

Title: Can the systematic use of non-invasive biomarkers in patients with lower gastrointestinal symptoms, help rationalise the demand for limited Endoscopy resources?

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By

Dr Alaa Alakkari

MB., BCh., BAO., MRCPI.



Department of Clinical Medicine, TUH/TCD

Supervisor: Dr Barbara M Ryan

Declaration:

I declare that this thesis is entirely my work and it has not been submitted as an exercise for a degree at this or any other Institution.

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Thesis Summary, (methods and major findings):

Three independent studies were carried out as part of this thesis, which specifically focusses on lower GI symptoms and Colonoscopy procedures. The first study retrospectively reviewed Colonoscopy outcomes in terms of mucosal inflammation, adenoma detection and colorectal cancer in symptomatic and asymptomatic (surveillance) patients over 12 months. The results of symptomatic patients were correlated with the *ad hoc* clinical use of serum and faecal biomarkers to calculate the NPV, PPV, sensitivity and specificity of FCal and CRP in predicting inflammation.

The second study prospectively explored the use of serum and faecal biomarkers in combination with diagnostic symptom-based questionnaire in the assessment of patients with lower gastrointestinal symptoms referred for Colonoscopy. Univariate and logistic regression analyses were performed to identify the variables strongly associated with mucosal inflammation. Models of clinical assessment were extrapolated and examined using ROC curves to assess their capability to predict inflammation. FCal-CRP score was also created to predict mucosal inflammation, comparing those with score=0 (normal CRP and FCal), score=1(either raised CRP or FCal), and score=2 (both CRP and FCal were raised).

The third study prospectively researched the use of a multi target stool DNA test (Cologuard™) in clinical practice for the investigation of both symptomatic patients and asymptomatic surveillance patients. The NPV, PPV, sensitivity and specificity of the stool test for the detection of advanced adenoma and CRC were calculated.

We also performed a patient survey to explore patient compliance with stool testing and potential reasons for the observed low rates of compliance in clinical practice and in our studies.

Findings:

Both retrospective and prospective studies in symptomatic patients showed that mucosal inflammation was significantly more common in patients with diarrhoea, but most patients who underwent Colonoscopy had no evidence of

mucosal inflammation (79 - 86.6 % and 90.4 - 93%, in the non-diarrhoea and diarrhoea predominant groups respectively).

Univariate and logistic regression analyses in the prospective study showed that raised FCal (>50 ug/g) was strongly associated with inflammation (OR = 10.59, 95% CI: 3.65-30.75) with increasing risk for each additional unit increase in FCal. It also showed a statistically significant association between mucosal inflammation and having frequent loose or mushy stool, as part of the questionnaire (p value = 0.008) and frequent abdominal pain (p value = 0.029).

Preliminary analysis using ROC curve suggested that model 1 (includes age, gender, F Cal and the questions relating to frequent loose/ mushy stool and less frequent hard stool) was the best at predicting mucosal inflammation. FCal-CRP score was found to be very strongly associated with mucosal inflammation.

The incidence of adenomas was low in young patients (6.4 - 8.7% adenoma detection rate (ADR)) with no cases of CRC in both the retrospective and prospective studies. Older patients had a higher incidence of adenomas (24.7% ADR) and 5 cases of CRC in the retrospective study. The ADR was higher in asymptomatic surveillance patients (35.7%) in keeping with international standards. Faecal Cologuard™ performed poorly in our study with NPV and PPV of 77.5% and 40%, with a sensitivity and specificity of 30.8% and 83.8%, respectively.

A potential model of diagnostic algorithm extrapolated from our data, for young patients with diarrhoea predominant lower GI symptoms is as follows; exclude Coeliac disease and hyperthyroidism, check FCal-CRP score, and if all of these are negative, do not refer for Colonoscopy, and consider further management in primary care setting or refer to a dietician-led functional GI clinic if available. For older patients with diarrhoea predominant symptoms the same model can be applied, however further consideration has to be given to the risk of adenomatous polyps and CRC. Unfortunately, Faecal Cologuard performed poorly in our study, and does not appear to be a feasible test for use in Ireland with the logistical limitations of the current service provisions. There are alternative validated faecal biomarkers available such as FIT that can be added

to the above diagnostic pathway for older patients regardless of the predominant symptom. This can be used to prioritize and expedite Colonoscopy for those with positive results especially with the current national constraints on resources and capacity limitations.

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Abbreviations:

National Treatment Purchase Fund	NTPF
Inpatient Day-case and Planned Procedure	IDPP
Did Not Attend	DNA
Tallaght University Hospital	TUH
General Practice	GP
Oesophago-Gastro-Duodenoscopy	OGD
Coeliac Disease	CD
Peptic Ulcer Disease	PUD
Helicobacter Pylori	HP
Inflammatory Bowel Disease	IBD
Barrett's Epithelium	BE
British Society of Gastroenterology	BSG
Gastroesophageal Reflux Disease	GORD
Oesophageal Adenocarcinoma	OAC
American College of Gastroenterologists	ACG
Waist Hip Ratio	WHR
National Colon Cancer Screening Program	NCCSP
Faecal Immunochemical Test	FIT
Functional Gastrointestinal Disorders	FGIDs
Irritable Bowel Syndrome	IBS
Chronic Idiopathic Constipation	CIC
Functional Dyspepsia	FD
Confidence Intervals	CI
Odds Ratio	OR
Microscopic Colitis	MC

Transglutaminase Antibodies	TGA
Endomysial Antibodies	EMA
Ulcerative Colitis	UC
Crohn's Disease	CrD
Indeterminate Colitis	IC
Computerised Tomography	CT
Magnetic Resonance Imaging	MRI
Non-Steroidal Anti-inflammatories	NSAIDs
Proton Pump Inhibitors	PPIs
National Cancer Registry of Ireland	NCRI
Colorectal Cancer	CRC
Clinical Outcomes Research Initiative	CORI
World Health Organisation	WHO
Joint Advisory Group on GI Endoscopy	JAG
Association of Coloproctology of Great Britain and Ireland	ACPGBI
Quality Assurance	QA
Key Performance Indicator	KPI
C Reactive Protein	CRP
Interleukin	IL
Faecal Calprotectin	FCal
Enzyme Linked Immunosorbent Assay	ELISA
Diverticular Disease	DD
Faecal Occult Blood Test	FOBT
Guaiac Faecal Occult Blood Test	gFOBT
Multi-target Stool DNA	MT-sDNA
Polymerase Chain Reaction	PCR

Xenonucleic Acid	XNA
Sessile Serrated Polyp	SSP
Adenoma Detection Rate	ADR
Food and Drug Administration	FDA
Positive Predictive Value	PPV
Negative Predictive Value	NPV
Full Blood Count	FBC
Renal Function Test	RFT
Liver Function Test	LFT
Thyroid Function Test	TFT
Formalin Fixed Paraffin Embedded	FFPE
Receiver Operating Characteristics Curve	ROC Curve
Area Under the Curve	AUC

Chapter 1

Introduction and Aims

1.1. The challenges of national endoscopy capacity meeting population demand:

There is increasing demand and pressure on endoscopy services internationally and locally. Despite significant annual increase in the number of endoscopy procedures performed across the public hospitals nationwide, demand continues to outstrip capacity leading to expanding waiting lists, and in some cases delay in significant diagnoses. In 2016 the Department of Health commissioned the National Treatment Purchase Fund (NTPF) [1] to lead the development of a guidance protocol to ensure that there is a consistent and standardised approach to the management and scheduling of patients on Inpatient, Day case and Planned Procedure (IDPP) waiting lists within each hospital and across hospital groups. This is to ensure the safe, timely and effective treatment of patients in a fair and equitable manner. When the demand for a service exceeds the capacity, there is a so-called “capacity gap”.

One of the fundamentals of waiting list management is capacity and demand planning, which should be under continuous review. In Ireland, demand constantly outstrips capacity in most areas in the public health system, and as a result several initiatives have been developed across the hospital groups to deal with the waiting list problem. These include; the outsourcing of patients to undergo their procedures within the same hospital group or to private hospitals within the local area, and adoption of stricter policies in relation to patients who do not attend for their appointments (so-called ‘did not attend’ patients or DNAs). Capacity has also been maximised by extending the working day and weekend-working for routine day case procedures.

Despite all these measures, waiting lists continue to grow in most services due to the increasing demand and population growth, and this has been seen particularly with regard to the endoscopy service. Much of the demand is driven

by increased clinical need in symptomatic patients and by the growth in population. There is also increased public awareness of colon and other cancers due to better public education and increased media coverage. A downside to this increased coverage is that there is often a degree of fear regarding gastrointestinal symptoms.

1.2. Trends in endoscopy activity and waiting lists:

Within our institution, Tallaght University Hospital (TUH), the number of patients undergoing endoscopy procedures annually has increased by 60% over the past 10 years, with almost 10,000 procedures being done each year. According to the NTPF database the total number of gastroenterology patients awaiting a day case endoscopy nationally was 6252 at the end of July 2015 compared to 7241 at the end of the same month in 2017, with an increase of almost 1000 patients still waiting to undergo their endoscopy procedure at the end of the specified month, compared over a two-year period despite national and local initiatives to deal with the increasing demand. This reflects the increasing trend nationwide [1]. There have been a number of endoscopy waiting list initiatives in TUH over the past few years to deal with the increasing pressures on limited endoscopy resources. Despite this the total number of patients awaiting an endoscopy procedure in TUH did not change significantly over 2 years, with 1592 patients on the waiting list at the end of July 2015 compared to 1585 patients in July 2017, see Figure1.1.

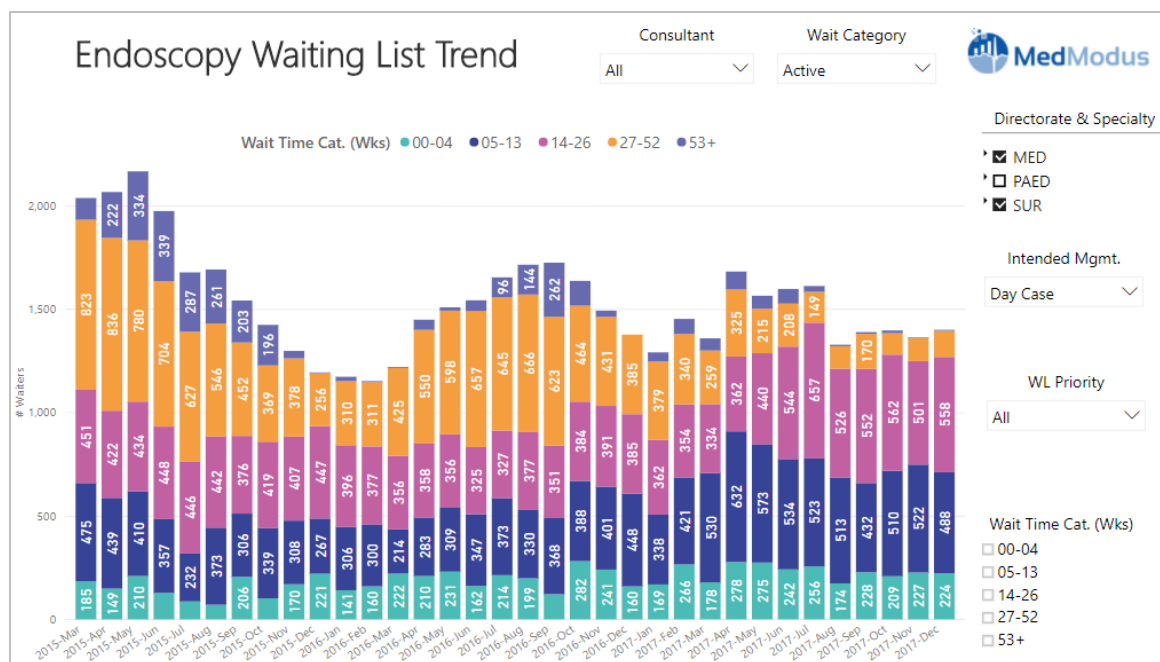


Figure 1.1. Active endoscopy waiting list trends in Tallaght hospital.

On the other hand, the average number of months on the waiting list has decreased significantly over the two years, with 728 patients waiting for longer than 6 months in July 2015 compared to 76 in July 2017 [1]. This significant reduction in the number of patients waiting for more than 6 months for their procedure over the last 2 years is a direct effect of the increased endoscopy capacity as a result of the local implementation of the NTPF protocol for waiting list management including the utilisation of outsourcing initiatives. Despite increasing capacity as a result of this outsourcing initiative, the total number of patients on the waiting list has not changed significantly over the 2 years reflecting the increasing demand on the service. TUH endoscopy unit needs to complete approximately 3,700 more patients or about 4,500 more procedures per year to meet waiting time standards for index and surveillance patients, which represents an increase of 55% on current procedure activity. The capacity gap in TUH is illustrated in Table 1.1.

Area	Gap (# patients per year)
Quantity needed to stop waiting list growth	1,000
+ Quantity needed to meet 13 week maximum	700
+ Quantity needed to maintain surveillance targets	1,000
+ Quantity of additional surveillance from doing 1,700 more index scopes per year	500
+ Quantity needed to improve inpatient wait times	500
Total Capacity Gap per year	3,700

Table 1.1. Endoscopy Capacity Gap in Tallaght University Hospital Endoscopy Unit.

In addition to the patients on the day case endoscopy waiting lists across hospital groups, there is also a large number of patients waiting to be seen in the gastroenterology clinics nationwide. There was a national total of 8719 patients on the gastroenterology outpatient clinic waiting list at the end of July 2015 compared to 10389 at the end of the same month in 2017 [1]. In TUH, although an average number of 200 patients are seen in the gastroenterology clinics on a weekly basis, a large number of patients remain on the outpatient waiting list at the end of every month. According to the NTPF database there were 1030 patients waiting to be seen in a gastroenterology clinic in AMNCH at the end of July 2017, with 345 patients waiting for more than 6 months [1].

1.3. The burden of gastrointestinal complaints in society:

The increasing demand on endoscopy resources is due in part to the burden of gastrointestinal complaints within a growing and aging population. Gastrointestinal complaints are very common in the general population, accounting for 1 in 12 general practice (GP) consultations in the UK, with 29% of these patients being referred for specialist assessment by a gastroenterologist or surgeon [2]. This leads to huge numbers of referrals to specialist clinics at secondary and tertiary care centres nationwide. Fortunately, most people with gastrointestinal symptoms have benign conditions, with functional disorders accounting for almost 50% of GP attendances with gastrointestinal symptoms [2]. Current medical practice in gastroenterology in our institution, but also nationally and internationally, relies too heavily on invasive procedures – endoscopies including Oesophago-Gastro-Duodenoscopy (OGD) and Colonoscopy- to help make a diagnosis, although in the majority of cases they yield normal results. There is a wide spectrum of organic conditions that present with gastrointestinal complaints with an age dependent distribution. In young patients, investigations are often carried out to check for potential inflammation in different parts of the gastrointestinal tract suggestive of treatable underlying conditions such as Coeliac Disease (CD), Peptic Ulcer disease (PUD), Helicobacter Pylori (HP) infection, Inflammatory Bowel Disease (IBD), and more rarely cancer. In the case of older patients > 50 years of age, while the same disease spectrum seen in young patients can be diagnosed, malignancies are more common, and can present in protean ways with varied symptoms. Therefore, endoscopy in patients over 50 years of age yields more significant pathology and can also be considered a screening opportunity for premalignant lesions such as polyps and Barrett's epithelium (BE).

1.4. Opportunistic screening for premalignant abnormalities:

Recommendations for screening are provided by the different international bodies. In the case of BE, the British Society of Gastroenterology (BSG) recommends endoscopic screening in patients with chronic gastroesophageal reflux disease (GORD) symptoms and multiple risk factors (at least three of age 50 years or older, white race, male sex, obesity) [3]. However, the threshold of multiple risk factors should be lowered in the presence of family history including at least one first-degree relative with Barrett's or oesophageal adenocarcinoma (OAC) (Recommendation grade C). The American College of Gastroenterologists (ACG) on the other hand recommends screening for BE in men with chronic (>5 years) and/or frequent (weekly or more) symptoms of gastroesophageal reflux (heartburn or acid regurgitation) and two or more risk factors for BE or OAC [4]. These risk factors include: age >50 years, Caucasian race, presence of central obesity (waist circumference >102 cm or waist-hip ratio (WHR) >0.9), current or past history of smoking, and a confirmed family history of BE or OAC (in a first-degree relative) (strong recommendation, moderate level of evidence). Given the substantially lower risk of BE and OAC in females with chronic gastroesophageal reflux symptoms (when compared with males), screening for BE in females is not recommended. However, screening could be considered in individual cases as determined by the presence of multiple risk factors for BE or OAC (age >50 years, Caucasian race, chronic and/or frequent GORD, central obesity: waist circumference >88 cm, WHR >0.8, current or past history of smoking, and a confirmed family history of BE or OAC (in a first-degree relative)) (strong recommendation, low level of evidence). Screening of the general population is not recommended (conditional recommendation, low level of evidence) [4].

With regards to screening for early colorectal cancer and/ or premalignant polyps in low risk population, the recommendations are quite variable. In Ireland, the National Colon Cancer Screening Program (NCCSP) recommends

population screening for patients aged 55 – 75 years using Faecal Immunochemical Test (FIT) for blood in stool samples, with subsequent Colonoscopy only offered to those with a positive stool test [5]. The US multi-society task force on colorectal cancer screening recommends screening in the general population should begin at age 50 years, with 10 yearly Colonoscopy or annual FIT as first consideration for screening [6]. There are no specific guidelines for opportunistic screening in patients presenting with common benign gastrointestinal symptoms, nonetheless there is an argument for a lower endoscopy referral threshold in older symptomatic patients, especially in view of the reported high prevalence of adenoma in western screening populations (age 50 – 70 years), which can be as high as 40%, with advancing age and male gender associated with higher prevalence [7].

1.5. Causes of gastrointestinal complaints in the general population:

1.5.1. Functional gastrointestinal disorders:

The most common causes of gastrointestinal symptoms are functional gastrointestinal disorders (FGIDs). These are characterised by persistent and recurring gastrointestinal symptoms caused by abnormalities in the function of the gastrointestinal tract, rather than any structural, histological or biochemical abnormality. Therefore, routine medical investigations in these patients tend to be normal. FGIDs can affect different parts of the gastrointestinal tract leading to a wide range of symptoms. FGIDs occur as a result of morphological and physiological abnormalities that often exist in combination including motility disturbances, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota and altered central nervous system processing.

Irritable bowel syndrome (IBS) is one of the most common FGIDs worldwide, with somewhere between 5 – 15% of the general population suffering from IBS [8][9]. The global pooled prevalence of IBS is approximately 11%, with all regions of the world affected by IBS at similar rates, see Figure 1.2 [8]. The diagnosis of IBS can be difficult to make due to the variation of symptoms over time and the overlap of symptoms with other conditions such as lactose intolerance. There is no specific biomarker or gold standard diagnostic test for IBS, therefore clinicians and researchers have relied on different criteria that have been developed over the years such as the Manning and Rome criteria, but none have proved perfect. The Rome IV criteria define IBS as a functional bowel disorder in which recurrent abdominal pain is associated with defecation or a change in bowel habits. Disordered bowel habits are typically present (i.e., constipation, diarrhoea or a mix of constipation and diarrhoea), as are symptoms of abdominal bloating/distension. Symptoms should have started at least 6 months prior to the diagnosis and should be present during the previous 3 months [10].

IBS patients are classified into different subtypes based on the predominant bowel habit present. There are 3 IBS subtypes; IBS with predominant constipation (IBS-C), IBS with predominant diarrhoea (IBS-D), IBS with mixed bowel habit (IBS-M). Patient who do not fit into any of the above categories are considered to have IBS unclassified [10]. Furthermore, IBS subtypes are not separate conditions. The quantity, intensity, and severity of symptoms varies from patient to patient. The IBS subtype can be re-categorized as a person's bowel habits change [11].

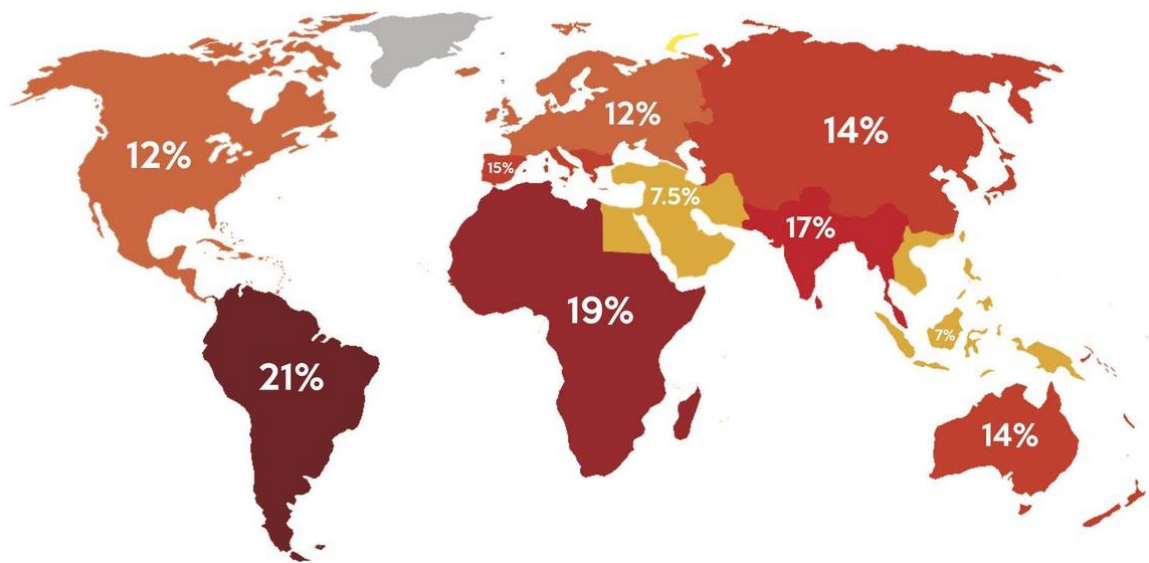


Figure 1.2. The prevalence of IBS in the regions of the world (based on pooled data from 81 studies reported by Lovell and Ford) [8].

