TITLE

Approaches to Diagnosing Irritable Bowel Syndrome

Title Page

Title: Approaches to diagnosing irritable bowel syndrome

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Chapter 1: Introduction.

- Sood R, Ford AC. 2017. Editorial: Volatile Organic Compounds in Irritable Bowel Syndrome – technology for an Accurate and Reliable Point-of-care Test? *Alimentary Pharmacology and Therapeutics*. **45**(4),pp.563-564. The first author wrote the manuscript, following which it was critically reviewed by the remaining author.

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Chapter 3: Systematic Review and Meta-Analysis: Accuracy of Diagnosing Irritable Bowel Syndrome with Symptoms, Biomarkers and Psychological Markers.

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Chapter 4: Enhancing Diagnostic Performance of Symptom-based Criteria for Irritable Bowel Syndrome by Additional History and Limited Diagnostic Evaluation.

- Sood, R., Camilleri, M., Gracie, D.J., Gold, M.J., To, N., Law, G.R. and Ford, A.C. 2016. Enhancing Diagnostic Performance of Symptom-Based Criteria for Irritable Bowel Syndrome by Additional History and Limited Diagnostic Evaluation. *The American journal of gastroenterology*. **111**(10),pp.1446–1454. The first author collected the data, undertook all analyses and wrote the manuscript, following which it was critically

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Chapter 5: Derivation and Validation of a Diagnostic Test for Irritable Bowel Syndrome Using Latent Class Analysis.

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My own contributions, fully and explicitly indicated in the thesis, have been:

- Design of the study protocol
- Data collection- UK study
- Database construction- UK study
- Statistical analysis of data
- Drafting of all manuscripts. First author and guarantor of all published articles
- Design and drafting of thesis

The other members of the group and their contributions have been as follows:

- Assistance in study protocol design (ACF and GRL)
- Assistance in collecting data- UK study (DJG)
- Assistance in database construction- UK study (MJG, NT)
- Collection of data and database construction- Canadian study (PB, ACF, PM, MIPS)
- Assistance in analysing data (ACF, GRL)
- Critical review of drafted manuscripts for publication (PB, MC, ACF, GRL, PM, MIPS)

Abstract

Introduction: Differentiating irritable bowel syndrome (IBS) from organic disease is inherently challenging as symptoms can overlap. Symptom-based diagnostic criteria were developed to aid the clinician in making a positive diagnosis of IBS, and therefore avoid unnecessary invasive investigations. However, previous studies have shown these criteria perform only modestly in differentiating IBS from organic disease.

Aim: The aim of this thesis is to assess the accuracy of the symptom-based diagnostic criteria, as well as address some of the limitations in their performance.

Methods: A systematic review and meta-analysis was conducted in order to summarise the approaches that are currently available to aid in the diagnosis of IBS, including symptoms, biomarkers, psychological markers, and combinations thereof, as well as to understand the strengths and weaknesses of the available diagnostic tests for IBS. Using these findings, two diagnostic test studies were designed and undertaken with the intention of creating accurate, inexpensive, and easily administrable tests for clinicians consulting in routine clinical care.

Results: A meta-analysis undertaken showed that symptom-based diagnostic criteria, biomarkers, and psychological markers perform only moderately well in diagnosing IBS. Combining symptoms with markers of organic disease or psychological affect seemed to represent the best way forward in improving the accuracy of diagnosing IBS. The first diagnostic test study undertaken confirmed

this finding, and showed that modifications to the symptom-based diagnostic criteria with the addition of symptoms, markers of affect, and simple laboratory tests resulted in improved diagnostic accuracy. The second diagnostic test study used latent class analysis to derive and validate a model that performs with similar accuracy to the symptom-based diagnostic criteria, but importantly this method has the potential for improvement in its accuracy through the addition of clinical markers, such as faecal calprotectin.

Conclusions: This thesis has shown that combining symptoms with clinical markers, markers of affect, and/or novel biomarkers leads to greater accuracy in diagnosing IBS. The novel findings of two diagnostic test studies undertaken suggests that this approach may represent the best way forward in developing an accurate and non-invasive diagnostic test for IBS.

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Glossary of Terms

Ab	antibodies
AIC	Akaike information criterion
ANCA	anti-neutrophil cytoplasmic antibody
AUC	area under the curve
BAM	bile acid malabsorption
BIC	Bayesian information criterion
BMI	body mass index
CBT	cognitive behavioural therapy
CdtB	cytolethal distending toxin B
Cg	chromogranin
CI	confidence interval
CIC	chronic idiopathic constipation
CLE	confocal laser endomicroscopy
CRP	C-reactive protein
DNA	deoxyribonucleic acid
ESR	erythrocyte sedimentation rate
FGID	functional gastrointestinal disorder
FISH	fluorescent in-situ hybridisation
FGF-19	fibroblast growth factor
fMRI	functional magnetic resonance imaging
FODMAP	fermentable oligosaccharide, disaccharide, monosaccharide, and polyols
GC-C	guanylate cyclase-C

GCD	gluten-containing diet
GFD	gluten-free diet
GI	gastrointestinal
GP	general practitioner
HADS	hospital anxiety and depression scale
HC	healthy control
IBD	inflammatory bowel disease
IEL	intraepithelial lymphocytes
IgE	immunoglobulin E
IgG	immunoglobulin G
IL	interleukin
IBS	irritable bowel syndrome
IBS-C	irritable bowel syndrome with constipation
IBS-D	irritable bowel syndrome with diarrhoea
IBS-M	mixed constipation and diarrhoea IBS
IBS-U	unsubtyped irritable bowel syndrome
LCA	latent class analysis
LR	likelihood ratio
LR^2	likelihood ratio chi-squared
MCC	midcingulate cortex
MRI	magnetic resonance imaging
NCGS	non-coeliac gluten sensitivity
NNT	number needed to treat
NPV	negative predictive value
OR	odds ratio

PCR	polymerase chain reaction
PEG	polyethylene glycol
PHQ-12	patient health questionnaire-12
PHQ-15	patient health questionnaire-15
PI-IBS	post-infectious irritable bowel syndrome
PPV	positive predictive value
PROs	patient reported outcomes
ROC	receiver operator characteristics curve
RR	relative risk
SCC	somatic symptom checklist
SD	standard deviation
SeHCAT	tauroselcholic (75 selenium) acid
Sg	secretogranin
SNP	single nucleotide polymorphism
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
TNF-α	tumour necrosis factor alpha
UC	ulcerative colitis
UK	United Kingdom
VAS	visual analogue scale
VOC	volatile organic compound
VOM	volatile organic metabolite
7α-C4	7α-4-cholesten-3-one

CHAPTER 1

Introduction

This chapter will provide an overview of how the definition of irritable bowel syndrome (IBS) has developed over time, in particular the history and development of symptom-based diagnostic criteria, the current gold standard for diagnosing IBS, will be discussed. Our understanding of the likely pathophysiological mechanisms that underpin IBS, as well as possible treatment options, will also be reviewed. Finally, the current published literature on the available methods used to diagnose IBS will also be evaluated, in order to understand the rationale for conducting this body of work.

1.1 History and Definition of the Irritable Bowel Syndrome

It was in 1892 that Sir William Osler's textbook "*The Principals and Practice of Medicine"* first made reference to a condition known as mucous colitis, which was described as "a tenacious mucus, which may be slimy and gelatinous, like frog spawn…" Patients with mucous colitis were also considered at the time to be hysterical, depressed, suffering from hypochondria, and likely to complain of colicky abdominal pain (Christensen, 1992). In the 1920's, the term colonic spasm was coined, and it was thought to be a common feature of mucous colitis, in which patients complained of lower abdominal pain that was made worse by anxiety, smoking, menses, and defaecation. However, it was soon realised that colonic spasm and mucous colitis were the same condition, and it was felt that this was likely to occur as a consequence of autonomic nerve dysfunction related to a specific personality disorder (Christensen, 1992).

It was not until the 1940's that the term irritable bowel syndrome began to replace previous nomenclature in the literature (Peters and Bargen, 1944). This was defined as colonic dysfunction in the absence of either an organic colonic or extra-

colonic disease causing the symptoms. Although “personality disorders” were thought to be associated with IBS, they were no longer considered as a primary cause, with autonomic disturbances, endocrinopathies, and allergies considered to have more important contributory roles (Christensen, 1992).

In 1962, the first attempt at classifying IBS was undertaken (Chaudhary and Truelove, 1962). In this retrospective study of 130 patients, two main subtypes of IBS were recognised; the spastic colon group and the painless diarrhoea group. The predominant symptom in the spastic colon group was abdominal pain, which was considered to be colonic in origin. Bowel habit could vary between being normal, exclusively constipation, exclusively diarrhoea, or alternating between constipation and diarrhoea. The second group, the painless diarrhoea group, was considered to consist of individuals without abdominal pain, and whose predominant symptom was loose and frequent stools. Psychological comorbidity was thought to play a significant role in both the development and continuation of symptoms in both groups.

1.1.1 The Manning Criteria

A seminal paper published in 1978 by Manning and colleagues (Manning et al., 1978) was later to form the first symptom-based diagnostic criteria for IBS, now known as the “Manning criteria”. In this cross-sectional study, 65 patients referred to a gastrointestinal (GI) clinic for investigation of their symptoms were asked to complete a symptom questionnaire prior to their clinic appointment, and followed through until a final diagnosis was established. In total, 32 (49.2%) patients were finally diagnosed with IBS, and four individual symptom items were found to be markedly more common in these patients, compared with those found to have

organic GI disease after investigation. These were abdominal distension; abdominal pain relieved by defaecation; increased stool frequency associated with the onset of abdominal pain; and looser stools associated with the onset of abdominal pain. Combining these symptoms together led to a greater ability to discriminate between IBS and organic disease, with three or four of these symptoms present in 20 (63%) of the IBS patients, but in only five (15%) of the 33 patients with organic disease. Manning and colleagues concluded that the more of these symptoms that were present in combination, the more likely the diagnosis of IBS. The addition of two other symptoms, mucus per rectum and a feeling of incomplete stool evacuation, further increased the likelihood of IBS, with all six symptoms present in six (19%) of the patients with IBS, but in only one (3%) patient who had organic disease. The importance of this work is reflected by the fact that symptom-based diagnostic criteria still remain the gold standard for diagnosing IBS to this day.

1.1.2 The Rome Criteria

The Manning Criteria were superseded in the 1990's, when a working committee for the Rome foundation produced the first comprehensive diagnostic criteria for all functional gastrointestinal disorders (FGIDs), including IBS, now known as the Rome I criteria (Drossman et al., 1990). The aim of these criteria were firstly to enable clinicians to make a positive diagnosis of IBS with the use of minimal investigations, secondly to help select homogeneous patients for clinical trials, and thirdly to ensure standardisation when investigating pathophysiological mechanisms of IBS. The working committee chose to use a more restrictive criteria to define IBS than had been previously used, with the condition defined as "a functional gastrointestinal disorder attributable to the intestines and associated with

symptoms of abdominal pain, and/or altered defaecation, and/or bloatedness or distension" (Drossman et al., 1990). This differed from the Manning criteria, in that it was recognised that the change in stool consistency or frequency could be towards harder or less frequent stools, as well as looser or more frequent stools. Furthermore, the Rome criteria introduced, for the first time, a minimum frequency and duration of symptoms required to diagnose IBS. Less emphasis was also placed on abdominal bloating or distension and mucus per rectum, which were now considered as supportive, rather than diagnostic, of IBS.

Further modifications were made to the Rome criteria in 1999, published as the Rome II criteria (Thompson et al., 1999), and again in 2006, the Rome III criteria (Longstreth et al., 2006). In particular, changes were made to the minimum duration of symptoms required. The most recent criteria, Rome IV, published in 2016 (Mearin et al., 2016), now define IBS as recurrent abdominal pain associated with a change in stool form and/or frequency, with the term "abdominal discomfort", which was present in the Rome III criteria, removed as it was considered an ambiguous term for patients, and does not exist in certain languages. There was a change in the frequency of abdominal pain required to meet the threshold for IBS, from at least 3 days per month in the preceding 3 months in Rome III, to at least one episode per week in the preceding 3 months in Rome IV, as well as a change from "improvement of abdominal pain with defaecation" to "abdominal pain related to defaecation", in an attempt to acknowledge that some patients with IBS report a worsening of abdominal pain following defaecation (Sood and Ford, 2016). The original Manning criteria and the four iterations of the Rome criteria are shown in Table 1.

Table 1. Symptom-based Diagnostic Criteria for Irritable Bowel Syndrome.

Criteria	Symptom items included	Minimum symptom duration
Manning	1. Abdominal pain relieved by defaecation 2. More frequent stools with onset of pain 3. Looser stools with onset of pain 4. Mucus per rectum 5. Feeling of incomplete emptying 6. Patient-reported visible abdominal distension	None
Rome I	Abdominal pain or discomfort relieved with defaecation, or associated with a change in stool frequency or consistency, plus two or more of the following on at least 25% of occasions or days: 1. Altered stool frequency 2. Altered stool form 3. Altered stool passage 4. Passage of mucus 5. Bloating or distension	≥3 months

Rome II	Abdominal discomfort or pain that has two of three features: 1. Relieved with defaecation 2. Onset associated with a change in frequency of stool 3. Onset associated with a change in form of stool	≥12 weeks (need not be consecutive) in last 12 months
Rome III	Recurrent abdominal pain or discomfort ≥3 days per month in the last 3 months associated with 2 or more of the following: 1. Improvement with defaecation 2. Onset associated with a change in frequency of stool 3. Onset associated with a change in form of stool	Symptom onset ≥6 months prior to diagnosis

Rome IV	Recurrent abdominal pain, occurring on average, at least 1 day per week in the last 3 months, and associated with 2 or more of the following: 1. Related to defaecation 2. Associated with a change in frequency of stool. 3. Associated with a change in form (appearance) of stool.	Symptom onset ≥6 months prior to diagnosis

1.1.3 Subtypes of IBS

The Rome II criteria, for the first time, also allowed classification of patients according to the dominant stool consistency. The IBS subtype definitions have also been updated with each iteration of the Rome criteria, and the Rome IV criteria classify IBS in to four subtypes consisting of: constipation predominant IBS (IBS-C), whereby Bristol stool form types 1 and 2 (Lewis and Heaton, 1997) are experienced in >25% of bowel movements, and Bristol stool form types 6 and 7 are experienced in <25% of bowel movements; diarrhoea predominant IBS (IBS-D), whereby stool form types 6 and 7 are experienced in >25% of bowel movements and stool form types 1 and 2 are experienced <25% of the time; mixed constipation and diarrhoea IBS (IBS-M), whereby stool form types 1 and 2 and stool form types 6 and 7 are experienced >25% of the time; and unsubtyped IBS (IBS-U), where there is insufficient abnormality of stool consistency to meet the three previously described subtypes (Mearin et al., 2016).

However, a patient's subtype is not necessarily stable, and it is possible to move between these subtypes over time. In a study that evaluated the stability of Rome III IBS subtypes over a 10-week period (Engsbro et al., 2012), 126 patients who were recruited to two placebo-controlled treatment trials of probiotics conducted in Denmark and Sweden, were also asked to complete a daily diary in which they scored all defaecations according to the Bristol stool form scale (Lewis and Heaton, 1997). IBS subtypes were calculated according to the information provided in the diary at 1- and 2-weekly intervals. Irrespective of the interval used, the distribution of IBS subtypes remained stable, with approximately one-third of the study population meeting the criteria for IBS-C, IBS-D and IBS-U respectively. IBS-M was the least prevalent subtype. However, only 18% to 35% of patients were

considered as having a stable subtype. There was a tendency for IBS-C and IBS-D patients to have the most stable subtype, and between 65% and 82% of patients changed subtype at least once during the 10-week period. Patients changing subtype most often changed between IBS-C or IBS-D and IBS-U.

1.2 The Incidence and Prevalence of IBS

In a systematic review and meta-analysis published in 2012 (Lovell and Ford, 2012), the pooled prevalence of IBS in 80 study populations, containing 260,960 subjects, was found to be 11.2% (95% confidence interval (CI) 9.8% to 12.8%). In this same study, the prevalence of IBS was also shown to differ according to geographical location, with the highest pooled prevalence of IBS in Northern Europe of 12.0% (95% CI 9.0% to 15.0%), compared with the lowest IBS prevalence in South-East Asia of 7.0% (95% CI 5.0% to 9.0%). The prevalence of IBS also depended on the criteria used to define it. Prevalence was highest when three or more of the Manning criteria (Manning et al., 1978) were used to define it (14.0% (95% CI 10.0% to 17.0%)), and lowest when the Rome I criteria (Drossman et al., 1990) were used to define IBS (8.8% (95% CI 6.8% to 11.2%)). In this same meta-analysis, the prevalence of IBS was found to be higher in women (14.0% (95% CI 11.0% to 16.0%)) compared with men (8.9% (95% CI 7.3% to 10.5%)), with an odds ratio (OR) of 1.67 (95% CI 1.53 to 1.82) favouring women.

Furthermore, IBS is more prevalent in individuals with other FGIDs, including dyspepsia, gastro-oesophageal reflux disease, and chronic idiopathic constipation (CIC) (Ford et al., 2010; Lovell and Ford, 2012; Suares and Ford, 2011). In a systematic review and meta-analysis of 19 papers, incorporating 18,173 patients, the prevalence of IBS in individuals with dyspepsia was 27% (95% CI 23%

to 31%) compared with 7% (95% CI 5% to 10%) in those without dyspepsia (Ford et al., 2010). Similarly, in a systematic review and meta-analysis conducted by the same group, the prevalence of CIC was higher in those patients who also reported IBS (OR 7.98; 95% CI 4.58 to 13.92) (Suares and Ford, 2011). IBS is also more common in those with non-FGIDs, such as fibromyalgia and chronic fatigue syndrome (Riedl et al., 2008), and these patients are also more likely to experience extra-intestinal and somatic symptoms when compared with healthy controls (HCs), as well as undergo a worse course of disease (Riedl et al., 2008). Symptoms such as chronic headache or migraine (Azpiroz et al., 2000; Cole et al., 2006), temporomandibular joint dysfunction (Aaron et al., 2000), urogenital syndromes including dysmenorrhoea and dyspareunia (Prior et al., 1989), back pain (Longstreth and Yao, 2004), palpitations (Vandvik et al., 2004), sleep disturbance (Patel et al., 2016), and bronchial hyper-reactivity (Kennedy et al., 1998), are also more common in IBS patients when compared with healthy individuals.

The incidence of IBS is estimated to be 1% to 2% per year in the West in representative community samples (Ford et al., 2008), meaning that many people will report symptoms compatible with IBS at some point in their lives, and up to 60% of those who experience symptoms will consult a primary care physician as a result (Koloski et al., 2002; Koloski et al., 2003).

1.3 The Cost of IBS

Partly as a consequence of the high incidence of IBS, as well as its chronic relapsing course, there are substantial costs to health-care services globally. A comprehensive burden-of-illness study in the USA estimated that IBS cost almost US $1 billion in direct costs such as hospital and physician services, endoscopy,

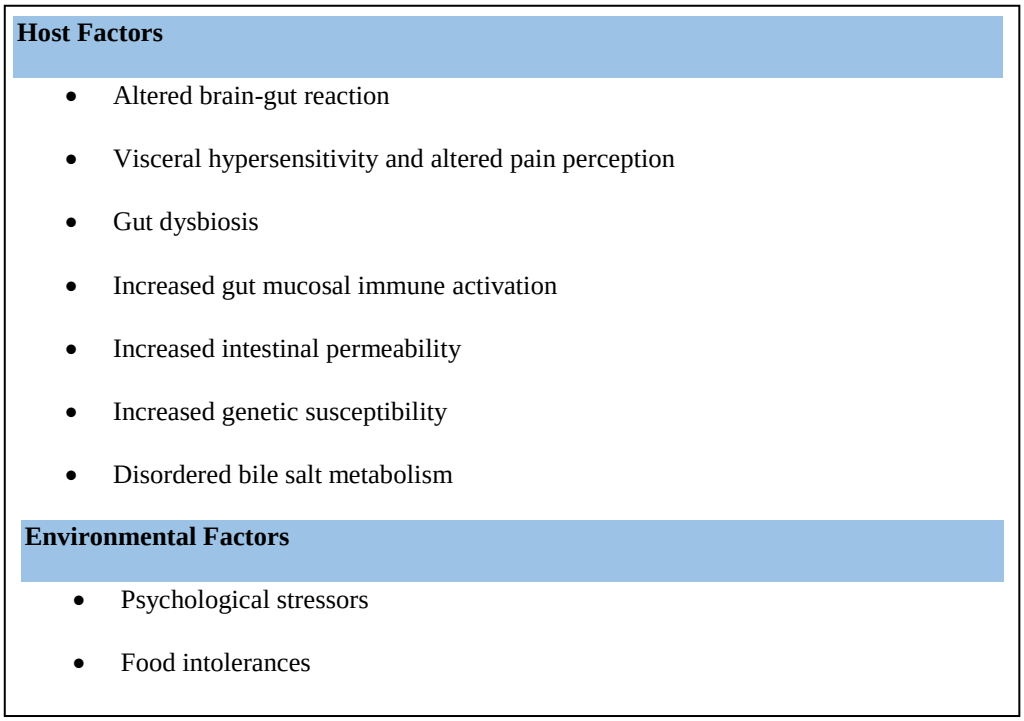
prescription and over-the-counter medications, and another $50 million in indirect costs such as loss of earnings (Everhart and Ruhl, 2009). IBS patients are also more likely to undergo surgery when compared with non-IBS patients, with rates of cholecystectomy, appendicectomy, and hysterectomy two to three times higher than non-IBS patients, further exacerbating direct and indirect costs (Longstreth and Yao, 2004; Agreus, 1993). The effect of IBS on society as a whole is substantial, due to factors such as absenteeism from work and reduced quality of life (Drossman et al., 1993). In a comprehensive search of the literature of six databases, a more recent narrative review looked at the costs incurred by the patient, such as the intangible cost of reduced quality of life (Canavan et al., 2014). This review reported on a survey conducted in the USA, in which 68% of IBS patients who responded missed the equivalent of one social activity per week over a 3-month period, directly as a result of their symptoms (Hulisz, 2004). In other studies conducted in Europe and North America, patients with IBS were more likely to report anxiety, depression, and lower perceptions of their own health, when compared with non-IBS patients (Bushnell et al., 2006; El-Serag et al., 2002). There are also direct out-of-pocket costs and loss of earnings to consider, predominantly through the cost of over-the-counter medications with 15% to 43% of IBS patients buying medications such as analgesia and laxatives, as well as paying privately for medical consultations and alternative therapies (Pare et al., 2006; Silk, 2001).

1.4 Aetiology of IBS

To date, no structural or physiological cause of IBS has been established, and it is unlikely that there is a single unifying explanation. Several potential mechanisms have been implicated in the pathogenesis of the condition, which are

discussed below. These can be broadly defined as host factors contributing to IBS, such as altered brain-gut reaction, increased visceral hypersensitivity, and gut dysbiosis, and environmental factors, such as psychological stressors and food intolerances (Figure 1).

Figure 1. Possible Pathophysiological Mechanisms of IBS.



1.4.1 Host Factors

1.4.1.1 Altered Brain-Gut Reaction

The importance of brain-gut interactions in IBS and other FGIDs are increasingly being recognised. These interactions in health play an important role in regulating digestive processes such as appetite and the gut-associated immune system, and alterations in central and peripheral brain-gut interactions are likely to be part of the explanation underpinning the symptoms of IBS (Mayer and Tillisch, 2011).

Findings from studies conducted in primary and secondary care show anxiety, depression, and somatisation are more prevalent in patients with IBS when compared with non-IBS patients. These associations have led some to hypothesise that the brain drives the gut manifestations of IBS (Tanaka et al., 2011). Studies that give credence to this theory include one large study of patients referred to secondary care for investigation of their GI symptoms (Patel et al., 2015). Of 4224 patients recruited, 840 met the Rome III criteria for IBS. The number of individual somatic symptoms and the mean somatisation score were found to be higher in IBS patients when compared with non-IBS patients ($P < 0.001$). In addition, high levels of somatisation were associated with a greater frequency of bloating and abdominal distension.

In one prospective population-based questionnaire study (Koloski et al., 2012), 1775 of 4500 patients who had responded to and completed a survey were contacted again 12 years later. Of these 1775 patients, 1002 completed the follow-up survey. In patients who did not have a diagnosis of IBS at the time of initial recruitment, a clinically elevated level of anxiety (OR 1.11; 95% CI 1.03 to 1.18) or depression

(OR 1.10; 95% CI 1.03 to 1.18) were found to be predictors of a new diagnosis of IBS in the follow-up survey.

Further evidence to support a brain-gut pathophysiological mechanism is supported by a meta-analysis that looked at the association between medically unexplained symptoms and anxiety and depression in 244 observational studies. In this meta-analysis, IBS was significantly associated with both anxiety and depression, when compared with HCs, and controls with known organic disorders that cause GI symptoms (Henningsen et al., 2003).

Although an association between IBS and mood disorders is supported by findings from studies such as those described above, what is not always certain is if the relationship is exclusively unidirectional, or whether in some cases, IBS symptoms may arise prior to the manifestation of mood disorders. In one prospective cross-sectional study, 1900 patients were recruited and asked to complete a survey at baseline and at 1 year. Of those with a diagnosis of IBS at baseline, and normal anxiety and depression levels, significantly higher levels of anxiety ($P = 0.002$) and depression ($P < 0.001$) were found 1 year later, suggesting a primary gut-brain axis pathway in this subset of IBS patients (Koloski et al., 2016).

A bi-directional brain-gut theory is further supported by the findings of a matched cohort study conducted across 123 primary care practices in the UK (Jones et al., 2006), in which 13% of patients with IBS had consulted a primary care physician with depression, or had been prescribed antidepressants in the 2 years prior to the diagnosis of IBS, compared with 5% of controls. Consultation rates for anxiety and depression were found to be higher in the IBS group, in the 6 years after the diagnosis was made.

The sizable psychosocial component of IBS has resulted in considerable interest in patient reported outcomes (PROs). These PROs, such as the IBS quality of life scale, are usually questionnaire-based, and are used to encourage clinicians to examine the mental, social, and physical impact of symptoms, with the aim of improving patient outcomes and satisfaction through targeted therapy (Marshall et al., 2006; Spiegel et al., 2011). PROMIS, a US National Institute of Health programme, was launched in 2004, with the aim of developing and validating a standardised databank of PROs for health-related illness, and a GI version is being developed currently (Spiegel, 2013). Although there is certainly an unmet need in terms of identifying and addressing the psychological and social needs of patients with IBS, it is unclear how PROs could be utilised in primary care and general gastroenterology clinics, where the majority of these patients are managed (Sood et al., 2014). One possible avenue of interest could be to use PROs to enhance a clinician's ability in making a positive diagnosis of IBS, and therefore avoid the need for expensive and invasive investigations.

1.4.1.2 Visceral Hypersensitivity and Altered Pain Perception

Visceral hypersensitivity is defined as altered sensation in response to a physiological stimuli (Farzaei et al., 2016), and a subset of IBS patients have been found to have increased visceral sensitivity following colonic distension, exhibited as a reduced threshold for pain and/or increased intensity of sensation (Mayer and Gebhart, 1994; Bouin et al., 2002). There are thought to be two major components of visceral hypersensitivity; allodynia and hyperalgesia. Allodynia is enhanced nociceptive sensation in response to normal stimuli, and hyperalgesia is intensified

pain sensation in response to a stimuli that would normally be expected to cause some pain (Farzaei et al., 2016).

Visceral afferent nerve response is thought to be provoked by chemical, mechanical, and luminal stimuli and normally silent nociceptors can be activated when tissue injury occurs, such as when bile salts are instilled in to the colon (Zhou and Verne, 2011). There is evidence to suggest that pro-inflammatory cytokines, such as interleukin-one beta (IL-1ß) and tumour necrosis factor-alpha (TNF-α), are involved in the sensitisation of the nociceptive system following an inflammatory stimuli, which in turn leads to hyperalgesia (Eijkelkamp et al., 2012). There is also increasing evidence to suggest that chronic hyperalgesia may be a result of persistent tissue injury (Zhou and Verne, 2011). In a model of chronic visceral hypersensitivity, neonatal male rats were exposed to either mechanical stimuli in the form of balloon distension of the colon, or chemical stimuli through the intracolonic injection of mustard oil. The control group consisted of rats that received neither of these stimuli. Colonic irritation with chemical or mechanical stimuli resulted in chronic visceral hypersensitivity, as determined by abdominal withdrawal reflex, with characteristics of both allodynia and hyperalgesia, when compared with controls (Al-Chaer, 2000).

Other researchers have suggested that visceral hypersensitivity may occur as a result of disturbances of the central nervous system, an observation supported by functional magnetic resonance imaging (fMRI) studies. Several studies have addressed the role of placebo analgesia in IBS using oesophageal and rectal distension as pain models. In one study, 17 IBS patients and HCs, matched for age and sex, underwent rectal distension. Brain activity was measured using fMRI during the placebo and control phases. IBS patients showed a lower visceral

perception threshold following rectal distension when compared with HCs. fMRI of the IBS patients during the placebo analgesia phase showed increased activity of the cingulate cortex and other areas of the brain involved in pain modulation, as well as increased activity in the areas of the brain involved in anticipatory mechanisms (Lee et al., 2012).

A second study aimed to determine whether changes in central pain modulation following administration of visceral placebo analgesia were specific to IBS, and secondly to observe the relationship between negative affect and central pain modulation during visceral placebo analgesia (Schmid et al., 2015). Seventeen patients with IBS who were diagnosed using the Rome III criteria, 15 patients with quiescent ulcerative colitis (UC), and 36 HCs were recruited. On the study day, rectal distension was performed using a pressure-controlled barostat system to determine rectal perceptual and pain thresholds. This was followed by a structural magnetic resonance imaging (MRI). There were then three 7-minute sessions, during which eight rectal distensions of 16.8 seconds each were delivered. The first session served as an adaptation period and in the following two sessions, an intravenous catheter was inserted, and the patients were administered an intravenous saline solution. In order to induce the placebo condition or the control condition, patients were informed that they had either received an anti-spasmodic (placebo) or saline solution (control) intravenously, although in both instances they received saline. Brain imaging was performed using fMRI, and data were collected during each of the three sessions. Salivary cortisol samples were collected from each patient upon arrival on the day of the study, prior to the rectal threshold assessment session, and prior to each of the three fMRI sessions.

There were no differences in rectal pain thresholds between the groups, measured using an online visual analogue scale (VAS), nor were there any differences during the adaptation phase in anxiety, tension, cortisol concentration, or expected and perceived pain. There was a significant reduction in expected and perceived pain on the expectation of receiving an analgesic (placebo), when compared with the expected administering of saline (control). Following analysis of variance (ANOVA), a significant placebo response for expected pain was found in IBS patients when compared with UC patients ($P < 0.001$) and HCs ($P = 0.003$). There was a significant reduction in perceived pain intensity following administration of placebo in both UC patients and HCs, when compared with IBS patients (IBS vs UC, $P < 0.001$; IBS vs HCs, $P < 0.001$). fMRI of the IBS patients revealed significantly less downregulation of pain-induced neural activation during pain anticipation and following placebo analgesia, when compared with UC patients and HCs. In particular, there was less effective downregulation of neural activity in the midcingulate cortex (MCC) of IBS patients, when compared with HCs, and in the posterior cingulate cortex of IBS patients, when compared with UC patients. These areas of the brain are involved in pain modulation and orientation in response to nociceptive stimuli respectively. Additionally, IBS patients showed enhanced neural activation in the parietal cortex, when compared with HCs, and dorsolateral prefrontal cortex, when compared with UC patients, suggesting that anticipatory mechanisms may play a role in central pain processing in IBS patients.

1.4.1.3 Intestinal Dysbiosis

The microbiota of the human GI tract comprises a complex ecosystem that is important for the health and physiological functions of the individual. It has a role in immune regulation, including development of the immune system, and as part of the host defence against pathogens and toxins, as well as emerging roles in metabolic regulation, by means of support of digestion through the provision of enzymes (Tap et al., 2016). Healthy individuals harbour approximately 100 different species of bacteria in faecal microbiota, with dominant species including *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Collins, 2014). Alterations in the constituents and diversity of the microbiota have been observed following use of antibiotics, prebiotics, and in obesity, and there is continuing interest in the gut microbiome for the cause, prevention, and treatment of conditions such as inflammatory bowel disease (IBD) (Prosberg et al., 2016), colorectal cancer (Brennan and Garrett, 2016), and diseases of the liver (Bluemel et al., 2016).

Variances in the intestinal microbiota between patients with IBS and HCs have mainly been studied using faecal material. The emergence of culture-independent methods, primarily through analysis of ribosome ribonucleic acid for bacteria and single-celled microorganisms (Suau et al., 1999), complementary methods such as fluorescent in-situ hybridisation (FISH), and quantitative polymerase chain reaction (PCR) testing, have also improved our understanding of the gut microbiome. Studies have suggested an increase in some bacteria such as *Firmicutes, Enterobacteriaceae*, and *Clostridium* species, and a decrease in other bacteria such as *Bacteroidetes, Bifidobacteria*, and *Eubacterium* species, as well as an overall decrease in heterogeneity, in faecal samples of IBS patients when compared with HCs.

Furthermore, the microbiota of IBS-D patients appear to deviate the most from HCs, whilst the microbiota of IBS-C patients deviate the least (Salonen et al., 2010).

In one study that examined the faecal microbiota of 14 IBS-C patients and 12 matched HCs, using FISH to quantify main groups of bacteria, statistically significant lower numbers of lactate-producing, lactate-utilising, and hydrogen-consuming bacteria were found in IBS patients (Chassard et al., 2012). Other studies have shown a predominance for methane-producing bacteria in IBS patients (Kim et al., 2012). In a larger study containing 114 IBS patients and 33 HCs, intestinal fermentation was measured using intestinal intraluminal pH and short-chain fatty acids (Ringel-Kulka et al., 2015). Colonic intraluminal pH was decreased, suggesting higher colonic fermentation in IBS patients when compared with HCs.

