, 9). The risk of developing IBD is greater for those who migrate during childhood suggesting that age at the time of migration is influential.

Figure 1.3 Temporal trends in incidence rates (cases per 100,000 person-years) of Crohn's disease in selected areas (Olmsted County; Cardiff; Rochester; Iceland; Aberdeen; Helsinki; Florence). Taken with permission from Loftus, Gastroenterology, 2004 (4).

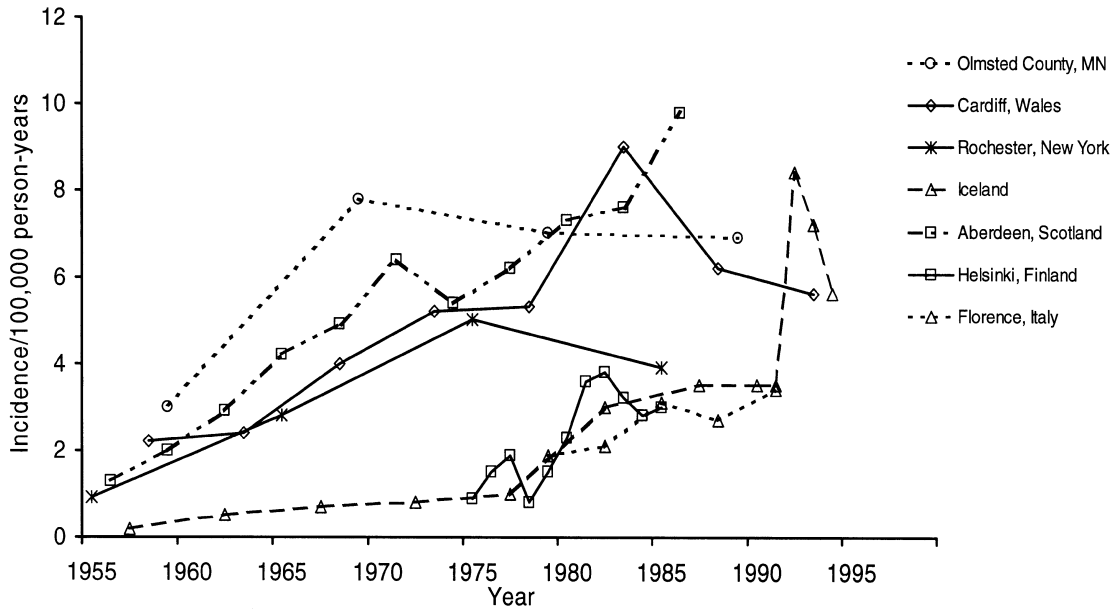
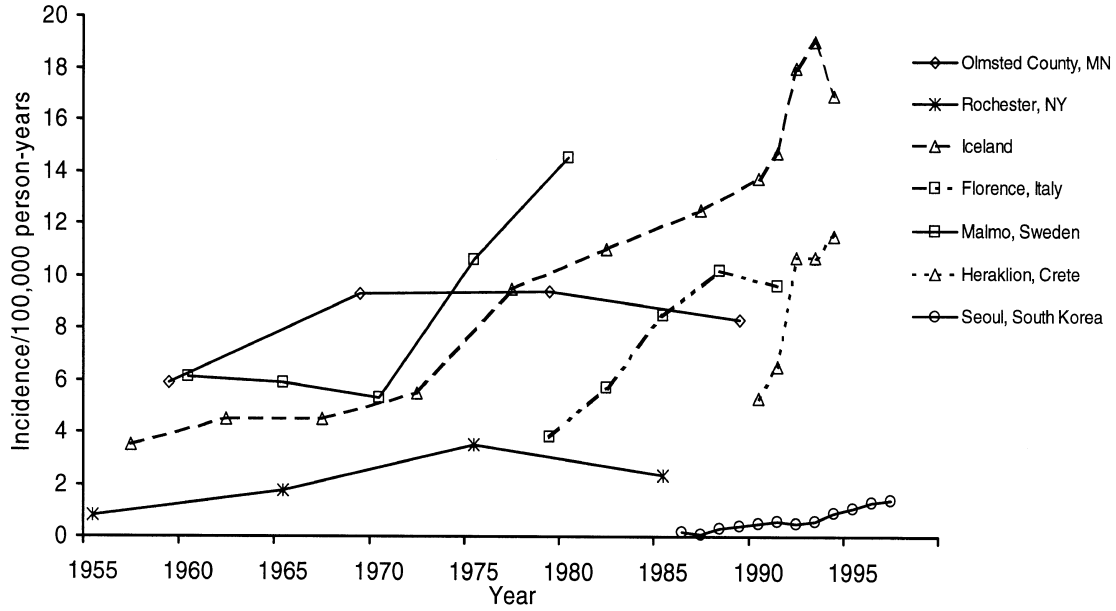


Figure 1.4 Temporal trends in incidence rates (cases per 100,000 person-years) of ulcerative colitis in selected geographic regions (Olmsted County; Rochester; Iceland; Florence; Malmo; Heraklion; Seoul). Taken with permission from Loftus, Gastroenterology, 2004 (4).



1.1.3 Pathophysiology

In his first description, Thomas Dalziel wrote: "*I can only regret that the etiology of the condition remains in obscurity, but I trust that ere long further consideration will clear up the difficulty*" (1). A century later, and after much consideration, the exact pathophysiology of IBD still remains unclear, but several factors including the environment, genetics, intestinal microbes, and a dysregulated immune response have been implicated as having major causative roles.

1.1.3.1 Environmental Factors

The variation in IBD incidence, both geographically and chronologically, has led to many potential environmental causes being studied. Diet, antibiotic usage, vaccinations, appendectomy, oral contraception, cigarette smoking, and perinatal factors have all been examined, but currently only cigarette smoking (predisposing in CD, but protective in UC) is offered as lifestyle advice (4).

Both patients and clinicians have frequently considered diet as an environmental cause of IBD, but most of the studies have been retrospective and therefore prone to recall bias. Physicians typically inform patients to eat a 'normal diet' without any specific exclusion, on the basis that there is no proven connection with any food group. However two recent prospective studies have suggested firstly an association between the intake of polyunsaturated fatty acids and onset of UC (10), and secondly an association between higher vitamin D levels and a reduced risk of developing Crohn's disease (11). Further prospective studies are underway, and

together with randomised controlled trials, the results will enable further insight into whether or not a protective diet for IBD patients can be constructed (12).

The introduction and escalation in use of antibiotics throughout the 20th century coincided with the increasing incidence of IBD, and a retrospective study of general practice records has demonstrated a significant association between CD and the use of antibiotics 2 - 5 years prior to the onset of diagnosis (odds ratio = 1.53; 1.12-2.07). Yet the lack of specificity to any particular subgroup of antibiotics raises uncertainty as to whether this relationship was actually causal or may reflect reverse-causation or the side-effect of other concurrent medication (13).

The appendix may play a role in the developing mucosal immune system. Meta-analysis has demonstrated that appendicectomy reduces the risk of developing ulcerative colitis by 69% (OR = 0.31, 95% CI 0.26-0.37) but possibly increases the risk of CD (4, 14). In UC the benefits are mainly limited to those in whom it is performed for acute appendicitis under the age of 20 years (15).

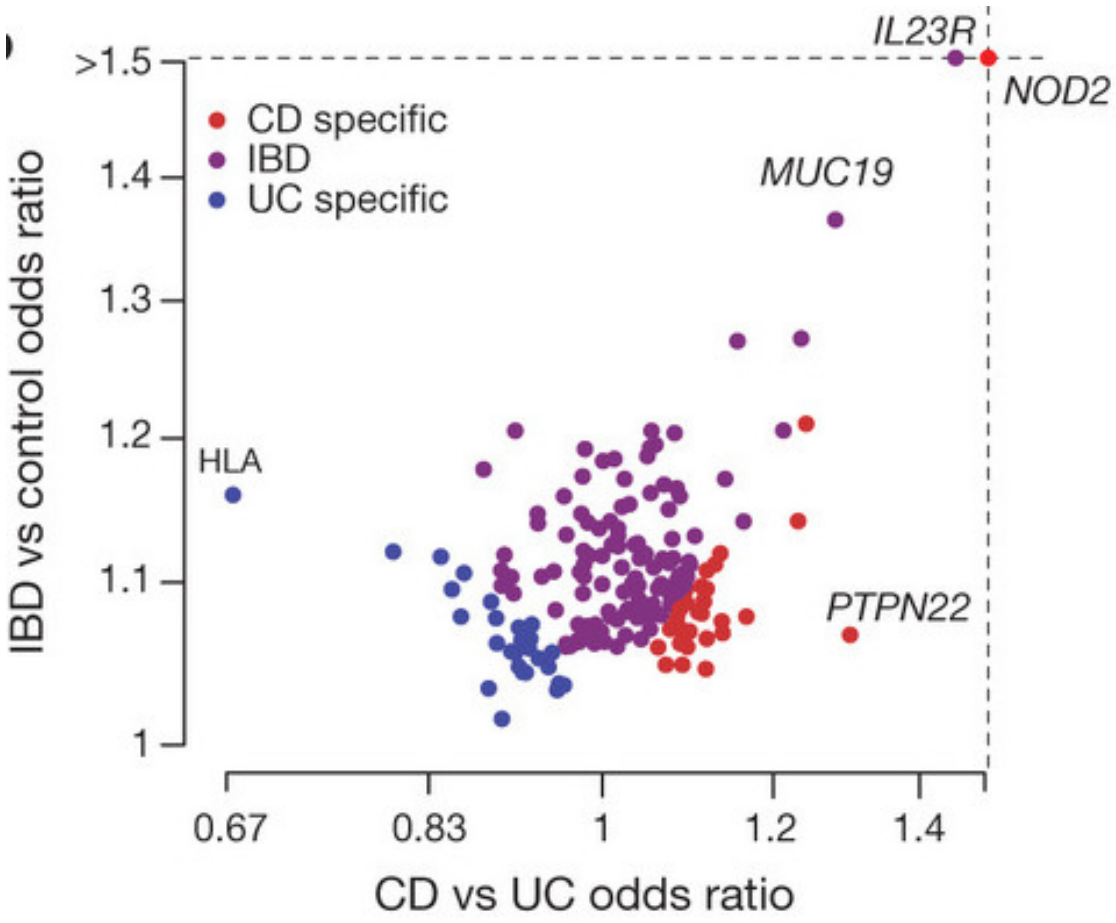
A meta-analysis to evaluate the relationship between smoking and IBD found that current smoking had a positive association with CD (OR = 1.76, 95% CI 1.40-2.22), and a negative association with UC (OR = 0.58, 95% CI: 0.45-0.75) (16). However it is clear that smoking is neither necessary nor sufficient to cause CD, and it is notable that countries with the highest smoking rates frequently have amongst the lowest rates of CD (17). As a result, it seems likely that smoking interacts with other non-environmental factors to influence the development and course of CD.

1.1.3.2 Genetics

A genetic component to IBD was implicated by the familial aggregation of cases, and studies confirmed a higher concordance rate amongst monozygotic twins (36% in CD, and 16% in UC) compared to dizygotic twins (4% in CD and UC) (18). In 1996, genome-wide linkage analysis examining multiple affected families discovered several susceptible loci for Crohn's disease (19). Five years later, fine mapping of the IBD1 locus on chromosome 16 identified the first susceptibility gene for CD in the form of nucleotide-binding oligomerisation domain 2 (NOD2), a gene that codes for an intracellular receptor for bacterial cell wall peptidoglycan (20, 21).

Since then, the development of genome-wide association studies (GWAS), in which the entire genome of cases and controls are compared, has demonstrated that IBD is a complex genetic condition, with many genes involved. A meta-analysis of Crohn's disease and ulcerative colitis GWAS identified a total of 163 IBD susceptibility loci, a substantially higher number than that reported for any other complex disease (22). Interestingly, most of the loci were associated with both CD and UC (Figure 1.5), suggesting that in terms of genetic variations they are similar conditions, and that other factors such as rarer genetic variation (not identified by GWAS) or environmental aspects make a considerable contribution to determining phenotype. The genes identified imply the interaction between host mucosal immune system and intestinal microbes is integral to IBD pathogenesis.

Figure 1.5 The 163 independent signals plotted by total IBD odds ratio and phenotypic specificity (measured by the odds ratio of CD relative to UC), and coloured by their IBD phenotype classification. Taken with permission from Jostins, Nature, 2012 (22).



1.1.3.3 Intestinal Microbes

The human gut is colonised by thousands of species of predominantly anaerobic bacteria that fall into four major phyla (Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria). Several specific micro-organisms have been proposed as pathogenic in IBD, with *Mycobacterium avium* subspecies *paratuberculosis* being the most intensively investigated, but none have any definitive evidence to prove their causative role (23).

Variations in the intestinal microbial composition have been identified when comparing healthy controls to IBD patients, but it has been difficult to determine whether these alterations contribute to the disease or simply reflect secondary changes due to inflammation (23). Studies examining the siblings of CD patients suggest that it is likely to be a primary event with changes occurring prior to the onset of inflammation (24). However many studies have used faecal microbiota as a representation of mucosal-associated microbiota and yet these populations differ significantly. Indeed, it remains uncertain as to which population or location is the most critical in causing IBD (23).

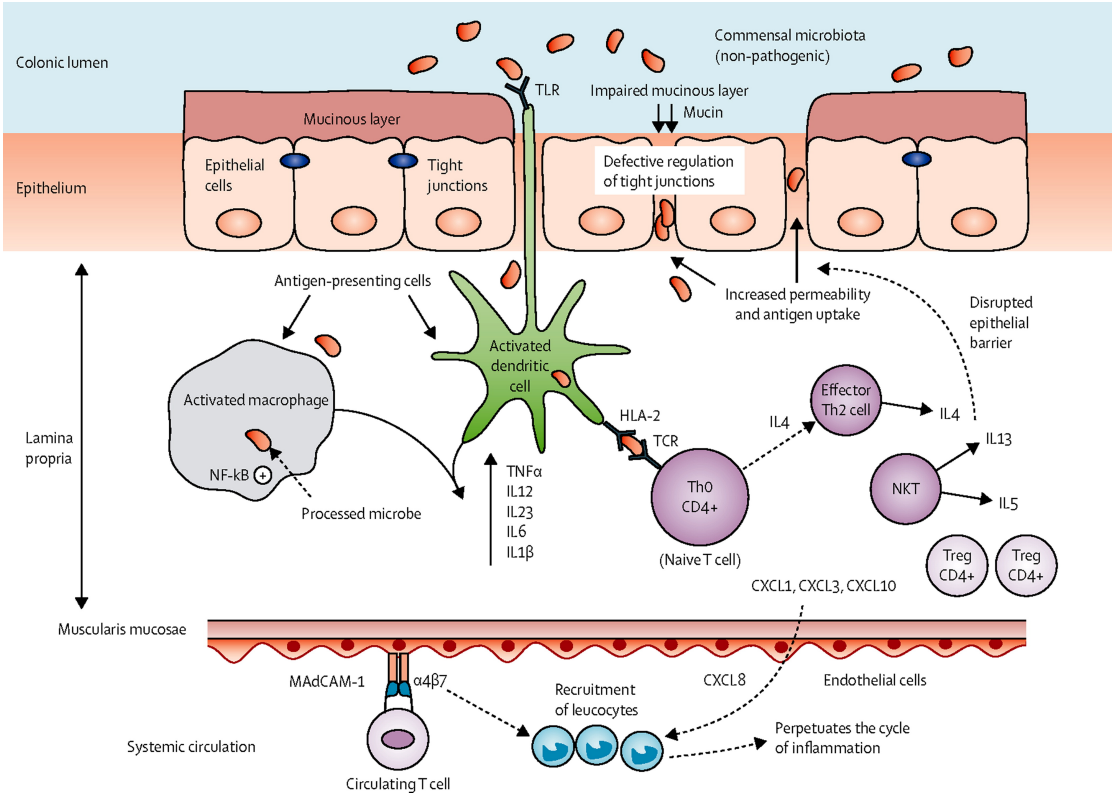
The most striking evidence for the role of gut bacteria in the development of IBD is from animal models, in which genetically susceptible animals reared in a sterile environment only developed colitis after the introduction of bacteria into the intestines (25). However the fact that IBD responds to immunosuppression indicates that bacteria do not seem to be acting as conventional pathogens.

1.1.3.4 Dysregulated Immune Response

The intestinal mucosa is constantly exposed to a great number of microbial antigens, and its immune response involves a complex balance between tolerance of normal commensal organisms and an ability to respond to an infectious insult. In normal function, pathogenic microbes are identified from commensals through pattern recognition receptors that recognise specific molecular markers. Toll-like receptors and nucleotide oligomerisation domains are receptors that can initiate signalling cascades including the nuclear factor-κB pathway provoking a pro-inflammatory

response. In IBD abnormalities have been observed in both the innate and adaptive immune systems and these result in a dysregulated response against the commensal bacteria of the gut (26, 27). An illustration of the pathophysiology of ulcerative colitis is shown in Figure 1.6 (28).

Figure 1.6 Pathophysiology of ulcerative colitis. TLR, toll-like receptor; HLA, human leucocyte antigen; IL, interleukin; TNF, tumour necrosis factor; NF-κB, nuclear factor-κB; Th, T-helper; NKT, natural killer T-cell; CXCL, chemokine; Treg, regulatory T cell, MAdCAM-1, mucosal addressin-cell adhesion molecule 1. Taken with permission from Ordas, Lancet, 2012 (28).



In the innate immune system the epithelial barrier represents the first line of mucosal defence and patients with IBD exhibit a reduction in the overlying mucus layer and deficits in the tight junctions between cells (29, 30). Consequently there is increased epithelial permeability and exposure of the luminal antigens to host

immune cells situated in the lamina propria. Deletion of the MUC2 gene that codes for mucin has been shown to predispose to the onset of experimental colitis in mice (31).

Defects in the function of specialised innate immune cells have also been identified. These include Paneth cells which secrete antimicrobial peptide granules, and dendritic cells which analyse the molecular pattern of microbes and determine whether to evoke an immune response or follow a path of tolerance. Their malfunction can lead to inappropriate immune responses to non-pathogenic insults (32). Both of these cell-types express NOD2 and provide functional examples of the genetic risk factors that have been identified in IBD.

A variety of macrophages provide a further level of innate mucosal immunity. Normally intestinal macrophages exert bactericidal and phagocytic functions but are largely refractory to inflammatory stimulation by microbial antigens, and actually express anti-inflammatory molecules. However in CD macrophages have been observed to produce large amounts of pro-inflammatory cytokines such as IL-23, TNFα, and IL-6, thereby escalating the inflammation cascade (33).

When an immune response is evoked, abnormalities in the adaptive immune system appear to maintain the response. Imbalances in the amounts of effector T-cells (predominantly T-helper cells) compared to regulatory T-cells have been reported and this results in abnormal cytokine secretion. Excessive T cell responses occur, with T helper 1 cells producing interferon-γ in CD, and natural killer cells producing

IL-13 in UC. The release of pro-inflammatory cytokines and chemokines recruits more leucocytes from the systemic circulation. These enter the inflamed mucosa, amplifying tissue injury and perpetuating the inflammatory cycle (28, 34).

1.1.4 Clinical Features and Diagnosis

Ulcerative colitis is an inflammatory disorder of the colonic mucosa. Typically the inflammation starts in the rectum and extends proximally in a continuous manner. The extent of colon affected varies between individuals and the disease has been graded accordingly (Table 1.1) (35). In general, patients present with a history of diarrhoea and visible blood loss. Inflammation limited to the rectum causes rectal bleeding, mucous discharge and urgency to defecate.

Inflammation in Crohn's disease may occur at any location in the gastro-intestinal tract, and consequently the presentation is more variable. It is classified with regards to age of onset, location and behaviour (Table 1.2) (35). These factors help to predict disease course and guide management. The most common sites for CD to affect are the ileum and colon, and so patients can present with abdominal pain, weight loss, or diarrhoea. In contrast to UC, inflammation in Crohn's disease extends deep to the mucosa and may penetrate through the intestinal wall. Complications such as fistulae, abscesses and strictures may occur as a result.

Both UC and CD are associated with several extra-intestinal manifestations that may occur during the initial presentation or later on in the disease course. These include

oral ulcers, iritis, uveitis, erythema nodosum, and arthritis of the peripheral or axial joints.

Table 1.1 Montreal classification of ulcerative colitis (35).

Classification	Maximal extent of inflammation at colonoscopy
E1	Proctitis (Limited to rectum)
E2	Left-sided (Limited to proportion of colon distal to splenic flexure)
E3	Extensive (Inflammation extends proximal to splenic flexure, including pan-colitis)

Table 1.2 Montreal classification of Crohn's disease (35).

Age of onset	Location	Behaviour
A1: ≤ 16 years	L1: Terminal Ileum	B1: Non-stricturing, Non-penetrating
A2: 17 – 40 years	L2: Colon	B2: Stricturing
A3: > 40 years	L3: Ileo-Colonic	B3: Penetrating
	L4: Upper Gastrointestinal	Addition of 'p' denotes peri-anal disease

Note: For location a combination of codes can be used (e.g. L1+L4 for proximal and distal ileal disease)

In diagnosing IBD, clinical symptoms need to be integrated with the results of endoscopic, radiological, histopathology, and biochemical investigations. Its initial presentation can be difficult to differentiate from alternative non-inflammatory gastrointestinal (GI) disorders such as irritable bowel syndrome (IBS). Consequently, a variety of non-invasive markers of gut inflammation have been examined (36, 37). Potentially the most applicable of these is calprotectin, a calcium and zinc binding

protein found predominantly in neutrophils that can be quantitatively measured in faeces by enzyme linked immunosorbent assay (ELISA) (38). Faecal calprotectin (FC) has been shown to have a sensitivity and specificity of over 80% in distinguishing organic bowel disease from functional bowel disease, and it has been recommended for screening patients with symptoms suggestive of irritable bowel syndrome in primary care (39). A raised FC level in this setting indicates the possibility of intestinal inflammation and so diagnoses other than IBS should be considered.

Examination and biopsy of the colon is achieved through flexible sigmoidoscopy or colonoscopy. Occasionally the macroscopic and microscopic features identified do not allow a definite diagnosis of either UC or CD, and such cases are labelled 'unclassified IBD'. Evaluation of the small bowel can be achieved via radiological or endoscopic means, with choice of investigation depending on patient factors and the availability of facilities. Barium studies involve a significant amount of radiation, a particular concern in young patients at risk of repeated investigations, and this together with its higher quality images make magnetic resonance imaging the preferred option. Ultrasound and computed tomography scanning are also used to evaluate IBD, the latter especially useful in excluding extraluminal complications such as abscess formation. In addition, capsule endoscopy can examine the small bowel, although it is not advisable if stenosis is suspected (40). An assessment of disease activity, together with knowledge of the phenotypic classification, enables clinicians to direct appropriate therapy for their patients.

1.1.5 Management

The aims of therapy are to induce and maintain a state of disease remission, thereby improving quality of life and preventing disability.

In ulcerative colitis 5-aminosalicylates represent the first-line therapy for mild to moderate disease. They act topically, and can be administered via oral or rectal routes. If symptoms do not improve then corticosteroids are introduced with the aim of using a short course that gradually tapers over a period of weeks. Approximately one half of patients will have a prolonged response to corticosteroids, remaining in remission at 1 year (41). Patients who need repeated courses of corticosteroids to maintain remission are considered for thiopurine immunosuppressive medication. In cases that are refractory to this regimen, anti-tumour necrosis factor (anti-TNF) medication can be used, although there are restrictions on its use in the UK (42).

In acute severe colitis patients are admitted to hospital and receive intravenous steroids. If there is a poor response after 3-5 days then 'rescue therapy' with ciclosporin, anti-TNF, or surgery is indicated. In the first 10 years after diagnosis 16% of patients with UC require colectomy (43). This generally involves a proctocolectomy with either ileostomy or the formation of an ileal-pouch anal anastomosis. In the acute setting, a two-stage surgical procedure is usually carried out, with sub-total colectomy performed initially and then after recovery a completion proctectomy or pouch formation is achieved.

The management of Crohn's disease is influenced by multiple factors and should be tailored to the individual patient. Traditionally a 'step-up' approach to medical therapy has been implemented, in which more potent medication is gradually introduced if disease activity is not sufficiently controlled. However, the recognition of patient and disease characteristics that can predict an unfavourable course has led to some centres adopting an 'accelerated step-up' or even a 'step-down' regimen with the aim of reducing long-term morbidity (44, 45). For example, a trial of budesonide (a moderate-strength steroid preparation) may be appropriate first-line treatment in an elderly patient with only mild terminal ileal inflammation, but in a young patient with extensive ileo-colonic disease then early anti-TNF therapy should be considered.

Most CD patients will require long-term immunosuppressive medication (thiopurine, methotrexate, or anti-TNF) to prevent disease recurrence once remission has been achieved. In severe CD, a combination of thiopurine and anti-TNF medication may achieve better results (46), however the benefits of these potent immunosuppressive therapies need to be balanced against their associated risks of infection and malignancy (47, 48).

The rate of surgical intervention is higher in CD compared to UC, with 47% of CD patients undergoing surgery in the first 10 years after diagnosis (43). Indications for surgery include fibrostenotic strictures causing obstructive symptoms, complex perianal or internal fistulas that do not respond adequately to medical therapy, and abscess formation. Unfortunately surgery is not curative in Crohn's disease as it

recurs in a great number of patients. The recurrence rate varies depending on the definition used, but in those patients not on therapy clinical recurrence rates of 20-25% per year have been reported (49). Patients are at risk of recurrent surgical resection and so judicious decision-making is required to avoid the nutritional complications of extensive small bowel resection. Stricturoplasty can offer a safe alternative to resection for short ileal strictures.

Other important aspects of management include nutritional support, smoking cessation in Crohn's disease, and psychological care.

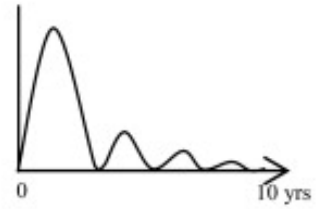
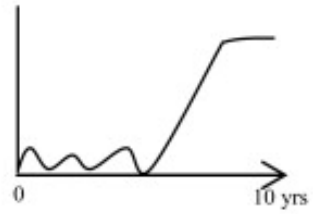
1.1.6 Disease Course and Monitoring

The course of IBD varies greatly between individuals. At one end of the spectrum it will occur as a single mild episode that does not recur, and at the other end it presents as a rapidly progressive colitis unresponsive to medical therapy. The different patterns of disease activity are illustrated in Figure 1.7.

In the majority of cases IBD is a lifelong condition that consists of episodes of active intestinal inflammation known as a relapse or flare, followed by periods of remission during which the inflammation is quiescent and symptoms improve. A Danish population-based study observed that in the initial 5 years after diagnosis of UC 13% had no relapses, 74% had two or more relapses but not in every year, and 13% had active disease every year (50). The proportions were similar in CD patients, with 18%, 57%, and 25% in the same respective groups.

With regards to overall mortality rates, a meta-analysis reported the standardised mortality ratio for UC was 1.19 (95% CI = 1.06-1.35) and for CD was 1.38 (95% CI = 1.23-1.55) indicating higher rates of death in both types of IBD relative to the general population (51).

Figure 1.7 Differing patterns of disease activity in IBD in terms of severity of bowel symptoms from diagnosis to 10-year follow-up. Taken with permission from Solberg, Clinical Gastroenterology and Hepatology, 2007 (52).

Curve (1-4):	Definitions:
	**Curve 1:** Decrease in the severity of bowel symptoms during the follow-up period
	**Curve 2:** Increase in the severity of bowel symptoms during the follow-up period
	**Curve 3:** Chronic continuous bowel symptoms during the follow-up period
	**Curve 4:** Chronic relapsing bowel symptoms during the follow-up period

The relapsing-remitting nature of IBD dictates that patients frequently seek advice from health professionals when they experience an increase in their abdominal symptoms. In this situation, an accurate assessment of disease activity is essential to planning appropriate management. Clinicians face a difficult balance between prescribing empirical immunosuppressive medication and organising multiple investigations, some of which may be invasive or involve exposure to radiation. In general treatment is guided by patients' symptoms, however there is evidence that the correlation between symptom-based assessment and disease activity is limited (53). More accurate assessment may be achieved by using a non-invasive marker of inflammation such as faecal calprotectin, which has been shown to correlate well with disease activity in UC and CD, especially in colonic disease (54-57). If diagnostic uncertainty still remains then endoscopic or radiological investigation may be required.

Several non-inflammatory conditions that can mimic active disease may need to be excluded, particularly in patients with Crohn's disease. Bile salt malabsorption is common in patients with terminal ileal resection. Small bowel bacterial overgrowth is associated with CD, especially in the presence of fistula or previous bypass surgery with blind loop formation. High rates of lactose malabsorption have also been reported (58). Lastly, there are functional disorders such as irritable bowel syndrome, and these chronic conditions have the potential to cause diagnostic uncertainty in patients with IBD.

1.1.7 Psychological Factors in Inflammatory Bowel Disease

Psychological disorders commonly exist in association with inflammatory bowel disease. During remission 29-35% of patients are reported to have anxiety or depression, and in active disease rates as high as 80% for anxiety and 60% for depression have been observed (59). Whilst these rates are higher than that found in the general community, they are actually quite similar to levels present in patients with other chronic illnesses such as rheumatoid arthritis and diabetes (60). Much debate has occurred as to whether the psychological comorbidity occurs as a result of having IBD, or whether it may actually have an aetiological role in the condition.

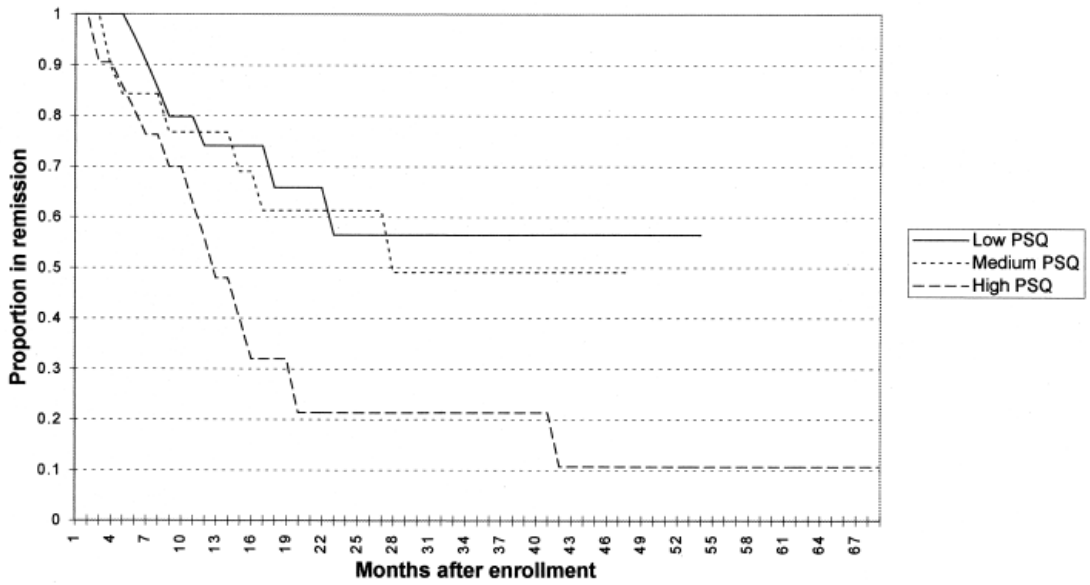
Animal models have enabled the physiological mechanisms by which stress may adversely impact on IBD to be examined in more detail. In rats, stress increased the severity of hapten-induced colitis compared to controls (61), and in mice that had recently recovered from colitis a period of stress increased the susceptibility to its reactivation (62). In the latter study stress was associated with increased intestinal permeability. Interestingly stress did not reactivate colitis in athymic or immunodeficient mice, however when CD4 T-cells taken from mice with previous colitis were injected into immunodeficient mice, the colitis could be reactivated by stress. This suggests that colitis reactivation by stress is immune mediated, and indicates the ability of psychological, immune and luminal factors to interact and reactivate quiescent colitis.

Opinions on IBD have changed since the 1950s when IBD was regarded by some as a psychosomatic disorder. Early studies investigating the temporal relationship

between psychological factors and IBD produced mixed conclusions and many of these have subsequently been reviewed and found to contain significant methodological flaws (63). Investigating psychiatric illness as a risk factor for disease onset ideally requires the prospective study of affected individuals and matched controls to identify if a difference in IBD incidence occurs, but the low prevalence of IBD makes this impractical and so no such studies have been performed. Consequently no convincing evidence exists to support psychological illness as a risk factor for IBD onset (60).

Examining the relationship between psychological factors and the course of IBD is less complex and several prospective studies have suggested an unfavourable association (64). In a study of 62 UC patients, high levels of long-term perceived stress more than tripled the risk of relapse in the following 8 months (Figure 1.8) (65). Similarly in Crohn's disease, perceived stress increased the risk of relapse, whereas certain styles of coping mechanism reduced the risk (66). The presence of depression has also been shown to predict a poorer course of disease (67). However there are still problems with these studies in that frequently disease activity is defined using clinical activity scores that can be influenced by subjective rating of symptoms, as opposed to using objective markers of inflammation such as endoscopy or faecal calprotectin. It has been suggested that the increased relapse rate observed in those groups with high levels of anxiety and depression may actually represent functional symptoms (known to be associated with psychological comorbidity), rather than true inflammatory bowel disease activity (68).

Figure 1.8 Kaplan-Meier analysis of cumulative rates of exacerbation in ulcerative colitis patients with high, middle, and low tertile scores on long-term Perceived Stress Questionnaire Scores (PSQ) at enrolment. Risk of exacerbation was higher among patients with high long-term stress levels than among those with low levels (p=0.03). Taken with permission from Levenstein, American Journal of Gastroenterology, 2000 (65).



1.2 Irritable Bowel Syndrome

1.2.1 Introduction

The expression 'functional disorder' has conventionally been used by physicians to describe those symptoms that appear to lack a structural pathology. These disorders are frequently encountered in the general population and prove less amenable to explanation or effective treatment. In the field of gastroenterology they have been classified using symptom based diagnostic criteria, produced by the Rome Foundation, an organization set up to assist on the diagnosis and treatment of functional gastrointestinal disorders (FGIDs). In adults, twenty-eight FGIDs have been presented in six domains; oesophageal, gastroduodenal, bowel, abdominal pain, biliary, and anorectal (69).

The most common FGID in the bowel domain is irritable bowel syndrome. It is a chronic gastrointestinal condition characterised by abdominal pain, bloating, and alterations in bowel habit. It has been defined as "a functional bowel disorder in which abdominal pain or discomfort is associated with defecation or a change in bowel habit, and with features of disordered defecation" (70).

Within this diagnosis of IBS, patients can be further classified according to the consistency of their stools: IBS with constipation (IBS-C), IBS with diarrhoea (IBS-D), mixed IBS (IBS-M), or unsubtyped IBS (IBS-U), (Table 1.3). These subtypes are unstable and in a patient population with equal proportions of IBS-D, IBS-C and IBS-M, approximately 75% of patients changed subtypes over a 1-year period (71).

Table 1.3 Subtyping IBS by predominant stool pattern (70).

IBS Subtype	Symptom Classification
IBS with constipation	Hard stools ≥25%, Loose stools <25% of bowel movements
IBS with diarrhoea	Loose stools ≥25%, Hard stools <25% of bowel movements
Mixed IBS	Hard stools ≥25% + Loose stools ≥25% of bowel movements
Unsubtyped IBS	Insufficient abnormality of stool consistency to subtype

Hard Stools = Bristol Stool Form Scale 1-2
Loose Stools = Bristol Stool Form Scale 6-7

The Rome foundation has created a symptom-based questionnaire to provide a clinical standard for diagnosis (70). Although this questionnaire is a useful tool for research, many patients will undergo a series of investigations before being diagnosed with IBS, and consequently in clinical practice it is often a 'diagnosis of exclusion'.

Epidemiological studies have estimated the prevalence of IBS in western society to be 10-20% (72). Prevalence is higher in females (odds ratio = 1.67; 95% CI: 1.53-1.82) (73), and it is frequently associated with co-existing mood disorders (74-76). Whilst there is no association between IBS and increased mortality, it does have a negative impact on quality of life and leads to significant healthcare expenditure (77, 78).

1.2.2 Pathophysiology

Several physiological abnormalities have been identified in patients with IBS. Alterations in colonic motility have been observed and appear to depend on IBS subtype. Increased motility and a greater number of high amplitude propagating contractions occur in diarrhoea-predominant IBS, with the opposite occurring in constipation-predominant patients (79, 80). However these patterns of motility can also occur in the asymptomatic population and so it is thought that visceral hypersensitivity has a fundamental role in causing IBS symptoms (81, 82). This is supported by several studies in which IBS patients exhibited enhanced pain sensitivity compared to controls in response to distension of the gut lumen (83, 84).