Another equally common FGID is chronic idiopathic constipation (CIC), with a global pooled prevalence of 14% in the general population [12]. It is more common in women and its prevalence increases with age. In addition to infrequent bowel movements, the definition of constipation includes excessive straining, a sense of incomplete evacuation, failed or lengthy attempts to defecate, use of digital manoeuvres for evacuation of stool, abdominal bloating, and hard consistency of stools. A diagnosis of CIC is made after exclusion of causes of secondary constipation such as poor diet, drug treatment, neurological or metabolic conditions. There are three subtypes of CIC: dyssynergic defaecation, slow transit constipation and normal transit constipation, which is the most common subtype. Both CIC and IBS have a significant socioeconomic impact due to their high prevalence, frequency of symptoms, impact on quality of life, and work absenteeism.

Among functional disorders affecting the upper gastrointestinal tract, Functional Dyspepsia (FD) has very high prevalence throughout the world. In a study of employees in the US, it affected 29% of the population [13]. In a large

population-based European study, epigastric symptoms were experienced by 20.6% of subjects in the previous 12 months [14]. Dyspeptic symptoms are more common in women (24.4% versus 16.6% in men) [14][15]. The prevalence of FD was found to increase significantly with age (7.7% at age 15–17 years; 17.6% at 18–24 years, 18.3% at 25–34 years, 19.7% at 35–44 years, 22.8% at 45–54 years, 23.7% at 55–64 years, and 24.4% above 65 years; $q = 0.084$; $p < 0.0005$) [14]. FD has been defined by the Rome IV criteria as determined by bothersome clinical symptoms [11]. It is classified into; epigastric pain syndrome, post-prandial distress syndrome and overlap of epigastric pain and post-prandial distress syndrome. The latter is more frequent in the hospital-based population than in the general population [16].

There is large overlap between FD and IBS, making it difficult sometimes to differentiate between the two, especially since abdominal pain is common in both. Ford et al have also reported that the incidence of IBS is 8 times higher in subjects with FD and suggests that IBS and FD share the process of the progression of their diseases [17]. Constipation delays gastric emptying and is commonly accompanied by upper gastrointestinal symptoms; on the other hand, bowel symptoms are common in FD. In a longitudinal study with over 12 years follow up, 40% of subjects with FD or IBS had switched symptomatology [18]. In a recent meta-analysis, the prevalence of IBS in subjects with dyspepsia was 37% (95% confidence interval (CI), 30–45%) compared with 7% (95% CI, 5–10%) in subjects without dyspepsia. The pooled odds ratio (OR) for IBS in subjects with dyspepsia was 8 (95% CI, 5.74–11.16). The degree of overlap between the two conditions varied from 15 to 42%, depending on diagnostic criteria used for each [19].

Diagnosing FGIDs can be challenging due to the overlap in presentation between functional and organic gastrointestinal diseases such as CD and IBD. This led to the development of symptom-based diagnostic criteria such as the ROME and Manning criteria to help in the diagnosis of FGIDs [20]. Current guidelines encourage the use of symptom-based diagnostic criteria to diagnose

FGIDs i.e. a positive diagnosis rather than a diagnosis of exclusion in an attempt to avoid extensive investigations. However, in validation studies these criteria tend to perform modestly in distinguishing IBS from organic disease and as a result clinicians tend to rely on the use of medical investigations including endoscopy to exclude organic causes [21]. Ford et al found that despite the high prevalence of IBS in the population, the positive predictive value of all the criteria investigated in the study (Manning, ROME I, II, and III) was < 50%, suggesting that more accurate ways of detecting IBS are required if the condition is to be diagnosed with any certainty, and the potential for unnecessary investigations avoided [19].

Scientific expansion and evolution in the understanding of FGIDs has led to further review and update of the Rome criteria. The Rome IV criteria were published in 2016 and translated into questions that can be understood and reported by patients and research subjects (The Rome IV Diagnostic Questionnaire for FGIDs in Adults). The Rome VI Diagnostic Questionnaire is a self-report integrated questionnaire for the diagnosis of all functional gastrointestinal disorders in adults, including alarm symptoms or red flags to alert clinicians to consider testing for alternative medical disorders. It includes six modules that can be used to screen for a number of diagnoses including; IBS, gastroduodenal disorders, oesophageal disorders, gallbladder and sphincter of Oddi disorders and anorectal disorders. It also includes flags for mental health involvement. Palsson et al tested the diagnostic sensitivity of the Rome VI Questionnaire in 843 patients from 9 gastroenterology clinics, with a focus on clinical diagnoses of irritable bowel syndrome (IBS), functional constipation (FC), and functional dyspepsia (FD) [22]. Sensitivity was 62.7% for IBS, 54.7% for FD, and 32.2% for FC. Specificity, assessed in a population sample of 5,931 adults, was 97.1% for IBS, 93.3% for FD, and 93.6% for FC. Excess overlap among IBS, FC, and FD was a major contributor to reduced diagnostic sensitivity, and when overlap of IBS with FC was permitted, sensitivity for FC diagnosis increased to 73.2% [22].

1.5.2. Organic causes of gastrointestinal symptoms:

Lower gastrointestinal symptoms can be caused by a range of organic diseases affecting the gastrointestinal tract. However, these account for a small percentage of the referrals to the gastroenterology clinics nationwide. These organic diseases include CD, IBD, Microscopic Colitis (MC), malignancy, other causes of colitis such as infectious, ischaemic and medication related (non-steroidal anti-inflammatory drugs, antibiotics, etc.), and gastrointestinal manifestations of systemic disease.

1.5.2.i. Coeliac Disease (CD):

CD is a disorder of the small bowel characterized by mucosal inflammation, villous atrophy, and crypt hyperplasia, which occurs upon exposure of genetically susceptible individuals to dietary gluten and which demonstrates improvement after withdrawal of gluten from the diet, Figure 1.3 [23].

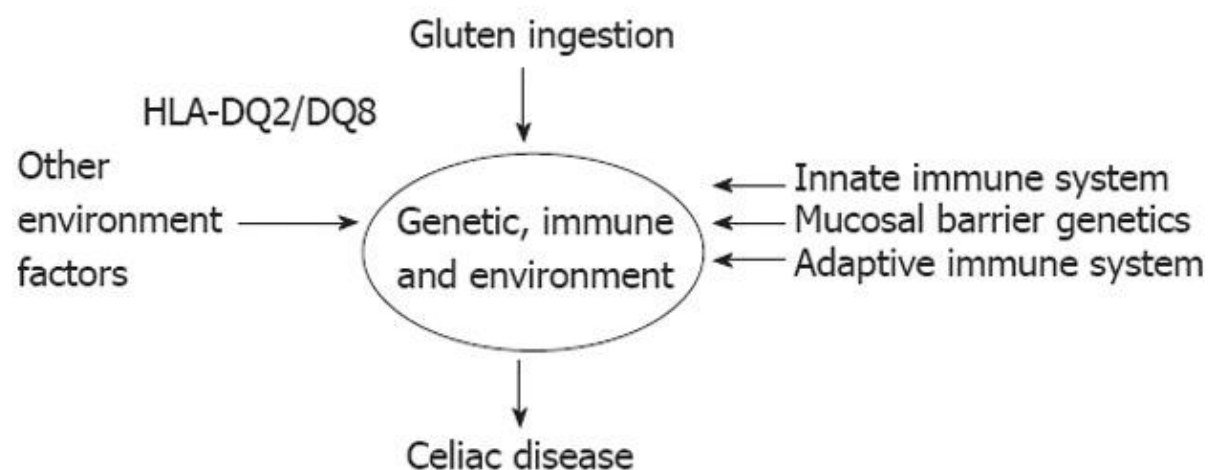


Figure 1.3. Factors necessary for Coeliac disease development (adapted). HLA: Human leukocyte antigen [23].

The condition has a number of different phenotypes. These include; active symptomatic, silent asymptomatic, and latent CD. Clinical manifestations can be both intestinal and extra-intestinal and include; diarrhoea, bloating, abdominal pain, weight loss, iron deficiency anaemia, dermatitis herpetiformis, dental enamel hypoplasia, metabolic bone disease and neurological problems [24]. During the 1980s, before advances in testing for CD were made, the condition was mistakenly thought to be rare. However, CD is now known to be a common condition, and the incidence in Ireland is one of the highest in the world, with the disease affecting approximately 1 in every 100 Irish people [25]. There is an increased prevalence in women compared to men (ratio of 2.8:1) [26], with men often diagnosed at an older age [27].

CD was originally thought to be almost exclusively a disease of white Europeans, however, it is now known to be widely distributed worldwide [28], Figure 1.4 [23].

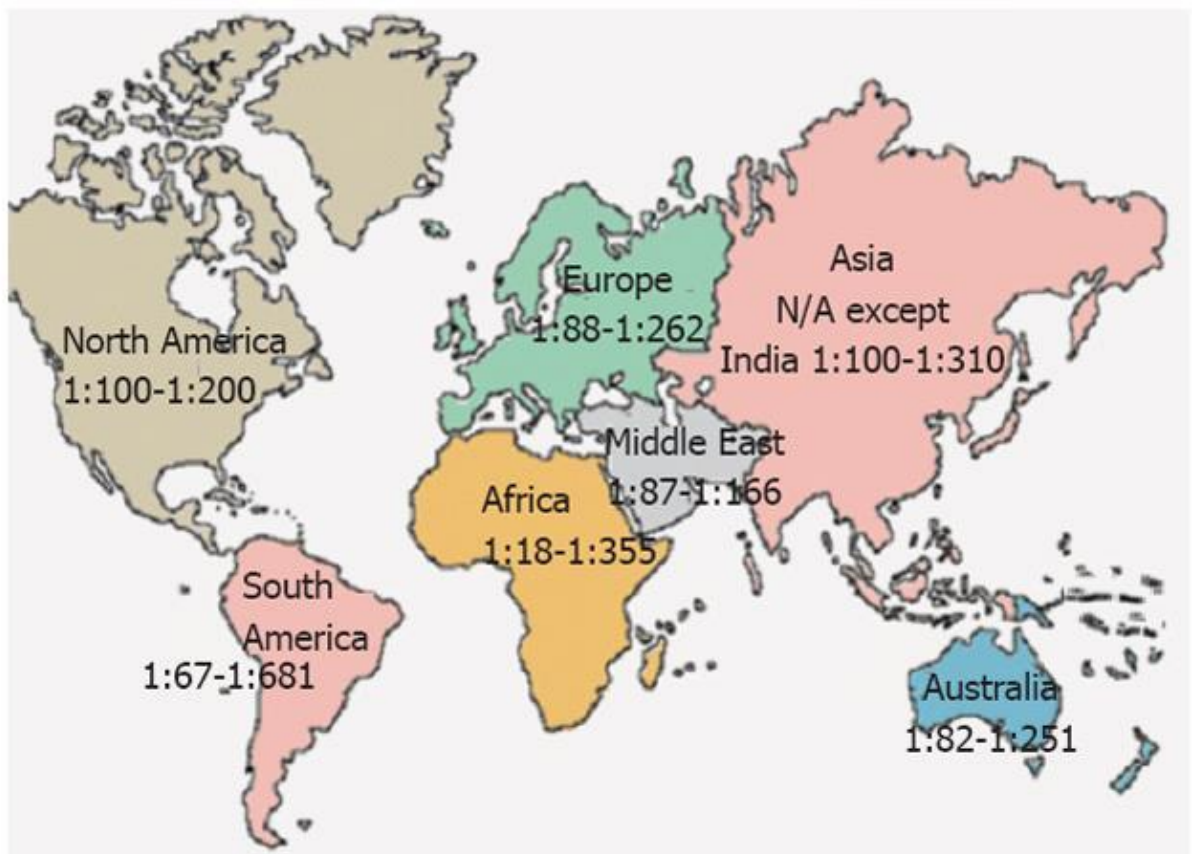


Figure 1.4. Prevalence of Coeliac disease worldwide. N/A: Not available [23].

Epidemiological studies conducted in areas supposedly free of CD, including Africa, the Middle East, Asia, and South America, show that the disease was previously underdiagnosed [29]. This provides evidence that CD is one of the most common genetic diseases, resulting from both environmental (gluten) and genetic (HLA and non-HLA genes) factors [23].

In some cases, CD does not cause any noticeable symptoms, or it causes very mild symptoms. As a result, it is thought that at least 50% or possibly as many as 90% of cases are either undiagnosed or misdiagnosed as other digestive conditions [30].

Diagnosis of CD is based on a combination of positive Coeliac specific serology and typical endoscopic and histologic findings on duodenal biopsy while on a gluten containing diet, Figure 1.5.



Figure 1.5. Endoscopic appearance of duodenum in Coeliac disease; scalloping and villous atrophy.

Assays for both transglutaminase antibody (TGA) and endomysial antibody (EMA) have high specificities and sensitivities both greater than 95% [31], and are good serological screening tools for diagnosis of CD, in the absence of Immunoglobulin A (IgA) deficiency [25]. Up to 2% of patients with CD have IgA

deficiencies, hence IgA level should always be checked concomitantly with CD serology, when relying on serology to screen for the disease [32].

1.5.2.ii. Inflammatory Bowel Disease (IBD):

IBD is an idiopathic chronic inflammatory condition, divided into two subtypes; Ulcerative Colitis (UC) and Crohn's Disease (CrD). UC is characterised by relapsing and remitting episodes of inflammation limited to the mucosal layers of the large bowel, Figure 1.6.

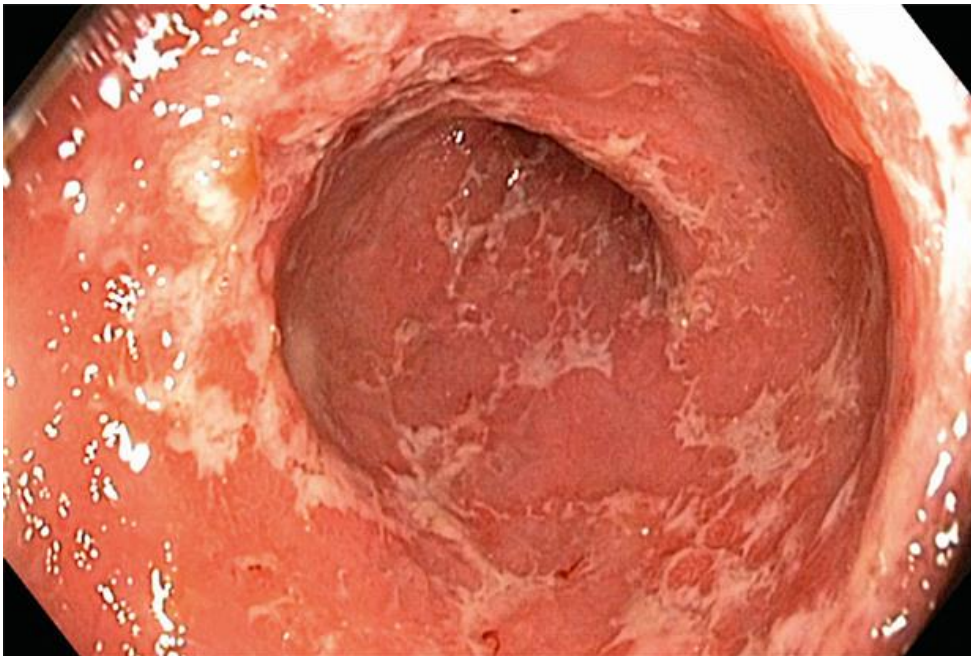


Figure 1.6. Colonic inflammation in Ulcerative Colitis.

It almost invariably starts in the rectum and extends proximally in a continuous fashion [33]. CrD on the other hand is characterised by transmural inflammation that can affect anywhere from the mouth to the perianal area, with skip lesions or discontinuous inflammation, Figure 1.7.



Figure 1.7. Terminal ileum ulceration in Crohn's Disease.

This transmural inflammation may lead to sinus tract formation giving rise to micro-perforations and fistulae, in addition to inflammatory masses, fibrosis and strictures.

There is no national database giving accurate figures about the incidence and prevalence of IBD in Ireland, however it is thought that at least 20,000 people are living with IBD in Ireland [34]. In 2011, there were 5.9 new cases of CrD and 14.9 new cases of UC in Ireland per 100,000 population, with both males and females equally affected. The peak age of diagnosis was 15-35 years with a second peak at 50-70 years [34].

In Europe there is significant geographic variation in the incidence and prevalence of IBD. The disease is more common in northern Europe and in industrialised countries. The highest incidence and prevalence rates of IBD are in Scandinavia [35]–[46] and the United Kingdom [47]–[50], however increasing incidence rates are being reported across Europe [51][52]. In North America, incidence rates of IBD range from 2.2 to 19.2 cases per 100,000 person-years for UC and 3.1 to 20.2 cases per 100,000 person-years for CrD [53][54], see Figure 1.8 [55].

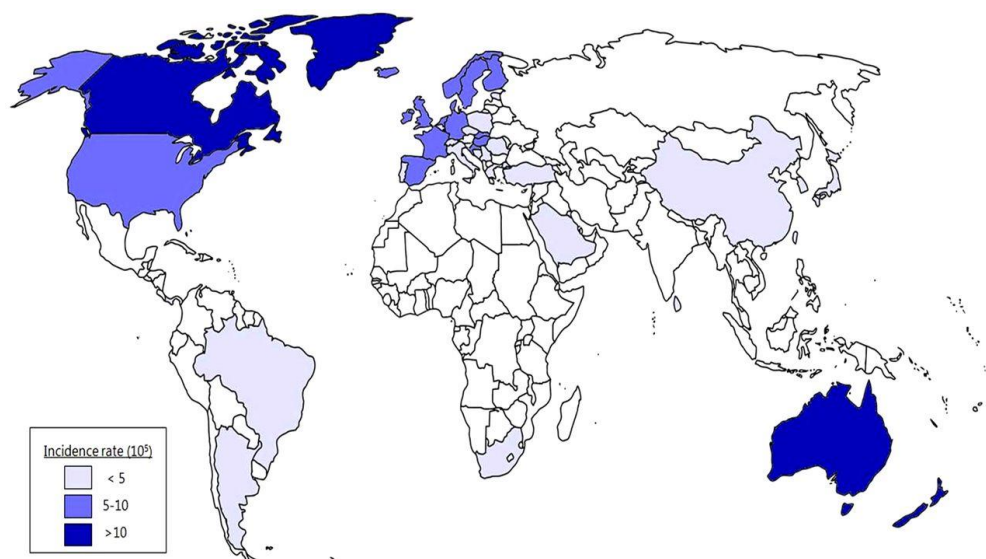


Figure 1.8. Incidence rates of IBD world-wide [55].

There are a number of both environmental and genetic risk factors for developing IBD. Ethnic and racial differences in disease prevalence may be related to environmental and lifestyle factors as well as due to underlying genetic differences [56]. Approximately 10 to 25% of individuals with IBD have a first degree relative with the condition [57]. Concordance for the same disease type within families has been demonstrated in some studies [58], but in clinical practice disease discordance within families is certainly seen (i.e. relatives with both UC and CrD). Smoking is a significant environmental and lifestyle factor and has been shown to have different effects on UC and CrD. Smoking is associated with an increased risk of CrD (hazard ration=1.4 to 1.9) [59], whilst in contrast it may have a protective effect in UC. Smoking cessation in patients with UC is associated with an increase in disease activity, hospitalisation and with first presentation of the disease [60].

Clinical manifestations of IBD are variable and depend on the disease phenotype. UC patients usually present with diarrhoea which is commonly associated with rectal bleeding. Associated symptoms include: colicky

abdominal pain, tenesmus, faecal urgency and incontinence. However, patients with distal disease may present with paradoxical constipation associated with blood and mucus per rectum. The clinical presentations of CrD are more variable than UC, and can be present for years before the diagnosis is made [61]. The manifestations depend on both CrD phenotype and distribution and include; abdominal pain, diarrhoea, rectal bleeding, perianal discharge, abscess/phlegmon, fistulae, malabsorption, oral ulceration, odynophagia, and dysphagia. Patients may also present with extra-intestinal manifestations of IBD including; vitamin B12 deficiency, osteoporosis, arthropathy / arthritis, eye involvement (e.g. uveitis, iritis) skin disorders (e.g. erythema nodosum, pyoderma gangrenosum), and primary sclerosing cholangitis, a chronic liver condition.

The diagnosis of IBD is based on endoscopic and histologic findings suggestive of the disease. In the case of CrD imaging modalities are also used in the diagnosis and assessment of disease extent. These include Computerised Tomography (CT) scans and Magnetic Resonance Imaging (MRI) of the small bowel and video capsule endoscopy. The assessment of patients with suspected IBD includes laboratory studies to out-rule gastrointestinal infection and assess the patient's inflammatory and nutritional status.

In about 10% of IBD patients it is unclear which classification of IBD is present at first diagnosis based on standard clinical testing, including colonoscopy, imaging, laboratory tests, and biopsy. This group of IBD patients are labelled as having Indeterminate Colitis (IC) or IBD unclassified (IBD-U). Some of these patients later declare themselves as having UC or CrD, but some continue to have durable IC. The measurement of perinuclear antineutrophil cytoplasmic antibodies (P-ANCA) and anti-*Saccharomyces cerevisiae* (ASCA) antibodies has been suggested as a method for differentiating UC from CrD [62]. The presence of ASCA has a specificity of approximately 90% for CrD and a positive predictive value of 88%, but this test seems to be most positive in patients in whom there is no real diagnostic dilemma—for example, those with small bowel

disease. In UC the presence of P-ANCA has a specificity approaching 90%, but may be a better marker of large bowel disease than any specific subtype [63]. The sensitivity of these tests is only 40–60%, limiting their usefulness in IC [64]. The clinical usefulness of these markers is controversial.

1.5.2.iii. Microscopic Colitis (MC):

Microscopic Colitis (MC) is a chronic, inflammatory disease of the colon that is characterized by chronic, watery diarrhoea. It typically occurs in middle-aged patients and tends to be more common in females. The endoscopic appearance of the colon in patients with MC is normal, but the diagnosis is established by typical histologic appearance of the colonic mucosa under the microscope. MC is comprised of two subtypes [65][66]:

- a. Collagenous Colitis; which is characterized by colonic sub-epithelial collagen band >10 micrometres in thickness.
- b. Lymphocytic Colitis; which is characterized by an intraepithelial lymphocytic infiltrate (>20 per high power field).