Although these studies suggest possible dysbiosis in IBS patients, with an overall decrease in heterogeneity of the intestinal microbiome and a predominance of bacteria that can result in increased intestinal fermentation, it should be acknowledged that there is currently a lack of clear consensus, and at present a specific microbiota profile for IBS patients has not been identified. One study has found a possible intestinal microbiota signature in patients with severe IBS symptoms (Tap et al., 2017). However, the investigators were unable to show any difference in the faecal microbiota of IBS patients, when compared with healthy patients. The lack of a specific microbiota profile for IBS is probably due to external factors including methodological differences, variances in diet, use of supplements and medications, such as antibiotics and proton pump inhibitors, and genetic variation (Zhuang et al., 2017). It is also likely that external influences, such as industrialisation, can alter the gut microbiota, as shown in a study that compared the microbiota of healthy children living in an Italian city, with a diet rich in fat and

protein, to those of healthy children living in a village in Burkina Faso, where the diet is predominantly fibre-based. The Burkina Faso cohort was found to have significantly higher levels of *Bacteroidetes* and significantly lower levels of *Firmicutes* (De Filippo et al., 2010).

In a more recent study undertaken in four Italian cities, heathy volunteers consisting of 51 vegetarians, 51 vegans, and 51 omnivores were recruited and asked to complete daily dietary information, as well as provide faecal samples on which metabolomic analysis was undertaken, using a technique known as gas-chromatography mass spectrometry-solid phase microextraction (De Filippis et al., 2015). The vegetarian and vegan volunteers were found to adhere to a mainly Mediterranean diet. Volunteers consuming more plant-based foods had higher faecal levels of *Bacteroidetes*, but also high levels of *Firmicutes*, which were also noted in those consuming mainly animal-based foods, which of course is not in keeping with the previously discussed study by De Filippo *et al*.

Studies in mice models, as well as in humans, have shown that antibiotics can lead to a disruption in gut microbiota (Anitha et al., 2012). In a small study of three patients, the gut microbiota was examined following two courses of ciprofloxacin. The effect of ciprofloxacin was profound, with an immediate decrease in the diversity of the microbiota (Dethlefsen and Relman, 2011). Evidence of an association between antibiotics and the development of IBS was seen in a large population based study of 26,107 patients (Villarreal et al., 2012). After controlling for gender and comorbidity, patients were more likely to develop IBS in the following 12 months after receiving a tetracycline (OR 1.48; P = 0.046). This finding has also been supported in another study of 421 patients who attended a health screening clinic and completed a symptom questionnaire. Forty-eight patients

had symptoms compatible with IBS, and antibiotic use was found to be strongly associated with IBS (OR 3.70; 95% CI 1.80 to 7.60) (Mendall and Kumar, 1998).

1.4.1.4 Increased Gut Mucosal Immune Activation

Low-grade gut mucosal inflammation as a cause of IBS was first proposed in the 1960's in a study in which the quantity of mast cells in the muscle layers of surgically resected colonic specimens were examined. Specimens included patients with a diagnosis of IBS, and these patients were found to have a similar number of mast cells to those with a diagnosis of UC (Hiatt and Katz, 1962). Following this landmark study, others have used endoscopic samples of colonic mucosa to compare the quantity of cells involved in inflammation such as eosinophils, lymphocytes, plasma cells, and neutrophils. Some of these studies have reported significantly higher levels of inflammatory cells in IBS patients, in particular IBS-D patients, when compared with HCs (Salzmann et al., 1989; Lee et al., 1988).

A previous systematic review has examined the role of surrogate markers of low-grade mucosal inflammation in IBS patients (Ford and Talley, 2011). Of 16 studies, 13 obtained colonic mucosa samples, one study obtained mucosa biopsy samples from the proximal jejunum, one study obtained full thickness samples from the proximal jejunum at laparoscopy, and one study took biopsies from the first and second part of the duodenum at upper GI endoscopy. Eligible studies were required to compare IBS patients with HCs, or asymptomatic individuals undergoing investigation for reasons other than GI symptoms. Surrogate markers of low-grade inflammation examined included mast cells, T-lymphocytes, B-lymphocytes, eosinophils, neutrophils, plasma cells, and mucosal cytokines. Of these markers, mast cells, B-lymphocytes, T-lymphocytes, and mucosal cytokines were shown to

be altered in IBS patients when compared with HCs. However, a number of the studies reported conflicting results. The strongest evidence appeared to exist for mast cells, which are involved in the immune system and, when activated, degrade and release inflammatory and immune mediators, such as histamine and tryptase (Holtmann et al., 2016). This in turn leads to abnormal GI sensitivity and mucosal secretions. Activation of mast cells can be through an array of stimuli including immunoglobulin E (IgE)-mediated pathways, neuro-hormonal pathways induced by psychological stress, and bacteria. However, even with mast cells, there is inconsistency in reported outcomes with variances according to gender, bowel segment sampled, and IBS subgroup, with IBS-D patients showing higher levels of mast cells when compared with IBS-C patients, and some studies suggesting no difference in mast cell quantity when comparing IBS patients with HCs (Zhang et al., 2016).

A more recent systematic review has examined the relationship between systemic and mucosal cytokines, immune cells, and IBS (Martin-Viñas and Quigley, 2016). This review showed that levels of the cytokine IL-10 were decreased in the systemic circulation of IBS patients, whereas levels of IL-6, IL-8, IL-1ß, and TNF-α were increased. Levels of mucosal IL-10 were decreased, and levels of IL-8, mast cells, enterochromaffin cells, and CD3+ and CD8+ T-lymphocytes cells were increased. However, once again, the results were not consistent across all studies, and the results of this systematic review were at odds with the results from a meta-analysis by Bashashati *et al.* that suggested there was no difference in circulating levels of IL-10 in IBS patients, when compared with HCs, therefore highlighting the inconsistency in available evidence (Bashashati et al., 2014). Therefore, although there is good evidence to support the hypothesis of increased gut mucosal immune

activation as a cause of IBS, there is not the irrefutable evidence required to classify IBS as an inflammatory bowel disorder.

One hypothesis that has been explored for persistent low-grade inflammation in IBS patients is exposure to inflammatory stimuli in genetically susceptible individuals. Multiple community studies have shown an increase in post-infectious IBS (PI-IBS) following outbreaks of enteric infection (Borgaonkar et al., 2006; Marshall et al., 2006), and up to 17% of patients believe their IBS symptoms commenced following an episode of gastroenteritis (Longstreth et al., 2001). In one large cohort study, the incidence of PI-IBS was calculated following an outbreak of *Escherichia coli* 0157:H7 and *Campylobacter jejuni* gastroenteritis in a small rural town in Ontario, Canada (Marshall et al., 2006). Two years following the outbreak, residents who had clinically suspected gastroenteritis, residents who had self-reported gastroenteritis, and non-affected residents (acting as controls) were asked to complete a modified questionnaire based on the Rome I criteria, allowing the incidence of PI-IBS to be calculated and its epidemiology characterised. Of 2069 eligible participants, 71 (10.1%) of 701 controls, 249 (27.5%) of 904 with self-reported gastroenteritis, and 168 (36.2%) of 464 with clinically suspected gastroenteritis, met the Rome I criteria ($P < 0.001$). Risk factors for PI-IBS were female gender, younger age, and features of systemic illness during the acute enteric infection, including weight loss, prolonged diarrhoea, and bloody stools.

In a recent systematic review and meta-analysis of 45 cohort studies reporting on the prevalence of IBS following infectious enteritis, the pooled prevalence of IBS at 12 months after infectious enteritis was 10.1% (95% CI 7.2% to 14.1%), and the prevalence of IBS more than 12 months after the index episode of infectious enteritis was 14.5% (95% CI 7.7% to 25.5%) (Klem et al., 2017).

A retrospective study has shown that the risk of PI-IBS is independent of the pathogen that causes the initial gastroenteritis, giving credence to the theory that there is a common pathway triggered by infection, which in turn leads to persistent low-grade inflammation (Neal et al., 1997). However, whether an enteric infection induces a mast cell response, which then triggers a sustained immune response, persistent low-grade gut mucosal inflammation and symptoms of PI-IBS remains to be established (Zhang et al., 2016).

The role of allergens precipitating mast cell activation is well established, and is one other hypothesis of persistent low-grade gut inflammation that has been investigated. Allergens are known to precipitate an immune-mediated response which in turn leads to mast cell activation, leading to the allergic response (Zhang et al., 2016). Some studies have suggested elimination diets based on immunoglobulin G (IgG) antibodies have resulted in a significant decrease in symptoms experienced by IBS patients, compared with those who undergo dietary restrictions not guided by IgG antibody levels (Kalliomaki, 2005). The role of diet and food tolerances in the pathophysiology of IBS is explored later in this chapter.

1.4.1.5 Increased Intestinal Permeability

The small intestine is lined with epithelial cells, with tight junctions between these cells. The tight junctions are composed of complex protein systems that allow absorption of nutrients by regulating transportation through the extra-cellular matrix. As tight junctions encircle epithelial cells, attaching them tightly to each other, this helps prevent antigens and unwanted solutes and microbes from entering the systemic circulation. Therefore, disruption to the tight junctions can result in increased intestinal permeability (Piche, 2014). Diseases such Crohn's and UC are

characterised by intestinal inflammation, and it is known that inflammation can compromise the integrity of the intestinal barrier, through epithelial damage and increased gaps between tight junctions (gap density), resulting in increased intestinal permeability (Landy et al., 2016).

There is increasing evidence that a similar process may be the cause of symptoms experienced in a subset of patients with IBS. Studies using a technique known as confocal laser endomicroscopy (CLE), a non-invasive imaging modality, have shown that the gap density is increased in IBS patients, when compared with HCs. A case-control study was performed, in which 16 patients with IBS (12 IBS-D, 4 IBS-C) and 18 HCs underwent CLE of the terminal ileum following colonoscopy and ileoscopy (Turcotte et al., 2013). The HCs consisted of patients undergoing colonoscopy for other reasons, including colorectal cancer screening and positive faecal occult blood testing. The study showed that IBS patients had a significantly increased gap density when compared with HCs (32 gaps/1000 cells in IBS patients vs. 6 gaps/1000 cells for HCs ($P < 0.001$)), thereby providing evidence for a hypothesis of low-grade gut mucosal inflammation causing IBS symptoms. Interestingly, no significant difference in gap density was found between the two IBS subtypes, although as the authors acknowledge, the study was not sufficiently powered to examine this.

However, other studies using alternate techniques, have found differences in intestinal permeability amongst IBS subtypes. In one study that recruited 15 patients with PI-IBS, 15 patients with IBS-C and 15 HCs, participants ingested 1.8MBq of chromium labelled ethylene-diamine-tetra-acetate (51Cr-EDTA) (Dunlop et al., 2006). Following this, urine was collected over 24 hours and small bowel permeability was expressed as a percentage of the total dose of 51Cr-EDTA

excreted. Small intestinal permeability was increased in PI-IBS, when compared with IBS-C ($P = 0.004$) and HCs ($P = 0.037$).

Another study has examined the differential expression of tight junction proteins (Bertiaux-Vandaële et al., 2011). In 50 IBS patients (19 IBS-D, 14 IBS-C, 15 IBS-M and 2 IBS-U) and 31 HCs, tight junction proteins were quantified in colonic mucosal biopsies using quantitative reverse transcriptase PCR, while localisation was determined by immunofluorescence. Expression of tight junction proteins was lower in IBS patients, when compared with HCs ($P < 0.05$). However, following sub-group analysis, tight junction protein expression was decreased in the IBS-D patients, when compared with HCs, but not in IBS-C or IBS-M, when compared with HCs, suggesting that increased intestinal permeability as a cause of IBS symptoms may be relevant only in IBS-D patients.

1.4.1.6 Increased Genetic Susceptibility

Evidence suggests that IBS aggregates strongly in families. In a case-control study, 50 patients with IBS and 53 controls randomly selected from a medical outpatient clinic completed a symptom questionnaire, and provided contact details for first degree relatives, who were also asked to complete the same symptom questionnaire (Saito et al., 2008). Relatives were considered to have IBS if they met the Rome I or II criteria and there was no alternative GI diagnosis as a cause of their symptoms. The IBS patients reported that 21% of family members had IBS, whilst 37% of family members of IBS patients met the Rome criteria for IBS. Controls reported that 4% of family members had IBS, whilst 16% of family members met the Rome criteria for IBS. Relatives of IBS patients were three times more likely than the relatives of the controls to have IBS ($P < 0.05$). In another case-control

study, 477 patients with IBS, 297 matched controls, 1492 relatives of IBS patients and 936 relatives of the matched controls were asked to complete a GI symptom questionnaire (Saito et al., 2010). At least one relative with IBS was observed in 50% of IBS patients and 27% of controls, with an OR of 2.75 (95 % CI 2.01 to 3.76, $P < 0.0001$).

Evidence from studies such as those described above, has led some to hypothesise that genetics may play a significant part in the pathophysiology of IBS, an assertion supported by twin studies. In one study, same-sex twins completed a structured interview on symptoms consistent with FGIDs (Morris-Yates et al., 1998). Of the 686 individuals, 33 (4.8%) had a diagnosis of a FGID. Genetic models were constructed, in which 56.9% (95% CI 40.6% to 75.9%) of the variance was accredited to genetic variance, with the remaining 43.1% attributed to environment. In another study, the contribution of genetic and environmental factors was assessed by comparing concordance for IBS in monozygotic and dizygotic twins with concordance between mothers and their children (Levy et al., 2001). Of 6060 twin pairs, concordance for IBS was significantly greater in monozygotic twins, when compared with dizygotic twins (17.2% vs. 8.4%, $P = 0.03$). Interestingly, the proportion of dizygotic twins with IBS, whose mother had IBS, was higher than the proportion of dizygotic twins who had co-twins with IBS (15.2% vs. 6.7%, $P < 0.001$). Following logistic regression, having a mother or father with IBS was a stronger predictor than having a twin with IBS ($P < 0.001$).

This possible genetic link has encouraged investigators to look for mutations that may result in susceptible individuals developing IBS. SCN5A and KCNH2 encode for sodium and potassium channels found on GI smooth muscle and interstitial cells of Cajal. However, these genes are also known to be associated with

congenital prolonged QT syndrome. In one study, patients who were referred to a clinic for prolonged QT syndrome genetic testing, and their family members who acted as controls, were contacted and asked to complete a GI symptom questionnaire (Locke et al., 2006). The association between ion channel genes and GI symptoms was assessed by logistic regression. Of 219 patients, 50% patients with the SCN5A mutation reported abdominal pain compared with 13% of controls (OR 5.7; 95% CI 1.3 to 24.4). More than 65% of those with the SCN5A mutation reported at least one GI symptom, compared with 28% of controls (OR 5.2; 95% CI 1.5 to 18.3). No association was found between the KCNH2 mutation and GI symptoms.

Other investigators have examined the role of single nucleotide polymorphisms (SNPs), which are deoxyribonucleic acid (DNA) sequence variations in nucleotides, in the pathophysiology of IBS (Swan et al., 2013). The authors hypothesised that IBS-D is characterised by a genetic susceptibility to over-react to an inflammatory insult. Twenty-one patients with *Campylobacter jejuni* infection in the previous 6 months, 37 patients with IBS-D, 19 patients with IBS-C and 25 HCs underwent flexible sigmoidoscopy and rectal biopsy. Following gene expression analysis, SNPs of the gene TNFSF15 were associated with IBS-D, whilst SNPs of TNF-α were associated with PI-IBS. A further study consisting of 1992 patients supports this association between SNPs of the TNFSF15 gene and the risk of developing IBS (OR 1.37; 95% CI 1.19 to 1.58) (Zucchelli et al., 2011). This study also showed that this association was greatest in IBS-C patients, when compared with other IBS subtypes (OR 1.79; 95% CI 1.41 to 2.26). A probable role for TNFSF15 in the development of IBS is supported by a meta-analysis that has looked at the association between 16 separate SNPs and the risk of developing IBS in 2894 patients (1073 IBS-D, 839 IBS-C and 502 IBS-M patients) and 3138 HCs (Czogalla et al., 2015). Only one

SNP linked to the TNFSF15 gene was found to increase the risk of IBS (OR 1.19; 95% CI 1.08 to 1.31), and in particular for the IBS-C subtype (OR 1.24; 95% CI 1.08 to 1.42).

Environmental factors are thought to have a significant effect on the function of genes. In a pilot study, 27 IBS patients and 23 age-matched and sex-matched HCs provided blood samples from peripheral blood mononuclear cells for measurement of DNA methylation (the change in activity of a DNA segment) (Mahurkar et al., 2016). Gene expression was measured using PCR, and the participants were also asked to complete a symptom questionnaire. The investigators identified 133 differentially methylated positions that were potentially related to oxidative stress and neuropeptide hormone activity. Furthermore, epigenetic changes in one of the genes, subcommissural organ-Spondin, were associated with elevated hospital anxiety and depression scale (HADS) scores in IBS patients, suggesting a role for DNA methylation in the pathogenesis of IBS. However, these findings have yet to be confirmed in larger independent studies.

1.4.1.7 Disordered Bile Salt Metabolism

Bile acids are synthesised in the liver before being released in the duodenum, where they are responsible for solubilisation of fatty acids and monoglycerides. Reabsorption occurs in the terminal ileum via the apical ileal bile acid transporter. Approximately 95% of bile acid is reabsorbed, with the remainder being recycled by hepatocytes via the portal vein (Holtmann et al., 2016). The mechanism of action underpinning bile acid malabsorption (BAM) is thought to be as a result of defective feedback in the inhibition of bile acid synthesis by fibroblast growth factor (FGF-19). FGF-19 normally works by feeding back negatively on hepatocytes, reducing

the production of new bile acid when recycled bile acids are returned to the hepatocytes.

Abnormalities in bile acid metabolism have been recognised for many years in Crohn's disease (Beigel et al., 2014), or following surgery such as cholecystectomy (Sauter et al., 2002) and ileal resection (Walters, 2010), although a significant proportion of patients presenting with lower GI symptoms may have idiopathic BAM. In a systematic review of 18 studies, reporting on 1223 patients presenting with symptoms consistent with IBS-D, 10% (95% CI 7% to 13%) had confirmed severe idiopathic BAM on tauroselcholic [75 selenium] acid (SeHCAT) scan, 32% (95% CI 29% to 35%) had moderate BAM, whilst 26% (95% CI 23% to 30%) had mild BAM (Wedlake et al., 2009). These findings were confirmed in a large retrospective study of 373 patients that showed of those patients with IBS-D, 27.3% had evidence of BAM (Gracie et al., 2012).

Although there is sufficient evidence to confirm a link between BAM and IBS-D, what is less clear is whether BAM is a cause or a consequence of IBS. Studies have shown that faecal levels of primary bile acids are higher in IBS-D patients when compared with HCs (Duboc et al., 2012; Shin et al., 2013). Another study examined the interplay between faecal microbiota composition, serum and faecal bile acid compositions, 7α-4-Cholesten-3-one (7α-C4) (a metabolite that reflects hepatic bile acid synthesis and whose increase closely correlates with BAM), and FGF19 (Dior et al., 2016). In this study of 15 HCs, 16 IBS-D patients and 15 IBS-C patients, an increase in *Escherichia coli* was found in IBS-D patients, and an increase in *Bacteroides* and *Bifidobacterium* found in IBS-C patients, which are bacteria known to metabolise bile acid. Faecal bile acids were significantly higher in the IBS-D patients when compared with HCs, whilst serum bile acids were

significantly increased in both IBS-D and IBS-C patients when compared with HCs, with no significant difference in 7α-C4 or FGF19 levels between the three groups. This study therefore showed that serum and faecal bile acid profiles differ between IBS patients and health, which may be secondary to dysbiosis, and gives credibility to the hypothesis that BAM is a consequence of, rather than a cause of IBS.

1.4.2 Environmental Factors

1.4.2.1 Psychological Stressors

The associations between psychological life stressors such as abuse and trauma, and medical illness, such as IBS, have been well established for several decades (Drossman, 2011). In one of the first studies to report on the link between sexual and physical abuse in women and FGIDs, a self-administered questionnaire was completed by 206 consecutive patients referred to a university based gastroenterology clinic for investigation of GI symptoms (Drossman et al., 1990). Of these patients, 89 (44%) reported a history of physical or sexual abuse. Patients with a FGID diagnosis were more likely to report physical abuse (OR 11.39; 95% CI 2.22 to 58.48), sexual abuse (OR 2.08; 95% CI 1.03 to 4.21), chronic abdominal pain (OR 2.06; 95% CI 1.03 to 4.12), and an increased number of surgical procedures (2.7 procedures vs. 2.0 procedures, $P < 0.03$). The increased prevalence of physical and sexual abuse in IBS and other FGIDs has been confirmed in subsequent studies, in both community and tertiary referral populations (Longstreth and Wolde-Tsadik, 1993; Delvaux et al., 1997).

An increased prevalence of FGIDs has also been reported in patients who experience other forms of trauma, such as psychological trauma (Ablin et al., 2010).

Although the mechanisms of why patients who experience trauma report an increased prevalence of IBS is not completely understood, some experts have hypothesised that this may be due to enhanced activity of the MCC (Drossman, 2011), as previously discussed.

1.4.2.2 Food Intolerances

There is limited evidence for a true immune-mediated allergy to certain food groups that may precipitate IBS symptoms. It is estimated that between 20% and 65% of IBS patients believe that their symptoms can be attributed to adverse food reactions (Nanda et al., 1989; Dainese et al., 1999), and one study has previously observed a high prevalence of functional dyspepsia and IBS in patients with food reactions (Ciprandi and Canonica, 1988). A systematic review examined this issue and found that, in a subset of IBS patients, a true food allergy may exist mediated by IgE and IgG, with elevated levels of IgE and IgG4 reported in IBS patients when exposed to known food intolerances (Park and Camilleri, 2006).

A study that examined food intolerances in IBS patients using the CLE technique provides further evidence for food acting as an allergen. Thirty-six patients with suspected food intolerances, and 10 patients with Barrett's metaplasia, who acted as controls, underwent CLE (Fritscher-Ravens et al., 2014). An increase in intraepithelial lymphocytes (IELs) and epithelial breaks were seen in 14 of the 36 IBS patients when exposed to an allergen (CLE+ patients). Baseline IELs were higher in these patients when compared with CLE- patients, and IELs and epithelial gaps increased significantly, when compared with baseline, following exposure to antigens ($P = 0.0008$ and $P < 0.001$ respectively). Following a 4-week exclusion diet, GI symptom scores improved by >50% in these patients.

1.5 Treatment of IBS

1.5.1 Physical Activity

Treatments are generally targeted towards the symptoms of IBS including abdominal pain, bloating, diarrhoea, and constipation. However, there may be some general measures that patients can take, which include increasing physical activity. In a RCT, 102 IBS patients were randomised to a physical activity group, which involved a physiotherapist instructing them to increase their physical activity, or the control group, whereby the patients were asked to maintain their current lifestyle (Johannesson et al., 2011). The study found a significant improvement in the IBS severity scoring system in the physical activity group, when compared with the control group ($P = 0.003$). Furthermore, the proportion of patients with an increase in IBS symptom severity during the study was significantly larger in the control group.

1.5.2 Diet

1.5.2.1 Dietary Fibre

Although true food allergies are uncommon in IBS, food intolerances are frequently reported (Chey et al., 2015; Eswaran et al., 2013). Historically, increasing dietary fibre was one of the recommended treatments for IBS. However, two diverse opinions currently exist. The first that believes a low-fibre western diet is a cause of IBS, and the second, that fibre may exacerbate the symptoms of IBS (Burkitt et al., 1972; Painter, 1972). Some early RCTs found a significant improvement in IBS symptoms, such as abdominal pain and stool form and frequency, with high fibre

diets consisting of wheat bran or isphagula husk (Manning et al., 1977; Prior and Whorwell, 1987; Lambert et al., 1991). However, patients with IBS often believe that fibre worsens symptoms, as highlighted in a survey of 100 patients which found that 55% of patients' symptoms were made worse by wheat bran, whereas only 10% thought fibre improved symptoms (Francis and Whorwell, 1994). This discrepancy between patient beliefs and evidence cited by clinicians was highlighted in a survey of IBS patients and their general practitioners (GPs), which found that patients often considered fibre as a food intolerance that exacerbated their symptoms, whilst GPs regarded a lack of fibre as an aetiological cause of symptoms (Bijkerk et al., 2003).

A systematic review and meta-analysis sought to clarify the role of fibre in IBS (Ford et al., 2008). In 12 RCTs, 155 of 300 (52%) patients who were assigned to a high fibre diet had no improvement, or persistent symptoms, compared with 168 of 291 (57%) patients assigned to placebo or a low fibre diet (RR 0.87; 95% CI 0.76 to 1.00, P = 0.05). The number needed to treat (NNT) with fibre to prevent persistent symptoms was 11. When wheat bran and isphagula husk were considered separately, this statistically significance benefit persisted only for isphagula husk (RR 0.78; 95% CI 0.63 to 0.96). The NNT was 6 to prevent one patient having persistent symptoms.

1.5.2.2 Short-Chain Carbohydrates

Other investigators have looked at the role of short-chain carbohydrates in the role of IBS. These are small and osmotically active molecules in the intestinal lumen, which can cause increased intestinal luminal water volume if absorbed slowly. These molecules also rapidly ferment which leads to the production of carbon dioxide, hydrogen, and methane. It is these two mechanisms that are thought

to cause symptoms in IBS patients when exposed to these molecules (Shepherd et al., 2013). The discovery of these mechanisms has led to the development of the fermentable oligosaccharide, disaccharide, monosaccharide and polyol (FODMAP)-restricted diet, which essentially eliminates all classes of poorly absorbed short-chain carbohydrates from the diet.

In a single-blind, cross-over interventional trial, 15 IBS patients and 15 HCs were asked to consume diets that were either high or low in FODMAP content for 2 days, following which, breath samples for hydrogen and methane tests were collected (Ong et al., 2010). Higher levels of hydrogen were produced by patients consuming a high FODMAP diet, with statistically higher levels found in IBS patients when compared with HCs. Levels of methane were reduced in the HCs with a high FODMAP diet, but unchanged in the IBS patients. IBS patients reported higher levels of all GI symptoms ($P < 0.01$), worsening heartburn ($P = 0.025$), and tiredness ($P = 0.012$) with a high FODMAP diet. A recent systematic review has looked at the efficacy of a low FODMAP diet in clinical trials, and the investigators found that a restricted FODMAP diet improved symptoms in IBS patients in the four studies included (Rao et al., 2015). However, because of the significant heterogeneity between the studies, and poor methodological quality, further high quality studies are required before a definitive conclusion can be made.

1.5.2.3 Gluten

Uptake of gluten-free diets (GFD) amongst the general population has increased, and in one study, 13% of 1002 patients surveyed in the UK reported a sensitivity to gluten (Aziz et al., 2014). More recently the term non-coeliac gluten sensitivity (NCGS) has been coined to encompass individuals who do not have

coeliac disease, but who report GI symptoms that improve following withdrawal of gluten from their diet (Lebwohl et al., 2015). IBS patients who have tested negative for coeliac disease have also reported GI symptoms on a gluten-containing diet (GCD) that improve on a GFD. In a double-blinded RCT in which IBS patients received either gluten-containing food or placebo for 6 weeks, patients who received the gluten-containing food were more likely to report poorly controlled symptoms (68% vs 40%, P = 0.0001) (Biesiekierski et al., 2011).

The pathophysiology of NCGS in IBS patients is unclear, although studies have reported an increase in small bowel intestinal permeability in those who report symptoms whilst on a GCD. In one RCT of 45 patients with IBS-D, of which 23 were placed on a GFD and 22 placed on a GCD, the GCD patients had a greater number of bowel movements per day and higher small bowel permeability. Of note, those who tested positive for the genes HLA-DQ2 and HLA-DQ8, had statistically greater number of bowel movements and increased small bowel permeability, compared with those who did not (Vazquez-Roque et al., 2013). In a second RCT that recruited 34 patients with IBS, although a significant worsening of GI symptoms were reported in those on a GCD, there was no significant change in small bowel permeability (Biesiekierski et al., 2011).

1.5.3 Pharmacological Treatments for IBS-D

1.5.3.1 Medications Acting on Opioid Receptors

Loperamide is the only anti-diarrhoeal that has been evaluated in RCTs for the treatment of IBS. Two studies have shown an improvement in stool frequency and consistency, but with no overall improvement in global IBS symptoms.

Therefore, in the recently published American College of Gastroenterology guidelines (Ford et al., 2014), loperamide was not recommended for this indication.

Eluxadoline is a mixed mu-opioid receptor agonist and delta-opioid receptor antagonist that is orally administered and acts locally in the GI tract (Keating, 2017). In two phase 3 trials, 2427 patients with IBS-D were randomised to eluxadoline (75mg or 100mg) or placebo, for 26 weeks in the first trial (IBS-3001), and 52 weeks in the second trial (IBS-3002). The primary end point was the proportion of patients who had a composite response of a decrease in abdominal pain and an improvement in stool consistency on the same day for at least 50% of the days. In the IBS-3001 trial, efficacy was sustained from 1 to 26 weeks in patients who received eluxadoline 100mg when compared with placebo (29.3% vs. 19.0%, $P < 0.001$), and in the IBS-3002 trial, for eluxadoline 75mg and 100mg when compared with placebo (30.4% and 32.7% vs. 20.2%, $P = 0.001$ and $P < 0.001$ respectively) (Lembo et al., 2016).

1.5.3.2 Antispasmodics

Antispasmodics work by relaxing gut smooth muscle through anticholinergic mechanisms, or through the blocking of calcium channels. A Cochrane review in 2011 of 29 RCTs (2333 patients) showed a beneficial effect for antispasmodics over placebo for improvement in abdominal pain (RR 1.32; 95% CI 1.12 to 1.55, $P < 0.001$, NNT = 7), global assessment (RR 1.49; 95% CI 1.25 to 1.77, $P < 0.0001$, NNT = 5), and symptom score (RR 1.86; 95% CI 1.26 to 2.76, $P < 0.01$, NNT = 3) (Ruepert et al., 2011). This finding was subsequently confirmed in a systematic review in 2014, which also showed there was a class effect (Ford et al., 2014). Hyoscine bromide, dicyclomine hydrochloride, pinaverium bromide, and

cimetropium bromide were all shown to be beneficial. Mebeverine, alverine, trimebutine, pirenzipine, rociverine, propinox, and prifinium did not have a significant effect on IBS symptoms, although many of the trials were hampered by the small number of patients recruited.

1.5.3.3 Serotonin Receptor Antagonists

Serotonin, a hormone produced in the gut, is known to influence gut motility and sensitivity (Mawe and Hoffman, 2013). Alosetron was shown to be effective in improving global IBS symptoms, when compared with placebo in a meta-analysis containing 8 RCTs (RR = 0.79, 95% CI 0.69 to 0.90, NNT = 8) (Ford et al., 2014). The drug was licensed for the treatment of IBS-D in the USA, but during post-marketing surveillance, there were several reports of ischaemic colitis, and it was therefore withdrawn. It is now available again for women with severe IBS-D, under a restricted access programme. (Camilleri and Boeckxstaens, 2017)

More recently, ondansetron has recently been evaluated in a RCT for the treatment of IBS-D, and was found to improve stool consistency, frequency, urgency, and abdominal bloating when compared with placebo, although abdominal pain did not improve significantly. Sixty-five percent of patients reported adequate relief of symptoms with ondansetron, compared with 14% in the placebo arm of the trial (RR 4.7; 95% CI 2.6 to 8.5, $P < 0.001$) (Garsed et al., 2014).

1.5.4 Pharmacological Treatments for IBS-C

1.5.4.1 Laxatives

Only two studies have compared the osmotic laxative polyethylene glycol (PEG) with placebo, with no evidence that PEG improves overall symptoms and pain in IBS (Ford et al., 2014). There are no trials involving IBS-C patients for the osmotic laxative, lactulose, or stimulant laxatives such as bisacodyl, sodium picosulfate, or senna.

1.5.4.2 Pro-Secretory Agents

1.5.4.2.1 Lubiprostone

Lubiprostone is a chloride channel activator that works by stimulating intestinal fluid secretion (Chey et al., 2015). Three studies, reporting on 1366 patients, have shown that lubiprostone is effective in the treatment of IBS-C when compared with placebo (NNT 12.5, 95% CI 8 to 25) (Ford et al., 2014).

1.5.4.2.2 Linaclotide

Linaclotide is a 14-amino-acid peptide guanylate cyclase-C (GC-C) agonist. It binds to, and activates, GC-C receptors, resulting in release of chloride and bicarbonate in to the intestinal lumen, and subsequently increased intestinal fluid secretion (Sood and Ford, 2013). A systematic review and meta-analysis of three RCTs (1773 patients), comparing linaclotide with placebo for IBS-C, has shown that linaclotide is moderately effective in improving symptoms in patients with IBS-C (Atluri et al., 2014). Using the Food and Drug Administration endpoint of an

improvement of ≥30% from baseline in the average of the worst abdominal pain scores, and an increase of ≥1 complete spontaneous bowel movements from baseline, 66% of patients receiving linaclotide failed to achieve this endpoint, compared with 82.6% of patients receiving placebo (RR of failure to respond 0.80; 95% CI 0.76 to 0.85).

1.5.5 Other Treatments

1.5.5.1 Probiotics and Antibiotics

Probiotics are live microorganisms that when administered may result in a benefit for the host. In a recent systematic review and meta-analysis of 21 RCTs, when compared with placebo, probiotics were associated with an overall improvement in symptom response (RR 1.82; 95 % CI 1.27 to 2.60), and quality of life (standardised mean difference 0.29; 95 % CI 0.08 to 0.50), but not in individual symptoms (Zhang et al., 2016).

Rifaximin is a poorly systemically absorbed antibiotic, that is derived from rifamycin, and is effective against both aerobic and anaerobic bacteria (Pimentel, 2016). A systematic review and meta-analysis of five RCTs, comparing rifaximin with placebo, showed an improvement in global IBS symptoms (OR=1.57; 95% CI 1.22 to 2.01, NNT = 10.2) and bloating (OR=1.55; 95% CI 1.23 to 1.96 NNT = 10.1) (Menees et al., 2012). The side effect profile of rifaximin has been shown to be similar to placebo, probably as a result of its poor systemic absorption (Schoenfeld et al., 2014).

1.5.5.2 Antidepressants

The efficacy of tricyclic antidepressants (TCAs) in IBS have been evaluated in 11 RCTs (744 patients). Of those patients receiving TCAs, 43.3% showed no improvement in IBS symptoms, compared with 63.7% receiving placebo. The RR of IBS symptoms not improving on TCAs was 0.66 (95 % CI 0.56 to 0.79), with a NNT of 4 (95% CI 3 to 6) (Ford et al., 2014). Seven RCT have compared selective serotonin reuptake inhibitors (SSRIs) with placebo, with a RR of IBS symptoms not improving on SSRIs of 0.68 (95% CI 0.51 to 0.91), and a NNT of 4 (95% CI 2.5 to 20) (Ford et al., 2014).