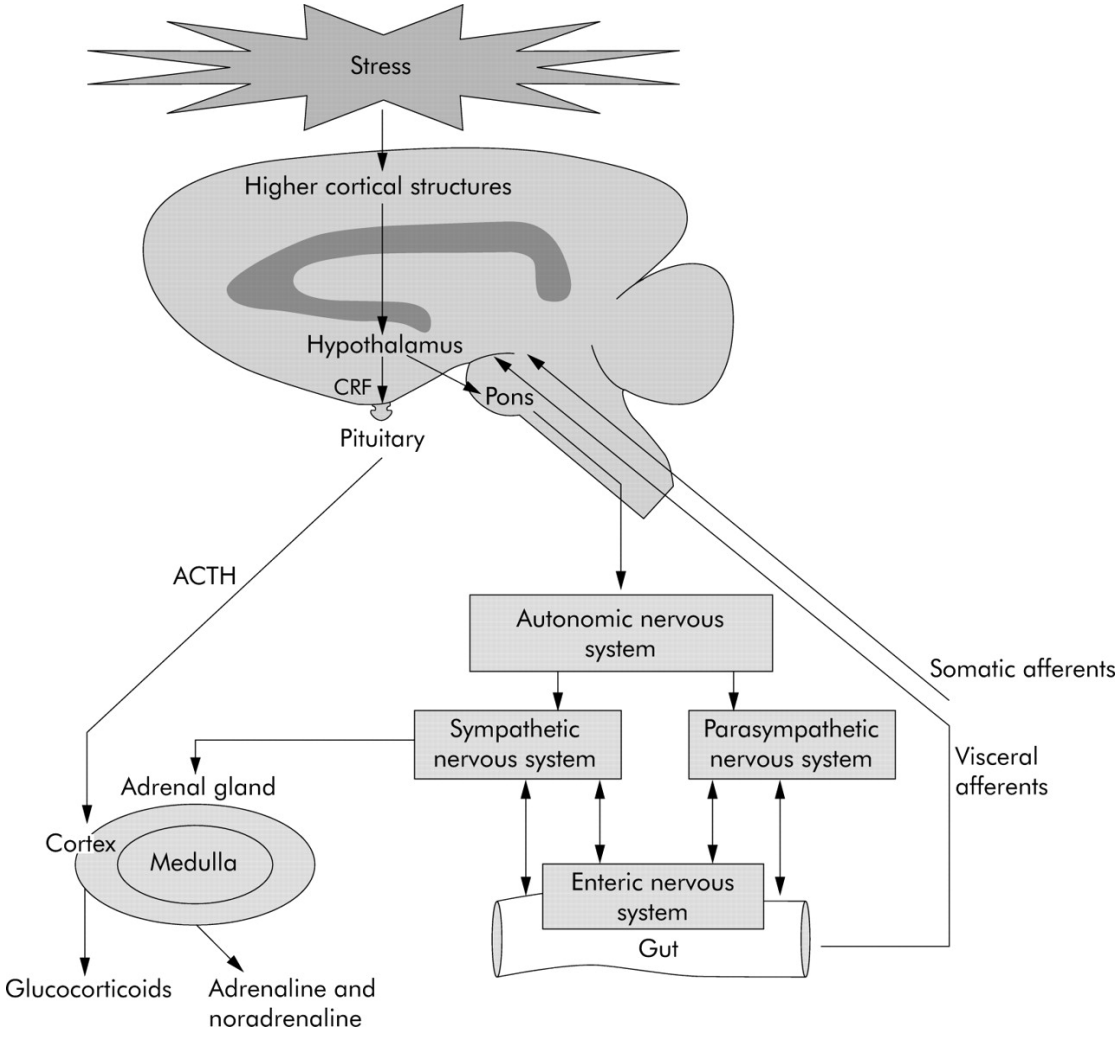
These physiological abnormalities were considered to be driven predominantly by central factors via a neurohumoral communication known as the 'brain-gut axis'. This pathway facilitates the influence of psychological stress on gastrointestinal physiology. However research during the last decade has raised awareness regarding the involvement of peripheral factors in the development of IBS.

1.2.2.1 Central Mechanisms: The Brain-Gut Axis

Bidirectional communication occurs between the central nervous system (CNS) and the gastrointestinal tract through a neurohumoral system that has been termed the brain-gut axis (Figure 1.9). Inside the wall of the gut exists a substantial amount of neural tissue which is known as the enteric nervous system (ENS). This consists of the myenteric and submucosal plexuses and contains around 500 million neurones. It possesses internal reflex circuits that can regulate digestive function without

direction from the brain, however this independence is modulated by the CNS through connections via the autonomic nervous system (ANS) (85). Through this pathway GI motility and secretions can be influenced.

Figure 1.9 Pathways mediating the effects of stress on the gastrointestinal tract. ACTH, adrenocorticotrophic hormone; CRF, corticotrophin releasing factor. Taken with permission from Mawdsley, Gut, 2005 (63).



The stress response is formed by elements located in the CNS and in peripheral organs. The two principal neuroendocrine systems involved in producing the stress response are the hypothalamic-pituitary-adrenal (HPA) axis and the ANS. Upon evaluating a situation to be stressful, inputs from both limbic circuits and brainstem centres instigate neurosecretory cells in the paraventricular nucleus of the hypothalamus to release corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) (86). In the HPA axis, CRH and AVP act on the anterior pituitary to increase adrenocorticotrophic hormone secretion into the systemic circulation, which consequently stimulates glucocorticoid production from the adrenal cortex. CRH also activates nuclei of the ANS in the brainstem. This leads to release of catecholamines, both from the adrenal medulla via the sympathetic-adrenal-medullary axis, and directly from postganglionic sympathetic nerve fibres. Glucocorticoids and catecholamines are the main peripheral mediators of the stress response and their actions are exerted on systems throughout the body (87).

In healthy human volunteers stress has been shown to enhance colonic motility, increase jejunal water and ion secretions, and intensify the sensation of urgency in response to rectal distension (88-90). Studies using animal models demonstrate that stress also increases intestinal permeability, potentially facilitating exposure of luminal macromolecules and antigenic factors to the mucosal immune system (91). It is apparent that both the HPA-axis and the ANS are involved in producing these changes (63, 92).

1.2.2.2 Peripheral Mechanisms

A direct relationship has been reported between episodes of gastroenteritis and the subsequent development of IBS. This specific form of IBS has been termed post-infectious IBS (PI-IBS). Risk factors for developing PI-IBS include toxicity of the infecting organism and the presence of psychological comorbidity. Typically infections associated with mucosal ulceration such as *Campylobacter jejuni* are associated with subsequent PI-IBS, whereas it occurs much less frequently after episodes of viral gastroenteritis. The observation that anxious and depressed patients are more likely to develop PI-IBS potentially suggests an interaction between central and peripheral factors in developing this condition. Examination of colonic mucosa in patients with PI-IBS reveals persistent enterochromaffin cell hyperplasia and raised levels of lymphocytes suggestive of ongoing inflammation; changes that are present even at 1 year after the initial infection (93).

Observations of intestinal dysbiosis in patients with IBS may provide further support for the pathological role of gut microbes, however it remains difficult to establish whether these differences in the microbiota are primary or secondary events. An increased ratio of firmicutes bacteria to bacteroidetes has been reported in patients with IBS, but this has also been shown to occur in rats after the administration of excess bile acids. (94-96). Potentially intestinal dysbiosis could have functional impacts in the form of an increased synthesis of short chain fatty acids (SCFAs) and intestinal gas (95, 97).

Dietary carbohydrates that are not digested or absorbed in the small bowel enter the colon and undergo bacterial fermentation. This produces SCFAs which are able to alter colonic motility and secretion. Animal models demonstrate that SCFAs stimulate colonic transit and initiate high amplitude propagating contractions through the release of serotonin from mucosal enterochromaffin cells (98, 99). In addition they induce transepithelial ion and fluid transport in the distal colon (100). Serotonin acts through receptors located on the submucosal and myenteric neurons of the enteric nervous system, and can stimulate contraction or relaxation of the intestinal smooth muscle.

Levels of serotonin have been observed to vary depending on IBS-subtype; with IBS-D patients having raised concentrations of plasma serotonin whereas IBS-C patients exhibited a reduced serotonin response to meal ingestion (101). Further variations in the gut endocrine system of IBS patients have been identified, in particular with regards to cholecystokinin activity, however reports have been inconsistent and more research into the role of enteroendocrine cells is required (102).

Low-grade mucosal inflammation appears to be a feature in post-infectious IBS, however its role in the general IBS population has yet to be established. Several studies have reported an increase in mucosal mast cell activity and plasma levels of pro-inflammatory cytokines, particularly in diarrhoea predominant cases, but these findings have not been consistently replicated (103). Activation of mast cells causes release of mediating compounds such as histamine and tryptase that can activate sensory nerves innervating the GI tract. The proximity of mast cells to mucosal nerve

fibres correlated with the frequency and severity of abdominal pain in IBS patients, and in rat models the release of mast cell mediators excited nociceptive neurones indicating a potential mechanism for visceral hypersensitivity (104, 105).

Visceral sensations are transmitted from the gut via afferent nerves travelling through the spinal cord to the brain. The sensation of pain arises when noxious stimuli activate ion channels located on nociceptor terminals and cause the nociceptive afferent neurone to be depolarised. These ion channels include the transient receptor potential vanilloid 1 (TRPV1) channel, a member of the transient receptor potential family of ion channels that mediate a variety of sensations. TRPV1 can be activated by capsaicin and inflammatory mediators, and data from animal studies have suggested a role in visceral hypersensitivity (106). Interestingly, TRPV1 nerve fibres have been found to be present in significantly greater numbers on the colonic biopsies of IBS patients compared to controls (107). They were observed in all IBS subtypes and their presence correlated with abdominal pain scores. This finding represents a further explanation for the visceral hypersensitivity that is present in IBS.

1.2.3 Management

1.2.3.1 Dietary Modification

A considerable proportion of patients with IBS believe that diet has a causative role in their symptoms and as a result many will exclude certain foods from their nutrition (108-110). There is recognition in the general public that 'dietary fibre' can influence bowel habit and so many will try increasing their levels of fibre intake as a first step in their management. The term 'fibre' refers to soluble and insoluble non-starch polysaccharides which are plentiful in fruit, vegetables and cereals. However there is actually little data supporting this approach, and paradoxically it may exacerbate symptoms. Current recommendations are that a trial of cereal fibre exclusion should be considered, but that if fibre supplementation is thought necessary then soluble forms such as ispaghula are probably the best choice (81).

Malabsorption of individual carbohydrates such as lactose and fructose is known to cause abdominal symptoms, but their respective exclusion diets have had limited success (111-113). Consequently, a new approach that involves excluding a much broader range of short chain carbohydrates has been developed. This reduces dietary intake of fermentable oligosaccharides, disaccharides, monosaccharides and polyols and has been called the FODMAP diet (114). In addition to lactose and fructose are fructo- and galacto-oligosaccharides, and sugar alcohols (sorbitol, mannitol, xylitol and maltitol). These compounds share three common functional properties in that they are poorly absorbed in the intestines, they are osmotically active, and they are rapidly fermented by gut bacteria. This leads to excess SCFA

production, together with an increase in the volume of intestinal fluid and gas that distends the lumen.

These physiological properties have been demonstrated. A high FODMAP diet has been shown to increase delivery of fluid and fermentable substrate to the proximal colon (115). It also led to increased hydrogen gas production in both IBS and healthy populations compared to the low FODMAP diet (116). Interestingly, in this latter study, patients with IBS produced significantly more hydrogen gas than healthy volunteers suggesting that there is indeed increased fermentation in this group, possibly as a result of altered motility or intestinal dysbiosis. Therefore it appears that FODMAPs produce gut distension in both healthy and IBS populations but symptoms are of a higher severity in the IBS group partly due to a greater amount of fluid and gas production and partly due to visceral hypersensitivity.

Whereas the success of exclusion diets which focused on just one form of carbohydrate may have been limited by the effects of other sugars, the emphasis of the low FODMAP diet is that it restricts all short chain carbohydrates that are poorly absorbed and in this way aims to improve efficacy in symptom control. Certainly early studies suggest a benefit in IBS patients (117-119).

1.2.3.2 Medication

The symptomatic treatment of abnormal transit with laxatives or anti-motility agents is relatively straightforward, but identifying an effective therapy for the cardinal symptoms of abdominal pain and bloating remains more difficult.

Antispasmodics such as mebeverine or hyoscine aim to reduce the increased contractility that is seen particularly in IBS-D patients. A meta-analysis showed 56% patients on active drug reported global improvement versus 38% for placebo (NNT = 5.5), and 53% v 41% for abdominal pain (NNT = 8.3) (120). A more recent meta-analysis reported similar results with a NNT to prevent a patient having persistent symptoms being five (121).

Antidepressant medication is used in the treatment of IBS due to their potential modulation of pain perception. Additional psychological benefits may occur in the presence of concurrent mood disorder. The most commonly used forms are tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs). Meta-analysis has reported that they are equally effective and provide significant benefit compared to placebo in patients with IBS, with a NNT of 4 (122).

Serotonin (5-HT) is involved in the regulation of gastrointestinal secretion, sensation and motility. Of the seven 5-HT receptor subtypes, 5-HT_3 and 5-HT_4 appear to be the most important in this particular role and have thus been identified as possible therapeutic targets (123). Alosetron, a 5-HT_3 receptor antagonist has been shown to improve symptoms in IBS-C compared to placebo with a NNT of 8 (122). Similarly,

Tegaserod, a 5-HT_4 agonist was reported to improve IBS-C with a NNT of 10 (122). However in both cases there are concerns regarding their long-term safety, with a small number of patients developing ischaemic colitis on alosetron, and an increased number of cardiovascular and cerebrovascular events with tegaserod. Highly specific 5-HT_4 agonists such as prucalopride have been developed for treating chronic constipation and may prove to be a safe effective treatment in constipation predominant IBS in the future (124).

Trials of antibiotics and probiotics have investigated manipulation of intestinal flora. A two-week course of the non-absorbable antibiotic rifaximin showed benefit over placebo with a NNT of 10 (125). Probiotics may offer some benefit with symptoms of bloating but larger trials are required before definitive conclusions are made (126).

Trials of anti-inflammatory agents have generally involved small numbers of participants and produced mixed results (127-129). They are not currently employed in normal clinical practice. Other potential new therapies include intestinal chloride secretagogues, bile acid modulation, and mast cell stabilisers (126).

Many of the therapeutic studies described in this section have used meta-analysis. It is important to reflect that despite the obvious advantages of this method there are also potential flaws, such as the studies included in the analysis being influenced by personal or publication bias. Thus, caution is required when interpreting the quoted NNT figures, particularly when it is being used in such a heterogeneous condition such as IBS.

1.2.3.3 Psychological Interventions

The theory that IBS is a centrally driven disorder facilitated by the brain-gut axis, together with the frequent co-existence of mood disorders, has led to psychological interventions being employed in its management.

A variety of techniques have been studied including cognitive behavioural therapy (CBT), relaxation training, hypnotherapy, mindfulness based therapy, dynamic psychotherapy, and a combined multi-therapy approach. Typically these consist of face-to-face sessions with therapists on an individual or group-based format, however more recently options for self-taught or internet-based intervention have been developed (130, 131).

A meta-analysis of psychological interventions in this setting concluded that a NNT to prevent IBS symptoms persisting in one patient was four (75). CBT had the most evidence available, but all forms appeared to have similar efficacy, except for relaxation training which showed no statistically significant benefit. There were several limitations of this analysis with the studies examined having inadequate power calculations, variable definitions of IBS, and short follow-up periods with the majority only being for 8 to 12 weeks.

The views of patients on the use of psychotherapy will vary and can influence the outcome. It is important to discuss the options available and elicit their preferences before referral. The most benefit is likely to come from those patients who are keen to pursue psychological intervention or those with concurrent anxiety or depression (81).

1.3 Irritable Bowel Syndrome in Inflammatory Bowel Disease

1.3.1 Introduction

Considering the prevalence of IBS in the general population, it is to be expected that a proportion of patients with IBD will also have co-existing IBS. Indeed, when patients whose IBD is in remission as defined by clinical criteria are assessed for the presence of IBS-type symptoms, the prevalence is 32-39% in UC and 42-60% in CD (132-134). Anxiety levels are higher, and quality of life scores are lower in this group when compared to those asymptomatic patients whose IBD is in remission (133-135).

1.3.2 Aetiology of IBS-type symptoms in patients with IBD

The reported prevalence of IBS-type symptoms in patients with IBD is higher than that observed in the general population and so there is uncertainty as to the exact nature of these symptoms. An obvious consideration in IBD is whether active inflammation may be responsible; even those patients who appear to be in remission clinically may have ongoing sub-clinical inflammation. The only previous study to evaluate this hypothesis found that FC levels were significantly higher in patients in clinical remission with IBS-type symptoms compared to those without (132). The authors concluded that IBS-type symptoms reflected subclinical inflammation, however no analysis of those patients with a normal FC level was reported.

Evidence that IBS-type symptoms in IBD patients are not simply due to ongoing inflammation has come from the study of pain receptors in these patients. The presence of TRPV1 receptors, known to be associated with IBS, has been studied in IBD patients who were confirmed to be in complete remission with normal clinical activity scores, FC level, and mucosal appearance on colonoscopy. Colonic biopsies revealed a significant increase in TRPV1 fibres in those patients with IBS-type symptoms compared to those who were asymptomatic. The increased TRPV1 levels were considered to be driven by nerve growth factor, which in turn can be influenced by psychosocial aspects, with higher levels found in stress. Anxiety and depression scores in this group were indeed significantly higher than in asymptomatic patients (136). It is clear that further investigation of IBS-type symptoms in IBD patients is required to establish the contribution of sub-clinical inflammation compared to 'true IBS'.

1.3.3 Pitfalls of IBS-type symptoms in IBD

The overlapping spectrum of symptoms that IBS and IBD share, make this cohort of patients that report bloating, discomfort and altered bowel habit despite their IBD being in remission, a complex situation to manage. This is particularly problematic given the fluctuating activity of IBD in which it can be difficult to determine if a true relapse is occurring. Significant functional symptoms in a patient with quiescent IBD may lead to the overuse of potent immunosuppressive medication, but alternatively a clinician's suspicion of IBS in a patient with persistent inflammation could lead to under-treatment of active disease. The possibility of coexistent IBS affecting clinical

activity indices for IBD such as the Harvey-Bradshaw index (HBI) or the simple clinical colitis activity index (SCCAI) that rely on clinical symptoms to determine whether patients are in remission is also a concern (137, 138). It has been speculated that this may account for apparent discrepancies in some therapeutic IBD trials.

Various potential biomarkers for IBS have been studied, including measurements of intestinal motility (139), visceral sensory perception (83), and imaging of the central nervous system (140). As knowledge of the pathophysiology of IBS has developed, interest has now been directed towards identifying molecular markers of gene expression and immune mediators such as cytokines (105). However these methods have not been introduced into routine practice as the techniques involved are complex and expensive, and have not yet produced consistent results in discriminating IBS from controls.

The benefit of identifying a reliable biomarker for IBS in the general population is obvious. However, in a similar fashion it would be extremely useful in the management of IBD patients in whom IBS-type symptoms were present. The ability to positively diagnose IBS in this setting would reduce the amount of invasive or radiological investigation, and enable treatment to be directed more effectively.

The management of IBS-type symptoms in patients with inflammatory bowel disease has never been addressed directly. Whether those strategies that are employed in managing IBS in the general population would also be effective in the IBD population is unclear. The quality of life in IBD patients who report IBS-type symptoms is

significantly lower than their asymptomatic counterparts and so potentially they should represent a therapeutic target. Further investigation is required to identify effective management options for this group of patients.

1.4 Objectives

The initial aim of the work described in this thesis was to establish the nature of IBS-type symptoms in patients with inflammatory bowel disease. The intention was to clarify the role of sub-clinical inflammation in causing these symptoms and to assess the impact they have on the clinical assessment of disease activity.

Having defined the patient group, the next objective was to examine potential biomarkers of irritable bowel syndrome and evaluate their use in the IBD population. Potentially these would enable a positive diagnosis of IBS to be made and thereby reduce the need for invasive investigations.

The final aim was to examine a therapy for improving IBS-type symptoms in patients with IBD. It was anticipated that an improvement in these symptoms would enhance overall quality of life.

1.5 Overall Hypothesis

A significant amount of morbidity in patients with inflammatory bowel disease is due to non-inflammatory mechanisms.

1.6 Study Design

Four studies were performed to achieve these objectives:

Study 1:

This was a cross-sectional observational study that determined the prevalence of IBS-type symptoms in the local IBD population. It investigated the characteristics of those IBD patients with IBS-type symptoms, examined the contribution of sub-clinical inflammation in producing IBS-type symptoms, and considered the impact these symptoms have on the clinical assessment of IBD activity. A total of 169 patients with IBD were assessed.

Study 2:

This laboratory-based study examined the hypothesis that toxic metabolites, produced by bacterial fermentation of dietary carbohydrates in the colon, play a role in causing the symptoms of IBS. The ability of metabolites to covalently modify plasma albumin and affect its enzymatic activity was examined to explore whether this property could be used as a biomarker of IBS. In this study samples of serum were collected from patients with IBS, patients with IBD, and healthy volunteers.

Study 3:

In this observational study the cognitive profile of patients with IBS and IBD were examined. Patients with IBS, patients with IBD, and healthy volunteers completed a series of neuropsychological performance tests that examined a range of cognitive

functions. The cognitive profiles of the three groups were compared to identify if a unique deficit existed that may act as a biomarker for IBS.

Study 4:

A randomised controlled trial of a mindfulness-based therapy was performed in inflammatory bowel disease patients with IBS-type symptoms or high perceived stress levels. The study aimed to explore whether this intervention was a therapeutic option in these patient groups. A total of 66 IBD patients were recruited to the trial.

Chapter 2

Methods

2.1 Introduction

This chapter describes methods that are applicable to the overall thesis. Those methods, including statistical analysis, that are specific to an individual study are described in the respective study chapter.

2.2 Ethical Approval

The research was approved by the South-East Wales research ethics committee in November 2010. Reference number: 10/WSE02/49.

2.3 Recruitment

Recruitment for the research studies took place between January 2011 and May 2012. All participants completed a consent form prior to participation.

Patients with inflammatory bowel disease and patients with irritable bowel syndrome were recruited from gastroenterology clinics at the University Hospital Llandough and University Hospital of Wales. These hospitals are part of the Cardiff and Vale University Health Board that provides healthcare for approximately 500,000 patients in Cardiff and the Vale of Glamorgan. Patients were supplied with information sheets on the research studies prior to their clinic appointments and were offered the opportunity to participate following their clinic consultation.

Healthy volunteers were recruited from the general population using a volunteer panel set up by Cardiff University. This panel includes contact details for members of the public who have expressed an interest in participating in research. They were emailed an information sheet regarding the studies and were asked to contact the research team if they were interested in participating. A fee of £10 was offered to cover time and travel expenses.

2.4 Participant Definitions

All participants were aged 18 – 65 years. They were excluded if they were pregnant, had a diagnosis of cognitive impairment, or if they had an ileostomy, colostomy, or previous colectomy performed.

Patients with inflammatory bowel disease:

Diagnosis of IBD was verified according to the European Crohn's and Colitis Organisation criteria (141, 142), and disease extent was defined according to the Montreal classification (35).

Patients with irritable bowel syndrome:

All patients had been reviewed in gastroenterology clinic by a physician whose clinical impression was that of IBS. In addition, they were required to meet the Rome III criteria for IBS (defined in section 2.4) (70). To exclude organic pathology as a cause of their symptoms, only those patients who had a normal colonoscopy as part of their diagnostic investigations were included. If colonoscopy had not been

performed, then patients supplied a stool sample for faecal calprotectin analysis and were excluded if the level was greater than 90μg/g.

Healthy volunteers:

Healthy volunteers were excluded if they reported symptoms of abdominal discomfort, diarrhoea, constipation or rectal bleeding. Specifically, they did not meet the Rome III criteria for IBS. They were excluded if there was a previous diagnosis of IBS, IBD, coeliac disease, lactose intolerance, or had a history of bowel surgery (other than appendicectomy).

2.5 Questionnaires

All participants completed a questionnaire that documented demographics, past medical history, and current medication (Appendix 1). Questionnaires that were used throughout the research are described below. Questionnaires that were used only in a specific study are described in the respective study chapters.

Rome III Criteria (Appendix 2):

The presence of IBS was assessed using the Rome III criteria (70). This symptom-based standard for diagnosing IBS has been produced by the Rome committee through a consensus approach. It defines IBS as the presence of abdominal discomfort on at least 3 days per month, occurring in the last 3 months, and with onset at least 6 months ago. The abdominal discomfort must be associated with two

or more of the following; improvement with defecation, onset associated with a change in frequency of stool, onset associated with a change in form of stool.

Patients that fulfilled Rome III criteria for IBS were classified into subtypes according to their responses on questions seven and eight of the questionnaire (Appendix 2). Patients reporting only loose stools were classified as diarrhoea-predominant, and those with only harder stools as constipation-predominant. If both forms of stools were experienced then patients were designated as having mixed symptoms, and if no change in stool consistency was present then they were classified as unsubtyped.

Irritable Bowel Syndrome Symptom Severity Scale (Appendix 3):

Patients who met the Rome III criteria for IBS completed the irritable bowel syndrome symptom severity scale (143). This measures the severity of IBS symptoms in five domains; frequency and severity of abdominal discomfort, severity of abdominal bloating, satisfaction with bowel habit, and impact of symptoms on life in general. Each domain is scored 0–100, and an overall score of 0–500 is obtained. A higher score indicates more severe symptoms.

Hospital Anxiety and Depression Scale (Appendix 4):

Levels of anxiety and depression were measured using the hospital anxiety and depression scale (144). This self-assessment scale consists of 14 statements (seven regarding anxiety, and seven for depression) which are graded 0–3 according to their relevance to the individual. A range of scores from 0 to 21 are provided for anxiety and depression, respectively.

Simple Clinical Colitis Activity Index (Appendix 5):

A clinical assessment of disease activity in patients with ulcerative colitis was performed using the simple clinical colitis activity index (138). This questionnaire is a symptom-based activity index that uses six questions to provide an immediate result, and does not require blood tests or endoscopy.

Harvey-Bradshaw Index (Appendix 6):

A clinical assessment of disease activity in patients with Crohn's disease was performed using the Harvey-Bradshaw index (137). Patients respond to five questions regarding clinical symptoms and disease activity is determined without the need for blood tests or endoscopy.

2.6 Measurement of Faecal Calprotectin:

Patients were asked to provide a stool sample within 1 week of their clinical assessment. They were supplied with a standard 30ml container for stool collection and advised to take the specimen to either their local general practice surgery or to specimen collection in University Hospital of Wales. They were asked to do this within 24 hours of collecting the specimen.

Samples were stored in freezers at -40°C. All samples were analysed within one month of collection using the CALPRO Calprotectin ELISA Test, a quantitative enzyme immunoassay. This was performed in the biochemistry department of the University Hospital of Wales. The faecal calprotectin laboratory reference level for screening

patients with functional abdominal symptoms to exclude intestinal inflammation is <90μg/g. This level was selected to define biochemical remission in IBD patients as it was considered important to confidently exclude active inflammation when evaluating the potential presence of functional symptoms in patients with IBD. A FC level of 100μg/g in IBD patients has been demonstrated to have a sensitivity of 72% and a specificity of 96% in predicting remission, with a positive predictive value of 91% and a negative predictive value of 84% (145).

2.7 Statistical Analysis:

Mean and standard deviations are shown for all normally distributed data, and comparisons were made using unpaired t–test or analysis of variance (ANOVA). Values of median and range are provided for non-normally distributed data, and comparisons were performed using Mann–Whitney or Kruskal–Wallis tests. Categorical data are presented with absolute numbers and percentages, and were analysed using Chi-squared tests. All analysis was performed using PASW Statistics 18.0 (IBM Corporation, Armonk, NY, USA).

Chapter 3

IBS-type symptoms in patients with IBD: the presence of sub-clinical inflammation and the impact on clinical assessment of disease activity

3.1 Introduction

A recent meta-analysis of patients with inflammatory bowel disease demonstrated that 25-46% of those in clinical remission have symptoms compatible with a diagnosis of IBS (146). This is higher than the prevalence of IBS found in normal western populations which is estimated to be 10-20% (72). There is uncertainty as to the cause of these apparent functional symptoms in IBD patients, and concern for the influence they may exert on clinical management (53, 147).

Several different mechanisms have been implicated in the pathogenesis of IBS-type symptoms in patients with IBD. Firstly, it may be the same IBS condition that occurs in the general population ('true IBS'), as there is no reason why IBS and IBD should be mutually exclusive. Secondly, there is the possibility that sub-clinical inflammation may be responsible. Finally, it has been proposed that chronic inflammation may modulate the physiology of the enteric nervous system and intestinal wall, such that subsequent altered motility and visceral hypersensitivity may produce IBS-type symptoms (135, 148).

Accurate assessment of disease activity in IBD is essential in order to provide appropriate treatment. A physician's clinical suspicion of a relapse, based on history and examination, may lead to further endoscopic or radiological investigation being performed, or alternatively to the introduction of empirical immunosuppressive therapy. However the presence of symptomatic IBS in patients with IBD could influence this initial clinical assessment. Patients who are in remission but experience considerable functional symptoms, may appear to have active disease,

and so undergo unnecessary invasive procedures or receive inappropriate and potentially harmful medication.

The aims of this study were to determine the different contributions of 'true IBS' and sub-clinical inflammation in producing IBS-type symptoms in IBD patients, and to ascertain the impact IBS-type symptoms have on the clinical assessment of IBD activity.

Hypothesis: A substantial proportion of IBD patients will have IBS-type symptoms despite being in remission as defined by a normal faecal calprotectin level. The presence of these symptoms will have a detrimental impact on the clinical assessment of IBD activity.

3.2 Methods

Patients with inflammatory bowel disease completed a series of questionnaires regarding disease activity, presence of IBS-type symptoms, and levels of anxiety and depression.

Symptom-based indices were used to assess clinical IBD activity; the simple clinical colitis activity index (SCCAI) for ulcerative colitis, and the Harvey-Bradshaw index (HBI) for Crohn's disease. Patients completed the irritable bowel syndrome section of the Rome III diagnostic questionnaire for adult functional disorders and were categorised as having IBS-type symptoms or not according to the Rome III criteria.

The presence of mood disorders, known to be associated with IBS, were assessed using the Hospital Anxiety and Depression Scale.

Clinical definitions of IBD activity:

A blood sample was taken from each patient to check the level of C-reactive protein (CRP), a protein that is produced in response to inflammation. 'Clinical remission' in UC was defined as SCCAI <3 points and CRP <10mg/l, and in Crohn's disease HBI <5 points and CRP <10mg/l. 'Clinically active' disease was defined as SCCAI ≥3 points or HBI ≥5 points. Those patients with a low activity score (SCCAI <3 or HBI <5) but a high CRP >10mg/l were defined 'unclassified'.

Faecal Calprotectin Measurement:

To establish if IBS-type symptoms were associated with active inflammation, an objective marker of intestinal inflammation in the form of FC was measured. The FC laboratory reference level for screening patients with functional abdominal symptoms to exclude intestinal inflammation is <90μg/g. This level was used to define biochemical remission in IBD patients. Faecal calprotectin levels were not available to clinicians at the time of categorising IBD activity or presence of IBS-type symptoms.

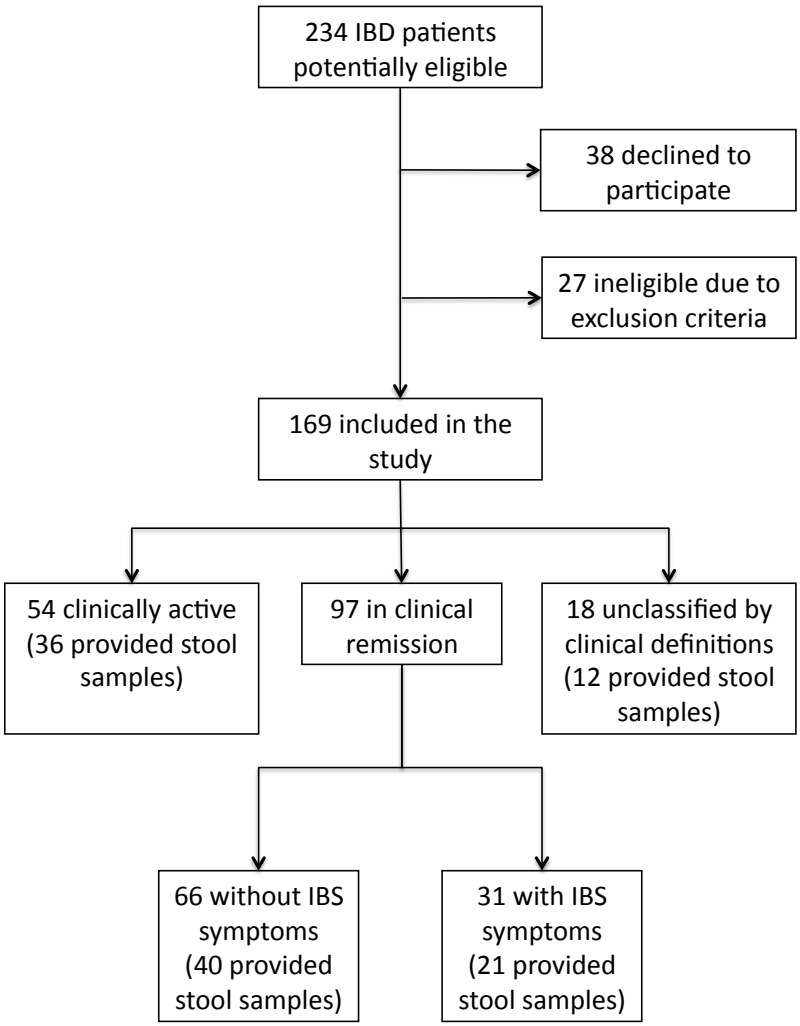
Statistical Analysis:

Logistic regression was used to examine factors associated with the presence of IBS. The Kappa statistic was used to assess the level of agreement between clinical and biochemical assessment of disease activity.

3.3 Results

A total of 169 patients with inflammatory bowel disease were recruited. The numbers of individuals at each stage of the study are illustrated by the flowchart in Figure 3.1 These included 108 (64%) females; the mean age of the group was 44 years. There were 101 cases of UC and 68 of CD.

Figure 3.1 Flowchart showing number of participants at each stage of the study. The 169 patients included in the study consisted of 101 cases of UC and 68 CD.



Using clinical criteria 97 patients were in remission, 54 had active disease, and 18 were unclassified as they had a low clinical activity index score but CRP>10mg/l. For those patients in clinical remission the overall prevalence of IBS-type symptoms was 32% (95% CI: 23 - 41%).

Patients in clinical remission:

The characteristics of patients in clinical remission, with and without IBS-type symptoms, are compared in Table 3.1. Symptoms meeting criteria for IBS were significantly more common in female patients and were associated with higher levels of anxiety and depression. Prevalence of IBS-type symptoms was similar in UC and CD, and did not seem to relate to patient's age, disease duration or smoking status. The proportion of CD patients who had a previous bowel resection was higher in those with IBS-type symptoms (62% v 33%) but the difference was not statistically significant. For disease location the number of patients was insufficient to perform analysis, however the proportions in each category suggest there was no significant relationship. When these factors were entered into stepwise logistic regression only gender (odds ratio = 4.64, 1.55–13.88) and anxiety score (odds ratio = 1.11, 1.01–1.21) were significantly associated with presence of IBS-type symptoms.

Of the 31 patients who were in clinical remission and reported IBS-type symptoms, 12 (39%) had diarrhoea-predominant symptoms, 2 (6%) were constipation-predominant, 14 (45%) had mixed symptoms, and 3 (10%) were unsubtyped.

Table 3.1 Demographic, psychological and disease characteristics of IBD patients in clinical remission, with and without IBS-type symptoms.