The estimated incidence of collagenous colitis and lymphocytic colitis are 1.1 to 5.2 and 3.1 to 5.5 per 100,000 per year, respectively [67]–[70]. While the mean age at diagnosis of MC is approximately 65 years, a quarter of the patients are actually diagnosed before the age of 45 years [71].

The pathogenesis of MC is unclear however it is likely multifactorial, involving mucosal immune responses to luminal factors in a genetically predisposed individual. MC has been associated with CD, autoimmune thyroiditis, type 1 diabetes mellitus and nonerosive arthritis [72]. It is not yet clear if these associations reflect an autoimmune pathogenesis of MC. Certain drugs have

also been implicated including Non-Steroidal Anti-inflammatories (NSAIDs), and Proton Pump Inhibitors (PPIs), amongst others.

MC is characterised by chronic watery diarrhoea which is often of insidious onset with nocturnal episodes, but maybe sudden in 40% of cases [67]. Abdominal pain occurs in 50% of cases, with associated faecal urgency and incontinence [73]. The diagnosis of MC is established by histology, therefore colonic biopsies at colonoscopy are essential to make the diagnosis, especially on the right side because the histologic severity declines distally in the colon. The endoscopic appearance tends to be grossly normal, however histology reveals characteristic inflammatory changes diagnostic of either lymphocytic or collagenous colitis.

1.5.2.iv. Infectious Colitis:

Infectious colitis secondary to common pathogens such as Norovirus, Campylobacter, Salmonella and Shigella tends to present with acute diarrhoea in immunocompetent patients, and do not generally cause chronic symptoms. Infectious colitis causing chronic symptoms is most likely caused by parasites, including protozoa like Giardria, Cryptosporidium, and Cyclospora. Bacteria are unlikely to cause chronic diarrhoea in immunocompetent individuals with the possible exception of Yersinia, Plesiomonas and Aeromonas [74].

1.5.2.v. Ischaemic Colitis:

Intestinal ischemia is caused by a reduction in blood flow to a level that is insufficient for the delivery of oxygen and nutrients required for cellular metabolism. The presenting symptoms can be acute or chronic depending on the cause of ischaemia such as acute arterial occlusion (embolic, thrombotic),

venous thrombosis, or hypo perfusion of the mesenteric vasculature causing non-occlusive ischemia. The incidence is estimated at 16 cases per 100,000 person-years, which has increased over time due to the aging population [75]. Colonic ischaemia is the most frequent form of intestinal ischaemia, most often affecting older adults [76]. Chronic symptoms are caused by chronic ischaemic colitis which should be suspected in high risk patients with lower abdominal pain and bloody diarrhoea or haematochezia; however, these symptoms are nonspecific. Risk factors for developing intestinal ischaemia include recent myocardial infarct, cardiopulmonary bypass, aorto-iliac instrumentation/surgery, haemodialysis, acquired or hereditary thrombophilia, extreme exercise, Mesenteric arteriovenous fistula or malformation, and drugs such as oestrogen, non-steroidal anti-inflammatories, antipsychotics and cocaine [77]–[87]. Patients with episodes of chronic recurrent colonic ischemia can present with recurrent abdominal pain, bloody diarrhoea, and weight loss from protein-losing enteropathy, recurrent bacteraemia, persistent sepsis, or symptomatic colonic strictures [88].

1.5.2.vi. Colorectal Cancer (CRC):

CRC is the most common malignancy of the gastrointestinal tract. According to the NCRI report on the incidence, mortality, treatment and survival of CRC in Ireland 1994-2010; 11% (in women) and 14% (in men) of all invasive cancers (excluding non-melanoma skin cancer) were CRCs in 2007-2009, which makes this the second most common tumour diagnosed in women (after breast cancer), and in men (after prostate cancer) [89]. CRC was the third leading cause of cancer death in women, after lung cancer and breast cancer, and the second leading cause of cancer death in men after lung cancer in 2007-2009. It accounted for 10% and 12% of cancer deaths in males and females respectively in 2007 [89]. Approximately 950 women and 1,330 men were diagnosed with colorectal cancer annually during 2007-2009. The incidence rate of colorectal cancer in Ireland was similar to the European average in 2008 [89]. The numbers of colorectal cancer cases are projected to increase by 34% in women and

45% in men between 2010 and 2020 [90]. 67% of women and 69% of men diagnosed with colorectal cancer were aged greater than 65 years, see Figure 1.9 [89].

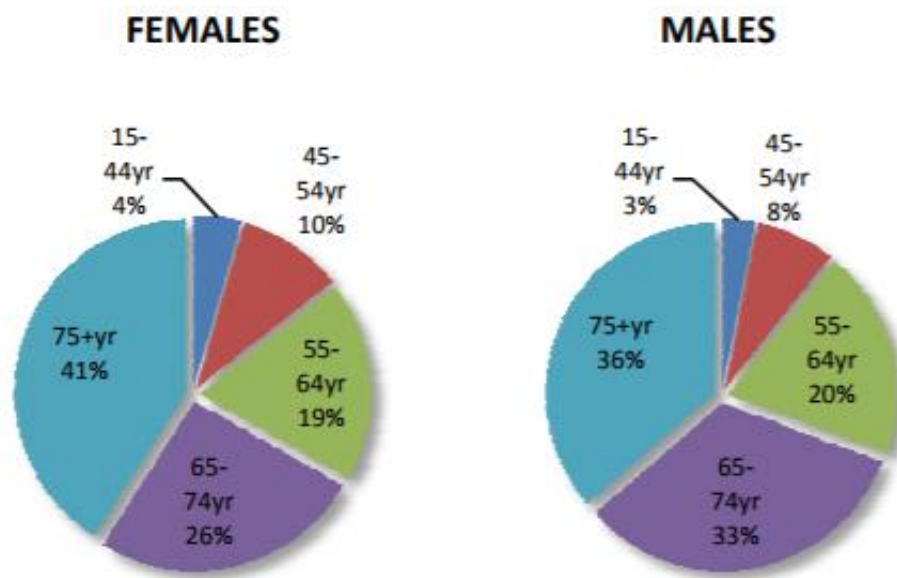


Figure 1.9. Age distribution of incident CRC 2006-2010 [89].

CRC is often asymptomatic until the late stages of the disease when it can present with symptoms secondary to the obstructive effects of the lesion, such as altered bowel habit, alternating constipation and diarrhoea, or metastatic disease. It can however manifest relatively early with iron deficiency anaemia.

The polyp-cancer sequence is well recognised in CRC, with cancer usually evolving within premalignant lesions/ polyps that, if detected early, can be resected to prevent progression to malignancy [91]. Since CRC and premalignant polyps are more common in patients over 50 years of age, there is an argument for having a low threshold to perform diagnostic Colonoscopy in older patients for the opportunistic detection of polyps.

The prevalence of adenomatous polyps increases substantially with age. A study by Corley et al found that at least one adenomatous polyp was found in

24.6% of all patients, and a colorectal cancer (including intramucosal cancer) was found in 1.5% [92]. Almost all patients with a cancer were also diagnosed with an adenoma. The estimated risk of an adenoma doubled from age 50-54 to age 70-74 (OR 2.00, 95% CI 1.76-2.26); the increase with age was similar for both men and women. Adenoma prevalence rose across all 5-year age categories, reaching a peak at 70-74 years of age ($P<0.001$). Among women, the crude prevalence increased from 15% in patients 50-54 years of age to a high of 26% by ≥ 75 years of age ($P<0.001$). Among men, the crude prevalence increased from 25% at 50-54 years of age to a high of 39% by 70-74 years of age ($P<0.001$) [92].

With the introduction of colonoscopy screening programs, the incidence of CRC and mortality in individuals over age 50 have both decreased. Alarming, a recent analysis from the National Cancer Institute's Surveillance, Epidemiology, and End Results Program reported that the incidence of CRC is increasing among adults younger than 50 years of age in the United States [55]. Colon cancer incidence rates increased by 1.0% to 2.4% annually since the mid-1980s in adults ages 20 to 39 years and by 0.5% to 1.3% since the mid-1990s in adults ages 40 to 54 years. The rise in cancer incidence is further pronounced in rectal cancers. Compared with adults born circa 1950, those born circa 1990 have double the risk of colon cancer and quadruple the risk of rectal cancer [93].

1.5.2.vii. Oesophageal Cancer:

According to the 2007 National Cancer Registry of Ireland (NCRI) report; cancer of the oesophagus was the thirteenth most common cancer in Ireland, accounting for 1.8% of all malignant neoplasms (excluding non-melanoma skin cancer) in women and 2.7% in men (Table 1.2) [94].

	Ireland female	Ireland male	ROI female	ROI male	NI female	NI male
% of all new cancer cases	1.3%	2.0%	1.3%	1.9%	1.3%	2.2%
% of all new cancer cases excluding non-melanoma skin cancer	1.8%	2.7%	1.8%	2.7%	1.7%	2.9%
Average number of new cases per year	182	301	122	202	60	98
Cumulative risk age 74	0.4%	1.0%	0.4%	0.9%	0.4%	1.0%
15-year prevalence (1994- 2008)	426	701	289	454	137	247

Table 1.2. Summary information for oesophageal cancer in Ireland, 1995-2007.

The average number of new cases diagnosed each year was 182 in women and 301 in men. During 1995-2007, the number of new cases diagnosed increased by approximately 2% per annum. The risk of developing oesophageal cancer up to the age of 74 was 1 in 258 for women and 1 in 105 for men and was similar in Northern Ireland and Republic of Ireland [94]. The age distribution at diagnosis was different for men and women, see Figure 1.10 [94].

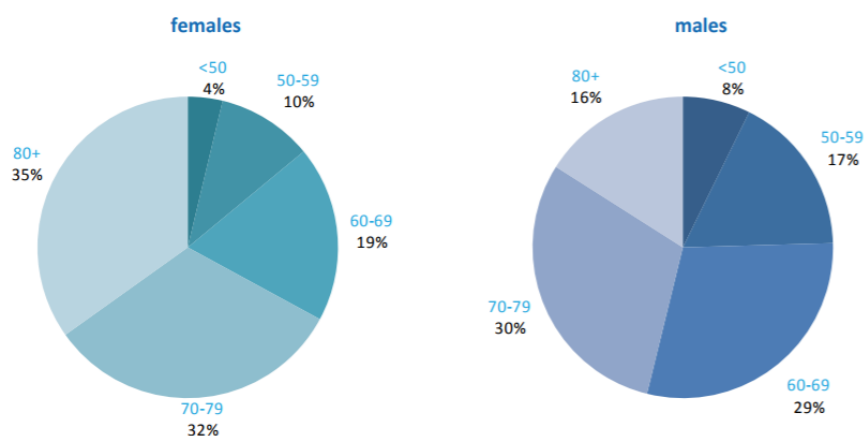


Figure 1.10. Age distribution of oesophageal cancer cases in Ireland, 1995-2007, by sex[94].

More than half of men, but only one-third of women, presented at under 70 years of age, while a further third of women, but only 16% of men, were aged 80 years or older at diagnosis. The two main types of oesophageal cancer are squamous cell carcinoma and adenocarcinoma. Symptoms of oesophageal cancer tend to occur late in the disease course, and include; dysphagia, odynophagia, upper gastrointestinal haemorrhage and iron deficiency anaemia. Therefore, regular endoscopic surveillance of premalignant lesions such as Barrett's epithelium is recommended for the early detection of malignant transformation in the oesophagus.

1.5.2.viii. Gastric Cancer:

According to the NCRI 2007 report; gastric cancer was the sixth most common cancer in Ireland, accounting for 2.7% of all malignant neoplasms (excluding non-melanoma skin cancer) in women and 4.0% in men, see Table 1.3 [95].

	Ireland female	Ireland male	ROI female	ROI male	NI female	NI male
% of all new cancer cases	2.0%	2.9%	1.9%	2.7%	2.1%	3.3%
% of all new cancer cases excluding non-melanoma skin cancer	2.7%	4.0%	2.6%	3.9%	2.8%	4.4%
Average number of new cases per year	278	442	181	294	97	148
Cumulative risk age 74	0.6%	1.4%	0.6%	1.3%	0.6%	1.4%
15-year prevalence (1994- 2008)	719	1096	476	746	243	350

Table 1.3. Summary information for stomach cancer in Ireland, 1995-2007 [95].

The average number of new cases diagnosed each year was 278 in women and 442 in men. During 1995-2007, the number of new cases diagnosed per annum remained fairly constant. More than 50% of all cases of stomach cancer were diagnosed at over 70 years of age—65% of women and 53% of men, see Figure 1.11 [95].

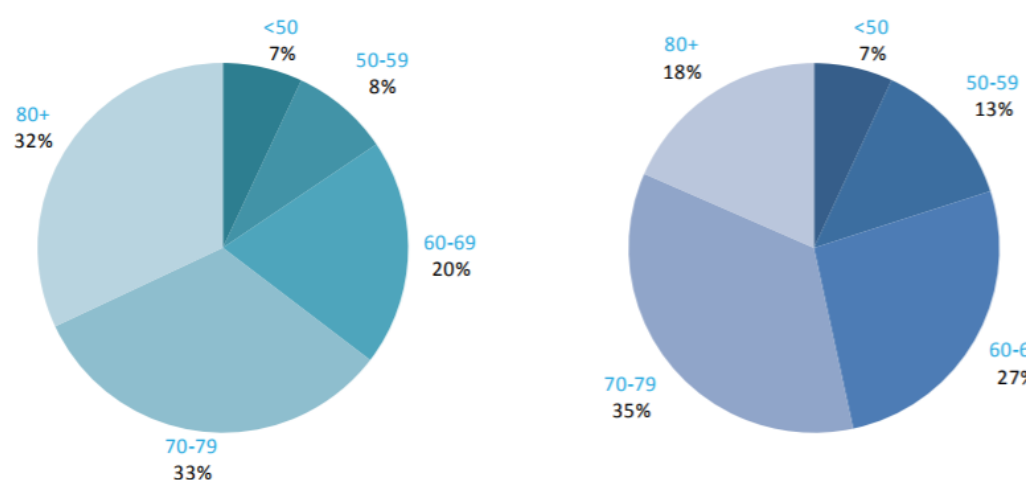


Figure 1.11. Age distribution of stomach cancer cases in Ireland, 1995-2007, by sex[95].

Only 7% of cases were aged under 50 years at diagnosis. Significant risk factors for stomach cancer include: helicobacter pylori (HP) infection, and smoking. HP colonises the stomach causing inflammation and ulcers, and is associated with a six-fold increased risk of stomach cancer [95]. Meta-analysis of intervention studies shows that stomach cancer risk is decreased by one-third in H pylori-positive patients randomised to eradication treatment [96]. Smoking is firmly established as a cause of stomach cancer. Compared to non-smokers, the risk in smokers is increased by 50% in those who have ever smoked and

70% in current smokers [97]. Gastric cancer is often asymptomatic in its early stages and may present late with a bleeding malignant gastric ulcer, iron deficiency anaemia, abdominal pain or weight loss.

1.5.2.ix. Gastrointestinal manifestations of Systemic Disease:

Upper and lower gastrointestinal symptoms can be part of the clinical manifestations of systemic disease. The pathophysiology of the gastrointestinal manifestations is often quite complex and depends on the disease type. For example, in hyperthyroidism 25% of patients have mild-to-moderate diarrhoea with frequent bowel movements [98][99]. This is often caused by a combination of fat malabsorption, intestinal hyper-motility, and a hyper-secretory state within the intestinal mucosa [100][101]. In hypothyroidism patients often have vague lower gastrointestinal symptoms that may be contributed to FGIDs [102]. The effect of hypothyroidism on the gastrointestinal tract seems to be multifactorial with possible alterations in hormone receptors, neuromuscular disorders and myopathy caused by infiltration of the intestinal wall. Reduction of peristalsis in hypothyroidism is the main pathophysiologic process [103], and constipation remains the most frequent gastrointestinal complaint [98].

Gastrointestinal complaints are common in the setting of poorly controlled Diabetes Mellitus. Poor glycaemic control with subsequent autonomic neuropathy in addition to structural smooth muscle remodelling can lead to impaired GI function [104]. This is associated with increased prevalence of oesophageal reflux, oesophageal dysmotility and delayed gastric emptying. Diabetes can also be associated with small bowel and colorectal dysfunction presenting with diarrhoea, constipation and/ or faecal incontinence.

Conditions that interfere with GI motility such as CREST syndrome and Scleroderma can manifest with an array of GI complaints including dysphagia, bloat-

ing, nausea, constipation, abdominal pain and diarrhoea due to small intestinal bacterial overgrowth. Conditions that affect the digestion and absorption of nutrients due to impaired pancreatic exocrine function can present with symptoms of bloating, diarrhoea, abdominal pain, excess flatulence and weight loss. These include chronic pancreatitis, cystic fibrosis, and pancreatic cancer.

1.6. Potential risks of Endoscopy:

Endoscopy procedures are relatively invasive and while they are generally very safe, they can be associated with some adverse effects. These are usually mild and transient, but they can be severe and life-threatening. The most serious complications tend to occur in high risk situations when a therapeutic procedure is being performed such as polypectomy and dilatation, or in high risk patients with medical comorbidities. Complications of endoscopy can be related to the sedation used or the actual endoscopic procedure.

1.6.1. Complications of Sedation:

Cardiopulmonary complications secondary to sedation have been variably defined to include events of unclear clinical significance, such as minor fluctuations in oxygen saturation or heart rate, to significant complications including respiratory arrest, cardiac arrhythmias, myocardial infarction, and shock [105]. In a study that used the Clinical Outcomes Research Initiative (CORI) database, cardiopulmonary complications occurred in 0.9% of procedures and made up 67% of the unplanned events during or after endoscopic procedures with sedation [106]. Transient hypoxemia occurred in 230 per 100,000 colonoscopies, but prolonged hypoxemia was reported in only 0.78 per 100,000 colonoscopies. Hypotension occurred in 480 per 100,000 colonoscopies. The risk of cardiopulmonary events associated with colonoscopy is increased with ad-

vanced age, higher American Society of Anaesthesiologists Physical Status Classification System scores, and the presence of comorbidities [107][108].

1.6.2. Complications of diagnostic OGD

Adverse event rates for OGD have been reported by large series ranging from 1 in 2000 to 1 in 10,000, with mortality rates from none to 1 in 2000 [109]–[114]. This variation in adverse event rates may be due to the method of data collection, patient cohort, follow up duration and definition of adverse events. Some studies report minor incidents like self-limited bleeding as an adverse event, while others only report significant adverse events that prevent procedure completion, or result in hospitalisation [105]. Infection is another potential adverse event of diagnostic OGD. It can result from the procedure itself or failure to follow guidelines for the reprocessing and use of endoscopic devices and accessories [115][116]. Transient bacteraemia has been reported at rates as high as 8%, but the frequency of infectious endocarditis and other clinical sequelae is extremely low [117][118].

A significant complication of OGD that may lead to high risk of morbidity and mortality is perforation. Prospective, multi-centre registries report perforation rates of 1 in 2500 to 1 in 11,000 [119]. Factors predisposing to perforation include the presence of anterior cervical osteophytes, Zenker's diverticulum, oesophageal stricture, malignancies of the upper gastrointestinal tract, and duodenal diverticula [119][120]. Perforation of the oesophagus is associated with a mortality rate between 2% and 36% [121]–[124]. Clinically significant bleeding is a rare complication of diagnostic OGD [125]. Mallory-Weiss tears occur in less than 0.5% of diagnostic OGDs and are not usually associated with significant bleeding [126].

1.6.3. Complications of diagnostic Colonoscopy:

Colonic perforation during colonoscopy may result from mechanical forces against the bowel wall, barotrauma, or as a direct result of therapeutic procedures. The rate of perforation reported in large studies is 0.3% or less and is generally less than 0.1% [127]. In a large study of screening colonoscopy, perforation was reported in 13 of 84,412 procedures (0.01%) [112]. Haemorrhage is most often associated with polypectomy, although it can occur during diagnostic colonoscopy. A number of large studies have reported haemorrhage in 1 to 6 per 1000 colonoscopies (0.1%-0.6%) [127]. Transient bacteraemia after colonoscopy, with or without polypectomy, occurs in approximately 4% of procedures, with a range of 0% to 25% [117]. However, signs or symptoms of infection are rare [116]. Although individual cases of infection after colonoscopy have been reported, there is no definite causal link with the endoscopic procedure and no proven benefit for antibiotic prophylaxis [116].

Mortality related to colonoscopy is rarely reported. However, in a 2010 review of colonoscopy complications based on prospective studies and retrospective analyses of large clinical or administrative databases there were 19 colonoscopy specific deaths among 284,097 patients (0.007%) [128]–[137]. Miscellaneous complications of colonoscopy include splenic rupture, acute appendicitis, diverticulitis, subcutaneous emphysema, and tearing of mesenteric vessels with intra-abdominal haemorrhage [138]–[142].

A well-recognised potential complication of Colonoscopy is missed lesions such as polyps. Missed adenomas have been reported by several studies at varying rates from 6% to 28% [143]–[146]. Multiple factors influence missed adenoma rates including polyp location and characteristics such as size, shape and number, and the quality of the examination which is influenced by bowel preparation, individual colonoscopist's technique and expertise, and the quality of the equipment used [147][148].

It is important to reduce polyp missed rates to improve the quality of the service and reduce healthcare costs by reducing the incidence of interval colon cancer and associated treatment costs. Quality in endoscopy has become an international priority in recent years. In the UK in 2013, the Joint Advisory Group (JAG) on GI endoscopy, the British Society of Gastroenterology (BSG), and the Association of Coloproctology of Great Britain and Ireland (ACPGBI) Commissioned a working group to review existing and define new Quality Assurance (QA) measures, and Key Performance Indicators (KPI) for Colonoscopy [149]. These measures define specific minimum standards to achieve and maintain to ensure an acceptable quality Colonoscopy service. If standards fall below the required level, then interventions must be implemented to improve the performance of those Colonoscopists. KPIs and QAs define minimum standards and aspirational targets relating to caecal intubation rates, adenoma detection rates, quality of bowel preparation, Rectal examination and retroversion, sedation used, patient comfort, the minimum number of endoscopy procedures performed per annum, Colonoscopy withdrawal times, Polyp removals and retrievals, post colonoscopy CRC rates, and rates of adverse events [149]. It is the responsibility of each endoscopy unit to measure, review and act on individual colonoscopists' KPIs on a regular basis to ensure that the agreed standards are met.