1.5.5.3 Psychological Therapies

Psychological therapies include gut-directed hypnotherapy, relaxation therapy, and cognitive behavioural therapy (CBT) (Eriksson et al., 2015). In a meta-analysis that included 41 RCTs (2290 IBS patients), which predominantly used CBT as the intervention, psychological therapy resulted in a significant improvement in GI symptoms in both the short-term (1 month to 6 months) and long-term (6 months to 12 months), when compared with a control group, which included sham treatment, online discussion forums, and symptom monitoring (Laird et al., 2016).

1.6 Diagnosing IBS

Diagnosing IBS can be challenging, not only because of the complex and overlapping pathophysiological mechanisms previously discussed, but also because the presenting symptoms of IBS can overlap with those of organic disease, such as colorectal cancer (Chang et al., 2015), IBD (Halpin and Ford, 2012), BAM (Gracie et al., 2012; Wedlake et al., 2009), and coeliac disease (Sainsbury et al., 2013;

Sanders et al., 2001), resulting in uncertainty for both the patient and doctor if a diagnosis is based on symptoms alone (Sood et al., 2014). Most people who report lower abdominal discomfort associated with a change in stool form or frequency will have IBS, which can, for the most part, be managed in a primary care setting (Yawn et al., 2001). However, as there is the potential for a missed diagnosis of organic GI disease, the difficulty arises for clinicians in distinguishing between IBS and organic causes of these types of symptoms, and in deciding on who will require investigation.

Part of the rationale for the development of the symptom-based diagnostic criteria was to encourage physicians to make a positive diagnosis of IBS, and minimise unnecessary investigations. Current guidelines for the management of IBS in both the UK and USA still advocate this approach (Brandt et al., 2009; National Institute for Health and Care Excellence, 2008; Ford et al., 2014), and state clearly that IBS is not a diagnosis of exclusion. However, it has been suggested that such criteria, although useful for recruiting homogeneous groups of patients into clinical trials of therapies for IBS, are less relevant to routine clinical practice (Shivaji and Ford, 2015). Studies that have developed and validated these types of criteria have also been hampered by the lack of an accepted reference standard for the diagnosis of IBS. Most investigators have used a normal colonoscopy as confirmation of a diagnosis of IBS, that is, physicians still regard IBS as a diagnosis of exclusion, which is perhaps justified by the modest performance of the different symptom-based criteria, as shown below (Dogan and Unal, 1996; Ford et al., 2013; Tibble et al., 2002).

1.6.1 Diagnostic Accuracy of Symptoms and Symptom-based Diagnostic Criteria

1.6.1.1 Symptoms

Patients with IBS report symptoms such as lower abdominal pain, change in stool form or frequency, passage of mucus per rectum, abdominal pain relieved by defaecation and visible abdominal distension, or a sensation of bloating. Several studies have assessed the accuracy of individual symptoms in terms of their accuracy in the diagnosis of IBS (Frigerio et al., 1992; Hammer et al., 2004; Jeong et al., 1993; Kruis et al., 1984; Manning et al., 1978; Rao et al., 1993). A systematic review and meta-analysis (Ford et al., 2008), which pooled the results of these six studies, containing between 188 and 915 patients for the analysis of each symptom item, demonstrated that individual symptoms performed poorly in predicting IBS, with pooled sensitivities in the range of 39% to 90% and specificities in the range of 32% to 77%, respectively (Table 2).

Table 2. Pooled Sensitivity and Specificity for Individual Symptoms in Diagnosing Irritable Bowel Syndrome (Ford et al., 2008).

Symptom item	Number of studies	Number of patients	Sensitivity (95% CI)	Specificity (95% CI)
Lower abdominal pain	4	915	90 (79-97)	32 (21-44)
Mucus per rectum	4	507	45 (22-69)	65 (47-81)
Incomplete evacuation	4	507	74 (66-82)	45 (31-60)
Looser stools at onset of pain	4	507	58 (46-69)	73 (64-81)
More frequent stools at onset of pain	4	188	53 (41-66)	72 (58-84)
Pain relieved by defaecation	4	507	60 (54-67)	66 (57-73)
Patient-reported visible abdominal distension	3	227	39 (20-60)	77 (64-88)

1.6.1.2 Symptom-based Diagnostic Criteria

The poor performance of individual symptoms in differentiating between IBS and organic disease is, perhaps, not unexpected. In a real-life setting, physicians rarely use a single item from the clinical history to formulate a diagnosis, and are more likely to combine various items. The groups of symptoms that constitute the Manning or Rome criteria cluster together, and demonstrate statistically significant associations with each other in community-based factor analysis studies (Talley et al., 1998; Drossman et al., 1990). These observations were taken as evidence to support the biological plausibility of IBS as a distinct clinical entity, and led to the development of some of the available symptom-based diagnostic criteria described earlier.

1.6.1.2.1 The Manning Criteria

A systematic review and meta-analysis (Ford et al., 2008) identified three studies that validated the Manning criteria (Dogan and Unal, 1996; Jeong et al., 1993; Rao et al., 1993). When data from these three studies were pooled with the original validation study, the Manning criteria demonstrated a sensitivity and specificity of 78% and 72%, respectively. A further study has been published since this meta-analysis (Ford et al., 2013), which showed that when three or more symptoms were used, sensitivity and specificity were 62% and 82%, respectively. Perhaps not unexpectedly, the Manning criteria performed well in the original validation study (Manning et al., 1978), but also demonstrated reasonable accuracy in a study from Turkey (Dogan and Unal, 1996).

1.6.1.2.2 The Rome Criteria

A systematic review and meta-analysis, published in 2008 (Ford et al., 2008), found only one eligible study containing 602 patients that reported on the accuracy of the Rome I criteria (Tibble et al., 2002) with sensitivity and specificity of 71% and 85%, respectively. A further study has been published in the intervening years (Ford et al., 2013), recruiting 1,848 individuals referred to secondary care for consideration of investigation of GI symptoms, with good sensitivity (96%), but a specificity of only 71%.

The accuracy of the Rome II and III criteria remained unknown, due to a lack of prospective validation studies until 2013, when a study of 1,848 individuals referred to secondary care in Canada was published, which validated all iterations of the Rome criteria and the Manning criteria simultaneously within the same dataset (Ford et al., 2013). The sensitivity and specificity of the Rome II criteria were 90% and 72%, respectively, whereas Rome III demonstrated a sensitivity of 69% and specificity of 79.5%. Importantly, the Rome III criteria did not perform better than any of the previous symptom-based criteria within this dataset (Ford et al., 2013). The Rome IV criteria have yet to be externally validated in an unselected population, although they have been internally validated by the Rome committee in differentiating IBS from other FGIDs such as functional dyspepsia and CIC and were found to have a sensitivity of 62.7% and a specificity of 97.1% (Palsson et al., 2016).

1.6.2 Biomarkers

Perhaps because of the modest performance of symptom-based diagnostic criteria, interest has focused on various biomarkers as a means of diagnosing IBS. Biomarkers are measurable biological characteristics including physiological responses, genes, metabolites, or proteins that can serve as an indicator of a disease state or condition. Some of the potentially available biomarkers that have been developed are based on our understanding of the pathophysiology of IBS.

1.6.2.1 Visceral Hypersensitivity as a Biomarker

Altered visceral perception in IBS patients, expressed as reduced sensory thresholds, has been demonstrated in a number of studies (Mertz et al., 1995; Naliboff et al., 1997). There has been one retrospective study that has evaluated the sensitivity and specificity of rectal sensitivity to distension in patients with IBS (Bouin et al., 2002). 86 patients with IBS, 25 HCs, and 78 controls with other FGIDs were recruited. All patients underwent rectal distension testing, which involved placement of a rectal probe, inflated with a barostat bag to a maximum pressure limit of 48mmHg. Discomfort and pain levels were scored. IBS patients were found to have a significantly lower threshold for pain, when compared with the other groups. The optimal level of distension to identify IBS was 40mmHg, which yielded a sensitivity of 95.5% and a specificity of 72% in differentiating IBS from health or other FGIDs (Table 3). In a similar study recruiting patients with IBS and HCs, a threshold pressure of 26mmHg was able to distinguish between IBS and health with 63% sensitivity and 90% specificity (Ludidi et al., 2012). As a potential biomarker, it would be more desirable if this test was able to differentiate accurately between IBS and organic disease, thus reassuring both patient and clinician, and avoiding

unnecessary investigation. However, this test fails to fulfil these criteria, particularly as visceral hypersensitivity has also been described in patients with UC (Delvaux, 2002). Additionally, the invasive nature of the test, and its lack of availability outside of a research setting, probably limit its role in clinical practice.

Table 3. Sensitivity and Specificity of Symptom-based Diagnostic Criteria, Biomarkers, Psychological Markers, or Combinations Thereof in Diagnosing Irritable Bowel Syndrome.

	Study and Year	Sensitivity (95% confidence interval)	Specificity (95% confidence interval)
Manning criteria	Ford *et al.* 2008	78 (62-90)	72 (55-87)
	Ford *et al.* 2013	62 (57-67)	82 (80-91)
Rome I	Tibble *et al.*	71 (66-76)	85 (80-89)
	Ford *et al.* 2013	96 (93-97)	71 (68-73)
Rome II	Ford *et al.* 2013	90 (87–93)	72 (69-74)
Rome III	Ford *et al.* 2013	69 (64-73)	79.5 (77-81.5)

Visceral hypersensitivity as a biomarker ≥48mmHg	Bouin *et al.* IBS from health and other FGIDs	95.5 (not reported)	72 (not reported)
≥26mmHg	Ludidi *et al.* IBS from health	63 (not reported)	90 (not reported)
Altered pain perception as a biomarker	Kim *et al.* IBS from health and other FGIDs	86 (not reported)	76 (not reported)

Serum biomarkers			
10-biomarker panel	Lembo *et al.*		
	IBS from non-IBS	50 (not reported)	88 (not reported)
34-biomarker panel	Jones *et al.*		
	IBS from health	81 (75-87)	64 (54-75)
Anti CdtB Ab	Pimentel *et al.*		
	IBS-D from IBD	44 (not reported)	92 (not reported)
	IBS-D from coeliac	33 (not reported)	81 (not reported)
	IBS-D from health	29 (not reported)	95 (not reported)
Anti-vinculin Ab	Pimentel *et al.*		
	IBS-D from IBD	33 (not reported)	84 (not reported)
	IBS-D from coeliac	44 (not reported)	79 (not reported)
	IBS-D from health	44 (not reported)	91 (not reported)

Faecal biomarkers			
Volatile organic metabolites	Ahmed *et al.*		
	IBS-D from Crohn's	94 (not reported)	82 (not reported)
	IBS-D from UC	96 (not reported)	80 (not reported)
	IBS-D from health	82 (not reported)	78 (not reported)
Volatile organic compounds	Aggio *et al.*		
	IBS from active IBD	93 (not reported)	90 (not reported)
	IBS from inactive IBD	89 (not reported)	80 (not reported)
	IBS from all IBD	92 (not reported)	78 (not reported)
	IBS from health	74 (not reported)	81 (not reported)
Secretogranin II (>0.16nmol/g)	Öhman *et al.*		
	IBS from health	80 (not reported)	79 (not reported)

Secretogranin III (>0.53nmol/g) Chromogranin b (<0.48nmol/g)	Öhman *et al.* IBS from health Öhman *et al.* IBS from health	80 (not reported) 78 (not reported)	68 (not reported) 69 (not reported)
Colonic mucosal immune biomarkers	Cremon *et al.* IBS from health IBS from UC	80 to 94 (not reported) 86 to 100 (not reported)	73.5 to 90 (not reported) 90 to 95 (not reported)
Tight junction disruption as a biomarker	Turcotte *et al.* IBS from health	62 (not reported)	89 (not reported)

Psychological markers	Jones *et al.* IBS from health	74 (not reported)	75 (not reported)
Biomarkers and psychological markers	Jones *et al.* IBS from health	85 (not reported)	88 (not reported)
Kruis scoring system	Ford *et al.*	77 (68-85)	89 (76-97)
Faecal calprotectin and small intestinal permeability ratio	Tibble *et al.*	69 (64-74)	92 (88-95)
Faecal calprotectin, small intestinal permeability ratio, and Rome I criteria	Tibble *et al.*	50 (45-56)	98 (96-99)

1.6.2.2 Altered Pain Perception as a Biomarker

As discussed earlier, visceral hypersensitivity is an important pathophysiological mechanism in the aetiology of IBS. Kim *et al.* investigated whether there were any differences in pain perception during colonoscopy between IBS and non-IBS patients (Kim et al., 2010). 217 patients, 101 with IBS, 37 with other FGIDs, and 79 HCs underwent colonoscopy for either investigation of GI symptoms, or screening purposes in the case of HCs. Those found to have organic pathology, including IBD, or who underwent procedures such as polypectomy, were excluded from the final analysis. Pain perception immediately post-procedure was evaluated by patients using a 100-mm VAS. Generally, IBS patients experienced more pain during colonoscopy. The optimal VAS pain score in differentiating between IBS and HCs, or other non-IBS FGIDs, was 31mm, with a sensitivity of 86% and 76% respectively (Table 3). Again, this study used an invasive test to make a positive diagnosis of IBS, perhaps defeating the purpose of a clinically useful biomarker. This study is also limited by its exclusion of patients with organic pathology.

1.6.2.3 Serum Biomarkers

In 2009, Lembo *et al.* validated a panel of biomarkers for differentiating IBS patients from non-IBS patients (Lembo et al., 2009). Following a review of the medical literature, 60,000 biomarkers were identified that were related to potential pathophysiological processes in IBS, or other organic GI diseases. Each represented a relationship between a gene, protein, cellular process, or physiological condition in the GI tract. When only those that were serum-based and had a viable commercial

assay were considered, this number fell to 140. Of these, 10 were chosen finally, among them IL-1β, anti-tissue transglutaminase, and anti-neutrophil cytoplasmic antibody (ANCA), based on their performance in differentiating IBS from non-IBS. These were then combined to form an algorithm. Serum samples collected from 1721 individuals with IBS, organic GI diseases, other FGIDs, non-GI functional disorders, and healthy people were then split into a derivation cohort of 1205 subjects, used to train the algorithm, and a validation cohort of 516 individuals. However, sensitivity and specificity in differentiating IBS from non-IBS were 50% and 88% respectively (Table 3), which is no better than existing available symptom-based criteria, and potentially far more expensive.

Jones and colleagues added another 24 biomarkers to the original panel, 14 of which were identified by analysing genes that were expressed differentially in IBS patients compared with healthy people, with the other 10 selected from pathways involved in pain, inflammation, serotonin metabolism, or mast cell activation (Jones et al., 2014). These included histamine, tryptase, serotonin, IL-12, IL-10, Il-6, IL-8, low density lipoprotein receptor, and vasoactive intestinal peptide receptor-1. However, performance of the 34 biomarker panel in 204 patients who met the Rome III criteria for IBS and 90 age and gender-matched healthy volunteers was again modest, with sensitivity and specificity of 81% and 64% respectively (Table 3). A subset of four biomarkers accounted for the majority of the performance. The major limitation of this study was that the serum biomarker algorithm was used to differentiate between IBS and health, whereas a biomarker that could differentiate between functional and organic GI disease would be far more relevant to clinical practice.

Recently, Pimentel *et al*. (Pimentel et al., 2015) validated two serum biomarkers, antibodies (Abs) to cytolethal distending toxin B (CdtB) and vinculin, primarily focusing on differentiating IBS-D from IBD. CdtB is a bacterial toxin commonly produced by *Campylobacter jejuni*, as well as *E.coli*, *Salmonella*, and *Shigella*. Infection of rats with C.*jejuni* can lead to PI-IBS, similar to humans, and the presence of CdtB appears to be positively associated with the likelihood of developing a PI-IBS phenotype. In addition, rats infected with a strain of C.*jejuni* that lacks CdtB exhibit a lower likelihood of developing this phenotype (Jee et al., 2010; Pokkunuri et al., 2012). Vinculin is a host cell adhesion protein, with which anti-CdtB Abs are known to cross react. The study recruited 2681 participants aged 18 to 65 years old from 180 centres. Of these, 2375 were Rome III IBS-D subjects recruited into a large RCT of rifaximin in IBS, 142 had IBD (73 with Crohn's disease and 69 with UC), 121 had coeliac disease, and 43 were healthy volunteers. Participants in the IBS-D group were approximately 4 years older, when compared with the non-IBS participants, but there was no difference in sex distribution between IBS-D and non-IBS participants. ELISA testing was performed on plasma samples collected from all participants, using recombinant *Campylobacter* CdtB protein, and full length human vinculin protein, as antigens. Levels of anti-CdtB and anti-vinculin Abs were then calculated and compared from optical densities, and the sensitivity, specificity, and likelihood ratios (LRs) of these were assessed, and receiver operating characteristics (ROC) curves constructed.

Levels of anti-CdtB Ab titres were found to be significantly higher in participants with IBS-D (2.53 $\pm$ 0.69) when compared with Crohn's disease (1.72 $\pm$ 0.81), UC (1.54 $\pm$ 0.68), coeliac disease (2.23 $\pm$ 0.70), or healthy subjects (1.81 $\pm$ 0.73) ($P < 0.001$). Furthermore, levels of anti-vinculin Abs were significantly higher

in IBS-D subjects (1.34 ± 0.85) when compared with Crohn's disease (1.05 ± 0.91), UC (0.96 ± 0.77), coeliac disease (1.07 ± 0.98), or healthy subjects (0.81 ± 0.59) ($P < 0.0001$).

When determining optimum cut-off points on ROC curves using anti-CdtB and anti-vinculin Ab levels to distinguish between IBS-D and non-IBS subjects, the authors elected to maximise specificity over sensitivity, reducing the number of false positive results, and therefore improving the positive LR. When distinguishing IBS-D from IBD, the area under the curve (AUC) was higher for anti-CdtB Abs when compared with anti-vinculin Abs (0.81 vs. 0.62). Using a cut-off point of levels ≥2.80 for anti-CdtB Abs, sensitivity was 43.7%, specificity 91.6%, and positive and negative LRs were 5.2 and 0.6 respectively. Using a cut-off point of levels ≥1.68 for anti-vinculin Abs, sensitivity and specificity were 32.6% and 83.8%, with a positive and negative LR of 2.0 and 0.8 respectively.

ROC curves constructed for anti-CdtB Abs were also able to differentiate IBS-D from coeliac disease and healthy volunteers, with an AUC of 0.63 and 0.76 respectively. At levels ≥1.68 of anti-CdtB Abs, IBS-D was differentiated from coeliac disease with a sensitivity of 32.6% and a specificity of 81.0%. Positive and negative LRs were 1.7 and 0.8 respectively. At levels ≥1.80 of anti-CdtB Abs, IBS-D was differentiated from healthy volunteers with a sensitivity of 28.9% and specificity of 95.4%, with positive and negative LRs of 6.2 and 0.7 respectively.

The AUCs for anti-vinculin Abs in differentiating IBS-D from coeliac disease, and IBS-D from healthy volunteers, were 0.61 (sensitivity 43.7%; specificity 79.3%; positive LR 2.1; negative LR 0.7, at levels ≥2.80) and 0.68 (sensitivity 43.7%; specificity 90.7%; positive LR 4.7; negative LR 0.6, at levels

≥2.80) respectively. Sensitivity and specificity were not reported for either marker in differentiating IBS subjects from all non-IBS subjects.

This study is important for two reasons. Firstly, the fact that these two antibodies were able to differentiate IBS-D from IBD and HCs, with a reasonable degree of accuracy, suggests that a substantial proportion of individuals with IBS may have an overt or sub-clinical post-infectious trigger, resulting in intestinal microbial disturbances, and the development of IBS-D. Secondly, the ability of these tests, if positive, to rule in IBS-D and rule out IBD is encouraging, especially when compared with the accuracy of previously validated serum-based biomarkers. Their ability to make a positive diagnosis is in direct contrast to other available biomarkers such as faecal calprotectin, in which a negative test rules out IBD, and therefore only reaches a "diagnosis" of IBS-D via the exclusion of an organic disease. However, a major limitation of this study, as well as previous studies assessing the accuracy of serum-based biomarkers, is that they have all been conducted in secondary or tertiary care with an enriched sample of cases, resulting in an artificially high prevalence of IBS, meaning that the diagnostic accuracy reported may not be reproducible in an unselected primary care population, where the prevalence of IBS is likely to be lower. This is also the setting in which an accurate diagnostic test is probably required the most.

1.6.2.4 Faecal Biomarkers

Other biomarkers have also been assessed as a potential diagnostic tool in IBS. A recent study examined the accuracy of volatile organic metabolites (VOMs) in differentiating 30 patients with IBS-D from 110 patients with active IBD (Ahmed et al., 2013). VOMs are chemicals that are released in faeces, and which then

undergo changes as a result of organic disease or alterations in the intestinal microbiota, and these were extracted from faeces using solid-phase microextraction fibres, after faecal samples had been heated in a water bath at 60°C for 1 hour. Following univariate analysis, 44 VOMs were found to be able to differentiate IBS-D from active IBD, of which 35 were more abundant in IBS-D. Sensitivity and specificity of the model in differentiating IBS-D from Crohn's disease was 94% and 82% respectively, and sensitivity and specificity of differentiating between IBS-D and UC was 96% and 80% respectively. Finally, the model was able to differentiate between IBS-D and HCs with a sensitivity of 82% and specificity of 78% (Table 3).

Aggio *et al.* (Aggio et al., 2016) conducted a study that used a prototype device based on gas chromatography to separate volatile organic compounds (VOCs) (products from digestion and fermentation by the intestinal microbiota), from faecal gas, in order to identify patterns that could be used to differentiate between IBS, IBD, and health. Patients were recruited prospectively between October 2010 and October 2011, and faecal samples were obtained from 28 patients with IBS, as defined by the Rome II criteria (26 patients with IBS-D), 33 patients with active IBD, 50 patients with inactive IBD, and 41 HCs. A simple clinical activity index score $\geq$3 or a Harvey Bradshaw index $\geq$4 were used to define active UC and active Crohn's disease, respectively.

Faecal samples were stored at -20ºC, and samples were analysed by gas chromatography, which works by characterising the VOCs contained in the faecal samples. Patterns in the VOCs were then detected for each of the individual medical disorders. This prototype device had a runtime of only 40 minutes, therefore potentially providing a means for a point-of-care test. The device was able to distinguish between IBS and active IBD with a maximum sensitivity and specificity

of 93% and 90% respectively; between IBS and inactive IBD with a maximum sensitivity and specificity of 89% and 80% respectively; between IBS and all IBD patients with a maximum sensitivity of 92% and specificity of 78%; and between IBS and HCs with a maximum sensitivity and specificity of 74% and 81% respectively. Following construction of a ROC curve, the AUC for all comparisons ranged between 83.2% for differentiating between IBS and HCs and 96.5% for differentiating between IBS and active IBD.

Granins, such as chromogranins (Cg) and secretogranins (Sg), are proteins found in the secretory cells of the enteric, endocrine, and immune system, which are thought to reflect activity of these systems. In one study of 82 IBS patients and 29 HCs, higher levels of faecal CgA, SgII, and SgIII were found in IBS patients relative to healthy individuals, and levels of CgB were found to be lower in IBS patients (Ohman et al., 2012). However, these faecal biomarkers performed only modestly in differentiating IBS from HCs, with SgII performing the best (sensitivity 80%, specificity 79%) (Table 3). Again, this study was limited by the small sample size and the biomarker being used to differentiate between IBS and health, rather than IBS and organic disease. Granins are also unlikely to be specific markers of IBS, as increased levels are also associated with organic GI diseases, such as lymphocytic colitis and coeliac disease (Camilleri, 2012).

1.6.2.5 Colonic Mucosal Biomarkers

As previously discussed, studies have reported increased mast cells and T-lymphocytes within the colonic mucosa of patients diagnosed with IBS (Cremon et al., 2009; Akbar et al., 2008). In one study, Cremon *et al.* (Cremon et al., 2013) examined whether colonic mucosal immune biomarkers, including mast cells,

immune cells, or immune gene expression, could be used to diagnose IBS. In total, 144 patients with IBS, 32 with UC, and 68 HCs underwent colonoscopy and colonic mucosa sampling for histological examination. Using quantitative real time reverse transcriptase PCR the expression of nerve growth factor, interferon-ɣ, toll-like receptor-4, and pre-haptoglobin-2 were measured. These were all significantly increased in IBS patients, compared with HCs, but were significantly lower than among UC patients. Sensitivity and specificity of the biomarkers in distinguishing between IBS and HCs were 80% to 94% and 73.5% to 90% respectively (Table 3). Sensitivity and specificity, when distinguishing between IBS and UC, were 86% to 100% and 90% to 95%, respectively. However, there have been no other studies replicating these findings and, at the time of writing, the results of this study have not been fully published.

1.6.2.6 Exhaled Organic Compounds as a Biomarker

Hundreds of VOCs are present in human exhaled breath, some of which can be associated with pathophysiological processes such as lung cancer and asthma (Tang et al., 2017; Oguma et al., 2017). In one study, the researchers collected breath samples and symptom data from 170 IBS patients, as well as 153 age- and gender-matched HCs in whom GI symptoms and disorders were excluded following medical consultation (Baranska et al., 2016). A further 1307 participants were enrolled from a large general population cohort, and provided exhaled breath samples and a 7-day GI symptom diary. Analysis of breath was by thermal desorption-gas chromatography, combined with mass spectrometry, in order to determine a combination of VOCs that best discriminated IBS patients from HCs,

following which a ROC curve was constructed to determine the sensitivity and specificity of this biomarker panel.

The investigators identified 16 VOCs that best discriminated between 123 IBS patients and 123 HCs. The accuracy of this panel was then validated in the remaining 47 IBS patients and 30 HCs, and was able to differentiate between the two with a sensitivity of 89% and specificity of 73%. Positive and negative predictive values (PPV and NPV) were 84% and 82% respectively, and the AUC was 0.83. Analysis of breath-o-grams from the IBS patients and HCs demonstrated visible separation between the two in both the derivation and validation cohorts. However, some IBS patients had VOC profiles that overlapped with the HCs, which could not be explained either by medical history or baseline characteristics.

In the IBS cohort, a significant correlation was found between the panel of VOCs and abdominal pain, discomfort, belching, and flatulence ($r = 0.55$, $P = 0.0003$). In the general population cohort, only participants with 7-day mean scores for abdominal pain or discomfort >1.5 on a 1 to 5-point scale were used in the correlation analysis, and a moderate but significant correlation was found between the panel of VOCs and abdominal pain, belching, bloating, flatulence, nausea, and diarrhoea ($r = 0.54$, $P = 0.0004$). The authors concluded that breath analysis may be useful in both the diagnosis and monitoring of IBS, but could also be used as a screening tool to detect the condition in the general population. The study is important for two reasons. Firstly, this is the only study that has attempted to identify and then validate a panel of exhaled VOCs that can differentiate IBS from health, and which can potentially be applied as a simple and non-invasive clinical test. Secondly, in utilising multiple VOCs, this is more likely to encompass the multifactorial aetiology of IBS, compared with individual markers. Finally, in

showing that a panel of VOCs appears to correlate with GI symptoms, an issue that has not been explored in previous studies, there is the potential for developing a means to monitor IBS symptoms following instigation of medical therapy. However, the major limitation of this study is that once again the VOC panel was validated only in terms of its ability to differentiate IBS from health.

1.6.2.7 Tight Junction Disruption as a Biomarker

As previously discussed, disruption to the tight junctions resulting in increased intestinal permeability may be an important pathophysiological mechanism in IBS. In one small study, 17 IBS patients and 18 HCs underwent colonoscopy and ileoscopy, with the aim to identify presence of epithelial gaps using CLE (Turcotte et al., 2013). IBS patients were found to have significantly higher epithelial gap densities in the terminal ileum compared with controls. Using a cut-off of 30 gaps per 1000 cells as the threshold to define abnormal gap density, sensitivity and specificity were 62% and 89% respectively (Table 3). However, the usefulness of this test is probably limited, as increased epithelial gap density has also been demonstrated in IBD patients (Liu et al., 2011). In addition, the performance of the test is likely to be highly dependent on the operator, and the required skills may not be available in other centres.

1.6.2.8 Faecal Bile Acid, Colonic Transit, and Intestinal Permeability as a Biomarker

In one study, the ability of three quantitative traits, total faecal bile acids, colonic transit, and intestinal permeability, were assessed in order to identify treatable processes that may discriminate between IBS-D, IBS-C, and HCs

(Camilleri et al., 2014). The study recruited 64 patients with IBS-D, 30 patients with IBS-C, and 30 HCs, and all study participants completed a validated bowel disease questionnaire, the somatic symptom checklist (SSC), HADS questionnaire, and underwent measurement of colonic transit, testing for faecal BAM, evaluation of bile acid synthesis, and also assessment of intestinal permeability. Colonic transit was evaluated by scintigraphy, using methods validated previously. Evidence of BAM was assessed using serum 7α-C4, and a combination of liquid chromatography and mass spectrometry was used to measure faecal bile acid excretion. Daily faecal fat excretion was estimated by nuclear magnetic resonance spectrometry. Bile acid synthesis was quantified by measuring serum FGF19, and intestinal permeability, via urinary excretion of lactulose and mannitol, after oral ingestion.

Psychosomatic and depression scores, according to the SSC and HADS, were statistically higher among IBS patients when compared with HCs. Faecal fat, serum C4 levels, and total faecal bile acid levels were higher in the IBS-D patients, compared with both IBS-C patients and HCs. Colonic transit studies at 48 hours showed a higher geometric centre in the IBS-D patients ($P < 0.001$). Urine mannitol levels at 2 hours were also higher in the IBS-D group, when compared with IBS-C patients and HCs ($P = 0.039$). Significant correlations were demonstrated between total faecal bile acids and colonic transit (at both 24 hours and 48 hours), and between total faecal bile acids and intestinal permeability.

Following logistic regression analysis and using ROC curves, with estimation of AUC, total faecal bile acids were found to be a predictor for IBS-D vs. HCs ($P = 0.025$, $ROC_{AUC} = 0.70$), and for IBS-D vs. IBS-C ($P = 0.024$, $ROC_{AUC} = 0.81$). The addition of C4 and FGF19 measurements did not improve the accuracy of total faecal bile acids in discriminating between the three study groups. Colonic transit at

48 hours was also able to discriminate IBS-C from HCs ($P = 0.03$, ROC_{AUC} 0.70), and IBS-C from IBS-D ($P < 0.001$, $ROC_{AUC} = 0.78$). Small intestinal permeability on its own was not a significant predictor. However, combining total faecal bile acids, colonic transit, and small intestinal permeability led to greater accuracy in differentiating IBS-C from HCs when compared with each of these variables independently ($ROC_{AUC} = 0.73$).

A two-item model, consisting of colonic transit measurement at 48 hours, and total faecal bile acids, was assessed in terms of its accuracy to discriminate IBS from health. Using a fixed threshold of 80% sensitivity on the ROC curve, the specificity of the model in differentiating IBS-D from HCs was 43%, in differentiating IBS-C from HCs it was 57%, and in distinguishing between IBS-D and IBS-C it was 81%. When using a reduced sensitivity threshold of 60%, these specificities increased to 75%, 85%, and 90% respectively.

Limitations of the study include the fact that 10 of the 64 IBS-D patients (15.6%) had undergone cholecystectomy, a slightly higher proportion than in both the IBS-C group (10%) and HCs (6.7%). In a study which has previously been discussed, 190 (50.9%) of 373 patients with chronic diarrhoea who underwent SeHCAT scan were found to have BAM, with cholecystectomy associated with an increased risk for an abnormal SeHCAT scan (OR 2.51; 99% CI 1.10 to 5.77) (Gracie et al., 2012). Therefore, in the current study, the higher cholecystectomy rate in the IBS-D group could have contributed to the higher level of total faecal bile acids observed in this group. In addition, once again the biomarker combination was used to differentiate IBS from health but, as previously stated, a test that differentiates IBS from organic GI disease presenting with similar symptoms, would

be more clinically useful. Finally, the study was conducted in a tertiary centre with a relatively small, and potentially highly selected, patient group.

1.6.3 Diagnosing IBS Using Psychological Markers

The link between psychological life stressors and IBS has previously been discussed. In one study that assessed the accuracy of psychological markers in diagnosing IBS (Jones et al., 2014), 244 individuals were recruited, 168 of whom had IBS and 76 were HCs. All participants were asked to complete three validated measures of psychological wellbeing, the HADS scale, the patient health questionnaire-15 (PHQ-15), and the perceived stress scale. Mean scores across all three measures were significantly higher among those with IBS, compared with HCs. Psychological measures alone had a sensitivity and specificity of 74% and 75% in differentiating IBS from health (Table 3).

1.6.4 Diagnosing IBS Using Combinations of Symptoms, Biomarkers, and Psychological Markers

None of the methods described above, with the exception of faecal VOMs/VOCs in two relatively small studies and anti-CdtB Abs in a case-control study with a high IBS prevalence, seem to be able to predict the presence of IBS with any particular accuracy, or are hampered by their applicability in a clinical setting. Combining clinical items, biomarkers, and psychological markers together may lead to a greater ability to discriminate between IBS and organic disease. As early as 1984, Kruis and colleagues developed a statistical model to aid in the diagnosis of IBS (Kruis et al., 1984), based on a scoring system which incorporated

the clinical history, physical examination, and the results of some simple blood tests, including a full blood count and an erythrocyte sedimentation rate (ESR) (Table 4).

Table 4. The Kruis Statistical Model.

Symptom items included	Minimum symptom duration
Symptoms (reported by the patient using a form): Abdominal pain, flatulence, or bowel irregularity Description of abdominal pain as "burning, cutting, very strong, terrible, feeling of pressure, dull, boring, or 'not so bad'" Alternating constipation and diarrhoea **Signs (each determined by the physician):** Abnormal physical findings and / or history pathognomonic for any diagnosis other than IBS ESR >20mm/2h Leucocytosis >10,000/μL Anaemia (Haemoglobin <12 g/dL for females or <14 g/dL for males) Impression by the physician that the patient's history suggests blood in the stools	>2 years

This was assessed in 479 patients in the original study, with the items described above combined to produce a scoring system, and a score of ≥44 used as the threshold to define IBS. The Kruis statistical model has been validated subsequently in three studies (Dogan and Unal, 1996; Frigerio et al., 1992; Bellentani et al., 1990), meaning that its accuracy has been assessed in 1171 patients in total. A meta-analysis pooled the results of these four studies (Ford et al., 2008), and reported sensitivity and specificity of 77% and 89% respectively (Table 3). However, there have been few other investigators who have used this type of approach, until recently.