	IBS-type symptoms (n=31)	No IBS-type symptoms (n=66)	*P* value
Gender: Male Female	5 (16%) 26 (84%)	34 (52%) 32 (48%)	<0.01* (Chi-Square)
Mean age, years (s.d.):	46 (11)	46 (12)	0.82 (t-test)
Diagnosis: UC CD	18 (58%) 13 (42%)	39 (59%) 27 (41%)	0.92 (Chi-Square)
Disease Location:			
UC (n= 57): Proctitis Left-Sided Pan-Colitis	6 (33%) 7 (39%) 5 (28%)	7 (18%) 21 (54%) 11 (28%)	N/A
CD (n=40): Ileal Colonic Ileo-colonic	5 (38%) 3 (23%) 5 (38%)	7 (26%) 10 (37%) 10 (37%)	N/A
Median disease duration, years (s.d.):	9 (1-36)	8 (1-47)	0.99 (Mann-Whitney)
Current Smoker: Yes No	4 (13%) 27 (87%)	9 (14%) 57 (86%)	1.00 (Fisher's Exact)
Currently on immunosuppressant[#]:			
Yes: No:	11 (35%) 20 (65%)	19 (29%) 47 (71%)	0.51 (Chi-Square)
Previous bowel resection[†]: Yes (CD patients only, n=40) No	8 (62%) 5 (39%)	9 (33%) 18 (67%)	0.09 (Chi-Square)
Psychological indices:			
Mean Anxiety score, (s.d.) Mean Depression score, (s.d.)	9.0 (5.2) 5.8 (4.2)	5.8 (5.0) 3.5 (3.7)	<0.01 (t-test)* <0.01 (t-test)*

N/A: Not Applicable due to insufficient numbers to perform analysis

* Indicates statistically significant difference with $p<0.05$

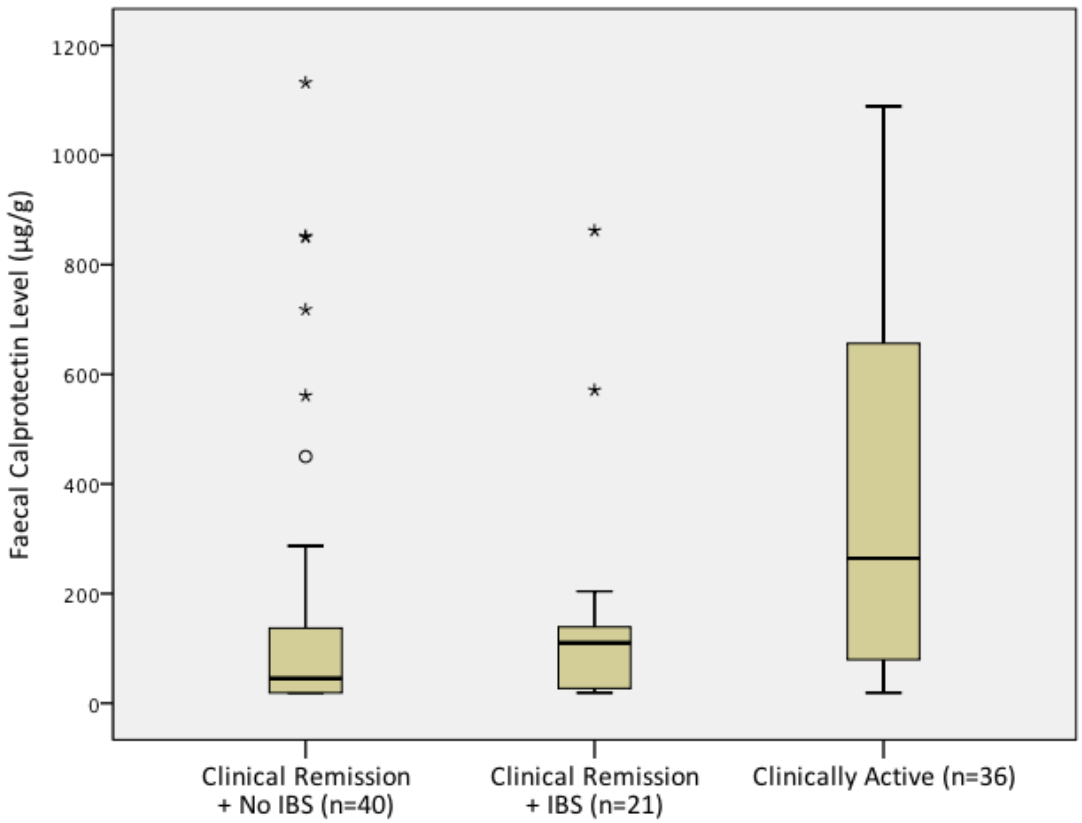
Immunosuppressants include thiopurines, methotrexate, and anti-TNFs

† Data only presented for CD patients as no UC patients had prior bowel resection

Faecal calprotectin levels:

FC was measured in the 109 patients that provided a stool sample. The box plot in Figure 3.2 illustrates the distribution of FC levels in the three clinically defined groups of IBD patients. There was no statistical difference between the FC levels of patients in clinical remission with IBS-type symptoms compared to those without IBS-type symptoms (median values = 111μg/g v 45.5μg/g respectively, p=0.17). However FC levels in the clinically active group were significantly higher, (median value = 233μg/g, p<0.01).

Figure 3.2 Box plot of the faecal calprotectin levels in respective patient groups.



For patients in clinical remission the prevalence of IBS-type symptoms was higher in those with raised FC level (42%) compared to those with normal FC level (29%) but the difference did not reach statistical significance, p=0.20.

Faecal calprotectin levels of patients in clinical remission were analysed separately for ulcerative colitis and Crohn's disease. In UC the median FC level for patients with IBS-type symptoms was 71μg/g and in those without 35μg/g, (p=0.32). In CD the median FC level for patients with IBS-type symptoms was 111μg/g and in those without 50μg/g, (p=0.32).

Overall, 48 (44%) of the 109 patients that provided a stool sample had a FC level <90μg/g confirming they were in biochemical remission. The prevalence of IBS-type symptoms in this group was 31% (95% CI: 19 - 46%).

Assessments of disease activity:

The relationships between the clinical and biochemical assessments of disease activity are shown for those patients with IBS-type symptoms (Table 3.2) and without IBS-type symptoms (Table 3.3). Only those patients who provided stool sample are included in this analysis. The kappa statistic, measuring agreement between the two assessments of disease activity, was 0.26 for patients with IBS-type symptoms and 0.25 for those without.

Table 3.2 Relationship between clinical and biochemical definitions of remission in patients with IBS-type symptoms.

	Biochemical Remission FC <90μg/g	Biochemical Active FC ≥90μg/g
Clinical Remission	10 (67%)	9 (33%)
Clinical Active	5 (33%)	18 (67%)

Table 3.3 Relationship between clinical and biochemical definitions of remission in patients without IBS-type symptoms.

	Biochemical Remission FC <90μg/g	Biochemical Active FC ≥90μg/g
Clinical Remission	25 (86%)	15 (63%)
Clinical Active	4 (14%)	9 (38%)

3.4 Discussion

This observational study demonstrates that IBS-type symptoms are significantly more common in female IBD patients, are associated with high anxiety levels, and can occur in patients with no active inflammation. Together, these features are similar to those exhibited by 'true' IBS occurring in the general population, and suggests that in some IBD patients the same condition may be responsible for producing their symptoms.

The absence of an objective biomarker for diagnosing IBS has meant that observational studies examining the phenomenon of IBS in IBD patients have all used

symptom-based criteria to define its presence (132-135, 149-151). As a result, it is unclear whether this is 'true IBS' or whether there are alternative pathologies causing similar symptoms that simply meet the criteria for a diagnosis of IBS.

It has been proposed that IBS-type symptoms reflect subclinical inflammation, based on the observation in a previous study that patients in clinical remission with IBS-type symptoms had significantly higher FC levels compared to those without (132). However these findings are in contrast to the results described above, in which there was no significant difference between the respective clinical remission groups. The current study was not powered directly towards testing this hypothesis and so the analysis may be subject to a type II statistical error, however further information regarding the distribution of FC values can be gained from inspection of the box plot in Figure 3.2. This demonstrates that there is a higher proportion of patients with IBS-type symptoms who have mildly elevated FC levels (100 - 200μg/g), and it is feasible that this low level inflammation may account for the symptoms experienced in some patients. Nevertheless, the observation that IBS-type symptoms occurred in 31% of the 48 patients with very low FC levels (<90μg/g) suggests that sub-clinical inflammation does not account for a substantial number of cases in the cohort studied.

Interestingly, the duration and extent of disease were not associated with IBS-type symptoms; a finding that is shared by other surveys (132, 134, 151). This appears to counter the theory that functional symptoms result from chronic inflammation modulating the intestinal physiology. In this scenario it would be expected that more

extensive inflammation, occurring over a longer period of time would yield increased IBS-type symptoms.

It has previously been observed that IBS-type symptoms occur more commonly in female IBD patients (prevalence range 43-58%) compared to males (25-45%), but the results of the current study are the first in which this difference has been statistically significant (132, 133, 151). The 45% prevalence found in female patients is similar to earlier reports but the 13% prevalence in males is much lower. This was the case in both CD (6%) and UC (17%). The positive association between concurrent IBS-type symptoms and mood disorders identified in IBD patients has been replicated in several other research papers (132, 134, 135, 151). This alludes to the fundamental role of the brain-gut-axis in producing these symptoms in IBD patients. The higher prevalence of IBS-type symptoms observed in IBD patients compared to the general population may partly be due to the increased anxiety levels that are recognised in this patient group (59, 60).

There has been concern that IBS-type symptoms may influence the clinical assessment of disease activity, with patients exhibiting a high burden of functional symptoms appearing to have active disease when they are actually in remission (53, 147). The results of this study show that the frequency of a correct clinical diagnosis of remission was slightly higher in those patients without IBS symptoms (86% versus 67%), however the overall level of agreement between clinical and biochemical assessments were very similar in patients with IBS-type symptoms compared to those without.

Faecal calprotectin has been shown to perform well in distinguishing active from inactive disease in both UC and CD, and to correlate with the endoscopic assessment of disease activity (37, 55, 152-154). Yet uncertainty remains as to the optimum FC cut-off value that should be used to determine remission in IBD. The level of 90μg/g was applied in this study as it is the value used to screen young adults with functional symptoms and has a high sensitivity for excluding inflammation in this situation. The relationship between this biochemical definition of remission and the clinical assessment equated to a less than moderate level of agreement as measured by the kappa statistic (155). This limited correlation between symptom based activity indices and actual mucosal inflammation has been highlighted previously, especially in CD, and emphasizes the importance of also using objective markers of inflammation such as FC to improve clinical judgement (37, 55, 152).

The generalisation of the results of this study may be limited by several factors. It is a single centre study and 16% of the potentially eligible patients declined to participate. In addition, 36% of the 169 patients included in the study did not provide a stool sample for FC analysis. Although faecal calprotectin is an established diagnostic tool, the gold standard for assessing IBD activity is ileo-colonoscopy with biopsy specimens and this was not performed in patients at the time of the study to confirm disease activity. Lastly, complications of IBD including bile acid malabsorption (22), lactose intolerance, and small bowel bacterial overgrowth (SBBO) were not excluded, and it is recognised that these conditions may lead to symptoms similar to IBS (58, 156). BAM and SBBO are more common following small bowel resection and could potentially account for the higher rate of IBS-type

symptoms found in patients with a history of previous surgery (47% v 22%, p=0.09). Indeed, over 80% of the patients with a prior history of surgery had right hemicolectomy performed, thereby predisposing to BAM.

In summary, the results of this study have shown that IBD patients with IBS-type symptoms share similar characteristics to people diagnosed with IBS in the general community, thereby suggesting that these conditions may not be mutually exclusive and might co-exist in a considerable number of IBD patients. Sub-clinical inflammation may play a role in a proportion of cases, and it is likely that other conditions such as BAM and SBBO will also contribute. This multifactorial nature may account for the apparent increased prevalence of IBS-type symptoms in IBD patients compared to that seen in the normal western population. The results highlight the substantial number of patients that experience IBS-type symptoms despite having normal calprotectin levels. Clinician's need to be aware that healing inflammation is not necessarily the end-point in therapy, and that further management of symptoms may be required.

Recognising IBS in these circumstances may help to direct interventions more appropriately. Dietary adjustments, antispasmodics, antidepressants, and psychotherapy have all been shown to be effective in treating IBS (75, 81, 121). The effectiveness of these strategies in unselected IBD patients has been limited but their therapeutic efficacy may be increased if suitable target populations are identified (157, 158). Dietary modifications and psychotherapy have already shown promise in improving specific symptoms such as fatigue and abdominal pain (159,

160). Randomised controlled trials are required to determine whether those therapies that are effective in treating IBS are also useful in the management of IBS-type symptoms in patients with IBD.

Additional research is also needed to evaluate the contribution of non-inflammatory factors such as SBBO, BAM and lactose intolerance in causing IBS-type symptoms. Until the development of objective biomarkers that enable clinicians to positively diagnose IBS, this will remain a complex scenario to assess and patients will require a systematic diagnostic approach.

Chapter 4

Albumin catalysed coelenterazine chemiluminescence as a biomarker of IBS

4.1 Introduction

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (161). They are an essential aspect of current medical practice; providing information on aetiology, acting as a diagnostic tool, predicting prognosis, and monitoring the efficacy of a treatment. For clinical use a biomarker needs to demonstrate high sensitivity and specificity, and should ideally be inexpensive and non-invasive. Examples of commonly used biomarkers include blood glucose concentration in diabetes mellitus, arterial blood pressure in cardiovascular disease, and prostate-specific antigen in prostate cancer.

In current clinical practice there are no biomarkers available to assess patients with IBS. Diagnosis relies on the evaluation of clinical symptoms and the exclusion of other gastrointestinal disorders. The development of a biomarker in this setting would clearly be a useful tool for clinicians, enabling a positive diagnosis to be made and directing therapy more effectively.

An aspect of IBS, that has to date only received minimal consideration, is its association with a range of non-gastrointestinal symptoms (162-164). These are referred to as the 'non-colonic' or 'systemic' symptoms of IBS and are more prevalent in IBS patients than in healthy controls (Table 4.1) (162). They are varied in their nature and include headaches, urinary symptoms, cognitive dysfunction and muscular aches. The cause of these diverse symptoms has been debated. Some suggest they result from a hypervigilant state of mind, however others propose that

a molecular mechanism underlies both the colonic and systemic symptoms of IBS (164). This latter theory is considered to relate to the absorption of toxic metabolites produced by bacterial fermentation of dietary carbohydrates in the colon. Certainly a similar range of non-colonic symptoms have been reported to occur in association with lactose intolerance, a limited form of carbohydrate malabsorption (165). If this mechanism is confirmed, it may represent an important opportunity to develop a biomarker for IBS.

Table 4.1 Non-colonic symptoms in IBS patients and controls (162).

Non-colonic Symptoms	Prevalence in IBS (%)	Prevalence in Control (%)	p-value
Back Pain	68	28	<0.001
Constant Tiredness	70	20	<0.001
Bad Breath	65	16	<0.001
Frequent Headaches	34	3	<0.001
Urinary Urgency	41	9	<0.001
Nocturia	48	17	<0.001
Incomplete Voiding	50	18	<0.001
Dyspareunia	41	5	<0.001

Dietary carbohydrates that are not digested and absorbed in the small intestine enter the colon. In the anaerobic conditions present, bacterial fermentation occurs and a range of metabolites are produced. Predominantly these consist of short chain

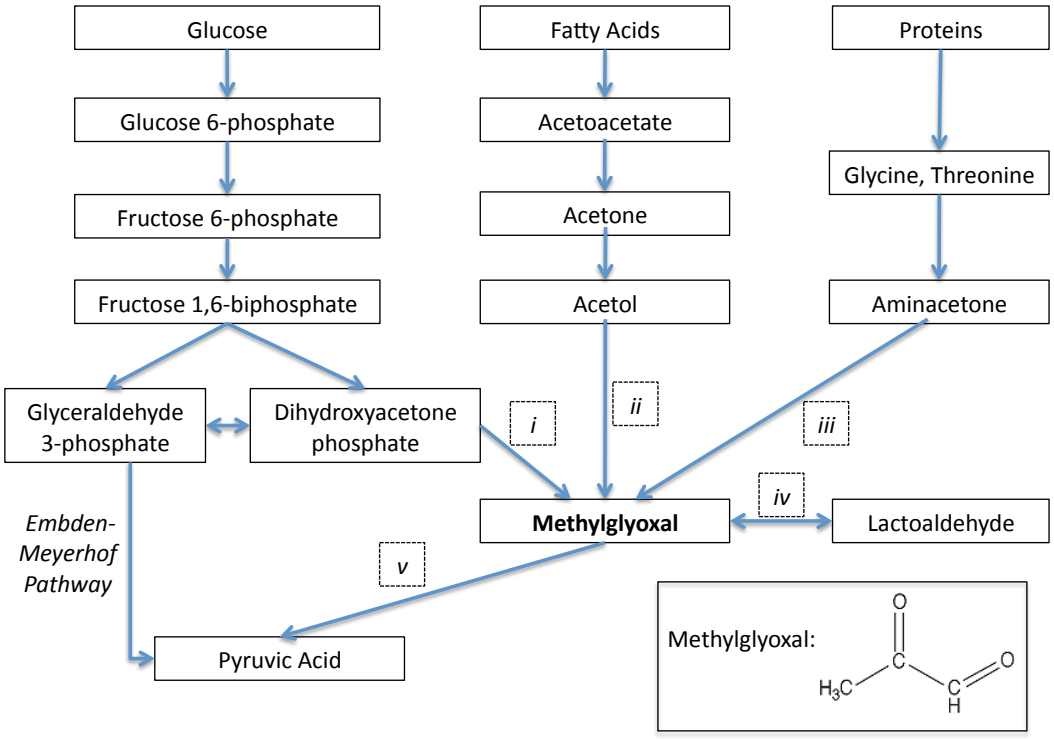
fatty acids (acetate, propionate, butyrate) that provide nutrition to the colonic epithelium and an energy supply to the rest of the body. Other fermentation products include ethanol, lactate, succinate and gases in the form of hydrogen, methane, and carbon dioxide. However the fermentation process also produces highly reactive electrophilic compounds that could potentially have toxic adverse effects (166, 167).

One such compound is methylglyoxal. This is an α-oxoaldehyde, predominantly formed from intermediates of glycolysis, but also in lesser quantities from the metabolism of fatty acids and protein (Figure 4.1) (168, 169). Only a very small amount exists in its free form in plasma, with the majority (as much as 99%) bound to plasma proteins such as albumin (170). A covalent bond is formed between the carbonyl group on methylglyoxal and free amino acid residues on the protein. This glycation process is one of several that leads to advanced glycation endproducts which have been implicated in the pathogenesis of diabetic complications (171).

Methylglyoxal is of interest as it has been shown to have several toxic effects on eukaryotic cells; inhibiting cell growth and affecting the activity of ion channels. Through the formation of covalent bonds it can modify albumin, insulin, serotonin and adrenaline, with potential sequelae on their biological activity (164). Other hormones and neurotransmitters yet to be investigated may be similarly affected. Methylglyoxal is one of several highly reactive metabolites that could potentially cause the variety of symptoms observed in patients with IBS. It is therefore hypothesised that metabolites from colonic bacterial fermentation of dietary

carbohydrates are absorbed into the bloodstream, and play an important role in causing the symptoms of IBS – providing a molecular mechanism for both the abdominal and the non-colonic features.

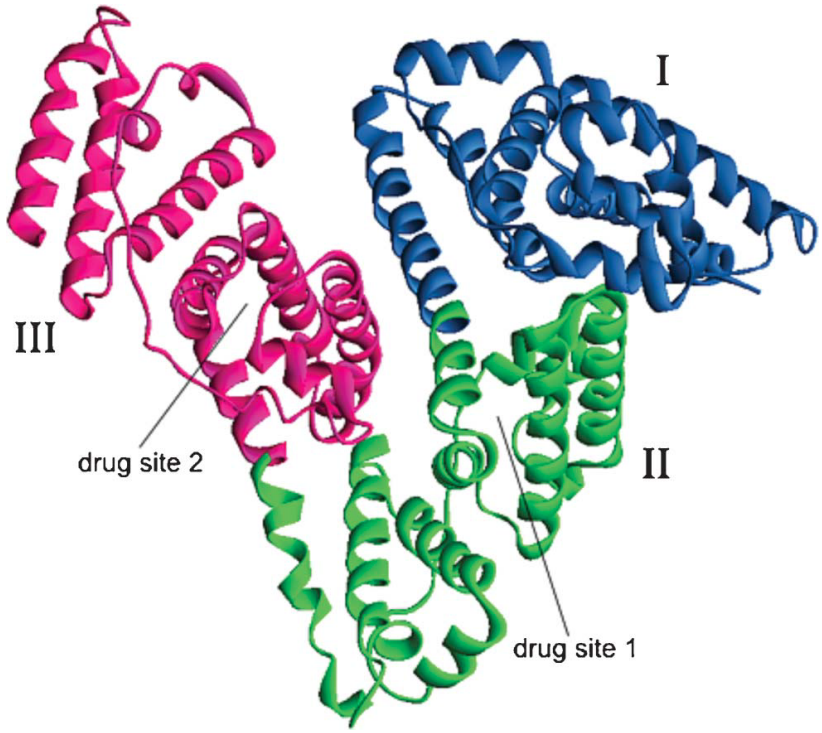
Figure 4.1 Pathways of methylglyoxal metabolism. Enzymes involved in the reactions: (i) methylglyoxal synthase; (ii) acetol monooxygenase; (iii) amine oxidase; (iv) methylglyoxal reductase (v) α-oxoaldehyde dehydrogenase. Adapted from Kalapos, Toxicology Letters, 1999 (168).



Further investigation of this mechanism and of its potential use as a biomarker in IBS utilises the property of these highly reactive compounds to bind with albumin in plasma. Albumin is the most abundant protein in human plasma with a concentration of 35 – 50 g/l. It has a molecular weight of 66kDa and is comprised of three structurally homologous domains (I, II, and III), each of which is formed by two smaller subdomains (A and B). The main functions of albumin are maintenance of

colloid osmotic blood pressure and transportation of multiple ligands including fatty acids, hormones and minerals (172). It has two principal binding sites for ligands, and these have been located on subdomains IIA (drug site 1) and IIIA (drug site 2) as shown in Figure 4.2.

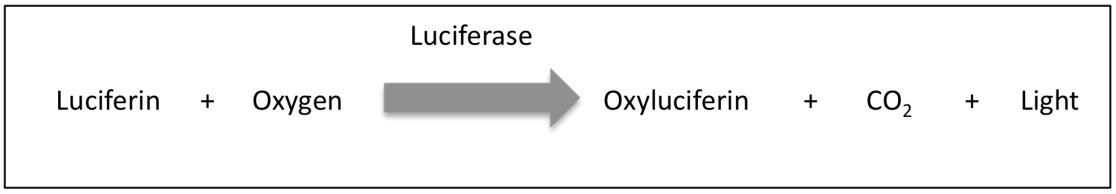
Figure 4.2 Schematic diagram of albumin showing the three domains and two principal binding sites. Taken with permission from Berti, Organic and Biomolecular Chemistry, 2011 (175).



It has recently been demonstrated that albumin exhibits enzymatic activity in a type of reaction known as chemiluminescence (173). This term describes a chemical reaction that results in the emission of light. It requires a luciferin (a compound that when oxidised produces light) and a luciferase (an enzyme that increases the rate at which the luciferin is oxidised) (174). Once this occurs an electron in an excited state

is produced, and it is when the electron decays to a ground state that energy is produced in the form of light (Figure 4.3).

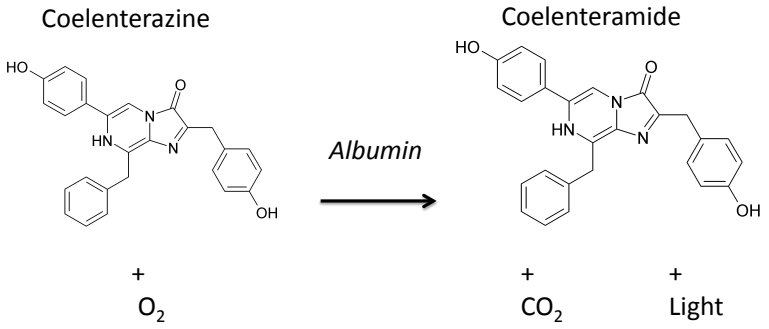
Figure 4.3 The chemiluminescence reaction



An example of a luciferin is coelenterazine. This naturally occurring compound is responsible for the chemiluminescence reactions that occur in many aquatic organisms (176). Early experiments using coelenterazine required it to be extracted from these marine animals, but it has since been synthetically manufactured and is now available commercially. Several luciferases have been identified in the luminescence of coelenterazine including albumin (Figure 4.4) (173, 177).

It has been demonstrated that the ability of albumin to catalyse the oxidation of coelenterazine is influenced by methylglyoxal, with an approximate 50% reduction in chemiluminescence (173). This suggests that the binding of methylglyoxal to albumin causes a functional alteration that affects the chemiluminescence enzymatic site. This is in keeping with a previous report that used quantitative mass spectrometry to identify several sites at which methylglyoxal binds to albumin, together with their relative affinity. The highest affinity was located at binding site 1, and a functional alteration was confirmed with a reduction in the modified albumin's affinity for warfarin (178).

Figure 4.4 Coelenterazine chemiluminescence catalysed by albumin



Therefore it is proposed that methylglyoxal and other toxic metabolites produced by bacterial fermentation in the colon are involved in the pathophysiology of IBS. This will be investigated by measuring albumin catalysed coelenterazine chemiluminescence. If the levels of these highly reactive metabolites are raised in patients with IBS, it would be expected that their covalent modification of plasma albumin will affect its activity as a luciferase. This will lead to a reduction in the amount of light emitted by coelenterazine chemiluminescence, and in this way may act as a biomarker for IBS.

Hypothesis: Methylglyoxal and other toxic metabolites produced by bacterial fermentation in the colon are involved in the pathophysiology of IBS. Higher levels of these compounds will be present in patients with IBS and will cause covalent modification of plasma albumin. This will lead to a reduction in the coelenterazine chemiluminescence of plasma samples from patients with IBS and thereby act as a biomarker for the condition.

4.2 Methods

Materials:

The coelenterazine used in this study was a gift from Bruce Bryan (Prolume Ltd., Pinetop, AZ, USA). The other reagents were obtained from either Sigma Corp (Sigma-Aldrich Company Ltd., England) or Fisher Scientific UK.

Buffer:

50mM HEPES solution with pH 7.4 was used as a buffer in all of the experiments. 50ml of 50mM HEPES solution was made by adding 49ml of distilled water to 0.59575g of HEPES (mr=238.30). Using a pipette 0.1mM NaOH was added to the HEPES solution to adjust the pH to 7.4. The solution was stored in the refrigerator.

Coelenterazine:

20nM aliquots of coelenterazine were stored in vials in a freezer at -20°C. At the start of an experiment a 20nM coelenterazine aliquot was dissolved using 100µl of methanol and 100µl of buffer solution, thereby giving a 100µM concentration. During the experiment this solution was stored in an airtight container that was placed on ice and wrapped in foil to shade it from light.

Clinical Samples:

IBS patients, IBD patients and healthy volunteers who gave informed consent for phlebotomy had two 4ml blood samples collected in EDTA vacutainers. Samples were centrifuged and the plasma was pipetted into a separate 5ml tube. These were

stored in a freezer at -40°C. At a later date the plasma samples were thawed and aliquotted into 200µl samples before re-freezing at -40°C. At the start of the experiment the selected plasma samples were removed from the freezer and thawed.

Chemiluminometer:

Chemiluminescence was quantified digitally using a custom-built chemiluminometer. Uniform laboratory conditions with a room temperature of 20°C and low-level lighting were used throughout the experiments to ensure consistency. A 'machine background' chemiluminescence reading was recorded at the start of each experiment.

Experiments:

All experiments were performed in the school of pharmacy and pharmaceutical sciences at Cardiff University. The initial experiments were performed to identify the optimal conditions for measuring albumin catalysed coelenterazine chemiluminescence, thus preparing the assay for future analysis of clinical samples.

1. Preparing the assay: Identifying optimal machine temperature

Aim: To identify the optimal machine temperature for reducing background noise.

Method: Chemiluminescence readings were checked for background noise (dark count with casing closed) and signal (light count with casing open) at 5°C temperature intervals from 20°C (room temperature) to -20°C. The signal to noise ratio was calculated for each temperature interval.

2. Preparing the assay: Determining appropriate time intervals for measuring human serum albumin (HSA) catalysed coelenterazine chemiluminescence

Aim: To determine the time period over which measurements of chemiluminescence should be taken.

Method: Chemiluminescence measurements were taken over a 5-minute period to determine if light emission remained stable over time.

i). 10µl of HSA (10% w/v) was added to 90µl of buffer solution containing 10µM coelenterazine.

ii). Chemiluminescence counts were taken every 10 seconds for 5 minutes.

3. Preparing the assay: Dose response for coelenterazine in HSA catalysed coelenterazine chemiluminescence

Aim: To determine the effect of coelenterazine concentration on chemiluminescence

Method:

i). 10µM and 100µM coelenterazine solutions were prepared using methanol and 50mM HEPES buffer.

ii). Chemiluminescence was measured for four different final concentrations of coelenterazine. The coelenterazine concentrations and the solution contents are outlined in the Table 4.2.

Table 4.2 Composition of the final solutions used to test chemiluminescence for the four different coelenterazine concentrations.

Final concentrations of coelenterazine (µM)	HEPES buffer volume, (µl)	10% HSA solution volume, (µl)	Coelenterazine solution volume, (µl)
1	80	10	10 (10µM)
5	40	10	50 (10µM)
10	80	10	10 (100µM)
20	70	10	20 (100 µM)

4. Preparing the assay: Dose response of HSA and bovine serum albumin (BSA) in catalysing coelenterazine chemiluminescence

Aim: To determine the effect of HSA and BSA concentration on coelenterazine chemiluminescence

Method:

i). 100mg of HSA was dissolved in 1ml of distilled water to prepare a 10% solution. Samples of this solution were used to prepare 1% and 0.1% solutions by dissolving with 50mM HEPES buffer. The same method was used to prepare corresponding solutions of BSA.

ii). Chemiluminescence measurements were taken for five different final concentrations of HSA and BSA. The concentrations and their contents are outlined in the Table 4.3.

Table 4.3 Composition of the final solutions used to test chemiluminescence for the five different HSA and BSA concentrations.

Final concentrations of HSA and BSA (w/v)	HEPES buffer volume, (µl)	100 µM coelenterazine volume, (µl)	HSA or BSA solution volume, (µl)
1%	80	10	10 (10% w/v)
0.5%	40	10	50 (1% w/v)
0.1%	80	10	10 (1% w/v)
0.05%	40	10	50 (0.1% w/v)
0.01%	80	10	10 (0.1% w/v)

5. Identification of coelenterazine's binding site on albumin

Aim: To identify the site on albumin that coelenterazine uses to bind

The specific albumin binding sites of several drugs have been identified and are shown in Table 4.4. Certain medications will only bind to one location, for example warfarin at subdomain IIA and ibuprofen at subdomain IIIA, whereas others such as aspirin show nearly equal distribution between the two binding sites (172). This information can be used to identify the albumin binding site of other ligands such as coelenterazine. The addition of a drug that uses the same binding site as coelenterazine would be expected to reduce the chemiluminescence of an albumin + coelenterazine solution as there will be competition for the enzymatic site.

Table 4.4 Ligand binding locations to HSA for different medications (172).

Ligand	Subdomain location of binding site
Aspirin	IIA, IIIA
Warfarin	IIA
Diazepam	IIIA
Digitoxin	IIIA
Clofibrate	IIIA
Ibuprofen	IIIA

Method:

i). The chemiluminescent count of 1% w/v HSA, 10µM coelenterazine solution was recorded.

ii). 10µl of 10mM warfarin was added to 90µl of 1% w/v HSA, 10µM coelenterazine solution and the chemiluminescent count was recorded.

iii). 10µl of 10mM ibuprofen was added to 90µl of 1% w/v HSA, 10µM coelenterazine solution and the chemiluminescent count was recorded.

6. Analysis of clinical samples from IBS patients, IBD patients, and healthy volunteers

Aim: To examine the coelenterazine chemiluminescence of clinical samples

Method:

i). Plasma samples were classified into the following categories:

- Healthy volunteers
- IBS patients
- IBD patients with active disease
- IBD patients in remission without IBS-type symptoms
- IBD patients in remission with IBS-type symptoms

(Note that in this section IBD activity is determined by faecal calprotectin level such that those with FC<90µg/g are defined as in remission and those with FC>90µg/g are defined as active).

ii). No blood samples from diabetic patients were included in the analysis as it has been reported that plasma methylglyoxal levels are higher in diabetic patients (169).

iii). Plasma samples were analysed in sets of five. Every set contained a sample from each clinical category (although later sets were limited by the number of samples in the IBD categories).

iv). As part of every set a HSA sample (0.4% w/v final concentration) was also analysed in order to act as a control sample for that set.

v). 10µl of 100µM coelenterazine solution was added to 80µl of 50mM HEPES buffer.

vi). 10µl of the plasma sample was added to the coelenterazine/buffer solution and a chemiluminescence measurement was recorded.

vii). Within every set the process was repeated in reverse order for the plasma and HSA samples using a separate 10µl specimen. Consequently, two distinct chemiluminescence measurements were recorded for each sample and a mean result calculated.

viii). For each set of samples the HSA control result was compared to that performed in the initial set and a ratio calculated. Using this ratio the results of the plasma samples were adjusted so that inter-set variation in conditions could be controlled for.

iv). The chemiluminescence results of samples from healthy volunteers, IBS patients and all IBD patients were compared using ANCOVA with serum albumin level as a covariate.

7. Analysis of non-colonic symptoms in healthy volunteers, IBS and IBD patients

Aim: To examine the occurrence of non-colonic symptoms in patients with IBS and IBD, and to explore if patients with these symptoms represent a specific sub-group of IBS patients for which coelenterazine chemiluminescence may act as a biomarker.

It has been established that non-colonic symptoms are more prevalent in patients with IBS compared to the normal population. Considering that IBS may be regarded as a heterogeneous condition in which separate pathologies are occurring to create similar symptoms, it is possible that the presence of non-colonic symptoms may represent a sub-division of IBS patients in which a systemic pathology is occurring. To explore this hypothesis in the context of carbohydrate metabolites a further analysis of non-colonic symptoms and their relationship with chemiluminescence results was performed.

Methods:

a. Non-colonic symptoms in healthy volunteers, IBS patients and IBD patients:

i). Healthy volunteers, IBS patients and IBD patients completed a questionnaire that documented whether they regularly (every 1-2 weeks) experienced a range of non-colonic symptoms (Appendix 7). The questionnaire was based on those non-colonic symptoms that have been reported to be associated with IBS and lactose intolerance (162, 165).

ii). Non-colonic symptoms that occurred significantly more common in IBS patients compared to healthy volunteers were identified.

iii). IBS patients were sub-divided according to whether they reported 3 or more of the commonly experienced non-colonic symptoms.

iv). Analysis of the plasma chemiluminescence results was performed using the new sub-division of IBS patients.

b. The relationship of individual non-colonic symptoms with coelenterazine chemiluminescence:

i). For each non-colonic symptom the plasma chemiluminescence results of those participants who reported experiencing the symptom were compared to those who did not. This comparison was performed within respective participant categories rather than grouping all participants together, (for example the chemiluminescence result of healthy volunteers that reported headaches was compared to the result of those healthy volunteers that did not report headaches).

ii). Those non-colonic symptoms which were associated with a significant reduction in coelenterazine chemiluminescence were identified.

iii). IBS patients were sub-divided according to whether they reported these symptoms.

iv). Analysis of the plasma chemiluminescence results was performed using this new sub-division of IBS patients.

c. Non-colonic symptoms in IBD patients who are in remission:

i). IBD patients in remission were divided into those with IBS-type symptoms and those without.

ii). The prevalence of non-colonic symptoms was compared between these two groups.

d. The relationship between presence of non-colonic symptoms and anxiety levels:

i). Healthy volunteers, IBS patients and IBD patients all completed the hospital anxiety and depression scale as detailed in the overall thesis methods (Section 2) (144).

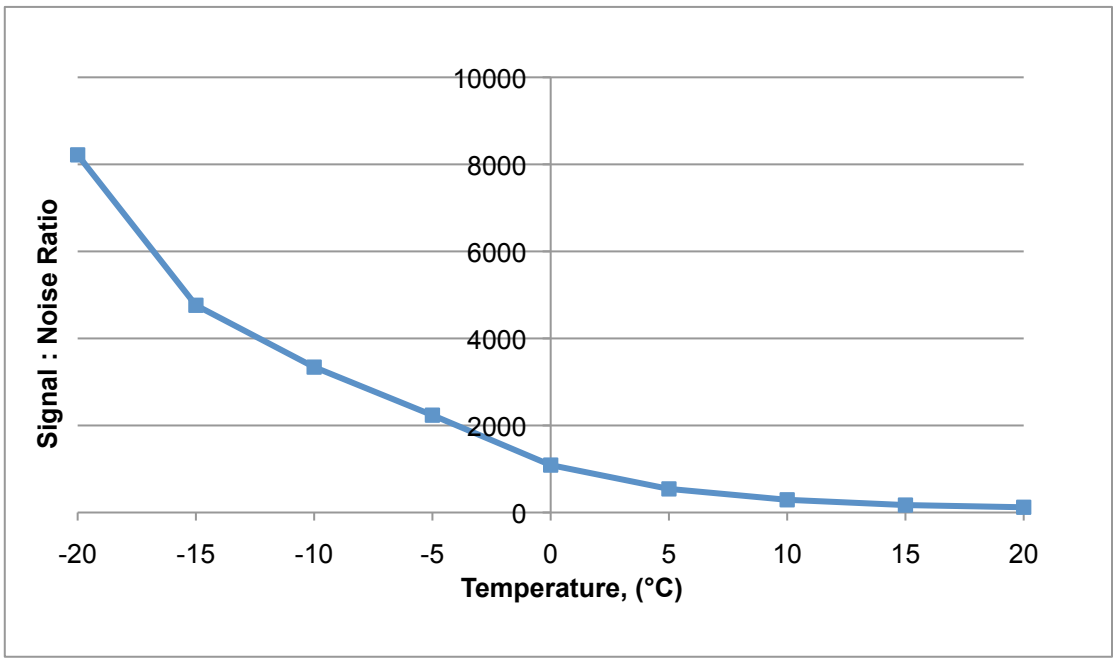
ii). For each non-colonic symptom the anxiety score of those participants who reported experiencing the symptom was compared to those who did not. This comparison was performed within respective participant categories.

4.3 Results:

1. Preparing the assay: Identifying optimal machine temperature

The signal to noise ratio increased with a reduction in temperature of the chemiluminometer (Figure 4.5). Consequently, all experiments were performed with the machine temperature set at -20°C.

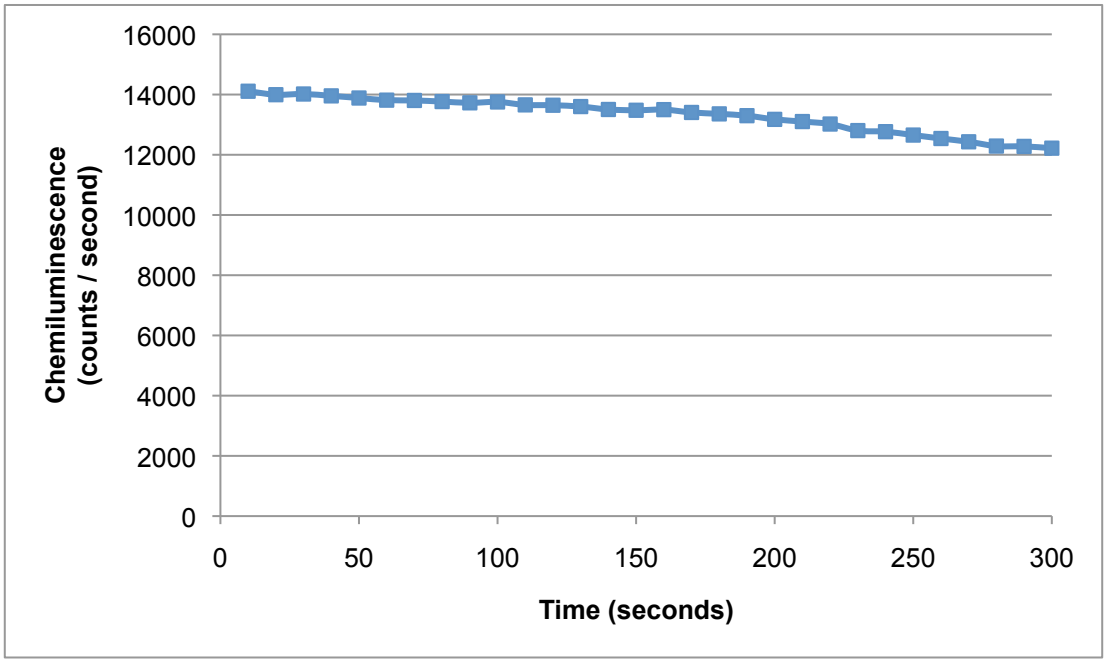
Figure 4.5 The signal to noise ratio for chemiluminescence at 5°C temperature intervals from 20°C to -20°C.



2. Preparing the assay: Determining appropriate time intervals for measuring HSA catalysed coelenterazine chemiluminescence

The HSA catalysed chemiluminescence count gradually decreased throughout the 5 minute time period (Figure 4.6). At 60 seconds it had decreased by 2.1%, and at 300 seconds it had fallen by 13.4%. As the decrease at 60 seconds was less than 5% this was deemed acceptable. In further experiments the mean of the first 6 x 10 second counts was used.

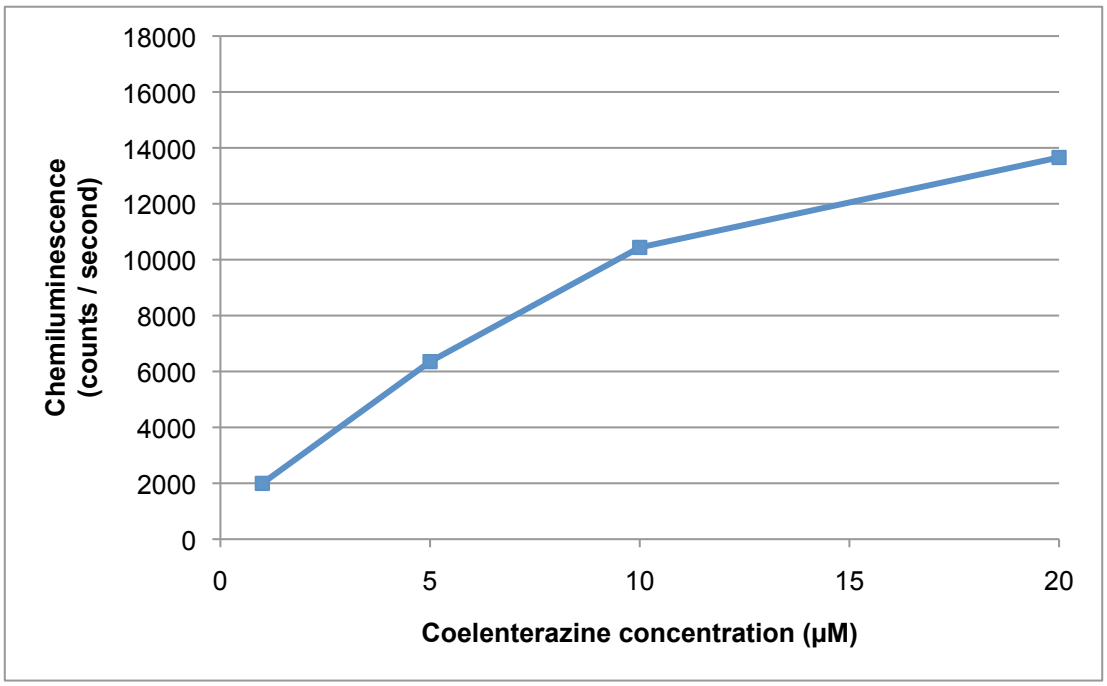
Figure 4.6 HSA catalysed chemiluminescence measured over a 5 minute time period.



3. Preparing the assay: Dose response for coelenterazine in HSA catalysed coelenterazine chemiluminescence

The chemiluminescence count significantly increased with the concentration of coelenterazine. For 20μM coelenterazine there was an eight-fold increase in light emission compared to 1μM coelenterazine, $p<0.01$ (Figure 4.7).

Figure 4.7 Dose-response curve for coelenterazine in HSA catalysed coelenterazine chemiluminescence.

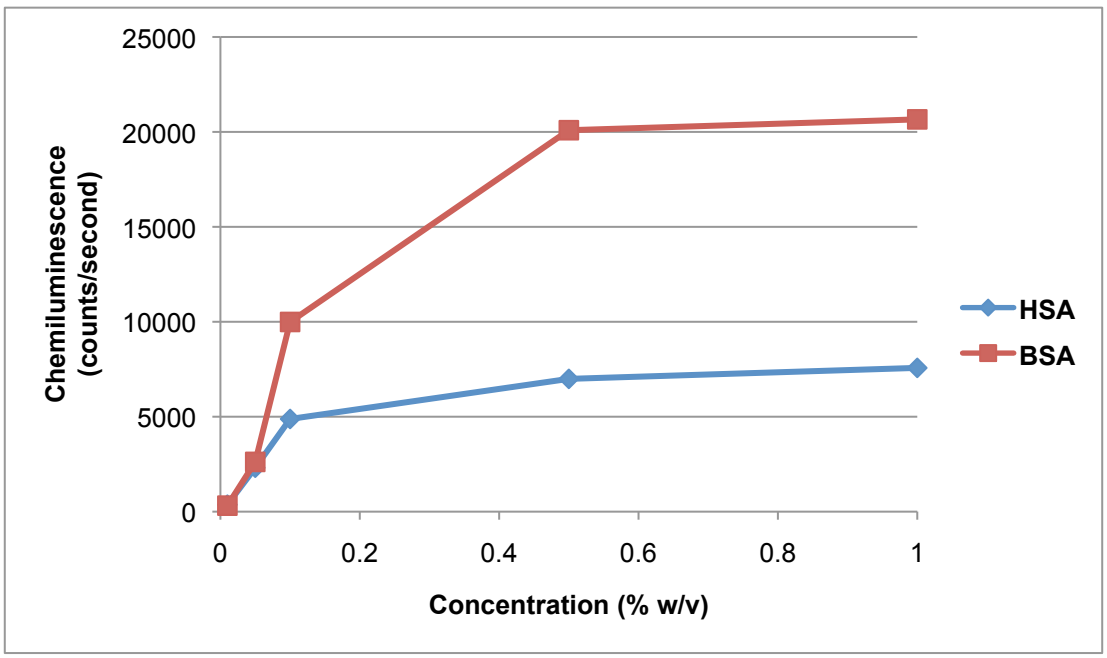


4. Preparing the assay: Dose response of human serum albumin and bovine serum albumin in catalysing coelenterazine chemiluminescence

Concentration of HSA and BSA were observed to have a significant effect on chemiluminescence count (Figure 4.8). Light emission for 1% w/v HSA was 7572c/s which was significantly higher than 367c/s for 0.01% w/v HSA, p=0.02. This indicated that albumin concentration would need to be included as a covariate in the analysis of the clinical samples.

Chemiluminescence count was significantly higher for BSA compared to HSA. At concentrations of 1% w/v the mean BSA count was 20660c/s compared to HSA 7572c/s, p<0.01.

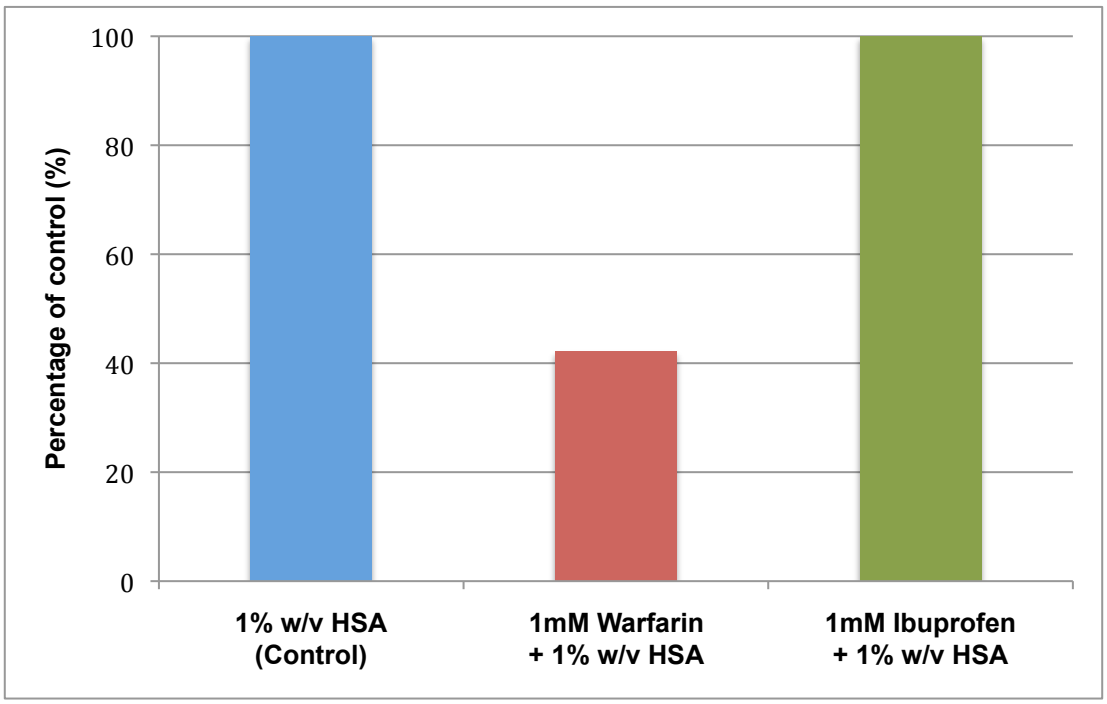
Figure 4.8 Dose-response curve for HSA and BSA in catalysing coelenterazine chemiluminescence.



5. Identification of coelenterazine's binding site on albumin

The addition of warfarin to the albumin + coelenterazine solution reduced the chemiluminescence count by 57.9%, whereas the addition of ibuprofen did not cause any significant change (Figure 4.9). These results show that warfarin competes with coelenterazine for the same binding site, therefore indicating that coelenterazine uses binding site 1 on albumin.

Figure 4.9 Changes in chemiluminescence caused by the addition of warfarin and ibuprofen to an albumin/coelenterazine solution.



6. Analysis of clinical samples from IBS patients, IBD patients, and healthy controls

A total of 81 clinical samples were collected and analysed. This comprised 20 healthy volunteers, 20 IBS patients, 17 IBD patients with active disease, 17 IBD patients in remission without IBS-type symptoms, and 7 IBD patients in remission with IBS-type symptoms. Characteristics of the respective participant groups are shown in Table 4.5. There were no significant differences in age or gender between the groups, but IBD patients with active disease had a significantly lower serum albumin level.

Initially the chemiluminescence results of samples from healthy volunteers, IBS patients and all IBD patients were compared using ANCOVA with serum albumin level as a covariate (Table 4.6). There was no significant difference observed between these clinical groups, ($F=0.650$, $df=80$, $p=0.525$).

The results were then analysed with IBD patients split into their respective categories (Table 4.7). ANCOVA demonstrated a significant difference between the groups ($F=3.409$, $df=80$, $p=0.013$). Post-hoc analysis identified that the chemiluminescence result of IBD patients in remission with IBS-type symptoms (19015c/s) were significantly lower than IBD patients with active disease (23907c/s), $p=0.017$. However there were no significant differences between any of the other group comparisons (Table 4.8).

Table 4.5 Demographic details, albumin levels, and IBS-subtypes in those patients that clinical samples were obtained from.

	Healthy (n=20)	IBS (n=20)	IBD Active (n=17)	IBD Remission without IBS (n=17)	IBD Remission with IBS (n=7)	p value
Age, years (mean, s.d.)	45 (11)	41 (13)	49 (11)	45 (10)	42 (10)	0.310
Sex Male Female	8 (40%) 12 (60%)	6 (30%) 14 (70%)	5 (29%) 12 (71%)	6 (35%) 11 (65%)	0 (0%) 7 (100%)	0.394
Smoker Yes No	2 (10%) 18 (90%)	5 (25%) 15 (75%)	2 (12%) 15 (88%)	3 (18%) 14 (82%)	1 (14%) 6 (86%)	N/A
Alcohol > 28 units/week 1-28 units/week Nil	 1 (5%) 14 (70%) 5 (25%)	 1 (5%) 11 (55%) 8 (40%)	 2 (12%) 9 (53%) 6 (35%)	 4 (24%) 13 (77%) 0 (0%)	 0 (0%) 5 (71%) 2 (29%)	N/A
Serum Albumin (g/l) (mean, s.d.)	39 (4)	40 (3)	37 (2)	40 (2)	39 (2)	0.008*
IBS-Subtypes IBS-D IBS-C IBS-M IBS-U	 N/A	 11 (55%) 1 (5%) 8 (40%) 0 (0%)	N/A	N/A	 2 (29%) 0 (0%) 5 (71%) 0 (0%)	N/A

* Indicates statistically significant difference with $p<0.05$

IBS-D = Diarrhoea-predominant IBS; IBS-C = Constipation-predominant IBS;
IBS-M = Mixed IBS; IBS-U = Unsubtyped IBS

Table 4.6 Chemiluminescence results (counts/second) for healthy volunteers, IBS patients, and total IBD patients.

Group	Mean (s.d.)	95% confidence interval	Minimum	Maximum
Healthy (n=20)	21136 (3293)	(19595, 22677)	16486	27895
IBS (n=20)	21933 (3062)	(20500, 23366)	14791	27129
IBD (n=41)	22254 (3956)	(21005, 23503)	15469	32018

Figure 4.10 Box plot of the chemiluminescence results for healthy volunteers, IBS patients and total IBD patients.

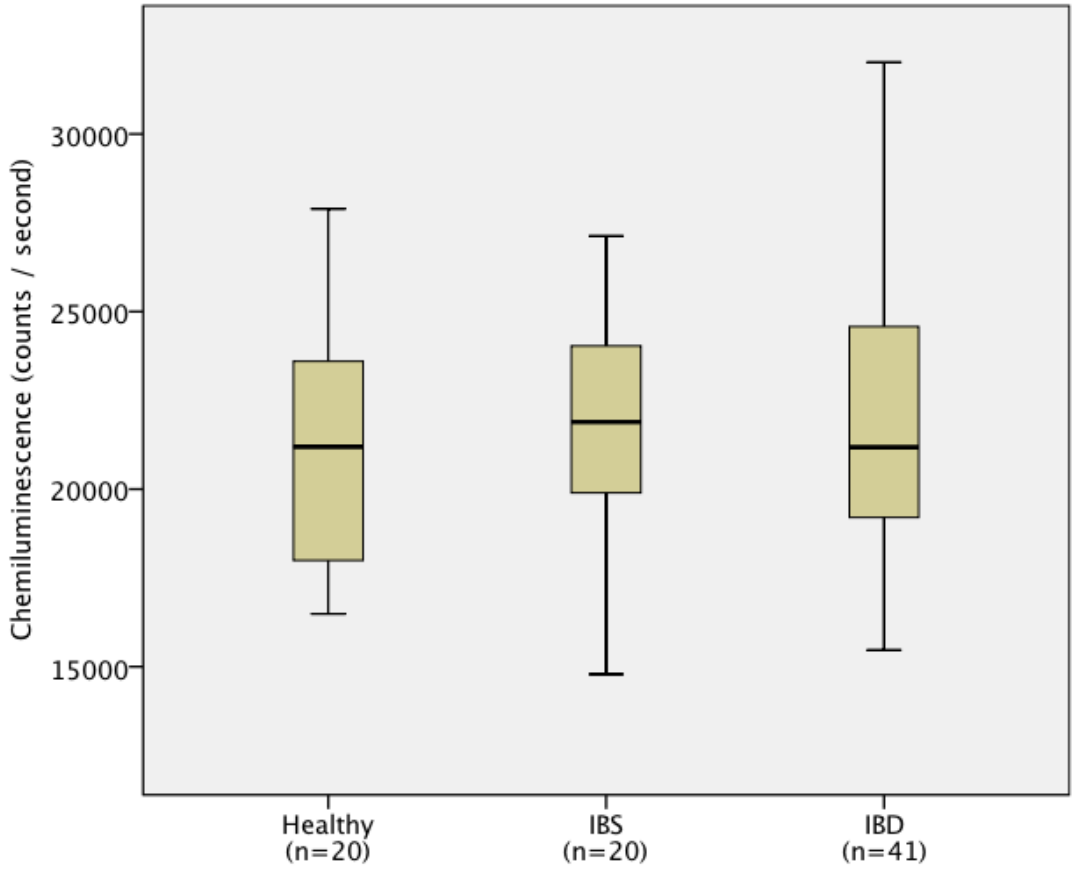


Table 4.7 Chemiluminescence results (counts/second) for healthy volunteers, IBS patients, and the sub-divisions of IBD patients.

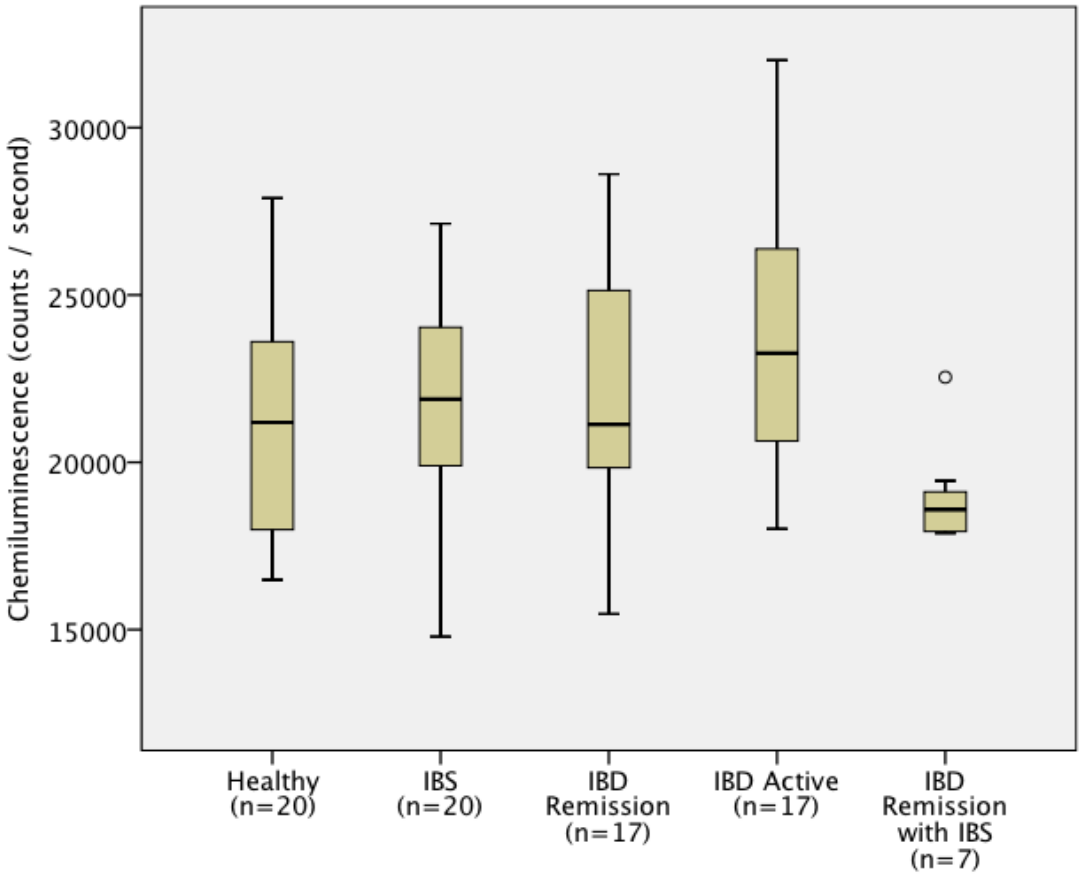
Group	Mean (s.d.)	95% confidence intervals	Minimum	Maximum
Healthy (n=20)	21136 (3293)	(19595, 22677)	16486	27895
IBS (n=20)	21933 (3062)	(20500, 23366)	14791	27129
IBD active (n=17)	23907 (4440)	(21624, 26189)	18013	32018
IBD remission without IBS-type symptoms (n=17)	21935 (3283)	(20247, 23623)	15469	28613
IBD remission with IBS-type symptoms (n=7)	19015 (1658)	(17481, 20549)	17878	22542

Table 4.8 Post-hoc analysis of the chemiluminescence results.

Groups		Mean difference	p value
Healthy	IBS	-797	0.947
	IBD active	-2771	0.112
	IBD remission without IBS	-799	0.954
	IBD remission with IBS	2121	0.622
IBS	Healthy	797	0.947
	IBD active	-1973	0.411
	IBD remission without IBS	-2	1.000
	IBD remission with IBS	2918	0.304
IBD active	Healthy	2771	0.112
	IBS	1973	0.411
	IBD remission without IBS	1972	0.452
	IBD remission with IBS	4892	0.017*
IBD remission without IBS	Healthy	799	0.954
	IBS	2	1.000
	IBD active	-1972	0.452
	IBD remission with IBS	2920	0.325
IBD remission with IBS	Healthy	-2121	0.622
	IBS	-2918	0.304
	IBD active	-4892	0.017*
	IBD remission without IBS	-2920	0.325

* Indicates statistically significant difference with $p<0.05$

Figure 4.11 Box plot of the chemiluminescence results for healthy volunteers, IBS patients, IBD patients with active disease, IBD patients in remission without IBS-type symptoms, and IBD patients in remission with IBS-type symptoms.



7. Analysis of non-colonic symptoms in healthy volunteers, IBS and IBD patients

a. Non-colonic symptoms in healthy volunteers, IBS patients and IBD patients:

A total of 231 participants completed the questionnaire. This included 41 healthy volunteers, 40 patients with IBS, and 150 patients with IBD. The prevalence of each non-colonic symptom in the respective participant categories is illustrated in Table 4.9. Overall myalgia, fatigue, cognitive impairment, pruritus, palpitations, mouth ulcers, sore throat, sleep disturbance all showed a significant difference in prevalence between groups. All of these, except for mouth ulcers, were more frequently experienced in both IBS and IBD patients compared to healthy volunteers. Mouth ulcers were more common in IBD patients than healthy volunteers, however when IBS patients were compared to healthy volunteers the difference was non-significant ($p=0.432$). There was no significant difference in the prevalence of any non-colonic symptoms when IBS and IBD patients were compared.

Myalgia, fatigue, cognitive impairment, pruritus, and palpitations all had p-values <0.01. IBS patients were sub-divided into those that had 3 or more of these symptoms – "IBS with non-colonic symptoms". The plasma chemiluminescence results were analysed again using this sub-division of IBS patients (Table 4.10). ANCOVA showed no significant difference between the groups ($F=1.147$, $df=3$, $p=0.336$).

Table 4.9 Percentage of participants in each category that reported non-colonic symptoms.

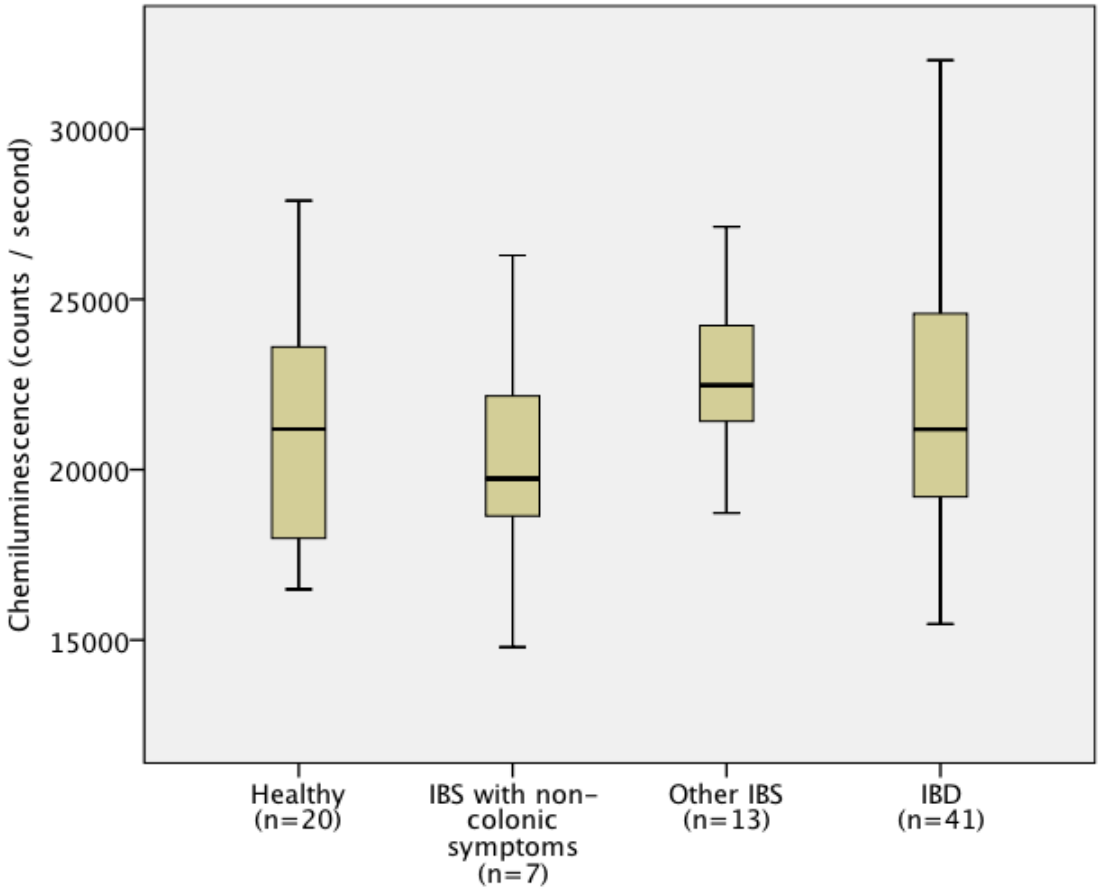
Symptom	Healthy (n=41)	IBS (n=40)	IBD (n=150)	p-value
Headache	29%	48%	37%	0.236
Myalgia	17%	40%*	43%*	0.009*
Fatigue	5%	55%*	54%*	<0.001*
Cognitive Impairment	15%	55%*	49%*	<0.001*
Rhinitis	10%	13%	17%	0.493
Pruritus	5%	35%*	21%*	0.004*
Palpitations	2%	30%*	21%*	0.005*
Mouth Ulcers	5%	10%	19%*	0.044*
Sore Throat	2%	20%*	16%*	0.047*
Sleep Disturbance	27%	48%*	51%*	0.024*

* Indicates a statistically significant difference ($p<0.05$) in the frequency of a non-colonic symptom when compared to the frequency in the healthy population

Table 4.10 Plasma chemiluminescence (counts/second) of clinical groups with new sub-division of IBS patients with 3 or more non-colonic symptoms.

Group	Mean (s.d.)	95% confidence interval	Minimum	Maximum
Healthy (n=20)	21136 (3293)	(19595, 22677)	16486	27895
IBS with non-colonic symptoms (n=7)	20347 (3715)	(16912, 23783)	14791	26290
Other IBS (n=13)	22788 (2385)	(21346, 24228)	18727	27129
IBD (n=41)	22254 (3956)	(21005, 23503)	15469	32018

Figure 4.12 Box plot of the chemiluminescence results for healthy volunteers, IBS patients with non-colonic symptoms, other IBS patients, and IBD patients.



b. The relationship of individual non-colonic symptoms with coelenterazine chemiluminescence:

For IBS patients the chemiluminescence results were significantly lower in those who regularly experienced myalgia (p=0.049) or fatigue (p=0.027) compared to those who did not. In healthy volunteers and IBD patients there were no significant differences present for any of the respective non-colonic symptom comparisons (Table 4.11).

IBS patients were sub-divided into those with myalgia and fatigue, and those without these symptoms. The plasma chemiluminescence results were compared between

groups using this new IBS sub-division. However ANCOVA demonstrated there was no significant difference between groups (F=1.372, df = 3, p=0.258).

Table 4.11 A comparison of the chemiluminescence results for participants with and without each non-colonic symptom.

	Healthy (n=20)		IBS (n=20)		IBD (n=41)	
	Chem Res	p-value	Chem Res	p-value	Chem Res	p-value
Headache						
Yes	20483	0.530	22416	0.451	21856	0.594
No	21487		21344		22536	
Myalgia						
Yes	22027	0.499	20124	0.049*	21949	0.713
No	20839		22908		22430	
Fatigue						
Yes	23520	0.473	20463	0.027*	22221	0.956
No	21010		23404		22292	
Cognitive impairment						
Yes	22423	0.574	21166	0.176	21059	0.058
No	20993		23084		23393	
Rhinitis						
Yes	21884	0.823	22233	0.833	22142	0.909
No	21097		21858		22300	
Pruritus						
Yes	16999	0.206	22326	0.652	21557	0.502
No	21354		21671		22509	
Palpitations						
Yes	N/A	N/A	22121	0.879	22408	0.875
No	21136		21871		22190	
Mouth Ulcers						
Yes	23538	0.289	19145	0.181	21784	0.692
No	20869		22243		22386	
Sore Throat						
Yes	N/A	N/A	19360	0.117	22135	0.944
No	21136		22387		22271	
Sleep Disturbance						
Yes	21309	0.882	21461	0.361	21934	0.619
No	21062		22811		22559	

* Indicates a statistically significant difference ($p<0.05$) in the chemiluminescence count for those patients with a specific non-colonic symptom compared to those without the symptom. (Note comparisons are performed within the respective participant groups and not between groups)

c. Non-colonic symptoms in IBD patients who are in remission:

Non-colonic symptoms were generally observed to occur more frequently in those IBD remission patients with IBS-type symptoms compared to those without (Table 4.12). However the difference was only significant in headache (0.015) and fatigue (p=0.046).

Table 4.12 Prevalence of non-colonic symptoms in IBD remission patients with and without IBS type symptoms.

Symptom	IBS-type Symptoms (n=23)	No IBS-type symptoms (n=34)	p-value
Headache	65%	32%	0.015*
Myalgia	48%	32%	0.239
Fatigue	65%	38%	0.046*
Cognitive Impairment	65%	44%	0.118
Rhinitis	13%	9%	N/A
Pruritus	30%	27%	0.744
Palpitations	17%	12%	N/A
Mouth Ulcers	13%	29%	0.148
Sore Throat	17%	12%	N/A
Sleep Disturbance	57%	35%	0.113

* Indicates statistically significant difference with $p<0.05$

d. The relationship between presence of non-colonic symptoms and anxiety levels:

In patients with IBS and IBD, the presence of each individual non-colonic symptom was associated with a higher anxiety level, although the differences were not always statistically significant (Table 4.13). Overall, healthy volunteers reported lower anxiety levels compared to those with IBS and IBD.

Table 4.13 A comparison of anxiety levels for participants with and without each non-colonic symptom.

	Healthy (n=41)		IBS (n=40)		IBD (n=150)	
	Anxiety	p-value	Anxiety	p-value	Anxiety	p-value
Headache						
Yes	4.2	0.205	12.3	0.011*	9.7	0.032*
No	5.6		9.1		8.2	
Myalgia						
Yes	5.9	0.520	11.9	0.120	10.1	0.001*
No	5.0		9.8		7.7	
Fatigue						
Yes	11.5	0.002*	10.7	0.957	10.5	<0.001*
No	4.8		10.6		6.6	
Cognitive impairment						
Yes	7.7	0.033*	11.7	0.062	10.4	<0.001*
No	4.7		9.3		7.2	
Rhinitis						
Yes	5.0	0.924	11.0	0.840	9.8	0.163
No	5.2		10.6		8.5	
Pruritus						
Yes	4.0	0.605	12.6	0.026*	10.0	0.056
No	5.2		9.6		8.4	
Palpitations						
Yes	8.0	0.367	12.4	0.071	11.2	<0.001*
No	5.1		9.9		8.1	
Mouth Ulcer						
Yes	6.0	0.700	13.3	0.180	9.7	0.171
No	5.1		10.4		8.5	
Sore Throat						
Yes	3.0	0.498	13.0	0.066	11.5	0.001*
No	5.2		10.1		8.2	
Sleep Disturbance						
Yes	5.6	0.554	12.4	0.007*	9.7	0.004*
No	5.0		9.1		7.7	

* Indicates a statistically significant difference ($p<0.05$) in the anxiety score for those patients with a specific non-colonic symptom compared to those without the symptom. (Note comparisons are performed within the respective participant groups and not between groups)

4.4 Discussion:

The results of this study do not support the hypothesis that albumin catalysed coelenterazine chemiluminescence is a useful biomarker in irritable bowel syndrome. There was no significant difference in mean chemiluminescence count when healthy volunteers, IBS patients and IBD patients were compared. On sub-division of the IBD group it was found that patients in remission with IBS-type symptoms had a lower chemiluminescence count than patients with active disease. However the implication of this result is unclear, as neither group was significantly different to healthy volunteers, IBS patients, or those IBD patients in remission without IBS-type symptoms.

The lack of any significant difference in chemiluminescence count between groups does not substantiate the theory that metabolites produced by colonic bacterial fermentation are involved in the pathophysiology of IBS, although several potential confounding factors need to be considered when interpreting these results. Firstly, a dietary history was not recorded. As patients with IBS frequently avoid specific food-products it is possible that carbohydrate intake may have been reduced in this group. Secondly, carbohydrate metabolites may not bind with and structurally alter albumin *in vivo* to such an extent that its ability to catalyse coelenterazine chemiluminescence is sufficiently impaired. Patients' medication could potentially bind to albumin altering its capacity to act as a catalyst. Finally, plasma may contain other substances with enzymatic activity that compete with albumin to act as a catalyst for coelenterazine chemiluminescence.

A limitation of the chemiluminescence assay is the transient nature of the signal. A molecule of the substrate will only produce light once, and as the substrate is used up the signal will diminish. At room temperature coelenterazine is oxidised in the atmosphere, and for this reason it was stored in a sealed container on ice during each experiment. However, as each clinical sample was tested in duplicate, it was noted that the signal produced on the second round of testing was generally lower compared to the initial round, suggesting that some of the substrate may have been oxidised between rounds. Relatively large standard deviations are noted on the mean calculations of the chemiluminescence counts. To minimise this effect the order of sample testing was reversed on the second round, but it is still possible that the results may have been influenced. A further technique to minimise the effect of variations in substrate was the use of a HSA control sample that was performed with each round of clinical samples. Chemiluminescence counts for clinical specimens were adjusted according to the result of the HSA control sample.

Consideration must be given to the fact that IBS may be a heterogeneous condition in which several different pathologies act to cause a similar constellation of symptoms. Sub-categories of IBS are starting to be recognised. An example is post-infectious IBS, in which symptoms occur following an episode of gastroenteritis and appear to be due to persisting low grade inflammation (81). Therefore it is feasible that carbohydrate metabolites may not be a factor in all patients with IBS but may only be a feature in a small proportion.