1.7. Non-invasive tests

Many endoscopic procedures are absolutely necessary, but many might be avoided if other, non-invasive forms of investigation were available which could reliably exclude significant pathology. This in turn could lead to reduced demand on endoscopy units with resultant reduced waiting times, improved access for those who really need endoscopic procedures, and reduced unnecessary exposure of patients to the aforementioned risks of endoscopy. Investigations to detect levels of biomarkers in the human body can be used in clinical practice to assess patients for possible underlying diseases. The term 'Biomarker' a portmanteau of biological marker was defined by a joint venture on

chemical safety, the International Programme on Chemical Safety, led by the World Health Organization (WHO) and in coordination with the United Nations and the International Labour Organization, as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease”[150].

1.7.1. Serum Biomarkers

1.7.1.i. C Reactive Protein (CRP):

Acute phase proteins are often up- or down regulated in the acute phase response to infection, inflammation, necrosis, and neoplasia. CRP is one of the best defined acute phase proteins in humans [151]. During an acute phase reaction, the hepatocyte production of CRP is rapidly increased under the influence of interleukin (IL)-6 and tumour necrosis factor alpha and IL-1 beta. CRP has a short half-life (19hours) and will therefore rise early after the onset of inflammation and rapidly decrease after resolution of the inflammation [152]. Although CRP is upregulated in most inflammatory disease including IBD, there is significant heterogeneity in its response between UC and CrD. UC tends to have a modest to absent CRP response whereas CrD tends to be associated with a strong CRP response [153]. Moreover, there is a large variation in CRP response amongst individuals. Some patient will mount a larger response than others, for example some patients will have no detectable CRP response to mild inflammation.

Florin et al found that ~10% of examined patients with active CD by clinical assessment were found to have a CRP of <10 [154]. A retrospective review of Mayo Clinic Data found a greater clinical disease severity (OR 4.5), active disease on Colonoscopy (OR 3.5) and severe inflammation on histology (OR 10.6) is associated with a raised CRP [155]. Elevated CRP was detected in 51% of

UC patients with active disease on Colonoscopy. Follow up CT enterography correlated with CRP when peri-enteric inflammation was present, but not inflammation limited to the small bowel wall [156]. Across studies, CRP is less sensitive and has a lower correlation with mucosal inflammation by endoscopy than stool based biomarkers calprotectin and lactoferrin [157]. This is the case in both UC and CrD patients.

1.7.1.ii. Other serum tests:

Other laboratory markers for inflammation in the gastrointestinal tract include white cell count, platelets and albumin. During inflammation or infection, white cell count and platelets usually rise while albumin decreases. The haemoglobin might be low because of underlying inflammation or malabsorption. Electrolytes and renal function may be abnormal reflecting the complications of gastrointestinal disease such as dehydration, hypo- or hypernatremia and hypo- or hyperkalaemia.

Coeliac antibodies; TGA and EMA have very high sensitivity and specificity, both exceeding 95% in patients with active coeliac disease [31], but are unreliable in patients with IgA deficiency which occurs in 2% of patients with CD[32]. Therefore, total IgA should be measured to identify patients with IgA deficiency in whom TGA is not reliable to diagnose Coeliac disease. Thyroid hormone levels and calcium levels may be abnormal in patients whose symptoms are caused by thyroid disease or diseases of calcium metabolism.

1.7.2. Faecal Biomarkers:

Specific stool tests allow the detection of several potential causes of gastrointestinal symptoms, including infection, inflammation, premalignant polyps or established malignancy. Stool culture and sensitivity and microscopy

for ova and parasites can be used to identify pathogens that can cause symptoms such as diarrhoea, abdominal cramps and bloating.

1.7.2.i. Faecal Calprotectin (FCal) in IBD:

FCal is used to detect inflammation in the gastrointestinal tract. Calprotectin is an antimicrobial protein found in neutrophils, which is released into the GI tract during inflammation. 'FCal' Immunoassay test has been validated as a sensitive marker of inflammation within the bowel [158]. A diagnostic meta-analysis of six adult studies, found that FCal has a pooled sensitivity and specificity of 93% and 96% respectively in detecting IBD, and using it as screening tool would result in a 67% reduction in the number of adults requiring endoscopy [158]. A systematic review which included twenty-eight studies looking at using FCal for differentiating between inflammatory and non-inflammatory bowel disease gave a pooled sensitivity and specificity of 93% and 94% respectively at a cut off level of 50µg/g using enzyme-linked immunosorbent assay (ELISA) tests [159]. Another study looking at clinical and cost-effectiveness of measuring FCal in the diagnosis of IBD in adults and children found that increasing the FCal cut-off level to ≥ 50 µg/g increases diagnostic accuracy without substantially increasing total cost of patient assessment and management [160].

1.7.2.ii. Faecal Calprotectin in other gastrointestinal conditions (Coeliac Disease (CD), Diverticular Disease (DD), Microscopic Colitis (MC) and Colorectal Cancer (CRC)):

FCal can be elevated in other conditions affecting the gastrointestinal tract. A study in 2010 looking at FCal concentration in CD found that its concentration is increased in childhood CD, related to the severity of histopathologic findings and responsiveness to gluten free diet [161]. However, an adult study in 2014 of CD reported no significant correlation between FCal levels and symptoms, Marsh grade or antibody level [162]. Studies looking at the role of FCal in patients with DD have demonstrated elevated FCal levels in patients with colonic inflammation secondary to DD, and suggested it may be useful in

differentiating diverticulitis from IBS [163][164]. Elevated FCal levels by ELISA have also been demonstrated in some patient with active MC, suggesting it as a potential non-invasive surrogate marker in differentiating patients with active MC from those with IBS [165]. FCal may also have a role in the assessment of patients with suspected CRC. A prospective diagnostic accuracy study reported FCal negative predictive value for CRC of 98.6% and 97.2% when including polyps $\geq 10\text{mm}$ [166].

1.7.2.iii. Faecal Immunochemical Test (FIT)/ Occult Blood Test (FOBT):

Faecal Immunochemical Test detects the presence and quantity of blood in a stool sample by a human haemoglobin specific immunoassay [167]. It is semiquantitative allowing the use of different thresholds to modify the sensitivity and specificity of the assays for detecting polyps and CRC [168]. Multiple studies have demonstrated the superior sensitivity and specificity of FIT assays for CRC and advanced adenomas compared to guaiac faecal occult blood test (gFOBT), which detects blood in faeces based on the oxidation of alpha-guaiaconic acid by hydrogen peroxide to a blue coloured quinone [169][170].

A meta-analysis exploring the diagnostic accuracy of FIT for CRC detection found a pooled sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of FITs for CRC were 0.79 (95% CI, 0.69 to 0.86), 0.94 (CI, 0.92 to 0.95), 13.10 (CI, 10.49 to 16.35), 0.23 (CI, 0.15 to 0.33), respectively, with an overall diagnostic accuracy of 95% (CI, 93% to 97%) [171]. Sensitivity for CRC improved with lower assay cut-off values for a positive test result (for example, 0.89 [CI, 0.80 to 0.95] at a cut-off value less than 20 $\mu\text{g/g}$ vs. 0.70 [CI, 0.55 to 0.81] at cut-off values of 20 to 50 $\mu\text{g/g}$) but with a corresponding decrease in specificity [171].

One hospital based study found the sensitivity of guaiac and immunochemical tests for the detection of CRC in the screening group was 50.00% (95% confidence interval [CI] =6.76–93.24) and 75.00% (95% CI =19.41–99.37), respectively [172]. For comparison, the sensitivity of the gFOBT for detecting

CRC among the control group was 65.00% (95% CI =40.78–84.61) while that of FIT was 80.00% (95% CI =56.34–94.27). The specificity of the guaiac and immunoassay tests was 77.87% (95% CI =72.24–82.83) and 90.12% (95% CI =85.76–93.50), respectively. The positive likelihood ratio of guaiac and immunochemical tests for the detection of CRC was 2.26 (95% CI =0.83–6.18) and 7.59 (95% CI =3.86–14.94) [172].

Community based studies have demonstrated higher patient participation and higher detection rates for advanced adenoma and cancer with FIT than gFOBT [173][174]. A cohort-based Markov state transition model of CRC disease progression and screening using UK BCSP data and national sources, including a recent large pilot study of FIT screening in the BCSP demonstrated the cost-effectiveness of FIT at all thresholds considered [175].

In 2008, several American professional societies endorsed the use of FIT to replace FOBTs because of the former's improved performance characteristics and potential for higher participation rates [176]. Countries in Europe and Asia have also adopted widespread CRC screening programs using FIT, including the National Colon Cancer Screening Program (NCCSP) in Ireland (Bowel Screen) [177][178].

Despite the superiority of FIT, it only detects 20-30% of adenomas > 1cm in diameter [179]–[181]. Furthermore, occult blood testing detects significantly more lesions in the left than right colon, which is a significant issue given the increased incidence of right-sided CRCs that has developed over the last two decades [182]. Due to these limitations in the commonly used methods for CRC screening, there has been intense effort to develop a more sensitive, non-invasive stool-based CRC screening test that relies on specific molecular alterations observed in colon polyps and CRCs (e.g. gene mutations, aberrantly methylated DNA loci, micro RNAs, etc.) [183].

1.7.2.iv. Faecal Multi-Target DNA Tests:

Over the last 20 years, numerous molecular assay approaches have been explored for potential use in CRC screening. However, few have achieved high clinical accuracy or become available for patient use [184]. ColoScape™ is a multitarget stool DNA (MT-sDNA) test developed by DiaCarta [185]. It is a real-time Polymerase Chain Reaction (PCR)-based diagnostic assay for qualitative detection of colorectal cancer associated biomarkers in Formalin Fixed Paraffin Embedded (FFPE) tissue samples. The kit utilizes DiaCarta's proprietary QClamp® TaqMan-based PCR technology which leverages a sequence-specific clamp made by xeno-nucleic acid (XNA) to suppress PCR amplification of wild-type DNA template and selectively amplify only mutant DNA template [185]. The detection kit identifies the presence or absence of mutations in the targeted regions.

Cologuard™ is a multitarget stool DNA (MT-sDNA) test developed by a company called Exact Sciences® based in the USA [186]. It includes quantitative molecular assays for KRAS mutations, aberrant NDRG4 and BMP3 methylation, and β actin, plus a haemoglobin immunoassay. Two recent, large, multi-centre case-control studies have evaluated MT-sDNA performance [187][188]. In the first study, sensitivity for stage I and II CRC was 87% and adenoma detection ranged from 54%–92%, depending on size (54% for lesions ≥ 1 cm; 77% for >2 cm, 86% for >3 cm and 92% for >4 cm ($p < 0.0001$ for trend)) [187]. Importantly, the sensitivity for detection of either CRC or adenoma was not affected by location (proximal vs. distal colon) [187]. In the second study, sensitivity for CRC was 98% (91 of 93) overall and 97% (74 of 76) for stages I-III at a nominal 90% specificity cut-off. Sensitivity for advanced adenomas and polyps was significantly correlated with size; sensitivity for adenomas and sessile serrated polyps ≥ 1 cm was 57% (65 of 114), >2 cm was 73% (27 of 37), and >3 cm was 83% (20 of 24). Lesions with high-grade dysplasia were detected at 83% (15 of 18) sensitivity; 94% (16 of 17, where size was recorded) of these were >2 cm.

The performance of the MT-sDNA test in the case-control studies described above led to its evaluation in a large cross-sectional study in asymptomatic patients undergoing routine screening colonoscopy in the United States , which served as the criterion standard. This study compared MT-sDNA to FIT and colonoscopy in nearly 10,000 average-risk patients enrolled at 90 sites in North America [189]. Test specificity was based on the detection of CRC and advanced pre-cancerous lesions. By MT-sDNA, the overall sensitivity for CRC was 92% (95% CI, 83–97.5%) and 93% (95% CI 83.8–98.2%) for stage I-III CRC, compared to FIT sensitivities of 74% (95% CI, 61.5–84%) and 73% (95% CI, 60.3–83.9%), respectively (p=0.002). For advanced adenomas and sessile serrated polyps (SSP), the sensitivity of the MT-sDNA test increased proportionately with lesion size and grade. Detection of polyps with high grade dysplasia was 69% by MT-sDNA vs 46% by FIT (p=0.004). MT-sDNA was also significantly more sensitive than FIT for advanced adenomas: 42% (95% CI, 38.9–46%) vs 24% (95% CI, 20.8–27%), respectively, for those ≥ 1 cm and 66% vs 43% for those ≥ 2 cm (p< 0.001). SSPs ≥ 1 cm were detected at a rate of 42% for MT-sDNA compared 5% for FIT (p< 0.001); MT-sDNA was 67% sensitive for those >2 cm compared to 11% by FIT [190]. The overall specificity for the detection of either CRC or advanced pre-cancers was 87%; however, when not including other lesions detected at colonoscopy, including polyps <1 cm, the specificity was 90%. As a result, Cologuard™ has been approved for use for screening of colon cancer by the Food and Drug administration (FDA) in the USA. This test has not been validated or approved for use in symptomatic or surveillance patients and is not in use outside the USA at the current time.

1.8. Hypothesis and Aims:

We hypothesise that the demand on endoscopy, in particular Colonoscopy, as this constitutes the biggest burden on endoscopy services, can be optimised using algorithms for the investigation of patients with common gastrointestinal complaints through the systematic use of a combination of non-invasive serum and faecal biomarkers. Patients with so-called 'red-flag' or alarm symptoms were excluded from the study. These tests may identify patients likely to have underlying organic disease and prioritise them for endoscopy. Furthermore, patients with normal biomarkers can be reassured or treated without recourse to endoscopy. There are 3 independent studies carried out as part of this thesis, which specifically focusses on lower GI symptoms and Colonoscopy procedures. The aims of these studies were as follows:

- i. Retrospectively review Colonoscopy outcomes and the *ad hoc* clinical use of serum and faecal biomarkers over 12 months in symptomatic patients and asymptomatic surveillance patients to assess the diagnostic yield of these investigations for the detection of mucosal inflammation, colonic polyps (adenoma and SSPs) and CRC.
- ii. Prospectively explore the use of serum and faecal biomarkers in combination with diagnostic symptom-based questionnaire in the assessment of patients with lower gastrointestinal symptoms. The diagnostic yield of these tests for the detection of mucosal inflammation will be evaluated compared to the gold standard of Colonoscopy.
- iii. Prospectively research the use of a multi target stool DNA test (Cologuard™) in clinical practice for the investigation of both symptomatic patients and asymptomatic surveillance patients. The diagnostic yield of this test for the detection of colonic polyps and CRC will be evaluated compared to the gold standard Colonoscopy.

Chapter 2

Materials and Methods

2.1. Ethics Approval:

Ethics approval for this clinical research study was obtained from St. James's Hospital/ TUH Research and Ethics Committee.

2.2. Study 1; Retrospective review of Colonoscopy outcomes and the *ad hoc* clinical use of non-invasive tests in symptomatic patients and asymptomatic surveillance patients:

2.2.i. Study 1: Part 1. In younger symptomatic patients

Part 2. In older symptomatic patients

For all retrospective studies the Unisoft Endoscopy Reporting System was interrogated to obtain a list of all Gastroenterology day case Colonoscopies performed over 12 months between October 2015 and September 2016. Patient charts were reviewed, and data collected and analysed for all the patients in relation to age, gender, indication for Colonoscopy, endoscopic findings, and histology (including inflammation CRC, adenomatous polyps, and SSPs). For studies on symptomatic patients looking at correlation of Colonoscopic findings in a retrospective cohort in whom biomarkers had been assessed *ad hoc*, the inclusion and exclusion criteria are shown in Table 2.1:

Inclusion criteria	Exclusion criteria
Age $16 \leq 70$ years	Age > 70 years
Presenting complaint: diarrhoea, constipation and abdominal pain/ bloating	Indication: Overt or obscure GI bleeding, Iron deficiency anaemia, assessment/ surveillance of known IBD, Weight loss. Investigation of abnormality detected on other imaging modalities.
Complete diagnostic Colonoscopy	Planned therapeutic Colonoscopy
	Surveillance Colonoscopy due to a personal history of polyps and/ or family history of CRC or polyps.

Table 2.1. Inclusion and exclusion criteria of study 1, parts 1 and 2.

For the retrospective study of symptomatic patients CRP and FCal results were reviewed where available. An elevated CRP or FCal was taken as a positive result, and this composite when available was used to calculate the combined (CRP + FCal) positive predictive values (PPV), negative predictive values (NPV), sensitivity and specificity of these non-invasive tests. Results of Coeliac testing were also reviewed. The adenomatous polyp and SSP detection rates were calculated.

For the purpose of analysis, the results of this Study 1 were analysed for two separate age groups; younger cohort (aged 16 – 45 years) and older cohort (aged 46 – 70 years) given the likely differences in expected disease and polyp incidence in these different age groups.

2.2.ii. Study 1 part 3. In asymptomatic surveillance patients:

Colonoscopy data were also collated in the same way for asymptomatic surveillance patients who underwent Colonoscopy during the period of retrospective review (Oct 2015 – Sept 2016). None of these patients had biomarkers assessed. The inclusion and exclusion criteria for this study are shown in Table 2.2:

Inclusion Criteria	Exclusion criteria
Age 40-70 years	Age < 40 years or >70 years
Complete Colonoscopy procedure	Incomplete Colonoscopy procedure
Asymptomatic patients	Symptomatic patients
Surveillance procedure due to a personal history of polyps, and/ or a family history of CRC or polyps.	Surveillance due to high risk of CRC due to a familial genetic disorder such as Lynch syndrome.
	Investigation of an abnormality detected on another imaging modality

Table 2.2. Inclusion and exclusion criteria of study 1, part 3.

The purpose of this study was to record the adenoma and SSP detection rates, and to assess the diagnostic yield of surveillance Colonoscopy in our unit over the period of the retrospective review.

2.3. Study 2. Prospective study of symptomatic patients exploring the use of non-invasive biomarkers in combination with symptom-based diagnostic questionnaire in patients with lower gastrointestinal symptoms.

Patients were recruited for the prospective study from the new referrals to the Gastroenterology service; both outpatients and referrals that had been triaged to direct access. The inclusion and exclusion criteria are shown in Table 2.3:

Inclusion Criteria	Exclusion criteria
Patient age ≥ 16 years.	Investigation of Iron deficiency anaemia.
Patient able to give informed consent.	History of recent active GI bleed or bleeding per rectum.
Patient referred for diagnostic Colonoscopy as deemed appropriate by the assessing gastroenterologist based on standard clinical practice.	Known history of IBD.
Indication for Colonoscopy as follows: diarrhoea predominant, constipation predominant, abdominal pain, bloating.	History of recent unintentional weight loss.
	Pregnancy.
	Cognitive impairment.

Table 2.3. The inclusion and exclusion criteria of study 2.

As can be shown from Table 2.3, only those patients who had altered bowel habit (either constipation or diarrhoea), abdominal pain or bloating were

included in the study. These would constitute the most common indications for Colonoscopy apart from rectal bleeding, which was an absolute exclusion criterion for this study as, particularly in older patients, this is a 'red-flag' or alarm symptom and warrants Colonoscopy.

Patients who fulfilled inclusion criteria were invited to participate in the study. Once these patients reviewed the study information sheet, and the details of the study were explained to them by a medical professional, patients who indicated that they would be interested in participating in the study were then asked to give their informed consent. The eligible participants were then asked to complete the following;

1. The World Gastroenterology Organisation IBS questionnaire [191], used to help diagnose IBS in patients with lower gastrointestinal symptoms (appendix 1). The questionnaire elaborates on the patient's complaints relating to abdominal pain, abdominal bloating, distention and details of bowel movements including frequency, consistency, straining and urgency, and their effects on the abdominal pain. It also includes age group, red flag symptoms and relevant family history. Once completed, the answers are marked to give a total score that suggests whether a patient is more likely to have IBS, or whether other conditions should be considered. This questionnaire is relatively new and has not yet been validated. Information was also collected on smoking status and the regular use of Non-Steroidal Anti-Inflammatories (NSAIDs).
2. Routine blood tests: full blood count (FBC), renal function tests (RFTs), liver function test (LFTs), CRP (a level of < 5.0 mg/L was considered normal), Calcium, thyroid function tests (TFTs), and Coeliac serology where indicated.

3. Faecal Calprotectin FCal; samples taken in our hospital are sent for analysis to Laboratory Medicine Directorate, Cardiff, United Kingdom. The samples are analysed by Firefly Calpro ELISA. A positive result of $> 90\mu\text{g/g}$ faeces is suggestive of possible underlying IBD. Normal values are $< 50\mu\text{g/g}$, and values between $50 - 90\mu\text{g/g}$ are considered indeterminate. However, for this study we considered $\text{FCal} < 50$ to be normal, and $\text{FCal} \geq 50$ to be abnormal, in an attempt to reduce false negatives and maximise the sensitivity of the assay.
4. Colonoscopy (where indicated as part of the current standard clinical practice in Gastroenterology clinics). Only procedures completed to the caecum or terminal ileum, with at least adequate quality bowel preparation were included for analysis.

The results were reviewed and analysed in relation to patient demographics, indication for Colonoscopy, endoscopy findings, and histology. The final results regarding the presence or absence of inflammation or other findings were correlated with the collected blood and stool biomarkers, and the results of the IBS questionnaire using the statistical analysis described below.

Statistical analysis:

The patient cohort was stratified based on symptoms into 2 groups; diarrhoea predominant symptoms (group A) and non-diarrhoeal symptoms (group B). Faecal Calprotectin and CRP results for each group were used to calculate the sensitivity, specificity, PPV and NPV of the biomarkers based on symptoms.

Further statistical analysis was performed to assess the relationships between gastrointestinal inflammation diagnosed at Colonoscopy, the different biomarker variables analysed and patient demographics using the following statistics models:

- a. Univariate analysis was performed on the data collected using Chi-square for categorical and t-test for normally distributed continuous variables and Wilcoxon for non normally distributed continuous variables. A p value <0.05 was considered statistically significant
- b. Logistic regression analysis was performed to assess the same relationships.

Univariate and logistic regression analyses were also performed to examine the relationship between GI inflammation at Colonoscopy and the questions asked in the IBS symptoms-based questionnaire. The relationship with each individual question was assessed using logistic regression adjusting for the previously identified statistically significant variables.