In the study conducted by Jones *et al.* (Jones et al., 2014), the authors combined the markers of psychological effect described in the previous section above, with the 34-biomarker panel they had validated. This led to a greater ability to distinguish between patients with IBS and healthy volunteers, with improvement in sensitivity and specificity of 85% from 81% and 88% from 64% respectively, when compared with biomarkers alone (Table 3).

In the previously discussed study from Tibble *et al.* (Tibble et al., 2002), patients provided a stool sample for measurement of faecal calprotectin levels, in addition to undergoing a lactulose/L-rhamnose small intestinal permeability test. Using a faecal calprotectin level of <10 mg/L and a permeability ratio of <0.05, this biomarker combination was able to identify IBS patients with a sensitivity of 69% (95% CI 64% to 74%) and specificity of 92% (95% CI 88% to 95%).

In the same study, a positive result for the Rome I criteria was incorporated with faecal calprotectin levels of <10mg/L and permeability ratio of <0.05, resulting in a sensitivity of 50% (95% CI 45% to 56%) and a specificity of 98% (95% CI 96% to 99%) (Tibble et al., 2002).

1.7 Possible Future Directions

Gastroenterologists and primary care physicians consulting in clinic need diagnostic tests that distinguish between IBS and other organic disorders that may produce similar symptoms, and which could be confused with IBS, such as coeliac disease or IBD, rather than between IBS and health. Limitations of the methods of diagnosing IBS studied above include their conduct in referral populations, meaning that the results may not be generalisable to primary care where the prevalence of IBS is lower. The studies have had relatively small sample sizes in some instances, with a lack of other studies validating the more novel approaches prospectively, such as faecal VOMs/VOCs, and several of the techniques described have been used to discriminate between people with IBS and healthy individuals. One of the striking observations is the relatively modest performance of all the available symptom-based diagnostic criteria. This is probably not surprising, given that they are, for the most part, derived from each other, meaning that the same strengths and weaknesses have been passed on from one set of criteria to another.

The performance of biomarkers is also disappointing, particularly given their potentially expensive nature. However, faecal VOMs/VOCs appear promising, although the results reported by Ahmed *et al.* (Ahmed et al., 2013) and Aggio *et al.* (Aggio et al., 2016) need to be replicated by other investigators before their accuracy is confirmed. Perhaps combining demographic data such as age, gender, upper, as well as lower, GI symptoms, biomarkers, and psychological markers, using methodology, akin to the Kruis statistical model, as a means of improving IBS diagnostics is one future direction to consider. However, one drawback to this approach is increasing complexity, and a diagnostic test that may become too cumbersome to use in routine clinical practice. This is probably one of the reasons

why the Kruis scoring system has never been adopted widely, despite its reasonable performance in predicting IBS.

The diagnostic ability of any of these methods (symptom-based, demographic information, and biomarkers) could be improved through the use of more complex statistical methods. Latent class analysis (LCA) is one such method. LCA uses patterns in measured variables, such as symptoms, to develop an estimate of an unmeasured attribute, such as IBS, which has no specific biomarker. These methods are widely applied in other fields, such as psychiatry, and have been shown to be particularly valuable when, as is the case for IBS, an accurate and accepted gold standard test is lacking (Rindskopf and Rindskopf, 1986; Kato et al., 2010; Schur et al., 2007). LCA methods are designed to use the sets of measurements that we do have access to, in order to construct an appropriate pattern of measurements that most closely represents the latent construct IBS. They are a type of categorical data analysis, which define groups known as classes, and could be used to improve the predictive power of methods used to diagnose IBS, and therefore to discriminate between IBS and non-IBS symptom profiles.

For example, in a group of diseases that share some of the same symptoms, LCA can be used to detect patterns of association in the disease entities, and therefore determine the likelihood of belonging to a particular group. There are few instances of this type of modelling being used in FGIDs. Two such examples in the literature have examined the association between the most common functional somatic symptoms and syndromes, including IBS, which often show considerable overlap, and are thought to share similar aetiologies (Kato et al., 2010; Schur et al., 2007). Given the limitations of current approaches, better methods to diagnose IBS are required.

CHAPTER 2

Aims and Objectives

The aim of this thesis is to explore diagnostic approaches to IBS, to try to enhance the limited ability of symptom-based diagnostic criteria to correctly identify patients with IBS, and to assess the performance of novel methods to differentiate IBS from organic diseases that may present with similar symptoms, such as colorectal cancer, IBD, and coeliac disease. The following pieces of work have been conducted:

2.1 The Accuracy of Diagnosing Irritable Bowel Syndrome with Symptoms, Biomarkers, and Psychological Markers

As described previously, symptom-based diagnostic criteria appear to perform only moderately well in in differentiating IBS from organic pathology. Given their modest performance, more accurate ways of diagnosing IBS are required, and as discussed, interest has increased in developing biomarkers as a diagnostic tool to aid in this. A previous systematic review has already examined the accuracy of symptom-based diagnostic criteria in differentiating IBS from organic disease (Ford et al., 2008), but since this systematic review further individual studies have been published examining the accuracy of these criteria, as well as studies describing more novel methods to diagnose IBS, including biomarkers and psychological markers. A systematic review and meta-analysis was therefore conducted in order to summarise all available approaches in diagnosing IBS, including symptoms, biomarkers, psychological markers, and combinations thereof (Chapter 3). The aim was to understand the strengths and weaknesses of the available diagnostic tests for IBS. Using these findings, two diagnostic test studies were designed and undertaken with the intention of creating accurate, inexpensive, and easily administrable tests for clinicians consulting in routine clinical care.

2.2 Validating the Rome III Criteria in Secondary Care and Enhancing their Performance

There has been only one study to date, published in 2013 and undertaken in Canada (Ford et al., 2013), that has validated the Rome III criteria, despite these criteria being first described in 2006. A further validation of the gold standard in diagnosing IBS is therefore required in a demographically different study population.

As previously discussed, combining symptoms with clinical biomarkers and/or markers of psychological effect may lead to an increased ability to discriminate between IBS and organic disease. To date, no study has assessed the effect of combining the Rome III criteria with one or more relevant biomarkers or psychological markers, such as haemoglobin, C-reactive protein (CRP), anxiety and depression scores, somatisation scores, and other symptoms such as nocturnal passage of stools, to assess if this improves accuracy in differentiating IBS from organic disease. A diagnostic accuracy study was therefore conducted among consecutive new patient referrals with lower GI symptoms in Leeds, UK, in order to assess the performance of the Rome III criteria, as well as the effect of modifications to them. The aim was to develop an accurate, inexpensive, and potentially easily administrable test in routine clinical care.

2.3 A Novel Approach to Diagnosing IBS Using Latent Class Analysis

There are few examples in the literature of LCA being used to aid in the diagnosis and management of FGIDs (Kato et al., 2010; Schur et al., 2007), and only one study that has used this approach in IBS patients. In this study by Koloski *et al.* LCA was used to differentiate between IBS-C and CIC (Koloski et al., 2015). No studies, to date, have used LCA as a diagnostic test to aid in distinguishing

between IBS and organic disease. Using the database from the only study that has validated the Rome III criteria (Ford et al., 2013), we aimed to use LCA to derive a model that identified predictors of IBS, which could then be used as a diagnostic test for the disorder. This model was then validated in the database of consecutive patients referred with lower GI symptoms in Leeds recruited in the previous study.

CHAPTER 3

Systematic Review and Meta-Analysis: Accuracy of Diagnosing Irritable Bowel Syndrome with Symptoms, Biomarkers and Psychological Markers

3.1 Introduction

As discussed, symptom-based diagnostic criteria perform only modestly in differentiating IBS from organic disease. Furthermore, these criteria have been criticised for being overly complex and impractical in a clinical setting, particularly in primary care where the majority of IBS patients are diagnosed and managed (Shivaji and Ford, 2015; Thompson et al., 1997). Partly as a consequence of this, interest has grown in developing novel biomarkers, some of which have been reviewed in chapter one. However, a comprehensive systematic review and meta-analysis of novel biomarkers for IBS, incorporating several literature databases, has not previously been undertaken.

Additionally, although the accuracy of symptom-based diagnostic criteria in predicting IBS has previously been examined (Ford et al., 2008), since this meta-analysis was performed, there have been more studies published relating in particular to the Rome II and Rome III criteria that were not included in this meta-analysis.

Patients with IBS are more likely to have higher levels of anxiety, neuroticism, or mood instability when compared with healthy individuals and those with other lower GI disorders (Creed et al., 2001; Henningsen et al., 2003; Koloski et al., 2006; Lee et al., 2008). As a result, studies have also been conducted to assess whether measures of psychological wellbeing can aid in the diagnosis of IBS. However, no systematic review has assessed the accuracy of markers of psychological affect in predicting a diagnosis of IBS.

A systematic review and meta-analysis was therefore conducted to examine all the available methods that use symptoms, biomarkers, markers of psychological affect, or combinations thereof, to aid in the diagnosis of IBS.

3.2 Methods

3.2.1 Search Strategy and Study Selection

The systematic review was performed according to the Cochrane Methods Group on screening and diagnostic tests guidelines. A search of the medical literature was conducted using MEDLINE (January 1946 to February 2015), EMBASE, and EMBASE classic (1947 to February 2015). Eligible studies were required to report prospectively on adult (≥16 years of age) patients with lower GI symptoms, and had to assess the accuracy of one or more of the available accepted symptom-based diagnostic criteria for IBS, biomarkers, psychological markers, or combinations thereof, in diagnosing IBS against an accepted reference standard, taken as being a physician's diagnosis of IBS, another set of accepted diagnostic criteria, or the absence of an organic explanation for these symptoms, such as IBD, microscopic colitis, or colorectal cancer, following lower GI endoscopy (Box 1).

Box 1. Eligibility Criteria.

- Adult patients (aged ≥16 years) with lower GI symptoms
- Cross-sectional design or case-control
- Applied a diagnostic test for IBS to all patients, including one or more of: symptom-based diagnostic criteria†, biomarkers, psychological markers, or combinations thereof
- Confirmed presence of IBS using an accepted reference standard‡
- Results of diagnostic test for IBS compared with the reference standard
- ≥50 patients included

†Manning, Rome I, Rome, II, or Rome III criteria

‡Normal colonoscopy, barium enema, CT colonography, physician's opinion that this was IBS, or accepted symptom-based diagnostic criteria for IBS

When assessing the accuracy of symptom-based diagnostic criteria, the reference standard was mandated as negative lower GI investigations, but when assessing the accuracy of novel biomarkers this could either be accepted symptom-based diagnostic criteria or a physician's diagnosis of IBS. Studies that applied an accepted test for organic disease, such as faecal calprotectin, and therefore effectively reached a diagnosis of IBS by exclusion of the specific organic disease that the test was designed to detect, were not considered as eligible for inclusion in this meta-analysis.

Search terms used to identify potentially relevant publications were: *irritable bowel syndrome, IBS, functional diseases, colon,* or *functional adj5 bowel.* These were combined, using the set operator AND, with the following search terms: *Kruis, Manning, Rome 1, Rome I, Rome 2, Rome II, Rome3, Rome III, biomarker, f$ecal biomarker, psychological marker, metabolite, transit time, colonic motility, small intestinal motility, visceral hypersensitivity, pain, bile acid, cytokine, mast cell, intestinal permeability, chromogranin,* or *secretogranin.* These were again combined using the set operator AND with the search terms *sensitivity* or *specificity.* There were no language restrictions and abstracts of the papers identified by the initial search were evaluated by the lead author for appropriateness to the study question. All potentially relevant papers were obtained and evaluated in detail. Foreign language papers were translated. Abstract books of conference proceedings between 2007 and 2014 were hand-searched to identify potentially eligible studies published only in abstract form. The bibliographies of all identified relevant studies were used to perform a recursive search of the literature. Articles were assessed independently by two reviewers using pre-designed eligibility forms, according to

the prospectively defined eligibility criteria. Any disagreement between investigators was resolved by consensus.

3.2.2 Data Extraction

All data were extracted independently by two reviewers on to a Microsoft Excel spreadsheet (XP professional edition; Microsoft Corp, Redmond, WA, USA) as total number of patients with IBS, total number of IBS patients testing positive for IBS using the diagnostic criteria, biomarker, psychological marker, or combination thereof under study, total number of non-IBS patients, and the total number of non-IBS patients testing positive for IBS using the same diagnostic test. In addition, the following clinical data were extracted for each study: setting (primary or secondary care), number of centres, country of origin, and diagnostic test applied.

3.2.3 Data Synthesis and Statistical Analysis

The degree of agreement between investigators, in judging study eligibility, was measured using the Kappa statistic. The accuracy of diagnostic tests is often summarised using sensitivity and specificity. Although these are useful measures of a test's performance, they provide the probability of the test being positive if the disease of interest is present, or the probability of the test being negative if the disease is absent. However, for a physician consulting with a patient it is more useful to know the probability of the patient truly having the disease if the test is positive, or truly not having the disease if the test is negative. These are the PPV and NPV of the test.

One of the limitations of predictive values is that their magnitude varies according to the prevalence of the disease under study. For this reason, more useful summary measures of the diagnostic accuracy of a test are the positive and negative LRs. These are derived from the sensitivity and specificity of a test, which are fixed, and therefore do not vary according to the prevalence of the disease of interest (Moayyedi and Axon, 1999). The positive LR is derived from the formula: $\frac{sensitivity}{1-specificity}$ while the negative LR is derived from the formula: $\frac{1-sensitivity}{specificity}$ As a rule of thumb, positive LRs above 10 are very useful in ruling in a disease, and negative LRs below 0.1 are very useful in ruling out a disease.

For each study identified in the literature search, the raw data from the paper was extracted, as described above, into a Microsoft Excel spreadsheet (XP professional edition; Microsoft Corp, Redmond, WA, USA), in order to calculate the sensitivity, specificity, PPV and NPV of each of the diagnostic tests for IBS under study. The positive and negative LRs, and their 95% CIs, were also calculated within the same spreadsheet, using the aforementioned formulas. These calculations were checked using Meta-DiSc® version 1.4 (Universidad Complutense, Madrid, Spain). Where data were not extractable, the authors of the original paper were contacted, if possible, in order to obtain further information.

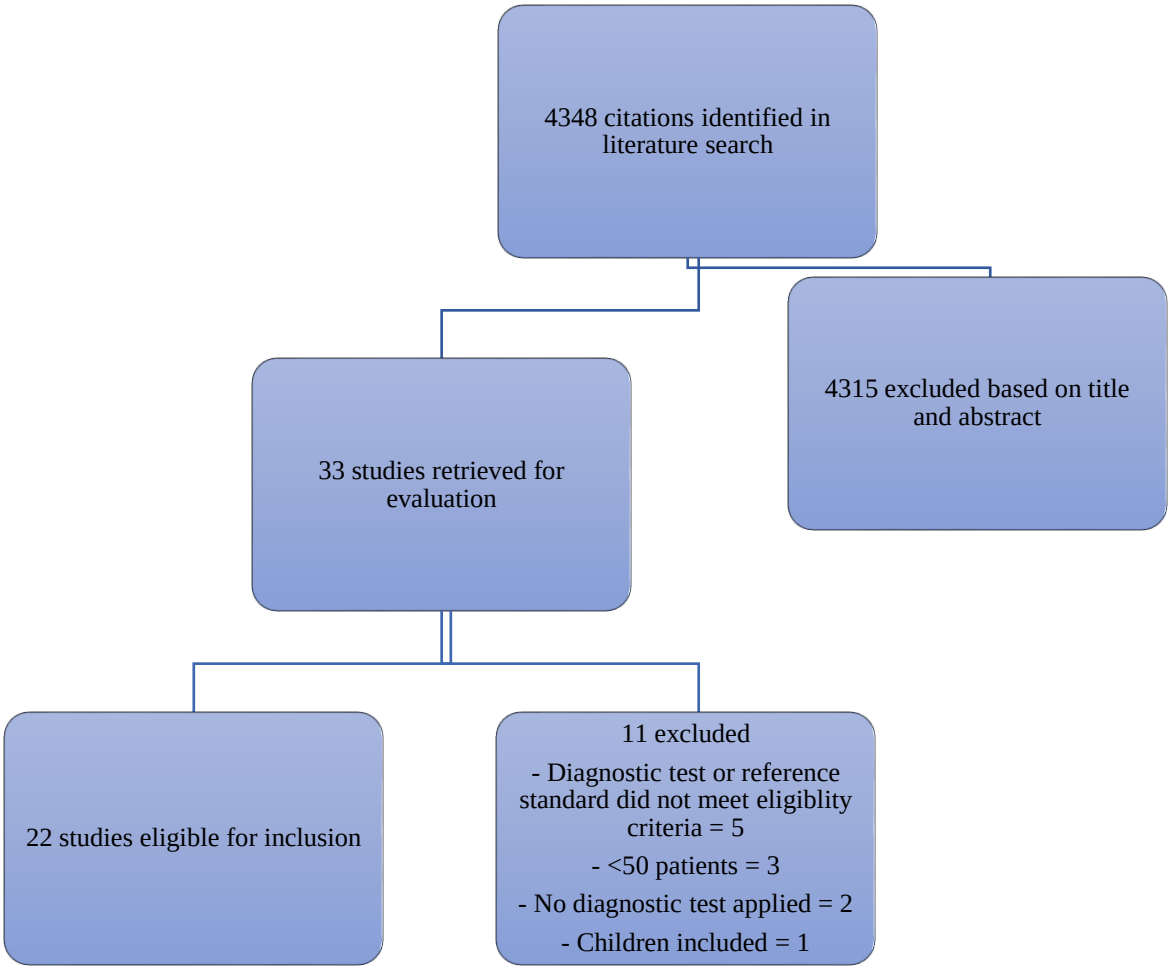
Where the accuracy of identical symptom-based criteria, biomarkers, psychological markers, or combinations thereof, were reported by more than one study, LRs were combined from each study using StatsDirect version 2.7.7 (StatsDirect Ltd, Sale, Cheshire, England), in order to generate pooled positive and negative LRs with 95% CIs. Results were also pooled from all studies in order to obtain pooled positive and negative LRs with 95% CIs for each approach used to diagnose IBS, including symptom-based criteria alone, biomarkers alone,

psychological markers alone, and combinations of these. A random effects model was used to provide a more conservative estimate of the accuracy of the various methods, allowing for heterogeneity between studies. Heterogeneity was assessed using the χ^2 and I^2 statistic, with a value >50% indicating significant heterogeneity. QUADAS-2, a quality assessment tool for primary diagnostic accuracy studies, was used to assess the risk of bias and any applicability concerns in the eligible studies (Whiting et al., 2003). All eligible studies were judged against four key domains covering patient selection, the diagnostic test applied, the reference standard, and the flow of patients through the study.

3.3 Results

The search strategy identified 4348 citations, of which 33 studies appeared to be eligible and were retrieved for evaluation. Twenty-two of these met all eligibility criteria (Ahmed et al., 2013; Bellentani et al., 1990; Bouin et al., 2002; Camilleri et al., 2014; Crade and Pham, 2006; Dogan and Unal, 1996; El-Salhy et al., 2014; El-Salhy et al., 2014; Ford et al., 2013; Frigerio et al., 1992; Hammer and Talley, 2008; Jeong et al., 1993; Jones et al., 2014; Kang et al., 1994; Kim et al., 2010; Kruis et al., 1984; Lembo et al., 2009; Manning et al., 1978; Ohman et al., 2012; Rao et al., 1993; Spiller et al., 2010; Tibble et al., 2002) (Figure 2).

Figure 2. Flow Diagram of Studies Identified in the Systematic Review.



Agreement between investigators when assessing eligibility was excellent (94% agreement, K = 0.86). The 22 included studies evaluated a total of 7106 patients with a pooled IBS prevalence of 53.5% (95% CI 47.4% to 59.6%). Eleven of the studies were conducted in Europe, six in North America, four in Asia, and one in Australasia. Thirteen of the studies were of cross-sectional design, and nine were case-control. Twenty-one were conducted in secondary care, with one in both primary and secondary care. Individual study characteristics are summarised in Table 5. The diagnostic tests utilised in the eligible studies are shown in Table 6, along with the number of studies assessing the accuracy of each test, total number of patients included, and the positive and negative LRs with 95% CIs (pooled where appropriate). Study bias and applicability outcomes assessed, according to the QUADAS-2 tool, are shown in Table 7. Fourteen of the 22 studies were judged as high risk in one or more of the four key domains.

Table 5. Characteristics of all Eligible Studies.

Study	Country	Study design	Setting (no. of centres)	No. of patients	No. with IBS (%)	Diagnostic test applied	Reference standard used	Overall risk of bias according to QUADAS-2 tool
Manning 1978	England	Cross-sectional	Secondary care (1)	65	32 (49.2)	≥2 Manning criteria ≥3 Manning criteria ≥4 Manning criteria	Normal colonoscopy or barium enema	High
Kruis 1984	Germany	Cross-sectional	Secondary care (1)	317	108 (34.1)	Kruis statistical model score ≥ 44 to diagnose IBS	Normal colonoscopy or barium enema	Low
Bellentani 1990	Italy	Cross-sectional	Primary and secondary care (15)	254	152 (59.8)	Kruis statistical model Own statistical model	Normal colonoscopy or barium enema	Low

Frigerio 1992	Italy	Cross-sectional	Secondary care (1)	253	52 (20.6)	Kruis statistical model Modified Kruis model with lowering of the predetermined cut-off point of haemoglobin level	Normal colonoscopy	Low
Jeong 1993	South Korea	Cross-sectional	Secondary care (1)	74	86 (77.5)	≥2 Manning criteria ≥3 Manning criteria ≥4 Manning criteria	Normal colonoscopy or barium enema	Low
Rao 1993	India	Cross-sectional	Secondary care (1)	88	65 (73.9)	≥2 Manning criteria ≥3 Manning criteria ≥4 Manning criteria	Normal colonoscopy or barium enema	Low
Kang 1994	Singapore	Case-control	Secondary care (1)	138	128 (92.8)	Pain perception during rectal insufflation at diagnostic sigmoidoscopy or colonoscopy	Manning criteria	High

Dogan 1996	Turkey	Cross-sectional	Secondary care (2)	347	165 (47.6)	≥2 Manning criteria ≥3 Manning criteria ≥4 Manning criteria Kruis statistical model	Normal colonoscopy or barium enema	High
Bouin 2002	Canada	Case-control	Secondary care (1)	189	86 (77.5)	Visceral hypersensitivity: rectal distension using a barostat bag with optimum performance determined using a cut-off of 40mmHg	Rome II criteria	High

Tibble 2002	England	Cross-sectional	Secondary care (1)	602	339 (56.3)	Rome I criteria Faecal calprotectin (<10mg/L) and intestinal permeability ratio (<0.05) Faecal calprotectin, intestinal permeability ratio, and Rome I criteria	Normal colonoscopy or barium enema	Low
Crade 2006	USA	Cross-sectional	Secondary care (1)	175	27 (15.4)	Sigmoid muscularis propria thickness: cut-off ≥3mm used to diagnose IBS	Physician's diagnosis	High
Hammer 2008	Australia	Cross-sectional	Secondary care (1)	538	233 (43.3)	≥3 Manning criteria ≥4 Manning criteria Rome I criteria Rome II criteria	Normal colonoscopy or barium enema	High

Lembo 2009	USA	Cross-sectional	Secondary care (6)	516	256 (49.6)	10 serum-based biomarker panel	Rome II and Rome III criteria	High
Kim 2010	South Korea	Cross-sectional	Secondary care (1)	217	101 (46.5)	Visceral hypersensitivity: VAS to record pain perception during colonoscopy	Rome III criteria	Low
Spiller 2010	England	Case-control	Secondary care (2)	470	319 (67.9)	Psychological markers: optimum performance determined using a cut-off >6 for PHQ-12 and >7 when assessing individual components of HADS	Rome II criteria	High

Öhman 2012	Sweden	Case-control	Secondary care (1)	111	82 (73.9)	Faecal chromogranins and secretogranins: optimum performance determined using a cut-off of >0.16 nmol/g of SgII, >0.53 nmol/g of SgIII and <0.48 nmol/g of CgB	Rome II criteria	High
Ahmed 2013	England	Case-control	Secondary care (1)	140	30 (21.4)	VOMs: optimum performance determined using a ROC curve	Manning criteria	High

Ford 2013	Canada	Cross-sectional	Secondary care (2)	1878	379 (20.2)	≥2 Manning criteria ≥3 Manning criteria ≥4 Manning criteria Rome I criteria Rome II criteria Rome III criteria	Normal colonoscopy	Low
Camilleri 2014	USA	Case-control	Secondary care (1)	124	94 (75.8)	Bile acid secretion and colonic transit: optimum performance determined using an ROC curve	Rome III criteria	High

Jones 2014	USA	Case-control	Secondary care (35)	244	168 (68.9)	10 serum-based biomarker panel 34 serum-based biomarker panel Psychological markers alone 34 serum-based biomarker panel and psychological markers	Rome III criteria	High
El-Salhy 2014	Norway	Case-control	Secondary care (3)	289	203 (70.2)	Mucosal intestinal endocrine cells: optimum performance determined using a cut-off of <200 cells/mm^2 of duodenal CgA	Rome III criteria	High

El-Salhy 2014	Norway	Case-control	Secondary care (1)	77	50 (64.9)	Mucosal intestinal endocrine cells:- optimum performance determined using an ROC curve	Rome III criteria	High

Table 6. Pooled Positive and Negative Likelihood Ratios (LRs) of Diagnostic Tests for Irritable Bowel Syndrome.

	Diagnostic test applied	No. of studies	No. of patients	Positive LR	95% CI	Negative LR	95% CI
Symptom-based diagnostic criteria	Manning ≥2 criteria	5	2452	2.20	1.54-3.14	0.18	0.10-0.32
	Manning ≥3 criteria	6	2966	2.85	1.95-4.15	0.36	0.24-0.53
	Manning ≥4 criteria	6	2986	3.43	2.49-4.71	0.66	0.60-0.73
	Rome I	3	3006	3.20	2.29-4.47	0.22	0.10-0.49
	Rome II	2	2402	2.56	1.64-4.00	0.25	0.08-0.85
	Rome III	1	1848	3.35	2.97-3.79	0.39	0.34-0.46
Biomarkers	Visceral hypersensitivity	2	328	3.71	2.74-5.02	0.16	0.10-0.24
	Pain perception	1	138	0.98	0.80-1.60	1.09	0.40-3.98
	Serum-based 10 biomarker panel	2	760	3.03	1.49-6.17	0.52	0.43-0.64
	Serum-based 34 biomarker panel	1	244	2.28	1.71-3.17	0.30	0.21-0.42
	VOMs in faeces	1	140	4.83	3.36-7.14	0.04	0.01-0.21

	Chromogranins and secretogranins in faeces:						
	Secretogranin II (SgII)	1	111	3.89	2.07-8.23	0.25	0.15-0.39
	Secretogranin III (SgIII)	1	111	2.59	1.61-4.70	0.28	0.17-0.47
	Chromogranin B (CgB)	1	111	2.51	1.56-4.56	0.32	0.20-0.51
	Duodenal chromogranin A (CgA)	1	289	18.5	7.58-47.3	0.14	0.10-0.20
	Rectal endocrine cells:						
	Peptide YY	1	77	7.56	2.96-21.9	0.18	0.09-0.33
	Oxyntomodulin	1	77	4.32	2.14-9.87	0.25	0.14-0.43
	Somatostatin	1	77	7.20	2.81-20.9	0.23	0.12-0.38
	Sigmoid muscularis propria thickness	1	175	14.9	7.07-31.5	0.31	0.17-0.51
	Faecal calprotectin and small intestinal permeability ratio	1	602	8.64	5.76-13.1	0.34	0.28-0.39
	Bile acid secretion and colonic transit	1	124	2.78	1.55-5.58	0.46	0.33-0.65
Psychological markers	PHQ-12 score	1	470	12.5	6.55-24.6	0.35	0.30-0.41
	Anxiety component of the HADS	1	470	2.88	2.20-3.86	0.37	0.30-0.45

	Depression component of the HADS	1	470	5.44	3.01-10.1	0.68	0.62-0.75
	Combination of HADS, PHQ-15, and the perceived stress scale	1	244	2.95	2.04-4.48	0.35	0.26-0.46
Combinations of symptoms, biomarkers, and/or psychological markers	Kruis *et al.* statistical model	4	1171	8.63	2.89-25.8	0.26	0.17-0.41
	Modified Kruis statistical model	1	253	7.73	4.83-12.4	0.34	0.22-0.49
	Bellentani *et al.* statistical model	1	254	4.29	2.86-6.66	0.30	0.22-0.39
	Faecal calprotectin, small intestinal permeability ratio, and Rome criteria	1	602	26.4	11.4-61.9	0.51	0.45-0.56
	Serum-based 34 biomarker panel and psychological markers	1	244	7.14	4.01-13.3	0.18	0.12-0.25

Table 7. QUADAS-2 Risk of Bias and Applicability for All Eligible Studies.

	Risk of bias				Applicability concerns		
Study	**Patient selection**	**Index test**	**Reference standard**	**Flow and timing**	**Patient selection**	**Index test**	**Reference standard**
Manning 1979	Low	Low	Low	Low	High	Low	Low
Kruis 1984	Low	Low	Low	Low	Low	Low	Low
Bellentani 1990	Low	Low	Low	Low	Low	Low	Low
Frigerio 1992	Low	Low	Low	Low	Low	Low	Low
Jeong 1993	Low	Low	Low	Low	Low	Low	Low
Rao 1993	Low	Low	Low	Low	Low	Low	Low
Kang 1994	High	Low	Low	High	Low	Low	Low
Dogan 1996	Low	Low	Low	Low	High	Low	Low
Bouin 2002	High	Low	Low	Low	Low	Low	Low

Tibble 2002	Low	Low	Low	Low	Low	Low	Low
Crade 2006	Low	Low	Unclear	Unclear	High	Low	Low
Hammer 2008	Low	Low	Low	Low	High	Low	Low
Lembo 2009	High	Low	Low	High	Low	Low	Low
Kim 2010	Low	Low	Low	Low	Low	Low	Low
Spiller 2010	High	High	Low	Low	Low	Low	Low
Öhman 2012	High	Low	Unclear	Unclear	Low	Low	Low
Ahmed 2013	High	Low	Low	Low	Low	Low	Low
Ford 2013	Low	Low	Low	Low	Low	Low	Low
Camilleri 2014	High	Low	Low	Low	Low	Low	Low
Jones 2014	High	Low	Low	Low	Low	Low	Low
El-Salhy 2014	High	High	Low	Low	Low	Low	Low
El-Salhy 2014	High	High	Low	Low	Low	Low	Low

3.3.1 Symptom-based Diagnostic Criteria

Five studies evaluated ≥2 of the Manning criteria (Dogan and Unal, 1996; Ford et al., 2013; Jeong et al., 1993; Manning et al., 1978; Rao et al., 1993), six studies ≥3 (Dogan and Unal, 1996; Ford et al., 2013; Hammer and Talley, 2008; Jeong et al., 1993; Manning et al., 1978; Rao et al., 1993), and six studies ≥4 (Manning et al., 1978; Jeong et al., 1993; Rao et al., 1993; Dogan and Unal, 1996; Hammer and Talley, 2008; Ford et al., 2013). The Rome I criteria were evaluated in three studies (Ford et al., 2013; Hammer and Talley, 2008; Tibble et al., 2002), the Rome II criteria in two studies (Ford et al., 2013; Hammer and Talley, 2008) and the Rome III criteria in only one study (Ford et al., 2013). All studies were cross-sectional in design, collected symptom data using a questionnaire completed by the patient, and utilised a reference standard of a normal colonoscopy or barium enema to confirm the diagnosis of IBS.

3.3.1.1 The Manning Criteria

Pooled positive and negative LRs when using ≥2 of the Manning criteria in a total of 2452 patients were 2.20 (95% CI 1.54 to 3.14; $I^2 = 90.5\%$; $P < 0.001$) and 0.18 (95% CI 0.10 to 0.32; $I^2 = 72\%$; $P = 0.006$) respectively (Dogan and Unal, 1996; Ford et al., 2013; Jeong et al., 1993; Manning et al., 1978; Rao et al., 1993). In studies assessing ≥3 of the Manning criteria (Dogan and Unal, 1996; Ford et al., 2013; Hammer and Talley, 2008; Jeong et al., 1993; Manning et al., 1978; Rao et al., 1993), accuracy was best in the study conducted by Dogan *et al.* (Dogan and Unal, 1996), with a positive LR of 7.15 (95% CI 4.93 to 10.57) and a negative LR of 0.11 (95% CI 0.07 to 0.17). However, this was not replicated in the five other studies, including in the original validation study (Manning et al., 1978). The pooled positive

and negative LRs, in a total of 2966 patients, were 2.85 (95% CI 1.95 to 4.15; I^2 = 89%; P < 0.001) and 0.36 (95% CI 0.24 to 0.53; I^2 = 89%; P < 0.001) respectively (Figure 3 and 4). Finally, when data were pooled from the six studies assessing the accuracy of ≥4 of the Manning criteria (Dogan and Unal, 1996; Ford et al., 2013; Hammer and Talley, 2008; Jeong et al., 1993; Manning et al., 1978; Rao et al., 1993), positive and negative LRs, in a total of 2986 patients, were 3.43 (95% CI 2.49-4.71; I^2 = 59%; P = 0.03) and 0.66 (95% CI 0.60-0.73; I^2 = 47%; P = 0.09) respectively (Figure 5 and 6).

Figure 3. Pooled Positive Likelihood Ratios of ≥3 Manning Criteria.

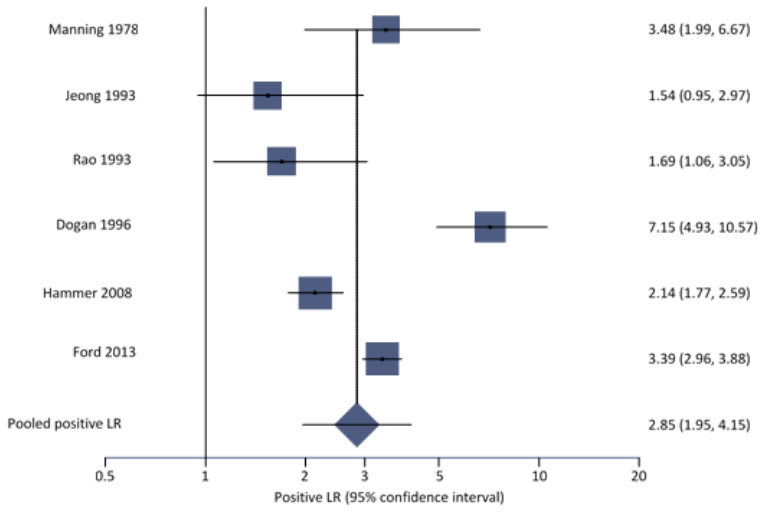


Figure 4. Pooled Negative Likelihood Ratios of ≥3 Manning Criteria.