This factor was considered when deciding to analyse the non-colonic symptoms associated with IBS. The proposed hypothesis that fermentation products may be a causative factor in IBS through covalent modification of hormones such as serotonin, suggests that the symptoms experienced would be systemic rather than being restricted to only the gastrointestinal system. Therefore further analysis of the chemiluminescence results was performed using the sub-group of IBS patients that reported multiple non-colonic symptoms. The mean plasma chemiluminescence result of this sub-group of IBS patients was lower than in other groups, however the difference was not statistically significant.

The association of non-colonic symptoms with carbohydrate intolerance has been observed in the setting of lactose and fructose breath testing, in which symptoms are recorded following ingestion of a carbohydrate load (165). Those patients who develop abdominal symptoms are termed lactose or fructose intolerant. However recent studies have cast doubt on whether the mechanism of intolerance is related to carbohydrate malabsorption. Intolerance is observed in patients with no evidence of malabsorption on breath testing, and indeed rates of lactose intolerance are similar in both lactose 'absorbers' and 'mal-absorbers' (179). Patients with functional gastrointestinal disorders have similar rates of malabsorption to asymptomatic healthy controls, and symptomatic improvement after dietary adjustment is predicted by presence of symptoms during breath testing rather than evidence of malabsorption (180). The presence of non-colonic symptoms is also reported to be associated with intolerance rather than malabsorption (181). These findings suggest

that malabsorption may not be the main driver of symptoms and that alternative mechanisms may be responsible.

In accordance with previous reports, the results demonstrate that a range of non-colonic symptoms are more commonly experienced in IBS patients compared to healthy volunteers (162). However the novel finding is that these symptoms are also prevalent in patients with IBD. Furthermore, for the majority of non-colonic symptoms their prevalence in IBS and IBD patients were remarkably similar. These findings suggest that the mechanism responsible for causing non-colonic symptoms may be shared in both disorders.

One factor that is common to both IBS and IBD is the high prevalence of mood disorders (59, 76). The results show that in patients with IBS, and in patients with IBD, the presence of non-colonic symptoms was generally associated with higher levels of anxiety. In IBD patients the majority of non-colonic symptoms were associated with significantly higher mean anxiety scores, and in IBS patients the trend was similar but the differences were not always significant, possibly due to a type II statistical error as the number of patients included was much smaller. These findings contrast with the conclusions of the previous study on non-colonic symptoms in IBS, which reported that these symptoms occurred irrespective of any associated psychiatric comorbidity (162). This conclusion was made from a sub-group analysis of IBS patients who were deemed not to have any psychiatric disorder based on a clinical interview schedule score less than fourteen. However their method in using a 'cut-off' value for interval data to diagnose psychiatric

comorbidity ignores the fact that there is likely to be a graded severity of disorder rather than a simple 'presence or absence'. In the healthy volunteer population anxiety levels were not always higher in those experiencing non-colonic symptoms, however it should be noted that the overall anxiety levels in this group were much lower than in patients with IBS or IBD. Further research is required to establish whether non-colonic symptoms frequently occur in those individuals with high anxiety levels but without a chronic gastrointestinal disorder.

Although the data suggests an association between high anxiety levels and the presence of non-colonic symptoms in IBS and IBD, this does not indicate causality. However it is interesting that lactose intolerance has also been reported to be associated with psychological factors (and not malabsorption), with patients demonstrating a tendency towards somatisation (179, 182). It is postulated that symptoms of intolerance are amplified and interpreted catastrophically in the somatising patient, and that cognitive-behavioural therapies may be of benefit (179).

In summary, despite previous *in vitro* tests suggesting a potential mechanism for the role of colonic bacterial fermentation metabolites in causing the symptoms of IBS, this study has found no evidence to support this theory. In its current form albumin catalysed coelenterazine chemiluminescence does not appear to be a useful biomarker in IBS. More recent evidence seems to suggest that malabsorption may not be the key factor in carbohydrate intolerance. Non-colonic symptoms may result from somatisation although further research is required to confirm this.

Chapter 5

Cognitive function in IBS and IBD patients

5.1 Introduction

Interactions between the brain and the gut occur continuously through a bidirectional communication pathway known as the brain-gut axis. This involves a complex interplay between neural, immune and endocrine systems, and is thought to play a key role in the pathophysiology of IBS. The nature of this pathway, together with the fact that both IBS and IBD share several risk factors for developing impaired cognition, has led to speculation that patients with these conditions may exhibit an altered cognitive profile (183). Indeed, in the previous chapter (Section 4.3, Table 4.9) it was observed that 55% of IBS patients and 49% of IBD patients subjectively reported cognitive impairment compared to only 15% of healthy volunteers ($p<0.001$).

Abnormal serum cytokine levels are an established feature of IBD and present to a lesser extent in IBS, and this may have implications for cognitive function (184, 185). The term 'sickness behaviour' describes a collection of neuropsychiatric symptoms that occur during illness and are thought to result from the effects of pro-inflammatory cytokines on the brain (186). Administration of interferon-alpha can induce depression that shares a similar profile to idiopathic depression occurring in otherwise healthy adults, the main difference being that cytokine-induced depression exhibits a greater level of psychomotor retardation, possibly reflecting effects on basal ganglia function (187). Long-term alterations in cytokine levels are believed to impair neuronal plasticity, thereby promoting mood disorders and cognitive dysfunction. Potential mechanisms include effects on the hypothalamic-pituitary-axis, and serotonergic or dopaminergic pathways (186). In healthy states

the immune system positively regulates learning and memory, however in pro-inflammatory conditions cytokines appear to have deleterious effects on these same domains, and have been implicated in contributing to states of cognitive decline such as dementia (188).

Mood disorders are prevalent in both IBS and IBD, and can have diverse effects on cognition, facilitating certain processes whilst impairing others. These actions occur predominantly through changes in glucocorticoid levels, and affect areas of the brain involved with memory and learning including the hippocampus, amygdala and pre-frontal cortex (189, 190).

A further risk factor for impaired cognition is the presence of chronic pain. This is a hallmark of IBS and is frequently present in patients with IBD. It has been associated with a wide range of cognitive deficits including impairments in memory, attention, speed of information processing, and executive function (191). Potential mechanisms include persistent nociceptive inputs competing with other sensory afferents, alterations in neurochemical substrates, or neuroplastic changes occurring as a result of chronic pain.

Finally, the intestinal microbiota has been implicated in the pathogenesis of both IBS and IBD. It represents another possible cause of cognitive dysfunction and has been studied in animal models. Changes in the memory and learning behaviour of mice have been demonstrated when alterations in their gut bacteria are produced through administration of antibiotics or dietary modification (192-194).

Deficits in cognition have been identified to occur in a wide range of chronic illnesses including systemic lupus erythematosus, hepatitis C, diabetes mellitus, chronic fatigue syndrome and lactose intolerance (165, 195-199). There is a paucity of data on the cognitive profile of IBS and IBD patients, but a relative reduction in verbal IQ, both to controls and to their own performance IQ, has previously been demonstrated (200, 201).

In view of the potential risk factors for impaired cognition, an explorative study was performed aiming to determine if cognitive deficits were present in patients with IBS or IBD, and to characterise their nature. It was anticipated that the presence of any specific deficits could potentially be utilised as a biomarker, and provide an opportunity to improve quality of life.

Hypothesis: Specific cognitive deficits are present in patients with IBS, and also in patients with IBD.

5.2 Methods

Healthy volunteers, IBS patients and IBD patients completed questionnaires detailing demographics, current medication, and past medical history. Level of education was also recorded and classified according to whether university was attended or not. Levels of anxiety and depression were measured using the hospital anxiety and depression scale. The presence of IBS was defined by Rome III criteria and the severity of symptoms was assessed using the irritable bowel syndrome symptom

severity scale (IBS-SSS). Faecal calprotectin levels were used to determine disease activity in IBD patients, with remission defined as FC <90μg/g.

Neuropsychological Test Battery:

Participants completed the "Cardiff Cognitive Battery", a series of computerised neuropsychological tests assessing a range of cognitive domains using well-established testing paradigms (202). The tests were available online and so participants were provided with a website address and asked to complete the assessments within one week of the initial recruitment meeting. The time and date of every participants assessment was automatically logged by the website. Advice was given to avoid alcohol on the day of the assessment.

Psychomotor speed: A two-choice reaction time test in which participants were shown two black boxes on the computer screen using a range of response-time intervals. The target (a white spot) appeared in either box and a button was pressed as fast as possible corresponding to the correct box. The mean overall response time was calculated and the number of correct responses recorded.

Working memory: In this forward digit-span task, participants were asked to recall a sequence of numeric digits. The number of digits increased by one with every successful round. The maximum number of digits remembered was recorded.

Episodic memory: In this paired associates learning task, target images were presented and covered, and the location of the images had to be recalled. The

number of target images increased with each successful round. The maximum number of cards correctly identified was recorded.

Attention Test: In this stroop task a coloured box was shown on screen. Below the box were written the names of two colours, one of which correctly described the colour of the box. Participants had to select the correct option. This was performed 30 times and candidates were asked to perform the task as quickly as possible. Mean response time and the number of correct responses were recorded.

Interference Test: In this interference condition of the stroop task, the name of a colour appeared on screen but the ink in which it was written was a different colour to that which it described. The names of two colours appeared below and participants had to select the one that described the colour of the ink. For example the word 'WHITE' written in black ink would be followed by the options 'black' or 'white', and the correct answer would be black. The mean response time and the number of correct responses were recorded.

Fluid Intelligence: Fluid intelligence is the capacity to think logically and solve problems that are independent of acquired knowledge. This was assessed using timed verbal and numeric reasoning test, which required participants to answer a series of questions. Each question was followed by five possible answers from which one was selected. The number of correct responses given within two minutes was recorded.

Crystallised Intelligence: Crystallised intelligence is the ability to use skills, knowledge and experience. It is often assessed using vocabulary and general knowledge. In the present study participants completed the National Adult Reading Test (NART) a test of the pronunciation of irregularly spelled words (203). The raw error score is transformed to estimate the full scale Wechsler adult intelligence score (204).

Sample Size:

The values for standard deviation and clinically significant difference varied between the seven neuropsychological performance tests. A pragmatic approach indicated that a sample size of 40 participants in each group would detect clinically significant levels of difference (set at 20% difference in mean values between groups) with a power of 80% and a confidence of 95%. A higher number of patients with IBD were recruited so that the effects of disease activity, duration of disease, and type of IBD could be analysed.

Statistical Analysis:

Results of the neuropsychological performance tests were initially compared between groups using ANOVA, and subsequently using analysis of covariance (ANCOVA). In view of the exploratory nature of the analysis a progressive model of ANCOVA was used, with respective covariates being sequentially introduced. This enabled the effect of specific variables or groups of variables to be studied in more detail. Further sub-group analysis was undertaken on IBD patients assessing effect of disease activity, duration of disease and type of IBD; and on IBS patients assessing effect of severity of symptoms.

5.3 Results

A total of 231 participants were recruited comprising 41 healthy volunteers, 40 patients with IBS, and 150 patients with IBD. Characteristics of the respective participant groups are shown in Table 5.1. Healthy volunteers tended to be better educated, show less anxiety and less depression than the patient groups.

Of the 40 patients with IBS, 29 had undergone colonoscopy as part of their diagnostic investigations and the remaining 11 patients provided a stool sample with FC level less than 90μg/g. The group consisted of 23 diarrhoea-predominant patients, two constipation-predominant patients, 14 were mixed subtype, and one was unsubtyped.

In the IBD population, 96 patients had ulcerative colitis and 54 had Crohn's disease. In total, 23% of IBD patients were taking immunosuppressant medication (azathioprine, 6-mercaptopurine, and methotrexate) and 7% were taking steroids. When UC patients were compared to those with CD there were no significant differences in any of the demographic details recorded, including the anxiety and depression scores.

One-way ANOVAs found significant differences in cognitive function between groups for fluid intelligence, crystallised intelligence, psychomotor speed and attention. However there were no significant differences between groups for either memory test or for the interference test.

Table 5.1 Summary of participant characteristics.

	Healthy Volunteers (n=41)	IBS patients (n=40)	IBD patients (n=150)	p-Value
Age (Mean, s.d.)	43.8 (13.4)	37.9 (11.7)	45.7 (11.3)	0.001* (ANOVA)
Gender				
Male	39% (n=16)	33% (n=13)	37% (n=55)	0.823
Female	61% (n=25)	67% (n=27)	63% (n=95)	(Chi-Square)
Married / In Relationship				
Yes	46% (n=19)	38% (n=15)	72% (n=108)	<0.001*
No	54% (n=22)	63% (n=25)	28% (n=42)	(Chi-Square)
Education Level				
School	37% (n=15)	47% (n=19)	62% (n=93)	0.009*
University	63% (n=26)	53% (n=21)	38% (n=57)	(Chi-Square)
Employment				
Employed	76% (n=31)	73% (n=29)	76% (n=114)	0.900
Unemployed	24% (n=10)	28% (n=11)	24% (n=36)	(Chi-Square)
Current Smokers				
Yes	7% (n=3)	22% (n=9)	12% (n=18)	0.105
No	93% (n=38)	78% (n=31)	88% (n=132)	(Chi-Square)
Alcohol				
< 28 units/week	90% (n=37)	95% (n=38)	89% (n=134)	0.554
> 28 units/week	10% (n=4)	5% (n=2)	11% (n=16)	(Chi-Square)
HAD score (Median, Range)				
Anxiety	5 (0-14)	11 (0-20)	9 (1-20)	<0.001*
Depression	2 (0-8)	5 (0-16)	5 (0-16)	<0.001* (Kruskal-Wallis)

* Indicates statistically significant difference with p<0.05

When the results were adjusted for participants age and sex using ANCOVA the variation between groups for fluid intelligence and crystallised intelligence remained significant (respective ANCOVA p-values: 0.019 and 0.021), however for psychomotor speed and attention the differences became non-significant (respective ANCOVA p-values: 0.469 and 0.318).

For fluid and crystallised intelligence, performance declined progressively from healthy volunteers (respective mean scores 5.3 and 119) to IBS patients (4.5 and 116) to IBD patients (4.2 and 115). On post-hoc analysis IBD patients had significantly lower scores on both intelligence tests compared with healthy volunteers (fluid intelligence p=0.01, and crystallised intelligence p=0.028). However IBS patients did not differ significantly from healthy volunteers on either intelligence test.

For fluid intelligence, adjustment by age and sex had little effect on the strength of association with disease group, (Model 1 in Table 5.2). When additional factors including relationship, employment, smoking status and alcohol consumption were inserted as covariates into the same analysis the association with groups remained significant, (Model 2 in Table 5.2). However with further adjustment for depression score the association became null (F=2.38, df=231, p=0.095), (Model 3 in Table 5.2). For crystallised intelligence a similar picture was observed (Table 5.3), with the addition of depression as a covariate again substantially attenuating the association (F=2.28, df=226, p=0.104).

Overall, education level had the greatest association with fluid and crystallised intelligence scores (Model 5 in Tables 5.2 and 5.3). When education was added into the ANCOVA analysis the significant association of depression with fluid and crystallised intelligence was removed. It is apparent from table 1 that there was a disparity in level of education between the groups (63% of healthy volunteers attending university compared to only 38% of IBD patients, p=0.023). To examine if this difference was secondary to illness, the age of IBD onset was analysed. The mean age of IBD onset was 35 years, with 83% of patients having onset after the age of 20 years. Those IBD patients who did not attend university had a significantly later age of disease onset than those who went to university (mean age 37 v 32 years, p=0.023).

Table 5.2 Progressive ANCOVA model comparing differences between groups for Fluid Intelligence as respective covariates are introduced.

	Model 1 (F-value, p-value)	Model 2 (F-value, p-value)	Model 3 (F-value, p-value)	Model 4 (F-value, p-value)	Model 5 (F-value, p-value)
Group (df=231)	4.03, 0.019*	3.60, 0.029*	2.14, 0.121	2.30, 0.103	1.17, 0.312
Age (df=231)	1.69, 0.195	1.34, 0.247	1.09, 0.298	0.83, 0.363	0.01, 0.965
Gender (df=231)	2.65, 0.105	3.00, 0.085	2.76, 0.10	3.06, 0.082	1.42, 0.234
Relationship (df=231)		0.64, 0.800	0.08, 0.774	0.09, 0.766	0.01, 0.932
Employment (df=231)		0.145, 0.704	0.06, 0.804	0.05, 0.817	0.21, 0.651
Smoking (df=231)		0.252, 0.616	0.10, 0.753	0.09, 0.759	0.04, 0.850
Alcohol (df=231)		0.387, 0.535	0.39, 0.534	0.45, 0.501	0.47, 0.496
Depression (df=231)			2.40, 0.123	2.60, 0.108	0.87, 0.351
Anxiety (df=231)				0.45, 0.505	0.05, 0.830
Education (df=231)					26.84, 0.00*

* Indicates statistically significant difference with $p<0.05$
df = Degrees Freedom

Model 1 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender)
Model 2 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender, Relationship, Employment, Smoking, Alcohol)
Model 3 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender, Relationship, Employment, Smoking, Alcohol, Depression)
Model 4 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender, Relationship, Employment, Smoking, Alcohol, Depression, Anxiety)
Model 5 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender, Relationship, Employment, Smoking, Alcohol, Depression, Anxiety, Education)

Table 5.3 Progressive ANCOVA model comparing differences between groups for Crystallised Intelligence as respective covariates are introduced.

	Model 1 (F-value, p-value)	Model 2 (F-value, p-value)	Model 3 (F-value, p-value)	Model 4 (F-value, p-value)	Model 5 (F-value, p-value)
Group (df=231)	3.95, 0.021*	3.42, 0.034*	1.87, 0.156	1.91, 0.150	0.80, 0.451
Age (df=231)	4.59, 0.033*	5.25, 0.023*	6.41, 0.012*	6.79, 0.010*	16.91, 0.00*
Gender (df=231)	4.90, 0.028*	4.91, 0.028*	4.63, 0.033*	4.96, 0.027*	2.24, 0.136
Relationship (df=231)		0.22, 0.639	0.28, 0.599	0.29, 0.594	0.03, 0.864
Employment (df=231)		0.80, 0.372	0.53, 0.466	0.53, 0.467	1.18, 0.278
Smoking (df=231)		0.01, 0.95	0.06, 0.805	0.07, 0.796	0.23, 0.630
Alcohol (df=231)		0.02, 0.891	0.02, 0.891	0.04, 0.523	0.04, 0.852
Depression (df=231)			5.76, 0.017*	4.94, 0.027*	1.52, 0.219
Anxiety (df=231)				0.41, 0.523	0.01, 0.944
Education (df=231)					55.90, 0.00*

* Indicates statistically significant difference with $p<0.05$
df = Degrees Freedom

Model 1 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender)
Model 2 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender, Relationship, Employment, Smoking, Alcohol)
Model 3 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender, Relationship, Employment, Smoking, Alcohol, Depression)
Model 4 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender, Relationship, Employment, Smoking, Alcohol, Depression, Anxiety)
Model 5 = ANCOVA (Fixed factor = Group; Covariates = Age, Gender, Relationship, Employment, Smoking, Alcohol, Depression, Anxiety, Education)

Sub-Group analysis:

There was no significant difference in the results of cognitive function tests for UC patients compared to CD patients. Similarly, regression analysis demonstrated that there was no significant effect from disease activity (as measured by faecal calprotectin level) or disease duration on the results of the neuropsychological performance tests in IBD patients.

Sixty-four of the IBD patients had symptoms compatible with a diagnosis of IBS and 86 patients did not. However there was no significant difference on any of the neuropsychological performance tests when these two groups of IBD patient were compared.

Regression analysis found no significant effect of the severity of IBS symptoms (as measured by IBS-SSS score) on cognitive performance.

5.4 Discussion

The results of this observational study do not support the hypothesis that IBS or IBD have an intrinsic disease process which causes cognitive dysfunction. However it is possible that concurrent mood disorders, in particular depression, are associated with impaired performance of patients with IBD in specific tasks.

Following adjustment of the initial ANOVA results to account for variations in simple demographics the only significant difference between groups was for scores of the

two intelligence tests. Healthy volunteers scored significantly higher than IBD patients on both intelligence tests, however IBS patients did not differ significantly from either group. Once depression score was added as a covariate the difference between healthy volunteers and IBD patients was nullified implying that the higher rate of mood disorder in IBD patients was partially responsible for their inferior performance. However, considering the association between depression and intelligence scores became non-significant when education level was added as a covariate, it appears that the initial group effects were more related to variations in education than depression.

It seems unlikely that the difference in education level between groups was a result of IBD as the vast majority of patients had disease onset after finishing their education, and the mean age of disease onset was significantly younger in those patients that attended university. This earlier age of disease onset may reflect differing patterns of sickness behaviour rather than any specific disease trait, with highly educated people tending to seek medical attention at an earlier stage.

Intellectual deficits have been reported in patients with IBS and IBD previously (200, 201). Both studies, performed by the same research team, identified a reduction in verbal IQ relative to performance IQ in both IBS and IBD patients compared to controls. Interestingly, these differences remained significant after education and mood disorder were added as covariates. Patients in these studies were recruited from community advertising and research databases rather than gastroenterology clinic. A separate study that examined cognition in IBS sufferers, found no difference

in intelligence scores compared to healthy volunteers, but observed a difference in constructive thinking, a concept which reflects problem solving style rather than problem solving ability (205).

Reduced concentration and poor short-term memory are amongst a range of non-gastrointestinal symptoms that have been described in association with both IBS and lactose intolerance (162, 165). These subjective reports are similar to the observations described in Chapter 4 of this thesis. The absence of any objective evidence for impaired cognition, together with the association of these symptoms with mood disorders, potentially adds further support to the hypothesis that these features are due to somatisation rather than an underlying systemic pathophysiology.

A strength of this study was the detailed measures taken to verify the diagnosis of patients with IBS and IBD. This is particularly important in cases of IBS, where the term is frequently applied to patients with a variety of abdominal symptoms that do not meet diagnostic criteria. Incorporating a normal faecal calprotectin or colonoscopy into the inclusion criteria provided further confidence in the diagnosis of IBS. However it is recognised that the IBS patients in this study were recruited from a secondary referral clinic and so are likely to have a higher burden of symptoms than the majority of patients who either do not seek medical attention or are managed in primary care.

The study has several limitations. Firstly, the study was powered to detect a 20% difference in mean values between groups, which was deemed to be clinically significant. Consequently, smaller differences that could be of scientific and physiological importance may not be detected. Larger studies are required to identify with confidence the range and strength of association between cognitive performance and IBD and IBS. Secondly, only certain features of cognitive function were examined. The Cardiff Cognitive Battery has been developed primarily for epidemiologic use and whilst the results suggest there is not a generalised impairment of cognition, it is possible that other specific forms of memory, perception or language that were not assessed in this study could be affected. A further limitation is that the recruitment of participants was not strictly matched in terms of demographics. Healthy volunteers were predominantly recruited from a panel set up by Cardiff University, and therefore the higher prevalence of university education likely reflects this source. This was a confounding factor in the analysis that ideally would have been matched for at recruitment, however using education as a covariate enabled these differences to be controlled for.

A final limitation reflects the chronic relapsing nature of IBS and IBD, in which activity varies over time. In IBS, symptoms can be particularly volatile, with episodes lasting only several hours before resolving. It is feasible that in IBS, cognitive dysfunction may only occur transiently whilst the illness is at its most active and that the effects are fully reversible. Consequently, a one-off neuropsychological assessment as performed in this study may not detect abnormalities. Although IBS-

SSS is a validated measure of severity, it considers symptoms over the previous ten days and so does not necessarily reflect activity at the time of cognitive testing.

Recruitment of participants in this study was restricted to between the ages of 18 to 65 years. Therefore, the question as to whether IBS or IBD impacts on the rate of cognitive decline that occurs in older age has not been explored. It is possible that any adverse effects of pro-inflammatory cytokines on brain function may be more apparent in the older population. Future research should examine the effect of IBS and IBD on cognition in this age group and establish if there is any association with the onset of dementia.

IBS patients in this study were not sub-classified into those with constipation (IBS-C), diarrhoea (IBS-D), or mixed symptoms (IBS-M) (70). Therefore further studies are also required to explore this population in greater detail and determine whether cognition varies between these sub-types.

In summary, this study has not found any evidence to suggest that a generalised cognitive impairment occurs in patients with IBS or IBD. Consequently, there does not appear to be any capacity for cognitive function testing to be used as a biomarker for either disorder. Anxiety and depression are common manifestations in patients with these conditions, and it appears that their presence may affect performance in certain situations. The management of concurrent psychological illnesses should remain an important therapeutic target in maintaining the global well-being of patients with IBS and IBD.

Chapter 6

A randomised controlled trial of mindfulness-based therapy for IBD patients with IBS-type symptoms or high perceived stress levels

6.1 Introduction

A variety of psychotherapeutic interventions have been studied in patients with IBD. These strategies include stress management, cognitive behavioural therapy, psychodynamic psychotherapy and hypnosis (206-209). Meta-analysis of these trials has been limited due to diversity in the interventions used, patients included and outcomes analysed, nevertheless it appears that moderate improvements in mood disorders and quality of life scores may result whereas impact on disease activity seems minimal (160, 210-212). A Cochrane review of psychological interventions performed in unselected IBD patients concluded that psychotherapy should not be administered to all patients, but may be of benefit in specific circumstances and that further research should identify those sub-groups most likely to benefit (157).

Psychological therapies have been shown to be an effective form of treatment in irritable bowel syndrome and have been included in management guidelines (75, 81, 213, 214). It is feasible that IBD patients with IBS-type symptoms may represent a sub-group of patients that will benefit from psychotherapeutic intervention. These patients are recognised to have higher anxiety levels and report lower quality of life scores than their counterparts without IBS-type symptoms (134, 135).

A second sub-group that could potentially benefit are those IBD patients with raised perceived stress levels. Several prospective studies have demonstrated that mood disorders and high perceived stress levels are associated with an increased risk of IBD relapse (65, 67, 215). However these studies did not use objective markers of intestinal inflammation to define relapse and instead relied on clinical activity index

scores. These indices can be influenced by IBS-type symptoms, which occur more commonly in the presence of mood disorders, and so it is possible that disease activity and therefore relapse rate may have been over-estimated as a result (53). Improving coping mechanisms and reducing perceived stress levels in this group might enhance outcomes by reducing the burden of functional symptoms.

Multi-convergent therapy (MCT) is a form of psychotherapy that combines mindfulness meditation together with aspects of cognitive behavioural therapy. Mindfulness is an awareness of the present moment experience, and emphasises attention on ones thoughts, bodily sensations and emotions. Through meditation, an ability to non-judgementally appreciate these aspects is developed with the aim of gaining a deeper perspective on one's own response to stress (216). The clinical effectiveness of MCT has been demonstrated for the treatment of IBS, tinnitus, and chronic fatigue syndrome but its applicability and efficacy in an IBD population has not previously been assessed (217-219).

IBD patients with IBS-type symptoms or high perceived stress levels represent two sub-groups that could potentially benefit from psychological therapy. The aim of this study is to assess the feasibility and efficacy of using multi-convergent therapy in the management of these two groups of IBD patients.

Hypothesis: Multi-convergent therapy will improve quality of life in IBD patients who are in clinical remission and have either IBS-type symptoms or high perceived stress levels.

6.2 Methods

Inclusion and Exclusion Criteria:

Patients with inflammatory bowel disease that met the following inclusion criteria were invited to participate:

(i) age 18 - 65 years

(ii) diagnosis of UC or CD that was in clinical remission based on the respective SCCAI and HBI scores, and a C-reactive protein level <10mg/l

(iii) the presence of IBS-type symptoms or a high perceived stress level.

(Definitions of these criteria are provided below).

The following were listed as exclusion criteria:

(i) pregnancy

(ii) presence of ileostomy or colostomy

(iii) previous colectomy

(iv) change in IBD medication (including use of steroids) in previous three months

(v) change in psychotropic medication in previous three months

(vi) diagnosis of cognitive impairment

(vii) previous psychological therapy.

Intervention:

Patients were randomly allocated to either the MCT course plus standard medical therapy (active group) or standard medical therapy alone (control group).

MCT employs a range of behavioural and cognitive techniques with mindfulness meditation as its central component. In this trial the therapeutic approach was standardised to follow the session plan summarised in Table 6.1. The MCT course consisted of six face-to-face sessions, each lasting 40 minutes, and took place over a 16-week period. A single experienced therapist conducted the course, which was performed at the University Hospital of Wales, Cardiff.

Table 6.1 A summary of the session plan for the multi-convergent therapy course

Session	Topic	Contents
1	Motivational interview	Explore biopsychosocial model + stress response Patient to keep diary of stressors / behaviours / symptoms
2	Treatment rationale	Identify stressors + explore coping mechanisms Introduction to MM – written / audio material
3	Mindfulness meditation	Reflection on behaviour patterns Application of MM
4	Theme exploration	Teaching patient to become their own therapist Role of graded exercise and breathing exercises
5	Relapse prevention	Use of meditation to influence physiological responses Complement lifestyle to maintain and consolidate gains
6	Final Review	Review of internal locus of control Reflect on techniques and patient preferences

MM = mindfulness meditation

Assessments and Definitions:

The presence of IBS-type symptoms was determined using Rome III criteria, and the severity was assessed by the IBS-SSS. The presence of mood disorders was evaluated using the hospital anxiety and depression scale (HAD).

Further questionnaires were used to assess measures of stress and coping mechanisms, quality of life, intelligence, personality, and availability of social resources:

Revised Daily Hassles Scale (RDHS): This measure of minor life stressors uses an ordinal scale of 0 to 3 to grade the degree of hassle caused by each of 53 minor common events (220). A cumulative score is calculated (range = 0-159). (Appendix 8).

Perceived Stress Questionnaire (PSQ): Levels of perceived stress were evaluated using a questionnaire that has been based on observations of an IBD population (221). An ordinal scale of 1 to 4 is applied to 30 questions regarding the level of perceived stress experienced in the previous month. A cumulative total is calculated. This total has 30 points subtracted, and then is divided by 90 (final score = 0.00-1.00). In a prospective study of patients with UC a PSQ score of >0.44 significantly increased the risk of an exacerbation in the following eight months (65). Therefore the entry criteria cut-off for defining a high level of perceived stress was set at >0.44. (Appendix 9).

Ways of Coping Checklist (WCC): Participants indicate how frequently they use certain behaviours and coping mechanisms in response to stressful scenarios (222). The questions are split into five coping styles (wishful thinking, positive thinking, avoidance, seek advice, and self blame). A mean score for each coping style is calculated (range = 0-3) with a higher score representing more frequent use of that particular style. (Appendix 10).

Inflammatory Bowel Disease Questionnaire (IBDQ): This is a validated quality of life assessment tool specifically for patients with IBD (223). An ordinal scale of 1 to 7 is used to respond to 32 questions concerning quality of life (overall score = 32-224). The questions are split into 4 domains (bowel, emotional, systemic, and social) and a mean score can be calculated for each domain. (Appendix 11).

National Adult Reading Test (NART): This is a literacy assessment in which participants are tested on the pronunciation of 50 irregularly spelled words. The number of correct responses are counted (score = 0-50). It is a measure of crystallised intelligence (203). (Appendix 12).

Big Five Inventory (BFI): Using a self-rating scale participants answer 44 statements regarding their personality (224). The responses are split into five domains; extraversion, agreeableness, conscientiousness, neuroticism and openness. A mean score (range = 1-5) is calculated for each domain with a high score signifying a strong correlation with that particular type of personality. (Appendix 13).

Interpersonal Support Evaluation List (ISEL): This assesses the perceived availability of social resources (225). It consists of 40 statements that are rated from 1 to 4 based on their applicability to the individual (overall score = 40-160). (Appendix 14).

Assessment of Disease Activity:

Activity of IBD was determined using two respective definitions:

(i). Clinical Indices: For UC patients, the simple clinical colitis activity index was modified such that the 'general well being' score was excluded (it was considered that IBS-type symptoms and high perceived stress levels would disproportionately affect this element) (138). Remission was defined as a score less than three. Similarly in CD a modified Harvey-Bradshaw index score was used with the 'general well being' score excluded, and remission defined as less than five points (137).

(ii). Faecal Calprotectin: FC levels were monitored to provide an objective marker of intestinal inflammation. They were not used as inclusion criteria for the study as samples were stored in batches before analysis and so results were not immediately available. Patients were asked to provide a stool sample within one week of their clinical assessment.

Changes in patients' medication during the follow-up period were also recorded. An escalation in IBD therapy included any initiation or increase in dosage of anti-inflammatory, steroid, immunosuppressant, or biological medication.

Trial Protocol:

A series of questionnaires were completed at baseline (time = 0) including Rome III Criteria, IBS-SSS, IBDQ, RDHS, PSQ, and WCC. Assessments of personality (BFI), social resources (ISEL), intelligence (NART), and mood disorder (HAD) were also performed at baseline in consideration of their potential to influence the outcome of therapy.

Patients in active and control groups were assessed at four, eight, and twelve months during the one year follow-up period using postal questionnaires. At each assessment their disease activity was assessed (together with providing a stool sample for FC level), and questionnaires were completed including Rome III Criteria, IBS-SSS, IBDQ, RDHS, PSQ, and WCC.

Patients in both groups continued to receive standard medical care for their IBD throughout the trial. They were asked to report any changes in medication at each four monthly assessment.

Randomisation:

Patients were randomised to either an active or control group once the eligibility criteria had been fulfilled and consent had been obtained. A blocked randomisation process, using random permuted blocks of size four and six (selected at random), was generated by the South East Wales Trials Unit. The sequences were put into sequentially numbered sealed opaque envelopes for use in the clinic. Patients with IBS-type symptoms were stratified according to type of IBD (UC or CD) and severity of IBS (IBS-SSS < or ≥300). Patients with high perceived stress levels who did not

have IBS-type symptoms were stratified according to type of IBD. At the end of the study an audit of the randomisation record was completed.

Sample Size:

A power analysis was performed using α = 0.05 and β = 0.80. The mean IBDQ score for patients in clinical remission has previously been reported as 183 with a standard deviation of 27.6 (226). A clinically significant improvement in quality of life as measured by the IBDQ was taken to be 20 (227). This indicated that 30 patients would be needed in each trial arm. A 10% drop-out rate was predicted and so a recruitment target of 66 patients was set.

Outcome Measures:

The primary outcome measure in this study was IBDQ score at four months analysed in the complete case population. Secondary outcomes included descriptive analysis of the acceptability and feasibility of administering MCT to an IBD population, and the effect of MCT on disease activity, levels of perceived stress and coping mechanisms. Separate exploratory sub-group analyses were performed on those patients with FC <90μg/g at baseline, those recruited with IBS-type symptoms at baseline, and those recruited with a high perceived stress level at baseline.

Study Population Definitions:

Screening population: Patients approached to participate in the trial.

Intention-to-treat (ITT) population: Patients randomised into the trial.

Complete-case population: Those patients from the ITT population that completed the follow-up assessments.

Per-protocol population: Patients that fully complied with the protocol and completed the follow-up assessments.

Statistical Analysis:

Assessment of IBDQ was performed for the complete-case-population and the per-protocol-population using analysis of covariance (ANCOVA) with baseline IBDQ as covariate. ANCOVA was also used to compare stress scores, coping mechanisms, and in the sub-group analysis of IBS-SSS scores. Regression analysis was used to evaluate factors associated with failure to complete the MCT course, and also characteristics associated with an improvement in IBDQ after the MCT course. For questionnaires that had less than 50% of a domain completed the missing data was replaced with the mean result for that domain, otherwise they were regarded as missing data.

Trial Registration:

This trial was registered at ClinicalTrials.gov; trial identifier NCT01426568.

6.3 Results

A total of 66 patients were randomised into the trial. The demographic details and clinical characteristics of these patients are outlined in Table 6.2.

Of the 33 patients in the active arm, eight did not attend the intervention and six dropped out during the course. The follow-up assessment at four months was completed by 27 patients. In the control group only one patient was lost to follow-up during the initial phase and so 32 patients completed the four-month assessment. The progression of patients through the trial and the reasons for drop-out are shown in Figure 6.1.

The only significant disparity between patients lost to follow-up and the complete-case-population was a younger age (respective mean age of 33 years v 47 years, p=0.04). Logistic regression analysis of those participants in the active arm of the trial did not identify any patient characteristics that were significantly associated with failure to complete the MCT course.

Primary Outcome

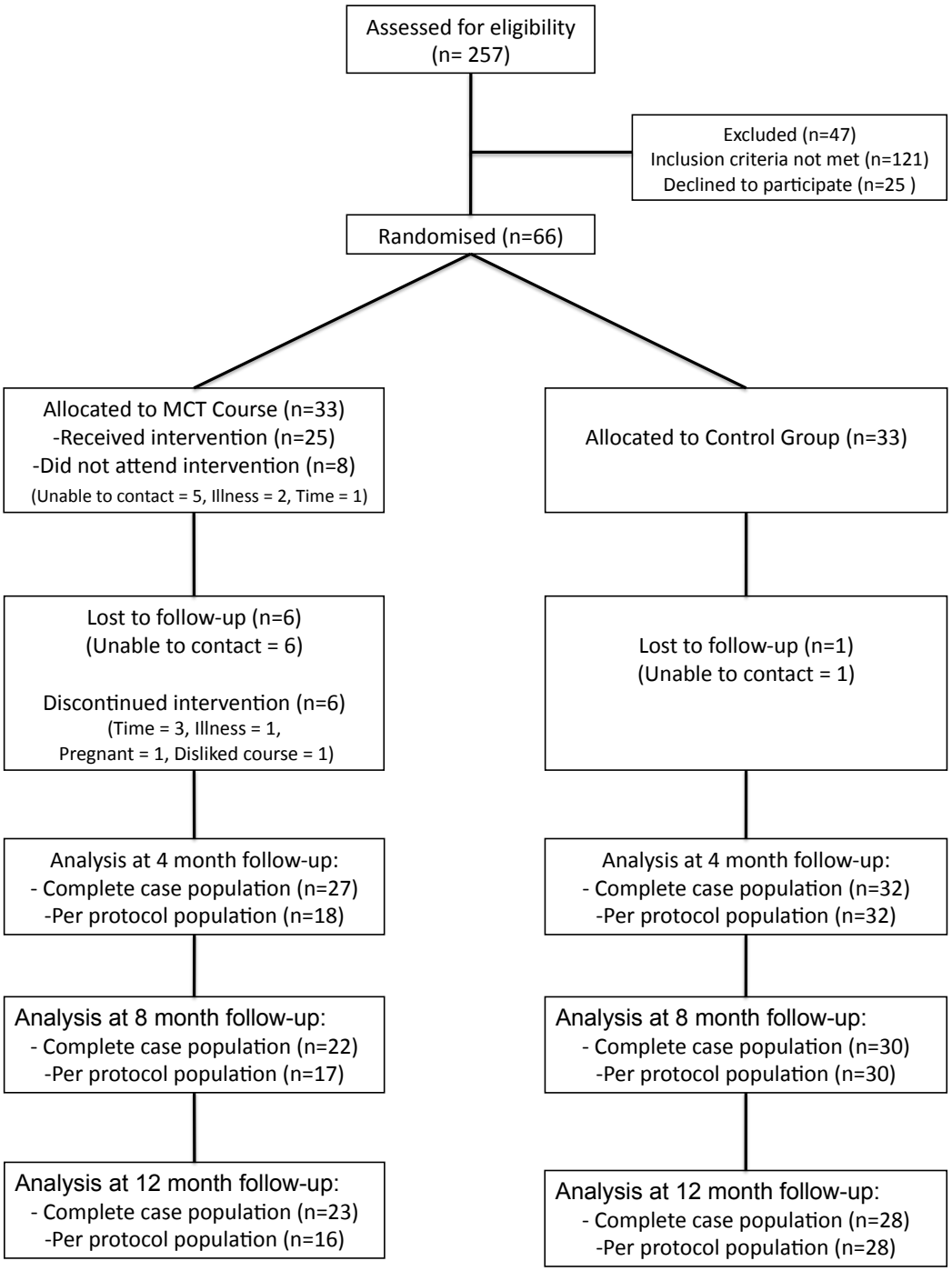
Analysis of the complete-case-population found that the mean IBDQ score at four months had improved to 167 in those patients randomised to the MCT course, whereas it remained unchanged at 156 for those in the control group. However the difference between the groups was not statistically significant ($F(1,58)=3.165$, $p=0.081$).

Table 6.2 Demographic details and clinical characteristics of the ITT population at baseline

		Active (n=33)	Control (n=33)
Age, Years		44.4 (11.7)	45.4 (10.6)
Gender:	Male	24% (8)	21% (7)
	Female	76% (25)	79% (26)
Diagnosis:	Ulcerative Colitis	73% (24)	64% (21)
	Proctitis	25% (6)	24% (5)
	Left-sided	58% (14)	67% (14)
	Pan-colitis	17% (4)	10% (2)
	Crohn's Disease	27% (9)	36% (12)
	Ileal	22% (2)	33% (4)
	Ileo-colonic	33% (3)	33% (4)
	Colonic	44% (4)	33% (4)
IBD Flare In Last Year		52% (17)	55% (18)
IBD Medication:	5-ASA	70% (23)	67% (22)
	Immunosuppressants	24% (8)	39% (13)
	Biologics	9% (3)	0% (0)
Current Smoker		9% (3)	6% (2)
Current Antidepressant Use		18% (6)	12% (4)
National Adult Reading Test Score		33 (13 - 45)	37 (10 - 47)
ISEL Score		82 (19)	85 (13)
Personality:	Extraversion	3.1 (0.8)	3.1 (0.8)
	Agreeableness	4.0 (0.6)	4.0 (0.8)
	Conscientiousness	4.0 (0.6)	3.9 (0.7)
	Neuroticism	3.5 (0.7)	3.5 (0.8)
	Openness	3.4 (0.6)	3.6 (0.7)
HAD Scale	Anxiety Score:	10.0 (3.5)	11.6 (4.4)
	Depression Score:	6.2 (2.9)	6.9 (3.4)
IBS-Type Symptoms Present (Meeting Rome III criteria)		58% (19)	58% (19)
Severity of IBS-Type Symptoms (IBS-SSS)		237 (101)	221 (83)
Inflammatory Bowel Disease Questionnaire Score		152 (33)	156 (20)
Faecal Calprotectin Level (μg/g)		105 (0-1019)	85.5 (0-1089)
Faecal Calprotectin <90μg/g		48% (14)	53% (16)

ISEL = Interpersonal Support Evaluation List; HAD = Hospital Anxiety + Depression Scale; IBS-SSS = Irritable Bowel Syndrome Symptom Severity Score

Figure 6.1 Patient flow through the study



The improvement in IBDQ observed in the active group appeared to be of a global nature with increased scores in all four domains of the assessment (Table 6.3). The progression of IBDQ score over the one year follow-up period is illustrated for active and control groups in Figure 6.2. There was no significant difference on repeated measures analysis over time, ($F(1,46)=1.77$, $p=0.190$).

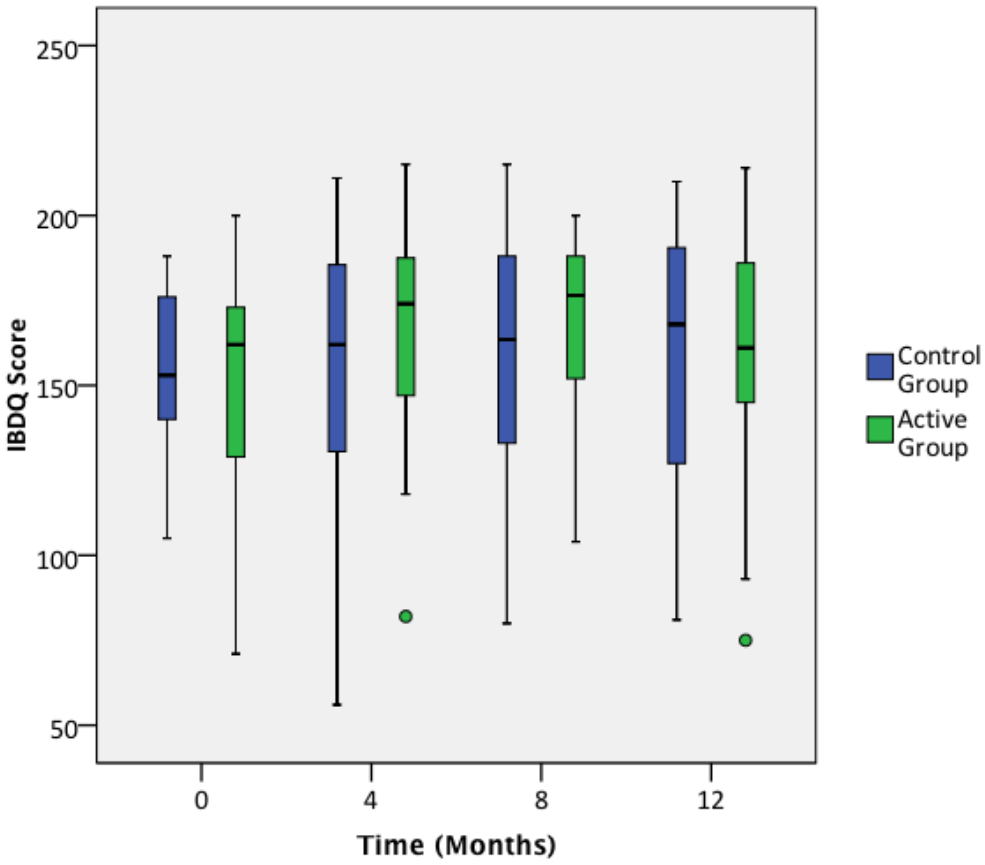
When the per-protocol population was analysed the IBDQ score at four months was significantly higher in the active trial group compared to controls (176 v 156, ($F(1,49)=4.547$, $p=0.038$) reaching the pre-specified clinically significant difference of 20. However the difference became non-significant at the eight and twelve month assessments.

Table 6.3 Respective domains of the IBDQ at baseline and four months

	Time = 0 months	Time = 4 months
Total IBDQ: Active (n=27) Control (n=32)	 156 (32) 156 (20)	 167 (30) 156 (37)
Bowel IBDQ: Active (n=27) Control (n=32)	 5.0 (1.1) 5.3 (0.7)	 5.4 (1.1) 5.2 (1.2)
Emotional IBDQ: Active (n=27) Control (n=32)	 4.7 (1.1) 4.5 (0.8)	 5.0 (1.0) 4.5 (1.3)
Systemic IBDQ: Active (n=27) Control (n=32)	 3.9 (1.1) 4.1 (1.2)	 4.3 (1.0) 4.2 (1.4)
Social IBDQ: Active (n=27) Control (n=32)	 6.0 (1.3) 6.0 (0.9)	 6.2 (0.9) 5.7 (1.4)

IBDQ = Inflammatory Bowel Disease Questionnaire

Figure 6.2 Progression of IBDQ score over the one year follow-up period



Secondary Outcomes

Using clinical indices to define relapse the active intervention group appeared to have a slightly lower rate of relapse at eight and twelve months compared to the control group, although the difference was not statistically significant (Table 6.4). However when FC levels were used to determine flare-ups the rate of relapse appeared to be similar in both groups. The kappa statistic, assessing level of agreement between relapse measures, was 0.13 when comparing clinical activity indices to FC, indicating only a slight level of agreement (155). Patients in the active intervention arm also appeared to require less frequent escalations in IBD medication during the follow-up period (25% v 41%, p=0.210).

Table 6.4 Cumulative rates of relapse and medication escalations over the 1 year follow-up period.

	Active	Control	P-value
Cumulative relapse rate: (Defined by CAI)			
4 months	27% (7)	28% (9)	0.919
8 months	27% (7)	39% (12)	0.347
12 months	35% (9)	48% (15)	0.294
Cumulative relapse rate: (Defined by FC)			
4 months	38% (6)	37% (10)	0.355
8 months	61% (14)	50% (14)	0.921
12 months	74% (17)	66% (18)	0.815
Medication escalations			
4 months	12% (3)	19% (6)	0.495
8 months	16% (4)	29% (9)	0.251
12 months	25% (6)	41% (12)	0.210

Levels of perceived stress reduced in both groups over the follow-up period, with a marginally greater reduction observed in the active group (Table 6.5). A comparison of coping mechanisms at the end of follow-up showed significantly more advice seeking behaviour in the treatment group (p=0.009) and also a trend towards positive thinking, although the latter was non-significant (p=0.102). The trends for using avoidance behaviour, wishful thinking and self blame were similar in both groups.

Table 6.5 Progression of hassles scores, perceived stress, and coping mechanisms over the follow-up period

	Active	Control	P-value
Hassles Score			
0 months	38 (14)	42 (18)	N/A
4 months	35 (15)	35 (17)	0.579
8 months	33 (15)	37 (20)	0.509
12 months	36 (14)	40 (23)	0.421
Perceived Stress Questionnaire			
0 months	0.43 (0.14)	0.46 (0.16)	N/A
4 months	0.37 (0.14)	0.43 (0.17)	0.343
8 months	0.35 (0.10)	0.41 (0.17)	0.380
12 months	0.35 (0.11)	0.41 (0.17)	0.164
Coping Mechanisms			
Wishful Thinking			
0 months	1.31 (0.72)	1.44 (0.58)	N/A
4 months	1.13 (0.69)	1.35 (0.82)	0.392
8 months	0.95 (0.74)	1.27 (0.77)	0.364
12 months	1.21 (0.81)	1.24 (0.77)	0.914
Positive Thinking			
0 months	1.47 (0.43)	1.37 (0.56)	N/A
4 months	1.55 (0.62)	1.36 (0.65)	0.689
8 months	1.56 (0.62)	1.39 (0.5)	0.685
12 months	1.60 (0.58)	1.30 (0.52)	0.102
Avoidance			
0 months	1.09 (0.53)	0.98 (0.49)	N/A
4 months	1.05 (0.62)	1.01 (0.61)	0.909
8 months	0.78 (0.52)	0.89 (0.54)	0.216
12 months	0.86 (0.56)	0.96 (0.67)	0.242
Seek Advice			
0 months	1.32 (0.64)	1.33 (0.73)	N/A
4 months	1.36 (0.77)	1.23 (0.93)	0.658
8 months	1.44 (0.71)	1.12 (0.61)	0.078
12 months	1.44 (0.54)	1.05 (0.65)	0.009*
Self Blame			
0 months	1.19 (0.58)	1.38 (0.67)	N/A
4 months	1.04 (0.40)	1.08 (0.80)	0.616
8 months	0.79 (0.46)	1.13 (0.68)	0.147
12 months	0.98 (0.50)	1.18 (0.82)	0.369

* Indicates statistically significant difference with $p<0.05$

Sub-Group Analysis

Of the 59 patients that provided a stool sample at baseline, 30 (51%) had faecal calprotectin <90μg/g indicating that they were in biochemical remission (as well as clinical remission) on entering the trial. When only those patients with FC <90μg/g were analysed the mean IBDQ scores at four months remained similar to that of the complete-case population analysis for both active and control groups (166 v 155, p=0.770).

A total of 38 patients from the ITT population had IBS-type symptoms at baseline. ANCOVA was used to analyse IBDQ score at four months in this sub-group and confirmed that it was significantly higher in the active group compared to the controls (161 v 145, p=0.021), (Table 6.6 and Figure 6.3). There was also a trend for IBS-type symptoms to occur less frequently and with less severity in the active group during the follow-up period, although these differences were not statistically significant, (Figure 6.4). When only those patients in this sub-group with FC <90μg/g at baseline were analysed (n=20) the mean IBDQ score at four months was 21 points greater in the active group compared to the controls (163 v 142, p=0.326).

Forty-eight of the patients recruited had a PSQ >0.44. The IBDQ score at four months was higher in those in the active group but the difference was not statistically significant (164 v 153, p=0.095). At four months the PSQ score had reduced in both groups but they did not differ significantly, p=0.417.

Table 6.6 Sub-group analysis of patients with IBS-type symptoms at baseline.

	Active	Control	P-value
% with persistent IBS-type symptoms			
0 months	100% (18)	100% (19)	N/A
4 months	69% (11)	89% (16)	0.214
8 months	62% (8)	82% (14)	0.242
12 months	85% (11)	81% (13)	1.000
IBS-SSS score			
0 months	237 (101)	221 (83)	N/A
4 months	160 (99)	206 (108)	0.219
8 months	166 (103)	221 (119)	0.213
12 months	187 (97)	224 (111)	0.234
IBDQ			
0 months	143 (32)	149 (99)	N/A
4 months	161 (35)	145 (39)	0.021*
8 months	155 (32)	147 (38)	0.304
12 months	150 (41)	137 (38)	0.059

* Indicates statistically significant difference with $p<0.05$

IBS-SSS = Irritable Bowel Syndrome Symptom Severity Score; IBDQ = Inflammatory Bowel Disease Questionnaire

Figure 6.3 Sub-group analysis for patients with IBS-type symptoms at baseline: Progression of IBDQ score during the follow-up period

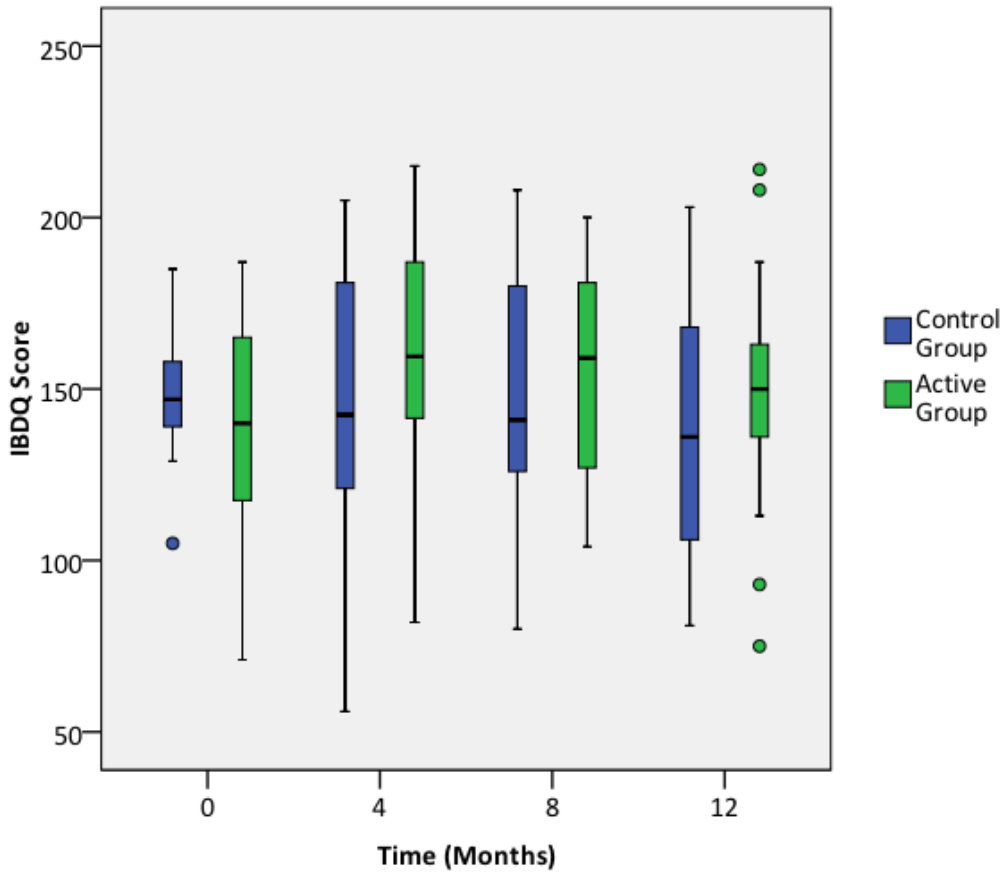
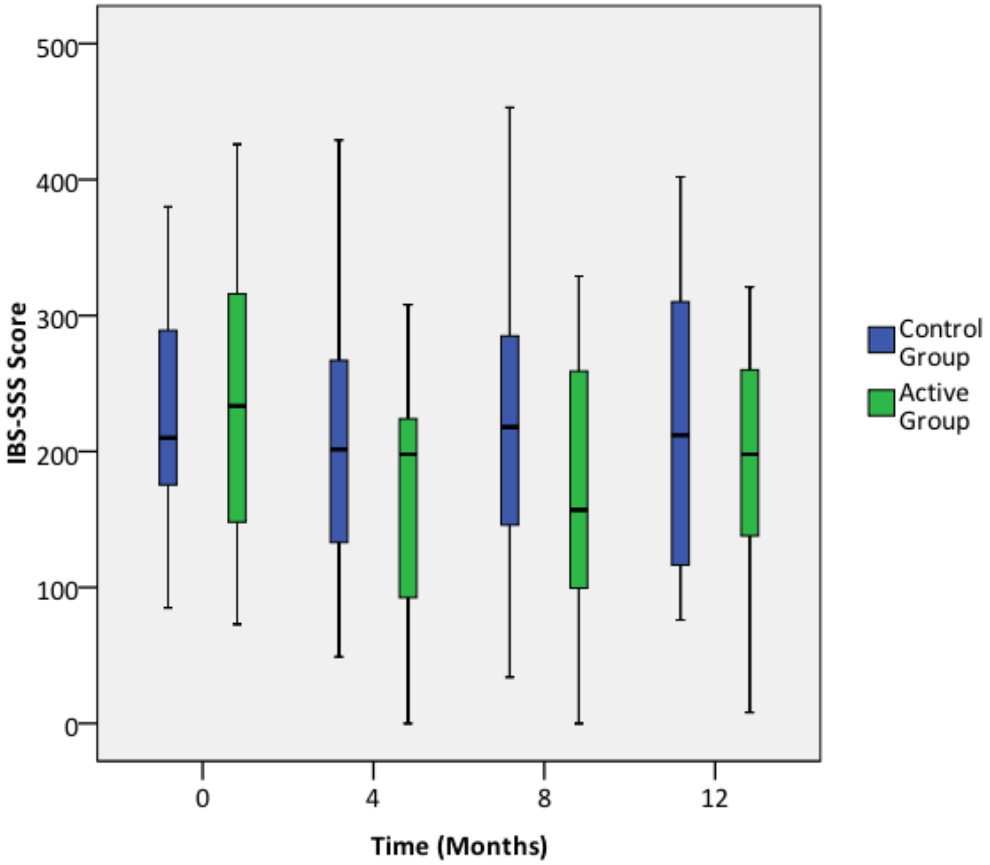


Figure 6.4 Sub-group analysis for patients with IBS-type symptoms at baseline: Severity of IBS symptoms in active and control groups during the follow-up period



Linear regression was used to determine those characteristics of patients in the MCT group that were associated with an improvement in IBDQ score at four months. The factors analysed included age, gender, type of IBD, NART score, presence of IBS-type symptoms, baseline PSQ score, baseline FC level, and MCT course compliance (Table 6.7). Presence of IBS-type symptoms (p=0.016) and baseline FC level (p=0.022) were the only factors with a significant association. However when stepwise linear regression was used in the analysis the only significant association was found to be presence of IBS-type symptoms, (p=0.038).

Table 6.7 Linear regression of patient factors associated with a significant change in IBDQ at four months

	B	Standard Error	p-value
(Constant)	-53.77	23.19	0.034
Age	0.43	0.32	0.198
Gender	9.36	8.18	0.269
Type of IBD	11.89	7.32	0.124
NART score	-0.14	0.34	0.689
IBS-type symptoms	17.88	6.61	0.016*
Baseline PSQ score	29.69	20.94	0.175
Baseline FC level	0.027	0.011	0.022*
MCT compliance	12.32	6.67	0.083

* Indicates statistically significant difference with $p<0.05$

NART = National Adult Reading Test; PSQ = Perceived Stress Questionnaire; FC = Faecal Calprotectin; MCT = Multi-Convergent Therapy

6.4 Discussion

This study assessed the feasibility of using a predominantly mindfulness-based therapy in an IBD population, and examined sub-groups of patients to identify those that may gain the most benefit. Whilst the increase in IBDQ score observed in the active arm of the ITT population was not statistically significant, the sub-group analysis identified that there was a significant improvement in quality of life in those IBD patients who were experiencing IBS-type symptoms. The improvement appeared to be due to a decline in the severity of symptoms. This study suggests that IBS-type symptoms in IBD patients represent a potential therapeutic target to improve quality of life. Further studies are required to confirm the efficacy of mindfulness-based therapy in treating these symptoms, and also to examine the use of alternative IBS therapies in this setting.

Analysis of mean IBDQ score at four months in the complete case population demonstrated an 11-point improvement in the active arm compared to no change in the controls. This difference was not statistically significant, and was below the pre-defined 20-point standard that represented a clinically relevant improvement in quality of life. However when the per-protocol population was analysed, the IBDQ score was significantly greater in the active group with a mean difference of 20 points, suggesting that patients completing the course did initially gain a substantial benefit. Subsequently, at the eight and twelve month assessments the difference between groups became non-significant indicating that the effects of intervention may decline over time. However it is feasible that this decline in efficacy may be

averted with the use of extra 'booster sessions' which are commonly employed in psychological interventions.

The high drop-out rate suggests that the intervention may not be acceptable to all patients. Eight patients randomised to the active group did not attend a single appointment and six withdrew during the course. Five of the patients that failed to attend any appointment did not respond to a number of attempts at communication. It is possible that these individuals lacked motivation for attending the course and perhaps outside of the trial setting may have declined participation. Several participants had genuine medical reasons for non-attendance or withdrawal including illness and pregnancy (an exclusion criteria). Four patients reported that they were unable to attend due to time constraints related to work or family commitments. Detailed communication prior to starting therapy is clearly required to optimise attendance and efficacy. The MCT course will not be suitable for everyone and there is no problem in patients withdrawing if they are not gaining benefit.

IBS-type symptoms are common in IBD patients who appear to be in remission and are associated with a reduced quality of life. Yet so far they have been the target of very few therapeutic trials. In this study the presence of IBS-type symptoms at baseline was associated with an improvement in IBDQ score following the MCT course. In the active group the percentage of patients with IBS-type symptoms initially decreased but at the end of follow-up the number was similar to that in the control group. However the main impact seemed to be on the severity of IBS

symptoms, which remained lower in the active group throughout the study. It is proposed that MCT may reduce the severity of IBS-type symptoms in IBD patients and in this way improve their quality of life.

This improvement in functional abdominal symptoms may explain the discrepancy in results for relapse rates. The trend for lower relapse rates in the active group based on clinical indices was not apparent when faecal calprotectin was used to define relapse. A recent study has demonstrated that functional symptoms can mimic active inflammation when clinical indices alone are used to assess disease activity (53), and so it is possible that the more severe IBS-type symptoms in the control group may account for the higher relapse rates observed based on clinical indices. The FC levels represent objective markers of intestinal inflammation and it does not appear that the MCT course had any effect on this. Interestingly, there was also a trend for fewer medication escalations in the active group. Therapeutic clinical decisions are frequently guided by patients' symptoms and so a further consequence of a reduction in functional symptoms may be to lower use of medication.

Faecal calprotectin levels were not used in the inclusion criteria to define remission as specimens were analysed in batches with results unavailable for up to a month following collection. Remission was defined using clinical indices together with a normal CRP level, however 50% of the patients had FC >90μg/g indicating ongoing inflammation. The proportion of patients with raised FC was similar in both active and control groups. As demonstrated in Chapter 3, sub-clinical inflammation may play a role in causing IBS-type symptoms in patients with IBD, and for this reason a

separate sub-group analysis was performed including only those patients with IBS-type symptoms and FC <90μg/g. The active group had a 21-point greater IBDQ score compared to the controls at four months, and although the difference was not statistically significant this may reflect the smaller number of participants involved in the analysis. In clinical practice, FC analysis should help to determine those patients with active inflammation who are likely to benefit from an escalation in medical therapy before considering management of potential functional symptoms.

Three aspects of stress and its management were assessed during the follow-up period, but the study was not directly powered to detect statistically significant differences in these secondary outcomes and so only trends could be observed. Both groups reported similar amounts of daily hassles throughout the follow-up period. Levels of perceived stress appeared to reduce in both groups but a slightly greater reduction was observed in the active group. However MCT's principal effect appeared to be on coping mechanisms for which a trend towards greater use of positive thinking and advice seeking behaviour was observed. These changes would generally be regarded to represent a healthier style of coping, leading to a reduction in perceived stress in the longer-term.

This trial has several limitations that need to be considered when interpreting the results. Participants were not blinded as to their allocation following randomisation and there was no placebo therapy used in the control group. As a result, the placebo effects of an expectation to improve and contact attention are unable to be determined. This is particularly relevant in the setting of IBS in which the mean

placebo response is reported to be 47% with a range from 0 to 84% (228, 229). Studies of similar psychological interventions have used support groups and online forums for the control groups in an attempt to account for these factors (131, 230). Although a placebo group would be necessary to assess the specific impact of mindfulness as a therapy, the current study format does provide information on the efficacy of using an intervention in this clinical setting.

Patients with IBS-type symptoms were recruited on the basis that they met the Rome III criteria for IBS, regardless of the severity of their symptoms. Similarly, patients were recruited irrespective of their baseline IBDQ score. Consequently there may have been patients with very mild IBS-type symptoms or with a very good quality of life at baseline included in the study. These patients are less likely to benefit from treatment compared to those with severe symptoms that are substantially impacting quality of life, and their inclusion in the study may have reduced its efficacy. In routine clinical practice patients with mild symptoms or good quality of life are unlikely to seek psychological intervention.

It is feasible that patients in remission who had severe functional symptoms were clinically assessed to have active disease and therefore excluded from the study. The use of clinical activity indices in the inclusion criteria rather than faecal calprotectin, may have prevented the participation of those patients that would have gained the most benefit.

The intervention arm experienced a high drop-out rate, with 24% of those randomised into the MCT course failing to attend even a single appointment, and in the majority of cases they were also lost to follow-up. An extra 10% of patients were recruited in view of potential drop-outs, however this under-estimated the actual numbers, and as a result the power of the study has been reduced. High drop-out rates are a recognised phenomenon in trials of psychological intervention, and recent trials of mindfulness-based therapy for IBS have experienced drop-out rates of 23-26% (131, 230, 231). Patients subjective need for psychological support is increased in IBD compared to other chronic inflammatory diseases, possibly due to the greater social restrictions associated with the disorder (232). However careful patient selection remains essential as a patient's motivation to participate in psychological therapy is a key factor in determining its success (233).

The generalisation of the results of this study is also limited by the fact that a single therapist administered the MCT course. In this type of intervention the relationship between the patient and therapist is vital in determining outcome, and so further studies with multiple therapists and recruiting across several sites would be required to evaluate the effects of MCT more thoroughly.

In the general population psychological therapy is only used for the management of IBS in a minority of cases, typically after other forms of intervention such as dietary modification, antidepressant medication, and serotonin receptor agonists have failed to provide adequate symptom relief. Further trials are needed to examine the role of these treatments in managing IBS-type symptoms in patients with IBD. A pilot

study of dietary modification in which patients reduced their intake of short-chain carbohydrates has already demonstrated an improvement in abdominal symptoms in this setting (159).

IBS-type symptoms in inflammatory bowel disease can have a variety of causes. Sub-clinical inflammation, bile salt malabsorption, and small bowel bacterial overgrowth need to be identified and managed appropriately. By excluding these pathologies the efficacy of interventions directed towards improving 'true IBS symptoms' should be improved. This study suggests that IBD patients with IBS-type symptoms may represent a sub-group that will benefit from psychotherapeutic intervention, however a multi-centre trial with adequate provision of placebo in the control arm is needed to confirm this.

Chapter 7

General Discussion

7.1 Overall Conclusions

A substantial proportion of patients with inflammatory bowel disease experience symptoms that are compatible with a diagnosis of irritable bowel syndrome. These occur even when the inflammatory bowel disease appears clinically to be in remission. Both IBS and IBD are chronic disorders that frequently follow a relapsing remitting course and both conditions overlap in their symptom profiles. Consequently their concurrent presence represents a diagnostic challenge and places the individual at a clinical disadvantage. Patients are at risk of additional invasive investigations or empirical immunosuppressive therapy, whilst their functional symptoms may be neglected. The work described in this thesis has focused on determining the nature of these IBS-type symptoms, examining potential biomarkers for IBS, and conducting a therapeutic trial. These are important issues that have received little attention thus far, despite IBD patients with IBS-type symptoms reporting lower quality of life scores compared to their asymptomatic counterparts.

Previously, it has been suggested that IBS-type symptoms in patients with IBD should be regarded as a marker of sub-clinical inflammation (132). Although it is important to consider this as one of several potentially reversible causes, the results from the initial study described in Chapter 3, demonstrated that IBS-type symptoms frequently occur in patients confirmed to be in remission based on their clinical symptoms and a normal faecal calprotectin level. Subsequent reports have also shared this observation that FC levels are similar in IBD patients with or without IBS-type symptoms (234, 235).

It was identified that IBD patients with IBS-type symptoms exhibit similar characteristics to IBS patients in the general population, with a significant association with female gender and higher anxiety levels. However the prevalence of IBS-type symptoms in patients with a normal FC level was 31%, which is higher than that observed in the general population. There are several possible explanations for this. Firstly, this study was limited by not measuring the occurrence of bile acid malabsorption and small bowel bacterial overgrowth, both of which commonly occur in Crohn's disease. These conditions may cause symptoms similar to IBS, and yet if they were responsible for a substantial number of cases then IBS-type symptoms would be expected to occur more frequently in CD compared to UC, which was not the case in the cohort of patients examined.

A second possibility is that IBS is more common in IBD patients compared to the general population. It has since been hypothesised that visceral hypersensitivity occurs in IBD patients due to the upregulation of nociceptive receptors that are induced by the acute inflammatory phase and persist during remission (235). Mucosal neurobiological changes have been demonstrated in IBD patients who experience IBS-type symptoms, with a higher number of TRPV1 nerve fibres present in rectosigmoid mucosa (136, 236).

The limitations of using symptom-based indices alone to assess IBD activity was highlighted in this research. The correlation between clinical activity indices and faecal calprotectin levels in defining remission was relatively weak, although this appeared to be the case irrespective of whether IBS-type symptoms were present.

However the potential for functional symptoms to influence clinical activity indices has been demonstrated recently in a study in which patients with IBS were asked to complete the Crohn's disease activity index, and their scores were significantly raised suggestive of active inflammation (53). These findings highlight the importance of using objective markers of gut inflammation such as faecal calprotectin.

Direct visualisation of the intestinal mucosa via endoscopy is regarded as the gold standard for assessing disease activity in IBD, but this is invasive, time-consuming and expensive. Faecal calprotectin levels have been shown to correlate with endoscopic disease activity scores in IBD, particularly in colonic disease, and are generally accepted as a surrogate marker of mucosal inflammation (152, 154). Studies have attempted to determine the 'cut-off' value that indicates the presence of significant inflammation but there is debate as to the optimum level. A value of 250μg/g has been proposed as having optimal sensitivity and specificity for predicting the presence of mucosal inflammation in UC and the presence of more severe lesions in CD (154). In the research reported in this thesis, a much lower FC level of <90μg/g was used to define remission. This was because it was considered important to confidently exclude active inflammation when evaluating the potential presence of functional symptoms in patients with IBD. This value was based on the laboratory reference range recommended to screen patients with functional abdominal symptoms to exclude intestinal inflammation. However, as a result it is possible that some IBD patients who were in remission may have been judged to have active disease based on a FC level that was slightly higher than 90μg/g.

This research found no evidence to support the hypothesis that toxic metabolites produced by the bacterial fermentation of dietary carbohydrates in the colon have a role in causing IBS symptoms. Although there were several potential confounding factors that limit the interpretation of these results, the recent reports that malabsorption is not present in many people with carbohydrate intolerance implies that the initial hypothesis seems unlikely. It is recognised that in all humans a proportion of dietary carbohydrate will not be absorbed and will proceed to the colon where bacterial fermentation will occur. Fluid will be drawn into the lumen, gas will be produced, and the lumen will become distended. It seems likely that visceral hypersensitivity to this luminal distension is integral to determining the severity of symptoms.

A variety of non-colonic symptoms were subjectively reported to occur more frequently in IBS patients and IBD patients compared to healthy volunteers. These symptoms included cognitive impairment, and yet no substantial deficit in cognition was detected on direct testing. Potentially this may add support to the theory that non-colonic symptoms are related to somatisation, especially considering that in many instances their presence was associated with high anxiety levels. However it is acknowledged that there were several limitations to the cognitive function study and that further research is required to confirm this hypothesis.

Many patients with IBD experience substantial morbidity during the course of their illness. Chronic active disease, fistula formation, intestinal strictures and malnutrition are all serious complications that may be encountered. As a result,

there is potential for IBS-type symptoms in these patients to appear trivial in comparison. Nevertheless, patients with IBS-type symptoms do report lower quality of life scores compared to their asymptomatic counterparts, and the results of the trial described in Chapter 6 suggest that they may be a sub-group that will benefit from additional therapeutic intervention.

In recent years, the improved outcomes that result from achieving mucosal healing in patients with inflammatory bowel disease have been recognised (237-239). Consequently, clinicians have been encouraged to strive towards this goal. However the work described in this thesis suggests that even after mucosal healing has been achieved, there may still be scope for improvement. Some patients will continue to experience distressing abdominal symptoms despite being in remission, and it is important that clinicians are aware of the impact these symptoms can have and the therapeutic options that are available.

7.2 Future Prospects

There is increasing recognition that the reliance on symptom-based criteria to diagnose patients with irritable bowel syndrome, and subsequently sub-classify and direct their management is inadequate (240). The heterogeneity of patients diagnosed with IBS indicates that there are almost certainly several sub-groups within this population, each with a different pathophysiology. As a result it seems unlikely that one biomarker will be applicable for all IBS patients, and rather that multiple biomarkers will be required. Management strategies are similarly limited by

the lack of specific pathophysiologies to target. Therapeutic trials performed on groups of patients that simply share a similar symptom predictably only produce modest results. The molecular mechanisms of these respective conditions need to be fully understood before significant progress can be achieved.

Pending the development of reliable biomarkers, those IBD patients with IBS-type symptoms will continue to require a systematic diagnostic approach. The exclusion of sub-clinical inflammation and secondary complications of IBD remains essential. Therapeutic options should be offered to those patients in whom IBS-type symptoms are causing substantial morbidity.

The trial described in this thesis suggests that psychological intervention may be of benefit in this setting, however it is not typically a first-line treatment for IBS and is often reserved for more severe cases that have proved resistant to other therapies. Therefore future therapeutic studies aimed at managing IBS-type symptoms in IBD patients should examine alternative strategies including medications and dietary modification that have proven effective in the treatment of irritable bowel syndrome.

Appendices

Appendix 1: Demographics Questionnaire

Participant ID Number:

Marital Status:		Ethnic Origin:		Age:
Employment:	Nil		Part-time	Full-time
Smoking Status:	Never		Previous	Current
Alcohol Intake:	None		≤ 28 Units	> 28Units
Education Level:	No Qualific.	GCSE	A-Level	University

Medications:

Medical History:

For IBD Patients:

Diagnosis:	Ulcerative Colitis	Crohn's Disease

Age at Diagnosis:

Disease Duration (Years):

Extra-Intestinal Manifestations:	Yes	No

Duration of Remission (Months):

Number of Flare-ups in the last year:
(requiring a change in medication)

Previous Resection:	Yes	No

Operation Details:

Disease Behaviour (C.D.):	Inflammatory		Fistulising	Stricturing
Disease Site (C.D.):	Colonic		Ileo-colonic	Ileal
Disease Site (U.C.):	Proctitis		Left-Sided	Pan-colitis
Medication: None	5-ASA	Steroids	Aza / Meth	Anti-TNF
Medication Changes in the last 3 months:		Yes	No	

Appendix 2: Rome III Criteria for Irritable Bowel Syndrome

1. In the last 3 months, how often did you have discomfort or pain anywhere in your abdomen?

0 Never (If never do not answer the next questions)
1 Less than one day a month
2 One day a month
3 Two to three days a month
4 One day a week
5 More than one day a week
6 Every day

2. For women: Did this discomfort or pain occur only during your menstrual bleeding and not at other times?

0 No
1 Yes
2 Does not apply because I have had the change in life (menopause) or I am a male

3. Have you had this discomfort or pain 6 months or longer?

0 No
1 Yes

4. How often did this discomfort or pain get better or stop after you had a bowel movement?

0 Never or rarely
1 Sometimes
2 Often
3 Most of the time
4 Always

5. When this discomfort or pain started, did you have more frequent bowel movements?

0 Never or rarely
1 Sometimes
2 Often
3 Most of the time
4 Always

6. When this discomfort or pain started, did you have less frequent bowel movements?

0 Never or rarely
1 Sometimes
2 Often
3 Most of the time
4 Always

7. When this discomfort or pain started, were your stools (bowel movements) looser?

0 Never or rarely
1 Sometimes
2 Often
3 Most of the time
4 Always

8. When this discomfort or pain started, how often did you have harder stools?

0 Never or rarely
1 Sometimes
2 Often
3 Most of the time
4 Always

Diagnostic Criteria for IBS:

- Pain / discomfort at least 2 days per month (Question 1 > 2)

- For women pain / discomfort should not only occur with menstrual cycle (Question 2 = 0 or 2)

- Symptoms should be present for at least 6 months (Question 3 = 1)

- Recurrent abdominal pain / discomfort at least 2 - 3 days/month in last 3 months associated with *two or more* of criteria below:

    1. Improvement with defecation: Pain / discomfort gets better after defecation at least sometimes (question4 > 0)

    2. Onset associated with a change in frequency of stool: Onset of pain / discomfort associated with more stools at least sometimes (question 5 > 0)

       or

       Onset of pain / discomfort associated with fewer stools at least sometimes (question 6 > 0)

    3. Onset associated with a change in form (appearance) of stool: Onset of pain / discomfort associated with looser stools at least sometimes (question 7 > 0)

       or

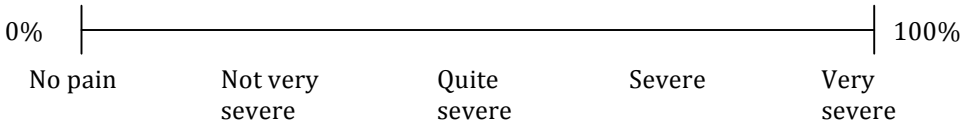
       Onset of pain / discomfort associated with harder stools at least sometime (question 8 > 0)

Appendix 3: Irritable Bowel Syndrome Symptom Severity Scale

1. a). **Do you currently suffer from abdominal (tummy) pain?** Yes ☐

No ☐

b). **If yes, how severe is your abdominal (tummy) pain?**



c). **Please enter the number of days that you get the pain in every 10 days**
(For example if you get enter 4 it means you get pain 4 out of 10 days)
(If you get pain every day enter 10) ☐

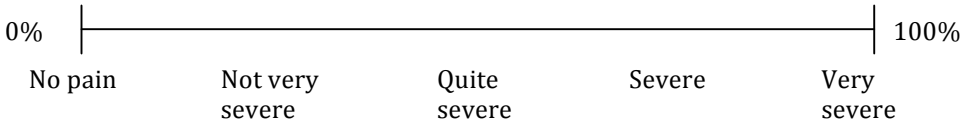
(x 10)

2. a). **Do you currently suffer abdominal distension (bloating or swollen tummy)?**
(Women please ignore distension related to your periods)

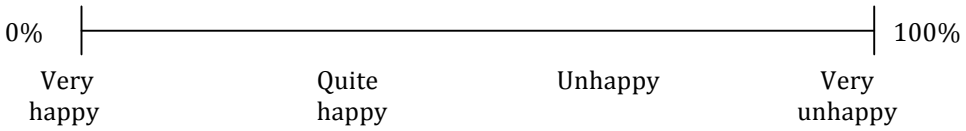
Yes ☐

No ☐

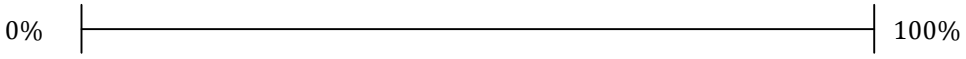
b). **If yes, how severe is you abdominal distension / tightness?**



3. **How satisfied are you with your bowel habit?**



4. **Please indicate with a cross on the line below how much your Irritable Bowel Syndrome is affecting or interfering with your life in general**



Overall IBS-SSS Score: ☐

For Office Use Only.
Score:

Appendix 4: Hospital Anxiety and Depression Questionnaire

Patient Advice:
Read each item and circle the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies; your immediate reaction to each item will probably be more accurate than a long thought out response.

1. I feel tense or 'wound up':	A
Most of the time	3
A lot of the time	2
From time to time, occasionally	1
Not at all	0
2. I still enjoy the things I used to enjoy:	D
Definitely as much	0
Not quite so much	1
Only a little	2
Hardly at all	3
3. I get a sort of frightened feeling as if something awful is about to happen:	A
Very definitely and quite badly	3
Yes, but not too badly	2
A little, but it doesn't worry me	1
Not at all	0
4. I can laugh and see the funny side of things:	D
As much as I always could	0
Not quite so much now	1
Definitely not so much now	2
Not at all	3
5. Worrying thoughts go through my mind:	A
A great deal of the time	3
A lot of the time	2
From time to time but not too often	1
Only occasionally	0
6. I feel cheerful:	D
Not at all	3
Not often	2
Sometimes	1
Most of the time	0
7. I can sit at ease and feel relaxed:	A
Definitely	0
Usually	1
Not often	2
Not at all	3

8. I feel as if I am slowed down:	D
Nearly all the time	3
Very often	2
Sometimes	1
Not at all	0
9. I get a sort of frightened feeling like 'butterflies' in the stomach:	A
Not at all	0
Occasionally	1
Quite often	2
Very often	3
10. I have lost interest in my appearance:	D
Definitely	3
I don't take so much care as I should	2
I may not take quite as much care	1
I take just as much care as ever	0
11. I feel restless as if I have to be on the move:	A
Very much indeed	3
Quite a lot	2
Not very much	1
Not at all	0
12. I look forward with enjoyment to things:	D
As much as ever I did	0
Rather less than I used to	1
Definitely less than I used to	2
Hardly at all	3
13. I get sudden feelings of panic:	A
Very often indeed	3
Quite often	2
Not very often	1
Not at all	0
14. I can enjoy a good book or radio or TV programme:	D
Often	0
Sometime	1
Not often	2
Very seldom	3

Interpretation of Answers:

The total score of the seven questions assessing anxiety, and the seven questions assessing depression will be calculated separately giving a score out of 21 for both respective conditions. There is no definitive 'cut-off' score to determine if anxiety or depression *are present* but the score will give a value for *how much* anxiety or depression exits. Scores from 0-7 suggest probable absence of anxiety or depression, 8-10 suggest possible presence, and 11-21 suggest probable presence.

Appendix 5: Simple Clinical Colitis Activity Index

Patients are assessed on each of the six questions below and a score is equated to their response. The six scores are added together to give the SCCAI result. Relapse is defined as a total score ≥ 5.

1.	Bowel frequency per day	Score
	1-3	0
	4-6	1
	7-9	2
	>9	3
2.	Bowel frequency per night	
	1-3	1
	4-6	2
3.	Urgency of defaecation	
	a. Hurry	1
	b. Immediate	2
	c. Incontinence	3
4.	Blood in stool	
	a. Trace	1
	b. Occasionally Frank	2
	c. Usually Frank	3
5.	General well being	
	a. Very well	0
	b. Slightly below par	1
	c. Poor	2
	d. Very poor	3
	e. Terrible	5
6.	Extra-colonic features	1 per manifestation

Appendix 6: Harvey Bradshaw Index

Patients are assessed on the following 5 aspects. The scores are added together to give the HBI result. Active disease is defined as a score ≥ 5.
Based on the previous day…..

1. General well-being (0=very well, 1=slightly below par, 2=poor, 3=very poor, 4= terrible) …..

2. Abdominal pain (0=none, 1=mild, 2=moderate, 3=severe) …..

3. Number of liquid stools per day …..

4. Abdominal mass (0=none, 1=dubious, 2=definite, 3=definite + tender) …..

5. Complications (arthralgia, uveitis, erythema nodosum, aphthous ulcers, pyoderma gangrenosum, anal fissure, new fistula, abscess) …..

Appendix 7: Non-Colonic Symptoms Questionnaire

Please indicate if you experience any of the following symptoms regularly:

Headache ☐

Muscular Aches ☐

Inappropriate severe fatigue ☐

Loss of concentration or poor memory ☐

Rhinitis ☐

Itching ☐

Palpitations ☐

Mouth Ulcers ☐

Sore Throats ☐

Sleep Disturbance ☐

Appendix 8: Revised Daily Hassles Scale

Hassles are irritants – things that annoy or bother you; they can make you upset or angry. Some hassles occur on a regular basis and others are rare. Some have only a slight effect, others have a strong effect. This questionnaire lists things that can be a hassle in your day-to-day life.

Please think about how much of a hassle each item has been for you in the last month, and circle the appropriate number.

0 = None, or Not Applicable
1 = Somewhat
2 = Quite a bit
3 = A great deal

1. Your child(ren)	0	1	2	3
2. Your parents or parents-in-law	0	1	2	3
3. Other relative(s)	0	1	2	3
4. Your spouse	0	1	2	3
5. Time spent with family	0	1	2	3
6. Health or well-being of a family member	0	1	2	3
7. Sex	0	1	2	3
8. Intimacy	0	1	2	3
9. Family-related obligations	0	1	2	3
10. Your friend(s)	0	1	2	3
11. Fellow workers	0	1	2	3
12. Clients, customers, patients, etc.	0	1	2	3
13. Your supervisor or employer	0	1	2	3
14. The nature of your work	0	1	2	3
15. Your workload	0	1	2	3
16. Your job security	0	1	2	3
17. Meeting deadlines or goals on the job	0	1	2	3
18. Money for necessities (e.g. food, clothing, housing)	0	1	2	3

19. Money for education	0	1	2	3
20. Money for emergencies	0	1	2	3
21. Money for extras (e.g. leisure, holidays)	0	1	2	3
22. Financial care for others not living with you	0	1	2	3
23. Investments	0	1	2	3
24. Your smoking	0	1	2	3
25. Your drinking	0	1	2	3
26. Recreational Drugs	0	1	2	3
27. Your physical appearance	0	1	2	3
28. Contraception	0	1	2	3
29. Exercise	0	1	2	3
30. Your medical care	0	1	2	3
31. Your health	0	1	2	3
32. Your physical abilities	0	1	2	3
33. The weather	0	1	2	3
34. News Events	0	1	2	3
35. Your environment (quality of air, noise, etc.)	0	1	2	3
36. Political or social issues	0	1	2	3
37. Your neighbourhood	0	1	2	3
38. Conserving Gas, Electricity, Water	0	1	2	3
39. Pets	0	1	2	3
40. Cooking	0	1	2	3
41. Housework	0	1	2	3
42. Home repairs	0	1	2	3
43. Yardwork	0	1	2	3
44. Car maintenance	0	1	2	3

45. Taking care of paperwork	0	1	2	3
46. Home entertainment (TV, Computer, etc.)	0	1	2	3
47. Amount of free time	0	1	2	3
48. Recreation outside the home	0	1	2	3
49. Eating	0	1	2	3
50. Church or community organisations	0	1	2	3
51. Legal matters	0	1	2	3
52. Being organised	0	1	2	3
53. Social commitments	0	1	2	3

Appendix 9: Levenstein's Perceived Stress Questionnaire

Patient Advice:
For each sentence, circle the number that describes how often it applies to you during the last month. Work quickly, without bothering to check your answers, and be careful to consider only the last month.

	Almost Never	Sometimes	Often	Usually
1. You feel rested	1	2	3	4
2. You feel that too many demands are being made on you	1	2	3	4
3. You are irritable or grouchy	1	2	3	4
4. You have too many things to do	1	2	3	4
5. You feel lonely or isolated	1	2	3	4
6. You find yourself in situations of conflict	1	2	3	4
7. You feel you're doing things you really like	1	2	3	4
8. You feel tired	1	2	3	4
9. You feel you may not manage to attain your goals	1	2	3	4
10. You feel calm	1	2	3	4
11. You have too many decisions to make	1	2	3	4
12. You feel frustrated	1	2	3	4
13. You are full of energy	1	2	3	4
14. You feel tense	1	2	3	4
15. Your problems seem to be building up	1	2	3	4
16. You feel you're in a hurry	1	2	3	4
17. You feel safe and protected	1	2	3	4
18. You have many worries	1	2	3	4
19. You are under pressure from other people	1	2	3	4
20. You feel discouraged	1	2	3	4
21. You enjoy yourself	1	2	3	4
22. You are afraid for the future	1	2	3	4
23. You feel you're doing things because you have to, not because you want to	1	2	3	4
24. You feel criticized or judged	1	2	3	4
25. You are light-hearted	1	2	3	4
26. You feel mentally exhausted	1	2	3	4
27. You have trouble relaxing	1	2	3	4
28. You feel loaded down with responsibility	1	2	3	4
29. You have enough time for yourself	1	2	3	4
30. You feel under pressure from deadlines	1	2	3	4

Interpretation of Answers:
Subtract the circled number from 5 for items 1, 7, 10, 13, 17, 21, 25, 29
Score the circled number for all other items
PSQ Index = (Overall score – 30) / 90

Appendix 10: The Ways of Coping Checklist

Please try and remember a stressful situation that you have experienced at work in the last month. If you can't think of a work situation please think of another situation. Now please read each of the following items and circle or underline the number next to each on the scale from 0 to 3, to show how much you used each approach to try and deal with the stress and to make yourself feel better.

0 = Used not at all; 1 = Used sometimes; 2 = Used often; 3 = Used all the time.

1. Bargained or compromised to get something positive from the situation [0] [1] [2] [3]

2. Concentrated on something good that could result [0] [1] [2] [3]

3. Tried not to burn my bridges behind me, tried to leave things open. [0] [1] [2] [3]

4. Changed myself to be a better person. [0] [1] [2] [3]

5. Made a plan of action and followed it. [0] [1] [2] [3]

6. Accepted the next best thing to what I wanted. [0] [1] [2] [3]

7. Came out of the experience a better person than when I went in. [0] [1] [2] [3]

8. Tried not to act too hastily. [0] [1] [2] [3]

9. Changed something so things would turn out all right. [0] [1] [2] [3]

10. Just took things one step at a time. [0] [1] [2] [3]

11. I knew what had to be done, so I tried harder to make things work. [0] [1] [2] [3]

12. Came up with a couple of different solutions to the problem. [0] [1] [2] [3]

13. Accepted my strong feelings, but didn't let them interfere with things [0] [1] [2] [3]

14. Changed something about myself so I could deal with situation better [0] [1] [2] [3]

15. Stood my ground and fought for what I wanted. [0] [1] [2] [3]

16. Talked to someone to find out more about the situation. [0] [1] [2] [3]

17. Accepted sympathy and understanding from someone. [0] [1] [2] [3]

18. Got professional help and did what they recommended. [0] [1] [2] [3]

19. Talked to someone who could do something about the problem. [0] [1] [2] [3]

20. Asked someone I respected for advice and followed it. [0] [1] [2] [3]

21. Talked to someone about how I was feeling. [0] [1] [2] [3]

22. Blamed myself. [0] [1] [2] [3]

23. Criticized or lectured myself. [0] [1] [2] [3]

24. Realised I brought the problem on myself. [0] [1] [2] [3]

25. Hoped a miracle would happen. [0] [1] [2] [3]

26. Wished I was a stronger person – more optimistic and forceful. [0] [1] [2] [3]

27. Wished that I could change what had happened. [0] [1] [2] [3]

28. Wished I could change the way that I felt. [0] [1] [2] [3]

29. Daydreamed or imagined a better time or place than the one I was in. [0] [1] [2] [3]

30. Had fantasies or wishes about how things might turn out. [0] [1] [2] [3]

31. Thought about fantastic things to make myself feel better. [0] [1] [2] [3]

32. Wished the situation would go away or somehow be finished. [0] [1] [2] [3]

33. Went on as if nothing had happened. [0] [1] [2] [3]

34. Felt bad that I couldn't avoid the problem. [0] [1] [2] [3]

35. Kept my feelings to myself. [0] [1] [2] [3]

36. Slept more than usual. [0] [1] [2] [3]

37. Got angry at the people or things that caused the problem. [0] [1] [2] [3]

38. Tried to forget the whole thing. [0] [1] [2] [3]

39. Tried to make myself feel better by eating, drinking, smoking, etc. [0] [1] [2] [3]

40. Avoided being with other people. [0] [1] [2] [3]

41. Didn't tell others how bad things were. [0] [1] [2] [3]

42. Refused to believe it had happened. [0] [1] [2] [3]

Appendix 11: Inflammatory Bowel Disease Questionnaire

This questionnaire is designed to find out how you have been feeling during the last 2 weeks. You will be asked about symptoms you have been having as a result of your inflammatory bowel disease, the way you have been feeling in general, and how your mood has been.

1. How frequent have your bowel movements been during the last two weeks? Please indicate how frequent your bowel movements have been during the last two weeks by picking one of the options from
1 Bowel movements as or more frequent than they have ever been
2 Extremely frequent
3 Very frequent
4 Moderate increase in frequency of bowel movements
5 Some increase in frequency of bowel movements
6 Slight increase in frequency of bowel movements
7 Normal, no increase in frequency of bowel movements

2. How often has the feeling of fatigue or of being tired and worn out been a problem for you during the last 2 weeks? Please indicate how often the feeling of fatigue or tiredness has been a problem for you during the last 2 weeks by picking one of the options from:
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

3. How often during the last 2 weeks have you felt frustrated, impatient, or restless? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

4. How often during the last 2 weeks have you been unable to attend school or do your work because of your bowel problem? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

5. How much of the time during the last 2 weeks have your bowel movements been loose? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

6. How much energy have you had during the last 2 weeks? Please choose an option from
1 No energy at all
2 Very little energy
3 A little energy
4 Some energy
5 A moderate amount of energy
6 A lot of energy
7 Full of energy

7. How often during the last 2 weeks did you feel worried about the possibility of needing to have surgery because of your bowel problem? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

8. How often during the last 2 weeks have you had to delay or cancel a social engagement because of your bowel problem? Please choose an option from:
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

9. How often during the last 2 weeks have you been troubled by cramps in your abdomen? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

10. How often during the last 2 weeks have you felt generally unwell? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

11. How often during the last 2 weeks have you been troubled because of fear of not finding a washroom? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

12. How much difficulty have you had, as a result of your bowel problems, doing leisure or sports activities you would have liked to have done during the last 2 weeks? Please choose an option from:
1 A great deal of difficulty; activities made impossible
2 A lot of difficulty
3 A fair bit of difficulty
4 Some difficulty
5 A little difficulty
6 Hardly any difficulty
7 No difficulty; the bowel problems did not limit sports or leisure activities

13. How often during the last 2 weeks have you been troubled by pain in the abdomen? Please choose an option from:
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

14. How often during the last 2 weeks have you had problems getting a good night's sleep, or been troubled by waking up during the night? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

15. How often during the last 2 weeks have you felt depressed or discouraged? Please choose an option from:
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

16. How often during the last 2 weeks have you had to avoid attending events when there was no washroom close at hand? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

17. Overall, in the last 2 weeks, how much of a problem have you had with passing large amounts of gas? Please choose an option from:
1 A major problem
2 A big problem
3 A significant problem
4 Some trouble
5 A little trouble
6 Hardly any trouble
7 No trouble

18. Overall, in the last 2 weeks, how much of a problem have you had maintaining or getting to, the weight you would like to be at? Please choose an option from:
1 A major problem
2 A big problem
3 A significant problem
4 Some trouble
5 A little trouble
6 Hardly any trouble
7 No trouble

19. Many patients with bowel problems often have worries and anxieties related to their illness. These include worries about getting cancer, worries about never feeling any better, and worries about having a relapse. In general, how often during the last 2 weeks have you felt worried or anxious? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

20. How much of the time during the last 2 weeks have you been troubled by a feeling of abdominal bloating? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

21. How often during the last 2 weeks have you felt relaxed and free of tension? Please choose an option from:
1 None of the time
2 A little of the time
3 Some of the time
4 A good bit of the time
5 Most of the time
6 Almost all of the time
7 All of the time

22. How much of the time during the last 2 weeks have you had a problem with rectal bleeding with your bowel movements? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

23. How much of the time during the last 2 weeks have you felt embarrassed as a result of your bowel problem? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

24. How much of the time during the last 2 weeks have you been troubled by a feeling of having to go to the bathroom even though your bowels were empty? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

25. How much of the time during the last 2 weeks have you felt tearful or upset? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

26. How much of the time during the last 2 weeks have you been troubled by accidental soiling of your underpants? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

27. How much of the time during the last 2 weeks have you felt angry as a result of your bowel problem? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

28. To what extent has your bowel problem limited sexual activity during the last 2 weeks? Please choose an option from
1. No sex as a result of bowel disease
2. Major limitation as a result of bowel disease
3. Moderate limitation as a result of bowel disease
4. Some limitation as a result of bowel disease
5. A little limitation as a result of bowel disease
6. Hardly any limitation as a result of bowel disease
7. No limitation as a result of bowel disease

29. How much of the time during the last 2 weeks have you been troubled by nausea or feeling sick to your stomach? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

30. How much of the time during the last 2 weeks have you felt irritable? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

31. How often during the past 2 weeks have you felt a lack of understanding from others? Please choose an option from
1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

32. How satisfied, happy, or pleased have you been with your personal life during the past 2weeks? Please choose one of the following options from
1 Very dissatisfied, unhappy most of the time
2 Generally dissatisfied, unhappy
3 Somewhat dissatisfied, unhappy
4 Generally satisfied, pleased
5 Satisfied most of the time, happy
6 Very satisfied most of the time, happy
7 Extremely satisfied, could not have been more happy or pleased

Appendix 12: National Adult Reading Test

Below is a list of words that you will be asked to read out aloud. Please read out one word at a time, and wait for the assessor to indicate when to move onto the next word. Most people will not recognise some of the words that follow but please guess at the pronunciation if you are unsure.

CHORD
ACHE
DEPOT
AISLE
BOUQUET
PSALM
CAPON
DENY
NAUSEA
DEBT
COURTEOUS
RAREFY
EQUIVOCAL
NAÏVE
CATACOMB
GAOLED
THYME
HEIR
RADIX
ASSIGNEE
HIATUS
SUBTLE
PROCREATE
GIST
GOUGE
SUPERFLUOUS
SIMILE
BANAL
QUADRUPED
CELLIST
FAÇADE
ZEALOT
DRACHM
AEON
PLACEBO
ABSTEMIOUS
DÉTENTE
IDYLL
PUERPERAL
AVER
GAUCHE
TOPIARY
LEVIATHAN
BEATIFY
PRELATE
SIDEREAL
DEMESNE
SYNCOPE
LABILE
CAMPANILE

Appendix 13: Big Five Inventory

Here are a number of characteristics that may or may not apply to you. For example, do you agree that you are someone who likes to spend time with others? Please circle a number next to each statement to indicate the extent to which you agree or disagree with that statement.

1 Disagree Strongly	**2** Disagree a little	**3** Neither agree nor disagree	**4** Agree a little	**5** Agree strongly

I am someone who:

1. Is talkative	1	2	3	4	5
2. Tends to find fault with others	1	2	3	4	5
3. Does a thorough job	1	2	3	4	5
4. Is depressed, blue	1	2	3	4	5
5. Is original, comes up with new ideas	1	2	3	4	5
6. Is reserved	1	2	3	4	5
7. Is helpful and unselfish with others	1	2	3	4	5
8. Can be somewhat careless	1	2	3	4	5
9. Is relaxed, handles stress well	1	2	3	4	5
10. Is curious about many different things	1	2	3	4	5
11. Is full of energy	1	2	3	4	5
12. Starts quarrels with others	1	2	3	4	5
13. Is a reliable worker	1	2	3	4	5
14. Can be tense	1	2	3	4	5
15. Is ingenious, a deep thinker	1	2	3	4	5
16. Generates a lot of enthusiasm	1	2	3	4	5
17. Has a forgiving nature	1	2	3	4	5
18. Tends to be disorganized	1	2	3	4	5
19. Worries a lot	1	2	3	4	5
20. Has an active imagination	1	2	3	4	5
21. Tends to be quiet	1	2	3	4	5

22. Is generally trusting	1	2	3	4	5
23. Tends to be lazy	1	2	3	4	5
24. Is emotionally stable, not easily upset	1	2	3	4	5
25. Is inventive	1	2	3	4	5
26. Has an assertive personality	1	2	3	4	5
27. Can be cold and aloof	1	2	3	4	5
28. Perseveres until the task is finished	1	2	3	4	5
29. Can be moody	1	2	3	4	5
30. Values artistic, aesthetic experiences	1	2	3	4	5
31. Is sometimes shy, inhibited	1	2	3	4	5
32. Is considerate and kind to almost everyone	1	2	3	4	5
33. Does things efficiently	1	2	3	4	5
34. Remains calm in tense situations	1	2	3	4	5
35. Prefers work that is routine	1	2	3	4	5
36. Is outgoing, sociable	1	2	3	4	5
37. Is sometimes rude to others	1	2	3	4	5
38. Makes plans and follows through with them	1	2	3	4	5
39. Gets nervous easily	1	2	3	4	5
40. Likes to reflect, play with ideas	1	2	3	4	5
41. Has few artistic interests	1	2	3	4	5
42. Likes to cooperate with others	1	2	3	4	5
43. Is easily distracted	1	2	3	4	5
44. Is sophisticated in art, music, or literature	1	2	3	4	5

Appendix 14: Interpersonal Support Evaluation List

This scale is made up of a list of statements, each of which may or may not be true about you. For each statement tick 'definitely true' if you are sure it is true about you and 'probably true' if you think it is true but are not absolutely certain. Similarly, you should tick 'definitely false' if you are sure the statement is false and 'probably false' if you think it is false but are not absolutely certain.

1. There are several people I trust to help solve my problem.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

2. If I need help mending something , (e.g. an appliance, car, clothes, furniture), there is someone who would help me.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

3. Most of my friends are more interesting than I am.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

4. There is someone who takes pride in my accomplishments.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

5. When I feel lonely, there are several people I can talk to.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

6. There is no one that I feel comfortable talking to about intimate personal problems.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

7. I often meet or talk with family or friends.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

8. Most people I know think highly of me.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

9. If I need a lift very early in the morning (e.g to the tube station, train station, or airport), I would have a hard time finding anyone to take me.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

10. I feel like I'm not always included in my circle of friends.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

11. There is really no one who can give me an objective view of how I'm handling my problems.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

12. There are several different people I enjoy spending time with.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

13. I think that my friends feel that I'm not very good at helping them solve their problems.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

14. If I were ill and needed someone (friend , family member, or acquaintance) to take me to the doctor, I would have trouble finding someone.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

15. If I wanted to go on a trip or outing for a day (e.g. to the seaside or countryside), I would have a hard time finding someone to go with me.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

16. If I needed a place to stay for a week because of an emergency (e.g. water or electricity not working in my flat or house), I could easily find someone who would put me up.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

17. I feel there is no one I can share my most private worries and fears with.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

18. If I were ill, I could easily find someone to help me with my daily chores.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

19. There is someone I can turn to for advice about handling problems with my family.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

20. I'm as good at doing things as most people are.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

21. If I decide one afternoon that I would like to go out (e.g. to the cinema) that evening, I could find someone to go with me.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

22. When I need suggestions on how to deal with a personal problem , I know someone I can turn to.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

23. If I needed an emergency loan of £100, there is someone (friend, relative or acquaintance) I could get it from.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

24. In general, people do not have much confidence in me.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

25. Most people I know do not enjoy the same things that I do.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

26. There is someone I could turn to for advice about making career plans or about changing my job.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

27. I don't get invited to do things with others.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

28. Most of my friends are more successful at making changes in their lives than I am.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

29. If I had to go away from home for a few weeks , there is someone I know who would look after my house or flat (the plants, pets, garden, etc.).

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

30. There is really no one I can trust to give me good financial advice.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

31. If I wanted to have lunch with someone , I could easily find someone to join me.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

32. I am more satisfied with my life than most people are with theirs.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

33. If I was stranded 10 miles from home, there is someone I could call who would come and collect me.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

34. No one I know would throw a birthday party for me.

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

35. It would be difficult to find someone who would lend me their car for a few hours. (If you don't drive, assume for the purpose of this question that you have someone to drive you, but no car).

4 = definitely true	1 = definitely false
3 = probably true	2 = probably false

36. If a family crisis arose, it would be difficult to find someone who could give me good advice about how to handle it.

4 = definitely true
3 = probably true
1 = definitely false
2 = probably false

37. I am closer to my friends than most people are to theirs.

4 = definitely true
3 = probably true
1 = definitely false
2 = probably false

38. There is at least one person I know whose advice I really trust.

4 = definitely true
3 = probably true
1 = definitely false
2 = probably false

39. If I needed some help in moving to a new house or flat, I would have a hard time finding someone to help me.

4 = definitely true
3 = probably true
1 = definitely false
2 = probably false

40. I have a hard time keeping pace with my friends.

4 = definitely true
3 = probably true
1 = definitely false
2 = probably false

References

References:

1. Dalziel TK. Chronic interstitial enteritis. BMJ. 1913;2(2756):1068-70.
2. Crohn BB, Ginzburg L, Oppenheimer GD. Regional ileitis: a pathologic and clinical entity. J Am Med Assoc. 1932;99(16):1323-9.
3. De Dombal FT. Ulcerative colitis: definition, historical background, aetiology, diagnosis, naturel history and local complications. Postgrad Med J. 1968 Sep;44(515):684-92.
4. Loftus EV, Jr. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology. 2004 May;126(6):1504-17.
5. Ponder A, Long MD. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. Clinical epidemiology. 2013;5:237-47.
6. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012 Jan;142(1):46-54.e42; quiz e30.
7. Rubin GP, Hungin AP, Kelly PJ, et al. Inflammatory bowel disease: epidemiology and management in an English general practice population. Aliment Pharmacol Ther. 2000 Dec;14(12):1553-9.
8. Probert CS, Jayanthi V, Pinder D, et al. Epidemiological study of ulcerative proctocolitis in Indian migrants and the indigenous population of Leicestershire. Gut. 1992 May;33(5):687-93.
9. Pinsk V, Lemberg DA, Grewal K, et al. Inflammatory bowel disease in the South Asian pediatric population of British Columbia. Am J Gastroenterol. 2007 May;102(5):1077-83.
10. Tjonneland A, Overvad K, Bergmann MM, et al. Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis: a nested case-control study within a European prospective cohort study. Gut. 2009 Dec;58(12):1606-11.
11. Ananthakrishnan AN, Khalili H, Higuchi LM, et al. Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. Gastroenterology. 2012 Mar;142(3):482-9.
12. Carbonnel F, Boutron MC. Ulcerative colitis: is it in the diet? Gut. 2009 Dec;58(12):1577-9.
13. Card T, Logan RF, Rodrigues LC, et al. Antibiotic use and the development of Crohn's disease. Gut. 2004 Feb;53(2):246-50.
14. Koutroubakis IE, Vlachonikolis IG, Kouroumalis EA. Role of appendicitis and appendectomy in the pathogenesis of ulcerative colitis: a critical review. Inflamm Bowel Dis. 2002 Jul;8(4):277-86.
15. Andersson RE, Olaison G, Tysk C, et al. Appendectomy and protection against ulcerative colitis. N Engl J Med. 2001 Mar 15;344(11):808-14.
16. Mahid SS, Minor KS, Soto RE, et al. Smoking and inflammatory bowel disease: a meta-analysis. Mayo Clin Proc. 2006 Nov;81(11):1462-71.
17. Bernstein CN. Why and where to look in the environment with regard to the etiology of inflammatory bowel disease. Dig Dis. 2012;30 Suppl 3:28-32.
18. Russell RK, Satsangi J. IBD: a family affair. Best Pract Res Clin Gastroenterol. 2004 Jun;18(3):525-39.

19. Hugot JP, Laurent-Puig P, Gower-Rousseau C, et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature. 1996 Feb 29;379(6568):821-3.
20. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature. 2001 May 31;411(6837):599-603.
21. Van Limbergen J, Russell RK, Nimmo ER, et al. The genetics of inflammatory bowel disease. Am J Gastroenterol. 2007 Dec;102(12):2820-31.
22. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012 Nov 1;491(7422):119-24.
23. Eckburg PB, Relman DA. The role of microbes in Crohn's disease. Clin Infect Dis. 2007 Jan 15;44(2):256-62.
24. Hedin C, McCarthy NE, Louis P, et al. A discriminant analysis demonstrates that siblings of patients with Crohn's disease have a distinct microbiological and immune phenotype compared with healthy controls: insights into disease pathogenesis. Gut. 2013;62(Supplement 1):A7.
25. Sellon RK, Tonkonogy S, Schultz M, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun. 1998 Nov;66(11):5224-31.
26. Turner JR. Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application. Am J Pathol. 2006 Dec;169(6):1901-9.
27. Torres MI, Rios A. Current view of the immunopathogenesis in inflammatory bowel disease and its implications for therapy. World J Gastroenterol. 2008 Apr 7;14(13):1972-80.
28. Ordas I, Eckmann L, Talamini M, et al. Ulcerative colitis. Lancet. 2012 Nov 3;380(9853):1606-19.
29. Buisine MP, Desreumaux P, Debailleul V, et al. Abnormalities in mucin gene expression in Crohn's disease. Inflamm Bowel Dis. 1999 Feb;5(1):24-32.
30. Soderholm JD, Olaison G, Peterson KH, et al. Augmented increase in tight junction permeability by luminal stimuli in the non-inflamed ileum of Crohn's disease. Gut. 2002 Mar;50(3):307-13.
31. Van der Sluis M, De Koning BA, De Bruijn AC, et al. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology. 2006 Jul;131(1):117-29.
32. Hart AL, Al-Hassi HO, Rigby RJ, et al. Characteristics of intestinal dendritic cells in inflammatory bowel diseases. Gastroenterology. 2005 Jul;129(1):50-65.
33. Cader MZ, Kaser A. Recent advances in inflammatory bowel disease: mucosal immune cells in intestinal inflammation. Gut. 2013 Nov;62(11):1653-64.
34. Baumgart DC, Sandborn WJ. Crohn's disease. Lancet. 2012 Nov 3;380(9853):1590-605.
35. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol. 2005 Sep;19 Suppl A:5-36.
36. Foell D, Wittkowski H, Roth J. Monitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage. Gut. 2009 Jun;58(6):859-68.

37. Langhorst J, Elsenbruch S, Koelzer J, et al. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. Am J Gastroenterol. 2008 Jan;103(1):162-9.
38. Roseth AG, Fagerhol MK, Aadland E, et al. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol. 1992 Sep;27(9):793-8.
39. Centre for Evidence-based Purchasing. Evidence review: Value of calprotectin in screening out irritable bowel syndrome. 2010.
40. Sidhu R, Sanders DS, Morris AJ, et al. Guidelines on small bowel enteroscopy and capsule endoscopy in adults. Gut. 2008 Jan;57(1):125-36.
41. Faubion WA, Jr., Loftus EV, Jr., Harmsen WS, et al. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. Gastroenterology. 2001 Aug;121(2):255-60.
42. National Institute of Health and Clinical Evidence. Ulcerative Colitis: Management in adults, children and young people. CG166. London: National Institute of Health and Clinical Evidence; 2013.
43. Frolkis AD, Dykeman J, Negron ME, et al. Risk of Surgery for the Inflammatory Bowel Diseases has Decreased Over Time: a Systematic Review and Meta-Analysis of Population-Based Studies. Gastroenterology. 2013 Jul 26.
44. D'Haens G, Baert F, van Assche G, et al. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. Lancet. 2008 Feb 23;371(9613):660-7.
45. Colombel JF, Sandborn WJ, Rutgeerts P, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gastroenterology. 2007 Jan;132(1):52-65.
46. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med. 2010 Apr 15;362(15):1383-95.
47. Siegel CA, Marden SM, Persing SM, et al. Risk of lymphoma associated with combination anti-tumor necrosis factor and immunomodulator therapy for the treatment of Crohn's disease: a meta-analysis. Clin Gastroenterol Hepatol. 2009 Aug;7(8):874-81.
48. Toruner M, Loftus EV, Jr., Harmsen WS, et al. Risk factors for opportunistic infections in patients with inflammatory bowel disease. Gastroenterology. 2008 Apr;134(4):929-36.
49. Van Assche G, Dignass A, Reinisch W, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. J Crohns Colitis. 2010 Feb;4(1):63-101.
50. Jess T, Riis L, Vind I, et al. Changes in clinical characteristics, course, and prognosis of inflammatory bowel disease during the last 5 decades: a population-based study from Copenhagen, Denmark. Inflamm Bowel Dis. 2007 Apr;13(4):481-9.
51. Bewtra M, Kaiser LM, TenHave T, et al. Crohn's disease and ulcerative colitis are associated with elevated standardized mortality ratios: a meta-analysis. Inflamm Bowel Dis. 2013 Mar;19(3):599-613.

52. Solberg IC, Vatn MH, Hoie O, et al. Clinical course in Crohn's disease: results of a Norwegian population-based ten-year follow-up study. Clin Gastroenterol Hepatol. 2007 Dec;5(12):1430-8.
53. Lahiff C, Safaie P, Awais A, et al. The Crohn's disease activity index (CDAI) is similarly elevated in patients with Crohn's disease and in patients with irritable bowel syndrome. Aliment Pharmacol Ther. 2013 Apr;37(8):786-94.
54. Costa F, Mumolo MG, Bellini M, et al. Role of faecal calprotectin as non-invasive marker of intestinal inflammation. Dig Liver Dis. 2003 Sep;35(9):642-7.
55. Sipponen T, Karkkainen P, Savilahti E, et al. Correlation of faecal calprotectin and lactoferrin with an endoscopic score for Crohn's disease and histological findings. Aliment Pharmacol Ther. 2008 Nov 15;28(10):1221-9.
56. Tibble J, Teahon K, Thjodleifsson B, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut. 2000 Oct;47(4):506-13.
57. Chang J, Kennedy N, Fasci Spurio F, et al. Correlation of clinical symptoms to current biomarkers of intestinal inflammation in patients with Crohn's disease. Journal of Crohn's and Colitis. 2013;7(S1):S116.
58. Eadala P, Matthews SB, Waud JP, et al. Association of lactose sensitivity with inflammatory bowel disease--demonstrated by analysis of genetic polymorphism, breath gases and symptoms. Aliment Pharmacol Ther. 2011 Oct;34(7):735-46.
59. Mikocka-Walus AA, Turnbull DA, Moulding NT, et al. Controversies surrounding the comorbidity of depression and anxiety in inflammatory bowel disease patients: a literature review. Inflamm Bowel Dis. 2007 Feb;13(2):225-34.
60. Graff LA, Walker JR, Bernstein CN. Depression and anxiety in inflammatory bowel disease: a review of comorbidity and management. Inflamm Bowel Dis. 2009 Jul;15(7):1105-18.
61. Gue M, Bonbonne C, Fioramonti J, et al. Stress-induced enhancement of colitis in rats: CRF and arginine vasopressin are not involved. Am J Physiol. 1997 Jan;272(1 Pt 1):G84-91.
62. Qiu BS, Vallance BA, Blennerhassett PA, et al. The role of CD4+ lymphocytes in the susceptibility of mice to stress-induced reactivation of experimental colitis. Nat Med. 1999 Oct;5(10):1178-82.
63. Mawdsley JE, Rampton DS. Psychological stress in IBD: new insights into pathogenic and therapeutic implications. Gut. 2005 Oct;54(10):1481-91.
64. Maunder RG, Levenstein S. The role of stress in the development and clinical course of inflammatory bowel disease: epidemiological evidence. Curr Mol Med. 2008 Jun;8(4):247-52.
65. Levenstein S, Prantera C, Varvo V, et al. Stress and exacerbation in ulcerative colitis: a prospective study of patients enrolled in remission. Am J Gastroenterol. 2000 May;95(5):1213-20.
66. Bitton A, Dobkin PL, Edwardes MD, et al. Predicting relapse in Crohn's disease: a biopsychosocial model. Gut. 2008 Oct;57(10):1386-92.
67. Mittermaier C, Dejaco C, Waldhoer T, et al. Impact of depressive mood on relapse in patients with inflammatory bowel disease: a prospective 18-month follow-up study. Psychosom Med. 2004 Jan-Feb;66(1):79-84.
68. Goodhand J, Rampton D. Psychological stress and coping in IBD. Gut. 2008 Oct;57(10):1345-7.
69. Drossman D. The Functional Gastrointestinal Disorders and the Rome III Process. Gastroenterology. 2006;130:1377-90.

70. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. Gastroenterology. 2006 Apr;130(5):1480-91.
71. Drossman DA, Morris CB, Hu Y, et al. A prospective assessment of bowel habit in irritable bowel syndrome in women: defining an alternator. Gastroenterology. 2005 Mar;128(3):580-9.
72. Drossman DA, Camilleri M, Mayer EA, et al. AGA technical review on irritable bowel syndrome. Gastroenterology. [Review]. 2002;123:2108-31.
73. Lovell RM, Ford AC. Effect of gender on prevalence of irritable bowel syndrome in the community: systematic review and meta-analysis. Am J Gastroenterol. 2012 Jul;107(7):991-1000.
74. Henningsen P, Zimmermann T, Sattel H. Medically unexplained physical symptoms, anxiety, and depression: a meta-analytic review. Psychosom Med. 2003 Jul-Aug;65(4):528-33.
75. Ford AC, Talley NJ, Schoenfeld PS, et al. Efficacy of antidepressants and psychological therapies in irritable bowel syndrome: systematic review and meta-analysis. Gut. 2009 Mar;58(3):367-78.
76. Whitehead WE, Palsson O, Jones KR. Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications? Gastroenterology. 2002 Apr;122(4):1140-56.
77. Halder SL, Locke GR, 3rd, Talley NJ, et al. Impact of functional gastrointestinal disorders on health-related quality of life: a population-based case-control study. Aliment Pharmacol Ther. 2004 Jan 15;19(2):233-42.
78. Longstreth GF, Wilson A, Knight K, et al. Irritable bowel syndrome, health care use, and costs: a U.S. managed care perspective. Am J Gastroenterol. 2003 Mar;98(3):600-7.
79. Bassotti G, Chistolini F, Marinozzi G, et al. Abnormal colonic propagated activity in patients with slow transit constipation and constipation-predominant irritable bowel syndrome. Digestion. 2003;68(4):178-83.
80. Chey WY, Jin HO, Lee MH, et al. Colonic motility abnormality in patients with irritable bowel syndrome exhibiting abdominal pain and diarrhea. Am J Gastroenterol. 2001 May;96(5):1499-506.
81. Spiller R, Aziz Q, Creed F, et al. Guidelines on the irritable bowel syndrome: mechanisms and practical management. Gut. 2007 Dec;56(12):1770-98.
82. Azpiroz F, Bouin M, Camilleri M, et al. Mechanisms of hypersensitivity in IBS and functional disorders. Neurogastroenterol Motil. 2007 Jan;19(1 Suppl):62-88.
83. Mertz H, Naliboff B, Munakata J, et al. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. Gastroenterology. 1995 Jul;109(1):40-52.
84. Kwan CL, Diamant NE, Mikula K, et al. Characteristics of rectal perception are altered in irritable bowel syndrome. Pain. 2005 Jan;113(1-2):160-71.
85. Furness JB. The enteric nervous system. Oxford: Blackwell; 2006.
86. Lightman SL. The neuroendocrinology of stress: a never ending story. J Neuroendocrinol. 2008 Jun;20(6):880-4.
87. Chrousos GP. Stress and disorders of the stress system. Nat Rev Endocrinol. 2009 Jul;5(7):374-81.

88. Rao SS, Hatfield RA, Suls JM, et al. Psychological and physical stress induce differential effects on human colonic motility. Am J Gastroenterol. 1998 Jun;93(6):985-90.
89. Barclay GR, Turnberg LA. Effect of psychological stress on salt and water transport in the human jejunum. Gastroenterology. 1987 Jul;93(1):91-7.
90. Gonlachanvit S, Rhee J, Sun WM, et al. Effect of acute acoustic stress on anorectal function sensation in healthy human. Neurogastroenterol Motil. 2005 Apr;17(2):222-8.
91. Saunders PR, Santos J, Hanssen NP, et al. Physical and psychological stress in rats enhances colonic epithelial permeability via peripheral CRH. Dig Dis Sci. 2002 Jan;47(1):208-15.
92. O'Malley D, Quigley EM, Dinan TG, et al. Do interactions between stress and immune responses lead to symptom exacerbations in irritable bowel syndrome? Brain Behav Immun. 2011 Apr 23.
93. Spiller R, Garsed K. Postinfectious irritable bowel syndrome. Gastroenterology. 2009 May;136(6):1979-88.
94. Rajilic-Stojanovic M, Biagi E, Heilig HG, et al. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology. 2011 Nov;141(5):1792-801.
95. Jeffery IB, O'Toole PW, Ohman L, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut. 2012 Jul;61(7):997-1006.
96. Islam KB, Fukiya S, Hagio M, et al. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology. 2011 Nov;141(5):1773-81.
97. Tana C, Umesaki Y, Imaoka A, et al. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. Neurogastroenterol Motil. 2010 May;22(5):512-9, e114-5.
98. Fukumoto S, Tatewaki M, Yamada T, et al. Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. Am J Physiol Regul Integr Comp Physiol. 2003 May;284(5):R1269-76.
99. Mitsui R, Ono S, Karaki S, et al. Neural and non-neural mediation of propionate-induced contractile responses in the rat distal colon. Neurogastroenterol Motil. 2005 Aug;17(4):585-94.
100. Karaki S, Kuwahara A. Propionate-induced epithelial K(+) and Cl(-)/HCO3(-) secretion and free fatty acid receptor 2 (FFA2, GPR43) expression in the guinea pig distal colon. Pflugers Arch. 2011 Jan;461(1):141-52.
101. Atkinson W, Lockhart S, Whorwell PJ, et al. Altered 5-hydroxytryptamine signaling in patients with constipation- and diarrhea-predominant irritable bowel syndrome. Gastroenterology. 2006 Jan;130(1):34-43.
102. Harrison E, Lal S, McLaughlin JT. Enteroendocrine cells in gastrointestinal pathophysiology. Curr Opin Pharmacol. 2013 Dec;13(6):941-5.
103. Ohman L, Simren M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. Nat Rev Gastroenterol Hepatol. 2010 Mar;7(3):163-73.
104. Barbara G, Stanghellini V, De Giorgio R, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology. 2004;126(3):693-702.

105. Barbara G, Stanghellini V. Biomarkers in IBS: when will they replace symptoms for diagnosis and management? Gut. 2009 Dec;58(12):1571-5.
106. Winston J, Shenoy M, Medley D, et al. The vanilloid receptor initiates and maintains colonic hypersensitivity induced by neonatal colon irritation in rats. Gastroenterology. 2007 Feb;132(2):615-27.
107. Akbar A, Yiangou Y, Facer P, et al. Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. Gut. 2008 Jul;57(7):923-9.
108. Monsbakken KW, Vandvik PO, Farup PG. Perceived food intolerance in subjects with irritable bowel syndrome-- etiology, prevalence and consequences. Eur J Clin Nutr. 2006 May;60(5):667-72.
109. Francis CY, Whorwell PJ. Bran and irritable bowel syndrome: time for reappraisal. Lancet. 1994 Jul 2;344(8914):39-40.
110. Biesiekierski JR, Newnham ED, Irving PM, et al. Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial. Am J Gastroenterol. 2011 Mar;106(3):508-14; quiz 15.
111. Campbell AK, Waud JP, Matthews SB. The molecular basis of lactose intolerance. Science Progress. 2009;92:241-87.
112. Shaukat A, Levitt MD, Taylor BC, et al. Systematic review: effective management strategies for lactose intolerance. Ann Intern Med. 2010 Jun 15;152(12):797-803.
113. Spanier JA, Howden CW, Jones MP. A systematic review of alternative therapies in the irritable bowel syndrome. Arch Intern Med. 2003 Feb 10;163(3):265-74.
114. Gibson PR, Shepherd SJ. Evidence-based dietary management of functional gastrointestinal symptoms: The FODMAP approach. J Gastroenterol Hepatol. 2010 Feb;25(2):252-8.
115. Barrett JS, Gearry RB, Muir JG, et al. Dietary poorly absorbed, short-chain carbohydrates increase delivery of water and fermentable substrates to the proximal colon. Aliment Pharmacol Ther. 2010 Apr;31(8):874-82.
116. Ong DK, Mitchell SB, Barrett JS, et al. Manipulation of dietary short chain carbohydrates alters the pattern of gas production and genesis of symptoms in irritable bowel syndrome. J Gastroenterol Hepatol. 2010 Aug;25(8):1366-73.
117. Shepherd SJ, Gibson PR. Fructose malabsorption and symptoms of irritable bowel syndrome: guidelines for effective dietary management. J Am Diet Assoc. 2006 Oct;106(10):1631-9.
118. Shepherd SJ, Parker FC, Muir JG, et al. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: randomized placebo-controlled evidence. Clin Gastroenterol Hepatol. 2008 Jul;6(7):765-71.
119. Staudacher HM, Whelan K, Irving PM, et al. Comparison of symptom response following advice for a diet low in fermentable carbohydrates (FODMAPs) versus standard dietary advice in patients with irritable bowel syndrome. J Hum Nutr Diet. 2011 May 25.
120. Poynard T, Regimbeau C, Benhamou Y. Meta-analysis of smooth muscle relaxants in the treatment of irritable bowel syndrome. Aliment Pharmacol Ther. 2001 Mar;15(3):355-61.

121. Ford AC, Talley NJ, Spiegel BM, et al. Effect of fibre, antispasmodics, and peppermint oil in the treatment of irritable bowel syndrome: systematic review and meta-analysis. BMJ. 2008;337:a2313.
122. Ford AC, Brandt LJ, Young C, et al. Efficacy of 5-HT3 antagonists and 5-HT4 agonists in irritable bowel syndrome: systematic review and meta-analysis. Am J Gastroenterol. 2009 Jul;104(7):1831-43; quiz 44.
123. Kim DY, Camilleri M. Serotonin: a mediator of the brain-gut connection. Am J Gastroenterol. 2000 Oct;95(10):2698-709.
124. Quigley EM, Vandeplassche L, Kerstens R, et al. Clinical trial: the efficacy, impact on quality of life, and safety and tolerability of prucalopride in severe chronic constipation--a 12-week, randomized, double-blind, placebo-controlled study. Aliment Pharmacol Ther. 2009 Feb 1;29(3):315-28.
125. Pimentel M, Lembo A, Chey WD, et al. Rifaximin therapy for patients with irritable bowel syndrome without constipation. N Engl J Med. 2011 Jan 6;364(1):22-32.
126. Camilleri M. Pharmacology of the new treatments for lower gastrointestinal motility disorders and irritable bowel syndrome. Clin Pharmacol Ther. 2012 Jan;91(1):44-59.
127. Klooker TK, Braak B, Koopman KE, et al. The mast cell stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. Gut. 2010 Sep;59(9):1213-21.
128. Leighton MP, Lam C, Mehta S, et al. Efficacy and mode of action of mesalazine in the treatment of diarrhoea-predominant irritable bowel syndrome (IBS-D): study protocol for a randomised controlled trial. Trials. 2013;14:10.
129. Dunlop SP, Jenkins D, Neal KR, et al. Randomized, double-blind, placebo-controlled trial of prednisolone in post-infectious irritable bowel syndrome. Aliment Pharmacol Ther. 2003 Jul 1;18(1):77-84.
130. Lackner JM, Jaccard J, Krasner SS, et al. Self-administered cognitive behavior therapy for moderate to severe irritable bowel syndrome: clinical efficacy, tolerability, feasibility. Clin Gastroenterol Hepatol. 2008 Aug;6(8):899-906.
131. Ljotsson B, Falk L, Vesterlund AW, et al. Internet-delivered exposure and mindfulness based therapy for irritable bowel syndrome--a randomized controlled trial. Behav Res Ther. 2010 Jun;48(6):531-9.
132. Keohane J, O'Mahony C, O'Mahony L, et al. Irritable bowel syndrome-type symptoms in patients with inflammatory bowel disease: a real association or reflection of occult inflammation? Am J Gastroenterol. 2010 Aug;105(8):1788, 9-94; quiz 95.
133. Minderhoud IM, Oldenburg B, Wismeijer JA, et al. IBS-like symptoms in patients with inflammatory bowel disease in remission; relationships with quality of life and coping behavior. Dig Dis Sci. 2004 Mar;49(3):469-74.
134. Simren M, Axelsson J, Gillberg R, et al. Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. Am J Gastroenterol. 2002 Feb;97(2):389-96.
135. Farrokhyar F, Marshall JK, Easterbrook B, et al. Functional gastrointestinal disorders and mood disorders in patients with inactive inflammatory bowel disease: prevalence and impact on health. Inflamm Bowel Dis. 2006 Jan;12(1):38-46.

136. Akbar A, Yiangou Y, Facer P, et al. Expression of the TRPV1 receptor differs in quiescent inflammatory bowel disease with or without abdominal pain. Gut. 2010 Jun;59(6):767-74.
137. Harvey RF, Bradshaw JM. A simple index of Crohn's-disease activity. Lancet. 1980 Mar 8;1(8167):514.
138. Walmsley RS, Ayres RC, Pounder RE, et al. A simple clinical colitis activity index. Gut. 1998 Jul;43(1):29-32.
139. Kellow JE, Phillips SF. Altered small bowel motility in irritable bowel syndrome is correlated with symptoms. Gastroenterology. 1987 Jun;92(6):1885-93.
140. Rapps N, van Oudenhove L, Enck P, et al. Brain imaging of visceral functions in healthy volunteers and IBS patients. J Psychosom Res. 2008 Jun;64(6):599-604.
141. Stange EF, Travis SP, Vermeire S, et al. European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. Gut. 2006 Mar;55 Suppl 1:i1-15.
142. Van Assche G, Dignass A, Panes J, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. J Crohns Colitis. 2010 Feb;4(1):7-27.
143. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther. 1997 Apr;11(2):395-402.
144. Zigmoid AS, Snaith RP. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand. 1983;67:361-70.
145. Dhaliwal A, Zeino Z, Tomkins C, et al. Utility of faecal calprotectin in inflammatory bowel disease (IBD): what cut-offs should we apply? Frontline Gastroenterology. 2014; doi:10.1136/flgastro-2013-100420.
146. Halpin SJ, Ford AC. Prevalence of symptoms meeting criteria for irritable bowel syndrome in inflammatory bowel disease: systematic review and meta-analysis. Am J Gastroenterol. 2012 Oct;107(10):1474-82.
147. Barratt HS, Kalantzis C, Polymeros D, et al. Functional symptoms in inflammatory bowel disease and their potential influence in misclassification of clinical status. Aliment Pharmacol Ther. 2005 Jan 15;21(2):141-7.
148. Collins SM. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. Gastroenterology. 1996 Dec;111(6):1683-99.
149. Mikocka-Walus AA, Turnbull DA, Andrews JM, et al. The effect of functional gastrointestinal disorders on psychological comorbidity and quality of life in patients with inflammatory bowel disease. Aliment Pharmacol Ther. 2008 Aug 15;28(4):475-83.
150. Isgar B, Harman M, Kaye MD, et al. Symptoms of irritable bowel syndrome in ulcerative colitis in remission. Gut. 1983 Mar;24(3):190-2.
151. Piche T, Ducrotte P, Sabate JM, et al. Impact of functional bowel symptoms on quality of life and fatigue in quiescent Crohn disease and irritable bowel syndrome. Neurogastroenterol Motil. 2010 Jun;22(6):626-e174.
152. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am J Gastroenterol. 2010 Jan;105(1):162-9.

153. Roseth AG, Aadland E, Jahnsen J, et al. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. Digestion. 1997;58(2):176-80.
154. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis. 2012 Dec;18(12):2218-24.
155. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977 Mar;33(1):159-74.
156. Camilleri M. Managing symptoms of irritable bowel syndrome in patients with inflammatory bowel disease. Gut. 2011 Apr;60(4):425-8.
157. Timmer A, Preiss JC, Motschall E, et al. Psychological interventions for treatment of inflammatory bowel disease. Cochrane Database Syst Rev. 2011(2):CD006913.
158. Mikocka-Walus AA, Turnbull DA, Moulding NT, et al. Antidepressants and inflammatory bowel disease: a systematic review. Clin Pract Epidemiol Ment Health. 2006;2:24.
159. Gearry RB, Irving PM, Barrett JS, et al. Reduction of dietary poorly absorbed short-chain carbohydrates (FODMAPs) improves abdominal symptoms in patients with inflammatory bowel disease-a pilot study. J Crohns Colitis. 2009 Feb;3(1):8-14.
160. McCombie AM, Mulder RT, Gearry RB. Psychotherapy for inflammatory bowel disease: A review and update. J Crohns Colitis. 2013 Mar 2.
161. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001 Mar;69(3):89-95.
162. Whorwell PJ, McCallum M, Creed FH, et al. Non-colonic features of irritable bowel syndrome. Gut. 1986 Jan;27(1):37-40.
163. Maxton DG, Morris J, Whorwell PJ. More accurate diagnosis of irritable bowel syndrome by the use of 'non-colonic' symptomatology. Gut. 1991 Jul;32(7):784-6.
164. Campbell AK, Matthews SB, Vassel N, et al. Bacterial metabolic 'toxins': a new mechanism for lactose and food intolerance, and irritable bowel syndrome. Toxicology. 2010 Dec 30;278(3):268-76.
165. Matthews SB, Waud JP, Roberts AG, et al. Systemic lactose intolerance: a new perspective on an old problem. Postgraduate Medical Journal. 2005;81(953):167-73.
166. Sadler MJ, Strain JJ, Caballero B. Encyclopedia of Human Nutrition. London: Academic Press; 1999.
167. Mortensen PB, Holtug K, Rasmussen HS. Short-chain fatty acid production from mono- and disaccharides in a fecal incubation system: implications for colonic fermentation of dietary fiber in humans. The Journal of nutrition. 1988 Mar;118(3):321-5.
168. Kalapos MP. Methylglyoxal in living organisms: chemistry, biochemistry, toxicology and biological implications. Toxicol Lett. 1999 Nov 22;110(3):145-75.
169. Thornalley PJ. Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification--a role in pathogenesis and antiproliferative chemotherapy. Gen Pharmacol. 1996 Jun;27(4):565-73.

170. Dhar A, Desai K, Liu J, et al. Methylglyoxal, protein binding and biological samples: are we getting the true measure? J Chromatogr B Analyt Technol Biomed Life Sci. 2009 Apr 15;877(11-12):1093-100.
171. Vander Jagt DL. Methylglyoxal, diabetes mellitus and diabetic complications. Drug Metabol Drug Interact. 2008;23(1-2):93-124.
172. He XM, Carter DC. Atomic structure and chemistry of human serum albumin. Nature. 1992 Jul 16;358(6383):209-15.
173. Vassel N, Cox CD, Naseem R, et al. Enzymatic activity of albumin shown by coelenterazine chemiluminescence. Luminescence. 2012 May-Jun;27(3):234-41.
174. Wiles S, Ferguson K, Stefanidou M, et al. Alternative luciferase for monitoring bacterial cells under adverse conditions. Appl Environ Microbiol. 2005 Jul;71(7):3427-32.
175. Berti F, Bincoletto S, Donati I, et al. Albumin-directed stereoselective reduction of 1,3-diketones and beta-hydroxyketones to anti diols. Organic & biomolecular chemistry. 2011 Mar 21;9(6):1987-99.
176. Shimomura O, Inoue S, Johnson FH, et al. Widespread occurrence of coelenterazine in marine bioluminescence. Comp Biochem Physiol. 1980;65B(2):435-7.
177. Shimomura O, Teranishi K. Light-emitters involved in the luminescence of coelenterazine. Luminescence. 2000 Jan-Feb;15(1):51-8.
178. Kimzey MJ, Yassine HN, Riepel BM, et al. New site(s) of methylglyoxal-modified human serum albumin, identified by multiple reaction monitoring, alter warfarin binding and prostaglandin metabolism. Chem Biol Interact. 2011 Jun 30;192(1-2):122-8.
179. Tomba C, Baldassarri A, Coletta M, et al. Is the subjective perception of lactose intolerance influenced by the psychological profile? Aliment Pharmacol Ther. 2012 Oct;36(7):660-9.
180. Barrett JS, Irving PM, Shepherd SJ, et al. Comparison of the prevalence of fructose and lactose malabsorption across chronic intestinal disorders. Aliment Pharmacol Ther. 2009 Jul 1;30(2):165-74.
181. Wilder-Smith CH, Materna A, Wermelinger C, et al. Fructose and lactose intolerance and malabsorption testing: the relationship with symptoms in functional gastrointestinal disorders. Aliment Pharmacol Ther. 2013 Jun;37(11):1074-83.
182. Casellas F, Aparici A, Casaus M, et al. Subjective perception of lactose intolerance does not always indicate lactose malabsorption. Clin Gastroenterol Hepatol. 2010 Jul;8(7):581-6.
183. Kennedy PJ, Clarke G, Quigley EM, et al. Gut memories: towards a cognitive neurobiology of irritable bowel syndrome. Neurosci Biobehav Rev. 2012 Jan;36(1):310-40.
184. Roberts-Thomson IC, Fon J, Uylaki W, et al. Cells, cytokines and inflammatory bowel disease: a clinical perspective. Expert Rev Gastroenterol Hepatol. 2011 Dec;5(6):703-16.
185. Bashashati M, Rezaei N, Bashashati H, et al. Cytokine gene polymorphisms are associated with irritable bowel syndrome: a systematic review and meta-analysis. Neurogastroenterol Motil. 2012 Aug 16.
186. Loftis JM, Huckans M, Morasco BJ. Neuroimmune mechanisms of cytokine-induced depression: current theories and novel treatment strategies. Neurobiol Dis. 2010 Mar;37(3):519-33.

187. Capuron L, Fornwalt FB, Knight BT, et al. Does cytokine-induced depression differ from idiopathic major depression in medically healthy individuals? J Affect Disord. 2009 Dec;119(1-3):181-5.
188. Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun. 2011 Feb;25(2):181-213.
189. McEwen BS, Sapolsky RM. Stress and cognitive function. Curr Opin Neurobiol. 1995 Apr;5(2):205-16.
190. Sandi C, Pinelo-Nava MT. Stress and memory: behavioral effects and neurobiological mechanisms. Neural Plast. 2007;2007:78970.
191. Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: a review of clinical and preclinical research. Prog Neurobiol. 2011 Mar;93(3):385-404.
192. Li W, Dowd SE, Scurlock B, et al. Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. Physiol Behav. 2009 Mar 23;96(4-5):557-67.
193. Collins SM, Bercik P. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology. 2009 May;136(6):2003-14.
194. Gareau MG, Wine E, Rodrigues DM, et al. Bacterial infection causes stress-induced memory dysfunction in mice. Gut. 2011 Mar;60(3):307-17.
195. Carbotte RM, Denburg SD, Denburg JA. Cognitive dysfunction in systemic lupus erythematosus is independent of active disease. J Rheumatol. 1995 May;22(5):863-7.
196. Hilsabeck RC, Perry W, Hassanein TI. Neuropsychological impairment in patients with chronic hepatitis C. Hepatology. 2002 Feb;35(2):440-6.
197. Brands AM, Van den Berg E, Manschot SM, et al. A detailed profile of cognitive dysfunction and its relation to psychological distress in patients with type 2 diabetes mellitus. J Int Neuropsychol Soc. 2007 Mar;13(2):288-97.
198. Thomas M, Smith A. An investigation into the cognitive deficits associated with chronic fatigue syndrome. Open Neurol J. 2009;3:13-23.
199. Waud JP, Matthews SB, Campbell AK. Measurement of breath hydrogen and methane, together with lactase genotype, defines the current best practice for investigation of lactose sensitivity. Ann Clin Biochem. 2008 Jan;45(Pt 1):50-8.
200. Attree EA, Dancey CP, Keeling D, et al. Cognitive function in people with chronic illness: inflammatory bowel disease and irritable bowel syndrome. Appl Neuropsychol. 2003;10(2):96-104.
201. Dancey CP, Attree EA, Stuart G, et al. Words fail me: the verbal IQ deficit in inflammatory bowel disease and irritable bowel syndrome. Inflamm Bowel Dis. 2009 Jun;15(6):852-7.
202. Gallacher J, Collins R, Elliott P, et al. A platform for the remote conduct of gene-environment interaction studies. PLoS One. 2013;8(1):e54331.
203. Nelson HE. National Adult Reading Test (NART): Test Manual: Windsor: NFER-Nelson; 1982.
204. Crawford JR, Parker DM, Stewart LE, et al. Prediction of WAIS IQ with the National Adult Reading Test: Cross-Validation and extension. British Journal of Clinical Psychology. 1989;28:267-73.
205. Rey E, Moreno Ortega M, Garcia Alonso MO, et al. Constructive thinking, rational intelligence and irritable bowel syndrome. World J Gastroenterol. 2009 Jul 7;15(25):3106-13.

206. Boye B, Lundin KE, Jantschek G, et al. INSPIRE study: does stress management improve the course of inflammatory bowel disease and disease-specific quality of life in distressed patients with ulcerative colitis or Crohn's disease? A randomized controlled trial. Inflamm Bowel Dis. 2011 Sep;17(9):1863-73.
207. Deter HC, Keller W, von Wietersheim J, et al. Psychological treatment may reduce the need for healthcare in patients with Crohn's disease. Inflamm Bowel Dis. 2007 Jun;13(6):745-52.
208. Garcia-Vega E, Fernandez-Rodriguez C. A stress management programme for Crohn's disease. Behav Res Ther. 2004 Apr;42(4):367-83.
209. Keefer L, Keshavarzian A. Feasibility and acceptability of gut-directed hypnosis on inflammatory bowel disease: a brief communication. Int J Clin Exp Hypn. 2007 Oct;55(4):457-66.
210. Goodhand JR, Wahed M, Rampton DS. Management of stress in inflammatory bowel disease: a therapeutic option? Expert Rev Gastroenterol Hepatol. 2009 Dec;3(6):661-79.
211. von Wietersheim J, Kessler H. Psychotherapy with chronic inflammatory bowel disease patients: a review. Inflamm Bowel Dis. 2006 Dec;12(12):1175-84.
212. Knowles SR, Monshat K, Castle DJ. The Efficacy and Methodological Challenges of Psychotherapy for Adults with Inflammatory Bowel Disease: A Review. Inflamm Bowel Dis. 2013 Jul 10.
213. Webb AN, Kukuruzovic RH, Catto-Smith AG, et al. Hypnotherapy for treatment of irritable bowel syndrome. Cochrane Database Syst Rev. 2007(4):CD005110.
214. NICE. Irritable bowel syndrome in adults: diagnosis and management of irritable bowel syndrome in primary care. 2008.
215. Bernstein CN, Singh S, Graff LA, et al. A prospective population-based study of triggers of symptomatic flares in IBD. Am J Gastroenterol. 2010 Sep;105(9):1994-2002.
216. Baer RA. Mindfulness training as a clinical intervention: a conceptual and empirical review. Clinical Psychology: Science and Practice. 2003;10:125-43.
217. Shaw G, Srivastava ED, Sadlier M, et al. Stress management for irritable bowel syndrome: a controlled trial. Digestion. 1991;50(1):36-42.
218. Sadlier M, Stephens SD, Kennedy V. Tinnitus rehabilitation: a mindfulness meditation cognitive behavioural therapy approach. J Laryngol Otol. 2008 Jan;122(1):31-7.
219. Thomas M, Sadlier M, Smith A. The effect of Multi Convergent Therapy on the psychopathology, mood and performance of Chronic Fatigue Syndrome patients: A preliminary study. Counselling and Psychotherapy Research. 2006;6(2):91-9.
220. DeLongis A, Folkman S, Lazarus RS. The impact of daily stress on health and mood: psychological and social resources as mediators. J Pers Soc Psychol. 1988 Mar;54(3):486-95.
221. Levenstein S, Prantera C, Varvo V, et al. Development of the Perceived Stress Questionnaire: a new tool for psychosomatic research. J Psychosom Res. 1993 Jan;37(1):19-32.
222. Vitaliano PP, Russo J, Carr JE, et al. The Ways of Coping Checklist: Revision and Psychometric Properties. Multivariate Behavioral Research. 1985;20:3-26.

223. Guyatt G, Mitchell A, Irvine EJ, et al. A new measure of health status for clinical trials in inflammatory bowel disease. Gastroenterology. 1989 Mar;96(3):804-10.
224. John OP, Robins RW, Pervin LA. Handbook of personality: Theory and research. New York: Guildford Press; 2008.
225. Cohen S, Hoberman H. Positive events and social supports as buffers of life change stress. Journal of Applied Social Psychology. 1983;13:99-124.
226. Vidal A, Gomez-Gil E, Sans M, et al. Health-related quality of life in inflammatory bowel disease patients: the role of psychopathology and personality. Inflamm Bowel Dis. 2008 Jul;14(7):977-83.
227. D'Haens G, Sandborn WJ, Feagan BG, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology. 2007 Feb;132(2):763-86.
228. Spiller RC. Problems and challenges in the design of irritable bowel syndrome clinical trials: experience from published trials. Am J Med. 1999 Nov 8;107(5A):91S-7S.
229. Patel SM, Stason WB, Legedza A, et al. The placebo effect in irritable bowel syndrome trials: a meta-analysis. Neurogastroenterol Motil. 2005 Jun;17(3):332-40.
230. Gaylord SA, Palsson OS, Garland EL, et al. Mindfulness training reduces the severity of irritable bowel syndrome in women: results of a randomized controlled trial. Am J Gastroenterol. 2011 Sep;106(9):1678-88.
231. Kearney DJ, McDermott K, Martinez M, et al. Association of participation in a mindfulness programme with bowel symptoms, gastrointestinal symptom-specific anxiety and quality of life. Aliment Pharmacol Ther. 2011 Aug;34(3):363-73.
232. Miehsler W, Weichselberger M, Offerlbauer-Ernst A, et al. Which patients with IBD need psychological interventions? A controlled study. Inflamm Bowel Dis. 2008 Sep;14(9):1273-80.
233. Keithly LJ, Samples SJ, Strupp HH. Patient motivation as a predictor of process and outcome in psychotherapy. Psychother Psychosom. 1980;33(1-2):87-97.
234. Jonefjall B, Strid H, Ohman L, et al. Characterization of IBS-like symptoms in patients with ulcerative colitis in clinical remission. Neurogastroenterol Motil. 2013 Sep;25(9):756-e578.
235. Keszthelyi D, Jonkers DM, Hamer HM, et al. Letter: the role of sub-clinical inflammation and TRPV1 in the development of IBS-like symptoms in ulcerative colitis in remission. Aliment Pharmacol Ther. 2013 Sep;38(5):560-1.
236. Keszthelyi D, Troost FJ, Jonkers DM, et al. Alterations in mucosal neuropeptides in patients with irritable bowel syndrome and ulcerative colitis in remission: a role in pain symptom generation? European journal of pain (London, England). 2013 Oct;17(9):1299-306.
237. Iacucci M, Ghosh S. Looking beyond symptom relief: evolution of mucosal healing in inflammatory bowel disease. Therap Adv Gastroenterol. 2011 Mar;4(2):129-43.
238. Bouguen G, Levesque BG, Pola S, et al. Endoscopic Assessment and Treating to Target Increase the Likelihood of Mucosal Healing in Patients With Crohn's Disease. Clin Gastroenterol Hepatol. 2013 Nov 15.

239. Bouguen G, Levesque BG, Pola S, et al. Feasibility of endoscopic assessment and treating to target to achieve mucosal healing in ulcerative colitis. Inflamm Bowel Dis. 2014 Feb;20(2):231-9.
240. McLaughlin JT. How should We Classify and Treat Patients with Functional Gastrointestinal Disorders? Therap Adv Gastroenterol. 2008 Nov;1(3):153-6.