Models for predicting mucosal inflammation:

Variables found to be statistically significant on the above analyses were further examined to extrapolate models or pathways of assessment tools that are best at predicting the presence and absence of inflammation. Their predictive power was examined using Receiver Operating Characteristics (ROC) curves to assess the capability of each of the models of predicting inflammation. An Area under the curve (AUC) near to 1 means the model has good predictive value, while AUC near 0 means the model has poor predictive value

A FCal-CRP score was also created to predict the presence of mucosal inflammation. If CRP was raised (>5) +1 was added to the score; similarly, if FCal was raised (>50), +1 was added to the score. This meant that if none were raised score was 0, if one was raised but not the other score was 1, and if both were raised score was 2. FCal-CRP score was included in the model and stepwise backwards regression performed to assess how good it is at predicting inflammation.

2.4. Study 3. Prospective study exploring the role of a novel non-invasive stool test (Faecal Cologuard™) in the assessment of symptomatic patients and asymptomatic surveillance patients.

The purpose of this study was to assess the performance of the Faecal Cologuard test in the detection of colonic neoplasia in both symptomatic and asymptomatic surveillance patients over the age of 40 years. Inclusion and exclusion criteria are shown in Table 2.4:

Inclusion Criteria	Exclusion criteria
Patient age $\geq$ 40 years	Patients < 40 years of age were excluded because the incidence of polyps is too low in this patient group, and the study would not be adequately powered.
Patient able to give informed consent.	Cognitive impairment
Indication for Colonoscopy: a. Symptoms of diarrhoea, abdominal pain, bloating, and/or constipation. b. Asymptomatic surveillance due to personal history of polyps. c. Asymptomatic surveillance due to family history of CRC or polyps.	Indication for Colonoscopy: a. Investigation of Iron deficiency anaemia. b. History of recent active GI bleed or bleeding per rectum. c. Known history of IBD. d. History of recent unintentional weight loss. e. High risk of CRC due to a familial genetic disorder such as Lynch syndrome.
	Pregnancy
	Incomplete Colonoscopy

Table 2.4. The inclusion and exclusion criteria of study 3.

Patients who fulfilled inclusion criteria were invited to participate in the study. Once these patients reviewed the study information sheet, and the details of the study were explained to them by a medical professional, informed consent was obtained. The eligible participants were then asked to complete the following;

1. Faecal Cologuard™; the kit was provided to the recruited patients at the hospital, where they were shown in detail how to collect, prepare and seal the stool sample, Figure 2.1. Detailed instructions of how to collect the sample are shown in Appendix 2.



Figure 2.1. Faecal Cologuard™ Kit

Once the sample was ready, the patients contacted Screenlink Healthcare on a specified contact number to arrange the sample pickup as soon as possible, ideally within 12 hours of collecting it. The samples were then delivered to the laboratory for initial preparation. Once prepared and frozen, the samples were shipped to the Exact Sciences laboratory in the United States for final analysis. The returned result was given as either positive or negative.

2. Colonoscopy (where indicated as part of the current standard clinical practice in Gastroenterology clinics).

The results were reviewed and analysed in relation to patient demographics, indication for Colonoscopy, Faecal Cologuard™ results, endoscopy findings, and histology. The adenoma polyp detection rate was calculated, and the results correlated with the Cologuard™ results to calculate PPV, NPV sensitivity and specificity of Faecal Cologuard™ in our patient population.

2.5. Patient survey to assess faecal testing compliance.

The purpose of this study was to explore reasons why patients are sometimes resistant to the provision of stool samples as we and others have found anecdotally, that patients often fail to provide stool samples even when they understand the clinical value and indication for the test.

An anonymous patient survey was randomly handed out by the outpatient nurses in the general Gastroenterology clinics over a 4 week period. The survey included information on patient age group, whether they were ever asked to provide a stool sample in any clinical setting and whether they were compliant with stool testing or not. The survey also enquired about whether they received adequate information on why the stool sample was needed and how to collect the sample. The patients who did not provide the stool sample were asked about their reasons and how their compliance could be improved. (See Appendix 3 for the patient survey)

Chapter 3

Results of Retrospective Studies

Chapter 3.1 Results of Study 1: Retrospective review of Colonoscopy and the *ad hoc* use of biomarkers in symptomatic patients.

A total of 2155 medical Gastroenterology Colonoscopy procedures were performed over 12 months between October 2015 and Sept 2016. Of these, 577 met the inclusion criteria for this study, 242 in the young patient cohort (part 1), and 235 in the older patient cohort (part 2), see Figure 3.1.

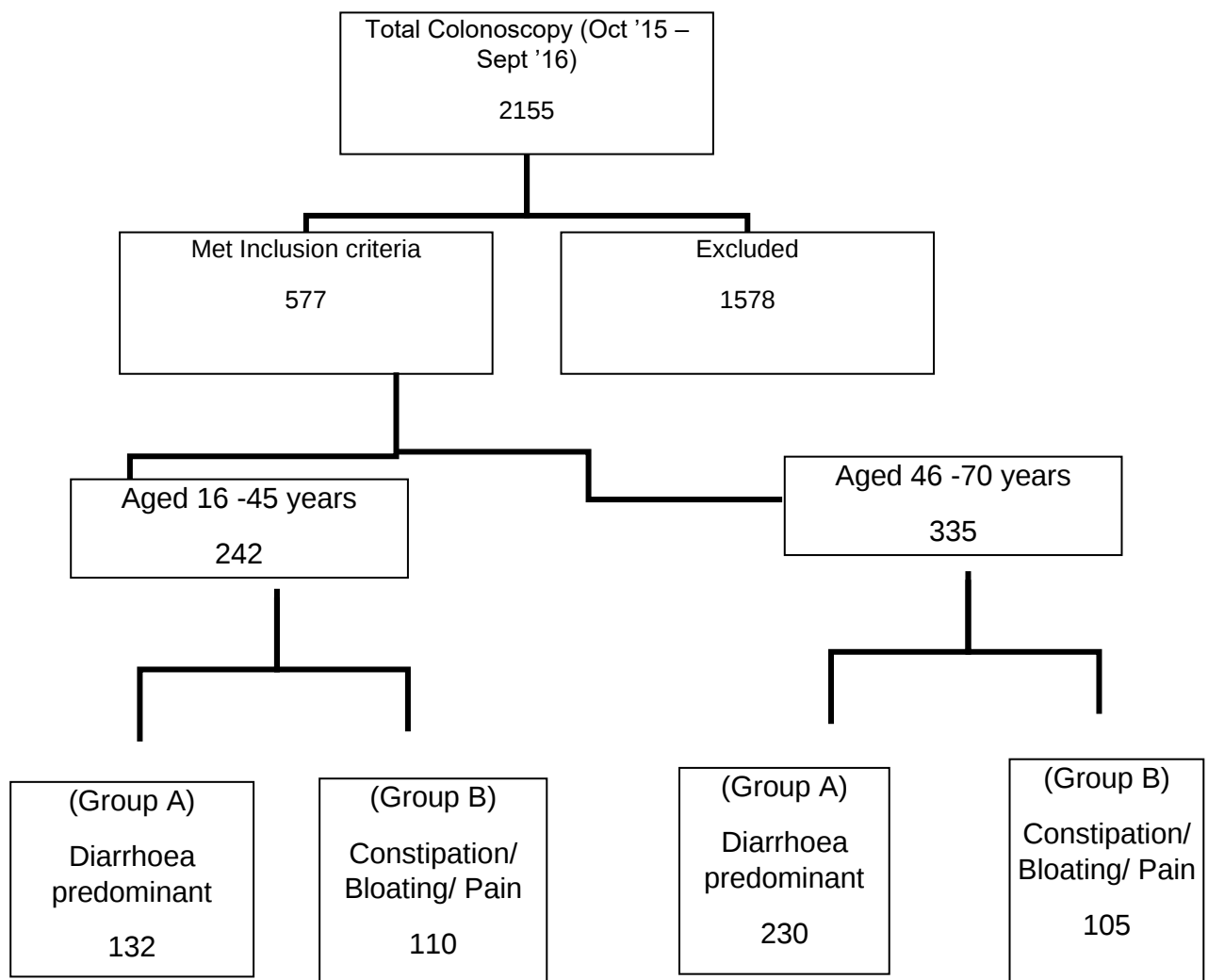


Figure 3.1. Results of Retrospective Study 1.

3.1.i. Study 1, part 1. Younger patient cohort ($16 \leq 45$ years of age).

The overall median age of the eligible patients was 34 years, with a range of 16 to 45 years of age. The number of female patients was 141 (58%), with 101 (42%) male patients. The patients were stratified into two groups based on the indication for Colonoscopy (see Table 3.1 for patient demographics and Colonoscopy results):

Group A: patients with diarrhoea predominant symptoms. There were 132 patients in this group, comprising 55% of the cohort. Median age 35 years, with 75 (57%) female patients, and 57 (43%) male patients.

Group B: patients with non-diarrhoeal symptoms. Symptoms in this group included constipation, abdominal pain and abdominal bloating. There were 110 patients in this group, comprising 45% of the cohort. Median age 35.5 years, with 66 (60%) female patients, and 44 (40%) male patients.

	Group A	Group B
Total number of patients, n	132	110
Female, n, (%)	75 (57%)	66 (60%)
Median age (years)	35	35.5
No inflammation	104 (79%)	102 (93%) (p 0.002)
Inflammation	28 (21%)	8 (7%)
Polyps (adenoma, or SSL)	15 (11%) (including 2 patients with SSP)	6 (5%) (including 1 patient with SSP)
Diverticulosis, n	8	2
Entirely normal Colonoscopy (i.e no inflammation, no polyps and no diverticulosis), n	80 (60.6%)	90 (81.8%)

Table 3.1. Younger patient cohort demographics and Colonoscopy results for Group A; diarrhoea predominant symptoms, and Group B; non -diarrhoea symptoms.

In total there were 206 colonoscopy procedures with no evidence of mucosal inflammation on biopsy (85%). Further stratification based on indications shows that in patients with diarrhoea predominant symptoms (Group A) 104 (79%) of Colonoscopies had no mucosal inflammation, while in patients with non-diarrhoeal symptoms (Group B) 102 (93%) of Colonoscopies showed no evidence of mucosal inflammation (p=0.002).

The total number of patients with confirmed mucosal inflammation on Colonoscopy was 36 (15%). Of these patients, 28 (78%) had diarrhoea

predominant symptoms, while only 8 patients with non-diarrhoeal symptoms had mucosal inflammation confirmed on histology ($p=0.0001$) (Table 3.2).

	Group A (diarrhoea predominant)	Group B (non-diarrhoeal symptoms)
Patients with mucosal inflammation, (total 36)	28 (77.8%)	8 (22.2%) (p 0.0001)
Colonic inflammation	18	4
Ileal Inflammation	6	1
Ileocolonic inflammation	4	3

Table 3.2. The results of younger patient cohort with mucosal inflammation for Group A and Group B.

The mucosal inflammation findings were divided into ileitis, colitis, and ileocolonic inflammation. There were 7 patients with active ileitis, including 2 patients with NSAID induced inflammation. Colitis was found in 22 patients; with 12 patients diagnosed with colonic IBD, 2 with Microscopic Colitis and 8 with non-specific colitis. Ileocolonic inflammation was found in 7 patients, all of whom were diagnosed with ileocolonic IBD.

Retrospective review of blood and stool tests revealed that as part of routine clinical care 158 of 242 (65.3%) patients had either a CRP or FCal performed. Of these 158 patients, 95 patients were in group A, and 63 patients in group B. These were patients who had attended the gastroenterology outpatient clinic prior to undergoing Colonoscopy. Patients who had been triaged/ referred to direct access Colonoscopy had no biomarkers tested. 35 patients had both CRP and FCal tests, 1 patient only had a FCal and 122 only had a CRP

performed. A CRP > 5 mg/L or FCal of ≥ 50 ug/g were considered abnormal. Of the 158 patients for whom CRP and/ or FCal were available, CRP was elevated in 21 patients while 14 patients had elevated FCal. Of the 95 patients in group A who had a CRP and/ or FCal performed, 26 (27.4%) patients had inflammation on Colonoscopy, 11 (42%) of whom had a raised CRP and/ or FCal. Of the 63 patients in group B who had a CRP and/ or FCal performed, 8 (12.7%) patients had inflammation on Colonoscopy, 3 (37.5%) of whom had a raised CRP and/ or FCal.

Patient who had a raised CRP or FCal or both was considered to have a positive result, and this was used to calculate the PPV, NPV, sensitivity and specificity for group A and group B (Table 3.3).

	Group A	Group B
NPV	79.1%	90.9%
PPV	47.8%	37.5%
Sensitivity	42%	37.5%
Specificity	82.6%	90.9%

Table 3.3. The correlation of CRP/ FC results with mucosal inflammation in Group A and Group B, in younger patients aged 16 – 45 years.

The PPV was 47.8% in Group A and 37.5% in Group B. NPV was 79% in group A and 90.7% in group B. The sensitivity was 42% in group A and 37.5% in group B, while the specificity was 83% and 90% in group A and B, respectively.

Investigations for CD (TGA and/ or duodenal biopsy) were carried out in 180 (74%) patients. The results were positive in 4 patients; 3 in group A, and 1 in group B. One patient in group A had both CD and MC.

Incidental early adenomatous polyps were found in 21 of 242 patients (8.7%) (Table 4). There were no cases of colorectal cancer or advanced adenoma in this patient cohort. Incidental colonic diverticulosis was diagnosed in 10 patients (4% of the study cohort).

3.1.i.a Discussion:

The results of this study confirm that most young patients with lower gastrointestinal symptoms tend to have no inflammation on colonoscopy, especially those with non-diarrhoeal symptoms; 79% of patients with diarrhoea predominant symptoms, and 93 % of patients with non-diarrhoeal symptoms had normal colonoscopies. Colonic mucosal inflammation was more common in patients with diarrhoea predominant symptoms (21% vs 7%, p value = 0.002), suggesting that this patient group may need more intensive investigation for possible underlying organic pathology. Therefore, colonoscopy may potentially be avoided in many cases if a reliable and validated non-invasive diagnostic pathway is available and implemented in clinical practice.

The limited number of FCal results available in this retrospective review highlights that FCal is still underutilised in clinical practice, and steps should be taken to increase awareness and accessibility of the test. The relatively high NPV (79.1% in the diarrhoea predominant cohort and 91% in the non-diarrhoeal cohort) of inflammatory blood and stool biomarkers suggests that they may play a vital role in the initial assessment of younger patients with lower gastrointestinal symptoms to identify those less likely to have underlying inflammation, and who can therefore be reassured without the need for invasive investigations. In this retrospective study the PPV and sensitivity of the combination of CRP/FCal for the detection of mucosal inflammation are relatively low, and this may be due in part to the limited number of FCal results available, and hence the results calculated were largely based on CRP results. The response of CRP to gastrointestinal inflammation is known to be variable,

and while it is quite reliable in CrD, in patients with UC the inflammatory response of CRP can be modest or absent [192][193].

Interestingly, incidental early adenomatous polyps were found in 8.7% of patients in this young cohort, but there was no advanced adenoma or CRC diagnosed in the study group.

3.1.ii. Study 1, part 2. Older symptomatic patient group (46 - 70 years of age).

As per Figure 3.1, 335 were eligible for inclusion in this study. The mean age of this patient cohort was 58 years, with a range of 46 to 70 years of age. There were 204 (61%) female patients, and 131 (39%) male patients. The cohort was stratified into two groups based on indication (see Table 3.4 for patient demographics and Colonoscopy results);

Group A: Patients with diarrhoea predominant symptoms. There were 230 patients in this group, comprising 69% of the cohort. Median age was 58 years, with a female preponderance of 63%.

Group B: Patients with non-diarrhoeal symptoms including constipation, abdominal pain and abdominal bloating. This group contained 105 patients, comprising 31% of the cohort. Median age was 60 years with a female preponderance of 55%.

	Group A	Group B
Total number of patients, n	230 (69%)	105 (31%)
Female, n, (%)	145 (63%)	58 (55%)
Median age (years)	58	60
Total mucosal inflammation:	23 (10%) divided into:	9 (9%) divided into:
Colonic,	14	7
Ileal,	7	2
Ileocolonic	2	0
Polyps (adenoma, or SSP)	51 (45 adenomas & 6 SSPs) (22.2%)	32 (31 adenomas & 1 SSP) (30%)
CRC	3 (1%)	2 (2%)

Table 3.4. Patient demographics and Colonoscopy results for Group A; diarrhoea predominant symptoms, and Group B; non -diarrhoea symptoms, in older patients age 46 - 70 years.

Mucosal inflammation confirmed on histology was found in 32 patients with a similar incidence in the two groups; 23 (10%) patients in group A, and 9 (9%) patients in group B. The inflammation was stratified based on distribution. For the entire cohort as a group; colonic inflammation was detected in 21 patients (including 4 cases of microscopic colitis). Isolated ileitis was found in 9 patients, and ileocolonic inflammation in 2 patients. The distribution of inflammation for each of groups A and B is as follows (Table 3.5):

- In group A; 14 patients had colonic inflammation, 7 had ileal inflammation and 2 had ileocolonic inflammation.

- In group B: 7 patients had colonic inflammation, 2 had ileal inflammation, with no cases of ileocolonic inflammation.

On review of inflammatory markers, CRP was available on 226 of 335 patients (67.4%), 160 in group A and 66 in group B. CRP was elevated in 40 patients (30 in group A and 10 in group B). When CRP was correlated with mucosal inflammation confirmed on histology (Table 3.5), it was found to have a NPV of 91.4% and 91%, with a PPV of 27.5% and 11% in group A and group B, respectively. The sensitivity of the test in group A and B was 42% and 16.6%, with a specificity of 84.7% and 86.4% respectively. Results for FCal were not available in this patient cohort indicating that this test was being under-utilised in clinical practice in older patients.

	Group A	Group B
NPV	91.4%	91%
PPV	27.5%	11%
Sensitivity	42%	16.6%
Specificity	84.7%	86.4%

Table 3.5. The correlation of CRP results with mucosal inflammation in Group A and Group B, in older patients age 46-70 years.

In total, 5 cases of CRC were diagnosed, 4 of which were diagnosed in patients over the age of 60 years, and the fifth patient was 49 years old. The number of patients with polyps confirmed on histology as either adenoma or SSP was 83 (including 76 adenomas and 7 SSPs), giving a detection rate of 24.7%. Adenomatous polyps with low grade dysplasia were detected in 76 patients, 7 of whom had advanced polyps of ≥ 10 mm in size. SSPs were found in 7 patients, all of whom had small lesions < 10 mm in size. Diverticulosis was seen in 70 (21%) patients.

There were 222 (66%) normal colonoscopies performed which had no evidence of mucosal inflammation, adenomatous polyps, SSP, or CRC. There was no significant difference in the percentage of normal procedures between the two groups. In Group A, 157 (68%) patients had a normal colonoscopy, while in group B, 65 (62%) patients had a normal colonoscopy.

3.1.ii.a. Discussion:

This retrospective study shows that the diagnostic yield of colonoscopy in this symptomatic patient cohort for mucosal inflammation is relatively low. In terms of potential non-invasive biomarkers for inflammation, CRP may be a reliable marker for the absence of inflammation due to its high NPV in both groups A and B. FCal was very underutilised in this patient age group compared to the young cohort. This requires further exploration and may be that clinicians are less comfortable relying on non-invasive tests in older patients.

On the other hand, the diagnostic yield of CRC and advanced adenomas in this patient group is higher than was observed in the younger patient cohort (12 cases of CRC or advanced adenomas in the older patient cohort compared to none in the younger patients). This observation supports an argument for having a low threshold for investigating older patients as an opportunity for colonic polyp screening. Furthermore, this can potentially be carried out by non-invasive means, using validated faecal tests with high sensitivity and specificity for CRC and advanced polyp detection.

The polyp detection rate in this study is in keeping with the current standards of quality assurance and key performance indicators set out by the joint working group commissioned in 2013 by the Joint Advisory Group (JAG) on GI endoscopy, the British Society of Gastroenterology (BSG), and the Association of Colproctology of Great Britain and Ireland (ACGBI) [149].

3.2. Results of Study 1, part 3; Retrospective review of CRC and polyp detection in asymptomatic surveillance patients undergoing Colonoscopy.

A total of 322 patients between the age of 40 and 70 years underwent a surveillance colonoscopy in 12 months between October 2015 and September 2016. The mean age was 57 years. Both genders were equally represented in the cohort with 162 (50.3%) female patients, and 160 (49.7%) male patients. The indications for surveillance colonoscopy were as follows; 142 (44%) for family history of colorectal cancer, and 180 (56%) for surveillance for adenomatous polyps. (Table 3.6).

	Patient number, n & %
Total number of patients in the study	322
Median Age (years)	57
Female (n, %)	162 (50.3%)
Male (n, %)	160 (49.7%)
Indications for Colonoscopy:	
Previous history of polyps (n, %)	180 (56%)
Family history of CRC or polyps (n, %)	142 (44%)
Polyps detected: Adenoma	115 (35.6%)
SSP	30 (9.3%)
CRC	1
Mucosal inflammation	4 (1.2%)

Table 3.6. Patient demographics and Colonoscopy results for asymptomatic surveillance patients.

Adenomatous polyps were found in 115 (35.7%) patients. Seven of these patients had advanced adenomatous polyps $\geq 10\text{mm}$ in size. SSPs were found in 30 (9.3%) patients, 4 of whom had polyps $\geq 10\text{mm}$ in size. There was only one case of CRC which was diagnosed in a 66 years old patient undergoing colonoscopy due to a previous history of adenomatous polyps.

Incidental mucosal inflammation confirmed on histology was diagnosed in 4 (1.2%) patients. Two patients had colonic inflammation, 1 patient had isolated ileitis, and 1 patient had ileocolonic inflammation.

3.2.i. Discussion:

This retrospective review was performed to assess the overall CRC and adenoma detection rates in our Unit as a Quality Assurance measure, and as a baseline for the prospective study looking at the detection of advanced adenomas and CRC in a similar cohort of asymptomatic surveillance patients using Faecal Cologuard.