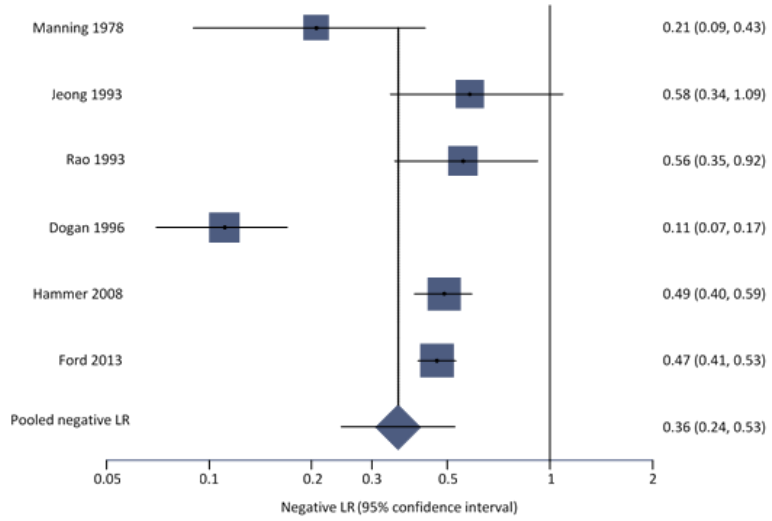


Figure 5. Pooled Positive Likelihood Ratios of ≥4 Manning Criteria.

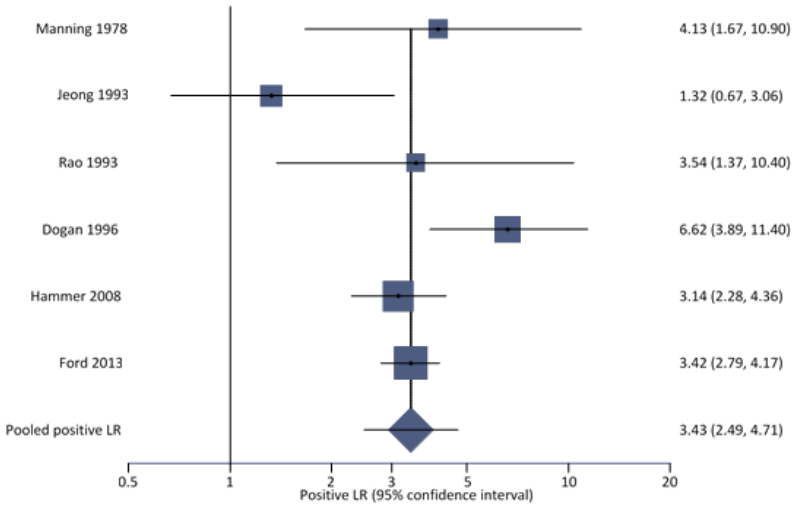
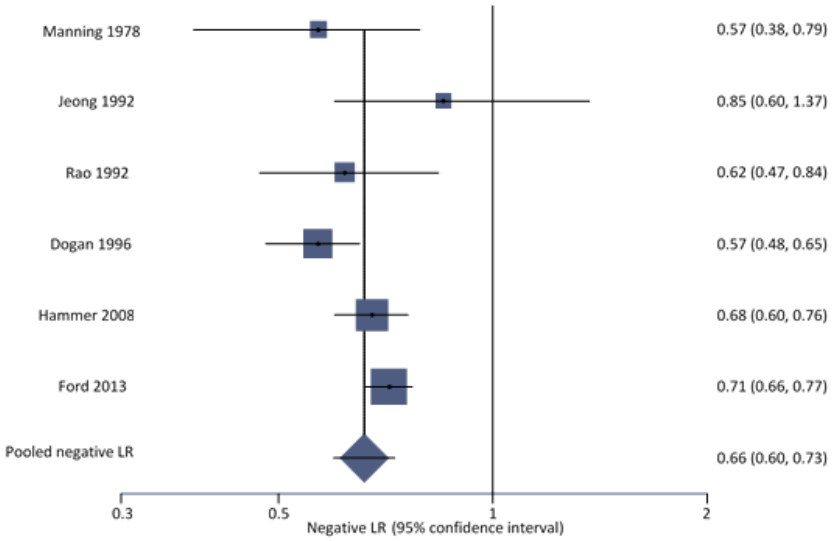


Figure 6. Pooled Negative Likelihood Ratios of ≥4 Manning Criteria.



3.3.1.2 The Rome Criteria

Pooled positive and negative LRs in the three studies (Ford et al., 2013; Hammer and Talley, 2008; Tibble et al., 2002), containing 3006 patients, that reported on the Rome I criteria were 3.20 (95% CI 2.29 to 4.47; $I^2 = 90.5\%$; $P < 0.001$) and 0.22 (95% CI 0.10 to 0.49; $I^2 = 97\%$; $P < 0.001$) respectively. Pooled positive and negative LRs of the two studies (Ford et al., 2013; Hammer and Talley, 2008), containing 2402 patients, that evaluated the Rome II criteria were 2.56 (95% CI 1.64 to 4.00) and 0.25 (95% CI 0.08 to 0.85) respectively. The positive and negative LRs in the one study (Ford et al., 2013), containing 1848 patients, that reported on the Rome III criteria were 3.35 (95% CI 2.97 to 3.79) and 0.39 (95% CI 0.34 to 0.46).

3.3.2 Biomarkers

3.3.2.1 Visceral Hypersensitivity and Pain Perception as a Biomarker

One case-control study and one cross-sectional study evaluated the role of visceral hypersensitivity using rectal barostat testing (Bouin et al., 2002) and pain perception (Kim et al., 2010) during colonoscopy, in differentiating IBS from HCs and miscellaneous GI and medical conditions, in a total of 328 patients. Pooled positive and negative LRs in the two studies were 3.71 (95% CI 2.74 to 5.02) and 0.16 (95% CI 0.10 to 0.24) respectively. One case-control study, containing 138 patients, reported on colonic air insufflation to reproduce typical abdominal pain experienced in IBS as a biomarker in differentiating the disorder from colonic structural disease (Kang et al., 1994). This test performed poorly, with positive and

negative LRs of 0.98 (95% CI 0.80 to 1.60) and 1.09 (95% CI 0.40 to 3.98) respectively.

3.3.2.2 Serum-based Biomarkers

The diagnostic accuracy of a serum-based 10 biomarker panel, which were selected by examining differences in biomarker expression between IBS patients and HCs (among them IL-1ß, anti-TTG and ANCA), was reported in one cross-sectional study (Lembo et al., 2009) and one case-control study (Jones et al., 2014) containing a total of 760 patients, with a pooled positive LR of 3.03 (95% CI 1.49 to 6.17) and pooled negative LR of 0.52 (95% CI 0.43 to 0.64). In the study conducted by Jones *et al.* (Jones et al., 2014), an additional 24 serum biomarkers, selected through a combination of gene chip human array, gene array data analysis and real-time quantitative PCR, were added to the original 10 biomarker panel. Positive and negative LRs of the 34 biomarker panel were 2.28 (95% CI 1.71 to 3.17) and 0.30 (95% CI 0.21-0.42) respectively.

3.3.3 Faecal Biomarkers

3.3.3.1 Volatile Organic Metabolites

The diagnostic accuracy of VOMs were assessed in one case-control study containing 30 IBS-D patients, 62 patients with active Crohn's disease and 48 patients with active UC (Ahmed et al., 2013). Using a ROC curve to determine optimum performance, the positive and negative LRs in differentiating IBS from active IBD were 4.83 (95% CI 3.36 to 7.14) and 0.04 (95% CI 0.01 to 0.21) respectively.

3.3.3.2 Chromogranins and Secretogranins

In one case-control study, CgB and SgII and SgIII levels were measured in faecal samples from 82 IBS patients and 29 HCs (Ohman et al., 2012). SgII and SGIII levels were higher in the IBS patients, and CgB levels were lower. SgII performed the most accurately using a cut-off of >0.16 nmol/g, with a positive LR of 3.89 (95% CI 2.07 to 8.23) and negative LR of 0.25 (95% CI 0.15 to 0.39).

3.3.4 Mucosal Intestinal Endocrine Cells as a Biomarker

Quantification of CgA cells was performed on biopsy samples taken from the duodenum during gastroscopy in one case-control study (El-Salhy al., 2014). Using a cut-off of <200 cells/mm^2, the positive and negative LRs in differentiating 203 IBS patients from 86 HCs, were 18.5 (95% CI 7.58 to 47.3) and 0.14 (95% CI 0.10 to 0.20). In a similarly designed study (El-Salhy et al., 2014), rectal biopsies were taken from 50 patients with IBS and 27 HCs. Endocrine cell content was quantified and three endocrine cells, (peptide YY, oxyntomodulin and somatostatin) were validated as diagnostic tests. Using optimum performance determined on an ROC curve, peptide YY performed the best, with a positive LR of 7.56 (95% CI 2.96 to 21.9) and a negative LR of 0.18 (95% CI 0.09 to 0.33) at a cut-off of <30 cells/mm^2.

3.3.5 Sigmoid Muscularis Propria Thickness as a Biomarker

In a cross-sectional study of 175 female patients who were undergoing trans-vaginal ultrasound for investigation of gynaecological symptoms, sigmoid muscularis propria thickness was measured (Crade and Pham, 2006). A diagnosis of IBS was made using a cut-off for abnormal muscularis propria thickness of ≥3 mm. A clinical diagnosis of IBS was confirmed with the primary physician and/or

Gastroenterologist following ultrasound. Positive and negative LRs were 14.9 (95% CI 7.07 to 31.5) and 0.31 (95% CI 0.17 to 0.51) respectively.

3.3.6 Combinations of Biomarkers

3.3.6.1 Faecal Calprotectin and Intestinal Permeability Ratio as a Biomarker

In the previously discussed study from Tibble *et al.* (Tibble et al., 2002), all patients provided a stool sample for measurement of faecal calprotectin levels, in addition to undergoing a lactulose/L-rhamnose small intestinal permeability test. Using a faecal calprotectin level of <10 mg/L and a permeability ratio of <0.05, this biomarker combination was able to identify IBS patients with a positive LR of 8.64 (95% CI 5.76 to 13.1) and a negative LR of 0.34 (95% CI 0.28 to 0.39).

3.3.6.2 Bile Acid Secretion and Colonic Transit as a Biomarker

One case-control study used a 2-item model consisting of total faecal bile acid excretion and colonic transit to differentiate between 64 IBS-D patients, 30 IBS-C patients, and 30 HCs (Camilleri et al., 2014). Using the optimum cut-off on an ROC curve, the 2-item model was able to differentiate IBS from HCs with a positive LR of 2.78 (95% CI 1.55 to 5.58) and a negative LR of 0.46 (95% CI 0.33 to 0.65).

3.3.7 Psychological Markers

The use of psychological markers in differentiating IBS from health was evaluated in two case-control studies containing 714 patients (Jones et al., 2014; Spiller et al., 2010). In the study conducted by Spiller *et al.* 319 IBS patients and 151 HCs completed the patient health questionnaire 12 (PHQ-12).

The PHQ-12 differs from the PHQ-15 in that the three specific GI-related questions are removed. Using a cut-off score of >6, the positive LR for the PHQ-12 in differentiating IBS from health was 12.5 (95% CI 6.55 to 24.6), and the negative LR was 0.35 (95% CI 0.30 to 0.41). Using a cut-off score of >7, the anxiety component of the HADS score was reported to have positive and negative LRs of 2.88 (95% CI 2.20 to 3.86) and 0.37 (95% CI 0.30 to 0.45) respectively. At a cut-off of >7, the depression component of the HADS demonstrated positive and negative LRs of 5.44 (95% CI 3.01 to 10.1) and 0.68 (95% CI 0.62-0.75), respectively.

In the previously described study conducted by Jones *et al.*(Jones et al., 2014), participants were asked to complete the HADS, PHQ-15, and the perceived stress scale. Positive and negative LRs of these measures of psychological well-being combined in differentiating between IBS and HCs were 2.95 (95% CI 2.04 to 4.48) and 0.35 (95% CI 0.26 to 0.46) respectively.

3.3.8 Combinations of Symptoms, Biomarkers and Psychological Markers

3.3.8.1 Kruis Statistical Model

The accuracy of the Kruis statistical model was assessed in four cross-sectional studies, including a total of 1171 patients (Bellentani et al., 1990; Dogan and Unal, 1996; Frigerio et al., 1992; Kruis et al., 1984). A score of ≥44 was used as the optimal cut-off to diagnose IBS, as validated in the original study. The pooled positive LR of these studies as assessed in a previous meta-analysis (Ford et al., 2008), as there have been no studies published in the interim, was 8.63 (95% CI 2.89 to 25.8; I^2 = 95%; P < 0.001) and the pooled negative LR was 0.26 (95% CI 0.17 to 0.41; I^2 = 84.5%; P < 0.001).

3.3.8.2 Other Statistical Models

Frigerio *et al.* (Frigerio et al., 1992), in a cross-sectional study, lowered the predetermined cut-off point of haemoglobin level in the Kruis statistical model from 14g/100ml to 13g/100ml in males and from 12g/100ml to 11g/100ml in females. Positive and negative LRs for this modified model, containing 253 patients, were 7.73 (95% CI 4.83 to 12.4) and 0.34 (95% CI 0.22 to 0.49) respectively.

Although differing from the Kruis model in the items included, the model validated in the cross-sectional study by Bellentani *et al.* (Bellentani et al., 1990), also incorporated the clinical history, physical examination, and an ESR and leucocyte count. Positive and negative LRs for this statistical model, containing 254 patients, were 4.29 (95% CI 2.86 to 6.66) and 0.30 (95% CI 0.22 to 0.39) respectively.

3.3.8.3 A Combination of Faecal Calprotectin, Intestinal Permeability Ratio, and the Rome I Criteria

In the previously described study by Tibble *et al.* (Tibble et al., 2002), if a positive result for the Rome I criteria was incorporated with faecal calprotectin levels of <10mg/L and permeability ratio of <0.05, the positive and negative LRs were 26.4 (95% CI 11.4 to 61.9) and 0.51 (95% CI 0.45 to 0.56) respectively.

3.3.8.4 A Combination of Serum-based Biomarkers and Psychological Markers

Finally, in the study conducted by Jones *et al.* (Jones et al., 2014), the serum-based 34 biomarker panel and psychological measures were combined to ascertain whether this improved accuracy in diagnosing IBS. Positive and negative LRs for

this combined approach in differentiating IBS from health were 7.14 (95% CI 4.01 to 13.3) and 0.18 (95% CI 0.12 to 0.25) respectively.

3.3.9 Pooled Positive and Negative LRs for Each Approach Used to Diagnose IBS

When individual study results were combined to obtain pooled positive and negative LRs for each of the approaches to diagnose IBS, there were significant differences in the pooled positive LR between studies using symptom-based criteria alone (positive LR 2.85; 95% CI 2.53 to 3.20), and studies that used a combination of symptoms, biomarkers, and psychological markers (positive LR = 8.48; 95% CI 4.64 to 15.5), but not between any of the other methods (Figure 7). Negative LRs were not significantly different for any of the four approaches (Figure 8). The sensitivities, specificities, PPVs, and NPVs for all the studies are shown in Table 8.

Figure 7. Pooled Positive Likelihood Ratios for All Approaches to the Diagnosis of IBS.

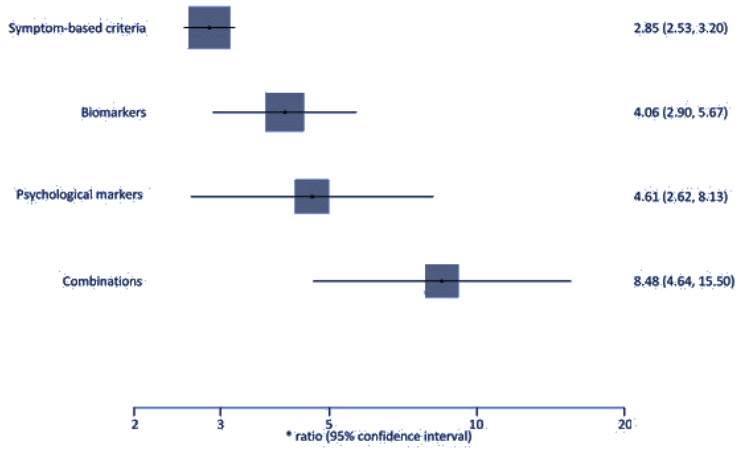


Figure 8. Pooled Negative Likelihood Ratios for All Approaches to the Diagnosis of IBS.

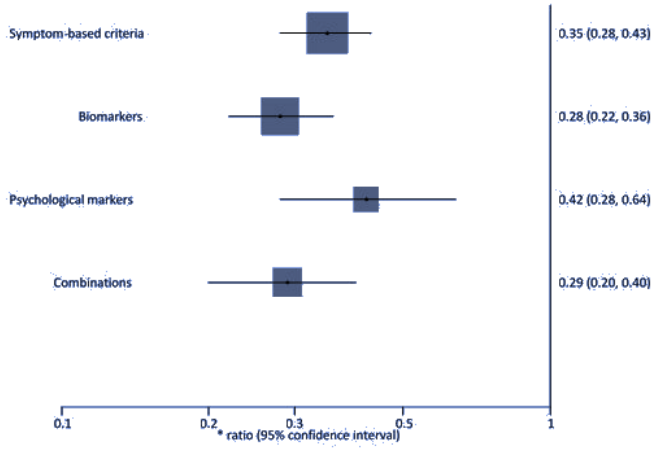


Table 8. Pooled Sensitivity, Specificity, and Predictive Values of Diagnostic Tests for Irritable Bowel Syndrome.

	Diagnostic test applied	No. of studies	No. of patients	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Symptom-based diagnostic criteria	Manning ≥2 criteria	5	2452	0.90 (0.84-0.96)	0.66 (0.57-0.76)	0.68 (0.53-0.88)	0.93 (0.88-0.99)
	Manning ≥3 criteria	6	2966	0.72 (0.61-0.86)	0.77 (0.70-0.84)	0.71 (0.57-0.90)	0.79 (0.71-0.88)
	Manning ≥4 criteria	6	2986	0.42 (0.38-0.47)	0.89 (0.87-0.92)	0.74 (0.59-0.92)	0.60 (0.50-0.73)
	Rome I	3	3006	0.77 (0.60-0.99)	0.75 (0.66-0.85)	0.63 (0.42-0.94)	0.80 (0.61-1.00)
	Rome II	2	2402	0.79 (0.61-1.00)	0.69 (0.64-0.75)	0.52 (0.39-0.70)	0.85 (0.65-1.00)
	Rome III	1	1848	0.69 (0.64-0.73)	0.80 (0.77-0.82)	0.45 (0.41-0.49)	0.91 (0.90-0.93)
Biomarkers	Visceral hypersensitivity	2	328	0.88 (0.84-0.94)	0.77 (0.70-0.84)	0.85 (0.68-1.00)	0.82 (0.70-0.97)
	Pain perception	1	138	0.78 (0.70-0.85)	0.20 (0.02-0.56)	0.93 (0.87-0.96)	0.07 (0.008-0.22)
	Serum-based 10 biomarker panel	2	760	0.59 (0.43-0.82)	0.78 (0.60-1.00)	0.82 (0.77-0.87)	0.58 (0.45-0.74)
	Serum-based 34	1	244	0.81 (0.74-0.87)	0.64 (0.53-0.75)	0.83 (0.77-0.89)	0.60 (0.49-0.71)

	biomarker panel						
	Volatile organic metabolites in faeces	1	140	0.97 (0.83-1.00)	0.80 (0.71-0.87)	0.57 (0.42-0.71)	0.99 (0.94-1.00)
	Chromogranins and secretogranins in faeces:						
	Secretogranin II	1	111	0.80 (0.70-0.88)	0.79 (0.60-0.92)	0.92 (0.83-0.97)	0.59 (0.42-0.74)
	Secretogranin III	1	111	0.80 (0.70-0.88)	0.69 (0.49-0.85)	0.88 (0.78-0.94)	0.56 (0.38-0.72)
	Chromogranin B	1	111	0.78 (0.68-0.86)	0.69 (0.49-0.85)	0.88 (0.78-0.94)	0.53 (0.36-0.69)
	Duodenal chromogranin A	1	289	0.86 (0.81-0.91)	0.95 (0.89-0.99)	0.98 (0.94-0.99)	0.75 (0.65-0.82)
	Rectal endocrine cells:						
	Peptide YY	1	77	0.84 (0.71-0.93)	0.89 (0.71-0.98)	0.93 (0.82-0.99)	0.75 (0.57-0.89)
	Oxyntomodulin	1	77	0.80 (0.66-0.90)	0.81 (0.62-0.94)	0.89 (0.76-0.96)	0.69 (0.50-0.84)
	Somatostatin	1	77	0.80 (0.66-0.90)	0.89 (0.71-0.98)	0.93 (0.82-0.99)	0.71 (0.53-0.85)
	Sigmoid muscularis	1	175	0.70 (0.50-0.86)	0.95 (0.90-0.98)	0.73 (0.52-0.88)	0.95 (0.90-0.98)

	propria thickness						
	Faecal calprotectin and small intestinal permeability ratio	1	602	0.69 (0.64-0.74)	0.92 (0.88-0.95)	0.92 (0.88-0.95)	0.70 (0.65-0.75)
	Bile acid secretion and colonic transit	1	124	0.65 (0.54-0.74)	0.77 (0.58-0.90)	0.90 (0.80-0.96)	0.41 (0.28-0.55)
Psychological markers	PHQ-12 score	1	470	0.66 (0.61-0.72)	0.95 (0.90-0.98)	0.96 (0.93-0.98)	0.57 (0.51-0.63)
	Anxiety component of the HADS	1	470	0.72 (0.67-0.77)	0.75 (0.67-0.82)	0.86 (0.81-0.90)	0.56 (0.49-0.63)
	Depression component of the HADS	1	470	0.36 (0.31-0.42)	0.93 (0.88-0.97)	0.92 (0.86-0.96)	0.41 (0.36-0.46)
	Combination of HADS, PHQ-15, and the perceived stress scale	1	244	0.74 (0.66-0.80)	0.75 (0.64-0.84)	0.87 (0.80-0.92)	0.56 (0.46-0.66)
Combinations of	Kruis *et al.* statistical model	4	1171	0.80 (0.73-0.87)	0.91 (0.86-0.96)	0.85 (0.77-0.94)	0.86 (0.80-0.92)

symptoms, biomarkers, and/or psychological markers	Modified Kruis statistical model	1	253	0.69 (0.55-0.81)	0.91 (0.86-0.95)	0.67 (0.53-0.79)	0.92 (0.87-0.95)
	Bellentani *et al.* statistical model	1	254	0.76 (0.68-0.82)	0.82 (0.74-0.89)	0.86 (0.79-0.92)	0.69 (0.60-0.77)
	Faecal calprotectin, small intestinal permeability ratio, and Rome criteria	1	602	0.50 (0.45-0.56)	0.98 (0.96-0.99)	0.97 (0.93-0.99)	0.60 (0.56-0.65)
	Serum-based 34 biomarker panel and psychological markers	1	244	0.85 (0.78-0.90)	0.88 (0.79-0.94)	0.94 (0.90-0.97)	0.72 (0.62-0.81)

3.4 Discussion

This study has examined the accuracy of symptom-based diagnostic criteria, biomarkers, psychological markers, or combinations thereof, in making a diagnosis of IBS. The Rome IV criteria have yet to be externally validated, and the Rome III criteria, the previous gold standard for the diagnosis of IBS, have only been validated in one study to date, and performed modestly and similarly to the other symptom-based diagnostic criteria that have been described previously, with a positive LR >3 and a negative LR of approximately 0.4. Proposed biomarkers, with the exception of abnormal sigmoid muscularis propria thickness in female patients, intestinal mucosal endocrine cells, and faecal VOMs, and a combination of faecal calprotectin and intestinal permeability, all examined in single or small cohort studies, appeared to perform no better than available symptom-based diagnostic criteria. The accuracy of psychological markers was also similar. Combining symptoms, biomarkers, and/or psychological markers in various permutations seemed to perform better generally in diagnosing IBS, and with a significantly greater pooled positive LR compared with symptom-based criteria alone.

Strengths of this study include a comprehensive search strategy, including a recursive search of the bibliographies of all eligible studies, and searching of conference proceedings to identify any potential studies published that may not have been included in the original search of the medical literature. This resulted in the identification of a wide range of potential methods for diagnosing IBS; specifically four different symptom-based diagnostic criteria evaluated in seven studies, eleven biomarkers evaluated in twelve studies, four psychological markers evaluated in two studies, and five different combinations of symptoms, biomarkers, and/or psychological markers evaluated in six studies. Pooling the data in some of our analyses resulted in a study population of 1800 patients or more for each of the symptom-based diagnostic criteria, and >1000 patients for

the Kruis statistical model. Furthermore, this is the first study that has attempted to summarise data from all available methods, including novel approaches, to diagnose IBS.

There are some limitations to this study. When data were pooled, there was significant heterogeneity between studies that evaluated the same diagnostic method in some analyses, which may be partly explained by differences in study design, recruitment, setting, and country, and differences in the reference standard used for diagnosing IBS. However, a random effects model was used when pooling study data in all these analyses, in order to provide a more conservative estimate of diagnostic accuracy. The cut-offs that were used to define presence of IBS for each of the diagnostic tests assessed in this meta-analysis were imposed by the reporting of the authors of the original studies. This is less relevant for studies employing diagnostic criteria, such as the Manning criteria, because data were obtained for several thresholds, but is an issue for studies using laboratory tests, such as faecal chromogranins or VOMs, or the measures of psychological affect, which were not always used at the threshold recommended by the original authors. Additionally, the pooled IBS prevalence of all studies was high at >50%, as the majority of studies were conducted in referral populations in secondary care, meaning that some of the findings may not be applicable to a primary care setting, where the majority of patients with IBS are diagnosed and managed, as the prevalence may well be lower. The inclusion of case-control studies may lead to an overestimation of the diagnostic performance of the test being examined, compared with studies using a clinical cohort, because these are subject to spectrum bias as the study design often omits mild cases that are difficult to diagnose (Lijmer et al., 1999). Finally, 14 of the 22 eligible studies were judged as high risk of bias, or had other applicability concerns, when assessing quality using the QUADAS-2 tool, highlighting the limitations of data from some of the studies.

Guidelines for the management of IBS recommend making a positive diagnosis of IBS based on symptoms, and discouraging a "diagnosis of exclusion" approach (Ford et al., 2014; National Institute for Health and Care Excellence, 2008). Symptom-based diagnostic criteria were developed to aid in this, and therefore avoid unnecessary and potentially invasive investigations. However, one of the most consistent findings of this study is the modest performance of all the available symptom-based criteria in identifying IBS. As stated previously, this comparable performance between the symptom-based criteria is perhaps not surprising, considering they are derivatives of each other, and therefore share the same strengths and weaknesses.

In general, the performance of biomarkers in the studies that were identified was similar to symptom-based diagnostic criteria, which is disappointing considering their potentially expensive nature. In some cases, the biomarkers would not be considered useful as a test outside of a tertiary referral centre, due to the invasive nature or complexity of the test applied. Furthermore, a number of the studies that assessed the accuracy of biomarkers used healthy volunteers as controls whereas, as highlighted previously, a biomarker that differentiates IBS from other organic disorders in which the symptoms are likely to overlap with those of IBS, would be more clinically useful. Sigmoid muscularis propria measurement using trans-vaginal ultrasound, appeared to perform well with a positive LR of 15. However, this study had a number of limitations, including the failure to exclude other causes of abnormal muscularis propria thickness, such as colorectal cancer, IBD, or diverticular disease, only 27 patients in the study population having a confirmed diagnosis of IBS, and the generalisability of the results, given that the test was applied in female patients only. Additionally, the results have yet to be validated by other investigators, despite the study being published 8 years ago. Duodenal mucosal CgA cell quantification also performed well in differentiating IBS

from health, but only in a single study, and the test is invasive. In addition, the effect of coeliac disease, duodenitis, duodenal ulcers or IBD on numbers of CgA cells in the duodenum has not been studied, and therefore further work in this area is required before any definitive conclusion can be drawn. Faecal VOMs showed some promise in differentiating IBS from active IBD in one small study, but again results of this study will require validating by others.

Given the degree of psychological co-morbidity in many IBS patients, it is perhaps surprising that the performance of psychological markers were, in general, no better than that of symptom-based criteria. Additionally, the two studies that have reported on the accuracy of psychological markers differentiated IBS from health, and therefore whether psychological markers can accurately discriminate between IBS and organic GI disorders is unclear. This would seem less likely, as there is evidence to suggest that many organic GI disorders are also associated with psychological impairment (Devlen et al., 2014; Iglesias-Rey et al., 2014; Sainsbury et al., 2013).

Combinations of symptoms, biomarkers, and/or psychological markers seemed to perform better, generally, in the six studies that assessed the accuracy of these approaches, and were superior to symptom-based criteria in terms of the pooled positive LR. This may be because IBS is a complex, heterogeneous disorder, for which there is no single unifying explanation, and for which numerous mechanisms have been proposed. Combining symptoms or examination findings, biomarkers and/or psychological markers may therefore be a more useful approach to diagnosing IBS, and perhaps points the way forward for future iterations of the Rome process.

In conclusion, this meta-analysis has shown that symptom-based diagnostic criteria, biomarkers, and psychological markers perform only moderately well in diagnosing IBS, and in the case of biomarkers many of these are potentially expensive or invasive, and are

not yet practical for clinical application. Combining symptoms with markers of organic disease or psychological affect, may represent the best way forward in improving the accuracy of diagnosing IBS.

CHAPTER 4

Enhancing Diagnostic Performance of Symptom-based Criteria for Irritable Bowel Syndrome by Additional History and Limited Diagnostic Evaluation.

4.1 Introduction

One of the limitations of current diagnostic test studies for IBS is the lack of an accepted reference standard. As previously stated, most investigators have used a normal colonoscopy as confirmation of a diagnosis of IBS (Ford et al., 2008), that is, physicians still regard IBS as a diagnosis of exclusion, which is perhaps justified by the modest performance of the different symptom-based criteria for IBS proposed over the last four decades (Manning et al., 1978; Ford et al., 2013). Indeed, the current level of diagnostic confidence, based exclusively on these criteria, has not reduced the performance of testing such as colonoscopy and biopsies in some settings (Spiegel et al., 2010), despite the desirability to enhance high-value care.

The systematic review and meta-analysis undertaken in the previous chapter evaluated approaches to diagnosing IBS, and this study showed that biomarkers alone performed similarly to symptom-based criteria, while probably adding to the cost of care. Interestingly, studies using combinations of symptoms with biomarkers and/or measures of psychological wellbeing reported improved diagnostic accuracy (Jones et al., 2014; Tibble et al., 2002). Other investigators have reported that the absence of ”red flag” features, such as nocturnal symptoms of abdominal pain or diarrhoea (Vanner et al., 1999; Macaigne et al., 2014), or, as previously discussed, incorporating the results of simple laboratory tests, including haemoglobin and ESR (Kruis et al., 1984), may increase the ability to distinguish between IBS and organic lower GI diseases.

The aim of this study, based on these observations, was therefore to conduct a diagnostic accuracy study to examine whether the performance of the Rome III criteria could be improved if combined with additional items from the history, results of simple blood tests, markers of anxiety, depression, or somatoform-type behaviour, or combinations thereof. Proof of enhancement in the diagnostic performance of symptom-

based criteria could result in a reliable, inexpensive, and easily administrable clinical test, and represent a considerable advancement in assisting clinicians to make a positive diagnosis of IBS.

4.2 Methods

4.2.1 Participants and Setting

Unselected, consecutive patients aged ≥16 years newly referred from primary care to secondary care for consideration of investigation of GI symptoms were considered as eligible for the study. All patients were approached in six of the medical gastroenterology outpatient clinics of Leeds Teaching Hospitals Trust, West Yorkshire, United Kingdom (UK). The hospitals provide secondary care services to a local population of almost 800,000 people in the North of England. The only exclusion criteria were an inability to understand written English, as the questionnaires utilised were self-administered. Potentially eligible subjects were given a study information sheet at their initial clinic visit, before consultation with a gastroenterologist. Those agreeing to participate provided written informed consent at that visit. The local ethics committee approved the study (reference 13/YH/0216), with recruitment commencing in January 2014, and continuing through to December 2015. During the 2-year recruitment period the six involved clinics saw approximately 2200 new outpatient referrals. As the study was conducted in routine clinical practice, the diagnostic evaluation of the recruited patients was not standardised, and was left at the discretion of the responsible physician. A minimum panel of blood tests, or collection of colonic biopsy specimens in all patients was not mandated.

Potential participants were provided with an information sheet. They were then given the opportunity to ask any questions they may have had about the study. If they agreed to take part, they were asked to provide written, informed consent.

4.2.2 Data Collection and Synthesis

4.2.2.1 Demographic and Symptom data

All demographic and symptom data were collected prospectively at the initial clinic visit. Demographic data of interest in this study included age, height (in metres), and weight (in kilograms), from which body mass index (BMI) was calculated, gender, tobacco and alcohol use, marital status, educational level, and ethnicity. The Rome III diagnostic questionnaire for adult FGIDs was used to collect data on GI symptoms (Whitehead WE, 2006). Patients were also asked as to whether they experienced nocturnal passage of stools, which was recorded as occurring never, rarely, sometimes, often, most of the time, or always, with a symptom frequency of sometimes or greater used to define its presence.

4.2.2.2 Mood and Somatisation Data

The HADS was used to collect information about mood (Zigmond and Snaith, 1983). This 14-item instrument contains seven questions concerning anxiety, and another seven depression. Each of these questions is scored from 0 to 3, giving a total possible score of 21 for anxiety or depression separately. A score of ≥8 was used to define possible anxiety or depression (see the appendix for individual items of the HADS).

The validated PHQ-15 was used to assess for evidence of somatisation-type behaviour (Kroenke et al., 2002). Each of these questions is scored on a scale from 0 to 2, giving a total possible score of 30. A score of ≥15 is the validated threshold used to define high levels of somatisation (see the appendix for individual items of the PHQ-15).

4.2.2.3 Baseline Tests in Diagnostic Evaluation

Information was also collected from patients' case notes and computerised records. Haemoglobin level (normal for males ≥13.5g/dL, normal for females ≥11.5g/dL) and CRP levels (normal <5mg/L) were recorded at the initial clinic visit. The initial diagnosis made by the physician who consulted with the patient at their first outpatient clinic appointment, as well as the final diagnosis made after investigation to the level deemed appropriate by each individual consulting physician, were also recorded.