This study has demonstrated adenoma detection rate within our Unit that meet international standards, while the rate of SSP detection is similar to the rates described in older studies that suggested the prevalence of SSPs ranged from 0.6%–5.3% [194]–[199]. However, many of these investigations did not use the current WHO standard terminology for the histological classification of serrated polyps. The true prevalence of SSPs is dependent on both the detection rate and the correct pathologic classification of these lesions, and there is evidence of considerable variability of both these factors [200]. One recent study tried to estimate a more accurate prevalence of SSPs by attempting to control for both variability in detection and pathologic classification. The results of 1,910 screening colonoscopies in average-risk, asymptomatic patients performed by a single, high adenoma-detecting endoscopist with histologic interpretation by a sin-

gle, experienced GI pathologist were reviewed. All serrated rectosigmoid polyps >5 mm and serrated polyps proximal to the sigmoid colon were included. One or more serrated polyps were found in 389 of 1,910 patients (20%). The prevalence of SSPs was calculated at 7.4% [201]. SSP characteristics make them difficult to detect. They are more prevalent in the right colon where the bowel preparation is often poor, they tend to be flatter with paler mucosa than conventional adenomas, and they are often covered by a mucus cap. As a result, these lesions have proved to be a challenging dilemma not just in our institution but to practicing endoscopists internationally.

Chapter 4

Results of Prospective Studies

4.1. Results of Study 2; Prospective study exploring the use of non-invasive biomarkers in combination with symptom-based diagnostic questionnaire in patients with lower gastrointestinal symptoms.

4.1.i Patient recruitment and characteristics

Over the 12 month period of the study we received approximately 500 referrals per month to our gastroenterology service (6000 referral over 12 months). Approximately 1800 (30%) of these were referrals for patients with lower GI symptoms. About 500 (27.7%) of the 1800 patients were triaged to direct access endoscopy, while 1300 were triaged for assessment in the general GI clinic. All new referrals to the unit over the study period were reviewed and patients who appeared to fulfil the study inclusion criteria based on their referrals were identified as potential candidates for the study. In total, 300 patients were deemed eligible for the study and were invited to participate (see Figure 4.1).

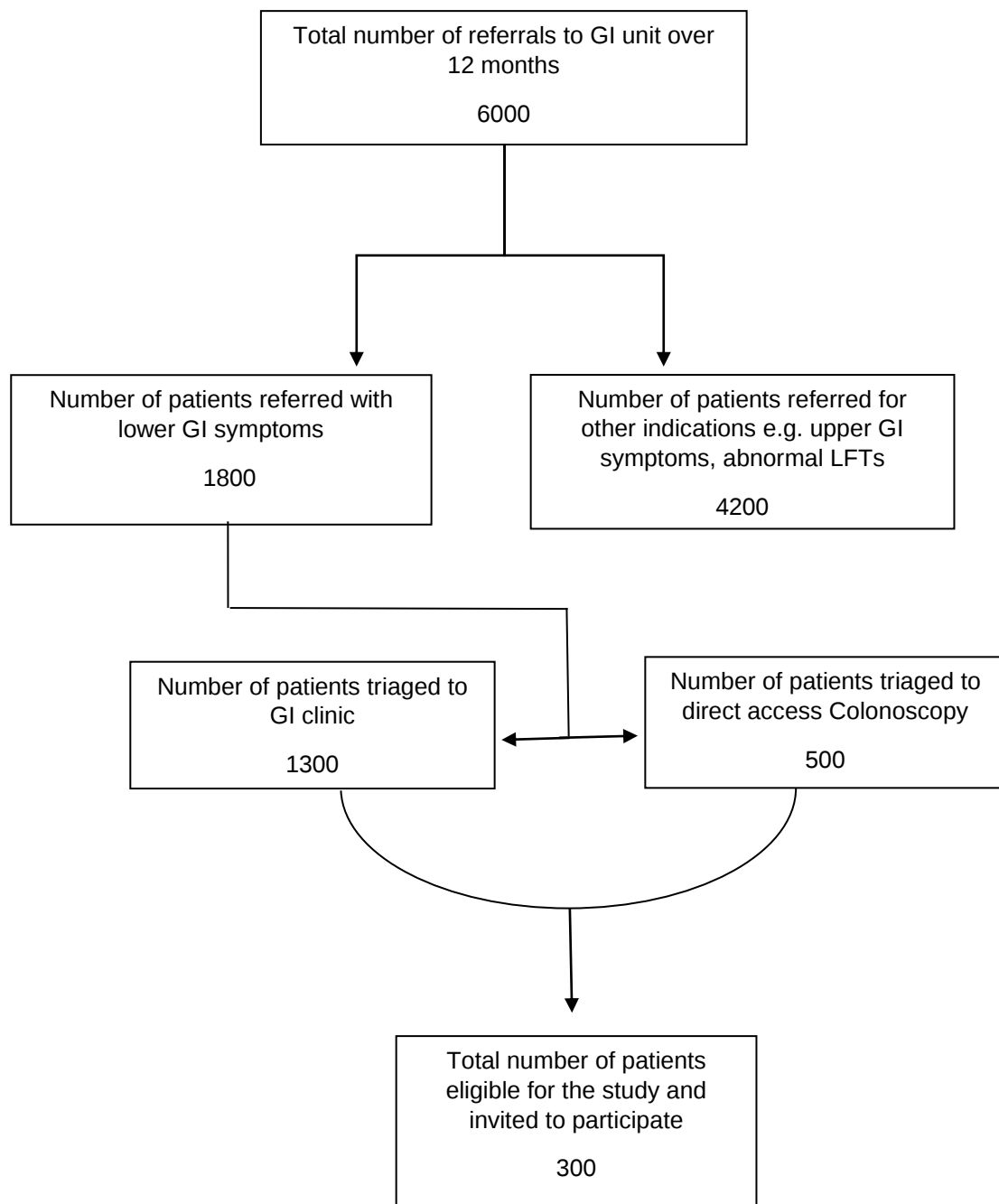


Figure 4.1. Results of patient assessed and invited to participate in the Study 2.

Of the 300 patients who were invited to participate, 209 (69.6%) gave informed consent to take part in the study. Of these, 22 patients were excluded from analysis; 7 did not attend for their Colonoscopy, 12 were not referred for Colonoscopy after study recruitment because they were reassured by the initial

normal non-invasive tests and improved with dietary and lifestyle measures, 1 patient became pregnant and was excluded as a result, 1 patient had positive Coeliac serology and 1 had hyperthyroidism, both of which accounted for their symptoms (see Figure 4.2).

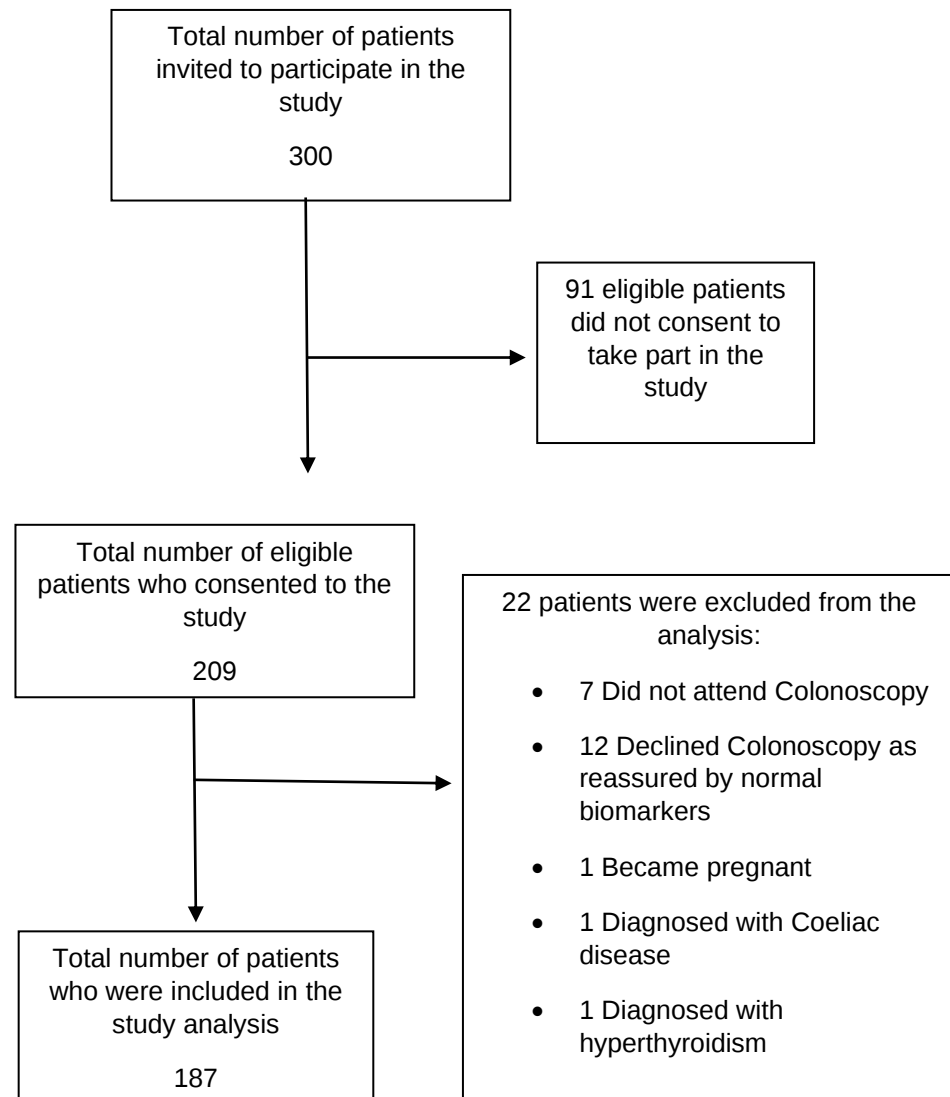


Figure 4.2. Results of patients recruited for study 2.

The 187 patients who completed the study and were included in the analysis ranged from 16 to 71 years of age, with a median age of 35 years. There was a female preponderance with 123 (66%) females and 64 (34%) males. Diarrhoea accounted for 135 (72%) cases, while non-diarrhoeal symptoms (constipation,

abdominal pain and bloating) accounted for 52 (28%) cases. 30 patients were using non-steroidal anti-inflammatories, and 39 (21%) patients were actively smoking at the time of the study (See Table 4.1).

	Patient number, n & %
Total number of patients in the study	187
Median Age (years)	35 (Range 16 – 71 years)
Female (n, %)	123 (66%)
Male (n, %)	64 (34%)
Indications for Colonoscopy:	
Diarrhoea predominant (n, %)	135 (72%)
Non- diarrhoeal symptoms (n, %)	52 (28%)
NSAID use	30 (16%)
Smokers	39 (21%)

Table 4.1. Patient demographics findings for study 2.

Of 135 patients with diarrhoea predominant symptoms 117 (86.6%) patients had a normal Colonoscopy, while 47 of 52 patients (90.4%) with non-diarrhoeal symptoms had a normal Colonoscopy (see Table 4.2).

4.1.ii Outcome of Colonoscopies

Mucosal inflammation was diagnosed on Colonoscopy in 23 (12.2%) patients. Of the 23 patients with inflammatory changes confirmed on histology, 5 patients had ileocolonic inflammation, 5 had colonic inflammation, 11 had ileitis and 2 patients had Microscopic Colitis (Table 4.2). Of the 23 patients with mucosal inflammation, 18 (78.2%) patients had diarrhoea predominant symptoms and 5

(21.8%) had non-diarrhoeal symptoms. Incidental adenomatous polyps were found and removed in 12 (6.4%) patients in this study (Table 4.2).

CRP was available for 178 patients, 45 (25.3%) of whom had a raised CRP. The remaining 9 patients did not complete their serum investigations despite being given the necessary blood forms and information. Faecal Calprotectin test was performed by 162 (86.6%) patients, 120 in group A (diarrhoea predominant), and 42 in group B (non-diarrhoeal symptoms). The other 25 patients did not provide their stool samples despite being given detailed information on the usefulness and indication of the test, and repeated contact by the study investigators to encourage them to provide the sample. Of the 162 patients who provided the stool sample, 45 (28%) patients had an elevated result (Table 4.2).

	Patient number, n & %
Raised Fcal	45 (28%)
Raised CRP	45 (25%)
Normal Colonoscopy in;	
Diarrhoea predominant group (n, %)	117 (86.6%)
Non- diarrhoeal group (n, %)	47 (90.4%)
Mucosal inflammation, total	23 (12.2%)
Diarrhoea predominant group	18 (78.2%)
Non-diarrhoeal group	5 (21.8%)
Mucosal inflammation comprising:	
Ileitis,	11
Colonic,	5
Ileocolonic,	5
Microscopic Colitis	2
Patients with incidental adenomatous polyps	12 (6.4%)

Table 4.2. Results of biomarkers and Colonoscopy findings for study 2.

4.1.iii Faecal Calprotectin Results

A total of 120/ 135 patients provided a stool sample for FCal in group A (diarrhoea predominant symptoms). Of these, 17 patients had inflammation on Colonoscopy, 12 (70.5%) of whom had a raised FCal, and 5 (29.5%) had a normal FCal level. The remaining 103 patients had no evidence of inflammation on Colonoscopy, 23 (22.3%) of whom had a raised FCal, with normal FCal levels detected in 80 (77.7%) of these patients. 42/52 patients provided a stool sample for FCal in Group B (non-diarrhoeal symptoms). Of these, 5 patients had inflammation on Colonoscopy, 4 (80%) of whom had a raised FCal and 1 had a normal FCal. The remaining 37 patients had no evidence of inflammation on Colonoscopy, 7 (19%) of whom had a raised FCal, and 30 (81%) had a normal FCal result.

The sensitivity and specificity of FCal in the diarrhoea predominant group A were 70.6% and 77.7%, respectively with NPV of 94.1% and PPV of 34.3%. In the non-diarrhoea group B; the sensitivity and specificity of FCal were 80% and 81% respectively, with NPV of 96.8 and PPV of 36.4% (Table 4.3).

	Diarrhoea predominant (group A)	Non-diarrhoea symptoms (group B)
Sensitivity	70.6%	80%
Specificity	77.7%	81%
PPV	34.3%	36.4%
NPV	94.1%	96.8%

Table 4.3. Correlation of FCal with mucosal inflammation in patients with diarrhoea predominant symptoms (group A) and those with non-diarrhoeal symptoms (group B).

4.1.iv CRP Results

Of the 178 patients who had CRP performed, 128 patients had diarrhoea predominant symptoms (group A), and 50 patients had non-diarrhoeal symptoms (group B). In group A, 18 patients had inflammation on Colonoscopy, 8 (44.4%) of whom had a raised CRP, and 10 (55.6%) had a normal CRP. 110 patients in group A had no evidence of inflammation on Colonoscopy, 25 (22.7%) of whom had a raised CRP and 85 (77.3%) had a normal CRP. In group B, 5 patients had inflammation on Colonoscopy, 2 (40%) of whom had a raised CRP and 3 (60%) had a normal CRP. 45 patients in group B had no evidence of inflammation on Colonoscopy, 10 (22.2%) of whom had a raised CRP and 35 (77.8%) had a normal CRP. The sensitivity and specificity of CRP in the diarrhoea predominant group A were 44.4% and 77.3% respectively, with NPV of 89.5% and PPV of 24.2%. While in the non-diarrhoea group B the sensitivity and specificity of CRP were 40 and 77.8% respectively, with NPV of 92.1% and PPV 16.6% (Table 4.4).

	Diarrhoea predominant group A	Non-diarrhoea symptoms group B
Sensitivity	44.4%	40%
Specificity	77.3%	77.8%
PPV	24.2%	16.6%
NPV	89.5%	92.1%

Table 4.4. Correlation of CRP with mucosal inflammation in patients with diarrhoea predominant symptoms (group A) and those with non-diarrhoeal symptoms (group B).

Overall the blood tests performed were largely within the normal range. The median values are as follows; White Cell Count 6.7, Haemoglobin 13.8,

Platelets 259, Sodium 140, Potassium 4.1, Albumin 45, Corrected Calcium 2.28, Thyroid Stimulating Hormone 1.5, TGA 1.0 (Table 4.5).

Blood test	Median, (Range)
White Cell Count	6.7 (2.9 – 14.7)
Haemoglobin	13.8 (11 – 17.6)
Platelets	259 (153 – 565)
Sodium	140 (135 – 145)
Potassium	4.1 (3.2 – 5.0)
Albumin	45 (38 – 51)
Corrected Calcium	2.28 (2.11 – 2.51)
Thyroid Stimulating Hormone	1.5 (<0.05 – 8.83)
TGA	1.0 (0.1 - >128), (1 positive, 0.5%)

Table 4.5. Patient blood results for study 2.

4.1.v Results of univariate and logistic regression analyses:

Univariate analysis to investigate the relationships between mucosal inflammation and the listed variable (including age, gender, symptoms, NSAID use, smoking history, F Cal, and CRP) showed a statistically significant difference in mucosal inflammation by gender, CRP and F Cal (Table 4.6). Mucosal inflammation was more likely to be found in male patients, and in those with a higher F Cal (>44) and higher CRP (>4.1).

	All		Mucosal inflammation,		No mucosal Inflammation,		P value	Test
	N	%	N	%	N	%		
N	187		23	12.3	164	87.7		
Female	123	65.7	10	43	113	68.9	0.016	Chi Square
Male	64	34.2	13	56.5	51	31.1		
Age, mean	37	13.4	41	13.97	36	13.2	0.12	t-test
NSAID use, Yes	30	16.5	3	1.30	27	16.9	0.86	Chi Square
No	152	83.5	20	86.9	132	83.0		
Smoking, Yes	39	21.4	5	21.7	34	21.3	0.96	Chi Square
No	143	75.6	18	78.2	125	78.6		
Diarrhoea, Yes	135	72.2	18	78.2	117	71.3	0.49	Chi Square
No	52	27.8	5	21.7	47	28.6		
F Cal	17.3	7.9-60.5	111	44-280.7	15.4	6.9-38.2	< 0.00001	Wilcoxon
CRP	1.7	1-5.05	4.6	1.5-8.75	1.6	1-4.1	0.042	Wlcoxon

Table 4.6. Univariate analysis (Chi-square for categorical and t-test for normally distributed continuous variables and Wilcoxon for not normally distributed continuous variables. A p value <0.05 was considered statistically significant).

Investigating the relationships between mucosal inflammation and FCal using logistic regression analysis showed that F Cal was statistically significantly associated with mucosal inflammation at Colonoscopy. A raised FCal (>50 ug/g) was strongly associated with inflammation (OR = 10.59, 95% CI: 3.65-30.75) and for each additional unit increase in FCal, inflammation risk increased 1.01 times (95%CI:1.005-1.016). Repeating the analysis comparing ranges of FCal (<50 ug/g, 50-100 ug/g and >100 ug/g) showed a stronger relationship

between inflammation and FCal >100 ug/g, OR=15.66 (95%CI: 4.77-51.37, p-val=0.0000056), compared to FCal 50-100 ug/g, OR=5.84 (95%CI: 1.48-23.11, p-val=0.012).

4.1.v (a) Statistical analysis using age stratification:

Statistical analysis was performed following age stratification of the Cohort (young cohort; age $\leq$ 45 years (n=143), and older cohort; age > 45 years (n=39)). In the young cohort 14 (9.8%) patients had intestinal inflammation and 129 (90.2%) had a normal Colonoscopy. An association was noted between inflammation and raised FCal, however this was only significant for FCal >100 ug/g, OR = 6.46 (95% CI: 1.81-23.09). Again, in continuous analysis, unit increase in FCal was associated with an increased risk of inflammation OR=1.01 (95%CI: 1.003-1.013).

In the older cohort, 7 (18%) patients had inflammation and 32 (82%) did not have inflammation. FCal was also associated with inflammation in this group; in continuous analysis we found that for a unit increase in FCal the risk of inflammation increased. [Due to low numbers the analysis could not be performed with FCal ranges.]

4.1.v (b) Statistical analysis using gender stratification:

Univariate analysis was performed following stratification of the study population into two cohorts based on gender. A strong association between raised FCal and colonic inflammation was observed in males. When compared to normal FCal of <50 ug/g, FCal in the 50-100 ug/g range was significantly associated with inflammation (OR=11.14, 95%CI: 1.46-85.07), and the relationship was even stronger for FCal >100 ug/g (OR=33.51; 95% CI: 4.41-254.74). An association was also present in the female cohort, albeit weaker. When compared to normal FCal of <50 ug/g, FCal in the 50-100 ug/g range was non-significantly associated with inflammation (OR=1.08, 95%CI: 0.18-

17.76), but this was significant for FCal >100 ug/g (OR=8.18; 95% CI: 1.90-35.22).

4.1.v (c) Statistical analysis of Symptom Based Questionnaire:

A total of 195 (93.3%) out of 209 recruited patients completed the questionnaire (included in Appendix 1). The summary of the results and analysis is shown in Table 4.7. For questions 1 to 15 relating to symptoms, the answers were marked as 0 if the response was never, 1 if the response was some of the time, and 2 if the response was most/all of the time. For question 16 relating to the age of the participant, the answer was marked as 0 if age is over 50 years and 2 if age is between 15 to 50 years. If the age is under 15 years then it is marked as 1, but none of the patients recruited were below the age of 16 years.

Univariate analysis assessing the relationship between mucosal inflammation and each individual question answered on the IBS questionnaire showed a statistically significant association between mucosal inflammation and having frequent loose or mushy stool (p value = 0.008) and frequent abdominal pain (p value = 0.029). Logistic regression analysis of the same relationship, adjusting for the variables which were previously found to be significant showed a statistically significant positive association between mucosal inflammation and having frequent loose/ mushy stool, and a negative association between it and having less frequent hard stool (Table 4.7).

Q1 (Frequency of abdominal pain)	Mucosal inflammation	0 (never)	1 (some of the time)	2 (most/all of the time)	P value
	no	1	98	54	
	yes	1	8	12	0.029047
Q2 (Duration of abdominal pain)		0	1	2	

	no	10	21	122	
	yes	1	3	17	0.951451
Q3_Female.only (Related to menstruation)		no	yes		
	no	96	4		
	yes	9	0		1
Q4 (Improvement of pain with bowel movements)		0	1	2	
	no	23	91	39	
	yes	4	14	3	0.519344
Q5 (Increased frequency of bowel movements)		0	1	2	
	no	35	54	64	
	yes	3	5	13	0.220494
Q6 (Decreased frequency of bowel movements)		0	1	2	
	no	84	57	12	
	yes	11	8	2	0.956088
Q7 (Looser stool)		0	1	2	
	no	18	71	64	
	yes	4	4	13	0.058938
Q8 (Harder stool)		0	1	2	
	no	64	75	14	

	yes	14	6	1	0.099656
Q9 (Frequency of lumpy/ hard stool)		0	1	2	
	no	49	90	14	
	yes	12	8	1	0.076382
Q10 (Frequency of watery/ mushy stool)		0	1	2	
	no	16	83	54	
	yes	3	4	14	0.008763
Q11 (Difficulty evacuating stool/ straining)		0	1	2	
	no	31	81	41	
	yes	8	8	5	0.175676
Q12 (Urgency)		0	1	2	
	no	25	79	49	
	yes	3	11	7	0.970341
Q13 (Bloating feeling)		0	1	2	
	no	11	62	80	
	yes	2	11	8	0.474975
Q14 (Visible abdominal distension)		0	1	2	
	no	30	71	52	
	yes	6	11	4	0.343434

Q15 (Increased wind/ gas)		0	1	2	
	no	28	59	66	
	yes	1	13	7	0.086926
Q16 (Age)		0 (> 50 years)	1 (< 15 years)	2 (15 - 50 years)	
	no	25	1	127	
	yes	4	0	17	0.892302
Q17_CRC.family.hx		no	yes		
	no	121	32		
	yes	18	3		0.674203
Q17_IBD.family.hx		no	yes		
	no	110	43		
	yes	13	8		0.491743
Q17_Coeliac.Family.hx		no	yes		
	no	131	22		
	yes	18	3		1
Q18 (Recent antibiotics)		no	yes		
	no	116	37		
	yes	18	3		0.462791
Q19 (Unintentional weight loss)		no	yes		
	no	129	24		
	yes	17	4		0.939074
Q20 (Rectal bleeding)		no	yes		
	no	101	52		
	yes	12	9		0.578906

Q21 (Nocturnal symptoms)		no	yes		
	no	59	94		
	yes	8	13		1

Table 4.7. Questionnaire; univariate analysis looking at the relationship between questions asked and colonoscopy inflammation.

4.1.vi Statistical Models predictive of inflammation:

Using the variables found to be significantly associated with mucosal inflammation in the analyses above, different models of clinical assessment variables were extrapolated to predict the presence of inflammation. As analysis was limited by the number of observations, only preliminary analysis was undertaken to determine the power of models in predicting inflammation using ROC curves.

4.1.vi (a) ROC curves

Variables found to be significantly associated with mucosal inflammation in the multivariate analysis using backwards stepwise regression were used to develop models predictive of inflammation. The variables used to create the models are; FCal, age, gender, Hb and questions in the symptom-based questionnaire (Q6, Q9, Q10 relate to the frequency and consistency of bowel movements and Q14 relates to abdominal distension). Although CRP was significantly associated with inflammation in Univariate analysis, this was not the case when analysed using multivariate analysis by backwards stepwise regression, and hence was excluded from these models. The models created are as follows:

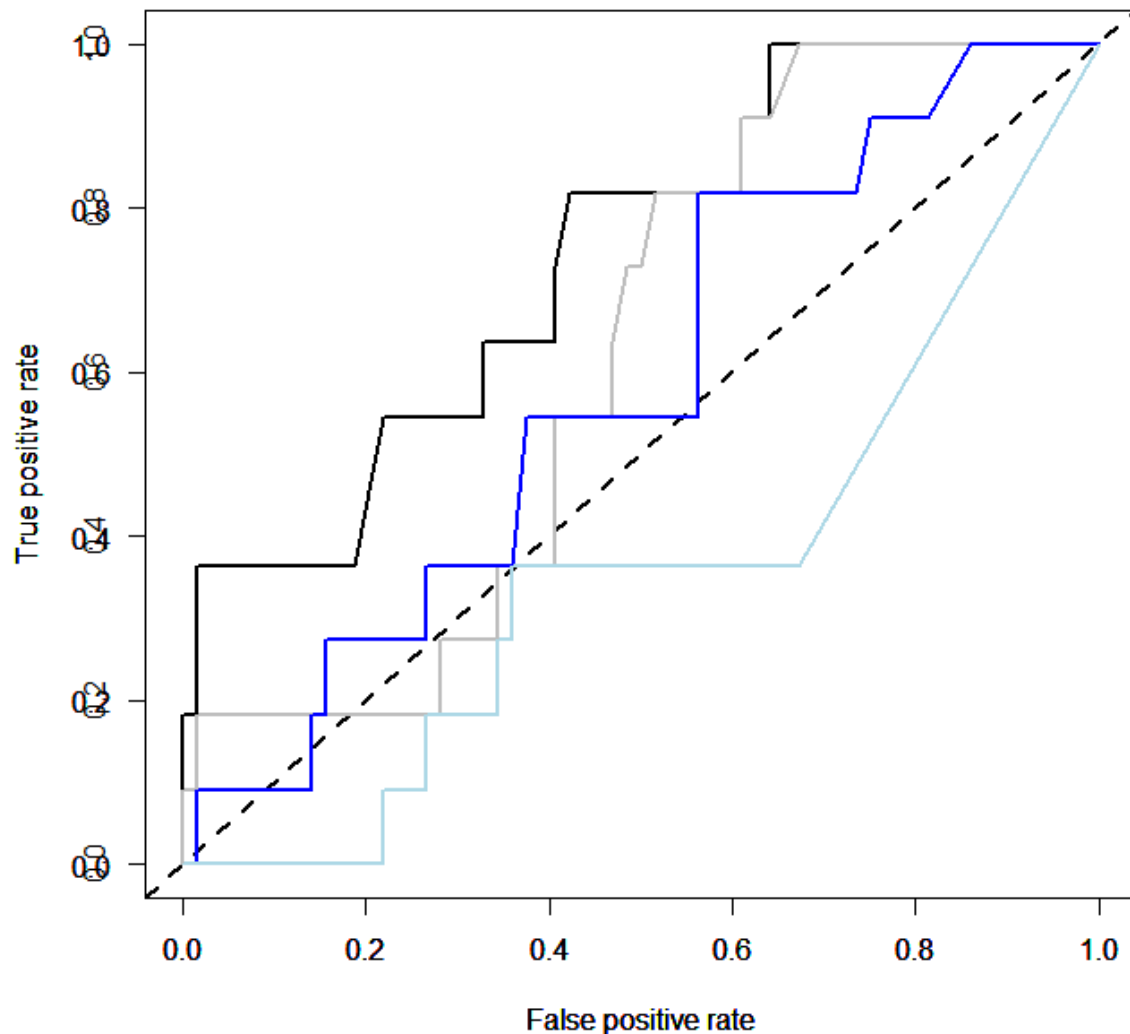
Model 1: Age + Gender + FCal + Hb + Q6 + Q9 + Q 10 + Q 14.

Model 2: Age + Gender + Hb + Q6 + Q9 + Q 10 + Q 14.

Model 3: Age + Gender + Q6 + Q9 + Q 10 + Q 14.

Model 4: Age + Gender.

These models were analysed using ROC curves to assess the contribution each makes to the overall ROC curve. Figure 4.3 shows the ROC curves for each of the models described above. Each model is represented by a coloured line. In the preliminary analysis, the black line corresponding to Model 1 has the largest AUC compared to the other models. This suggests that Model 1 (includes age, gender, F Cal and the questions relating to frequent loose/mushy stool and less frequent hard stool) was the best out of all the models at predicting mucosal inflammation. Although Model 1 performed best out of all the models on the ROC curve, it is still suboptimal as the AUC is not that close to 1. This is likely due to the relatively small number of patient with mucosal inflammation in this study. Further studies with larger numbers are required to improve the predictive power of the model.



Black - model 1
 Grey - model 2
 Blue - model 3
 Light blue - model 4

Figure 4.3. ROC curves assessing the predictive power of the different models.

4.1.vii FCal-CRP score:

Of 23 patients with mucosal inflammation; 10 had either an elevated FCal or CRP, 8 had both an elevated FCal and CRP, and in the remaining 5 patients both FCal and CRP were normal. Of 164 patients with no evidence of mucosal

inflammation; 109 had a normal FCal and CRP, 10 had a raised FCal and raised CRP, and 45 had either a raised FCal or raised CRP. See Table 4.8.

	Mucosal inflammation (23)	No mucosal inflammation (164)
Raised FCal or raised CRP, n (%)	10 (43.5%)	45 (27.4%)
Raised FCal and raised CRP, n (%)	8 (34.8%)	10 (6.1%)
Normal FCal and CRP, n (%)	5 (21.7%)	109 (66.5%)

Table 4.8. Results of FCal and CRP in patients with and without mucosal inflammation.

Based on logistic regression analysis, the FCal-CRP score was created and analyzed to assess its relationship with mucosal inflammation. FCal-CRP score was found to be very strongly associated with mucosal inflammation. The odds of mucosal inflammation in patients who scored 1 (i.e. had either a raised FCal or raised CRP) were 5 times increased compared to those with score of 0 (OR=5.48, 95%CI: 1.58-19.02), which means these patients are 5 times more likely to have mucosal inflammation than patients with normal serum and faecal inflammatory biomarkers. Furthermore, patient who scored 2 (i.e. had both a raised FCal and raised CRP) were 19 times more likely to have mucosal inflammation than those with normal biomarkers (OR=19.35, 95%CI: 4.70-79.64).

4.1.viii Discussion

This prospective study validates what was noted in our retrospective study that the majority of patients investigated for lower GI symptoms have normal Colonoscopy. If this cohort of patients can be identified by preliminary

screening tests that can predict the presence or absence of inflammation, unnecessary Colonoscopy procedures may be avoided, and the consequent available capacity used for patients who are likely to have an underlying organic diagnosis. Hence, this supports the use of non-invasive tests to investigate patients with lower gastrointestinal symptoms before considering further referral for more invasive tests. Initial non-invasive tests may allow for an early diagnosis of patients with alternative causes for their symptoms such as hyperthyroidism or Coeliac disease. Such patients can then avoid Colonoscopy and its associated potential complications.

The results show a significant association between frequent loose stool and the presence of mucosal inflammation. Thus, these patients can be more closely targeted with detailed testing using FCal and blood tests to determine whether they may have underlying inflammation which warrants an endoscopic examination.

The significant association of FCal and CRP with mucosal inflammation, especially in a paired model (FCal-CRP score) supports their use as initial screening tests in patients with lower GI symptoms to identify patients who need a Colonoscopy and those who can be reassured regarding the absence of mucosal inflammation or underlying organic disease without the need for invasive investigations.

In this prospective study, FCal had a high negative predictive value but relatively low positive predictive value. This is likely due to the low cut off value of FCal used to define a positive stool test. In this study and in clinical practice in our institution the cut off for raised FCal is $> 50 \text{ ug/g}$. This leads to a higher false positive rate but a lower false negative rate, which means more patients will be referred for further investigation but fewer cases of mucosal inflammation will be missed. Using investigation models that combine FCal and CRP with validated symptom-based questionnaires may result in higher sensitivity and specificity without increasing the false positive rate of the tests.

In our study, ROC curves were used to evaluate the predictive models extrapolated from the statistical analysis of the data. The ROC curve suggested that model 1 using age, gender, F Cal and the questions relating to frequent loose/ mushy stool and less frequent hard stool was the best out of all the created models at predicting mucosal inflammation, but it is still not ideal due to the small sample size. Repeating the analysis with a larger sample size may provide a stronger predictive model, however the major limiting factor to this is patient compliance with stool testing. Despite detailed face to face education and written information given to the recruited patients in this study, who consented to taking part, only 86.6% actually returned the stool sample. The compliance rate with providing stool samples is even lower in standard every day clinical practice. Henceforth, compliance will remain an obstacle to the wide spread utilisation of stool testing in clinical practice. Measures need to be taken to identify and tackle the reasons behind this compliance issue.

Although this study was designed to examine the use of non-invasive tests as a predictor of inflammation in a cohort of patients being referred for Colonoscopy, a proportion of patients in this study were adequately reassured by their blood and faecal tests and declined Colonoscopy. These patients reflect a large group of patients with functional symptoms referred for a specialist opinion to Gastroenterology units across the country. Furthermore, these patients may be managed in a primary care setting through the implementation of non-invasive testing pathways in primary care. These pathways may be used to triage patients who are likely to have functional symptoms and can thus be managed in primary care, or referred to a dietician-led functional GI clinic, and those who are likely to have underlying inflammation and require further investigation and management in secondary care.

4.2. Results of Study 3; Prospective study exploring the role of a novel non-invasive stool test (Faecal Cologuard™) in the assessment of older (>40 years) symptomatic and asymptomatic surveillance patients.

There were 81 patients identified as eligible and initially recruited for the study. All of these patients were given a Faecal Cologuard™ kit, educated by a physician on how to use the kit and scheduled for a Colonoscopy. However, only 50 patients completed the study. The age range was 42 years to 80 years with a mean of age of 61 years. The gender distribution was equally matched with 26 (52%) female patient and 24 (48%) male patients. The indications for colonoscopy were as follows; 17 (34%) patients had lower gastrointestinal symptoms (same exclusion criteria as outlined in Table 2.3) and 33 (66%) patients were asymptomatic and underwent a surveillance colonoscopy due to a history of adenomatous polyps or a family history of colorectal cancer (Table 4.9).

	Patient number, n & %
Total number of patients who completed the study	50
Median Age (years)	61
Female (n, %)	26 (52%)
Male (n, %)	24 (48%)
Indications for Colonoscopy:	
Lower gastrointestinal symptoms (n, %)	17 (34%)
Asymptomatic surveillance for CRC or polyps (n, %)	33 (66%)

Table 4.9. Patient demographics for study 3 (prospective Faecal Cologuard™ study)

Faecal Cologuard™ results were positive in 10 of 50 patients (20%) who completed the study. Four (40%) of these patients had adenomatous polyps at colonoscopy; 1 patient had a polyp > 10mm in size, and the remaining 3 patients had polyps ≤ 5mm in size. One patient had haemorrhoids, and 1 had angiodysplasia. No cases of CRC were diagnosed in this group.

Faecal Cologuard™ results were negative in 40 of 50 patients (80%) who completed the study; 9 (24%) patients had adenomatous polyps, only one of these had an advanced polyp ≥ 10mm in size. No cases of CRC were diagnosed in this group. Haemorrhoids were documented in 3 patients, and radiation proctitis in 2 other patients.

The NPV and PPV of Faecal Cologuard™ in this study were 77.5% and 40%, with a sensitivity and specificity of 30.8% and 83.8%, respectively (Table 4.10).

	Analysis of Faecal Cologuard™
Sensitivity	30.8%
Specificity	83.8%
PPV	40%
NPV	77.5%

Table 4.10. Results of correlating Faecal Cologuard™ with Colonoscopy outcome in study 3.

4.2.i. Discussion:

Faecal Cologuard™ performed poorly in this study of symptomatic and asymptomatic surveillance patients compared to previous studies performed in asymptomatic patients that reported overall sensitivity range of 92% - 98% for the detection of CRC by Faecal Cologuard™, with a sensitivity range of 57% - 92% for the detection of advanced polyps, which correlated with polyp size, with 87% overall specificity [187]–[189].

Our study has multiple limitations. It is important to note that a positive Cologuard™ results could be either due to the detection of DNA abnormalities or blood in the stool. Therefore, abnormalities that could bleed such as haemorrhoids or angiodysplasia could potentially lead to a false positive result. The most significant limitation is the small sample size, which was beyond the control of the investigators. A large sample size was intended at the start of the trial, however Exact Sciences® who provided the Cologuard™ kits unexpectedly withdrew from the trial due to technical difficulties encountered during the study. The test required a relatively large volume of faeces to be collected and transported to the laboratory in a short space of time. Some samples were insufficient, while others leaked during transport, and had to be repeated. Once the initial preparation was carried out in the Irish laboratory, the samples were frozen and transported on ice to the Exact Sciences® lab in the United States. Further difficulties arose in this process due to issues in U.S. customs, leading to delays in transport, rendering the samples inadequate. It is possible that extended preparation and transport process might have affected test accuracy leading to poor test performance. These issues would also arise if the faecal test was released into commercial use in Ireland, and this made it unfeasible for use in clinical practice in the country. Studies are under way exploring serum equivalents to detect DNA abnormalities suggestive of underlying colorectal cancer or advanced polyps.

4.3. Results of patient survey to assess faecal testing compliance

The anonymous surveys (Appendix 3) were distributed at the general gastroenterology clinics by the GI outpatient nurses over a 4-week period. 282 surveys were distributed to patients. Of these, 226 (80%) surveys were returned to the anonymous returns box in the outpatient department. Three surveys were excluded because they were incomplete. The remaining 223 surveys were stratified into two cohorts based on age group:

Group A contained 120 (53.8%) patients aged 50 years of age or less.

Group B contained 103(46.2%) patients aged more than 50 years.

The number of patients who had previously been asked to provide a stool sample by a medical professional was 87 (39%); of whom, 48 were in group A and 39 were in group B. There were 48 (55%) female patients and 39 (45%) male patients. The faecal test was completed by 81(93%) patients, with no statistically significant difference between the two groups. Of the 87 patients who had been asked to provide a faecal sample, 71 (82%) felt sufficiently informed about the indication of the faecal test, while 73 (84%) patients reported that they were given appropriate instructions on the collection and delivery of the sample.

The 6 patients who reported that they did not provide the faecal sample when requested, documented 2 main reasons;

1. They did not know how to collect the sample and found it technically difficult to do so.
2. They were uncomfortable with the idea of collecting a faecal sample.

4.3.i. Discussion

The results of this survey suggest a good compliance rate (93%) with stool testing which does not correlate with our previous clinical experience. This may indicate that patients who have not complied with providing stool tests were less likely to participate in the survey. The issues highlighted by the results of this survey arise from people's perceptions, beliefs, and attitudes which ultimately influence their behaviour.

In line with our results, a survey of South Asian women in England found that the most important factors affecting FOBt response related to the difficulty of collecting the stool specimen [202]. Other studies have also suggested that perceived provider attitudes play an extremely important role in how comfortable patients feel in returning a stool sample [203]–[205]. A qualitative study of patient perspectives on providing a stool sample to their GP found that barriers to stool collection included embarrassment, fear of results, concerns around hygiene and contamination, discretion and privacy, and lack of information [206]. Personal gain was identified as the main incentive to collecting and returning a stool sample. The need for an information leaflet on stool collection was emphasised by most patients.

Compliance with stool testing may be improved by better patient education in relation to what the test is for and how it can influence patient management to improve the quality of patient care. User friendly stool collection kits with gloves and information leaflet included could make it easier for patients to collect the sample especially where the patient is frail or debilitated. An opaque specimen bag with a clearly displayed drop-off box for specimens may reduce patient embarrassment regarding transporting the sample and handing it into the lab. People's perception and perspective can be improved by a public based strategy addressing attitudes, fears, and awareness of stool testing.

Chapter 5

General Discussion

5. General discussion:

Gastrointestinal complaints are very common in the general population, accounting for a large number of GP consultations and referrals for specialist assessment by a gastroenterologist or surgeon. There is an over reliance on invasive endoscopic procedures, with associated inherent risks, to investigate these patients, with the majority of them yielding a normal result. In most institutions demand continues to outstrip capacity leading to expanding waiting lists, which in turn can result in delay in significant diagnoses. This increase in demand is likely multifactorial. Some of these factors cannot be altered or influenced such as general population growth, and increase in life expectancy. Nevertheless, other factors may be amenable to modification such as rising prevalence of functional symptoms as a result of stress, dietary and lifestyle measures, and growing population fears of diseases and cancer. These factors may be addressed through national measures and policies to enhance health education of the general population and promote lifestyle and dietary improvements which ultimately impact on people's digestive system function and general well-being. Furthermore, the use of non-invasive screening tools in primary care as triage to identify patients likely to require further investigation and hence referral for a specialist opinion and those who can be reassured and managed in primary care.

We hypothesised that using sequential based testing combining non-invasive serum and faecal biomarkers may help identify patients likely to have underlying organic diseases and prioritise them for endoscopy. On the other hand, patients with normal biomarkers can be reassured without recourse to endoscopy. This in turn, avoids the risk of invasive procedures in patients who do not need them and optimises the demand for endoscopy to allow effective utilisation of resources and capacity.

In both our retrospective and prospective studies of symptomatic patients with lower gastrointestinal complaints, the majority of patients who underwent

Colonoscopy had no evidence of mucosal inflammation, especially those with non-diarrhoeal symptoms. In the retrospective review of young patients 93% of those with non-diarrhoeal symptoms and 79% of those with diarrhoeal symptoms had a normal Colonoscopy, and in our prospective study 90.4% of patients with non-diarrhoeal symptoms and 86.6% of patients with diarrhoea had normal Colonoscopy. The diagnostic yield of Colonoscopy for mucosal inflammation in older patient was also relatively low (10%). Therefore, Colonoscopy as a tool to investigate for underlying mucosal inflammation was unnecessary and may have been avoided in 80-90% of patients in our cohort if we used a non-invasive test that can accurately identify patients who are unlikely to have underlying inflammation (i.e. a test with a high negative predictive value and low false negative rate).

Furthermore, in both retrospective and prospective studies mucosal inflammation was significantly more common in patients with diarrhoea, suggesting that this group of patients may need more intensive investigation for possible underlying inflammation.

Non-invasive investigations include a plethora of tests that assess not only for potential inflammation but also other potential organic causes for the patient's symptoms. In our prospective study, two recruited patients were excluded from the analysis because they were found to have other organic causes for their symptoms. One patient was found to have Coeliac disease, and the other had hyperthyroidism. Both patients improved once their conditions were treated. These tests can be arranged in primary care prior to considering an appropriate specialist referral. In fact, 19 recruited patients in the prospective study declined Colonoscopy once they were reassured by their negative serum and stool tests, and reported improvement in their symptoms with dietary and lifestyle measures. This affirms that the application of non-invasive diagnostic models can play an important role in managing some patients without the need for Colonoscopy and may even facilitate the management of these patients in primary care.