4.2.2.4 Definition of IBS

The presence or absence of Rome III-defined IBS among individual patients was assigned according to the scoring algorithm proposed for use with the Rome III questionnaire (see the Rome III questionnaire in the appendix).

4.2.2.5 Colonoscopic and Histopathological Data

All included patients underwent complete colonoscopy to the caecum or terminal ileum. The endoscopy units in Leeds Teaching Hospitals Trust employ colonoscopes from both Olympus and Fujinon. Bowel preparation was either a combination of polyethylene glycol and sodium picosulfate, or polyethylene glycol alone, depending on renal function. All endoscopists performing colonoscopic examinations remained blinded to the

questionnaire data of the patient. Findings were recorded using the ADAM reporting system (Fujifilm, Europe), with reports accessed by study investigators in order to record the final colonoscopic diagnosis for each included patient. Findings classified as consistent with organic disease at colonoscopy are provided in Table 9. The presence of diverticular disease was not considered an organic disease finding at colonoscopy as there is a lack of evidence to associate the presence of uncomplicated diverticular disease with chronic GI symptoms. In a recent prospective study, no association was found between diverticulosis and symptoms of irritable bowel syndrome (OR 0.53; 95% CI 0.26 to 1.05) or chronic abdominal pain (OR 0.68; 95% CI 0.38 to 1.23) (Peery et al., 2017).

Table 9. Findings Consistent with Organic Disease at Colonoscopy, or After Histopathological Interpretation of Colonic Biopsies.

At Colonoscopy	After Histopathological Interpretation of Colonic Biopsies
Evidence of colitis Evidence of terminal ileitis (inflammation or ulceration) Colorectal carcinoma Colonic stricture Evidence of radiation-induced colorectal disease	Colonic adenocarcinoma Rectal adenocarcinoma UC Crohn's disease IBD-unclassifiable Microscopic colitis Ischaemic colitis Radiation enteropathy Ulceration seen macroscopically at colonoscopy with non-specific inflammation on histological examination Neuroendocrine tumour

Biopsy specimens were obtained at the discretion of the endoscopist performing the colonoscopy. Standard policy during these colonoscopies in any patient with chronic diarrhoea and a macroscopically normal colon is to take two biopsies from the right colon, two from the left colon, and two from the rectum. All biopsies were interpreted by experienced GI histopathologists, who remained blinded to the questionnaire data of the patient. Histopathological findings were accessed using computerised records to obtain the final histopathological diagnosis. Findings classified as being consistent with organic disease after histopathological examination of biopsy specimens are also provided in Table 9.

Using these data, patients were classified according to the presence or absence of organic lower GI disease. Individuals had to have no evidence of an organic explanation for their symptoms at both colonoscopy and histopathological examination of biopsy specimens in order to be classified as exhibiting no organic lower GI disease.

4.2.2.6 Reference Standard to Define the Presence of IBS

The reference standard used to define the presence of IBS was lower abdominal pain or discomfort occurring at least 3 days per month over the last 3 months, in association with a change in bowel habit, and in the absence of organic lower GI disease after colonoscopy and histopathological examination of colonic biopsies, if obtained, which would explain these symptoms. Exclusion of coeliac disease with distal duodenal biopsy was also undertaken, if coeliac serology was positive.

4.2.2.7 Statistical Analysis

In order to assess whether those who underwent colonoscopy and provided complete symptom data were representative of all patients recruited, demographic data were compared between those undergoing colonoscopy who completed the symptom questionnaire, and those who completed the symptom questionnaire but did not undergo colonoscopy, using a χ^2 test for categorical data, and an independent samples *t*-test for continuous data, with a mean and standard deviation (SD). Due to multiple comparisons a 2-tailed P value of < 0.01 was considered statistically significant for these analyses. Organic findings in those meeting the Rome III criteria for IBS were compared with those who did not, using Fisher's exact test, as numbers in each cell were relatively small. These statistical analyses were performed using SPSS for Windows version 21.0 (SPSS Inc, Chicago, IL, USA).

The first aim of the study was to ascertain the performance of the Rome III criteria for IBS in determining the presence of IBS versus the reference standard of symptoms suggestive of IBS and a negative colonoscopy, as described above. Sensitivity analyses were also performed, within which the reference standard was varied. We assessed how the Rome III criteria performed versus:

a. A physician's final diagnosis that this was IBS, after investigation to the level deemed appropriate, which may or may not have included complete colonoscopy depending on whether that individual physician used a positive diagnostic strategy for IBS (physician's final diagnosis of IBS), or;

b. A combination of symptoms suggestive of IBS and a negative colonoscopy described above, and a physician's final diagnosis of IBS as described in a.

To that end, sensitivity, specificity, PPVs and NPVs, and their 95% CIs, were calculated for the Rome III criteria versus the reference standard using StatsDirect version 2.8.0 (StatsDirect Ltd, Sale, Cheshire, England). The positive LR and negative LR, and their 95% CIs, were also calculated. These analyses were performed for all individuals recruited who underwent colonoscopy for investigation of their lower GI symptoms, and provided complete Rome III symptom data. However, in clinical practice there may be less uncertainty in the diagnosis of IBS-C as the degree of overlap of symptoms with those of an organic GI disease is probably smaller, so the challenge is often in distinguishing between IBS-D and other potential organic GI causes of diarrhoea. With this in mind, *post hoc* analyses were performed, including only those participants reporting either ≥4 stools per day, or loose, mushy, or watery stools.

As stated previously, the advantage of using LRs over predictive values is that LRs do not vary to the same degree as predictive values with a change in disease prevalence. As a rule of thumb, a positive LR of more than 10 is useful for ruling in a disease, and a negative LR of less than 0.1 is useful for ruling out a disease. However, in diseases of higher prevalence, the positive LR threshold required to cause a useful increase in probability that will result in a change of management may be lower. In a recently published systematic review, the authors assumed "medical certainty" for a novel biomarker in diagnosing IBS as a post-test probability (derived from the pre-test probability and positive LR) of ≥80% (Shah et al., 2015). At this threshold, in a secondary or tertiary care population with a prevalence of IBS of around 50%, a test with a positive LR of ≥5 would identify IBS with a post-test probability of 86.5%.

The second aim was to compare the performance of proposed modifications to the Rome III criteria, by including information on nocturnal passage of stools, a physician's working diagnosis that this was IBS at the initial consultation, laboratory results of

haemoglobin and CRP, and measures of anxiety, depression, or somatisation, against the best performing of the three reference standards described above. Again, sensitivities, specificities, PPVs and NPVs, and positive and negative LRs were calculated for each of these modifications both individually, and as combinations.

4.3 Results

There were 1002 consecutive patients (mean age 54.4 years (range 16 to 92 years), 638 (63.7%) female) who gave informed consent and were recruited into the study between January 2014 and December 2015. Of these, 318 (31.7%) patients (mean age 54.0 years (range 18 to 92 years), 216 (67.9%) female) underwent colonoscopy for investigation of their lower GI symptoms, and provided complete Rome III symptom data (Figure 9).

Comparison of the demographic data of this group with those who did not undergo colonoscopy is provided in Table 10. Patients providing complete symptom data and undergoing colonoscopy had a higher BMI and were more likely to meet the Rome III criteria for IBS, but there were no other significant differences between the two groups. Patients with IBS-D were more likely to undergo colonoscopy, but not patients with IBS-C or those with IBS-M.

Among the 318 individuals providing complete symptom and colonoscopy data, 98 (30.8%) met the Rome III criteria for IBS. The mean age of these 98 patients was 46.7 years, and 73 (74.5%) were female. There were 286 (89.9%) patients who had a haemoglobin check, 178 (56.0%) with a CRP measurement, 212 (66.7%) with coeliac serology, and 215 (67.6%) who had colonic biopsy specimens obtained. Relevant organic findings after colonoscopy and histopathological interpretation of biopsy specimens, plus duodenal biopsy in those with positive coeliac serology, in those that met the Rome III

criteria compared with the 220 patients that did not are detailed in Table 11. There were no significant differences in the prevalence of any of these organic findings between the two groups.

Figure 9. Flow of Study Participants.

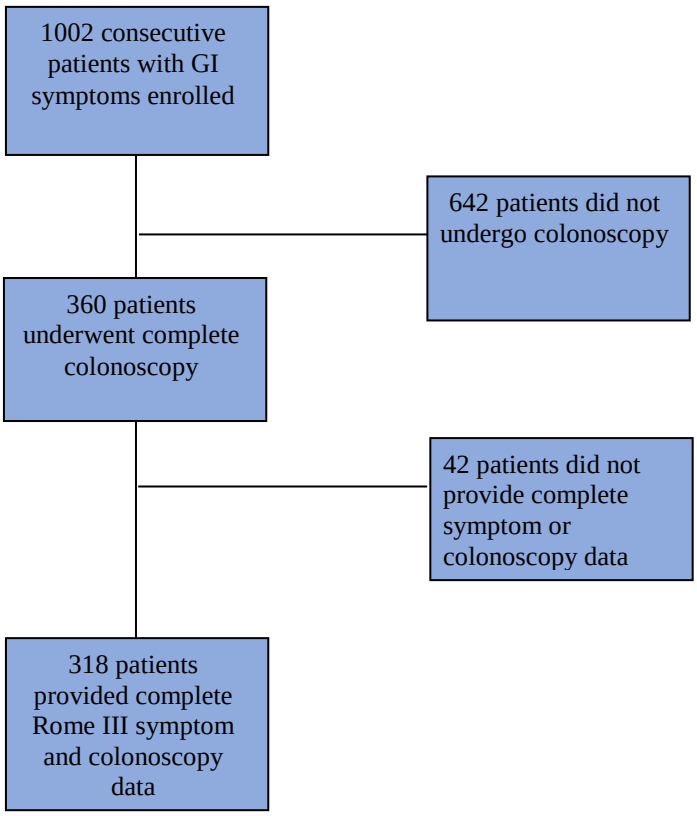


Table 10. Demographics and Baseline Characteristics of Patients Who Underwent Colonoscopy and Provided Complete Symptom Data Compared with Those That Did Not Undergo Colonoscopy.

	Underwent colonoscopy and provided complete Rome III symptom data (n = 318)	Did not undergo colonoscopy (n = 642)	P value*
Mean age in years (SD)	54.0 (16.3)	54.6 (18.1)	0.57
Mean BMI (SD)	27.2 (6.0)	26.2 (5.3)	0.02
Female gender (%)	216 (67.9)	402 (62.6)	0.11
Tobacco use (%)	74 (23.3)	149 (23.2)	0.99
Alcohol use (%)	171 (53.8)	351 (54.7)	0.87
Marital status (%)			
Married or cohabiting	177 (55.7)	354 (55.1)	
Divorced or separated	44 (13.8)	74 (11.5)	
Never Married	59 (18.6)	116 (18.1)	
Widowed	26 (8.2)	73 (11.4)	0.38
Educational level (%)			
Primary school	2 (0.6)	3 (0.5)	
Secondary school	144 (45.3)	277 (43.1)	
College or technical school	77 (24.2)	137 (21.3)	
University	47 (14.8)	91 (14.2)	
Postgraduate	29 (9.1)	55 (8.6)	0.98
White Caucasian ethnicity (%)	292 (91.8)	573 (89.3)	0.25

Met Rome III criteria for IBS (%)	98 (30.8)	126 (19.6)	< 0.001
IBS-D	46 (14.6)	32 (5.0)	< 0.001
IBS-C	5 (1.6)	25 (3.9)	0.08
IBS-M	45 (14.2)	60 (9.3)	0.03
HADS score ≥8 (n = 829)	144/292 (49.3)	278/537 (51.8)	0.50
High level of somatisation (n = 725)	57/258 (22.1)	99/467 (21.2)	0.78

*P value for independent samples *t*-test for continuous data and Pearson χ^2 for comparison of categorical data.

Table 11. Prevalence of Organic Disease in Patients Meeting the Rome III Criteria Compared With Those Who Did Not.

	Met Rome III criteria for IBS (n = 98)	Did not meet Rome III criteria for IBS (n = 220)	P value*
UC (%)	2 (2.0)	2 (0.9)	0.59
Crohn's disease (%)	4 (4.1)	2 (0.9)	0.08
IBD-unclassifiable (%)	0 (0)	2 (0.9)	0.52
Non-specific GI ulceration (%)	1 (1.0)	4 (1.8)	1.0
Collagenous colitis (%)	4 (4.1)	12 (5.5)	0.78
Lymphocytic colitis (%)	2 (2.0)	9 (4.1)	0.51
Colorectal cancer (%)	0 (0)	2 (0.9)	1.0
Coeliac disease (%)	2 (2.0)	5 (2.3)	1.0

*P value for Fisher's exact test for comparison of categorical data.

4.3.1 Performance of the Rome III Criteria for IBS Against All Reference Standards

Of 79 (24.8%) individuals meeting the reference standard of symptoms suggestive of IBS and a negative colonoscopy, 55 met the Rome III criteria, giving a sensitivity of 69.6% (Table 12). Among the 239 patients without IBS according to this reference standard, 196 did not meet the Rome III criteria, giving a specificity of 82.0%. Positive and negative LRs of the Rome III criteria were therefore 3.87 (95% CI 2.85 to 5.26) and 0.37 (95% CI 0.26 to 0.51) respectively. Altering the reference standard to one that used a physician's final diagnosis of IBS, or one that included a composite of symptoms suggestive of IBS, a negative colonoscopy, negative coeliac serology, and a physician's final diagnosis of IBS did not improve the performance of the Rome III criteria (Table 12), with lower positive LRs and higher negative LRs. A reference standard of symptoms suggestive of IBS and a negative colonoscopy was therefore used in all subsequent analyses.

Table 12. Diagnostic Accuracy of the Rome III Criteria for Irritable Bowel Syndrome versus Various Reference Standards.

Reference standard used	Number of patients included (% with IBS)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Symptoms suggestive of IBS and a negative colonoscopy	318 (24.8)	69.6% (58.3% – 79.5%)	82.0% (76.5% – 86.7%)	56.1% (46.3% – 65.5%)	89.1% (84.3% – 92.6%)	3.87 (2.85 – 5.26)	0.37 (0.26 – 0.51)
Physician's final diagnosis of IBS	545 (29.4)	58.8% (50.7% – 66.5%)	77.4% (72.9% – 81.5%)	51.9% (44.7% – 59.1%)	81.9% (77.6% – 85.5%)	2.60 (2.07 – 3.26)	0.53 (0.44 – 0.64)

Symptoms suggestive of IBS, a negative colonoscopy, and a physician's final diagnosis of IBS	215 (16.3)	71.4% (53.7% – 85.4%)	70.0% (62.7% – 76.6%)	31.6% (22.5% – 42.6%)	92.7% (87.0% – 96.0%)	2.38 (1.71 – 3.19)	0.41 (0.23 – 0.65)

4.3.2 Rome III Criteria and No Nocturnal Passage of Stools

The effect on the Rome III criteria by combining it with no nocturnal passage of stools was studied. When this was compared against the original reference standard, there were 311 patients with full data for analysis, of whom 78 had IBS according to the original reference standard. Of these, 26 met the combination of positive Rome III criteria and absence of nocturnal stools, resulting in a sensitivity of 33.3% (95% CI 23.1% to 44.9%) in detecting IBS. Of the 233 individuals that did not meet the original reference standard, 212 did not meet this combination, giving a specificity of 91.0% (95% CI 86.6% to 94.3%). Positive and negative LRs were therefore 3.70 (95% CI 2.21 to 6.14) and 0.73 (95% CI 0.61 to 0.84) (Table 13).

Table 13. Diagnostic Accuracy of the Rome III Criteria, and Modifications to the Rome III Criteria with the Inclusion of No Nocturnal Passage of Stools, Physician's Initial Impression that this was IBS, Biomarkers or Markers of Affective Disorders, or a Combination Thereof, versus the Reference Standard.

	Number of patients providing data in the analysis	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Rome III criteria alone	318	69.6% (58.3% – 79.5%)	82.0% (76.5% – 86.7%)	56.1% (46.3% – 65.5%)	89.1% (84.3% – 92.6%)	3.87 (2.85 – 5.26)	0.37 (0.26 – 0.51)
Rome III criteria and no nocturnal passage of stools	311	33.3% (23.1% – 44.9%)	91.0% (86.6% – 94.3%)	55.3% (41.3% – 68.6%)	80.3% (75.1% – 84.7%)	3.70 (2.21 – 6.14)	0.73 (0.61 – 0.84)

Rome III criteria and physician's initial impression that this was IBS	112	50.0% (33.4% – 66.6%)	79.7% (68.8% – 88.2%)	55.9% (39.5% – 71.1%)	75.6% (65.1% – 83.8%)	2.47 (1.42 – 4.27)	0.63 (0.43 – 0.84)
Rome III criteria and normal haemoglobin and CRP	208	49.0% (34.8% – 63.4%)	89.2% (83.2% – 93.6%)	59.5% (44.5% – 73.0%)	84.3% (78.0% – 89.1%)	4.53 (2.67 – 7.64)	0.57 (0.42 – 0.73)
Rome III criteria and a HADS score ≥8	292	47.2% (35.3% – 59.4%)	89.1% (84.2% – 92.9%)	58.6% (45.8% – 70.4%)	83.8% (78.5% – 87.9%)	4.33 (2.76 – 6.76)	0.59 (0.46 – 0.72)

Rome III criteria and a high level of somatisation	258	37.9% (26.2% – 50.7%)	94.8% (90.6% – 97.5%)	71.4% (55.0% – 83.7%)	81.6% (76.0% – 86.1%)	7.27 (3.74 – 14.2)	0.66 (0.53 – 0.77)
Rome III criteria, normal haemoglobin and CRP, and a HADS score ≥8	195	34.0% (20.9% – 49.3%)	93.2% (87.9% – 96.7%)	61.5% (42.5% – 77.6%)	81.7% (75.1% – 86.8%)	5.04 (2.48 – 10.2)	0.71 (0.55 – 0.84)

Rome III criteria, normal haemoglobin and CRP, and a high level of somatisation	165	24.4% (12.4% – 40.3%)	96.8% (92.0% – 99.1%)	71.4% (45.4% – 88.3%)	79.5% (72.3% – 85.1%)	7.56 (2.63 – 21.7)	0.78 (0.63 – 0.90)
Rome III criteria, no nocturnal passage of stools, and a HADS score ≥8	290	22.2% (13.3% – 33.6%)	95.4% (91.7% – 97.8%)	61.5% (42.5% – 77.6%)	78.8% (73.5% – 83.3%)	4.84 (2.33 – 10.0)	0.82 (0.70 – 0.91)

Rome III criteria, no nocturnal passage of stools, and a high level of somatisation	256	18.2% (9.8% – 29.6%)	99.0% (96.3% – 99.9%)	85.7% (60.1% – 96.0%)	77.7% (72.0% – 82.5%)	17.3 (4.45 – 67.6)	0.83 (0.72 – 0.90)

4.3.3 Rome III Criteria and a Physician's Initial Impression That This Was IBS

The Rome III criteria were then combined with a physician's initial impression that this was IBS at the first outpatient clinic visit (i.e. prior to any investigation being undertaken). Of 112 patients, 38 had IBS according to the original reference standard, of whom 19 met the combination of the Rome III criteria and a physician's initial impression that this was IBS, resulting in a sensitivity of 50.0% (95% CI 33.4% to 66.6%) in detecting IBS. Of the 78 that did not meet the original reference standard for IBS, 59 patients did not meet this combination, resulting in a specificity of 79.7% (95% CI 68.8% to 88.2%). Positive and negative LRs were 2.47 (95% CI 1.42 to 4.27) and 0.63 (95% CI 0.43 to 0.84) (Table 13).

4.3.4 Rome III Criteria and Normal Haemoglobin and CRP

When the Rome III criteria were combined with a normal haemoglobin and CRP, and compared with the original reference standard, 51 of 208 patients met the reference standard for IBS. Of these 51 patients, 25 met the combination of the Rome III criteria and a normal haemoglobin and CRP, resulting in a sensitivity of 49.0% (95% CI 34.8 to 63.4%). Of the 157 individuals that did not meet the original reference standard, 140 did not meet this combination, resulting in a specificity of 89.2% (95% CI 83.2% to 93.6%). Positive and negative LRs were 4.53 (95% CI 2.67 to 7.64) and 0.57 (95% CI 0.42 to 0.73) (Table 13).

4.3.5 Rome III Criteria and Psychological Markers

4.3.5.1 Rome III Criteria and a High Level of Somatisation

The Rome III criteria were combined with high somatisation levels, considered as a PHQ-15 score ≥15. Of 258 individuals, 66 met the original reference standard for IBS, of whom 25 met a combination of both the Rome III criteria and a high level of somatisation, giving a sensitivity of 37.9% (95% CI, 26.2% to 50.7%). Of the 192 individuals who did not meet the reference standard, 182 did not meet the combination of Rome III and somatisation, resulting in a specificity of 94.8% (95% CI 90.6% to 97.5%). Positive and negative LRs were 7.27 (95% CI 3.74 to 14.2) and 0.66 (95% CI 0.53 to 0.77) (Table 13).

4.3.5.2 Rome III Criteria and a HADS score ≥8

The Rome III criteria were then combined with markers for anxiety and depression, using a HADS anxiety or depression score of ≥8 to define possible anxiety or depression, and were compared against the original reference standard. Of 292 patients, 72 met the original reference standard for IBS, of whom 34 met the combination of Rome III criteria and a HADS anxiety or depression score ≥8, resulting in a sensitivity of 46.4% (95% CI 34.3% to 58.8%). Of the 220 patients that did not meet the original reference standard, 196 did not meet this combination, resulting in a specificity of 85.7% (95% CI 79.5% to 90.6%). Positive and negative LRs were 3.25 (95% CI 2.07 to 5.07) and 0.63 (95% CI 0.49 to 0.77) respectively (Table 13).

4.3.6 Combinations of the Rome III Criteria, Passage of Nocturnal Stools, Biomarkers, and Psychological Markers

4.3.6.1 Rome III Criteria, Normal Haemoglobin and CRP, and a HADS score ≥8

Of 195 individuals, 47 were considered to have met the original reference standard for IBS, and 16 met the combination of positive Rome III criteria, normal haemoglobin and CRP levels, and a HADS anxiety or depression score ≥8, giving a sensitivity of 34.0% (95% CI 20.9% to 49.3%). Of the 148 patients that did not meet the original reference standard, 138 did not meet this combination, giving a specificity of 93.2% (95% CI 87.9% to 96.7%). The positive and negative LRs were 5.04 (95% CI 2.48 to 10.2) and 0.71 (95% CI 0.55 to 0.84) respectively (Table 13).

4.3.6.2 Rome III Criteria, Normal Haemoglobin and CRP, and a High Level of Somatisation

Of 165 patients, 41 met the original reference standard for IBS, of whom 10 had a combination of positive Rome III criteria, normal biomarkers, and a high level of somatisation, resulting in a sensitivity of 24.4% (95% CI 12.4% to 40.3%). Of the 124 patients that did not meet the original reference standard, 120 did not meet this combination, giving a specificity of 96.8% (95% CI 92.0% to 99.1%). Positive and negative LRs were 7.56 (95% CI 2.63 to 21.7) and 0.78 (95% CI 0.63 to 0.90) (Table 13).

4.3.6.3 Rome III Criteria, No Nocturnal Passage of Stools, and a HADS ≥8

When combining the Rome III criteria with no nocturnal passage of stools and a HADS anxiety or depression score ≥8, 72 of 290 patients met the original reference

standard for IBS, of whom 16 met this combination of Rome III and markers, resulting in a sensitivity of 22.2% (95% 13.3% to 33.6%). Of the 218 patients that did not meet the original reference standard, 208 did not meet the combination, giving a specificity of 95.4% (95% CI 91.7% to 97.8%). Positive and negative LRs were 4.84 (95% CI 2.33 to 10.0) and 0.82 (95% CI 0.70 to 0.91) respectively (Table 13).

4.3.6.4 Rome III Criteria, No Nocturnal Passage of Stools, and a High Level of Somatisation

Finally, the Rome III criteria were combined with no nocturnal passage of stools and a high level of somatisation. Of 256 patients, 66 met the original reference standard for IBS, of whom 12 met the combination of the Rome III criteria, no nocturnal passage of stool, and a high level of somatisation, resulting in a sensitivity of 18.2% (95% CI 9.8% to 29.6%). Of the 190 patients that did not meet the original reference standard, 188 did not meet this combination, resulting in a specificity of 99.0% (95% CI 96.3% to 99.9%). Positive and negative LRs were 17.3 (95% CI 4.45 to 67.6) and 0.83 (95% CI 0.72 to 0.90) respectively. (Table 13)

4.3.6.5 Accuracy of the Rome III Criteria and Modifications in Patients Presenting with Diarrhoea

When the analyses were restricted to participants who reported either ≥4 stools per day, or loose, mushy, or watery stools, there were similar enhancements of positive LRs (in some instances, almost two-fold those for the Rome III criteria alone) with the incorporation of additional factors from the clinical history and simple laboratory tests into the Rome III criteria (Table 14).

Table 14. Diagnostic Accuracy of the Rome III Criteria, and Modifications to the Rome III Criteria with the Inclusion of No Nocturnal Passage of Stools, Physician's Initial Impression that this was IBS, Biomarkers or Markers of Affective Disorders, or a Combination Thereof, versus the Reference Standard Among Patients Presenting with Diarrhoea

	Number of patients providing data in the analysis	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Rome III criteria alone	252	68.0% (56.2% – 78.3%)	76.8% (69.9% – 82.4%)	55.4% (44.7% – 65.8%)	85.0% (78.5% – 90.2%)	2.94 (2.16 – 4.01)	0.42 (0.29 – 0.57)
Rome III criteria and no nocturnal passage of stools	251	30.7% (20.5% – 42.4%)	89.2% (83.7% – 93.4%)	54.8% (38.7% – 70.2%)	75.1% (68.7% – 80.8%)	2.84 (1.65 – 4.85)	0.77 (0.65 – 0.89)

Rome III criteria and physician's initial impression that this was IBS	100	54.3% (36.7% – 71.2%)	76.9% (64.8% – 86.5%)	55.9% (37.9% – 72.8%)	75.8% (63.6% – 85.5%)	2.35 (1.38 – 4.03)	0.59 (0.39 – 0.84)
Rome III criteria and normal haemoglobin and CRP	163	51.0% (36.3% – 65.6%)	85.1% (77.2% – 91.1%)	59.5% (43.3% – 74.4%)	80.2% (71.9% – 86.9%)	3.42 (2.05 – 5.72)	0.58 (0.41 – 0.75)
Rome III criteria and a HADS score ≥8	237	46.4% (34.3% – 58.8%)	85.7% (79.5% – 90.6%)	57.1% (43.2% – 70.3%)	79.6% (72.9% – 85.2%)	3.25 (2.07 – 5.07)	0.63 (0.49 – 0.77)
Rome III criteria and a high level of somatisation	207	38.1% (26.2% – 51.2%)	93.1% (87.6% – 96.6%)	70.6% (52.5% – 84.9%)	77.5% (70.5% – 83.5%)	5.49 (2.83 – 10.7)	0.67 (0.53 – 0.79)

Rome III criteria, normal haemoglobin and CRP, and a HADS score ≥8	158	34.8% (21.4% – 50.3%)	91.1% (84.2% – 95.6%)	61.5% (40.6% – 79.8%)	77.3% (69.2% – 84.1%)	3.90 (1.93 – 7.83)	0.72 (0.55 – 0.86)
Rome III criteria, normal haemoglobin and CRP, and a high level of somatisation	131	25.0% (12.7% – 41.2%)	95.6% (89.1% – 98.8%)	71.4% (41.9% – 91.6%)	74.4% (65.5% – 82.0%)	5.69 (2.00 – 16.3)	0.78 (0.62 – 0.91)
Rome III criteria, no nocturnal passage of stools, and a HADS score ≥8	237	20.3% (11.6% – 31.7%)	94.1% (89.3% – 97.1%)	58.3% (36.6% – 77.9%)	74.2% (67.8% – 79.9%)	3.41 (1.61 – 7.16)	0.85 (0.73 – 0.94)

Rome III criteria, no nocturnal passage of stools, and a high level of somatisation	207	17.5% (9.1% – 29.1%)	98.6% (95.1% – 99.8%)	84.6% (54.6% – 98.1%)	73.2% (66.4% – 79.3%)	12.6 (3.23 – 49.5)	0.84 0.72 – 0.92)

4.4 Discussion

This study has validated the symptom-based Rome III criteria for IBS against an accepted clinical reference standard. These criteria performed only modestly, with a positive and negative LR of 3.87 and 0.37 respectively. In addition, the effect of addition of nocturnal symptoms, factors related to somatisation, affective disorders, and haemoglobin and CRP measurements on the accuracy of the symptom-based Rome III criteria were examined. Sensitivities in diagnosing IBS ranged from 18.2% for presence of the Rome III criteria, no nocturnal passage of stools, and a high level of somatisation to 50.0% for presence of the Rome III criteria and a physician's initial impression that the diagnosis was IBS. Specificities ranged from 79.7% for presence of the Rome III criteria and a physician's initial impression that the diagnosis was IBS, to 99.0% for presence of the Rome III criteria, no nocturnal passage of stools, and a high level of somatisation. Furthermore, specificity approached 95% or more with a number of these modifications; thus the risk of a missed diagnosis of organic GI disease would be small, as the false positive rate was extremely low.

A combination of the Rome III criteria with any of a high level of somatisation, a normal haemoglobin and CRP with a HADS score of ≥8, a normal haemoglobin and CRP with a high level of somatisation, or no nocturnal passage of stools with a high level of somatisation all provided positive LRs of ≥5. Improved positive LRs were obtained by combining the Rome III criteria with a high level of somatisation alone (positive LR 7.27; 95% CI 3.74 to 14.2); a normal haemoglobin and CRP with a HADS score of ≥8 (positive LR 5.04; 95% CI 2.48 to 10.2); a normal haemoglobin and CRP with a high level of somatisation (positive LR 7.56; 95% CI 2.63 to 21.7); and no nocturnal passage of stools with a high level of somatisation (positive LR 17.3; 95% CI 4.45 to 67.6). In a secondary or tertiary referral population in a university hospital practice with a prevalence of IBS of

50% or more, a positive LR of this magnitude would be clinically useful for the diagnosis of IBS, identifying IBS with a post-test probability of >85% (Shah et al., 2015).

The performance of the Rome III criteria in this study is remarkably similar to that observed in the only other previous validation study, which also used a reference standard of the combination of symptoms suggestive of IBS and a negative colonoscopy (Ford et al., 2013). In that prior study from Canada, which included more than 1800 patients, the positive and negative LRs of the Rome III criteria were 3.35 (95% CI 2.97 to 3.79) and 0.39 (95% CI 0.39 to 0.46) respectively. Unlike the current study, the previous study did not incorporate other features of the clinical history or simple laboratory tests with the Rome III criteria. Sensitivity analyses were conducted in the Canadian study (Ford et al., 2013), where individuals reporting lower GI alarm symptoms, including rectal bleeding, anaemia, weight loss, or a family history of colorectal cancer were excluded. However, the addition of lower GI alarm symptoms resulted in only a small improvement in the positive LR. There have been few other studies that have attempted to modify the symptom-based Rome criteria. Vanner *et al.* examined the effect on the Rome I criteria of excluding patients with "red flag" features, including nocturnal GI symptoms (Vanner et al., 1999). An improvement was shown in the accuracy of the Rome I criteria in differentiating IBS from organic disease, with a sensitivity of 65% and a specificity of 100%.However, this was a small retrospective study, and the investigators did not attempt to separate nocturnal GI symptoms from other alarm symptoms, which are reported frequently by patients with functional bowel disorders (Whitehead et al., 2006).

As discussed previously, psychological or affective disorders have been shown to be strongly associated with IBS (Camilleri et al., 2008). There was an improvement in diagnostic test accuracy when other investigators added these to a biomarker panel, as shown in the recent study from Jones *et al.* (Jones et al., 2014). Rates of somatoform-type

behaviour, in particular, have been shown to be significantly higher in patients with IBS (Patel et al., 2015), and to differentiate IBS from health with greater accuracy, compared with markers of anxiety and depression, as shown in one of the studies discussed in the previous chapter (Spiller et al., 2010). The results of the current study support these findings, with a greater accuracy when a combination of the Rome III criteria and high level of somatisation was used, as compared with a combination of the Rome III criteria and HADS scores. Incorporating the presence of co-existent FGIDs into the modifications to the Rome III criteria may also have improved their performance. However, unlike in IBS, some other FGIDs are diagnoses of exclusion. For instance, a diagnosis of functional heartburn would not be made on symptoms alone, but only after a negative upper endoscopy and normal pH and impedance studies. As this study did not mandate the relevant investigations to confirm that, when the appropriate symptoms were reported, the cause was indeed another FGID this issue was therefore not examined.

The performance of the modifications to the Rome III criteria used in the current study can be best appreciated by comparing them with the accuracy of biomarkers. In general, biomarkers have been shown to perform no better than symptom-based diagnostic criteria in IBS, as shown in the preceding chapter and, in some cases, are probably not clinically useful outside of a research or tertiary care setting, due to their complex or invasive nature e.g. brain imaging, or endoscopy and biopsy with specialised histopathology.(Bouin et al., 2002; Kim et al., 2010; El-Salhy et al., 2014; El-Salhy et al., 2014). Furthermore, as previously discussed, many of the studies that have validated biomarkers have been limited by the fact that their utility in IBS was compared with HCs, when it would be more useful to assess the performance of the biomarker in distinguishing between IBS and organic disease. Alternatively, other appraisals of

biomarkers have used IBS-enriched populations, reducing their generalisability to a clinical setting, where the prevalence of IBS is likely to be lower.

One biomarker that is available for use in clinical practice currently was examined for its ability to differentiate IBS-D from IBD, coeliac disease, or health (Pimentel et al., 2015). The methodology of this study has previously been described, but to recap, antibodies to cytolethal toxin B and to vinculin performed best when differentiating IBS-D from IBD, with positive LRs of 5.2 and 2.0 respectively. However, as discussed, the authors used an enriched sample of cases, that consisted of a cohort of patients enrolled in a large randomised clinical trial of rifaximin, with >80% of participants having IBS-D. Thus, the LRs may not be reproducible in other populations, or in those with IBS-C or IBS-M. This underlines the importance of this study's findings in a consecutive, unselected secondary care population, where various combinations of the Rome III criteria, two routine blood tests, and a symptom-item checklist, appeared accurate and would be inexpensive to administer as a diagnostic test in the outpatient clinic.

The improved performance of the Rome III criteria when combined with relevant blood tests and markers of anxiety, depression, or somatisation is perhaps not surprising given the findings of other investigators, summarised in the meta-analysis in the previous chapter, which showed that studies that have used symptoms with clinical laboratory tests, biomarkers, and markers of psychological disorders, seem to lead to an increased ability to differentiate between IBS and organic GI disease.

There are methodological strengths to this study. First, it was conducted in a large, unselected population referred to secondary care, so the results are likely to be generalisable to patients with suspected IBS seen in usual clinical care by gastroenterologists. The sample size, although smaller than the previous validation study of the Rome III criteria (Ford et al., 2013) is larger than most other studies that have

assessed the accuracy of diagnostic tests for IBS. Second, it was designed to adhere to the STARD guidelines for the reporting of studies of diagnostic accuracy, with consecutive patients recruited, assessors blinded, and accepted references standard used. Third, it used inexpensive factors to modify the symptom-based criteria, and these lend themselves to application in either primary or secondary care.

There are some limitations to the study. Although the modifications made to the Rome III criteria result in an appreciable improvement in specificity and positive LR, this comes at the expense of worsening sensitivity and negative LR. This is likely to result in some patients who have IBS undergoing unnecessary investigation. However, clinicians need to have confidence that a test for IBS has a low probability of missing organic GI pathology. The modifications made to the Rome III criteria in this study succeed in this respect. Not all patients that underwent colonoscopy provided complete symptom data, and therefore these individuals were not included in the analyses. However, this number was relatively small, with almost 90% of patients providing full data. Most of the patients included in the study were White Caucasian, meaning that these results may not be applicable to other ethnicities. The mean age of included individuals was relatively high at 54 years, which probably reflects the use of a negative colonoscopy as a reference standard, meaning that there is some selection bias and that the results may therefore not be generalisable to a younger population. The study was conducted in secondary care as the reference standard used to define IBS mandated a negative colonoscopy. As a result, one possible criticism is that the study findings may not be reproducible in primary care where the majority of patients are diagnosed. However, the patient cohort referred to secondary care is more likely to contain mild cases that are difficult to diagnose. The expected result of this would be enhanced performance of the Rome III modifications in differentiating IBS from organic disease in a primary care setting. In addition, the reference standard used in the analyses included symptom data from

the questionnaire, which may have resulted in an overestimation of the accuracy of the Rome III criteria and its modifications, and a negative colonoscopy. Approximately one-third of patients included in the study did not have coeliac serology tested. In a recent systematic review and meta-analysis, the prevalence of positive coeliac serology in individuals presenting with symptoms suggestive of IBS was significantly higher when compared with HCs (OR 3.21; 95% CI 1.55 to 6.65) (Irvine et al., 2017). It is therefore possible that some patients who did not have a coeliac serology test were erroneously given a diagnosis of IBS. There are also other conditions that may mimic IBS, such as BAM, small intestinal bacterial overgrowth, or fructose and lactose intolerance (Aziz et al., 2015; Ford et al., 2009; Wilder-Smith et al., 2013), which are not excluded by a negative colonoscopy. These were not screened for routinely in this study, which was conducted within usual clinical practice. However, the prevalence of unequivocal small intestinal bacterial overgrowth in patients presenting with symptoms suggestive of IBS is probably less than 5% (Posserud et al., 2007). The modifications to symptom-based criteria in the current study enhance the diagnosis of IBS, but do not necessarily identify actionable features of the disorder. Thus, the recently validated additional measurements of colonic transit or of bile acid metabolism may still provide the best biomarkers to individualise therapy in subsets of IBS patients (Camilleri et al., 2014; Camilleri, 2015). Finally, the approaches suggested by these findings may not completely change physician behaviour, due to uncertainty or fear of a missed organic diagnosis, which is reflected by the fact that significantly more patients who met the Rome III criteria for IBS were referred for colonoscopy in this study. However, further proof of the validity of this approach in prospective cohorts will enhance the confidence with which physicians can make a positive diagnosis of IBS, which was the intent of the original symptom-based criteria proposed by Manning *et al.* (Manning et al., 1978).

In summary, the performance of the Rome III criteria in diagnosing IBS was similar to that observed in a previous validation study from a Canadian cohort (Ford et al., 2013). Important novel findings from this study were that modifying these criteria, with questionnaires concerning nocturnal symptoms, anxiety, depression, or somatisation, in addition to simple blood tests, improved their diagnostic performance. An inexpensive clinical test that combines symptoms with clinical markers, which is easily administered in a routine care setting, and accurate enough to allow the physician to confidently make a positive diagnosis of IBS would be highly desirable, and may have important implications for enhanced value care.

CHAPTER 5

Derivation and Validation of a Diagnostic Test for Irritable Bowel Syndrome Using Latent Class Analysis

5.1 Introduction

Although modifications to the Rome III criteria described in the previous chapter, in some instances, resulted in acceptable positive LRs for a diagnostic test for IBS, one criticism that can be made is that these modifications could result in a test similar to the Kruis statistical model (Kruis et al., 1984), which although shown to be accurate, was not widely used, probably as a result of its complexity.

As previously discussed, LCA is a statistical method that hypothesises the existence of one or more unobserved groups (latent classes) among a set of observed categorical variables, such as patient-reported symptoms. For example, symptoms that are reported by patients with IBS, or are known to be associated with IBS, could be incorporated into a latent class model, and it can then be observed how individuals cluster into IBS or non-IBS groups naturally, based on these variables. Individuals are classified according to their most likely latent class membership probabilities, that is the probability for a randomly selected member of a given latent class, a given response pattern will be observed. Although this method may initially appear overly complex for a diagnostic test, in the modern era of smartphones, an easy to use app could be developed, in which symptoms are inputted by the patient in the outpatient waiting room whilst waiting to see a physician, and then the relevant physical findings and blood test results are incorporated into the app during the clinician's assessment, which would then provide an overall probability of the patient having IBS.

Although there are only a few examples of LCA being used in gastroenterology (Christensen et al., 1992; Koloski et al., 2015), this statistical technique has been used successfully in other medical conditions where, as is the case in IBS, a gold standard diagnostic test is lacking (Rindskopf and Rindskopf, 1986; Ferraz et al., 1995; LaJoie et al., 2005; Kato et al., 2010). LCA was therefore applied to two unselected patient cohorts,

recruited prospectively, who were referred to secondary care gastroenterology services for investigation of their GI symptoms in Canada and the UK, and who answered identical questionnaires. The aims of this study were to develop a latent class model that could detect previously unobserved IBS and non-IBS latent classes using intestinal and extra-intestinal symptom data, and then to assess the ability of the model to correctly identify IBS, if used as a diagnostic test.

5.2. Methods

5.2.1 Participants and Settings

In both studies, unselected patients ≥16 years of age referred from primary care to secondary care for investigation of lower GI symptoms were considered eligible. There were no exclusion criteria, other than an inability to understand written English, as the questionnaires were self-administered. Potentially eligible participants were approached at their first clinic visit, and those agreeing to participate provided written informed consent at that visit. All questionnaires were completed prior to the patient's consultation with a gastroenterologist. The questionnaires used in both studies collected the same demographic data, and in both studies we used the validated Rome III questionnaire to collect data concerning individual GI symptom items, using Likert scales (Whitehead WE, 2006). The PHQ-15 was used to assess for evidence of somatisation-type behaviour (Kroenke et al., 2002).

In Canada, patients were recruited at the outpatient clinics of McMaster University Medical Centre and St Joseph's Healthcare, two hospitals in Hamilton, Ontario, serving a local population of more than 500,000. The Hamilton Health Sciences and McMaster University research ethics board approved the study in January 2008 and recruitment

continued until December 2012. The data from these patients were used to derive a latent class model to predict the presence of IBS.

The UK patients were recruited at the outpatient clinics of Leeds Teaching Hospitals NHS Trust, West Yorkshire as previously described. We used these patients to validate the latent class model derived from the patients contained within the Canadian dataset.

5.2.2. Data Collection and Synthesis

5.2.2.1 Demographic and Symptom Data

All demographic and symptom data were collected prospectively from the questionnaire at the initial clinic visit. Questionnaire data were entered into a database by trained researchers who were not involved in the clinical care of the patients; therefore ensuring assessors were blinded to symptom status. Demographic data of interest included age, height (in metres), and weight (in kilograms), from which BMI was calculated, gender, tobacco and alcohol use, marital status, educational level, and ethnicity.

5.2.2.2 Colonoscopic and Histopathological Data

In both studies, all patients underwent colonoscopy to the caecum or terminal ileum, using Pentax colonoscopes in the Canadian study, and Olympus or Fujinon colonoscopes in the UK study. All endoscopists performing colonoscopic examinations were blinded to the questionnaire data of the patient. Biopsy specimens were obtained at the discretion of the endoscopist performing the colonoscopy, and were interpreted by experienced GI histopathologists, who were again blinded to the questionnaire data of the patient. Findings classified as being consistent with organic disease at colonoscopy, or after

histopathological examination of biopsy specimens, in both studies are the same as those described in Table 9.

5.2.2.3 Data Incorporated into the Latent Class Model

Individual symptoms were used to identify naturally occurring clusters within the data. All intestinal symptoms that have been reported to be associated with IBS were considered, such as upper and lower abdominal pain or discomfort, abdominal bloating (Ryu et al., 2016), dyspepsia (Rasmussen et al., 2015; Ford et al., 2010), gastro-oesophageal reflux-type symptoms (Lovell and Ford, 2012) and nausea (Van Oudenhove et al., 2016). The absence of alarm signs or symptoms, such as weight loss or rectal bleeding, were not considered in the model, as it has been shown previously that these do not differentiate between IBS and organic disease with any great accuracy (Whitehead et al., 2006; Ford et al., 2013). Nor were demographic data, such as age or gender, included as the aim of the model was to identify naturally occurring clusters in patient-reported symptoms that could be used to distinguish between IBS and non-IBS latent classes. LCA assumes that there is no dependent association between variables entered into the model (Moayyedi et al., 2004). An example of this would be when the presence of one symptom is dependent on the presence or absence of another symptom, such as a change in stool frequency or form, in association with abdominal pain, as seen in the Rome IV criteria for IBS (Mearin et al., 2016). The full criteria would therefore be unsuitable to incorporate into such a model. However, if an individual reported abdominal pain, or an increased stool frequency, as symptoms independent of each other, then these symptoms would be suitable to incorporate into the model.

Individual items from the PHQ-15 questionnaire were also considered, as a recent study showed that both mean somatisation scores and mean number of somatic symptom

items were higher in patients with IBS, when compared with other patients with GI symptoms that did not meet criteria for IBS (Patel et al., 2015). In particular, the prevalence of nine of the 12 extra-intestinal symptoms that are included in the PHQ-15 was found to be statistically significantly higher in IBS patients. These nine items were therefore included in the latent class model. The variables incorporated into the model are shown in Table 15.

Table 15. Individual Symptom Items Included in the Latent Class Model.

Presence of the following symptoms in the preceding 3 months
Heartburn
Fullness after a regular-sized meal
Early satiety
Upper abdominal discomfort
Nausea
Belching
Lower abdominal discomfort
Abdominal bloating
<3 bowel movements per week
Hard or lumpy stools
≥4 bowel movements per day
Loose, mushy, or watery stool
Mucus per rectum
PHQ-9 somatic symptom items
Back pain
Arm, leg, or joint pain
Menstrual cramps or other period problems
Headaches
Dizziness
Heart pounding or racing
Pain or problems during sexual intercourse
Tired or low in energy
Trouble sleeping

5.2.2.4 Reference Standard to Define the Presence of IBS

The reference standard used to define the presence of IBS in both study populations was lower abdominal pain or discomfort occurring at least once a week, in association with a change in bowel habit, and in the absence of organic lower GI disease after colonoscopy and histopathological examination of colonic biopsies, if obtained, which would explain these symptoms. Exclusion of coeliac disease with distal duodenal biopsy was also undertaken in both studies, if coeliac serology was positive.

5.2.3 Statistical Analysis

The statistical program LatentGOLD version 4.5 (Vermunt and Magidson, 2005, Statistical Innovations, Inc., Belmont, MA, USA) was used to perform LCA. The modal assignment, which places individuals in the latent class in which they have the highest probability of membership was used. The latent class model was derived using the Canadian dataset, and an identical model was then applied to the UK dataset as a means of validating it. In order to determine the optimum number of classes that best fit the data, up to six latent class models were used, and the number of classes that best fit the data was determined using the likelihood ratio chi-squared (LR^2) statistic, and parsimony indices, which help in maintaining a balance between goodness-of-fit and model complexity. The parsimony indices used were: the number of parameters, the Akaike information criterion (AIC), and the Bayesian information criterion (BIC). In general, smaller values correspond to more parsimonious models.

The Wald statistical test was used to determine the significance of the responses given by the participants in the Canadian study, when deriving the latent class model. A P value < 0.05 means that the knowledge of the response for a particular symptom in the

model contributes in a significant way to discriminating between the clusters (Vermunt and Magidson, 2005).

Once individual membership to a latent class was derived, and the IBS and non-IBS latent classes determined based on their characteristics, correct latent class membership for each individual was calculated by comparing against the reference standard for IBS. From this we calculated the sensitivity, specificity, PPVs, NPVs, and positive and negative LRs of the latent class model, when compared with the reference standard, using a Microsoft Excel spreadsheet (2013 Edition; Microsoft Corp, Redmond, WA).

As LCA was used to calculate the probability of having IBS, this means it was possible to vary the discrimination threshold utilised in the model. In a diagnostic test for IBS, it is important that the false positive rate of the test is minimised, and therefore the risk of missing organic GI disease is low. ROC curves were constructed, and were used to maximise specificity over sensitivity, in order to calculate the maximum positive LR available for the latent class model. The AUC was calculated to assess the accuracy of the model in differentiating IBS from organic disease. These analyses were performed using SPSS for Windows version 21.0 (SPSS Inc, Chicago, IL, USA).

5.3 Results

5.3.1 Demographics

There were 4224 consecutive patients recruited into the Canadian study between January 2008 and December 2012. Of these, 1981 (46.9%) underwent colonoscopy for investigation of their symptoms and therefore provided data for the derivation of the latent class model. Mean age of those undergoing colonoscopy was 49.3 years (SD 17.1 years),

1251 (63.1%) were female and 1787 (90.2%) were White Caucasian. The prevalence of IBS in the study population as defined by the reference standard was 19.9% (n = 394).

Of the 1002 consecutive patients recruited to the UK study between January 2014 and December 2015, 360 (35.9%) underwent colonoscopic investigation for their symptoms, and therefore provided data to validate the latent class model. The mean age of those who underwent colonoscopy was 53.9 years (SD 16.5 years), 236 (65.6%) were female and 329 (91.4%) were White Caucasian. The prevalence of IBS was higher in the UK study at 27.5% (n = 99; P = 0.001 vs. the Canadian dataset).

Demographics of those undergoing colonoscopic examination in the Canadian and UK studies are shown in Table 16. Those in the UK study were older, but there were no other significant differences between individuals in the two studies. The prevalence of organic GI disease in the Canadian study was 20.6% compared with 16.7% in the UK study (P = 0.10). The breakdown of organic GI diseases in the two patient cohorts is detailed in Table 17. The prevalence of all types of idiopathic IBD was significantly higher in the Canadian study, and the prevalence of microscopic colitis was significantly higher in the UK study.

Table 16. Demographics and Baseline Characteristics of Those Undergoing Colonoscopy in the Canadian and UK studies.

	Canadian study (n = 1981)	UK study (n = 360)	P Value*
Mean age in years (SD)	49.3 (17.1)	53.9 (16.5)	0.006
Mean BMI (SD)	27.3 (6.0)	27.1 (5.9)	0.61
Female gender (%)	1251 (63.1)	236 (65.6)	0.42
Tobacco use (%)	409 (20.6)	86 (23.9)	0.19
Alcohol use (%)	1165 (58.8)	195 (54.2)	0.11
Married or co-habiting (%)	1212 (61.2)	203 (56.4)	0.10
University graduate or Postgraduate level of education (%)	467 (23.6)	68 (18.9)	0.06
White Caucasian ethnicity (%)	1787 (90.2)	329 (91.4)	0.55

*P value for independent samples *t*-test for continuous data and Pearson χ^2 for comparison of categorical data.

Table 17. Prevalence of Organic Disease in the Canadian Study In Comparison With the UK Study.

	Canadian study (n = 1981)	UK study (n = 360)	P value*
UC (%)	95	4	0.0005
Crohn's disease (%)	136	7	0.0001
IBD-unclassifiable (%)	66	4	0.02
Non-specific GI ulceration (%)	7	5	0.03
Collagenous colitis (%)	8	17	0.0001
Lymphocytic colitis (%)	25	12	0.009
Colorectal cancer (%)	47	4	0.17
Coeliac disease (%)	24	7	0.31

*P value for Fisher's exact test for comparison of categorical data.

5.3.2 Assessment of Model Fit

In order to determine the optimum number of classes that best fit the data, up to six latent class models were used. Using trends in the LR^2 statistic, BIC, and AIC it was determined that a four-class solution best fitted the Canadian dataset, and maintained the optimum balance between goodness-of-fit and model complexity. The P value for the Wald statistical test was < 0.05 for all the symptoms entered into the latent class model derived from the Canadian dataset (Table 18).

Table 18. LR^2 Statistic and Parsimony Indices Values for Six Latent Classes Derived from the Canadian Dataset.

Number of classes	Likelihood ratio chi-squared statistic	Bayesian information criterion	Akaike information criterion	Number of parameters
2	66328.69	88393.42	87912.57	86
3	65089.70	87329.03	86719.57	109
4	64445.44	86859.37	86121.31	132
5	63995.82	86584.35	85717.69	155
6	63656.78	86419.91	85424.65	178

5.3.3 Description of the Latent Class Model Clusters

The clinical characteristics of each class in the model in the Canadian and UK studies are shown in Table 19 and Table 20 respectively. In the Canadian study, the latent class that was predominantly IBS represented 20.8% of the population (n = 412), and in the UK study, 26.1% of the study population (n = 94). In the Canadian study, in the preceding 3 months, the IBS class were more likely to experience the following symptoms: heartburn, feeling uncomfortably full after a meal, inability to finish a regular sized meal, pain or burning in the upper abdomen, bothersome nausea, and bothersome belching, all at a frequency of every day, as well as bloating or distension occurring most of the time, than when compared with the non-IBS classes, as well as experiencing many of the extra-intestinal somatisation symptoms at a greater severity. However, although the IBS class was more likely to experience discomfort or pain in the lower abdominal pain once a week or more, non-IBS class 3 was more likely to experience the symptom of frequent loose, mushy, or watery stools at a frequency of ≥75%, and to report always having ≥4 bowel movements per day, than when compared with the IBS class.

In the UK study, in the previous 3 months, the IBS class were more likely to experience the following: feeling uncomfortably full after a meal, inability to finish a regular sized meal, bothersome nausea, and discomfort or pain in the lower abdomen every day, loose, mushy, or watery stools, ≥4 bowel movements per day, and bloating and distension occurring always, or 100% of the time. The majority of somatisation symptoms were also more severe. The IBS class experienced similar levels of heartburn, pain or burning in the upper abdomen, and bothersome belching to that reported by non-IBS class 2, who also experienced frequent somatisation symptoms, although to a lesser severity than the IBS class.

Table 19. Clinical Characteristics of the IBS and non-IBS Latent Classes in the Canadian Model.

Proportion of patients in each latent class				
	IBS class (n = 412)	**Non-IBS class 1 (n = 648)**	**Non-IBS class 2 (n = 562)**	**Non-IBS class 3 (n = 359)**
Female gender	0.8141	0.4426	0.7188	0.6201
Age <40 years	0.4122	0.1665	0.2204	0.4372
Symptoms occurring within the last 3 months				
Heartburn				
Never	0.1867	0.6845	0.4041	0.4768
Less than once a week	0.2901	0.2413	0.3303	0.3198
Once a week or more	0.3549	0.0670	0.2126	0.1689
Every day	0.1682	0.0072	0.0530	0.0345

Uncomfortably full after a meal				
Never	0.0292	0.8096	0.3397	0.3757
Less than once a week	0.0815	0.1465	0.2537	0.2569
Once a week or more	0.3408	0.0397	0.2841	0.2634
Every day	0.5484	0.0041	0.1224	0.1040
Unable to finish a regular sized meal				
Never	0.1801	0.8682	0.4557	0.5038
Less than once a week	0.1809	0.1055	0.2306	0.2280
Once a week or more	0.3365	0.0237	0.2160	0.1910
Every day	0.3025	0.0026	0.0978	0.0773

Pain or burning in the upper abdomen				
Never	0.1568	0.8467	0.4794	0.4503
Less than once a week	0.1389	0.1071	0.1925	0.1928
Once a week or more	0.3718	0.0409	0.2334	0.2492
Every day	0.3325	0.0052	0.0946	0.1077
Bothersome nausea				
Never	0.0650	0.8230	0.3736	0.4128
Less than once a week	0.1740	0.1499	0.3157	0.3141
Once a week or more	0.4411	0.0259	0.2528	0.2264
Every day	0.3199	0.0013	0.0579	0.0467
Bothersome belching				
Never	0.2962	0.7834	0.4896	0.5646
Less than once a week	0.1752	0.1409	0.1942	0.1907
Once a week or more	0.2141	0.0524	0.1593	0.1331
Every day	0.3145	0.0234	0.1570	0.1117

Discomfort or pain in the lower abdomen				
Never	0.0372	0.5798	0.3027	0.1159
Less than once a week	0.1105	0.2583	0.2787	0.1989
Once a week or more	0.3932	0.1378	0.3073	0.4091
Every day	0.4591	0.0241	0.1112	0.2762
<3 bowel movements per week				
Never / rarely	0.5942	0.8520	0.6056	0.8681
Sometimes	0.1706	0.1090	0.1700	0.1006
Often	0.0890	0.0253	0.0867	0.0212
Most of the time	0.0762	0.0097	0.0726	0.0073
Always	0.0699	0.0040	0.0651	0.0027

Hard or lumpy stools				
Never / rarely	0.3712	0.6083	0.3025	0.6514
About 25% of the time	0.3119	0.2778	0.2991	0.2598
About 50% of the time	0.1596	0.0772	0.1801	0.0631
About 75% of the time	0.1178	0.0310	0.1565	0.0221
Always, 100% of the time	0.0395	0.0056	0.0617	0.0035
≥4 bowel movements per day				
Never / rarely	0.2879	0.6404	0.7211	0.1432
Sometimes	0.2738	0.2568	0.2185	0.1968
Often	0.1852	0.0732	0.0471	0.1924
Most of the time	0.1223	0.0204	0.0099	0.1837
Always	0.1307	0.0092	0.0034	0.2838

Mushy, watery, or loose stools				
Never / rarely	0.0964	0.4479	0.4717	0.0320
About 25% of the time	0.2017	0.3389	0.3354	0.1030
About 50% of the time	0.2149	0.1305	0.1214	0.1684
About 75% of the time	0.3147	0.0691	0.0604	0.3785
Always, 100% of the time	0.1723	0.0137	0.0112	0.3181
Mucus in the bowel movement				
Never / rarely	0.2473	0.7592	0.6902	0.2785
Sometimes	0.2978	0.2013	0.2432	0.3090
Often	0.2301	0.0343	0.0550	0.2201
Most of the time	0.1418	0.0046	0.0099	0.1249
Always	0.0831	0.0006	0.0017	0.0674

Bloating or distension				
Never / rarely	0.0173	0.6514	0.2150	0.1282
Sometimes	0.0799	0.2489	0.2823	0.2322
Often	0.3397	0.0878	0.3422	0.3882
Most of the time	0.5572	0.0119	0.1600	0.2503
Always	0.0059	0.0000	0.0005	0.0010
PHQ symptoms experienced during the past 4 weeks				
Back pain				
No	0.0487	0.5580	0.2445	0.2775
A little	0.3183	0.3681	0.4749	0.4750
A lot	0.6331	0.0739	0.2806	0.2475
Arm, leg, or joint pain				
No	0.1373	0.4995	0.2744	0.3550
A little	0.3414	0.3499	0.3882	0.3865
A lot	0.5212	0.1506	0.3373	0.2585

Period pain				
No	0.5491	0.8793	0.7275	0.6788
A little	0.2383	0.0981	0.1805	0.2001
A lot	0.2125	0.0225	0.0920	0.1212
Headache				
No	0.0925	0.6478	0.3674	0.3676
A little	0.4318	0.3158	0.4829	0.4828
A lot	0.4757	0.0363	0.1498	0.1496
Dizziness				
No	0.1457	0.8378	0.4801	0.5062
A little	0.5175	0.1568	0.4445	0.4276
A lot	0.3368	0.0054	0.0754	0.0662
Palpitations				
No	0.3059	0.8754	0.5658	0.6901
A little	0.4519	0.1188	0.3539	0.2712
A lot	0.2421	0.0058	0.0803	0.0387

Dyspareunia				
No	0.5091	0.9127	0.8073	0.8502
A little	0.2607	0.0763	0.1467	0.1204
A lot	0.2302	0.0110	0.0460	0.0294
Feeling tired or low in energy				
No	0.0018	0.4128	0.0478	0.0369
A little	0.0888	0.4739	0.3665	0.3340
A lot	0.9094	0.1133	0.5857	0.6291
Insomnia				
No	0.0345	0.5517	0.1918	0.2237
A little	0.2349	0.3480	0.4093	0.4186
A lot	0.7306	0.1003	0.3990	0.3577

Table 20. Clinical Characteristics of the IBS and non-IBS Latent Classes in the UK Model.

Proportion of patients in each latent class				
	IBS class (n = 94)	**Non-IBS class 1 (n = 106)**	**Non-IBS class 2 (n = 86)**	**Non-IBS class 3 (n = 74)**
Female gender	0.7849	0.5779	0.7345	0.5080
Age <40 years	0.3427	0.2332	0.2241	0.0008
Symptom occurring within the last 3 months				
Heartburn				
Never	0.2366	0.7052	0.2635	0.6704
Less than once a week	0.2861	0.2238	0.2946	0.2413
Once a week or more	0.2988	0.0614	0.2846	0.0750
Every day	0.1786	0.0096	0.1573	0.0133

Uncomfortably full after a meal				
Never	0.1224	0.7621	0.3158	0.7959
Less than once a week	0.1613	0.1695	0.2354	0.1525
Once a week or more	0.3179	0.0564	0.2626	0.0437
Every day	0.3983	0.0119	0.1862	0.0080
Unable to finish a regular sized meal				
Never	0.2976	0.7994	0.4529	0.7663
Less than once a week	0.1760	0.1287	0.1908	0.1411
Once a week or more	0.3000	0.0597	0.2317	0.0749
Every day	0.2264	0.0123	0.1246	0.0176

Pain or burning in the upper abdomen				
Never	0.2918	0.7845	0.2852	0.8659
Less than once a week	0.1801	0.1369	0.1788	0.0991
Once a week or more	0.3021	0.0649	0.3046	0.0308
Every day	0.2260	0.0137	0.2314	0.0043
Bothersome nausea				
Never	0.1920	0.7259	0.3290	0.8951
Less than once a week	0.2195	0.1871	0.2543	0.0896
Once a week or more	0.4126	0.0793	0.3234	0.0147
Every day	0.1759	0.0076	0.0933	0.0006
Bothersome belching				
Never	0.4103	0.7075	0.3600	0.7720
Less than once a week	0.1422	0.1309	0.1373	0.1175
Once a week or more	0.1932	0.0949	0.2053	0.0701
Every day	0.2544	0.0667	0.2974	0.0405

Discomfort or pain in the lower abdomen				
Never	0.0401	0.3249	0.3259	0.7766
Less than once a week	0.0712	0.1911	0.1912	0.1393
Once a week or more	0.3741	0.3325	0.3319	0.0739
Every day	0.5146	0.1515	0.1510	0.0103
<3 bowel movements per week				
Never / rarely	0.7608	0.8306	0.6299	0.8283
Sometimes	0.1403	0.1178	0.1595	0.1187
Often	0.0386	0.0249	0.0601	0.0253
Most of the time	0.0333	0.0165	0.0713	0.0170
Always	0.0270	0.0103	0.0792	0.0107

Hard or lumpy stools				
Never / rarely	0.6030	0.6052	0.3924	0.6935
About 25% of the time	0.2403	0.2398	0.2586	0.2106
About 50% of the time	0.1067	0.1058	0.1897	0.0712
About 75% of the time	0.0437	0.0431	0.1286	0.0222
Always, 100% of the time	0.0063	0.0062	0.0307	0.0024
≥4 bowel movements per day				
Never / rarely	0.0627	0.1740	0.5592	0.8671
Sometimes	0.1927	0.3281	0.3413	0.1256
Often	0.2252	0.2354	0.0792	0.0069
Most of the time	0.2328	0.1494	0.0163	0.0003
Always	0.2866	0.1130	0.0040	0.0000

Mushy, watery, or loose stools				
Never / rarely	0.0113	0.0545	0.2339	0.7259
About 25% of the time	0.1010	0.2639	0.4739	0.2559
About 50% of the time	0.1651	0.2348	0.1764	0.0166
About 75% of the time	0.4040	0.3126	0.0982	0.0016
Always, 100% of the time	0.3185	0.1341	0.0176	0.0001
Mucus in the bowel movement				
Never / rarely	0.2925	0.5501	0.6072	0.9193
Sometimes	0.3352	0.3184	0.2953	0.0772
Often	0.2029	0.0973	0.0758	0.0034
Most of the time	0.1122	0.0272	0.0178	0.0001
Always	0.0572	0.0070	0.0038	0.0000

Bloating or distension				
Never / rarely	0.0504	0.3775	0.1839	0.7181
Sometimes	0.0785	0.2513	0.1790	0.2214
Often	0.0026	0.0036	0.0037	0.0015
Most of the time	0.4511	0.2635	0.4014	0.0498
Always	0.4174	0.1042	0.2321	0.0091
PHQ symptoms experienced during the past 4 weeks				
Back pain				
No	0.1184	0.5704	0.2157	0.6349
A little	0.3956	0.3504	0.4457	0.3096
A lot	0.4860	0.0791	0.3386	0.0555
Arm, leg, or joint pain				
No	0.1891	0.5314	0.2952	0.5482
A little	0.2998	0.2939	0.3235	0.2885
A lot	0.5112	0.1747	0.3812	0.1633

Period pain				
No	0.7846	0.8719	0.7678	0.9495
A little	0.1433	0.0978	0.1507	0.0447
A lot	0.0721	0.0303	0.0815	0.0058
Headache				
No	0.2925	0.6792	0.2633	0.8891
A little	0.4326	0.2735	0.4320	0.1055
A lot	0.2749	0.0473	0.3046	0.0054
Dizziness				
No	0.2852	0.7748	0.3990	0.7797
A little	0.5066	0.2118	0.4719	0.2076
A lot	0.2082	0.0134	0.1292	0.0128
Palpitations				
No	0.3239	0.8167	0.6383	0.8838
A little	0.4404	0.1695	0.3045	0.1108
A lot	0.2357	0.0138	0.0572	0.0055

Dyspareunia				
No	0.6543	0.9143	0.9236	0.9981
A little	0.2063	0.0731	0.0662	0.0019
A lot	0.1395	0.0125	0.0102	0.0000
Feeling tired or low in energy				
No	0.0087	0.1771	0.0442	0.2995
A little	0.1442	0.4386	0.2886	0.4555
A lot	0.8472	0.3843	0.6672	0.2450
Insomnia				
No	0.1015	0.4616	0.2161	0.5852
A little	0.2765	0.3362	0.3405	0.2933
A lot	0.6220	0.2023	0.4434	0.1215

5.3.4 Accuracy of the Latent Class Model

In the Canadian dataset, the latent class model was able to predict a diagnosis of IBS with a sensitivity of 44.7% and specificity of 85.3%. Positive and negative LRs were 3.03 and 0.65 respectively (Table 21). Following construction of a ROC curve (Figure 10), specificity was maximised at 92.7%, with a sensitivity of 28.7%, resulting in a maximum positive LR of 3.93 and a negative LR of 0.77. The AUC was 0.77. Performance of the latent class model using data from the UK study was similar, with a sensitivity of 52.5% and specificity of 84.3%. Positive and negative LRs were 3.35 and 0.56 respectively (Table 21). Following construction of a ROC curve (Figure 11), specificity was maximised at 93.0%, with a sensitivity of 29.3%, resulting in a maximum positive LR of 4.15 and a negative LR of 0.76, with an AUC of 0.79.

Table 21. Sensitivity, Specificity, Likelihood Ratios, and Predictive Values of the Canadian and UK Latent Class Models.

	Sensitivity (%)	Specificity (%)	Positive LR	Negative LR	PPV (%)	NPV (%)
Canadian study	44.7	85.3	3.03	0.65	43.6	85.8
UK Study	52.5	84.3	3.35	0.56	56.5	82.1

Figure 10. Receiver Operating Characteristic Curve for the Canadian Latent Class Model.

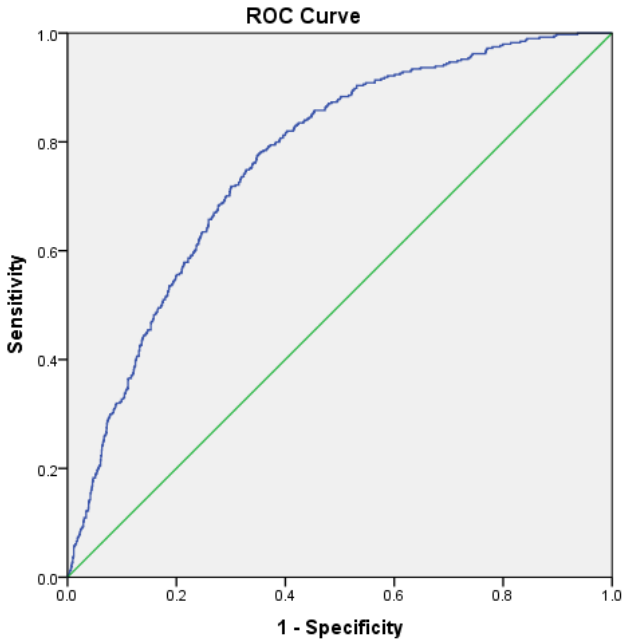
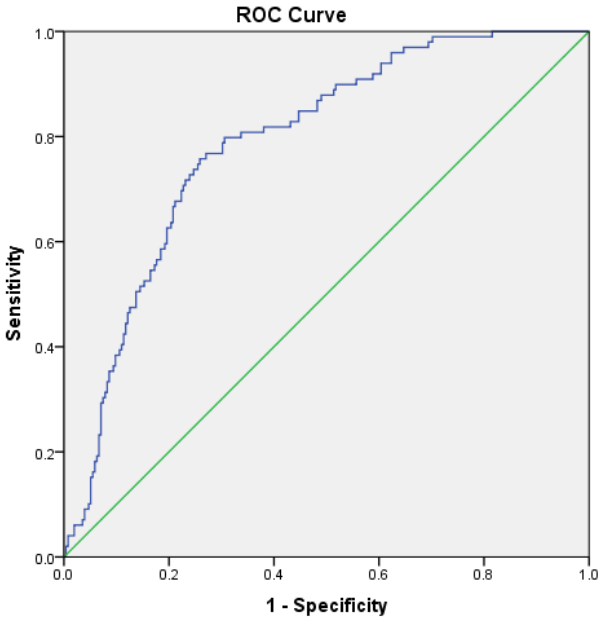


Figure 11. Receiver Operating Characteristic Curve for the UK Latent Class Model.



5.4. Discussion

This study has derived a latent class model for IBS in unselected patients referred to a secondary care hospital in Canada. The same model was then validated in unselected patients referred to a secondary care hospital in the UK. LCA was used to identify naturally occurring clusters in the data incorporated into the model, and it was then determined if correct latent class membership was obtained by comparing against the reference standard for IBS used in this study. In both cohorts of patients, the IBS class was more likely to experience post-prandial symptoms, nausea, lower abdominal pain or discomfort, and somatisation symptoms, compared with the non-IBS classes. In the Canadian study, following construction of an ROC curve, the model had a positive LR approaching 4 when used as a diagnostic test for IBS, and when specificity was maximised, whilst in the UK study the positive LR was 4.15. The discriminatory accuracy of the two models, as measured by the AUC, were good at 0.77 and 0.79 for the Canadian and UK models respectively.

The study has a number of strengths. The test performed similarly in two large cohorts of patients referred to secondary care. The patient groups studied were demographically distinct, with a significant difference in prevalence of both IBS and individual organic GI diseases, suggesting that the performance of the test is reliable. As the study was conducted in an unselected patient cohort, it means that the results are likely to be generalisable to gastroenterologists consulting with patients who have lower GI symptoms, including those suggestive of IBS, in usual clinical care. Furthermore, the test developed from this study is inexpensive, and should be considered at least as accurate as biomarkers, as demonstrated in the previous meta-analysis chapter, and these are potentially more expensive.

However, the study does also have limitations. The reference standard used to compare the accuracy of the latent class model included some of the symptoms that were also used in the model itself. This may have resulted in an overestimation of its accuracy. In both populations, the majority of the participants were White Caucasian and, in the UK study, tended to be older, meaning that our findings may not be applicable to different ethnicities or to younger individuals. Furthermore, not all patients that were recruited into the two studies underwent colonoscopy. These patients were therefore not included in the latent class model, and some of these excluded patients may have been given a diagnosis of IBS based on a physician's assessment alone. If the diagnosis of IBS were correct in all these patients then the true positive rate of the latent class model assessed would have been artificially reduced, leading to an underestimation of its accuracy. There was no mandate for serological screening to rule out coeliac disease, and the decision to obtain biopsy specimens at colonoscopy was at the discretion of the endoscopist, so it is possible that cases of coeliac disease or microscopic colitis may have been missed, meaning that these patients were incorrectly classified as having IBS. Lastly, as previously discussed, the study only used symptom data to differentiate between IBS and non-IBS. It would be interesting to note if the addition of clinical tests, such as normal haemoglobin and CRP, negative coeliac serology, or normal faecal calprotectin, resulted in improved accuracy of the test.

There are no other published studies that have used LCA to derive and then validate a diagnostic test for IBS. Only one other study has utilised LCA in the diagnosis of FGIDs. In a postal questionnaire conducted by Koloski *et al.* (Koloski et al., 2015), Rome III criteria symptoms for IBS-C and CIC were included in a latent class model, with the aim of identifying clinical and lifestyle factors that could be used to differentiate between the two. Of 3260 participants, 109 met the Rome III criteria for IBS-C, and 206 met the

threshold for CIC. A two class solution was yielded in which the first class was 75% CIC (n = 164) and 25% IBS-C (n = 54), whilst the other class was approximately half CIC (n = 42) and half IBS-C (n = 55). The CIC-dominant group had lower levels of abdominal symptoms, although the mixed group demonstrated higher levels of constipation symptoms. The model was unable to reproduce the Rome III criteria differentiation of individuals with IBS-C and CIC. The authors concluded that using these criteria to distinguish between the two disorders may be, to some degree, artificial and that differentiation between the two based on severity of abdominal symptoms may represent the way forward. This is in keeping with this study's findings, which suggest that IBS exists as a latent construct, consisting of a combination of both GI and non-GI symptoms. Given the significant associations with other GI symptoms, such as heartburn, post-prandial distress, and nausea that have been observed in this study, as well as extra-intestinal symptoms, attempting to define IBS as either a discrete FGID based on specific symptoms, or even as a purely GI disorder, may therefore be an overly simplistic approach.

The LCA model derived and validated in the current study compares favourably with currently available approaches to diagnosing IBS. As previously discussed, The Rome IV criteria are the preferred method for diagnosing IBS (Mearin et al., 2016), but have yet to be validated by an independent group, and the Rome III criteria (Longstreth et al., 2006) have been validated in one large study (Ford et al., 2013), and performed modestly in differentiating IBS from organic disease, with a sensitivity and specificity of 68.8% and specificity of 79.5%. Positive and negative LRs were 3.35 and 0.39 respectively.

The LCA model has a positive LR of 3.93 and 4.15 in the Canadian and UK cohorts respectively. However, the study was conducted in an unselected population referred to secondary care, with an IBS prevalence of 23.7% among the two cohorts combined, lower

than that used to calculate the post-test probability threshold of 86.5% recommended, in the previously discussed study (Shah et al., 2015), as useful for a diagnostic test in IBS. This suggests that the performance of the latent class model should be at least comparable to that of the previously aforementioned only commercial biomarker, which had a maximum positive LR of 5.2 when validated in an IBS-enriched population (Pimentel et al., 2015). Furthermore, the latent class model has the potential for an improvement in its accuracy. The addition of relevant biochemical tests, such as faecal calprotectin, may result in a reduction in the number of false positives, and therefore an improvement in the ability of the model to differentiate IBS from organic lower GI disease.

The used of statistical modelling to diagnose IBS may result in a more complex test, which is perhaps the reason why the Kruis model (Kruis et al., 1984) has never been widely used in routine clinical care. However, as mentioned previously, in the era of smartphone apps, data can be inputted into an online statistical model that uses techniques such as LCA, to give an accurate probability of IBS that can then be used to aid the physician consulting in routine clinical care.

Data from this study also provide some interesting insights into possible directions for the development of symptom-based diagnostic criteria in the future. The observation that patients in the latent class of IBS in both cohorts were more likely to report upper GI symptoms consistent with functional dyspepsia, such as early satiety and postprandial fullness suggest that, rather than making FGIDs discrete entities, the presence of these co-existent symptoms are likely to be supportive of a diagnosis of IBS. In addition, in this study bloating or distension seemed to be a key feature of IBS, present in over 80% of patients. This was first proposed as part of the diagnostic criteria for IBS by Manning *et al.* in 1978 (Manning et al., 1978), but is no longer required in the Rome criteria, and should perhaps be re-incorporated into the list of required symptoms for future iterations.

In conclusion, this study has identified the existence of a latent construct for IBS consisting of intestinal and extra-intestinal symptoms. Furthermore, a diagnostic test for IBS using LCA has been derived and validated. The study has shown that the resulting model performs similarly to the Rome III criteria, and is likely to perform as well as available commercial biomarkers. Importantly, the test has the potential for improvement in its accuracy, and future studies should consider the addition of biochemical markers to the model, in order to assess whether this is indeed achievable.

CHAPTER 6

Conclusions

There has been much progress in the methods employed to aid in diagnosing IBS since the condition was first recognised as a distinct entity, with the development of non-invasive approaches, such as the symptom-based diagnostic criteria for IBS, and some novel biomarkers, which have aided in our understanding of the pathophysiological mechanisms underpinning the disorder. However, despite this many physicians still consider IBS to be a diagnosis of exclusion. This may be due to the fear of missing organic pathology that can mimic IBS, such as colorectal cancer and IBD, but is also likely due to the lack of an accurate, simple, and non-invasive diagnostic test for IBS. This thesis has examined the accuracy and practicality of all approaches available to diagnose IBS, and used these findings to develop novel and non-invasive tests to aid in the diagnosis of IBS, which are potentially easily administrable in a clinical setting.

A previous systematic review and meta-analysis that assessed the accuracy of symptom-based diagnostic criteria showed that these performed only modestly in differentiating IBS from organic disease (Ford et al., 2008). However, at the time when the review was conducted, neither the Rome II or Rome III criteria had been externally validated. Furthermore, the authors did not assess the accuracy of other available diagnostic tests, which were out of the scope of the study. An up to date systematic review and meta-analysis that took in to account studies assessing the accuracy of the remaining iterations of the Rome criteria, as well as more novel techniques, was therefore timely.

This systematic review assessed the accuracy of symptom-based diagnostic criteria, biomarkers, psychological markers, and combinations thereof in diagnosing IBS against an accepted reference standard. The study showed that the symptom-based criteria performed comparably in accuracy to each other, whilst biomarkers, including novel serum and faecal biomarkers (Jones et al., 2014; Ahmed et al., 2013), on the whole

performed no better, or were only used to differentiate IBS from health, rather than from organic disease, which is more clinically useful. Interestingly, when combinations of symptoms, biomarkers and/or psychological markers were used, then for some of these tests, for example combining serum biomarkers with psychological markers (Jones et al., 2014), combining the Rome I criteria with intestinal permeability and faecal calprotectin (Tibble et al., 2002), or combining symptoms with blood test results in the Kruis statistical model (Kruis et al., 1984) the positive LRs were approaching what would be considered an acceptable accuracy for a diagnostic test for IBS (Shah et al., 2015). However, these tests have a number of limitations including only differentiating between IBS and health in the study from Jones *et al.* (Jones et al., 2014), and concerns regarding applicability for their use in routine clinical care due to the perceived complex nature of the tests in the studies from Tibble *et al.* (Tibble et al., 2002) and Kruis *et al.* (Kruis et al., 1984).

The findings from the completed systematic review aided in developing the hypothesis underpinning the final two results chapters of this thesis. Could the Rome III criteria, the accepted diagnostic test for IBS at the time the study was conducted, be improved by combining them with other relevant symptoms from the history, markers of psychological effect, and/or blood tests?

In the first diagnostic test study, questionnaire data from participants included in the Leeds study, along with relevant clinical blood test results, were used to modify the Rome III criteria, in an attempt to enhance the diagnostic ability of these criteria. Of 1002 patients recruited to the Leeds study, 318 provided the relevant questionnaire data and underwent colonoscopy. In keeping with the findings of the systematic review, combinations of different types of markers outperformed single markers alone (in this case the Rome III symptom-based diagnostic criteria). All modifications to the Rome III

criteria performed better than the Rome III criteria alone, irrespective of the combination used. In particular, when the Rome III criteria were combined with markers of high level of somatisation, the positive LR increased from 3.87 to 7.27, and this increase was more marked when the Rome III criteria were combined with no nocturnal passage of stools and high level of somatisation with a positive LR greater than 17. The impressive performance of psychological markers, in particular somatisation, in this study is in keeping with findings from other investigators, and highlights the large psychological component underpinning IBS, as discussed in the first chapter.

A potential weakness of combining multiple markers is the resulting increased complexity of the test derived. One way to overcome this complexity is to employ statistical models, which are able to give a probability of a diagnosis of IBS. LCA is one such method, which hypothesises the existence of one of more unobserved groups, so called latent classes, amongst a set of observed variables. In the second diagnostic test study, two separate patient cohorts from Canada and the UK were included. The LCA model was derived using the Canadian dataset, before being validated in the UK dataset. Patients were assigned to latent classes, according to the severity of GI symptoms associated with IBS, and individual answers from nine items of the PHQ-15 questionnaire. Each individual item incorporated in to the model was chosen based on evidence of an association with IBS. Correct latent class membership was confirmed by comparing against an accepted reference standard. In the Canadian cohort of 1981 individuals, the model had a sensitivity of 44.7% and a specificity of 85.3%. A maximum positive LR of 3.93 was achieved following construction of an ROC curve. The model performed similarly in the UK database of 360 individuals, with a sensitivity and specificity of 52.5% and 84.3%, with a maximum positive LR of 4.15. Performance of the diagnostic test was similar to the Rome III criteria.

The work undertaken in this thesis has also highlighted where future research is required. It has clearly been shown that tests consisting of one measurable aspect of the complex and multifactorial aetiology of IBS are unlikely to provide an accurate and acceptable performance. Therefore, combining more than one of these tests, or markers, appears to represent the optimum way forward. LCA could simplify the complex process of combining markers by the use of technological advances in computer software and applications. Although the LCA test derived performed no better than the current symptom-based diagnostic criteria, as previously stated, the test has the potential for improvement, and future studies could look at enhancing the derived LCA model through the addition of non-invasive tests such as faecal calprotectin, or other novel faecal and serum biomarkers.

This body of work also highlights how the existing symptom-based diagnostic criteria could be improved upon. It has been shown that minor modifications such as the addition of markers of somatisation and absence of nocturnal symptoms markedly improves the diagnostic accuracy of these criteria, and the results from this work could inform future iterations of the Rome criteria. Furthermore, findings from the LCA diagnostic study indicate that IBS is more likely to be associated with upper GI symptoms, such as early satiety and post-prandial fullness, and other symptoms such as abdominal bloating and distension, which suggests that there is likely to be significant overlap amongst the different FGIDs, and that attempts to classify them as distinct disorders is somewhat arbitrary. The model derived in this study not only shows the potential for an accurate diagnostic test for IBS, but also highlights that the presence of other FGIDs should be considered as supportive evidence for a diagnosis of IBS. This issue should therefore also be considered when developing future iterations of the Rome criteria.

In summary, this thesis has examined available methods to diagnose IBS and has shown that combining symptoms with clinical markers, markers of psychological affect, and/or novel biomarkers leads to greater accuracy in diagnosing IBS. The findings from the two diagnostic test studies undertaken confirm that this approach may represent the way forward in developing an accurate and non-invasive diagnostic test for IBS.

CHAPTER 7

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Appendices

Appendix A: Patient Information Sheet

The Leeds Teaching Hospitals NHS
NHS Trust

PATIENT INFORMATION SHEET

PART 1

Study Title: A Study to Validate and Test the Durability of the Rome III criteria for Functional Gastrointestinal Disorders Among Patients Referred to Secondary Care.

We would like you to take part in our research study. Before you decide we would like you to understand why the research is being done, and what it would involve for you. Please take time to read the following information carefully, and talk to others about the study if you wish. Ask us if there is anything that is not clear, or if you would like more information.

What is the purpose of the study?

Symptoms that are caused by problems with the gastrointestinal (digestive) tract are very common. This research study aims to find out how many of those people who suffer from these symptoms will have an obvious explanation for them when they are sent for an endoscopic examination (camera test) of either their stomach (upper gastrointestinal (GI) endoscopy) or large bowel (colonoscopy) or both, and how many people will have no explanation for their symptoms. If an obvious cause for the symptoms is found at upper GI endoscopy or colonoscopy this is usually known as “organic disease”, whereas if no cause is found doctors often call this “functional” disease. This study also aims to find out how good the criteria that Gastroenterologists have for classifying functional GI disorders, called the Rome III criteria, are at telling the difference between “organic” and “functional” disease. Finally, the study is interested in discovering how many people with symptoms that are felt to be due to a functional GI disorder when the person is first seen by a Gastroenterologist end up being found to have another, organic, cause for their

symptoms in the future. We intend to recruit around 4000 people who have these symptoms.

In order to help us perform this study we are asking you to answer questionnaires about your symptoms, about anxiety, depression, quality of life, other symptoms not related to the bowel, and to give us some other personal information, such as your age, gender, ethnicity, occupation, marital status, and tobacco and alcohol consumption. All this information will be collected at your outpatient clinic appointment or upper GI endoscopy or colonoscopy appointment. We will also ask for your permission to access your medical records in the future, so that we can see what happens to you, and whether your diagnosis changes in the future, and also your permission to contact you in the future with further questionnaires.

Why have I been invited?

You have been invited because you are suffering from symptoms that are caused by problems with the gastrointestinal tract, have been referred by your general practitioner for further tests because of this, and will be attending our clinic or upper GI endoscopy or colonoscopy list here at the Leeds General Infirmary or St. James's University Hospital.

Do I have to take part?

It is up to you to decide whether to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive. If you agree to take part, we will send a letter to your general practitioner informing them that you are involved in this research study.

What will happen to me if I take part?

If you agree to take part simply fill out the consent form and questionnaires, following the instructions provided on each, at your outpatient clinic appointment or your appointment for upper GI endoscopy or colonoscopy. Filling out the questionnaires should only take you about 30 minutes.

What are the possible disadvantages and risks of taking part?

Filling out the questionnaire may make some people worry about their bowel symptoms. If you find this to be the case you can discuss this with us during your visit to clinic. Please feel free to ask us any questions about your symptoms.

What are the possible benefits of taking part?

We cannot promise the study will help you, but the information we get from this study may help to increase our understanding of gastrointestinal symptoms and the causes of these symptoms, and may help us to know better how to investigate them in the future.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

Will my taking part in this study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The detailed information on this is given in part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

What will happen if I don't want to carry on with the study?

You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive. If you withdraw from the study we will destroy your questionnaire data.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the study doctors who will do their best to answer your questions (contact numbers are given below).
If you remain unhappy and wish to complain formally, you can receive further information from NHS Direct by calling 0845 4647.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential, and any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised. It will only be used for this study. Only the researchers involved in this study will have access to the data, which will be retained in a secure area, to which only researchers associated with this study have access for 5 years. Following this, it will be disposed of securely. If you do agree to take part, we will send a letter to your general practitioner informing them that you are involved in this research study.

What will happen to the results of this research study?

The results of this study may be published in a medical journal, but your identity will not be revealed.

Who is organising this study?

This study is being organised by Leeds General Infirmary and St. James's University Hospital.

Who has reviewed the study?

In order to protect your interests all research in the NHS is looked at by an independent group of people, called a Research Ethics Committee. This study has been reviewed and given a favourable opinion by Leeds Central Research Ethics Committee.

Further information and contact details:

Dr. Alex Ford (Investigator – Leeds General Infirmary): 0113 2068536

If you have any questions about your rights as a research volunteer, you may either contact your study doctors above, or look for further information on the UK clinical research collaboration website:
http://www.ukcrc.org/publications/informationbooklets.aspx
This website also provides a list of links to other useful websites.

Appendix B: Patient Consent Form

The Leeds Teaching Hospitals NHS
NHS Trust

CONSENT FORM

Title of project: A Cross-Sectional Survey to Validate and Test the Durability of the Rome III criteria for Functional Gastrointestinal Disorders Among Patients Referred to Secondary Care.

Name of researcher: Dr. Alex Ford.

	Please initial
I confirm that I have read and understood the information sheet dated 19th April 2013 (version 1.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected.	
I understand that relevant sections of my medical notes, and data collected during the study, may be looked at by individuals from Leeds Teaching Hospitals Trust, regulatory authorities, or the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to access my records.	
I understand that I may be contacted in the future to complete further questionnaires.	
I understand that if I agree to take part in this study you will contact my general practitioner to inform them of this.	
I agree to take part in the above study.	

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

Appendix C: Patient Symptom Questionnaire

Name:_____ Date of birth: _____

1. **What is your gender?**

   ☐ **Male** ☐ **Female**

2. **What is your age? _____**

3. **What is your current marital status?**

   ☐ **Married or co-habiting** ☐ **Divorced or separated**

   ☐ **Never married** ☐ **Widowed**

   ☐ **Civil partnership**

4. **Do you smoke tobacco?**

   ☐ **No** ☐**Yes**

   If yes, how many cigarettes per day? _____

5. **Do you drink alcohol?**

   ☐ **No** ☐**Yes**

   If yes, how many units per week? _____

6. **What is your height (in metres, or feet and inches)? _____**

7. **What is your weight (in kilograms, or pounds)?**

   _____

8. **What is your ethnic group?**

   ☐ **White Caucasian** ☐ **African**

   ☐ **South Asian** ☐ **South East Asian**

   ☐ **Middle-Eastern** ☐ **Latin-American**

   ☐ **Other (specify) _____**

9. **What is your occupation?_____**

10. What is your educational level?

- ☐ Some secondary school
- ☐ Completed secondary school
- ☐ Some technical school / college
- ☐ Tech school / college graduate
- ☐ Some university
- ☐ University graduate
- ☐ Postgraduate / professional

11. Is there any family history of:

	Yes	No
a. Oesophageal (gullet) cancer	☐	☐
b. Gastric (stomach) cancer	☐	☐
c. Coeliac disease (gluten intolerance)	☐	☐
d. Colorectal (bowel) cancer	☐	☐
e. Crohn's disease	☐	☐
f. Ulcerative colitis	☐	☐

12. In the last 3 months how often did you have a feeling of a lump, fullness, or something stuck in your throat?

- ☐ Never — **Go to question 15**
- ☐ Less than one day a week
- ☐ One day a week or more
- ☐ Every day

13. Have you had this feeling 6 months or 1onger?

- ☐ No
- ☐ Yes

14. Does this feeling occur between meals (when you're not eating)?

- ☐ No
- ☐ Yes

15. When you are eating or drinking does it hurt to swallow?

- ☐ Never or rarely
- ☐ Sometimes
- ☐ Often

☐ **Most of the time**

☐ **Always**

16. In the last 3 months how often did you have pain or discomfort in the middle of your chest (not related to heart problems)?

☐ **Never** **Go to question 19**

☐ **Less than one day a week**

☐ **One day a week or more**

☐ **Every day**

17. Have you had this chest pain 6 months or longer?

☐ **No**

☐ **Yes**

18. When you had your chest pain, how often did it feel like burning?

☐ **Never or rarely**

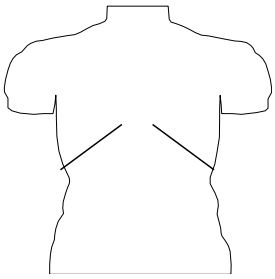
☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

19. In the last 3 months how often did you have heartburn (a burning discomfort or burning pain in your chest) (see picture)?



☐ **Never** **Go to question 21**

☐ **Less than one day a week**

☐ **One day a week or more**

☐ **Every day**

20. Have you had this heartburn (burning discomfort or pain in the chest) 6 months or longer?

- ☐ **No**
- ☐ **Yes**

21. In the last 3 months how often did food or drinks get stuck after swallowing or go down slowly through your chest?

- ☐ **Never** **Go to question 24**
- ☐ **Less than one day a week**
- ☐ **One day a week or more**
- ☐ **Every day**

22. Has this swallowing problem changed over the last 3 months?

- ☐ **Very much better**
- ☐ **Better**
- ☐ **Same**
- ☐ **Worse**
- ☐ **Very much worse**

23. Have you had this problem 6 months or 1onger?

- ☐ **No**
- ☐ **Yes**

24. In the last 3 months how often did you feel uncomfortably full after a regular-sized meal?

- ☐ **Never** **Go to question 26**
- ☐ **Less than one day a week**
- ☐ **One day a week or more**
- ☐ **Every day**

25. Have you had this uncomfortable fullness after meals 6 months or 1onger?

- ☐ **No**
- ☐ **Yes**

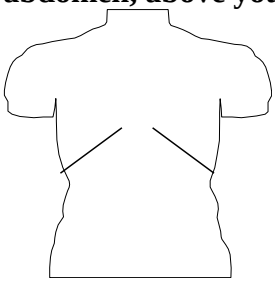
26. In the last 3 months how often were you unable to finish a regular sized meal?

- ☐ **Never** — **Go to question 28**
- ☐ **Less than one day a week**
- ☐ **One day a week or more**
- ☐ **Every day**

27. Have you had this inability to finish regular-sized meals 6 months or 1onger?

- ☐ **No**
- ☐ **Yes**

28. In the last 3 months how often did you have pain or burning in the middle of your abdomen, above your belly button but not in your chest (see picture)?



- ☐ **Never** — **Go to question 32**
- ☐ **Less than one day a week**
- ☐ **One day a week or more**
- ☐ **Every day**

29. Have you had this pain or burning 6 months or longer?

- ☐ **No**
- ☐ **Yes**

30. Did this pain or burning occur and then completely disappear during the same day?

- ☐ **Never or rarely**
- ☐ **Sometimes**
- ☐ **Often**
- ☐ **Most of the time**
- ☐ **Always**

31. Usually how severe was the pain or burning in the middle of your abdomen above your belly button?

- ☐ **Very mild**
- ☐ **Mild**
- ☐ **Moderate**
- ☐ **Severe**
- ☐ **Very severe**

32. In the last 3 months how often did you have bothersome nausea (feeling sick)?

- ☐ **Never** **Go to question 34**
- ☐ **Less than one day a week**
- ☐ **One day a week or more**
- ☐ **Every day**

33. Did this nausea start more than 6 months ago?

- ☐ **No**
- ☐ **Yes**

34. In the last 3 months how often did you vomit?

- ☐ **Never** **Go to question 40**
- ☐ **Less than one day a week**
- ☐ **One day a week or more**
- ☐ **Every day**

35. Have you had this vomiting 6 months or longer?

- ☐ **No**
- ☐ **Yes**

36. Did you make yourself vomit?

- ☐ **Never or rarely**
- ☐ **Sometimes**
- ☐ **Often**
- ☐ **Most of the time**
- ☐ **Always**

37. Did you have vomiting in the last year that occurred in separate episodes of a few days and then stopped?

- ☐ **Never or rarely**
- ☐ **Sometimes**
- ☐ **Often**
- ☐ **Most of the time**
- ☐ **Always**

38. Did you have at least three episodes during the past year?

- ☐ **No**
- ☐ **Yes**

39. In the last 3 months how often have you vomited blood?

- ☐ **Never or rarely**
- ☐ **Sometimes**
- ☐ **Often**
- ☐ **Most of the time**
- ☐ **Always**

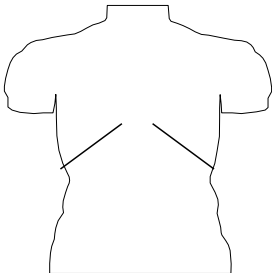
40. In the last 3 months how often did you experience bothersome belching?

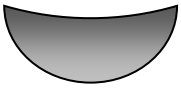
- ☐ **Never** **Go to question 42**
- ☐ **Less than one day a week**
- ☐ **One day a week or more**
- ☐ **Every day**

41. Did this bothersome belching start more than 6 months ago?

- ☐ **No**
- ☐ **Yes**

42. In the last 3 months how often did you have discomfort or pain anywhere in your lower abdomen below your belly button (see picture)?





☐ **Never** **Go to question 50**

☐ **Less than one day a week**

☐ **One day a week or more**

☐ **Every day**

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

☐ **No**

☐ **Yes**

☐ **Does not apply because I've had the menopause or I am male**

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

☐ **No**

☐ **Yes**

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

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

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

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

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

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

48. When this discomfort or pain started were your stools looser?

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

49. When this discomfort or pain started were your stools harder?

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

50. In the last 1 year have you had a change in your bowel habit?

☐ **No**

☐ **Yes**

51. In the last 3 months how often did you have fewer than 3 bowel movements (0-2) per week?

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

52. In the last 3 months how often did you have hard or lumpy stools?

☐ **Never or rarely**

☐ **About 25% of the time**

☐ **About 50% of the time**

☐ **About 75% of the time**

☐ **Always, 100% of the time**

53. In the last 3 months how often did you strain during bowel movements?

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

54. In the last 3 months how often did you have a feeling of incomplete emptying after bowel movements?

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

55. In the last 3 months how often did you have a sensation that the stool could not be passed (i.e. was blocked) when having a bowel movement?

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

56. In the last 3 months how often did you press on or around your bottom or remove stool in order to complete a bowel movement?

- ☐ **Never or rarely**
- ☐ **Sometimes**
- ☐ **Often**
- ☐ **Most of the time**
- ☐ **Always**

57. Did any of the symptoms of constipation listed in questions 51 – 56 above begin more than 6 months ago?

- ☐ **No**
- ☐ **Yes**

58. In the last 3 months how often did you have 4 or more bowel movements per day?

- ☐ **Never or rarely**
- ☐ **Sometimes**
- ☐ **Often**
- ☐ **Most of the time**
- ☐ **Always**

59. In the last 3 months how often did you have loose, mushy or watery stools?

- ☐ **Never or rarely** **Go to question 61**
- ☐ **About 25% of the time**
- ☐ **About 50% of the time**
- ☐ **About 75% of the time**
- ☐ **Always, 100% of the time**

60. Did you begin having frequent loose, mushy or watery stools more than 6 months ago?

- ☐ **No**
- ☐ **Yes**

61. In the last 3 months how often did you have to get up at night to have a bowel movement?

- ☐ **Never or rarely**
- ☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

62. In the last 3 months how often did you have to rush to the toilet to have a bowel movement?

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

63. In the last 3 months how often was there mucus in your bowel movement?

☐ **Never or rarely**

☐ **Sometimes**

☐ **Often**

☐ **Most of the time**

☐ **Always**

64. In the last 3 months how often did you have bloating or distension?

☐ **Never** **Go to question 66**

☐ **Less than one day a week**

☐ **One day a week or more**

☐ **Every day**

65. Did your symptoms of bloating or distension begin more than 6 months ago?

☐ **No**

☐ **Yes**

66. In the last 3 months have you noticed blood in your stools?

☐ **No** **Go to question 68**

☐ **Yes**

67. What colour was this blood?

- ☐ **Bright red**
- ☐ **Dark red**
- ☐ **Maroon**
- ☐ **Black**

68. In the last 3 months how often have you accidentally leaked liquid or solid stool?

- ☐ **Never**
- ☐ **Less than one day a week**
- ☐ **One day a week or more**
- ☐ **Every day**

69. In the last 3 months how often have you had aching, pain, or pressure in or around the back passage when you were passing a stool?

- ☐ **Never** **Go to question 73**
- ☐ **Less than one day a week**
- ☐ **One day a week or more**
- ☐ **Every day**

70. How long did the aching, pain, or pressure last?

- ☐ **Up to 20 minutes and then disappeared completely**
- ☐ **More than 20 minutes and up to several days or longer**

71. Did the aching, pain, or pressure in your back passage occur and then completely disappear during the same day?

- ☐ **No**
- ☐ **Yes**

72. Did the aching, pain, or pressure in your back passage begin more than 6 months ago?

- ☐ **No**
- ☐ **Yes**

73. In the last 1 year have you noticed any weight loss?

- ☐ **No**
- ☐ **Yes**

If yes, how much (in kilograms or pounds)? _____

74. Have you been told by your doctor that you are anaemic (a low blood count or low iron) (if female, not due to your menstrual period)?

☐ **No**

☐ **Yes**

75. In the last 3 months have you experienced bouts or spasms of coughing?

☐ **Never**

☐ **Less than one day a week**

☐ **One day a week or more**

☐ **Every day**

76. During the past 4 weeks how much have you been bothered by any of the following problems?

		No	A little	A lot
a.	**Stomach pain**	☐	☐	☐
b.	**Back pain**	☐	☐	☐
c.	**Arm, leg, joint (hip, knee etc) pain**	☐	☐	☐
d.	**Period pain / period problems**	☐	☐	☐
e.	**Headaches**	☐	☐	☐
f.	**Chest pain**	☐	☐	☐
g.	**Dizziness**	☐	☐	☐
h.	**Fainting spells**	☐	☐	☐
i.	**Heart pounding / racing**	☐	☐	☐
j.	**Shortness of breath**	☐	☐	☐
k.	**Pain / problems during intercourse**	☐	☐	☐
l.	**Constipation / diarrhoea**	☐	☐	☐
m.	**Nausea / gas / indigestion**	☐	☐	☐
n.	**Feeling tired or low in energy**	☐	☐	☐
o.	**Trouble sleeping**	☐	☐	☐

77. I feel tense or "wound up":

- ☐ **Most of the time**
- ☐ **A lot of the time**
- ☐ **From time to time, occasionally**
- ☐ **Not at all**

78. I still enjoy the things I used to enjoy:

- ☐ **Definitely as much**
- ☐ **Not quite so much**
- ☐ **Only a little**
- ☐ **Hardly at all**

79. I get a sort of frightened feeling as if something awful is about to happen:

- ☐ **Very definitely and quite badly**
- ☐ **Yes, but not too badly**
- ☐ **A little, but it doesn't worry me**
- ☐ **Not at all**

80. I can laugh and see the funny side of things:

- ☐ **As much as I always could**
- ☐ **Not quite so much now**
- ☐ **Definitely not so much now**
- ☐ **Not at all**

81. Worrying thoughts go through my mind:

- ☐ **A great deal of the time**
- ☐ **A lot of the time**
- ☐ **From time to time, but not too often**
- ☐ **Only occasionally**

82. I feel cheerful:

- ☐ **Not at all**
- ☐ **Not often**
- ☐ **Sometimes**

☐ **Most of the time**

83. I can sit at ease and feel relaxed:

☐ **Definitely**

☐ **Usually**

☐ **Not often**

☐ **Not at all**

84. I feel as if I am slowed down:

☐ **Nearly all the time**

☐ **Very often**

☐ **Sometimes**

☐ **Not at all**

85. I get a sort of frightened feeling like "butterflies" in the stomach:

☐ **Not at all**

☐ **Occasionally**

☐ **Quite often**

☐ **Very often**

86. I have lost interest in my appearance:

☐ **Definitely**

☐ **I don't take as much care as I should**

☐ **I may not take quite as much care**

☐ **I take just as much care as ever**

87. I feel restless as if I have to be on the move:

☐ **Very much indeed**

☐ **Quite a lot**

☐ **Not very much**

☐ **Not at all**

88. I look forward with enjoyment to things:

☐ **As much as I ever did**

☐ **Rather less than I used to**

☐ **Definitely less than I used to**

☐ **Hardly at all**

89. I get sudden feelings of panic:

☐ **Very often indeed**

☐ **Quite often**

☐ **Not very often**

☐ **Not at all**

90. I can enjoy a good book or radio or TV program:

☐ **Often**

☐ **Sometimes**

☐ **Not often**

☐ **Very seldom**