A common inflammatory biomarker used in primary care is CRP which is not a reliable marker for the diagnosis of Colonic inflammation, but it is still useful in monitoring inflammation in patients known to have a raised CRP in response to their inflammatory burden. Although CRP performed poorly in the prospective study in terms of sensitivity and PPV, it had a relatively good NPV (89.5 – 92.1%). Although univariate analysis showed that mucosal inflammation was more likely to be found in patients with a high CRP ($p=0.042$), multivariate analysis using backward stepwise regression did not show a significant association between CRP and mucosal inflammation. Therefore, CRP would appear to perform poorly as a screening tool for inflammation in patients with lower GI symptoms. This study excluded those patients with blood in their stools, so while CRP may be a useful marker of inflammation in patients with overt blood and hence more severe inflammation, this was not investigated in this study. We purposefully focussed on patients with milder symptoms and no alarm symptoms, as these form the bulk of referrals to our, and most clinical services.

Faecal Calprotectin is a stool biomarker for gastrointestinal inflammation. It performed well in predicting the absence of intestinal inflammation in patients with both diarrhoea and non-diarrhoeal symptoms, with 79% and 90.7% NPVs respectively in the retrospective review, and 95.2% and 96.8% NPVs respectively in the prospective study. On the other hand, it seemed to be a poor predictor of inflammation when combined with CRP (i.e a raised FCal and/ or CRP was considered a positive result for the purpose of the analysis) in the retrospective review with 42% and 37.5% sensitivities in the two cohorts. This is likely due to low numbers of patients who completed the test and were included in the analysis in the retrospective review leading to an over reliance on raised CRP as a positive result in the analysis. FCal performed better in the prospective study with sensitivities of 76.5% and 80% in the two groups respectively. These are lower than the previously reported pooled sensitivity and specificity of 93% and 94% respectively, at a cut off level of 50 μ g/g using ELISA tests [137]. This is likely due to the relatively small total sample size and

small number of patients with mucosal inflammation in the study cohort. Moreover, both univariate and logistic regression analyses in the prospective study confirmed a significant association between mucosal inflammation and raised FCal, supporting its use in the assessment of patients with suspected inflammation.

FCal at a cut off level of 50 µg/g had a high NPV but relatively low PPV in the prospective study, which means that it is very good in identifying patients with no mucosal inflammation when used as a screening tool, but less good at accurately diagnosing intestinal inflammation in patient with a positive result of >50 µg/g. Consequently, fewer patients with mucosal inflammation will be missed at the cost of performing more Colonoscopy procedures on patients who do not have mucosal inflammation and hence did not need the invasive investigation. The PPV of FCal could be improved by increasing the threshold of a positive test to >100 µg/g, and this is supported by the results of logistic regression analyses in the prospective study. In patients ≤ 45 years of age, there was a significant association between inflammation and raised FCal, however this was only significant for FCal >100, OR = 6.46 (95% CI: 1.81-23.09). The risk of inflammation increased with unit increase in FCal OR=1.01 (95%CI: 1.003-1.013). The association was also noted to be stronger for FCal >100 µg/g when using gender stratification; for male patients, OR=33.51 (95% CI 4.41-254.74) and for female patients, OR=8.18 (95% CI 1.90-35.22).

While increasing the threshold of a positive FCal test can increase the PPV of the test, it runs the risk of increasing the number of false negative results (i.e. some patient maybe falsely reassured with a negative FCal result). This can be tackled by the use of investigation algorithms that incorporate multiple variables found to be strongly associated with mucosal inflammation in the study analyses. Using the combination of both FCal and CRP as a screening tool for the potential presence of mucosal inflammation (FCal-CRP score) can increase the predictive power of the tests; OR=5.48 (95%CI: 1.58-19.02) if only one of either FCal or CRP is raised compared to OR=19.35 (95%CI: 4.70-79.64) if both CRP and FCal were elevated.

A number of potential investigation algorithms were extrapolated from our prospective study. The algorithm with the most reasonable performance on ROC curve included age, gender, F Cal, and the questions relating to frequent loose/ mushy stool and less frequent hard stool. Even though it was better at predicting mucosal inflammation than the other models, it was still not ideal. Repeating the same analysis with a larger study size that has more patients with mucosal inflammation might potentially produce a model with more accurate classification algorithm.

FCal has been underutilised in clinical practice and steps should be taken to increase awareness and accessibility of the test. In the retrospective review of young symptomatic patients FCal results were only available in 15% of cases. This could be due to a combination of inadequate awareness of the test among health care professionals and poor patient compliance with the stool test. In the prospective study exploring the use of non-invasive biomarkers and symptoms based diagnostic questionnaire in patients with lower gastrointestinal symptoms only 86.6% of recruited patients performed the requested stool test despite receiving face to face education and written instructions about the test, including how to collect the sample, in addition to a follow up phone call and written reminder to complete the test. Therefore, patient compliance remains a limiting factor to its successful use in clinical practice.

It is important to highlight that using non-invasive tests to predict the absence or presence of inflammation is particularly useful in younger patients as this group had a low incidence of adenomas (6.4 - 8.7% ADR) and no cases of CRC in both the retrospective and prospective studies. On the other hand, older patients had a higher incidence of adenomas (24.7% ADR) and 5 cases of CRC in the retrospective study. Therefore, the non-invasive diagnostic pathways for older patients need to incorporate predictors not only for mucosal inflammation but also adenoma/ CRC using validated faecal tests with high sensitivity and specificity for CRC and advanced polyp detection such as Faecal Cologuard or FIT.

Faecal Cologuard performed poorly in our study of symptomatic and asymptomatic surveillance patients compared to previous studies, likely due to the unexpected small sample size and specific technical and logistic problems encountered during the study. This study was conducted in real time clinical practice which highlighted logistical obstacles to its current use in Ireland, including patient compliance and technical difficulties with sample preparation and transport to Exact Sciences® lab in the United States. Also, it is possible that extended preparation and transport process might have affected test accuracy leading to poor test performance. Studies are under way exploring serum equivalents to detect DNA abnormalities suggestive of underlying colorectal cancer or advanced polyps. An alternative validated option available in clinical practice is FIT testing. This has been shown in previous studies to have high diagnostic accuracy in detecting advanced adenomas and CRC [171], and is currently being used by the National Colon Cancer screening program in Ireland [178]. This test can be incorporated into the investigation algorithm of older patients in view of the increased prevalence of adenomas and CRC in this cohort.

As alluded to earlier in the discussion, the major limiting factor in both prospective studies has been patient compliance with providing a faecal sample. Accordingly, to try to understand this further, we performed an anonymous short survey in the Gastroenterology outpatient clinics to explore this further. The results of the survey revealed good compliance with faecal testing which does not correlate with previous clinical experience. This may be due to behavioural factors leading to biased return of the surveys by patients who are likely to provide a stool sample, while those who are less likely to comply with the investigation did not return the survey. In order to improve compliance, behavioural factors which are often influenced by people's beliefs, attitudes and perceptions must first be addressed. Steps have to be taken to enhance patient education in relation to what the test is for and how it can influence patient management to improve the quality of patient care. User friendly stool collection kits with gloves and information leaflet included could

make it easier for patients to collect the sample especially where the patient is frail or debilitated. An opaque specimen bag with a clearly displayed drop-off box for specimens may reduce patient embarrassment regarding transporting the sample and handing it into the lab. People's perception and perspective can be ameliorated by a national strategy addressing attitudes, fears, and awareness of stool testing.

The results of both the retrospective reviews and prospective studies undertaken for this thesis, looking at Colonoscopy outcomes and non-invasive tests in patients with lower gastrointestinal symptoms, indicate that demand on gastroenterology clinics and endoscopy services could be optimised by the use of non-invasive diagnostic pathways as a screening tool. Moreover, patients with negative non-invasive tests could potentially be reassured and managed in the primary care setting without the need for Colonoscopy or specialist referral. This could lead to reduced demand on finite endoscopy resources, leading to improved capacity to investigate patients who are likely to have an underlying significant pathology in a timely and expedient manner. This in turn could lead to reduced delayed diagnoses of cancer and earlier instigation of treatment for patients with serious conditions.

Currently faecal calprotectin testing is not available to many GP practices in Ireland because of constrained resources. This needs to be addressed and access to this test should be broadened.

Chapter 6

Conclusions

6. Conclusions:

Both the retrospective review of Colonoscopy outcomes and the *ad hoc* clinical use of non-invasive tests in symptomatic patients, and the prospective study exploring the use of non-invasive biomarkers in combination with symptom-based diagnostic questionnaire in patients with lower gastrointestinal symptoms confirmed the negative yield of most Colonoscopy procedures performed in this patient cohort, in particular patients with non-diarrhoea predominant symptoms.

A normal CRP and FCal have good diagnostic accuracy for the absence of intestinal inflammation (ie. high negative predictive value), supporting their potential role in the initial assessment of patients with lower GI symptoms. A raised FCal and to lesser extent raised CRP were significantly associated with mucosal inflammation. This association is stronger with every unit increase in FCal. Increasing the threshold of a raised FCal to >100 µg/g increases the diagnostic power of the test for the detection of inflammation.

The main questions in the symptom-based questionnaire that were strongly associated with mucosal inflammation were relating to the presence of frequent loose stool with abdominal cramps. Furthermore, diarrhoea as an indication for Colonoscopy was more strongly associated with intestinal inflammation. Therefore, patients with diarrhoea predominant symptoms should be the main focus for the use of non-invasive biomarkers for the detection of inflammation. Colonoscopy should be avoided as much as possible in young patients with non-diarrhoeal symptoms (Constipation, abdominal pain and bloating).

The predictive power of non-invasive investigations can be enhanced by combining variables significantly associated with inflammation as verified by the FCal-CRP score and ROC curve. A potential model of diagnostic algorithm extrapolated from our data, for young patient with diarrhoea predominant lower

GI symptoms is as follows; exclude Coeliac disease and hyperthyroidism, check FCal-CRP score, and if all of these are negative, do not refer for Colonoscopy, and consider further management in primary care setting. Another option is to set up functional GI clinics delivered by dieticians and potentially a psychologist, both with a special interest in FGIDs.

In older patients with diarrhoea predominant symptoms the same model can be applied, however further consideration has to be given to the risk of adenomatous polyps and CRC. The detection rate for advanced adenoma and CRC was higher in the older patient cohort, highlighting the role for using non-invasive biomarkers for the detection of potential advanced adenoma and/ or CRC in older patients referred with lower GI symptoms. Unfortunately, Faecal Cologuard performed poorly in our study, and does not appear to be a feasible test for use in Ireland with the logistical limitations of the current service provisions. There are alternative validated faecal biomarkers available such as FIT that can be added to the above diagnostic pathway for older patients regardless of the predominant symptom. This can be used to prioritize and expedite Colonoscopy for those with positive results especially with the current national constraints on resources and capacity limitations.

In summary, based on the analysis of our studies, we recommend the use of integrated investigation pathways that include a validated clinical symptom questionnaire, non-invasive serum and faecal inflammatory biomarkers in the initial assessment of patients referred with lower gastrointestinal symptoms prior to referral for Colonoscopy. This will allow the accurate triage of patients who require further invasive assessment with Colonoscopy and those who can be reassured without the need for endoscopy, or who may have an alternative organic cause for their symptoms.

The main limiting factor to the successful implementation of diagnostic pathways that include faecal biomarkers is patient compliance with stool

testing. We propose consideration of a national strategy to ameliorate public perception and attitudes

towards faecal testing. Further work is required to explore alternative serum biomarker for both intestinal inflammation and CRC/ advanced adenoma with reliable sensitivity and specificity.

Chapter 7

References

7. References:

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Appendix 1: IBS Questionnaire; modified World Gastroenterology Organisation Q [191]

Please circle the answer that best describes your symptoms

Q1. In the last 3 months, how often did you have discomfort or pain anywhere in your abdomen?

- a. All the time
- b. Most of the time
- c. Some of the time
- d. Never

(If your answer is never please skip to question 10 below)

Q2. Have you had this discomfort or pain 6 months or longer?

- a. No
- b. Yes less than 6 months
- c. Yes more than 6 months

Q3. For women only; did this discomfort or pain occur only during your menstrual bleeding and not at other times? Yes or no

Q4. How often did this discomfort or pain get better or stop after you had a bowel movement?

- a. All/most of the time
- b. Some of the time
- c. Never

Q5. When this discomfort or pain started did you have more frequent bowel movements?

- a. All/most of the time
- b. Some of the time
- c. Never

Q6. When this discomfort or pain started, did you have less frequent bowel movements?

- d. All/most of the time
- e. Some of the time
- f. Never

Q7. When this discomfort or pain started, were your stools (bowel movements) looser?

- a. All/most of the time
- b. Some of the time
- c. Never

Q8. When this discomfort or pain started, how often did you have harder stools?

- a. All/most of the time
- b. Some of the time
- c. Never

Q9. In the last 3 months, how often did you have hard or lumpy stools?

- a. All/most of the time
- b. Some of the time
- c. Never

Q10. In the last 3 months, how often did you have loos, mushy or watery stools?

- a. All/most of the time
- b. Some of the time
- c. Never

Q11. In the last 3 months, how often did you have difficulty having a bowel movement (straining, feeling that you have not finished)

- a. All/most of the time
- b. Some of the time

- c. Never

Q12. In the last 3 months, how often did feel that you had to rush to the bathroom as soon as you got the urge to have a bowel movement?

- a. All/most of the time
- b. Some of the time
- c. Never

Q13. In the last 3 months, how often did you feel bloated?

- a. All/most of the time
- b. Some of the time
- c. Never

Q14. In the last 3 months, how often did you feel that your abdomen/ belly was actually distended?

- a. All/most of the time
- b. Some of the time
- c. Never

Q15. In the last 3 months, how often did you feel that you had a problem with passing too much gas/wind?

- a. All/most of the time
- b. Some of the time
- c. Never

Q16. What is your age?

- a. Under 15 years
- b. Between 15 and 50 years
- c. Over 50 years old

Q17. Are any of the following diseases present in your family?

(Please give details of you relation to family member with the condition)

- a. Colon cancer yes or no

- b. Coeliac disease yes or no
- c. Inflammatory bowel disease (crohns or ulcerative colitis)? Yes or no

Q18. Were you recently treated with antibiotics? Yes or no

Q19. Have you –unintentionally- lost weight? Yes or no

Q20. Did you lose blood with your stools? Yes or no

Q21. Did your symptoms wake you up at night? Yes or no

Appendix 2. Faecal Cologuard Instructions:

What Should I Know Before Using the Cologuard Collection Kit?

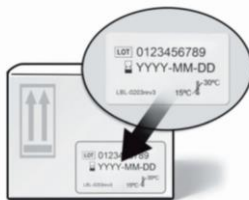


Only remove the items you need to collect your sample following the steps in this guide.

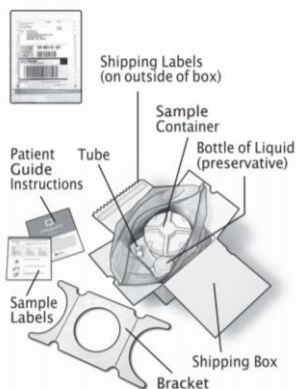
- **Leave the plastic bag inside the box.** The box, zippered bag and cardboard tray inside the bag will be used to send your sample to the lab.
- Use the kit before the expiration date printed on the side of the box.
- Avoid getting urine in the container when collecting your stool sample.
- Avoid getting toilet paper or other materials in the container when collecting your stool sample.
- **The lab must receive samples within 3 days.**
 - Collect a sample when you can ship your sample within a day of collection.
 - Make sure a holiday will not delay your shipment.
 - Review **Step 8: Ship Your Sample Using UPS** in this guide for detailed shipping information.

Step 1: Check the Expiration Date and the Kit

Check your kit to make sure the kit has not expired and you have all the parts of the kit.



- a. Check the expiration date on the outside of the box.
- b. Use the kit before the expiration date printed on the side of the box.
 - If the date has passed, do **not** use the kit. Close the box and contact Exact Sciences at 1-844-870-8878 for a new kit.



Leave the plastic bag and gray cardboard insert inside the box.

- c. Check the items in your box and make sure you have the following:
 - Bracket
 - Sample container
 - Tube
 - Bottle of liquid (preservative)
 - Shipping labels (attached to top of box)
 - Sample labels

If any items are missing from your kit, do **not** use the kit. Contact Exact Sciences at 1-844-870-8878 for a new kit.
 - d. If all items are included in your kit, your kit is ready to use.
- Follow the next steps when you feel ready to have a bowel movement.

Step 2: Prepare to Collect Stool Sample

Follow these steps in your bathroom to set up the bracket and stool sample container.



Timing
Your
Collection

- ✓ Ready to have a bowel movement
- ✓ Can ship sample within a day
- ✓ Use shipping schedule in Step 8

- a. Decide the best time to collect a stool sample.
 - Follow these steps when you feel ready to have a bowel movement.
 - Collect your sample on a day when you can ship your sample to arrive at the lab within 3 days.
 - o Ship your sample within a day of collection.
 - o See **Step 8: Ship Your Sample Using UPS** for a shipping schedule.
 - Do not collect a stool sample if you have:
 - o Bleeding hemorrhoids
 - o Bleeding cuts or wounds on your hands
 - o Rectal bleeding
 - o Menstrual period
 - o Diarrhea



b.

- b. Remove the bracket from the box and unfold the sides of the bracket



c.

- c. Raise the toilet lid and seat.



d.

- d. Place the bracket on the toilet as shown.
 - Place the bracket toward the back of the toilet.



e.

- e. Lower the toilet seat onto the bracket.
 - The entire opening of the bracket should be visible.



g.

- g. Turn the container lid and unscrew it.

- h. Set the container lid down.



i.

- i. Place the container into the top of the bracket.

Step 3: Collect the Stool Sample

- Make sure you have help if you have trouble sitting and standing when using the toilet.



- a. Sit on the toilet and have a bowel movement in the container.
- Try to keep urine from going into the container.
 - Do **not** put toilet paper or other items into the container.



- b. When your bowel movement is complete, stand up.
- c. Lift the stool sample container from the bracket and set the container on a flat, stable surface.
- Leave the container open.



- d. Remove the bracket from the toilet.
- The bracket can be recycled or thrown in the trash.



- e. Finish using the bathroom if needed.
- Follow the rest of the steps in this guide **immediately** after collecting your stool sample.

IMPORTANT: Complete the next step **before** you close the container.

Next Page

Step 4: Scrape the Stool Sample

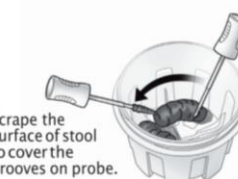
You must scrape the stool sample with the probe to get another small sample for the Cologuard test.



- a. Lift the tube out of the box.



- b. Turn the white tube cap and unscrew it.

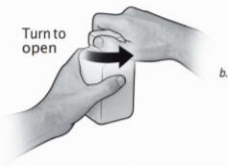


- c. Pull the probe from the tube.
- You may have to pull hard.

- d. Scrape the surface of your stool sample until the end of the probe has stool on it.
- Your stool sample may look different from the stool sample pictured.

Step 5: Prepare Stool Sample Container for Shipping

The stool sample must have a preservative poured onto it to make sure the lab can test it. Then, the container must be closed tightly for shipping.



b.

- a. Lift the bottle of liquid preservative out of the box.

- b. Hold the bottle and turn the cap to unscrew it.



Caution

- Do **not** drink the liquid.
- If the liquid from the bottle touches your skin or eyes, wash with water.



c.

- c. Pour **all** the liquid in the bottle into the container with the stool.
- The empty bottle and cap can be recycled or thrown in the trash.

Turn to close tight

e.

d. Hold the stool sample container on a hard, flat surface.

Tight seal
Correct!

f.

e. Place the lid on top of the container.

- Make sure the lid is straight so it will close tightly.

Lid with gap
Wrong:
loosen and tighten again

f. Turn the lid to tighten until it does not tighten anymore.

Step 6: Label Your Samples

You **must** label your samples to identify them. For best results, use a ballpoint pen and write the labels on a hard, flat surface.



- a. Find the page **Label Your Samples** that is included with this guide.

EXAMPLE

John
First Name

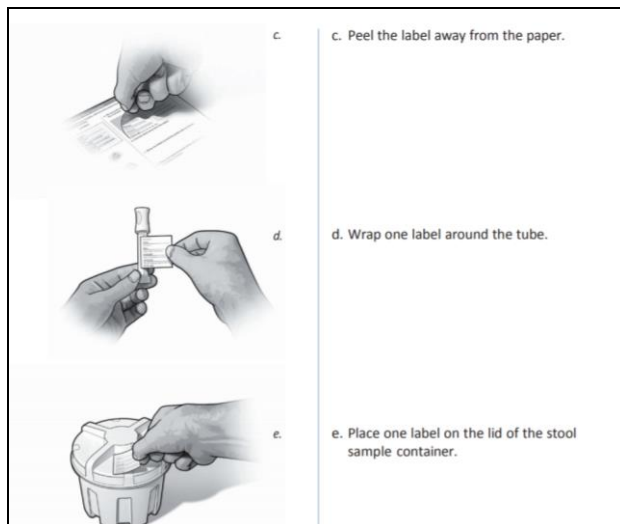
DOE
Last Name

05/16/99
Date of Birth (mm/dd/yy)

03/12/13
Date of Collection (mm/dd/yy)

09:15 AM
Time of Collection (0000) AM/PM

- b. Fill out **both** labels. **Print** the information in this order:
- Your first name
 - Your last name
 - Your birthdate (MM/DD/YY)
 - The date you collected your stool sample (MM/DD/YY)
 - The time you collected your stool sample



Appedix 3. Patient survey:

Gastroenterology Department, anonymous Patient Survey:

1. Please tick your (a) Age group 15-29 years

30-50 years

>50 years

(b) Gender Female

Male

2. Have you ever been asked by a doctor or nurse to provide a stool sample for testing?

Yes

No

(If your answer is No, please return the form to the yellow box now)

3. If yes, did you provide the sample?

Yes

No

4. Did you receive adequate information on what the test is for?

Yes

No

5. Did you receive adequate information on how to collect the sample?

Yes

No

6. If you were asked for a stool test but did not do it, which of the statements below best describes your reason:

- a. I did not know how to collect the sample
- b. I was not given enough information on why it was needed
- c. I am uncomfortable with obtaining and/or providing a stool sample
- d. I forgot to do it
- e. Other, please specify: