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Optimisation of Faecal Immunochemical Test (FIT) for Symptomatic Colorectal Cancer Diagnosis

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COVID-19 Impact Statement

The COVID-19 pandemic had a profound impact on my research, influencing various aspects of the work in a variety of ways. The pandemic brought significant disruptions to how my research was conducted and to the delivery of work alongside increased clinical commitments.

The pandemic necessitated a re-evaluation of my data collection methods. Travel restrictions, social distancing measures, and safety concerns made in-person data collection for clinical research projects extremely challenging. We had to adapt our clinical research plans, incorporate remote recruitment, and ensure the research complied with evolving ethical standards for pandemic-related research. These changes all ultimately brought about delays in completing our clinical research projects as originally envisaged.

The arrival of COVID-19 in March 2020 also brought changes to my clinical commitments. I joined a departmental COVID-19 rota for emergency general surgery work, which demanded more time be spent on-call at Nottingham University Hospitals and that I be available for work on a shadow rota when colleagues were unwell. Further details explaining how COVID-19 affected my studies are explained throughout Chapter 7 discussing my work.

In addition to clinical commitments, my personal circumstances also became more challenging during the pandemic. In May 2020 my mother was diagnosed with breast cancer. Nottingham's breast unit were able to offer treatment options not possible in Cornwall, so she came to live with me for the duration of her treatment, culminating with surgery in May 2021. This situation contributed additional stress given the social circumstances at the time, and certainly made focus on research work more challenging at times.

In summary, the COVID-19 pandemic had a significant impact on my research work, compelling me to adjust my methods, rethink my research objectives, and adapt to a rapidly changing academic and global landscape.

Abstract

Colorectal cancer (CRC) is one of the most common cancers in the UK and a leading cause of cancer death. Despite concerted efforts to detect more cases at an earlier stage in asymptomatic patients, most diagnoses are still made in patients with symptoms of the disease. The clinical features which typically prompt investigation for suspected CRC include a persisting change in bowel habit, iron deficiency anaemia, weight loss, presence of an abdominal mass or pain, and rectal bleeding or a rectal mass. The Two-Week-Wait (2WW) pathway was introduced as part of the National Health Service (NHS) Cancer Plan in 2000 to streamline the investigation and management of those at highest risk of cancer, and therefore improve cancer-related mortality. Referral on an urgent 2WW pathway is determined by age and symptom-based criteria set out by the National Institute for Health and Care Excellence (NICE) national guidelines. Unfortunately, the clinical features described are often vague and non-specific, and are more likely to be associated with benign disease than CRC.

The Faecal Immunochemical Test (FIT) is an investigation that identifies microscopic amounts of human haemoglobin in a stool sample which can indicate the presence of CRC. FIT has become the test of choice for the Bowel Cancer Screening Programme (BCSP) and has been endorsed by NICE guidelines to guide urgent secondary care referral in patients with "low-risk symptoms". Recently, FIT has been shown to accurately stratify risk of CRC across all symptom groups and can therefore be used to facilitate prioritisation of patients for invasive investigations.

This thesis seeks to explore how FIT can be optimally utilised to detect CRC in symptomatic patients within a 2WW pathway. Chapter 1 introduces the topic and gives context to the clinical pathways in Nottingham, where FIT has been incorporated since 2017.

The first study presented in this thesis in chapter 2 is an observational study reporting a service evaluation of the Rapid Colorectal Cancer Diagnosis (RCCD) pathway in Nottingham. Two years after the inception of the RCCD, FIT was shown to accurately stratify risk of CRC with excellent "rule out" performance at the lowest threshold. CRC detection in patients with a FIT less than 4 µg Hb / g faeces was 0.1%, and less than 0.3% for those with a FIT below 20 µg Hb / g faeces. Furthermore, patients with a faecal haemoglobin

(f-Hb) over 100 µg Hb / g faeces had a 20.7% risk of CRC, corroborating the value of FIT to identify patients who require urgent investigation.

The association between iron-deficiency anaemia and CRC is well established, but other blood tests can also identify those at risk of cancer. In chapter 3, a retrospective review of 2WW referrals between August 2014 and August 2017 examines the presence of thrombocytosis as an independent risk factor for CRC. A multivariate logistic regression analysis showed that patients with thrombocytosis were significantly more likely to have CRC than those with normal platelet values (OR 2.62, 95% CI 1.6-4.3). CRC diagnosis was significantly higher in males with thrombocytosis (16.1% vs 7.9%, χ^2 4.62, p=0.032) and females (10.3% vs 2.9%, χ^2 19.41, p<0.001), confirming the stratification value of thrombocytosis in a 2WW population.

In chapter 4, an observational study of a 2WW population in Nottingham is presented, evaluating the value of symptomatology in the assessment of symptomatic patients. 1784 patients were included in this analysis, with 181 CRC detected. CRC was diagnosed in 3.5% (24/684) with CIBH compared to 8.1% (6/74) with both CIBH and IDA. No individual or combination of referring clinical features were associated with an increased diagnosis of CRC (χ^2 8.03, p=0.155). 3 patients with negative FIT results (<4 µg Hb / g faeces) were diagnosed with CRC (3/1027, 0.3%). The highest proportion of cancers detected was in the ≥100 µg Hb / g faeces group (55/181, 30.4%). In the multivariate model presented, FIT outperformed age, gender and all symptoms prompting referral. FIT has greater stratification value than any referral symptoms and demonstrated value in patients with IDA.

Chapter 5 evaluates whether there are sociodemographic variations in the uptake of FIT when used in a primary care symptomatic pathway for CRC. A retrospective study of 38920 patients referred from primary care over a 4-year period was completed, with multivariate regression analysis performed to identify disparities in the age, sex, ethnicity and socioeconomic status of those referred for the investigation of CRC. Males accounted for 44% of the study population and had a significantly lower FIT return on multivariate analysis (OR 1.11, 95% CI 1.03-1.19). FIT return was significantly higher in patients ≥65 years compared to those aged 18-64 years (adjusted OR 0.78, 95% CI 0.72-0.83). The multivariate model showed the most socially deprived patients had more than double the rate of unreturned FIT compared to the least deprived (OR 2.20, 95% CI 1.99-2.43). Patients from

Asian (OR 1.82, 95% CI 1.58-2.10), Black (OR 1.21, 95% CI 0.98-1.49) and Mixed/Other ethnic groups (OR 1.29, 95% CI 1.05-1.59) were also more likely to not return their FIT kits compared to the White ethnic group after adjustment. This confirmed that FIT return varies by gender, age, ethnicity, and socioeconomic deprivation.

Chapter 6 is an examination of whether repeat FIT samples confer added diagnostic value compared to a single sample. A prospective clinical study evaluated 44 patients recently diagnosed with CRC by serially collecting FIT samples over a 4-week period. 4/44 (9.1%) of the first samples returned were below the local 20 µg Hb / g faeces threshold. Of the 4 who returned a falsely low FIT at this threshold, none of them returned a second sample which was below this positivity threshold.

Chapter 7 constitutes a summary discussion of the work undertaken for each chapter of this body of work and reports strengths and limitations of the thesis. Finally, a discussion of future research work required in the field of FIT for symptomatic patients is offered in Chapter 8.

This thesis provides evidence contributing towards the optimal utilisation of FIT for the investigation of CRC in symptomatic patients. Within this work the stratification value of FIT in both “high-risk” and “low-risk” symptoms is confirmed. Thrombocytosis is confirmed as an independent risk-factor for CRC which can be used synergistically with a haemoglobin level, ferritin, and FIT result to more accurately stratify risk of CRC. This thesis has presented evidence of sociodemographic variation in the uptake of FIT across a symptomatic population in Nottingham for the first time, identifying a need for strategies to mitigate differential impact as the use of FIT in primary care expands. Finally, the validity and added diagnostic performance potentiated by a further FIT sample is described, to further minimise the risk of a false-negative leading to a missed cancer.

Publications

1. GP access to FIT increases the proportion of Colorectal Cancers detected on urgent pathways in symptomatic patients
   Bailey JA, Khawaja A, Andrews H, Weller J, Chapman C, Morling J, Oliver S, Castle S, Simpson JA, Humes DJ, Banerjea A.
   The Surgeon. 2021 Apr;19(2):93-102.
   doi: 10.1016/j.surge.2020.03.002. Epub 2020 Apr 20. PMID: 32327303.
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2. FIT and blood tests for prioritisation of urgent colorectal cancer referrals in symptomatic patients: a two-year evaluation
   Bailey JA, Weller J, Chapman CJ, Ford A, Hardy K, Oliver S, Morling JR, Simpson JA, Humes DJ, Banerjea A.
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3. Thrombocytosis helps to stratify risk of colorectal cancer in patients referred on a Two-Week Wait Pathway
   Bailey JA, Hanbali N, Premji K, Bunce J, Simpson J, Humes DJ, Banerjea A.
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4. Quantitative FIT stratification is superior to NICE referral criteria NG12 in a High-Risk Colorectal Cancer population
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   doi: 10.1007/s10151-021-02466-z. Epub 2021 Jul 14. PMID: 34263362.
   Chapter 4 of thesis
   Citations: 8 Almetric: Unknown

5. Sociodemographic Variations in the Uptake of Faecal Immunochemical Tests (FIT) in a Primary Care Symptomatic Pathway for Colorectal Cancer
   Bailey JA, Jones J, Chapman CJ, Oliver S, Morling JR, Banerjea A, Humes DJ
   British Journal of General Practice. 2023 Sep.
   https://doi.org/10.3399/BJGP.2023.0033
   Chapter 5 of thesis
   Citations: 3 Altmetric: 13

Presentations

International

1. Post-Faecal Immunochemical Test (FIT) Colorectal Cancer Outcomes: Evaluation of a Symptomatic Pathway at 2 Years
   Association of Coloproctologists of Great Britain and Ireland (ACPGBI) Regional Meeting, 21 September 2020
   Oral presentation

2. Evaluation of Inequalities in Non-Responders to FIT in a Symptomatic Pathway for Colorectal Cancer
   16th Scientific and Annual Conference of the European Society of Coloproctology (ESCP), 22-24 September 2021
   Virtual Presentation

3. 3-Year Evaluation of Faecal Immunochemical Test (FIT) for Prioritisation of Urgent Colorectal Cancer Referral in Symptomatic Patients
   United European Gastroenterology (UEG) Week, 3-5 October 2021
   Virtual Presentation

4. Sociodemographic Variations in the Uptake of Faecal Immunochemical Tests (FIT) in a Primary Care Symptomatic Pathway for Colorectal Cancer
   17th Scientific and Annual Conference of the European Society of Coloproctology (ESCP), 21-23 September 2022
   Oral Presentation

5. Keeping Fit: A Comparison of Variable Fit Thresholds with a Single Cut-Off for Patients with Symptoms of Colorectal Cancer (CRC) in Nottingham Primary Care
   17th Scientific and Annual Conference of the European Society of Coloproctology (ESCP), 21-23 September 2022
   Poster Presentation

National

6. FIT and Blood Tests for Prioritisation of Urgent Colorectal Cancer Referrals in Symptomatic Patients: A Two-Year Evaluation
   British Society of Gastroenterology (BSG) Campus, 21-29 January 2021
   Virtual Presentation

7. FIT stratification in the COVID era - Is it safe for rectal bleeding?
   Association of Surgeons of Great Britain and Ireland (ASGBI) Virtual Congress 2021, 4-8 May 2021
   Virtual Presentation and Abstract Publication (DOI:10.1093/bjs/znab311.043)

Regional/Institutional

8. Utilisation of Faecal Immunochemical Test (FIT) For the Diagnosis of Colorectal Cancer in Symptomatic Patients
   University of Nottingham, Postgraduate Research Sandpit competition, 29 June 2021
   Oral Presentation – 1st Prize

9. Evaluation of Inequalities in Non-Responders to FIT in a Symptomatic Pathway for Colorectal Cancer
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   Oral Presentation – 2nd Prize

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Glossary of Terms

µg – Micrograms

µg Hb / g faeces – Micrograms of Haemoglobin per gram of faeces

2WW – Two-Week-Wait

5-FU – Fluorouracil

ACPGBI – Association of Colo-Proctologists of Great Britain and Ireland

AI – Artificial Intelligence

ANOVA – Analysis of Variance

APC – Adenomatous Polyposis Coli

ARMS – Amplification Refractory Mutation System

BCSP – Bowel Cancer Screening Programme

BJS – British Journal of Surgery

BRAF – Proto-oncogene B-Raf

BSG – British Society of Gastroenterology

CCG – Clinical Commissioning Group

CEA – Carcinoembryonic Antigen

cfDNA – Cell-Free Deoxyribonucleic Acid

CG27 – Clinical Guidance 27 "Referral Guidelines for Suspected Cancer", National Guidelines, NHS England, 2005

CI – Confidence Interval

CIBH – Change in Bowel Habit

COSD – Cancer Outcomes and Services Dataset

COVID-19 – Coronavirus Disease 2019

CRC – Colorectal Cancer

CRM – Circumferential Resection Margin

CT – Computed Tomography

CTC – Computed Tomography Colonography

CTCs – Circulating Tumour Cells

ctDNA – Circulating Tumour Deoxyribonucleic Acid

DCC – Deleted in Colorectal Cancer (oncogene)

DG30 – National Diagnostics Guidance, NHS England, 2017

DNA – Deoxyribonucleic Acid

DoH – Department of Health

DOI – Date of Issue

EBCSH – Easten Bowel Cancer Screening Hub

erBB2 – Epidermal Growth Factor Receptor B2 (oncogene)

ESCP – European Society of Colo-Proctology

F12 – System One referral template. Electronic Healthcare records system.

FAP – Familial Adenomatous Polyposis

FBC – Full Blood Count

FFPE – Formalin-Fixed Paraffin-Embedded

f-Hb – Faecal Haemoglobin

(f-Hb) – Faecal Haemoglobin Concentration

FIT – Faecal Immunochemical Test

FITS Study – Faecal Immunochemical Test in Surveillance Study

FITTER – checklist for the reporting of studies using FIT for haemoglobin

FOB Gold – Proprietary FIT-based occult blood test

FOBT – Faecal Occult Blood Test

g – Gram

GCP – Good Clinical Practice

GDG – Guideline Development Group

gFOBT – Guaiac based Faecal Occult Blood Test

GP – General Practitioner

Hb – Haemoglobin

HM-JACKarc – Proprietary FIT-based occult blood test

HNPCC – Hereditary Non-Polyposis Colorectal Cancer

HOT_ARMS – Optimised Polymerase Chain Reaction Amplification Refractory Mutation System

ICD – International Classification of Diseases

ICE – Electronic Healthcare system used by NHS trusts

IDA – Iron Deficiency Anaemia

IMD – Index of Multiple Deprivation

IoD19 – Index of Deprivation tool 2019

IQR – Inter Quartile Range

KRAS – Kirsten Rat Sarcoma Virus gene (oncogene)

LBO – Large Bowel Obstruction

MCED – Multi-Cancer Early Detection

MDT – Multi Disciplinary Team

MRI – Magnetic Resonance Imaging

MVA – Multivariate Analysis

myc – Regulator gene Myc (oncogene)

NCT – National Clinical Trial

NEQAS – The United Kingdom National External Quality Assessment Service

NG12 – Suspected cancer: recognition and referral. National guideline, NHS England, 2015

NHS – National Health Service

NICE – The National Institute for Health and Care Excellence

NIHR – National Institute for Health and Care Research

NPV – Negative Predictive Value

NUH – Nottingham University Hospitals

NUhCLEUS – Nottingham based software system

OC – Other Cancers

OC-Sensor™ – Proprietary FIT-based occult blood test

OR – Odds Ratio

PCR – Polymerase Chain Reaction

PIK3CA – phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha gene (oncogene)

PPV – Positive Predictive Value

RAS – Rat Sarcoma Virus gene (oncogene)

RCCD – Rapid Colorectal Cancer Diagnosis

REC – Research Ethics Committee

RfPB – Research for Patient Benefit

SEM – Standard Error of the Mean

SES – Socioeconomic Status

src – Tyrosine-protein kinase Src (oncogene)

STT – Straight To Test

TC – Treatment Centre

TNM – Tumour, Node, Metastasis

TX – Texas

UK – United Kingdom

UKAS – United Kingdom Accreditation Service

USA – United States of America

UVA – Univariate Analysis

VOC – Volatile Organic Compounds

WHO – World Health Organisation

χ^2 – Chi Squared

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Chapter 1 – Introduction

This thesis will present an evaluation of a clinical pathway for the Faecal Immunochemical Test (FIT) as an investigation for Colorectal Cancer (CRC). The use of FIT in clinical practice requires a well-designed and implemented clinical pathway to ensure that patients are properly informed, tested and managed.

This thesis will evaluate the effectiveness of the Nottingham clinical pathway in which FIT has been incorporated since 2017, and its evolution with regards to FIT positivity thresholds, the inclusion of blood test results, and patient concordance. By providing insights into the benefits and limitations of FIT within a clinical pathway, this thesis aims to inform policy and practice decisions related to the investigation of CRC in patients with symptoms.

1.1 – Epidemiology

Colorectal cancer (CRC) is one of the most common cancers in the UK and worldwide. Globally there are 1.3 million cases of CRC detected each year[1], accounting for approximately 10% of all cancer diagnoses. The disease is the second most common cancer in women and the third most common in men[1]. Rates of CRC vary geographically, with developed countries reporting a far higher risk than developing nations[2]. CRC is the second most deadly cancer worldwide, with an estimated 881000 deaths reported in 2018[1].

In the United Kingdom (UK), CRC is the third most common cancer and the second leading cause of cancer death, with approximately 43000 cases and more than 16000 deaths each year (2016-2018 average)[3]. Approximately 23900 of new cases in the UK were in males while females accounted for 19000 new cases[3]. Although advances have been made in the identification and management of CRC with concomitant improvements in mortality, the overall incidence in the UK remains largely unchanged over the past 20 years[4, 5].

1.2 – Aetiology

The clinical entity of colorectal cancer represents the culmination in a sequence of changes that occur at a sub-cellular level for many years before diagnosis. CRC may be classified according to the origin of these changes as either sporadic, inherited, or familial[6]. Sporadic cancers account for over 70% of all cases. Approximately 5% of cases are related to inherited syndromes causally associated with the disease, while the remaining 20% represent diagnoses in patients with a family history of CRC in a first-degree relative – without an identifiable genetic mutation[6].

Sporadic cases originate from a heterogenous group of genetic and epigenetic changes acquired through life and influenced by inherent genetic factors and environmental effects. The primary event in 70-90% of CRC is a mutation in the Adenomatous Polyposis Coli (APC) tumour suppressor gene of a stem-cell or stem-cell-like-cell in the base of colonic crypts[7], which initiates the formation of adenomatous polyps[2]. Secondary genetic events leading to RAS activation and/or p53 dysfunction potentiate further morphological changes of the aberrant colonic crypts leading to the progression of the adenoma and ultimately malignant transformation in a sequence described as the "Adenoma-Carcinoma Pathway"[8]. Advances in molecular techniques have revealed a far more complex network of gene mutations (including myc, ras, src, erBB2 and DCC) involved in sporadic CRC carcinogenesis than when this model was initially described[6, 9-12], but the premise that transformed adenomatous polyps are responsible for the majority of CRC cases remains true. The serrated neoplasia pathway describes a pathway where precursor lesions are hyperplastic, serrated polyps, with carcinogenesis mediated by early mutations in KRAS and BRAF, highlighting the varied landscape of sporadic cancers that has emerged[13]

Inherited cancers involve the inheritance of a mutation affecting one of the alleles of a relevant gene (such as those involved in cell growth regulation, tumour suppression and DNA repair), leaving the other allele vulnerable to a spontaneous point mutation which then precipitates the first changes of carcinogenesis[14]. Inherited cancers may be further subdivided based on the presence and number of adenomatous and hamartomatous polyps[15].

Familial Adenomatous Polyposis (FAP) is an inherited autosomal dominant condition associated with the development of hundreds of colonic polyps, as well as duodenal polyps and other extracolonic features. The basis of FAP is a mutation on the long arm of chromosome 5 (5q21) affecting the APC gene previously described[16].

In contrast to FAP, Lynch syndrome (formerly known as Hereditary Non-Polyposis Colorectal Cancer [HNPCC]) is an autosomal dominant condition not associated with polyposis (although polyps may also arise). Lynch syndrome is also associated with extracolonic malignancies as a consequence of mutations in DNA repair mechanisms, including endometrial, ovarian, gastric, and urinary cancers, among others[17]. A feature of Lynch syndrome not seen in sporadic CRC is the presence of microsatellite instability. Microsatellites are short repetitive DNA sequences which exist in thousands of areas of coding, non-coding and regulatory sections of the genome[18]. Microsatellite instability is not unique to Lynch syndrome, with loss of mismatch repair genes also caused by spontaneous events[19].

1.3 – Identification of CRC

Given the formidable global health challenge that CRC represents, optimal strategies for detection of the disease are imperative. The importance of early detection in CRC cannot be overstated, as it significantly influences prognosis and therapeutic outcomes[3, 4]. The detection of CRC may be broadly divided into asymptomatic and symptomatic categories. Asymptomatic patients are diagnosed by participation in screening programmes for the disease, while symptomatic patients may be diagnosed via several routes depending on their symptomatology. In 2020, The National Bowel Cancer Audit Report stated that of all patients diagnosed with CRC the previous year, 54% were diagnosed following referral by a General Practitioner (GP). 19% of CRC were detected following an emergency presentation to hospital, and less than 10% detected through the NHS Bowel Cancer Screening Programme (BCSP) for asymptomatic patients [20].

Early detection is pivotal in CRC management, as it allows for the identification and removal of precancerous polyps and early-stage malignancies, thereby preventing progression to advanced disease. The adenoma-carcinoma sequence described in section 1.2 which describes the transformation of normal epithelial cells into adenomatous polyps and subsequently into invasive carcinoma, underscores the potential for early intervention. Identification of these lesions at an early, non-invasive stage through screening can substantially reduce CRC incidence and mortality[21].

1.3.1 – Definitive Investigations

The diagnostic workup resulting in CRC detection depends largely on the presentation of the patient. The Association of Colo-Proctologists of Great Britain and Ireland (ACPGBI) guidelines recommend that histological diagnosis should be achieved prior to surgery for all CRC when possible[22]. Furthermore, complete staging of disease should be completed in non-emergent situations to ascertain the full anatomical extension of a neoplasm and identify any metastatic disease.

Endoscopic assessment completed via colonoscopy is the gold-standard investigation for diagnosis of CRC[23]. Colonoscopy allows direct visualisation of the colon to identify lesions and permits sampling of any lesion to provide histological diagnosis. Advanced

endoscopic techniques have also enabled therapeutic interventions for curative treatment of advanced adenoma and early-stage cancers[24]. Although colonoscopy is generally a safe procedure[25], it can be challenging to perform and unpleasant for the patient undergoing the procedure[26], as well as being associated with infrequent but serious complications[27-29]. These factors, in conjunction with resource limitations in diagnostic services, are pertinent considerations for clinical pathways responsible for the safe investigation of symptomatic patients[30, 31].

CT colonography (CTC) is an alternative "whole-colon" investigation which may be used to diagnose CRC. CTC uses low radiation Computed Tomography (CT) scanning to obtain and interior view of the colon, and may have a similar sensitivity to colonoscopy in the detection of CRC[32]. Although bowel preparation is still required for CTC, the procedure is generally considered to be safer than colonoscopy and more easily tolerated. CTC has the added benefit of being able to detect extraluminal disease where colonoscopy does not, which may be of particular use for elderly patients with vague or non-specific symptoms where alternative malignancies are also being considered[33].
Contrast-enhanced CT scanning of the chest, abdomen and pelvis is required in the staging of CRC to assess for metastatic disease. Guidelines recommend that all rectal cancers are pre-operatively staged with high resolution MRI to evaluate the circumferential resection margin (CRM) and guide operative planning.

1.3.2 – Staging

Systems to describe and classify the determinants of CRC survival have existed since British pathologist Cuthbert Dukes devised his eponymous classification system for rectal cancer in 1932 [34]. Although now superseded by modern staging systems, Dukes was the first to identify local and lymphatic spread as important prognostic indicators. The best-known modern staging system for CRC is the TNM system, which is based on 3 pieces of information – the size of the tumour (T) and its extension into the bowel wall; the spread of the cancer to nearby lymph nodes (N), and the spread of the cancer to distant sites (M)[35]. The goal of standard TNM assessment is to inform on the expected prognosis of disease, to aid with treatment planning and facilitate standardisation of treatment across different healthcare units. Full TNM classification details are summarised in table 1.

Whilst TNM staging provides important prognostic information, survival rates vary within each staging stratum. High-risk tumour characteristics include lymphovascular invasion and involvement of disease at tumour margins after a resection. The presence of these characteristics confers a poorer prognosis than identically staged disease without those factors – and is associated with increased risk of local and systemic tumour recurrence following treatment[36].

Table 1 – Full TNM Classification

pT – Primary Tumour

TX – Tumour cannot be assessed

T0 – No evidence of tumour

Tis – Carcinoma in situ. The cancer is at its earliest stage and only in the mucosa.

T1 – Invasion of submucosa

T2 – Invasion of muscularis propria

T3 – Invasion into subseroa/non-peritoneal pericolic or peri-rectal tissue

T4 – Subdivided into 2 stages, T4a and T4b:

T4a – Invasion into the serosa/visceral peritoneal surface

T4b – Invasion into nearby organs

pN – Regional Lymph Node Involvement

NX – Regional lymph node involvement cannot be assessed

N0 – No regional lymph node involvement

N1 – Involvement of 1-3 regional lymph nodes

N2 – Involvement of 4 or more regional lymph nodes

pM – Distant Metastatic Disease

MX – Distant metastatic disease cannot be assessed

M0 – No distant metastatic disease

M1 – Distant metastatic disease

Although the primary focus of this body of research is the utilisation of FIT in symptomatic patients, to appreciate the opportunities and challenges associated with FIT in this patient population it is important first to consider the evolution of screening for cancer, the history of screening for CRC and the emergence of FIT from more rudimentary occult blood testing for the disease.

1.3.3 – Asymptomatic Cancer Detection

The origins of cancer screening in the UK can be traced to the 1940s when Dr. George Papanicolaou established the effectiveness of the Pap smear test for detecting cervical cancer at an early, more treatable stage [37]. This innovation gained recognition over time, paving the way for future screening programmes as technological breakthroughs enabled reliable disease prevention programmes. Experimental work led to the development of screening for breast cancer in the 1960s and 1970s, with studies demonstrating the efficacy of mammography to detect breast cancer at earlier, more treatable stages[38].

Although important foundation work for screening programmes was undertaken as early as the 1940s, it was 1968 when the World Health Organisation (WHO) published what would become a landmark report on screening authored by James Wilson (Principal Medical Officer at the Ministry of Health in London, England) and Gunner Jungner (Chief of the Clinical Chemistry Department of Sahlgren's Hospital in Gothenburg, Sweden). Wilson and Jungner outlined principles to provide a robust framework for evaluating the appropriateness and efficacy of screening programmes[39]. These criteria have endured as a cornerstone of public health policy and continue to guide the implementation of effective screening initiatives. The core tenets of “Jungner's criteria” can be summarised as follows:

- Public health significance: The targeted condition must represent a significant public health burden, demonstrably impacting morbidity and mortality rates.
- Availability of effective treatment: A cornerstone of successful screening is the existence of efficacious treatment for the identified condition, particularly in its early stages.

- Diagnostic and treatment infrastructure: Adequate healthcare infrastructure is essential, ensuring confirmatory diagnosis and timely treatment for those identified through screening.
- Detectable early stage: The targeted condition should possess a recognisable pre-symptomatic or early symptomatic phase, allowing for intervention before significant clinical manifestations.
- Valid and acceptable screening test: The screening test must be demonstrably reliable, with high sensitivity and specificity, while also remaining culturally and socially acceptable to the target population.
- Natural history understanding: There must be a comprehensive understanding of the natural history of the disease in question, encompassing its progression from latent to overt stages, to inform optimal screening intervals and treatment protocols.
- Treatment guidelines: Clear and well-defined treatment guidelines, based on screening results, are essential for ensuring appropriate patient management.
- Cost-effectiveness: The economic implications of a screening programme necessitate a cost-effective approach, balancing the programme's cost with potential healthcare savings and improved patient outcomes.
- Continuous process: Screening programmes should be seen as ongoing initiatives, continuously identifying new cases and adapting to evolving knowledge and technologies.

Adherence to these principles can ensure that screening programmes are implemented strategically, maximising their public health impact while remaining fiscally responsible and promoting evidence-based decision making.

The late 20th century and early 21st century saw the establishment of systematic cancer screening protocols in Great Britain, including the NHS Cervical Screening Programme[40], NHS Breast Screening Programme[41], and NHS Bowel Cancer Screening Programme, which will be discussed in greater detail in section 1.3.4.

1.3.4 – Colorectal Cancer Screening

As described in section 1.1, CRC has emerged as one of the leading causes of cancer-related mortality, prompting public health authorities to explore strategies for its early detection and prevention[1]. Epidemiological insights, technological advancements, and evidence from clinical trials have progressively fulfilled aspects of Jungner's Criteria over recent decades to advance the argument for CRC screening as well as guiding its implementation.

There are several CRC screening tests available, broadly categorised into non-invasive stool or blood tests and more invasive imaging or endoscopy procedures. No single method is universally recognised as the optimal approach for CRC screening, leading to diverse strategies across different countries[42, 43]. The selection of a screening method depends in part on financial and endoscopic resources, as well as the population's willingness to undergo the screening test. Consequently, due to resource limitations and a preference for non-invasive tests, many organised screening programs adopt a two-step approach. It is important to consider the merits of non-invasive screening and invasive endoscopy-based screening methodologies to fully appreciate the landscape of CRC screening.

1.3.5 – Faecal Occult Blood Testing (FOBT)

Rectal bleeding is a well-established symptom which may be associated with CRC[44]. The presence of blood in faeces can serve as an effective marker for CRC because as colorectal neoplasms grow, they can erode the lining of the colon or rectum, leading to bleeding. This bleeding can be intermittent and may not always be visible to the naked eye, hence the term "occult" blood. Pre-malignant polypoidal neoplasms and early CRCs are also susceptible to bleeding, which creates a viable target that may be detectable by non-invasive means[43]. Testing for blood in the stool is a relatively simple and non-invasive procedure compared to other diagnostic methods such as colonoscopy and can be easily performed at home, not requiring specialised medical equipment.

Guaiac based tests emerged more than a century ago as a tool for detecting gastric blood loss stemming from ailments like peptic ulcers and gastric cancer, which afflicted a significant portion of the population[43]. In the 1970s, guaiac-based faecal occult blood tests (gFOBTs) were the inaugural method for widespread CRC screening initiatives. Operating on the principle of guaiac-impregnated paper coupled with hydro-peroxidase, these tests triggered a

chemical reaction upon contact with haem, resulting in a distinct blue discoloration—a qualitative indicator of blood presence. In its traditional form, the standard gFOBT comprised three paper cards, each housing two panels, necessitating sampling from three separate stool specimens.

Although FOBTs using a variety of methodologies were available from the 1970s[45, 46], the popularisation of gFOBT based CRC screening arose in the 1990s as several studies reported a reduction in the disease specific mortality of CRC[47-50]. The promising results from these studies led to several large-scale Randomised Controlled Trials (RCTs) in Funen, Goteborg, Minnesota and Nottingham which confirmed a reduction in CRC mortality with gFOBT-based screening, as well reporting a general shift towards the identification of earlier stage CRC with comparison groups[51-54]. The compelling evidence that repeated annual or biennial screenings correlated with a substantial reduction in CRC-related deaths was confirmed by a comprehensive Cochrane Systematic Review in 2007 which reported a pooled 15% reduction in CRC-related mortality compared to control groups[55]. This statistical synthesis underpinned the significance of gFOBT in combating CRC mortality.

In 2006, gFOBT was introduced as a means of population-based screening for CRC for those aged 60-74 years in England[56]. Unfortunately, gFOBT is vulnerable to false-positive results from a multitude of sources. In particular, haem derived from dietary sources (red meat), or from catalase activity (from ingested vegetables) may catalyse the oxidation reaction. False-negative results are not uncommon either, either from antioxidant activity inhibiting the reaction (particularly Vitamin C), or from the quantity of blood being insufficient to cause the colour change[57]. Limitations in the clinical efficacy of gFOBT ultimately incited the search for improvements to occult blood testing methodology which will be discussed in detail in section 1.4.

Due to limited resources and a preference for non-invasive screening among the population, many organised screening programs adopt a two-step approach. This approach involves initial screening with a non-invasive faecal test, followed by endoscopic evaluation for individuals who test positive. However, as stated above, there is no global consensus for the optimal screening methodology, and concurrent work into endoscopy-based screening in the 1990s added to uncertainty in this field.

1.3.6 – Endoscopy screening

Increasing knowledge base of the natural history of CRC[58] alongside advancements in techniques using optical colonoscopy[59] potentiated studies seeking to improve CRC mortality by the identification of cancerous lesions via endoscopic means before they became symptomatic, and the identification and removal of pre-cancerous lesions before malignant transformation[60, 61]. These studies were undertaken and reported concurrently with much of the work undertaken into FOBT screening.

Although the early studies reported above suggested that direct visualisation of lesions in a screening capacity represented a stepwise improvement in the management of adenomas and CRC compared to the erstwhile haphazard diagnostic landscape[47], concerns were raised about the paucity of evidence from prospective studies to support the efficacy of endoscopy-based screening[62] as well as the associated costs, and discomfort of sigmoidoscopy[63].

An evaluation of a once-only flexible sigmoidoscopy at 60 years old for prevention of CRC through identification of and removal of premalignant adenomas was one such prospective trial undertaken in the UK[64]. This study sought to confirm the validity of the screening methodology adopted in the United States of America (USA) at that time, where both annual FOBT and screening by flexible sigmoidoscopy every 3 to 5 years were recommended from 50 years old. It was felt that the benefit from the screening policy in the USA could be gained from a single flexible sigmoidoscopy examination at age 55 to 60 years with appropriate colonoscopy-based surveillance for the 3% to 5% found to have high-risk adenomas[64]. A subsequent multi-centre randomised controlled trial determined that once-only flexible sigmoidoscopy was “acceptable, feasible, and safe”[65].

Subsequent years saw the expansion and refinement of colorectal cancer screening strategies in the UK. In 2010, the results of the UK Flexible Sigmoidoscopy Screening Trial further supported an endoscopy-based screening methodology. This large-scale trial demonstrated the effectiveness of flexible sigmoidoscopy screening in reducing both the incidence of colorectal cancer and mortality rates. The findings provided compelling evidence for the inclusion of flexible sigmoidoscopy as a screening modality within the NHS BCSP.[66]

The invasive nature of endoscopy-based screening raises concerns around whether potential harms are mitigated by a possible decrease in disease specific morbidity or mortality. Whilst

colonoscopy is the gold standard investigation for the detection of colorectal cancer, it is not without risks and costs to both the NHS and patient[28]. Bowel perforation is the most serious complications and may require urgent surgical intervention. Other risks include post-procedural bleeding, cardiovascular events and effects related to the bowel preparation required[29].

A report of the outcomes from the BCSP in England after the first 1 million tests provided a comprehensive analysis of the effectiveness and impact of a gFOBT based screening protocol[56, 67]. The programme successfully identified a substantial number of early-stage bowel cancers, with early detection rates were markedly higher compared to the rates observed prior to the BCSP inception in 2006. Approximately 2% of those screened had positive results from the FOBT, necessitating further diagnostic procedures like colonoscopy. Among those referred for colonoscopy, about 10% were diagnosed with cancer, highlighting the effectiveness of FOBT as an initial screening tool. The report also highlighted the preventive aspect of the BCSP with a significant number of high-risk adenomas being identified. Overall report found that the BCSP not only improved cancer survival rates through early detection but also contributed to raising public awareness about bowel cancer and the importance of regular screening, underscoring the importance of organised screening programs in combating bowel cancer. In the past decade, efforts have been made to enhance the effectiveness and accessibility of colorectal cancer screening in the UK. It is this drive that led to the introduction of FIT on the CRC detection landscape.

1.3.7 – FIT in Screening

Faecal Immunochemical Tests for haemoglobin (FIT/FITs) are immunological assays which rely on the use of polyclonal antibodies to bind to the globin moiety of human haemoglobin[43].

FIT products have two main designs using different analytical techniques: lateral flow immunochromatographic analysis and immunoturbidimetric analysis[68]. Most qualitative FIT products are point-of-care tests used by clinicians outside a laboratory. These tests employ the lateral flow immunochromatographic system, similar to pregnancy tests and other point-of-care tests for drugs and hormones. This system separates soluble haemoglobin from faeces via lateral passive flow along a separation material, where antibodies capture the

haemoglobin and make it visible through various visualisation methods. The FIT systems that have been endorsed by NICE for use to detect CRC include the OC-Sensor (Eiken Chemical Co., Ltd., Tokyo, Japan), HM-JACKarc (Kyowa Medex Co., Ltd., Tokyo, Japan) and FOB Gold (Sentinel Diagnostics, Milan, Italy) FIT platforms, all of which utilise immunoturbidimetry principles to generate a quantitative result.

Faecal samples can degrade rapidly due to enzymatic activity and bacterial growth. The stabilizing buffer present in FITs help to preserve the integrity of the sample by preventing degradation of the analytes (haemoglobin) that is being assessed. The buffer also helps to minimise interference from substances present in the faecal sample that might affect the accuracy of the test results and standardises sample handling and processing which is crucial for ensuring consistent and reliable test results across different samples and testing instances[69].

FITs have a multitude of advantages over the gFOBT. Most FITs have a simple to use faecal specimen collection device which permits far easier sample collection for patients and does not require multiple samples from sequential bowel movements. As human haemoglobin is the target molecule of the antibodies used in FIT, the test is unaffected by dietary constituents and more specific for lower gastrointestinal bleeding. The quantitative FITs endorsed by NICE are more sensitive investigation than the gFOBT, with haemoglobin concentrations as low as 2 µg Hb / g faeces detectable by modern FIT systems, compared to comparative levels of 150 µg Hb / g faeces required to generate a positive FOBT result[70, 71].

The improved diagnostic performance of FIT led to it being adopted as the non-invasive test of choice in the BCSP[21]. Interest in the application of FIT in symptomatic patients increased after publication of NG12 in 2015[72], which will be discussed in greater detail in the proceeding section. Several peer-reviewed articles were published concerning the utilisation of FIT for patients with low-risk symptoms, as well as early study outcomes evaluating FIT for a broader set of symptoms than described in NICE guidelines[73-75] and systematic reviews on the topic [76-78].

1.4 – Identification of CRC in Symptomatic Patients

For patients with symptoms, the clinical features which trigger presentation and investigation are varied, depending largely on the site of the tumour and its size. Presenting complaints may include a persistent change in bowel habit; weight loss; rectal pain, rectal bleeding, or rectal mass; abdominal pain or abdominal mass, iron-deficiency anaemia or voiding symptoms such as tenesmus[2, 79]. Patients with left-sided cancers in the descending colon, sigmoid and rectum may more frequently present with a change in bowel habit or rectal bleeding, while right-sided cancers arising in the caecum and ascending colon are more typically associated with non-specific features like iron-deficiency anaemia and weight loss[80].

Patients diagnosed after emergency presentation to hospital may have a combination of these features, or may present with the urgent complications of CRC, including acute large bowel obstruction (LBO) or bowel perforation[81]. Emergency presentation of CRC is associated with poorer outcomes, necessitating strategies to minimise diagnosis through this route[82, 83].

1.4.1 – Two-Week-Wait Pathways

The Two-Week-Wait (2WW) pathway was first implemented in July 2000, as part of the NHS England Cancer Plan published by the Department of Health[84]. The policy sought to improve the efficiency of cancer diagnosis in the UK, reduce delays experienced by patients, and ultimately improve cancer survival. National policy mandated that all patients suspected by their GP of having cancer should be seen by a specialist within two weeks of the date of referral, with guidelines developed to support GPs decide who should be referred based on "higher risk criteria". The criteria set out by Clinical Guidelines 27 (CG27) for urgent referral were (1) rectal bleeding with a change in bowel habit, (2) a change in bowel habit in those over 60 years old, (3) persistent rectal bleeding in those over 60 years old, (4) a palpable abdominal mass, (5) a palpable rectal mass, or (6) unexplained IDA in men/postmenopausal women[85, 86]. The NHS England Cancer Plan estimated that these clinical features would identify 85-90% of all patients with CRC presenting to their GP[87]. Subsequent iterations of

the guidelines have sought to identify more patients at risk of CRC based on their age and symptomatology.

Over the past decade, organisations such as Cancer Research UK have launched campaigns to increase public awareness of symptoms which could represent CRC, such as "Be Clear on Cancer", which encouraged patients to attend their GP as soon as possible if they had the "key symptoms" of rectal bleeding or a change in bowel habit lasting at least 3 weeks [88, 89]. Unfortunately, whilst those "key symptoms" may represent the most common symptomatology experienced in CRC, they are also extremely common in primary care, and far more likely to be caused by benign conditions[78], necessitating further strategies to identify those requiring urgent investigation for colorectal cancer.

1.4.2 – NG12 – Suspected cancer: Recognition and Referral, 2015

Suspected cancer: Recognition and Referral [72] was published by NICE in 2015, replacing previous national guidelines CG27 for referral of suspected cancer. NG12 provided recommendations for healthcare professionals on the recognition and referral of suspected cancer in children, young people, and adults. The guideline aimed to ensure that patients with potential symptoms of cancer were identified and referred promptly to specialists, thereby improving diagnostic timeliness and patient outcomes. The key points of NG12 included guidance on identifying symptoms and signs that could indicate cancer (symptom recognition), criteria for urgent referrals to specialists based on specific symptoms and patient factors (referral recommendations), pathways for primary care practitioners to follow when cancer is suspected (diagnostic pathways), and information for patients and families about the referral process and what to expect (supportive information). The recommendations were developed using a "risk threshold" to determine whether referral and investigation was warranted. In consideration of the financial and clinical costs of broadening referral recommendations, the Guideline Development Group (GDG) agreed to use a 3% Positive Predictive Value (PPV) threshold value to underpin the recommendations for suspected cancer pathway referrals in adults[72].

NG12 perpetuated the age and symptom-based criteria established at the inception of 2WW, mandating urgent referral for anyone (1) aged 40 years and over with unexplained weight loss or abdominal pain, (2) aged 50 years and over with unexplained rectal bleeding, (3) aged 60

years and over with IDA or (4) change in bowel habit, or (5) with tests showing occult blood in their faeces. Additional advice to consider referral based on those with an abdominal or rectal mass or a combination of the aforementioned symptoms in younger adults was included[72]. This represented the first iteration of national guidelines to include occult blood testing (described further Chapter 1.5) for patients with symptoms.

The guidelines advised that occult blood testing could be considered for "low-risk" patients with symptoms not otherwise satisfying the criteria for referral to identify those who may be at higher risk of CRC. However, the guidelines did not specify the platform of occult blood testing to be used, nor did they include a recommended threshold to be considered a "positive" result which merited referral.

1.4.3 – DG30 – Quantitative faecal immunochemical tests to guide referral for colorectal cancer in primary care, 2017

NICE published a new diagnostic guidance document in 2017 to support the previously published NG12 [90]. "Quantitative faecal immunochemical tests (FIT) to guide referral for colorectal cancer in primary care" specifically recommended that the OC Sensor (Eiken Chemical Co., Ltd., Tokyo, Japan), HM-JACKarc (Kyowa Medex Co., Ltd., Tokyo, Japan) or FOB Gold (Sentinel Diagnostics, Milan, Italy) quantitative FIT platforms should be used to guide referral for people with suspected CRC without rectal bleeding, who do not otherwise meet the criteria for a 2WW referral. This document represented the first-time national guidelines designated that FIT should be utilised for patients with symptoms of CRC, albeit restricted to "low-risk" symptoms where their age and symptoms suggested a PPV for CRC less than 3%. A positivity threshold of 10µg Hb / g faeces was recommended based on a review of evidence from symptomatic FIT studies available at the time.

1.4.4 – DG56 – Quantitative faecal immunochemical testing to guide colorectal cancer pathway referral in primary care, 2023

NICE Diagnostics Guidance DG56, published in August 2023, provided updated recommendations for using quantitative faecal immunochemical testing (FIT) to guide colorectal cancer referral pathways in primary care[91]. This guidance built on previous

guidance (DG30) to improve early detection and maximise efficient use of healthcare resources.

DG56 stated that FIT should be used to guide referrals for suspected colorectal cancer in adults presenting with an abdominal mass, change in bowel habit, iron-deficiency anaemia, unexplained weight loss, abdominal pain, or rectal bleeding. Specifically, it recommended quantitative faecal immunochemical testing (FIT) using HM-JACKarc or OC-Sensor platforms to guide referral for suspected colorectal cancer in adults:

- with an abdominal mass
- with a change in bowel habit
- with iron-deficiency anaemia
- aged 40 and over with unexplained weight loss and abdominal pain
- aged under 50 with rectal bleeding and either of the following unexplained symptoms:
    - abdominal pain
    - weight loss
- aged 50 and over with any of the following unexplained symptoms:
    - rectal bleeding
    - abdominal pain
    - weight loss
- aged 60 and over with anaemia even in the absence of iron deficiency.

DG56 recommended that adults should be referred for colorectal cancer if their FIT result is at least 10 µg Hb / g faeces, consistent with the previous positivity threshold recommended in DG30. It stated that those with results below this threshold or who did not return a sample should be managed with safety netting processes and clinical judgment.

The guidance also called for additional research on higher thresholds for FIT, dual FIT usage, FIT in younger populations, and the impact of conditions or medications that increase gastrointestinal bleeding risk. Previous work in Nottingham reported large variations in faecal haemoglobin concentrations (f-Hb) when different FIT devices were used, and that analyser-specific (f-Hb) cut-offs may be valuable for clinical decision making[92]. DG56 recommended additional work to assess the diagnostic performance and effectiveness of

different FIT platforms. It was also highlighted that further research was needed to improve access and return rates for FIT samples, particularly in less engaged groups.

The guidance reported cost-effectiveness of using FIT at specified thresholds, allowing better allocation of colonoscopy resources. It emphasised that FIT can help prioritise patients who are most likely to have colorectal cancer, reducing unnecessary procedures for those at lower risk.

1.5 – Nottingham Clinical Pathway

1.5.1 – Pilot/Inception of FIT

After the inclusion of occult blood testing in NICE NG12, Nottingham University Hospitals (NUH) NHS Trust became one of the first centres in the UK to utilise FIT for symptomatic patients in 2016. In December 2015, a collaborative 'Getting FIT' working group was established with local GPs and commissioners, the Bowel Cancer Screening Hub, and Nottingham Colorectal Service. In September 2016, Nottingham City, Nottingham West, Nottingham North and East, and Rushcliffe Commissioning Groups commissioned "Getting FIT", thereby incorporating FIT as a triage tool in the 2WW pathway[93]. Early clinical results showed a PPV of 53.7 for those with the highest FIT results (≥ 150 µg Hb / g faeces), including patients with both "high-risk" and "low-risk" symptoms[93, 94].

1.5.2 – Description of Pathway

After a successful pilot, FIT was incorporated as a mandatory investigation for all referrals to secondary care – to identify those at highest risk of CRC who could benefit from expedited urgent investigations. Patients with rectal bleeding or palpable rectal mass were referred without FIT triage, given the high PPV recorded for these symptoms in the "Getting FIT" pilot, and consistent with the guidelines set out in NG12. At its inception, a twelve day "window" was maintained to alleviate fears expressed by the working group of FIT-associated delays compromising the speed of urgent referrals in the nascency of the pathway. Prospectively recorded audit data for the trust showed that FIT was not associated with delaying the patient journey through urgent referral and as such, this window was closed in June 2019. The pathway is displayed below in Figure 1. Concerns were raised about the ability of FIT to capture all colorectal cancers. Adjuncts to the diagnosis of colorectal cancer including blood markers such as thrombocytosis and anaemia were explored to improve the detection rate within the pathway.

Symptomatic FIT requests in Nottingham are administered by GPs, who have the option to request a Rapid Colorectal Cancer Diagnosis (RCCD) Pathway referral contemporaneously with the FIT request or await the FIT result and act accordingly. Patients with a FIT raised above the 10 µg Hb / g faeces threshold were triaged through to an urgent secondary care

review, and those with a FIT in the highest category (≥150 µg Hb / g faeces in the pilot and ≥100 µg Hb / g faeces thereafter) were contacted by the Straight-To-Test (STT) team which was previously shown to be an effective adjunct to the 2WW pathway[95].

Outcomes from Nottingham's pathway were continually reviewed using prospectively collated FIT results to determine the thresholds used locally, as recommended by NG12. In April 2020, the lower threshold for "FIT positivity" was raised to 20 µg Hb/g faeces, as the CRC risk in those with FIT results lower than this was consistently shown to be significantly lower than the NG12 risk threshold for urgent investigation. The updated pathway can be seen represented below in Figure 1. To mitigate the risk of a false-negative FIT, a lower threshold of 4 µg Hb / g faeces was applied for patients with other risk factors for CRC, such as anaemia or an abnormal ferritin. As part of this monitoring process return rates of FIT results were examined. There was a concern that patients with certain protected characteristics may not return kits and as a result experience delays in diagnosis as non-completion of a test resulted in a potential barrier to 2WW referral[96]. Similar disparities in use of FIT tests had been identified in National screening programmes[97].

1.5.3 – Use of FIT in Rectal Bleeding

Patients with rectal bleeding were not eligible for FIT stratification, in-keeping with NG12. In Nottingham between 2016 and 2020, patients with rectal bleeding were triaged to a secondary care review for direct assessment with flexible sigmoidoscopy. The notion was that rectal bleeding would cause faecal haemoglobin levels to be raised in all cases, eroding any stratification value of the test. However, studies evaluating the performance characteristics of FIT in patients with rectal bleeding subsequently confirmed FIT was safe to use in rectal bleeding and conferred useful stratification value [98, 99]. The absence of faecal haemoglobin in rectal bleeding still functions as an excellent "rule-out" test for CRC and thus was adopted in Nottingham's clinical practice in 2020.

Figure 1 – Clinical Pathway for secondary care referral November 2017 – April 2020 Pathway as originally introduced in November 2017: FIT used in all groups other than those with rectal bleeding or rectal mass (in Blue). All other symptoms eligible for FIT (in Red): Primary pathway on left where GPs request and action FIT independently. Secondary pathway on right (in Red) where GP's submit referral form and request FIT concomitantly. Referrals were initially held in a "window" until results were available up to maximum of 12 working days – this was closed with local agreement in June 2019.

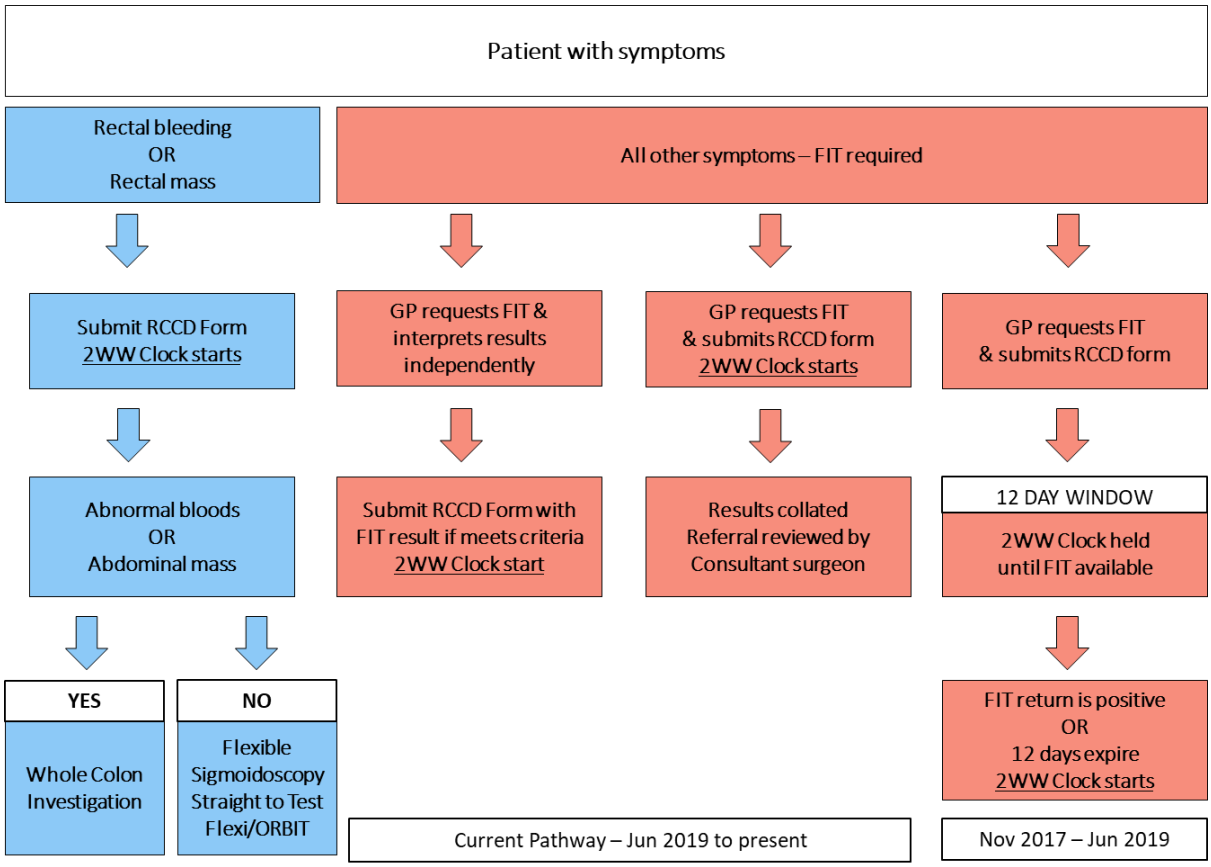
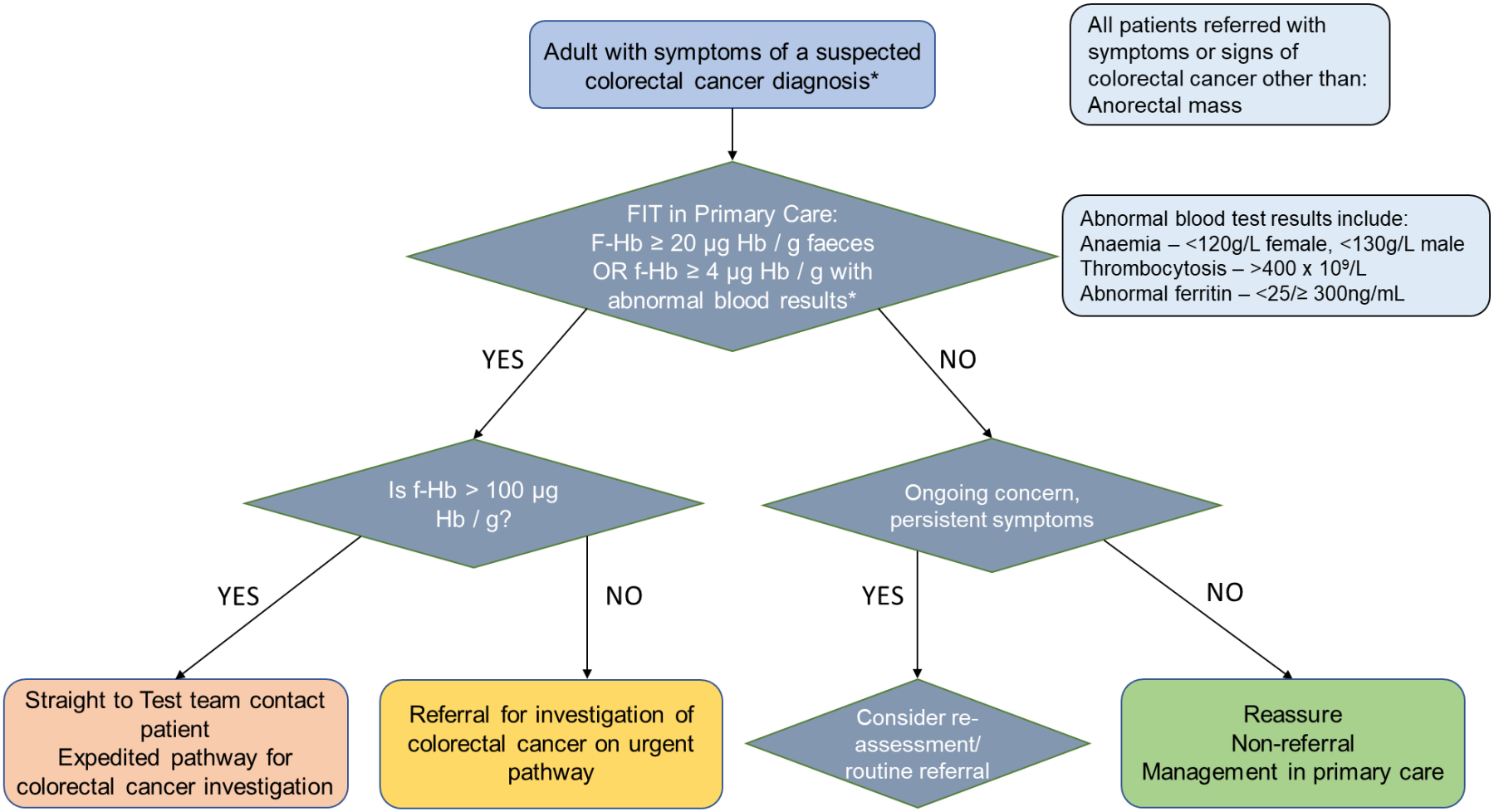


Figure 2 – Clinical Pathway for secondary care referral April 2020 to present

Pathway as of April 2020: FIT used in all symptom groups including with rectal bleeding.



1.6 – Thesis Aims and Chapter Outlines

- Assess the performance of Nottingham's Rapid Colorectal Cancer Diagnosis (RCCD) pathway and describe the clinical outcomes 2 years since its inception.

- Explore the utility of blood tests to aid in the identification of CRC in the RCCD pathway.

- Evaluate the stratification value of thrombocytosis for the detection of CRC in a symptomatic population.

- Compare the diagnostic value of clinical features within the NG12 referral criteria with FIT stratification.

- Evaluate sociodemographic variation between the base population of Nottinghamshire and patients referred for suspected CRC diagnosis on the RCCD pathway.

- Evaluate the sociodemographic variations in the return of FIT samples in an urgent suspected CRC pathway.

- Determine the occurrence of a falsely negative FIT result in the presence of CRC.

- Assess the diagnostic performance of additional FIT samples in the presence of CRC.

Chapter 2 – Faecal immunochemical testing and blood tests for prioritisation of urgent colorectal cancer referrals in symptomatic patients: a 2-year evaluation

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2.1 – Introduction

Colorectal cancer (CRC) is a common cancer diagnosis with over 40,000 new diagnoses each year and the second commonest cause of cancer death in the UK[100]. Improving outcomes remains a key healthcare policy aim[76]. Current criteria for urgent referral to secondary care are largely based on age and symptoms[101], but Bowel Cancer Screening Programme (BCSP) has demonstrated that CRC, and particularly early stage CRC, is often asymptomatic[67]. Faecal immunochemical testing (FIT) has replaced guaiac- based faecal occult blood testing (gFOBT) in the screening programme across the UK. The thresholds for positivity in the screening programme in England (120 ug Hb/g faeces) and Wales (150 ug Hb/g faeces) are higher (less sensitive) than in Scotland (≥ 80 µg Hb/g faeces) and many other countries around the world, and have been chosen to mitigate the demand on over-burdened diagnostic capacity in the NHS[21].

FIT has been shown to have value in patients with symptoms[74, 75, 93, 102-108] and in 2015, National Institute for Health and Care Excellence (NICE) guidance recommended testing for occult blood in faeces in low risk patients[101] and subsequently recommended a threshold of 10µg Hb/g faeces specifically in this context[109]. In September 2016, a locally commissioned year-long pilot of FIT in the two week wait (2WW) population (excluding those with rectal bleeding) was introduced and demonstrated clear stratification value in all symptom groups judged to be "high risk" by the local Primary Care colleagues[93]. The value of simple measures such as stratification of anaemia[110-112] and thrombocytosis[113, 114] from a Full Blood Count (FBC) has been also confirmed in the same local population. In November 2017, a Rapid Colorectal Cancer Diagnosis (RCCD) Pathway incorporating direct General Practitioner (GP) access to FIT and use of FIT, FBC and Ferritin results for "rule in", "rule out" and "first test" selection in secondary care was introduced[104]. This study aims to evaluate the CRC diagnoses from the first two years of this pathway stratified by FIT level[115].

2.2 – Methods

Rapid Colorectal Cancer Diagnosis Pathway (RCCD)

This "locally agreed" pathway was designed to incorporate FIT as a triage tool for all referral criteria in adult patients of any age, except rectal bleeding and rectal mass, as described elsewhere[104, 115], presenting to local GP practices within our catchment area. GPs were able to request FIT (and blood tests) independently and act on the result, or if clinical suspicion was high, they could submit an RCCD referral form contemporaneously. In the latter pathway, the form was held for 12 working days in a "window" and the 62-day clock only started either on receipt of FIT (and blood) results or on expiry of the "window". The outcomes from this pathway have been evaluated prospectively and in June 2019 the "window" was no longer required after local agreement that GPs were familiar with the pathway and contemporaneous audit data supported this change.

FIT requests and tests

FIT requests in Primary Care were made on an electronic request system (ICE) that also prompted requests for blood tests where indicated. Results were notified on the same electronic system with text guidance on how to interpret results and subsequent actions. An electronic guidance system F12 (SystemOne) was also used to guide GPs on the use of FIT and the new pathway in those practices that use this system, with direct links to the relevant referral form where appropriate. FIT dispatch and return were entirely postal and kits were analysed according to manufacturer's protocols as described elsewhere by an accredited BCSP Hub laboratory (Appendix I) [93, 104, 115]. The OC-Sensor™ platform (Eiken Chemical Co., Tokyo, Japan) was used as previously described.

Patients referred with a rectal mass were not subject to FIT testing but were seen in a one-stop flexible sigmoidoscopy clinic. Patients with rectal bleeding and no other symptoms and no anaemia were also seen in a one-stop clinic, as well as some patients with rectal bleeding deemed unlikely to be fit for colonoscopy at straight to test (STT) vetting of referrals[95]. Patients diagnosed with cancer in this one-stop pathway could have Computed Tomography (CT) Colonography (CTC) as part of their staging to exclude synchronous lesions if appropriate. This pathway has traditionally excluded rectal bleeding because CRC detection rate approaches 10% in this group locally, and the use of flexible sigmoidoscopy mitigates colonoscopy demand.

FIT, FBC and Ferritin (or Iron studies) were mandated for all other referrals irrespective of symptoms or age by local agreement with partners in Primary Care.

"Rule in"

Between November 2017 and June 2019 patients with a FIT result ≥150µg Hb/g faeces were considered "high risk" positive and the result was notified directly by BCSP laboratory to the Nottingham Colorectal Service Straight to Test Team (STT) team as well as to the GP, irrespective of whether an RCCD form had been submitted. The STT team contacted these patients directly for vetting and appropriate investigation on a "rapid" pathway according to local protocols. This threshold was lowered to ≥100µg Hb/g faeces in June 2019 as prospective evaluation demonstrated significant CRC detection rates at this threshold. Patients with a f-Hb ≥10µg Hb/g faeces or ≥4µg Hb/g faeces in the presence of anaemia, low Ferritin or thrombocytosis were also considered positive and were investigated on a two week wait (2WW) pathway.

"Rule out"

Patients with a FIT result <4µg Hb/g faeces were considered to have a "negative" FIT test and to be low risk for CRC. Patients with a FIT result ≥4 but <10µg Hb/g faeces were also considered "negative" if their Haemoglobin level was normal (≥130g/l in men; ≥120g/l in women), Ferritin was normal and Platelet count <400 ($x10^9$/l). GPs were advised that patients with negative FIT tests had low risk of CRC and management options were to consider an alternate urgent pathway, routine referral or repeat FIT testing.

Cohort and Data Collection

All patients that were subject of a FIT request between 7th November 2017 and 5th November 2019 were logged prospectively in the BCSP hub in order to ensure clinical governance of this novel pathway. All patients referred to the Nottingham Colorectal Service STT team on an RCCD form between these dates were logged prospectively in NUhCLEUS database that supports the STT pathway. Cancer Outcomes & Services Datasets (COSD) were used to evaluate diagnoses of CRC recorded using ICD codes C18-C20 (excluding C18.1 Appendix) with a censor date of 31st December 2019. NUH Trust data, electronic patient records and NUhCLEUS data were used for cross-checking and diagnosis data validation. Cancer diagnoses were related to any prior patient episodes that started from a FIT result and are

presented in that context. Further details around patients who underwent repeat FIT testing are presented in Appendix II.

Statistical Analysis

Histograms were used to check for normal distribution. Comparisons were made between continuous variables using the students t-test and ANOVA if normally distributed, with Tukey's multiple comparison test for multiple groups. Categorical data was summarised using frequencies and percentages. Comparisons were made between categorical data using Chi Squared tests (χ^2). All statistics were performed using GraphPad Prism, GraphPad Software, San Diego, CA, USA. Tests of significance were considered significant if a P-value of less than 0.05 was obtained.

Data was stratified and analysed by f-Hb according to the cut-offs we have used during a pilot study[93], and subsequent iterations of pathway as described above and elsewhere[94, 111]. For the primary analyses f-Hb <4µg Hb/g faeces, 4-9.9 µg Hb/g faeces, 10 – 99.9 µg Hb/g faeces and ≥100µg Hb/g faeces were used. This last group was further segmented into 100-149.9 µg Hb/g faeces and ≥150µg Hb/g faeces (original cut-off for high risk positive). Further stratification for sub-analysis of results between 10 and 99.9 µg Hb/g faeces was chosen empirically as follows: 10-19.9 µg Hb/g faeces, 20-39.9 µg Hb/g faeces, 40 – 59.9 µg Hb/g faeces, 60-79.9 µg Hb/g faeces and 80-99.9 µg Hb/g faeces.

Funding

The pathway was commissioned locally to allow direct access to FIT for local GPs and all four local CCGs (Nottingham City, Nottingham North and East, Nottingham West and Rushcliffe) approved and jointly funded this pathway. The cost of each FIT test was agreed as £17.50 per sample to CCGs – this included postage, analysis and administration costs. Nottingham University Hospitals NHS Trust audit number for service evaluation: 20-135C.

2.3 – Results

FIT Requests and Results

In total 15589 FIT test requests were made during the evaluation period (Figure 3). Some 564 (3.6%) requests were rejected as clinical details mentioned rectal bleeding as a symptom. There were 162 (1.0%) duplicate requests received and 73 requests (0.5%) were declined for other reasons. 14788 kits were dispatched, and 13395 kits were returned within 14 days (90.6%), 34 kits were spoiled on return or not suitable for analysis (0.2%).

Overall, 13361 FIT results were available, of which 9208 (68.9%) were <4 µg Hb/g faeces, 1583 (11.8%) were 4-9.9 µg Hb/g faeces, 1850 (13.8%) were 10-99.9 µg Hb/g faeces, and 720 (5.4%) were ≥100 µg Hb/g faeces. Table 2 shows the patient demographic characteristics in each sub-group. The majority (67.8%) of FIT testing occurred in symptomatic patients over the age of 60 years who currently meet the NICE guidance for referral[101]. Five-hundred and five FIT results (3.9%) were from patients under the age of 40 years and 81.6% (412 of 505) yielded f-Hb <4 µg Hb/g faeces. The mean age of patients with lower levels of f-Hb were significantly younger than the higher strata of f-Hb (ANOVA $p<0.0001$, Tukey's Multiple Comparison Test $p<0.01$). The cohort diagnosed with CRC was significantly older than those without (Unpaired t test, $p<0.0001$). There were significantly more males in the cohort with f-Hb ≥100 µg Hb/g faeces compared to those with lower f-Hb levels (53.1% v 43.5%, χ^2 25.2, $p<0.0001$) and also in those diagnosed with CRC compared to those without that diagnosis (59.5% v 43.7%, χ^2 22.4, $p<0.0001$).

CRC Diagnoses Post-FIT

The median follow-up of this cohort was 10.4 months (IQR 5.7-16.3) – Table 2. In total 227 CRCs were diagnosed after a FIT test (227/13361, 1.7%). Eight CRCs were diagnosed in 8920 patients (0.1%) following a FIT test with f-Hb <4 µg Hb/g faeces during follow-up, 10 CRC in 1568 patients (0.6%) with f-Hb 4-9.9 µg Hb/g faeces, 61 CRC in 1840 patients (3.5%) with f-Hb 10-99.9 µg Hb/g faeces and 148 CRC (21.4%) in 714 patients with f-Hb ≥100 µg Hb/g faeces. The known CRC detection rates were significantly lower in the cohort with f-Hb <4 µg Hb/g faeces compared to the rest of the cohort (0.1% v 5.3%, χ^2 449.7, $p<0.0001$) and in the cohort with f-Hb <10 µg Hb/g faeces (0.2% v 8.2%, χ^2 770.8, $p<0.0001$). The known CRC detection rate was significantly higher in the cohort with f-Hb ≥100 µg Hb/g faeces (20.7% v 0.6%, χ^2 1592.4, $p<0.0001$). Three diagnoses of CRC were

related to repeat FIT testing in 229 patients with sequential f-Hb results in different strata (Appendix II).

Overall, 86.7% (197 of 227) of CRC's were diagnosed in patients over the age of 60 years. One patient under the age of 40 years was diagnosed with cancer with f-Hb result ≥100 µg Hb/g faeces, and 29 were diagnosed in those ≥aged 40 to 59 years.

CRC diagnoses in "Negative FIT". Eight out of 227 CRCs (3.5%) were diagnosed after a f-Hb reported as <4 µg Hb/g faeces (Table 4). These patients were identified via referral through other pathways: 3 had CT scans arranged by the Upper GI team, 2 were seen by the medical gastroenterology team, 2 were diagnosed in routine colorectal clinics and 1 presented as an emergency with acute bowel obstruction. The median time from "negative" FIT test to CRC diagnosis was 41.5 days (IQR 31-72.25). One sample was analysed after 17 days and should have had a repeat FIT due to risk of a false-negative result[116]. In the population with f-Hb 4-9.9 µg Hb/g faeces all patients but one satisfied the Nottingham protocol whereby an abnormal blood parameter: anaemia, thrombocytosis or low ferritin (or iron), reduces the threshold to investigate to 4 µg Hb/g faeces (Table 4). The other case had abnormally high ferritin but was considered "negative" according to local protocol at the time of testing.

Blood results and palpable rectal mass. Detection rates for different strata of f-Hb within the range 10 – 99.9 µg Hb/g faeces are shown in Table 3. The CRC detection rate in the cohort with f-Hb 10-19.9 µg Hb/g faeces was 1.4% and below NICE's 3% threshold for urgent referral. These patients were all eligible for 2WW referral and investigation in the local protocol. Eight of 10 CRCs detected in this stratum had abnormal blood parameters or abnormal digital rectal examination prior referral (Table 4). 47 of 61 CRC's (77.0%) detected after f-Hb 10 – 99.9 µg Hb/g faeces had one or more abnormal blood results or a palpable rectal mass (latter not mentioned on referral from Primary care). Six CRCs were detected in 11194 patients with f-Hb <20 µg Hb/g faeces in whom there was no evidence of abnormal blood results or palpable rectal mass.

Figure 3 – Flow diagram of patients with FIT requests from referral to Colorectal Cancer (CRC) diagnosis. Numbers of patients lower than number of results in each stratum reflects repeat tests within one stratum. Additional data in Appendix II.

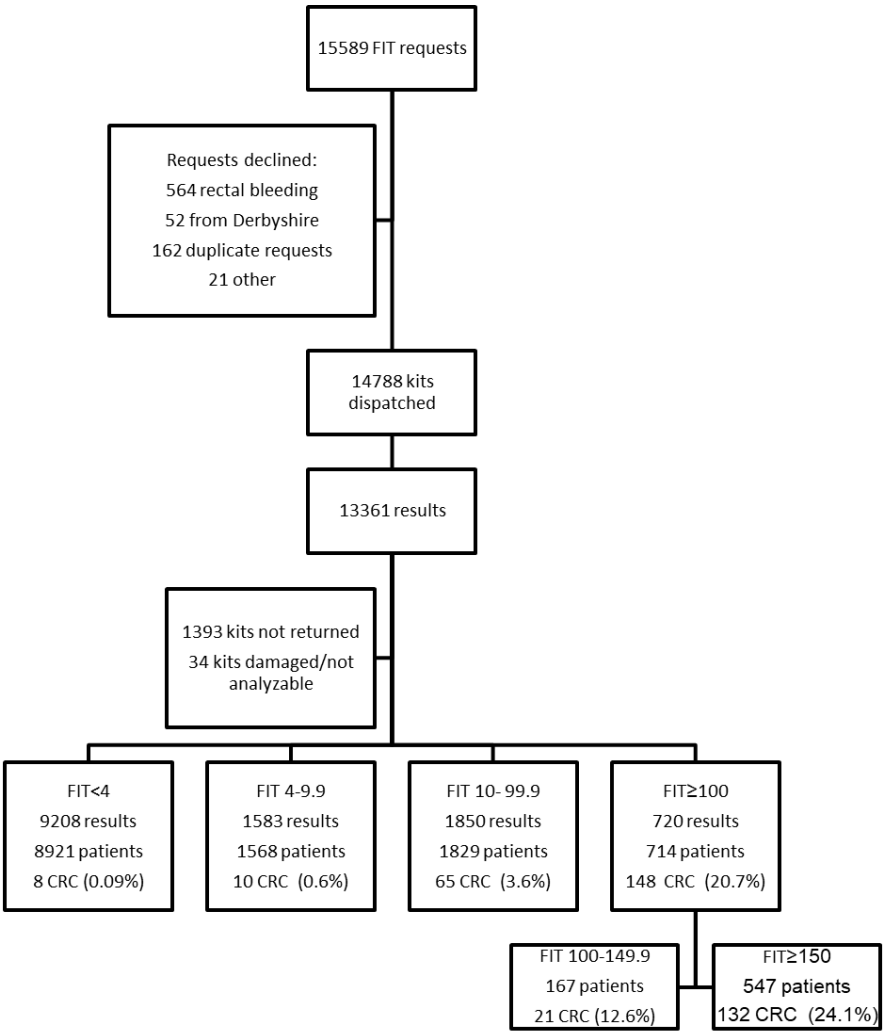

Table 2 – Demographics of all patients with a FIT result and CRC diagnosis stratified by f-Hb and age.

			Gender	Age(y)			
FIT stratum (µg Hb/g faeces)	Median Follow-up (months)	Patients with FIT results	Male: Female (%)	Mean Age years (SEM)	≤49 (% of stratum)	50-59 (% of stratum)	≥60† (% of stratum)
Total	10.4 (5.7-16.3)	13042	5740:7302 (44.0:56.0)	66.3 (0.2)	1658 (12.7)	2546 (19.5)	8838 (67.8)
Total CRC diagnosed (detection rate %)		227 (1.7)	135:92** (59.5:40.5)	74.1*** (1.2)			
<4	10.6 (5.8-16.5)	8920	3850:5070 (43.2:56.8)	64.5*** (0.1)	1329 (14.9)	2003 (22.5)	5588 (62.6)
CRC diagnosed (detection rate %)		8 (0.1)*	6:2 (75.0:25.0)	77.4 (3.1)	0	0	8 (0.1)
4-9.9	10.6 (5.0-15.0)	1568	656:912 (41.8:58.2)	69.0*** (0.3)	146 (9.3)	224 (14.3)	1198 (76.4)
CRC diagnosed (detection rate %)		10 (0.6)*	5:5 (50.0:50.0)	75.0 (3.6)	1 (0.7)	0	9 (0.7)
10-99.9	9.9 (5.7-15.8)	1840	855:985 (46.5:53.5)	71.4*** (0.3)	129 (7)	234 (12.7)	1477 (80.3)
CRC diagnosed (detection rate %)		61 (3.3)*	34:27 (55.7:44.3)	74.6 (1.4)	1 (0.8)	7 (3.0)	53 (3.6)
≥100	10.3 (5.9-16.2)	714	379:335** (53.1:46.9)	71.5*** (0.5)	54 (7.5)	85 (11.9)	575 (80.5)
CRC diagnosed (detection rate %)		148 (20.7)*	90:58 (60.8:39.2)	73.7 (0.9)	7 (13.0)	14 (16.5)	127 (22.1)

†Patients over the age of 60 years with symptoms commonly eligible for urgent referral according to NICE guidance (NG12) [3].

*Chi squared, p<0.0001. **Chi squared, p<0.0001. ***ANOVA, p<0.0001

Table 3 – Colorectal Cancers diagnosed in patients stratified by f-Hb result and detection rates above and below each lower limit.

FIT stratum (µg Hb/g faeces)	Patients with FIT results in stratum	CRC diagnoses	CRC detection rate within stratum (%)	CRC miss rate below lower limit of stratum (%)	CRC detection rate above lower limit of stratum (%)
<10	10488	18	0.2		
10-19.9	706	10	1.4	0.2	8.2
20-39.9	543	22	4.1	0.3	10.8
40-59.9	303	8	2.6	0.4	13.6
60-79.9	168	13	7.7	0.5	16.9
80-99.9	120	8	6.7	0.6	18.7
≥100	714	148	20.7	0.6	20.7

Table 4 – Colorectal cancers identified after a FIT test, laterality and objective measures at the time of diagnosis.

FIT result (µg Hb/g faeces)	Patients	CRC's	Site Right: Left: Rectum	Palpable rectal mass on investigation	Anaemia (Hb<130 male, Hb<120 female)	Thrombocytosis (Platelets ≥400)	Ferritin (<25)	Ferritin (≥350)	No abnormality on DRE or bloods
<4	8920	8	3:4:1	1	3	1	1	2	4
4-9.9	1568	10	6:2:2	0	5	0	6	1	0
10-19.9	706	**10**	5:3:2	2	6	2	5	1	**2**
20-39.9	543	**22**	8:10:4	1	15	7	10		**5**
40-59.9	303	**8**	4:3:1	0	6	1	4		**2**
60-79.9	168	**13**	5:4:4	2	6	3	6		**3**
80-99.9	120	**8**	4:2:2	1	4	0	4		**2**

This table shows the sites of cancer detection in our FIT pathway and other clinical or biochemical factors that were present. This is of relevance in the lower FIT strata where urgent investigation may not be indicated by the test. The presence of a palpable rectal mass or abnormal blood test results further improves risk stratification by selecting patients at higher risk of CRC that were not indicated by FIT.

This chapter has presented a high volume of data to demonstrate that FIT adds significant value to symptoms alone in aiding the decision to refer.

2.4 – Discussion

This is a large English dataset on Primary care access to FIT in symptomatic patients for all symptoms and all age groups. In previous studies a CRC diagnosis rate of 0.2% in patients undergoing 2WW investigation with f-Hb<4 µg Hb/g faeces was documented[93, 104, 115]. Data from Scotland suggests a similar "miss rate" with longer follow-up in those with unquantifiable f-Hb on a different manufacturer's platform[103]. The CRC diagnosis rate of 0.1% after a "negative" FIT test (as per local definition) in this population is consistent with these data, and appears well below the NICE threshold of 3% despite including the NG12 "high risk" population[101]. Other strengths of this study include a large dataset with optimal return rates and a "real world" analysis of FIT usage in a clinical setting. Use of a prospectively recorded database to log cancer diagnoses validates the accuracy of the retrospective study.

In evaluating this pathway, it would be important to stress that FIT should not be compared to colonoscopy, since in the UK, and many other countries, GPs do not have direct access to colonoscopy. Instead, 2WW pathways that use FIT in Primary Care should be compared with those that do not. Indeed, the "miss rate" of age and symptom based criteria in Primary Care – the number of CRC's detected in symptomatic patients after a routine referral- was historically around 50% on average[114] and even a "gold standard" investigation like colonoscopy is known to miss diagnoses of CRC[117].

Overall, 5588 patients over 60 years old with f-Hb<4 µg Hb/g faeces were tested by GP's. This would have equated to over 230 additional referrals per month over 2 years (if FIT had not been used for "rule out") to detect 8 CRC's. However, this methodology does not identify patients diagnosed with CRC at other trusts, which is a relative weakness. Concomitant analysis across the Cancer Alliance is ongoing and analysis of East Midlands Cancer Network data has not yet demonstrated additional cases of "post- negative FIT CRC", but this scenario may arise. Also, most patients have not been investigated after a "negative" FIT and some may yet present with CRC with ongoing follow-up.

In this study only 9 cancers were diagnosed in those under the age of 50 years and unsurprisingly the majority had FIT≥100 µg Hb/g faeces. The recommendation of f-Hb

threshold of 10 µg Hb/g faeces for "low risk" patients[109] appears questionable in this context. A threshold as low as 4 µg Hb/g faeces in patients with anaemia, low ferritin and thrombocytosis was driven by concern around the use of FIT in "high risk" patients but is vindicated by the detection of 9 such patients with CRC in the cohort with f-Hb <10 µg Hb/g faeces. Interestingly, these data suggest a similar principle may be applicable between 10 and 19.9 µg Hb/g faeces and perhaps at even higher levels.

The utility of anaemia[112] and thrombocytosis[114] in the local 2WW pathway were evaluated elsewhere, but not that of Ferritin. The protocol has hitherto mandated investigation only when Ferritin is low. However, studies have suggested that Ferritin may have value when abnormally high[118], thus there may be value in using high Ferritin to improve the sensitivity and specificity of the symptomatic pathway. In this cohort, only one additional CRC would have been missed if the threshold had been 20 µg Hb/g faeces for patients with normal rectal examination, FBC and Ferritin. FIT is a stratification tool and appears to be most useful when combined with other objective measures. The FAST score[119], combining FIT with age and gender, could be improved[120], and performance characteristics of such scoring systems might increase if FIT were combined with FBC, Ferritin and a digital rectal examination. These four "F's" appear likely to have greatest combined value as the level of f-Hb declines towards undetectable.

The use of FIT has been introduced in English BCSP with a threshold of 120 µg Hb/g faeces. This raises a number of interesting issues in relation to training, accreditation and workload of endoscopists. The challenge to reduce the FIT threshold or the screening age in England might be aided by the use of higher FIT thresholds, alongside blood tests, in symptomatic patients as greater diagnostic capacity is freed up. An alternative solution may be to invite screened patients with a FIT <120 µg Hb/g faeces to attend their GP for a FBC and Ferritin test, and to lower the threshold when such parameters are abnormal. Ultimately, raising symptomatic thresholds and lowering BCSP thresholds may help to yield more coherent and consistent use of FIT in all parts of the population.

Both symptomatic and asymptomatic pathways face unprecedented circumstances in the UK in relation to the Coronavirus pandemic. UK diagnostic services that were overstretched pre-pandemic shall doubtless struggle to cope with a backlog for many months afterwards.

Accordingly, FIT results and other objective measures such as blood results should be used to prioritise those individuals most likely to benefit from urgent investigation (Appendix III). Finally, other large service evaluation studies[103, 107] now demonstrate similar results to this dataset and the recently published NICE FIT study adds high volume multi-centre research data to this consensus. No test is perfect but there is now a high volume of data to demonstrate that FIT adds significant value to symptoms alone in aiding a possible difficult decision to refer from Primary Care.

Chapter 3 – Thrombocytosis helps to stratify risk of colorectal cancer in patients referred on a 2-week-wait pathway

Bailey JA, Hanbali N, Premji K, Bunce J, Simpson J, Humes DJ, Banerjea A.
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Citations: 4 Altmetric: 3

3.1 – Introduction

Colorectal cancer (CRC) is common with around 42000 new diagnoses made annually in the UK[3]. Outcomes in the United Kingdom lag behind the rest of Europe despite nearly two decades of two-week wait (2WW) pathways and other targets introduced to address this issue[121]. The desire for diagnosis at earlier stage led to the introduction of broader referral criteria for CRC in 2015 with the aim of investigating all those with risk of CRC ≥3%. However, these criteria for urgent referral to secondary care are largely based on patient age and symptoms[122] – the latter are often associated with later stage disease and are inherently non-specific. The search for objective markers that may help to stratify risk remains attractive in this context.

We introduced straight to test (STT) colonoscopy in 2014 as part of our 2WW pathway[95]. A Full Blood Count (FBC) was specified for inclusion with every referral. We have previously demonstrated the value of anaemia in those patients referred on an urgent pathway although compliance with submission of FBC results has been poor[93, 112]. A FBC also provides a platelet count and thrombocytosis (platelet count >400x109/L) appears to have value for risk stratification of colorectal cancer in Primary Care[113]. We aimed to evaluate its utility in the secondary care setting by undertaking a review of existing data for patients referred on a 2WW colorectal cancer pathway at our institution. We report on its value as a single marker of risk, as well as its association with other recognised parameters such as age, sex and anaemia.

3.2 – Methods

Data Sources

Adult patients referred to the Colorectal Service at Nottingham University Hospitals under the 2WW pathway for CRC are prospectively recorded on a local database in accordance with Best Practice guidance for audit of straight to test pathways. The name, date of birth, age, sex, Hospital ID, NHS number, date of referral and indication for referral is recorded for each patient.

Data for haemoglobin (Hb) and platelet counts at the time of referral were collected from the hospital electronic reporting system retrospectively. Cancer diagnoses, CRC and other cancer (OC) outcomes were collected from hospital electronic reporting system.

Cohort

All patients referred under the 2WW pathway between 01/08/2014 and 31/8/2017 for suspected CRC were identified from the referral database populated by specialist nurses at the Colorectal Service at Nottingham University Hospitals NHS Trust. Duplicate and rejected referrals were identified and excluded. Patients with no full blood count (FBC) on referral, no investigations or unknown outcome, were excluded from subsequent analysis of outcomes.

Exposure and covariates

Anaemia was diagnosed according to the WHO definitions of a haemoglobin of <120g/L in women or <130g/L in men, based on the most recent Hb at the time of referral. Thrombocytosis was defined as platelets >400x10^9/L in line with primary care studies[113]. The presence or absence of anaemia and thrombocytosis was evaluated for all patients.

Outcome definition

Colorectal cancer diagnosis was determined from investigation outcomes. Evidence of lower GI malignancy on colonoscopy, CT scans and histology reports reviewed at our cancer multi-disciplinary team (MDT) meeting that confirmed adenocarcinomas were reviewed for diagnosis. Non-colorectal cancer diagnoses were also recorded.

Statistical Analysis

Data were assessed for normality using histograms and a Shapiro-Wilk test. Comparisons were made between continuous variables using the students t-test if normally distributed or Mann-Whitney if not normally distributed. Categorical data was summarised using frequencies and percentages. Missing data were classified in a separate category and included in models. Comparisons were made between categorical data using Chi Squared tests (χ^2). Logistic regression analysis was used to test the association between diagnosis of colorectal cancer and thrombocytosis accounting for age, gender and anaemia. Univariate analysis was undertaken with age as a continuous variable; a multivariate model was then built including all factors associated with colorectal cancer in the univariate analysis. All statistics were performed using Stata Version 16 (Stata Corp, College Station, TX, USA). Tests of significance were considered significant if a P-value of less than 0.05 was obtained.

3.3 – Results

A total of 2991 patients referred for 2WW investigation during the study period were available for review, 2236 (74.8%) were included in the analysis. 755 patients (25.2%) were excluded from the study – In total 394 (13.2%) had no FBC available, 225 (7.5%) were not investigated due to clinical judgement/patient choice, 82 (2.7%) had missing clinic/blood test information, 45 (1.5%) did not attend their appointment and 9 (0.3%) were excluded due to database error/duplication.

Demographic characteristics are shown in Table 5. There was no significant difference in the age distribution of those with thrombocytosis and those without. 55.5% of the cohort were female, there were significantly more females in the thrombocytosis group than the group with normal platelets (72.1% v 53.9%, χ^2 24.63, p<0.0001).

A total of 130 CRCs (5.8%) and 52 other cancers (2.3%) were diagnosed in this cohort. CRC diagnosis was more likely in patients with thrombocytosis (12.4% vs 5.2%, χ^2 17.70, p=<0.0001) compared to those with a normal platelet count; significant for both females (11% v 2.8%, χ^2 23.70, p=<0.0001) and males (16.1% v 7.9%, χ^2 4.62, p=0.032). Thrombocytosis was significantly associated with advanced (stage 3/4) CRC diagnosis (19.1% vs 8.5%, χ^2 14.4, p<0.0001) and with diagnosis of right-sided cancers (34% vs 9.6%, χ^2 11.87, p=0.001) versus left-sided and rectal cancers. Patients with anaemia were also significantly more likely to be diagnosed with CRC (9.5% vs 3.5%, χ^2 35.33, p<0.0001) compared to those with a normal haemoglobin.

Univariate analysis identified sex, age, anaemia and thrombocytosis as significant risk factors for CRC diagnosis as summarised in Table 5. Multivariate logistic regression of the whole dataset confirmed the association of thrombocytosis with CRC diagnosis after adjustment for gender, age and anaemia (OR 2.62, 95% CI 1.60-4.30). Repeat analysis was completed with non-CRCs excluded, yielding the same results (OR 2.58, 95% CI 1.57-4.25). No association was found in a comparative analysis on thrombocytosis for the diagnosis of non-CRCs (OR 0.76, 95% CI 0.26-2.18).

Table 5 – Proportion of patients diagnosed with CRC, univariate and multivariate analysis for thrombocytosis accounting for age, gender and anaemia. Patients with thrombocytosis were more likely to have CRC (OR 2.62, 95% CI 1.60-4.30, p=<0.001) but not non-colorectal cancers (analysis not shown – OR 0.76, 95% CI 0.26-2.18). SEM= standard error of mean

Parameter	Total	CRC	Univariate analysis		Multivariate analysis	
Total patients	2236	130	OR (95% CI)	p value	OR (95% CI)	p value
Age (SEM)	68.19 (0.27)	74.22 (0.87)	1.05 (1.03-1.07)	<0.001	1.04 (1.02-1.06)	<0.001
Male (%): Female (%)	994 (44.5%): 1242 (55.5%)	83 (63.8%): 47 (36.2%)	2.32 (1.60-3.35)	<0.001	2.44 (1.67-3.58)	<0.001
Anaemia (%)	860 (38.5%)	82 (63.1%)	2.74 (1.92-3.93)	<0.001	1.83 (1.23-2.73)	0.003
Thrombocytosis (%)	201 (9.0%)	25 (19.2%)	2.51 (1.58-3.98)	<0.001	2.62 (1.60-4.30)	<0.001

3.4 – Discussion

This study demonstrates that thrombocytosis has value in stratifying risk for this cohort of patients after referral on a 2WW pathway for CRC. The study period mostly pre-dates the Bailey *et al*. paper[110] highlighting the value of platelets in Primary Care risk stratification, and it is unlikely that this publication would have affected our results. It is noted that thrombocytosis appears more significant in the female population – the reverse of the primary care study in which males showed a greater increase in risk. However, symptoms such as change in bowel habit are more common in females than males, leading to more of this group being referred on a 2WW pathway despite a lower risk of CRC. This population is therefore selected from the general population on the basis of symptoms, which may also explain the lack of increased risk for other malignancies. The higher average age of those diagnosed with CRC is understandable, given that risk of CRC increases with age. In the dataset presented there was no significant association between age and thrombocytosis. Overall, it is demonstrated that thrombocytosis confers an increased CRC risk in the referred population independent of gender, age and anaemia.

An FBC is a cheap easily accessible test that appears to provide two objective markers of risk in haemoglobin and platelet count[110, 112]. However, despite the referral form requesting submission of this data since 2014, only 82.8% of referrals complied with this. As a retrospective study of thrombocytosis, there was little choice but to exclude those without a recorded FBC (394), which is a limitation of the study. Local discussions with primary care commissioners identified a concern that waiting for pre-requisite test results to make a referral may cause delays in suspected cancer cases. The 2WW initiative in the UK requires that a GP suspecting a diagnosis of cancer send a referral to the secondary care provider within 24 hours[123]. As such, an FBC did not become a requirement in our pathway until 2019. Incomplete referrals made without an FBC are now returned, with the stratification value of anaemia confirmed[112]. Within this cohort, 5.8% of patients were diagnosed with CRC, satisfying the 3% NICE threshold for urgent investigation. However, the 2.9% detection rate in women without thrombocytosis suggests some subsets of this cohort may fall below that cut-off. The presence of thrombocytosis in 19.2% of CRC diagnoses reaffirms the notion that a single parameter is insufficient for risk stratification. Association between thrombocytosis and right-sided cancers is of great interest, given the increased likelihood of false-negative FIT results from right-sided cancers[124]. In the cohort presented

thrombocytosis was also significantly associated with later stage CRC, though not specifically metastatic cancer. In some cases within this dataset thrombocytosis was the only abnormal stratification parameter in early-stage CRC.

Identifying cohorts at increased risk of CRC is key to improving diagnosis at earlier stages when outcomes are favourable[125]. Increasing demand on 2WW diagnostic capacity requires more individualised risk stratification in order to improve rates of early diagnosis and clinical effectiveness of such pathways[126]. NUH NHS Trust have combined anaemia with faecal immunochemical testing (FIT) in the local pathway[104], and whilst FIT is useful at the extremes of (f-Hb), there is a broad range (locally between 4 and 100) that would benefit from additional discriminatory value. Scoring systems such as FAST hold promise in this regard[119]. Here it is postulated that adding values from an FBC such as Hb and platelet count may improve the performance characteristics of such a score. It might be argued that such parameters could be extrapolated to support individualised decision making in screening programmes to further decrease CRC mortality and minimise iatrogenic harm[127]. The English Bowel Cancer Screening Programme could recommend an FBC to identify anaemia or thrombocytosis meriting further investigation for patients with faecal haemoglobin concentrations below the 120µg Hb/g faeces cut-off threshold.

Chapter 4 – Quantitative FIT stratification is superior to NICE referral criteria NG12 in a High-Risk Colorectal Cancer population

Bailey JA, Ibrahim H, Bunce J, Chapman CJ, Morling JR, Simpson JA, Humes DJ, Banerjea A.

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4.1 – Introduction

Colorectal cancer (CRC) is a leading cause of cancer death in the UK and worldwide; the stage of disease at the time of treatment remains the most significant predictor of survival [128]. Whilst asymptomatic population-based screening programmes have been shown to identify a higher proportion of CRC at an earlier stage, advancements in the diagnosis of symptomatic patients has remained elusive despite concerted efforts over the past 20 years [100].

The Two-Week-Wait (2WW) referral pathway was introduced to decrease cancer-related mortality as part of the NHS England Cancer Plan in 2000. Publication of subsequent national guidelines have typically focused on optimising age and symptoms-based criteria to identify patients requiring definitive investigation [79]. Owing to the variable and non-specific symptoms which are typical of CRC (if any symptoms are present at all), challenges remain triaging patients correctly and mitigating the risk of iatrogenic harm during investigations [128].

Recently Faecal Immunochemical Tests (FIT) have been nationally endorsed to guide secondary care referral in patients with low-risk symptoms [109]. We have previously discussed the safe incorporation of FIT, alongside common blood test parameters, into "high-risk" urgent symptomatic pathways in Nottingham [104, 115]. The publication of multi-centre studies confirming the diagnostic accuracy of FIT in the UK may precipitate more widespread use of the test [129].

The COVID-19 pandemic is certain to burden diagnostic services in the immediate and medium-term future – with increasing referrals post-lockdown likely to be met with limited colonoscopy and CT colonography capacity. A guidance document for the second phase of the response to COVID-19 by NHS England recommended the use of FIT to help prioritise 2WW referrals but omits use of established risk-factors like iron-deficiency anaemia (IDA) [112, 130]. This study aims to compare the diagnostic value of clinical features and FIT result – to identify those at a higher or lower risk of CRC, thereby facilitating effective triage of patients.

4.2 – Methods

Patients were identified using a prospectively collected local database of 2WW referrals, with outcomes identified from a retrospective review of electronic hospital databases from September 2016 to June 2018. An independent provider (Circle Health, London, UK) at a neighbouring Treatment Centre (TC) received 2WW referrals during this period which were not included in this study. CRC diagnosis following 2WW referral to TC and routine referral to NUH NHS Trust are discussed elsewhere [115]. All patients returned a self-collected FIT sample (OC-Sensor™; Eiken Chemical Company, Tokyo, Japan) via a postal service as part of their clinical investigation, as described previously (Appendix I) [104].

Clinical features were recorded at the time of referral based on national 2WW referral guidelines. Abdominal pain, weight loss, abdominal mass, rectal mass, rectal bleeding, and referral prompted by FIT result were classified as "other symptoms" to facilitate comparison. Change in bowel habit (CIBH) was the most common clinical feature, and closest to the 3% risk-threshold recommended in NG12; thus, it was used as the reference for comparison of other clinical features prompting referral.

FIT results were categorised as "Negative" if < 4 µg Hb / g faeces was detected, the limit of reliable detectability on the analyser platform. This group was used as the reference for comparison of the other FIT categories: 4-9.9 µg Hb / g faeces, 10-99.9 µg Hb / g faeces and ≥ 100 µg Hb / g faeces. DG30 recommends a threshold of 10 µg Hb / g faeces in symptomatic patients. Our local pathway utilises a threshold of 4 µg Hb / g faeces where other risk factors are present (anaemia, thrombocytosis, and abnormal ferritin). Where there were more than one FIT/referral, only the first FIT was included.

Data were assessed for normality using histograms and a Shapiro-Wilk test. Levene's test was used to confirm equal variance. The predictive value of age, gender, clinical features, and FIT categorisation was assessed by Pearson's Chi-Squared Test/Fisher's Exact Test and calculating the positive predictive value (PPV), odds ratio (OR) and 95% confidence intervals (CI) as appropriate. Logistic regression models were used to assess the combination of all factors as predictors of CRC. Age was treated as a categorical variable (<60yrs/≥60yrs) in univariate and multivariate logistic regression models. All statistics were performed using

STATA v16 (Stata Corp, College Station, TX, USA). Tests of significance were considered significant if a P-value of less than 0.05 was obtained.

4.3 – Results

In total 1784 patients investigated via the 2WW pathway during the study period were included with 76 (4.3%) colorectal cancers diagnosed. 1727 patients (96.8%) had a Haemoglobin and 1419 (79.5%) had Ferritin/Iron Studies as part of their investigation. The median age was 71 years (range 18-96 years, inter-quartile range 61-79 years). The patients diagnosed with CRC were significantly older than those without (74.0yrs vs 68.9yrs, p=0.0007). Male patients were more likely to be diagnosed with CRC than females (6.5% vs 2.5%, χ^2 16.93, p<0.0001).

The most common referring clinical feature was CIBH alone with 684 patients (38.3% of referrals) with 24 CRCs detected (3.5%) (Table 6). The greatest proportion of colorectal cancers diagnosed by referring clinical feature was in the CIBH and IDA group with 74 referrals with 6 colorectal cancers diagnosed (8.1%). No single referring clinical feature or combination of clinical features was significantly associated with CRC diagnosis (χ^2 8.03, p=0.155). Patients with right-sided CRC were significantly more likely to be anaemic than those diagnosed with left-sided CRC (92.6% vs 30.6%, p=<0.0001).

A negative FIT result (< 4 µg Hb / g faeces) was found in 1027 (57.5%) patients, with 3 CRCs diagnosed (0.3%). Those found to have a malignancy from this subset returned a single FIT sample – 2 were referred with a change in bowel habit and 1 with IDA. The proportion of CRC detected increased with increasing FIT level from 0.3% (3/1027) in the < 4 µg Hb / g faeces group to 30.4% (55/181) in the >100 µg Hb / g faeces group (χ^2 345.62, p=<0.0001) (Table 6).

In the univariate analysis age over 60 years and male sex were associated with a 2- and 2.7-fold increased risk of diagnosis of CRC respectively (Table 6). The only symptom associated with an increased risk of CRC compared to CIBH were CIBH *and* IDA which were associated with a 2.5-fold increased risk (OR 2.43, 95% CI 0.96-6.14). Each increasing stratum of FIT was associated with an increased risk of CRC compared to the baseline of < 4 µg Hb / g faeces (Table 6).

Multivariate logistic regression showed that only increasing FIT level and male sex were associated with increased risk of CRC; accounting for age and referring clinical features.

Males in the cohort were more than twice as likely to be diagnosed with CRC compared to females (adjusted OR 2.30, 95% CI 1.33-4.00), whilst those with a FIT of 10-99.9 µg Hb / g faeces were more than 12 times more likely to be diagnosed with a CRC compared to those with a FIT of < 4 µg Hb / g faeces (adjusted OR 12.75, 95% CI 3.62-44).

Table 6 – Univariate and multivariate logistic regression of CRC diagnosis accounting for age, gender, clinical features and FIT-based categorisation

Parameter	Total (%)	CRC (%)	Univariate analysis OR (CI)	p value	Multivariate analysis OR (CI)	p value
Total Patients	1784	76 (4.3)				
< 60yrs	402 (22.5)	10 (2.5)	Reference			
≥ 60yrs	1382 (77.5)	66 (4.8)	1.96 (1.00-3.86)	0.050	1.56 (0.71-3.44)	0.267
Gender						
Female	996 (55.8)	25 (2.5)	Reference			
Male	788 (44.2)	51 (6.5)	2.69 (1.65-4.38)	<0.001	2.30 (1.33-4.00)	0.003
Clinical Features						
CIBH	684 (38.3)	24 (3.5)	Reference			
IDA	342 (19.2)	20 (5.8)	1.62 (0.87-3.00)	0.126	1.02 (0.50-2.07)	0.953
Other	362(20.2)	10 (2.8)	0.78 (0.37-1.66)	0.523	0.69 (0.30-1.58)	0.375
CIBH + IDA	74 (4.1)	6 (8.1)	2.43 (0.96-6.14)	0.061	2.79 (0.90-8.62)	0.075
CIBH + Other	260 (14.6)	13 (5)	1.45 (0.73-2.89)	0.294	1.37 (0.61-3.06)	0.449
IDA + Other	63 (3.5)	4 (6.3)	1.86 (0.63-5.55)	0.263	1.60 (0.46-5.53)	0.456
FIT result (µg Hb / g faeces)						
< 4	1027 (57.5)	3 (0.3)	Reference			
4-9.9	211 (11.8)	4 (1.9)	6.60 (1.47-29.69)	0.014	6.75 (1.49-30.59)	0.013
10-99.9	365 (20.4)	14 (3.8)	13.61 (3.89-47.65)	<0.0001	12.75 (3.62-44.92)	<0.0001
≥ 100	181 (10.1)	55 (30.4)	148.99 (45.94-483.28)	<0.0001	139.73 (42.77-456.50)	<0.0001

4.4 – Discussion

In this study we aimed to identify patients at increased risk of CRC within a 2WW population. We previously published our experiences incorporating FIT in a 2WW pathway, discussing its discriminatory value across both "high-risk" and "low-risk" patients [93, 115]. Here we confirm superiority of FIT stratification over clinical feature-based triage in a multivariate model accounting for age and gender. Although the overall detection of CRC in this cohort (4.3%) satisfies the 3% risk-threshold stipulated in NG12, further stratification of risk based on clinical features is of limited value; with no individual symptom or combination of clinical features conferring a significantly higher risk than CIBH on multivariate logistic regression.

The significant risk of CRC associated with a high FIT demonstrates the value of FIT for stratifying patients that need urgent investigation wherever diagnostic capacity is constrained, as well as in the challenging environment brought on by COVID-19. Conversely, a negative FIT corresponds with a 0.3% risk of CRC in this cohort which is consistent with previous service evaluation as well as emerging data from multicentre research studies [107, 129]. FIT appears safe for "rule out" and our data confirm its utility across all groups including IDA.

The CRC detection rates between 4-99.9 µg Hb / g faeces show that improvements in the PPV of FIT might be desirable. At these levels the risk of CRC is closer to the NG12 risk-threshold, and whilst further segmentation is possible – other stratification tools may be required to optimise diagnostic strategies. Our local pathway mandates an FBC for referral, informing whether risk factors like anaemia, thrombocytosis and abnormal ferritin are present. CRC-scoring systems which promise increased accuracy have been created [119], although their widespread applicability remains unproven [120] and blood parameters were not included. A limitation of our study is the cohort size and we have not analysed the value of thrombocytosis or abnormal ferritin as these results were not available for some of the cohort as highlighted above. However, we continue to use different FIT cut-offs for those with normal and abnormal blood test results. The increased frequency of anaemia in right-sided CRC who may theoretically return lower FIT results highlights the importance of blood tests in any risk stratification system. Whilst we offer further evidence of FITs value over symptoms in a clinical setting, we feel further improvements in stratification may arise if all

these factors could be combined. Age, gender and blood test results might be used to define a pre-test probability that adjusts the FIT threshold for urgent investigation and further work in this area would represent a significant development in diagnostic pathways.

Chapter 5 – Sociodemographic Variations in the Uptake of Faecal Immunochemical Tests (FIT) in a Primary Care Symptomatic Pathway for Colorectal Cancer

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5.1 – Introduction

Colorectal cancer (CRC) is a common disease and an important cause of cancer morbidity and mortality worldwide. There are more than 42000 new CRC cases and 16000 deaths in the UK each year, making it the second leading cause of cancer death [100]. The most important predictor of survival in CRC is the stage of disease at the time of treatment [78]. 90% of those diagnosed with an early-stage tumour can expect to survive for at least 5 years, compared to less than 10% diagnosed with the most advanced cancer [100]. Two main strategies have been employed in the UK to improve CRC outcomes – population-based screening of asymptomatic patients and expedited diagnostic pathways for patients with symptoms. Screening has shown to be cost-effective and reduce CRC mortality [131, 132] by diagnosing earlier stage disease, but the majority of CRC diagnoses are still made via symptomatic pathways in Primary Care, where similar improvements have not been achieved [100, 133].

The Faecal Immunochemical Test (FIT) is now the screening tool of choice for asymptomatic pathways in the UK, detecting microscopic quantities of blood, which indicate increased risk of CRC and other significant luminal pathology, with improved diagnostic performance compared to its predecessor the guaiac Faecal Occult Blood Test (FOBT) [68, 134]. FIT appears to be an acceptable test to patients, with increased participation rates compared to FOBT[21, 135]. More recently FIT has been used to stratify risk of CRC for patients with lower gastrointestinal symptoms following NICE guidance on occult blood testing for low-risk patients. Early adopters have used FIT more widely – identifying patients at the highest risk of CRC for expedited investigation on timed pathways, with formal research studies providing corroborating evidence for this approach [103, 108, 136-142]. Notably higher rates of FIT return have been reported by the centres utilising FIT for symptomatic patients than in screening populations [103, 142, 143].

The promising increase in screening participation disguises considerable gender-based, ethnic, and socio-economic variability in CRC diagnosis and treatment [134, 144]. Inequalities in participation have been observed in numerous disease prevention programmes [145] and appear to be intricately related to patient demographics and social deprivation [144]. Lower access to healthcare and significantly lower participation with bowel cancer screening programmes are reported in the most deprived areas – and often used to explain the

disparities seen in the stage of disease at diagnosis [146] and disease survival [147]. Disparate presentation, diagnosis and treatment outcomes have also been described in various ethnicities and screening programmes, suggesting a complex interaction between socioeconomic, cultural and physician factors [148, 149]. In the Bowel Cancer Screening Programme (BCSP) it is recognised that males, socially deprived groups and certain ethnic groups demonstrate lower uptake of both occult blood testing and colonoscopy [148, 150-152]. Furthermore, variations in cancer incidence add complexity as CRC is more common in men and deprived populations. By contrast, CRC incidence is lower in Asian and Black populations compared to White ethnic groups [153]. In this study we aim to evaluate whether sociodemographic factors affect FIT sample return, which remains an unknown in symptomatic pathways.

5.2 – Methods

Study Population/FIT Platform/Data Sources

We introduced a novel pathway incorporating FIT as a triage tool for all adult symptomatic patients in 2017, except those with rectal bleeding and/or rectal mass, as described elsewhere [143, 154]. The pathway was commissioned to provide direct access to FIT for General Practitioners (GPs), who were able to request and act upon results independently or submit a referral contemporaneously for secondary care review with the mandated FIT and blood test results included. All patients subject to a FIT request for symptoms from inception of the pathway 3 November 2017 and 31 December 2021 were recorded prospectively for clinical governance purposes. A retrospective review of FIT return was undertaken. FIT return was defined as the proportion of patients returning a sample for analysis after their first FIT request. FIT non-return was defined as not returning a sample for analysis 14 days after a request was made. General Practitioners were informed if a sample kit was not completed. Subsequent FIT requests made for patients not returning their first request were recorded and analysed as a sub-group.

FIT requests were submitted via an electronic request system (ICE) which includes prompts on the use of FIT, interpretation of results and whether a 2WW referral is recommended. FIT sample kits were sent and collected via a postal service and analysed in a BCSP-accredited laboratory. The OC-Sensor FIT System (Eiken Chemical, Tokyo, Japan) was used to analyse all samples (See FITTER Checklist information in Appendix I).

Demographic information was derived from the Patient Administration System (PAS) and outcomes were sourced from the Cancer Outcomes and Services Dataset (COSD). Base population data were obtained from NHS Nottingham and Nottinghamshire Clinical Commissioning Group (CCG). Patients with missing data were included in the final analysis in an “Unknown” category.

Exposures, Covariates and Outcomes

The age and gender of all patients receiving a FIT request for symptomatic investigation were recorded. 65 years was used as a cut-off threshold (<65/≥65 years) for analysis of FIT return and comparison with base population data. Gender was classified as female or male or unknown. Patient ethnicity was recorded as declared by the patient on PAS and can be seen

summarised in Appendix IV. The ethnicities were categorised into five broad ethnic groups as defined by the UK Government for Census research purposes and are as follows: (1) White; (2) Asian or Asian British; (3) Black, African, Caribbean or Black British; (4) Mixed/ multiple or other ethnic groups; and (5) Unknown. Socioeconomic data were obtained from 6-digit patient postcodes using the Index of Deprivation tool (IoD19) to derive Index of Multiple Deprivation (IMD) quintiles for all patients. Deprivation was classified from least deprived (5^{th} Quintile) to most deprived (1^{st} Quintile). The primary study outcome was FIT return/non-return.

Cancer Outcomes

Cancer Outcomes and Services Datasets (COSD) were used to evaluate the diagnosis of colorectal cancer, recorded using ICD codes C18-C20 (excluding C18.1). Nottingham University Hospitals NHS Trust data and electronic patient records were used for cross-checking and validation of diagnosis data.

Statistical Analysis

Cohort demographics were presented as proportions and stratified by those who returned a FIT test and those that did not. Histograms were constructed to identify normal distribution for continuous data. Means with standard deviations were calculated for parametric data and medians with interquartile range for non-parametric data as appropriate. Differences in proportions between groups were evaluated for statistical significance using χ^2 test statistic. Characteristics of the study population were compared with Nottinghamshire population data by χ^2 analysis.

Factors predicting FIT non-return were evaluated by χ^2 comparison and used to develop a logistic regression model to analyse FIT non-return, adjusting for confounders including age, gender, ethnicity and socioeconomic status. Univariate and multivariate logistic regression analyses were undertaken to evaluate FIT return/non-return by gender, age, ethnic group and socioeconomic deprivation. Age was treated as a categorical variable, divided into patients aged 18-64 years and patients 65yrs old or greater. All statistics were performed using Stata Version 17 (Stata Corp, College Station, TX, USA). Tests of significance were considered significant if a P-value of less than 0.05 was obtained. Clinical data was collected as part of service evaluation audit (NUH Registration Number: 20-135C).

CRC outcomes were examined first by χ^2 comparison and subsequently analysed within a univariate and multivariate model to report the probability of CRC in those not returning a requested FIT sample compared to the overall referred population and those returning a low or negative FIT.

5.3 – Results

Cohort demographics

A total of 49166 FIT requests were made for 40817 individual patients in the study period (Figure 4). 1897 ineligible requests were excluded (Table 7). The first FIT requests for 38920 individual patients were included in the main analysis. 35289 patients returned a FIT sample after the first request (overall return 90.7%), whilst 3631 patients did not return a sample after first requested (overall non-return rate 9.3%). Of the 3631 patients who did not return their first FIT request, 1637 had a subsequent FIT request within 6 months. After a second request, 1022 patients returned a FIT sample, while just 615 did not. For the overall cohort, median follow-up time was 17.9 months (IQR 8.8-30.4). Median follow-up for FIT non-returners was 14.2 months (IQR 6.2-26.6) and for those with a f-Hb < 4 µg Hb/g faeces it was 19.0 months (IQR 9.6-31.9). The median age of the cohort was 66yrs (IQR 54-77yrs). The largest ethnic population in the study population was White (27278, 70.1%). The largest socioeconomic group of the investigated population was the least deprived (5^{th}) quintile (11036, 28.4%).

CRC Diagnosis

599 CRC were detected in the overall study population (cohort risk 1.5%). 561 CRC were detected in those returning their FIT (1.6%) whilst 38 CRC (1.0%) were detected in 3631 patients who did not return the first requested FIT. Of the FIT non-returns, 20 CRCs were detected from 1826 patients via routine or emergency pathways after no further FIT requests were made and 18 CRCs were detected from 1805 patients who had a further FIT requested (16 CRCs from 1637 within 6 months of the initial request).

Patients not returning a FIT sample after their first request were significantly more likely to be diagnosed with CRC than patients returning a FIT<4 (1% vs 0.1%, χ^2=112.52, p<0.001) or a FIT<20 (1% vs 0.3%, χ^2=149.53, p<0.001). Inclusion of all FIT returns made from subsequent requests within a 6-month period made no difference to the significance of the results for FIT<4 (χ^2=26.62, p<0.001) or FIT<20 (χ^2=6.02, p<0.014).

Comparison with the Nottinghamshire population

The baseline characteristics of the study population compared to the Nottinghamshire population are summarised in Table 8. There were significantly more females in the study population compared to the Nottinghamshire population (56% vs 49.9%, χ^2= 564.29,

p<0.001). The study population were significantly older, with 53.7% at least 65yrs old compared to 21.9% of the base population (χ^2= 21247.3, p<0.001). There were differences between the ethnicities of the study and Nottinghamshire populations (χ^2= 3974.3, p<0.001), the largest of which was in the Unknown group, comprising 21.5% of the study population and just 11.4% of the Nottinghamshire population. Social deprivation differed significantly between the study and Nottinghamshire population (χ^2= 2544.4, p<0.001). The least deprived (5th Quintile) were overrepresented in the study population, accounting for 28.4% of all FIT requests whilst constituting just 19.7% of the Nottinghamshire population. The most deprived quintile accounted for 22.9% of all FIT requests and represented 19.6% of the Nottinghamshire population.

FIT Return

FIT return varied by gender, age, ethnicity, and social deprivation (Table 9). Males were at a higher risk of FIT non-return, with 90.2% responding compared to 91% of females (χ^2=6.8, p=0.01). FIT non-returners were younger on average (median 62 years, IQR 49-77) than those returning their FIT test (median 67 years, IQR 55-77). FIT return in patients under 65yrs was significantly lower than those over 65yrs old (89.2% vs 91.9%, χ^2=87.1, p<0.001). FIT return was significantly higher in patients from White ethnicities compared to ethnic minority groups (91.2% vs 83.8% for Asian patients, 86.6% for Black patients, and 87.2% for patients from mixed or other races, χ^2=124.8, p<0.001). FIT return also differed significantly according to socioeconomic status. FIT return in the least deprived quintile was 93.6% compared to just 86.3% in the most deprived quintile (χ^2=352.9, p<0.001). FIT return by gender, age category, ethnic group and social deprivation is summarised in Table 8.

Predictors of FIT return

Male patients were less likely to return a FIT request compared to female patients after adjustment for other factors (OR 1.11 [95% CI 1.03-1.19]). Patients over the age of 65yrs were more likely to return a FIT test prior to referral compared to those aged 18-64 years (OR 0.78 95% CI 0.72-0.83). People from Asian and Black ethnicities had a 1.8- and 1.2-fold increase in non-return compared to those from a White ethnicity (OR 1.82 95% CI 1.58-2.10, and OR 1.21 95% CI 0.98-1.49 respectively). Non-return was comparatively worse in the Mixed/Other ethnic group (OR 1.29 95% 1.05-1.59) but not the Unknown group (OR 0.99 95% CI 0.90-1.08) compared to those declaring a White ethnicity. Non-return of a FIT

request varied by social deprivation and showed a marked graded increase across deprivation quintiles. Those in the most deprived quintile were more than twice as likely not to complete a FIT request than those in the least deprived quintile when accounting for other confounding factors (OR 2.20 95% CI 1.99-2.43).

Figure 4 – Flow chart showing first FIT requests made per patient, returns and CRC diagnoses by FIT strata

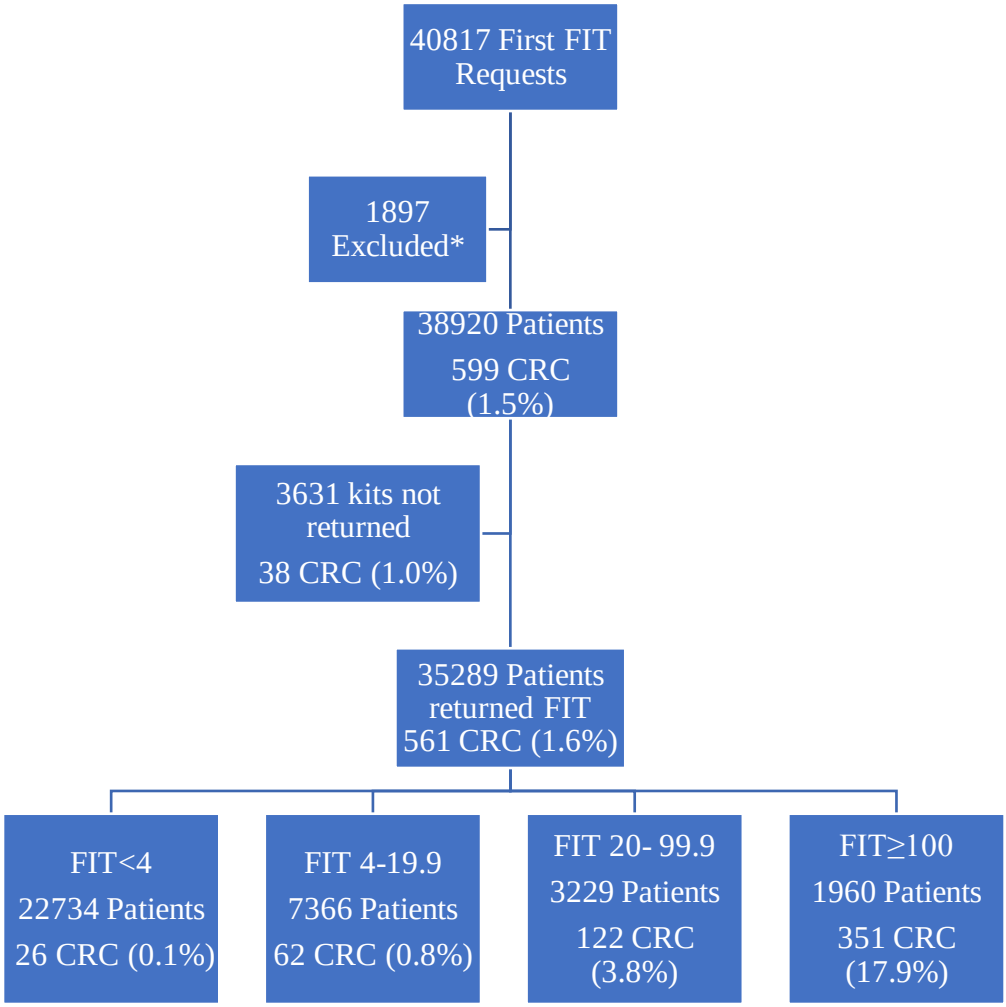

Table 7 – Excluded FIT Requests

Reason for Exclusion	Number Excluded
Rectal Bleeding	1218
Duplicate Request	315
Request from Out of Area	197
Sampling Error	101
Incomplete Request	39
Not indicated under 18 years	16
Incomplete Records	11
Total Excluded	1897

Table 8 – Baseline characteristics of patients who had a FIT request from November 2017 to December 2021 compared with Nottinghamshire population

		Base population	Investigated population	
Variable	**Categories**	Totals (%)	Totals (%)	CRC detected (%)
Gender	**Female**	496525 (49.9)	21800 (56)	252 (1.2)
	Male	498755 (50.1)	17112 (44)	347 (2)
	Unknown	35 (0.0)	8 (0.0)	0 (0.0)
Age	**<65 years**	777085 (78.1)	18029 (46.3)	130 (0.7)
	≥65 years	218195 (21.9)	20891 (53.7)	469 (2.2)
	Unknown	35 (0.0)	0 (0.0)	0 (0.0)
Ethnicity	**White**	753845 (75.7)	27277 (70.1)	439 (1.6)
	Asian	66220 (6.7)	1584 (4.1)	6 (0.4)
	Black	29565 (3.0)	801 (2.1)	7 (0.9)
	Mixed/Other	31750 (3.2)	876 (2.3)	8 (0.9)
	Unknown	113935 (11.4)	8382 (21.5)	139 (1.7)
Social Deprivation*	**5th Quintile**	195680 (19.7)	11036 (28.4)	183 (1.7)
	4th Quintile	204595 (20.6)	6278 (16.1)	124 (2)
	3rd Quintile	205315 (20.6)	6454 (16.6)	95 (1.5)
	2nd Quintile	194310 (19.5)	6177 (15.9)	95 (1.5)
	1st Quintile	195325 (19.6)	8927 (22.9)	102 (1.1)
	Unknown	90 (0.0)	48 (0.1)	0
* 5th Quintile refers to least deprived group, 1st Quintile refers to most deprived group				

Table 9: Univariate and Multivariate Logistic Regression Analysis of FIT Return by Gender, Age, Ethnicity and Social Deprivation

	Categories	Return (%)	Non-Return (%)	Univariate	Multivariate
				OR (95% CI)	OR (95% CI)
Gender*	**Female**	19841 (91)	1959 (9)	Reference	
	Male	15442 (90.2)	1670 (9.8)	1.10 (1.02-1.17)	1.11 (1.03-1.19)
Age	**<65 yrs**	16080 (89.2)	1949 (10.8)	Reference	
	≥65 yrs	19209 (91.9)	1682 (8.1)	0.72 (0.67-0.77)	0.78 (0.72-0.83)
Ethnicity	**White**	24864 (91.2)	2413 (8.8)	Reference	
	Asian	1328 (83.8)	256 (16.2)	1.99 (1.73-2.29)	1.82 (1.58-2.10)
	Black	694 (86.6)	107 (13.4)	1.59 (1.29-1.96)	1.21 (0.98-1.49)
	Mixed/Other	764 (87.2)	112 (12.8)	1.51 (1.23-1.85)	1.29 (1.05-1.59)
	Unknown	7639 (91.1)	743 (8.9)	1.00 (0.92-1.09)	0.99 (0.90-1.08)
Deprivation	**5th Quintile**	10328 (93.6)	708 (6.4)	Reference	
	4th Quintile	5808 (92.5)	470 (7.5)	1.18 (1.05-1.33)	1.18 (1.04-1.33)
	3rd Quintile	5885 (91.2)	569 (8.8)	1.41 (1.26-1.58)	1.39 (1.24-1.56)
	2nd Quintile	5521 (89.4)	656 (10.6)	1.73 (1.55-1.94)	1.68 (1.50-1.87)
	1st Quintile	7703 (86.3)	1224 (13.7)	2.32 (2.10-2.55)	2.20 (1.99-2.43)
	Unknown	44 (91.8)	4 (8.2)	1.30 (0.47-3.62)	1.28 (0.46-3.57)

*8 requests for patients of Unknown gender with 6 samples returned not displayed in table.

5.4 – Discussion

This is the first study to describe sociodemographic variations in FIT return in symptomatic patients from Primary care. Our study identified considerable demographic, ethnic and socioeconomic variation in FIT return. Male patients accounted for just 44% of the referred population despite constituting 50.1% of the base Nottinghamshire population, and males who were referred were less likely to return a FIT sample than females. FIT return also differed according to age, with younger patients (<65 years) significantly less likely to return a requested FIT compared to those over 65 years old. The referred population were considerably older than the base population, which was expected given the increased CRC risk at an older age. The ethnicities of those referred were broadly similar to the base population; and whilst the referred cohort contained a large number of patients of unknown ethnicity, this group appeared similar to the White ethnic group is its performance. Patients from ethnic minority groups had significantly lower FIT returns. The socioeconomic status (SES) of the base population was diverse, with a nearly equal split across all deprivation quintiles. This pattern was not mirrored in the referred population, which was most significantly over-represented by the least deprived/most affluent patients. Furthermore, FIT return decreased in a stepwise fashion across each deprivation quintile, with non-return more than twice as likely in patients from the most socially deprived areas compared to the most affluent.

The large cohort and high FIT return are strengths of the study discussed, which constitutes the first UK study to describe inequalities in FIT when used in a Primary Care setting to guide urgent referral practice for CRC. The data presented is from primary care and therefore represents an unselected real-life experience of FIT. Social deprivation data is more than 99% complete for the population and for those referred, which is a strength for identifying socioeconomic disparities pertaining to consultation/referral and to FIT return after referral. A relatively large proportion of the referred population did not declare their ethnic identity which is a weakness of this study. This unknown component, coupled with relatively low numbers of patients from Asian and Black ethnic groups somewhat limited further comparisons of outcomes with the base population. FIT stratification for rectal bleeding was not implemented during the study period, and as such this symptomatic subset has not been evaluated. Furthermore, some patients may have had subsequent cancer diagnoses in distant

trusts which has not been captured from our data collection. We primarily considered the first FIT request made for each patient to yield accurate cohort risks: subgroup analysis of additional FIT requests did not identify any divergence in FIT return or test performance.

The lower referral figures for males despite an equal gender split in the community, and furthermore their lower FIT return after referral represents a well described trend of lower male engagement with healthcare services. Numerous explanations exist for this trend, with masculinity ideologies [155], fearful health beliefs and lower health awareness [156] among men frequently cited as prohibitive factors. Simple solutions to redress this disparity are not immediately apparent, but strategies aimed at improving health education may encourage men to utilise health services in the long-term. This imbalance may require solving to meaningfully reduce CRC mortality given the higher incidence of CRC in the UK in males and a similar but more prominent disparity described in CRC screening participation[21].

Our study shows disparate results for FIT return by age, with patients under 65 years old less likely to return a FIT after it has been requested. The older referred population compared to the base is somewhat predictable given the increased CRC risk in older age groups, but still demands consideration to maximise engagement in younger patients in whom CRC incidence is rising in the UK and worldwide [157, 158]. Younger patients may be at risk of delayed referral in pathways reliant on symptoms, as a result of age-based referral criteria [159, 160] and perceptions of CRC as a disease of older individuals. FIT represents an opportunity to identify patients requiring investigation from this group to avoid missing curable pathology.

FIT return differed across different ethnic groups in this study. FIT return was highest in patients from White ethnic groups, and whilst White ethnic groups appear under-represented in the referred population, this may be artefactual; confounded by the large number of unknown ethnicities reported. After referral, patients from ethnic minorities were less likely to return a FIT, particularly when also from a lower socioeconomic quintile. Studies into barriers of CRC screening have shown that ethnic minority groups, non-English speakers, and those who do not engage with the information are less likely to complete the test kit [144], and similar factors may influence FIT return in symptomatic patients. Although rates of bowel cancer are lower in patients of Asian and Black ethnicity in the UK [153], this disparity demands we use insights from the literature and apply novel strategies to minimise ethnic inequalities, with appropriate safety-netting activities to support sample completion

[161]. Locally we have employed strategies including the provision of visual instructions and multi-lingual correspondence. Focused media campaigns including targeted social media activity may also have a role to play.

FIT return decreased in a stepwise fashion across each socioeconomic quintile, with non-return more than twice as likely in patients from the most socially deprived areas compared to the most affluent. The over-representation of the least deprived patients in the referred population is in line with CRC screening studies and the wider literature which reports lowest engagement in the most deprived patients. This may be due to patients from more deprived areas presenting less to primary care facilities, or due to GPs being less likely to refer patients from more deprived areas. The graded decrease in FIT return by SES appears less severe in our study, which may result from symptomatic patients being inherently more motivated to complete their investigations than asymptomatic patients due to a perceived threat to their health, which may overcome some of the negative emotions often associated with lower engagement such as embarrassment, disgust and fear [162, 163]. Wardle *et al*. reported that social deprivation was associated with more fearful and fatalistic attitudes towards cancer, and found an amelioration in the gradient of interest towards screening after accounting for these health beliefs in their analysis [162]. The differential return relating to negative health beliefs reinforces the need to counsel patients when the investigation is requested, and to promote a positive view of cancer outcomes to minimise fear-related avoidance.

There is understandable interest in the risk of missing CRC diagnoses when FIT is used as a stratification tool in Primary care and safety netting strategies to reduce post-negative FIT CRC rates are topical. We identify the need to consider "non-return FIT" CRC as another facet to this debate. The rate of "missed" CRC in this group at 1.0% appears to be lower than the 3% threshold defined by NICE for urgent referral but is much higher than the rate of CRC noted at follow-up for those with f-Hb below a threshold of 10µg Hb/g faeces (0.2%) or 20µg Hb/g faeces (0.3%). Thus, awareness in Primary Care of groups that are likely not to respond may address more potentially missed diagnoses than current concerns around "negative FIT" CRC. Frank conversations around willingness or ability to sample faeces in certain demographic groups may be useful and may also feed into safety netting strategies with follow-ups designed to ensure FIT has been returned. It is clear some means of accessing secondary care investigation should underpin implementation of FIT in Primary Care and the CRC rate presented here suggests that routine referral may be an appropriate safety net in the

event of FIT non-return. Whilst this may lead to a potential further disadvantage for those in whom a positive f-Hb would otherwise prompt urgent referral, the alternative of urgent referral for all non-returns would be unnecessary in the overwhelming majority and diminish the value of stratification by FIT. Evaluation in secondary care of demographics, symptoms, and other relevant factors, with clear information that the patient will not engage with FIT, could still allow upgrade to more urgent pathways when deemed clinically appropriate. In addition, multi-lingual correspondence and education resources may provide benefit in addressing ethnic variations and such strategies are well established in BCSP. Gender and deprivation disparities are more difficult to address but should still guide the initial consultation where FIT testing is agreed between doctor and patient.

Conclusions: Use of FIT in Primary Care is acceptable with over 90% of patients returning a kit. However, these data suggest that the population that fails to return kits has specific characteristics related to gender, age, ethnicity, and socioeconomic deprivation. These patterns appear similar to those reported in screening programmes based on faecal testing and should be considered as FIT for symptomatic patients continues to expand to ensure patients with these protected characteristics are not disadvantaged.

Chapter 6 – Serial Faecal Immunochemical Testing (FIT) in Patients with Colorectal Cancer

6.1 – Introduction

Colorectal cancer (CRC) is the 3rd most common cancer in males and females in the UK, and the 2nd most common cause of cancer death[1]. CRC is detected either in asymptomatic patients through the Bowel Cancer Screening Programme (BCSP), or more commonly in symptomatic patients referred to secondary care for investigation[3].

National guidelines to help General Practitioners (GPs) decide which patients should be referred for urgent investigations are currently based on age and symptom-based criteria, seeking to identify patients with at least a 3% risk of CRC (high-risk symptoms)[122]. A growing body of evidence supports the use of Faecal Immunochemical Testing (FIT) as a valid and objective risk stratification tool for patients considered to be symptomatically high or low risk with a far greater Positive Predictive Value (PPV) than clinical features alone[159, 164, 165], although national guidance has until recently recommended use of FIT only for those at "low-risk" of CRC[90].

The Association of Coloproctology of Great Britain and Ireland (ACPGBI) and the British Society of Gastroenterology (BSG) issued a joint guideline, recommending that FIT should be used by primary care clinicians to prioritise patients with clinical features of CRC for referral for urgent investigation, using a single test methodology and a positivity threshold of f-Hb ≥10 μg Hb/g[166]. This was reflected in updated diagnostic guidance published in 2023[91].

Although data strongly suggest a FIT-stratified triage significantly outperforms NG12 criteria on a population level, concern remains that a falsely negative FIT owing to one or more of a multitude of factors could prohibit individuals from accessing prompt investigation of their symptoms where a single-sample methodology is employed. Small studies undertaken by pioneer sites in the early phase of FITs incorporation into symptomatic pathways produced conflicting results on the added diagnostic performance of multiple FITs[105, 167]. The

ACPGBI/BSG recommendations found insufficient evidence to recommend use of repeat/second FIT to guide referrals in routine practice and found no studies examining the optimal period for undertaking a repeat FIT, suggesting also that further data are required to clarify the role of this approach[166].

Our aims in this study were to determine the occurrence of a falsely negative FIT result in the presence of CRC, and to identify the likelihood of sequential FITs yielding results in divergent strata used for clinical decision making.

6.2 – Methods

Patients diagnosed with colorectal cancer between October 2020 and August 2022 at Nottingham University Hospitals NHS Trust were considered for this study. All patients were identified at a specialist colorectal cancer multidisciplinary team (MDT) meeting. All FIT sample collection devices were sent to the patient to complete at home and return in a prepaid envelope. Baseline demographics and clinical information including age, gender, ethnicity, baseline haemoglobin, platelets, and ferritin, TNM staging, site of cancer and serial FIT results were recorded prospectively in a database stored on an encrypted NHS server. Patients were excluded if they were unable to give informed consent or if they were younger than 18 years old.

Five FIT sample kits were dispatched to participants with instructions for completion. The sample kits returned were analysed using the OC-Sensor FIT system (Eiken Chemical Co. Ltd) in the Eastern Bowel Cancer Screening Hub (EBCSH), adjoined to Nottingham University Hospitals NHS Trust. The EBCSH is UKAS (The United Kingdom Accreditation Service) accredited and participates in the UK NEQAS (The United Kingdom National External Quality Assessment Service) external quality assessment schemes. The research study was registered on www.clinicaltrials.gov (NCT04242901). IRAS number: 262746, REC reference number: 20/EM/0076.

The primary aim of the study was to determine the occurrence of a falsely negative FIT result in the presence of CRC. The number of participants yielding sequential FIT results in divergent strata used for clinical decision making were calculated to determine the variability of FIT and evaluate the efficacy of repeated FIT sampling.

6.3 – Results

44 participants out of 50 recruited returned their signed consent forms and FIT samples for inclusion in the final analysis. The median FIT result for the 1st sample returned was 552.4 (IQR 56.8-1916.8). A positivity threshold of 4 μg Hb/g faeces would have yielded no false negative results. A positivity threshold of 10 μg Hb/g faeces would have yielded 2 false negative results. 43 participants returned their 2nd FIT sample. The median FIT result for the 2nd sample was 2366 (87-1129.6). A positivity threshold of 4 μg Hb/g faeces would have yielded no false negative results. A positivity threshold of 10 μg Hb/g faeces would have yielded 2 false negative results. 43 participants returned their 3rd FIT sample. The median FIT result for the 3rd test was 361.8 (57.8-1271.6). A positivity threshold of 4 μg Hb/g faeces would have yielded 2 false negative results. A positivity threshold of 10 μg Hb/g faeces would have yielded 6 false negative results. 39 participants returned their 4th FIT sample. The median FIT result for the 4th test was 269.8 (42.8-1256.4). A positivity threshold of 4 μg Hb/g faeces would have yielded 2 false negative results. A positivity threshold of 10 μg Hb/g faeces would have yielded 2 false negative results. 30 participants returned their 5th FIT sample. The median FIT result for the 5th test was 180.3 (42.2-772.4). A positivity threshold of 4 μg Hb/g faeces would have yielded 3 false negative results. A positivity threshold of 10 μg Hb/g faeces would have yielded 5 false negative results.

40/44 participants also returned a FIT sample as part of their clinical investigations prior to CRC diagnosis. 25 of these participants completed the FIT after symptomatic presentation and 15 completed a FIT as part of the BCSP. The remaining 4 patients that were diagnosed with CRC did not complete a FIT during their initial investigations. A positivity threshold of 4 μg Hb/g faeces would have yielded 2/40 false negative results. A positivity threshold of 10 μg Hb/g faeces would have yielded 2/40 false negative results.

The median stage of disease at the time of diagnosis was T3 (IQR 2-3). 4 participants had been diagnosed with T1 disease, 14 with T2, 20 with T3 and 6 with T4 disease. 19 patients in the study had nodal disease (N1 or N2) and 5 patients had metastatic disease (M1).

20 patients in the study were diagnosed with rectal cancer (Table 11). 8 patients had sigmoid cancer, 7 had caecal cancer, 3 had CRC affecting their ascending colon, 4 hepatic flexure, 3 transverse colon, and 1 affecting the splenic flexure.

Table 11 shows the chance of a falsely negative test "missing" a CRC at different positivity thresholds. Participants had a 4.5% chance of a false negative from returning a single sample where a threshold of 10 µg Hb / g faeces was applied. This increased to 9.1% and 13.6% for thresholds of 20 µg Hb / g faeces and 40 µg Hb / g faeces respectively. The addition of a second sample reduced the occurrence of false negatives to 0 for thresholds of 10 µg Hb / g faeces and 20 µg Hb / g faeces. Further samples decreased the chance of a falsely negative result with a threshold of 40 µg Hb / g faeces but did not eradicate the risk entirely.

Table 10 – FIT result by Tumour Location after a single sample

	Tumour Location		
FIT	Right	Left	Rectal
<4	0	0	0
4-9.9	0	1	1
10-99.9	5	2	7
100+	9	9	10

Table 11 – Chance of Missed Cancer at Different Positivity Thresholds by Number of Tests

	Number of FIT samples				
Positivity Threshold	1 n=44	2 n=87	3 n=130	4 n =169	5 n=199
10 µg Hb / g faeces	4.5%	0	0	0	0
20 µg Hb / g faeces	9.1%	0	0	0	0
40 µg Hb / g faeces	13.6%	2.3%	2.3%	2.6%	3.3%

6.4 – Discussion

Potential outcomes of the study included added confidence in the ability of a single FIT sample to accurately diagnose CRC, and an ability to advise on the appropriate course of action in the event of a low/negative FIT result in a patient with lower-bowel symptoms. The study aimed to answer whether there was a benefit to repeating a FIT if the first sample did not meet the threshold for referral, and if there was an optimal time to do so to minimise the chance of a second false negative. This research was important to do to optimise FIT methodology possible before further comprehensive work into an individualised risk-scoring system can be considered.

In this study we aimed to examine the variability of FIT results in the presence of CRC over time to improve understanding of the performance characteristics of FIT, and whether additional FIT samples nullified the chance of falsely negative test results. Our study confirmed that the risk of a falsely negative result exists with a single sample, even with the application of a low positivity threshold such as that recommended by national advisory bodies[91]. Although no negative results were encountered in the first sample of this study using the lowest possible positivity threshold (4 µg Hb / g faeces), work in the preceding chapters of this thesis has demonstrated that such a low threshold does not completely eradicate the risk of false negatives on an individual level.

It is already known from research studies and clinical data in both screening and symptomatic populations that a single FIT sample confers a risk of a negative result falsely reassuring patients despite the presence of CRC[106, 124, 167, 168]. Units utilising FIT for symptomatic patients have arrived at disparate conclusions on how to use this information – many units continue to employ a single sample methodology (in line with national guidelines) whilst others have adopted a dual sample methodology[169, 170]. A recent systematic review of this subject confirmed the paucity of evidence for this approach, suggesting further research relating to replicate and repeat FIT testing[171].

Our study suggested that additional FIT samples appeared to ameliorate the occurrence of falsely negative results. Whilst no false negatives were evident after the return of at least 2 samples using a threshold of 10 or 20 µg Hb / g faeces, further rounds of testing paradoxically appeared to increase the risk of a falsely negative result for a threshold of 40

µg Hb / g faeces, as shown in Table 11. There is no suggestion that more tests increase the risk of falsely negative results, but rather this highlights a relative weakness of the study. Due to a single participant returning more than two samples below the threshold of 40 µg Hb / g faeces the number of "missed cancers" remains low; but with less participants returning third, fourth and fifth samples than the first two rounds of testing, the denominator used to calculate risk for more than two samples is lower, yielding the paradoxical increase in risk of missed cancers for this group. This also reinforces the notion that additional rounds of FIT sampling provide no guarantee of a higher FIT result for certain patients.

It is interesting to note that there did not appear to be a relationship between location of the tumour and the occurrence of a falsely negative result (table 10), although this may be explained by the relatively small cohort size evaluated in this study. The study was not designed to evaluate whether further samples was cost effective but does appear to suggest that a second sample confers increased sensitivity at positivity thresholds commonly used for clinical decision making. Real world clinical data with multiple FIT samples is required to conclude optimal testing strategy.

Chapter 7 – Discussion

This chapter constitutes an overall discussion of the thesis aims laid out in Chapter 1.6. The impact of the studies contained within this body of work are considered here alongside the relative merits and weaknesses of the studies undertaken.

The increasing incidence and significant associated mortality of colorectal cancer (CRC) necessitates effective and accessible diagnostic tools for early detection, particularly among symptomatic individuals. The body of work presented here aimed to optimise the efficacious use of FIT in the identification of CRC in symptomatic patients in Nottingham's RCCD pathway. The utility of blood tests and clinical features were examined, and sociodemographic variations in the pathway were evaluated. Finally, a clinical research study to determine the occurrence of falsely negative FIT results in the context of CRC was undertaken to assess the effect of additional samples on the diagnostic performance of FIT.

The first aims of this thesis were to assess the performance of Nottingham's RCCD pathway, report clinical outcomes 2 years since its inception, and to explore the utility of blood tests to aid in the identification of CRC. With regards to diagnostic accuracy, the FIT-stratified pathway demonstrated excellent discriminatory value as a "rule out" test, with a CRC risk less than 0.1% (8/8920) in patients with a FIT <4 µg Hb / g faeces. In the 2 years from its inception, patients who returned a FIT greater than 100 µg Hb / g faeces had a CRC risk of 21.4% (148/714), clearly identifying patients at the highest risk of CRC for urgent investigation as a "rule in" test.

Nottingham implemented a lower threshold for FIT positivity (4 µg Hb / g faeces) in the presence of anaemia, thrombocytosis, or an abnormal ferritin result from the pathway's inception in 2017. 9 cases of CRC were detected in patients from this subset, vindicating the lower threshold for those with aberrant blood tests. Conversely, only 1 CRC was detected with normal blood test results from this FIT stratum.

Over the first 2 years of the pathway, CRC detection in patients with a FIT of 10-19.9 µg Hb / g faeces was 1.4% (10/706). If the FIT positivity threshold had been 20 µg Hb / g faeces, 8 out of 10 of the CRCs detected in this group (10-19.9 µg Hb / g faeces) would have still been referred due to abnormal blood test results. The overall risk of CRC in patients with a FIT <20 µg Hb / g faeces was 0.1% (6/11194). These findings support the consideration of a higher positivity threshold than the nationally recommended 10 µg Hb / g faeces. In view of these results, the RCCD FIT positivity threshold for patients with normal blood test results was increased to 20 µg Hb / g faeces in April 2020 (and kept at 4 µg Hb / g faeces for those with anaemia/ thrombocytosis/ abnormal ferritin).

A relative weakness of the study reported in chapter 2 is the absence of a comparison with clinical outcomes and pathway effectiveness prior to the incorporation of FIT. This information is reported in another publication[172], albeit only from the first year of the pathway – with the authors concluding that FIT-stratification led to an increased ratio of CRC diagnoses being made on an appropriate 2WW pathway, and reporting that more than half of the 113 CRC cases diagnosed were early stage tumours (stage 1 or 2). Difficulties in obtaining reliable staging data and blood test results for all referred patients precluded further evaluation of any potential "stage shift" from the RCCD but did help to drive a change in FIT

data governance structures; and contributed to the expansion of the team responsible for symptomatic FIT audit with a dedicated data analyst.

It is clear from chapter 2 that readily available and cheap blood tests can be utilised alongside FIT stratification safely, with more CRC detected as compared to the nationally endorsed referral criteria. The "Four Fs" (FIT, Full blood count, Ferritin & Finger) recommended by Nottingham's RCCD exhibited a superior diagnostic performance than stratification with FIT alone.

In the examined cohort comprising of 13361 FIT results, the diagnostic performance of a 10 µg Hb / g faeces FIT positivity threshold with no blood tests would still fulfil the recommendations of the GDG in NG12, who advocated a 3% PPV risk-threshold for the urgent investigation of cancer. However, we have demonstrated that such a threshold would have missed more CRC than a FIT positivity threshold of 4 µg Hb / g faeces 20 µg Hb / g faeces depending on blood test results. A decision must therefore be made by those responsible for pathway development whether the risk of potentially missing cancers with less sensitive referral criteria is justified by operating within the boundaries of national clinical guidelines. This decision may be justifiable on a population-level but would seem especially unpalatable to an individual who is overlooked in the interstices of this dilemma.

The third aim of this thesis was to evaluate the stratification value of thrombocytosis for the detection of CRC in a symptomatic population. In chapter 3, a study of 2236 patients referred on a 2WW pathway between August 2014 and August 2017 is presented to achieve this aim. It was reported that CRC was significantly more likely in patients with thrombocytosis than those with a normal platelet count (12.4% vs 5.2%, χ^2 17.7, p=<0.0001). Thrombocytosis was significantly associated with more advanced (stage 3 or 4 CRC) and with the diagnosis of right-sided cancers compared with left-sided and rectal cancers. A multivariate logistic regression model of the dataset confirmed the associated of thrombocytosis with CRC diagnosis after adjustment for gender, age and anaemia (OR 2.62, 95% CI 1.60-4.30). Importantly, this study also found that thrombocytosis was associated with CRC diagnosis but not with the diagnosis of other cancers.

Whilst this report found that thrombocytosis was significantly associated with later stage disease, some patients diagnosed with early (stage 1 or 2) CRC in the study had no other abnormal blood parameters, suggesting that thrombocytosis does confer added stratification value for CRC. The association of thrombocytosis with right-sided CRC is also of relevance in this context, given an increased risk of falsely negative FIT results in patients with right-sided cancers [124]. The pathophysiology underlying this association has not been elucidated but may pertain to differences in cancer biology between right and left sided CRC [173].

A weakness of this study is the different patient population compared to that reported in chapter 2. The study to evaluate thrombocytosis had a considerably smaller cohort and a higher CRC risk of 5.8% (130/2236), compared to 1.7% (227/13361) from the RCCD 2-year outcomes. This suggests that the results examined in chapter 3 may not be representative of the current 2WW population and introduces a potential bias into any conclusions drawn. Unfortunately, accurate blood test results were not available for all patients in the 2-year RCCD analysis. This frustration helped motivate the aforementioned changes in FIT data governance structures but did limit further comparisons between study populations and the added value of thrombocytosis.

The fourth aim of this thesis was to compare the diagnostic value of clinical features within the NG12 referral criteria with FIT stratification. At the time of its authorship, NG12 was the relevant clinical guideline for urgent referral of suspected cancer in England, recommending referral based on age and symptoms-based criteria in primary care[72]. In the study presented in chapter 4, 1784 patients referred via 2WW pathway between September 2016 and June 2018 were included, with clinical features recorded at the time of referral. Male gender and being aged over 60 years were significantly associated with an increased risk of CRC diagnosis. Univariate logistic regression analysis found that patients presenting with both a change in bowel habit (CIBH) and IDA were the only symptom group significantly associated with an increased risk of CRC, compared to the reference group of patients presenting with a CIBH. Multivariate logistic regression showed that only an increased FIT result and male sex were significantly associated with an increased risk of CRC.

This study is important as it showed FIT stratification to be superior to clinical features at the time of referral in a multivariate model. Of interest, in this study population the risk of CRC

for patients with FIT <100 µg Hb / g faeces was 1.3% (21/1603). A single FIT stratified referral mechanism with a positivity threshold of 100 µg Hb / g faeces would have been concordant with the 3% PPV risk threshold recommended by the GDG in NG12. However, 21 CRCs would have been missed. Whilst nobody is advocating a positive threshold of 100 µg Hb / g faeces, this point reinforces the notion that fulfilling the obligations of a recommended risk threshold should not be the primary goal of any referral criteria.

The fifth aim of this thesis was to evaluate sociodemographic variation between the base population of Nottinghamshire and patients referred for suspected CRC diagnosis on the RCCD pathway. This study included 40817 patients who had FIT requests between November 2017 and December 2021 and compared them to the Nottinghamshire base population with 995315 patients.

There were sociodemographic differences between the base population and the referred population, with females significantly overrepresented in the referred population (56% vs 49.9%). The referred population were also significantly older than the base population (53.7% vs 21.9%). There were clear variations in the ethnicities of referred populations, although this was confounded by a large subset of patients in the referred population who had unknown or undeclared ethnicities. Finally, there were also disparities in the level of social deprivation in the referred population, with the least deprived patients significantly overrepresented as compared to the base population (28.4% vs 19.7%). Of note, the most deprived quintile was also overrepresented in the referred population (22.9% vs 19.6%).

As discussed in chapter 5, there are numerous potential reasons for the observed disparities. Given the increased risk of CRC at an older age, it is not surprising that the referred population were significantly older than the base population. The female overrepresentation in the referred population is consistent with literature that reports lower engagement with healthcare by males, both in CRC screening and in a broader context [174-176]; and is a concern given the higher incidence of CRC in men. The factors contributing to this imbalance are likely multi-factorial, with masculine attitudes, health literacy, social pressure, and cultural attitudes likely playing a part. Given the complex interplay of individual, interpersonal, organisational and societal factors involved, solutions are likely to be multifaceted and require context-specific interventions such as addressing health literacy

gaps, providing culturally sensitive care and engaging men in opportunistic settings such as the workplace [177-179].

Socioeconomic disparities between the base and referred population are also likely to be multifactorial. People with higher SES are more likely to be aware of available healthcare services, and more likely to have higher educational attainment with better health literacy to navigate the healthcare system[180, 181]. Furthermore, geographic barriers and time constraints limiting access to healthcare are more problematic for low-income communities[181]. The easy to use and typically postal-based nature of FIT assessment mitigates some of these difficulties, but still typically requires a healthcare consultation with a primary care physician to initiate investigations, so it is not surprising that disparity is evident in the referred population.

The sixth aim of this thesis sought to evaluate the sociodemographic variations in the return of FIT samples in the referred population previously discussed. The overall cohort returned the first FIT request more than 90% of the time, suggesting a test that is broadly acceptable to patients. However, the disparities described between the base population and referred population are largely matched by disparities in FIT return – with males, younger people, people from ethnic minorities and more deprived patients less likely to return a FIT request that has been made. The factors described previously are also relevant in this analysis, with similar strategies to improve engagement representing viable interventions to minimise inequality.

In Nottingham, visual instructions and multi-lingual correspondence have been introduced to minimise inequality. Focused median campaigns including targeted social media activity may also have a role to play. A FIT steering committee will continue to develop strategies to mitigate disparities, but clearly further work is required to better elucidate reasons for lower FIT return and to understand whether the disparities described are region-specific or broadly applicable to FIT pathways across the country.

The CRC incidence in the group of patients who did not return their FIT request was 1%, which is lower than the risk of CRC in the overall referred patient population, and far below the 3% risk threshold recommended for the urgent investigation of suspected cancer. This

suggests that an automatic urgent referral for people who do not engage with FIT is not necessarily indicated; but clearly patients who do not return FIT samples should still have access to secondary care investigation. We have suggested that an automatic routine referral may be an appropriate safety net in the referral machinery for patients who do not return a FIT sample, to minimise the risk of missing cancers whilst not diminishing the value of stratification provided by FIT.

As described, a weakness of the study described in chapter 5 is the large number of patients with undisclosed ethnicities. Whilst the White population appear to be underrepresented, the "Unknown" group have characteristics which mirror the White population. This confounding factor limited comparison between base and referred populations and requires consideration for future methods employed to evaluate inequality.

The seventh aim of this thesis was to determine the occurrence of a falsely negative FIT result in the presence of CRC and the final aim was to assess the diagnostic performance of additional FIT samples in the presence of CRC. These aims were presented in Chapter 6, reporting a prospective clinical research project in a study cohort recently diagnosed with CRC. 44 patients completed consent forms and multiple FIT samples over a month period to be included in this study. A "false negative" in the context of this study was considered as any result that would have been below the studied threshold for referral for urgent investigation.

Small studies undertaken by pioneer sites who incorporated FIT into symptomatic pathways have produced conflicting findings on the added diagnostic performance of multiple FIT samples [105, 167, 182]. Our study reported a 4.5% chance of a false negative FIT result where a single sample methodology was used with a positivity threshold of 10 µg Hb / g faeces. This false-negative rate increased to 9.1% and 13.6% with a positivity threshold of 20 µg Hb / g faeces and 40 µg Hb / g faeces respectively. The addition of a second FIT sample reduced the occurrence of a false-negative, with no false-negatives detected with positivity thresholds of 10 µg Hb / g faeces and 20 µg Hb / g faeces. Further samples decreased the chance of a falsely negative result with a threshold of 40 µg Hb / g faeces but did not eradicate the risk entirely.

False-negative results can occur for a multitude of reasons with FIT[124, 164, 183]. It has been suggested that stenosing lesions may confer a higher risk of falsely low F-Hb results[76]. Other authors have reported that medications such as proton pump inhibitors may reduce the accuracy of FIT for reasons yet to be elucidated[184]. Sampling or distribution errors may also account for false-negative results. Our findings show that although the risk of a falsely negative FIT result can be reduced with the low positivity threshold endorsed by national advisory bodies, a small risk of missing CRC remains, a finding echoed by other centres[185].

In our study, the addition of a second FIT sample would decrease the risk of a false-negative where the highest result was accepted. A second FIT sample costing less than £20 would appear to be a reasonable addition if it resulted in a lower chance of missing a CRC. However, consideration must be given to the additional burden on patients to complete multiple samples and the possibility that this could lead to lower FIT return rates. Such a change would also likely lead to a higher number of patients referred for urgent investigation with a concomitant increase in the demand on diagnostic services.

The Serial FIT study undertaken as part of this thesis had several weaknesses. 39/44 participants returned 4 FIT samples, but only 30/44 returned their 5th FIT sample. This may be due to patients starting treatment before being due to return their final sample. Furthermore, there is clearly a burden on patients who have recently been diagnosed with cancer to complete so many samples, which may have contributed. A study requesting just 2 or 3 FIT samples may have led to more successful recruitment, yielding more meaningful results. The small study size limited further analysis into whether tumour location or the stage of disease affected the likelihood of a false-negative result.

Whilst no false-negative FIT results were evident after the return of at least 2 samples using a threshold of 10 or 20 µg Hb / g faeces, further rounds of testing paradoxically appeared to increase the risk of a false-negative using a threshold of 40 µg Hb / g faeces, as shown in Table 11. There is no possibility that additional FIT samples increase the risk of a false-negative if a single result above the positivity threshold is considered a positive result for an individual. As less participants returned their 3rd, 4th and 5th samples compared to the number returning their first 2 samples, the denominator used to calculate risk for 3, 4 and 5 samples were lower, yielding a paradoxical increased in the rate of false negatives in this subset.

Unfortunately, the study reported here was devised at the height of the COVID-19 pandemic. The re-allocation of Research and Innovation department staff led to delays in the study obtaining ethical approval and being greenlit. Additionally, changes to the methodology were mandated by the sponsoring organisation to adopt phone-based recruitment. The initial study protocol had gained approval for participants to be consented and given information packs for the study in person after their MDT clinic appointments, which would have removed a source of delay associated with phone-based recruitment and may have potentiated more efficient recruitment of participants.

Overall, the study sample size was too small to derive statistically meaning conclusions around multiple sample methodology. The study into sociodemographic variation in chapter 5 that included a study population of 38920 patients contained several thousand patients who returned multiple FIT samples across the 4-year period that results were available for. Although there was significant heterogeneity in the rationale for these additional samples and in the period between them, research into this subset could be considered to evaluate whether there are any identifiable benefits for a multiple sample methodology. A recent systematic review concerning additional samples for symptomatic patients found that current evidence for repeat FIT is both minimal and conflicting[171]. This review supports the notion that further research in the field is required, as highlighted by NICE in DG56 [91, 171].

The foundation of this thesis rests upon a comprehensive and methodologically rigorous approach, affording it a series of notable strengths. The results reported in this body of work represent some of the most significant “real-world” clinical research in the field, with the largest datasets in the country. A thorough assessment of the RCCD incorporating FIT is offered, alongside a justification for the inclusion of additional risk stratifying blood tests. The first evidence of sociodemographic inequality in a symptomatic cohort is provided, where there is otherwise a paucity of data. Furthermore, the clinical research study undertaken on Serial FIT analysis is the first study designed to show variation of FIT results over a period of weeks, compared to studies which have previously examined FIT variability from a single stool or sequential bowel movements.

This body of work does have some limitations, described in the respective chapters and in the overall discussion. The COVID-19 pandemic significantly influenced the scope of data collection and analysis, limiting the depth of insights that could be deduced. Whilst it is acknowledged that utilising both quantitative and qualitative methodologies to impart a multifaceted perspective in the field of study would have enabled a richer comprehension of the concepts investigated, it was not possible to incorporate qualitative work with the time and resource constraints that were experienced. Although rigorous steps were taken to mitigate bias are described in the respective chapters, the presence of certain inherent biases within the research instruments and respondent pool cannot be entirely discounted. Additionally, the focus on a specific geographic region may curtail the transferability of findings to different contexts.

The relative strengths and limitations of this thesis have been openly discussed, engendering a renewed perspective on the significance of the work. Transparency in acknowledging the limitations demonstrates a commitment to intellectual honesty, prompting future researchers to address these areas with greater scrutiny. The strengths, on the other hand, accentuate our contribution to the field, reinforcing the potential for our work to be a catalyst for informed discussions and further investigations into the optimisation of FIT for the diagnosis of CRC in symptomatic patients. In this juncture, the strengths and limitations converge to spotlight the intricacies of our research, illuminating the path toward more refined inquiries.

Chapter 8 – Future work

The findings presented in this thesis represent a comprehensive appraisal of safely incorporating FIT into clinical pathways for the investigation of CRC in symptomatic patients.

Although it has been demonstrated that patient triage using FIT represents a generational improvement of diagnostic performance in comparison to previous referral criteria, it is patently clear that FIT alone cannot satisfy the requirements of an ideal instrument for risk stratification, with falsely positive and falsely negative results possible irrespective of positivity threshold or testing methodology. It is therefore imperative that work continues to optimise the utilisation of FIT, whilst seeking out novel solutions for the investigation of CRC.

Regarding the optimisation of FIT, future improvements may exist in a multitude of forms. Advancements in technology and further research may lead to improved sensitivity and specificity of the test, enhancing its ability to detect CRC and other gastrointestinal conditions accurately. Scoring systems to calculate individual risk of CRC already exist, as described in previous chapters, but have not been reliably proven in a clinical setting and are yet to be adopted on a wider basis. The use of large-scale health datasets and electronic health records could be analysed by traditional methods and by the training of Artificial Intelligence (AI) algorithms to identify trends and risk factors for CRC, ultimately leading to more efficient diagnostic strategies.

The RCCD findings presented in this thesis reported a very small number of CRC detected in patients with anaemia and either a negative or a lowly-positive FIT result. Work to determine whether non-colorectal cancers were more prevalent than CRC in this population of patients would be illuminating. If this is the case, further research into more efficient diagnostic strategies could be undertaken, potentially revealing a broader role for CTC in this subset. Conversely, the implications for patients with a high FIT and no detectable disease on "definitive" investigations remains unknown.

Research is needed to ascertain whether the diagnostic capabilities of FIT can be enhanced by the addition of established or novel biomarkers to provide more comprehensive information about the patient's present or future risk of CRC. Combining FIT results with molecular profiling could offer a more detailed assessment and improve diagnostic performance compared to FIT and the simple blood tests outlined in earlier chapters.

The use of liquid biopsy in CRC is a novel concept where specific genetic aberrations known to be associated with the disease could be identified in patients' blood samples, using highly optimised Polymerase Chain Reaction (PCR) techniques to potentially identify CRC before the disease is even radiologically evident. Multi-Cancer Early Detection (MCED) techniques such as the Galleri assay may yet aid in the detection of cancer, although they may overwhelm diagnostic services if widely adopted, by identifying genetic abnormalities which are common to multiple cancers, leading to increased referrals through 2WW pathways.

A successful grant application was made to the Mason Medical Research Foundation to facilitate a research study exploring the use of liquid biopsies for the diagnosis of CRC in symptomatic patients (Appendix VII). Unfortunately, time and resource constraints combined with institutional challenges meant that this work was not able to be completed for the thesis presented here. It is possible that these techniques may one day be used in conjunction with FIT, to tailor positivity thresholds for individual patients based on their specific risk factors or genetic profiles, allowing for more precise diagnostic approaches.

Prior to the incorporation of FIT in clinical pathways for symptomatic patients, it was widely thought that effectively ruling out CRC using FIT would free up diagnostic capacity, allowing for more sensitive positivity thresholds to be applied to screening programmes – the only route which has consistently identified early-stage disease. As FIT for symptomatic patients has become more widely utilised in primary care, the number of referrals on urgent pathways has increased, so diagnostic services have not been liberated from the demands imposed on them by 2WW pathways as had been hoped. In years to come it may be possible to recover some diagnostic capacity, enabling the attribution of more sensitive thresholds in the BCSP using additional tests as described above, but this concept is yet to be verified.

Finally, FIT may also be utilised in novel populations if its performance characteristics are proven in this capacity. A successful proposal was made to NIHR for an observational study exploring the utility of FIT to identify disease recurrence after a CRC resection (Appendix VIII). This pioneering work could be instrumental to ultimately liberating much-needed diagnostic capacity.

This thesis has presented a compelling argument that FIT stratification represents a generational improvement for the detection of symptomatic CRC. It has also demonstrated that significant further work is required to determine the optimal strategy for diagnosis of CRC.

Appendices

Appendix I: FIT Testing Methodology

Additional FIT methodology in line with FITTER checklist

All patients referred without rectal bleeding were sent (by normal UK Post Office mail system) a faecal sample collection device (OC-Sensor™, Eiken Chemical Co, Tokyo, Japan) within 2 days of the 2WW referral being received. The haemoglobin concentration in the OC-Sensor FIT is determined in nanograms of haemoglobin per millilitre of buffer in the sample tube (ng/ml). Each sample tube contains 2 ml of stabilising sample buffer in which, with the aid of the test-wand, 10 mg of stool sample is suspended. Final results are reported in ug Hb / g faeces.

The device was pre-labelled with the patient's name, NHS number, a unique laboratory ID number and a space to add the sample date. An instruction leaflet for using the sampling device, a letter outlining the purpose of the test and clarifying that the results would not be used for diagnostic purposes in isolation, and a prepaid first class return envelope were also included. Participants were asked to sample their faeces according to instructions, date the sampling device, and return it to the laboratory as soon as possible within 14 days of receipt of the letter. The process for kit dispatch and return was entirely postal.

All returned samples were logged prospectively at the receiving laboratory and analysed once for f-Hb using the automated OC-Sensor™-iO (Eiken Chemical Co., Tokyo, Japan) according to manufacturer's protocols, alongside f-Hb controls. The analyser was calibrated once a month, and 2 levels of controls were validated at the beginning and end of each run. Returned samples were stored in a refrigerator at 4°C upon arrival until analysis. All samples were analysed within 1 week of receipt.

If sample values were above the linearity of the assay (200ug Hb /g faeces) they were diluted in OC Calibration Diluent (1 in 10 and 1 in 100) in order to obtain a quantitative result.

Appendix II: Repeat FIT results in different strata

Colorectal cancer (CRC) diagnoses were related to any prior patient episodes that started from a FIT result and are presented in that context. There were six possible repeat FIT result scenarios possible:

1. single positive result was counted as a single positive patient
2. single negative result was counted as a single negative patient
3. multiple negative results for an individual were counted as a single negative patient
4. multiple positive results were counted as multiple positive patients
    a. allowing individual patients to be presented more than once only if multiple referral episodes were completed
    b. or as a single positive patient if only one referral was made
5. one or more negative results followed by a positive result for an individual were counted as a single negative patient
6. one or more positive results followed by a negative result for an individual were counted as a single positive patient if referral and investigation was completed prior to negative test.

Scenarios 1-3 are presented in Table 2. 229 patients had results in keeping with scenarios 4-6 with 3 related CRC diagnoses are shown below.

Appendix Table: 229 patients underwent repeat testing with f-Hb results in different strata.

Appendix Table: 229 patients underwent repeat testing with f-Hb results in different strata.

f-Hb (µg Hb/g faeces)		4-9.9	10-99.9	≥100
<4	Number of repeats Number of CRC's	101 0	71 1[†]	11 0
4-9.9	Number of repeats Number of CRC's		30 1[‡]	4 1[§]
10-99.9	Number of repeats Number of CRC's			12 0

[†]FIT test undertaken after resection of Colorectal cancer (diagnosed following "positive" FIT) returned a "negative" result during follow up (Scenario 6)

[‡]Initial FIT result not acted upon by GP but subsequent FIT also positive prompted referral (Type 4b).

[§]Initial FIT result prompted referral with IDA and f-Hb of 4 µg Hb/g faeces but CT abdomen and pelvis (CT AP) was undertaken as patient was considered unsuitable for colonoscopy. Patient was reassured after normal CT result. Repeat FIT seven months later yielded f-Hb>100 µg Hb/g faeces and prompted colonoscopy and diagnosis of CRC (Type 4a).

A fourth patient had "negative" FIT during our pilot study[9] that was not used for decision making. The patient had CTAP only as clinical review concluded that whole colon investigation was inappropriate. A subsequent FIT during this evaluation period showed 4-9.9 µg Hb/g faeces with low Ferritin only and was found to have CRC on colonoscopy. The patient declined resection but had potentially curable disease at the time of diagnosis.

Appendix III: A proposal for prioritisation during COVID

During lockdown local practice was amended to reflect national and association guidelines. Limited endoscopy and CT colonography was maintained at a local Private Provider site.

Rectal mass	Flexible sigmoidoscopy only – rectal cancer is treatable with RT
Rectal bleed	Patients <40yo should have a telephone consultation – bright red bleeding with pain/perianal symptoms put on "hold" During COVID Nottingham Colorectal Service shall request a FIT test for patients and send the attached letter. Those with FIT<4 should be placed on "hold". FIT>4 should proceed as follows: Flexible sigmoidoscopy only in patients >70y with underlying health conditions irrespective of blood results – rectal cancer treatable with RT Flexible sigmoidoscopy only in all other patients with normal bloods CTC or Colonoscopy if Hb<120 M, Hb<110 F OR Platelets>400 OR Ferritin<25 or >350
All other symptoms	**FIT result >100 and rectal bleeding excluded at telephone vetting:** FIT 100-149.9 CTC (Targeted endoscopy only if biopsy needed) FIT 150+ CTAP first for patients >60yo and all patients with platelets ≥400 to identify: Identifiable colorectal pathology and need for endoscopy accordingly Identifiable metastasis – to be discussed at MDT Extra-colonic malignancy to be passed on to other MDTs If negative: CTC for >60yo with underlying health conditions (targeted endo for bx if needed). Colonoscopy for all other patients. FIT 20-99.9 Hold FIT 4-19.9 with abnormal bloods Hold

Post-lockdown exit strategy:

Prioritise new referrals with FIT>100 as above until backlog cleared.

Return all FIT results below 20 if FBC and Ferritin normal as agreed locally with usual advice.

Revert to pre-COVID blood thresholds for new referrals:

Hb<130 M, Hb<120 F OR Platelets>400 OR Ferritin<25 or >350

Prioritisation of FIT 20-99.9 backlog as follows:

1. FIT 20-99.9 with abnormal bloods in declining FIT order:
    a. Colonoscopy if not on anticoagulants/antiplatelets
    b. CTC if on anticoagulants/antiplatelets (or not suitable for colonoscopy)
2. When above cleared CTC for FIT 4-20 with abnormal bloods
3. When above cleared FIT 20-99.9 with normal bloods
    a. Colonoscopy if not on anticoagulants/antiplatelets
    b. CTC if on anticoagulants/antiplatelets (or not suitable for colonoscopy)

Prioritisation of rectal bleeding new and backlog as follows:

1. Colonoscopy for rectal bleeding with abnormal bloods and FIT>4 - CTC if not suitable for colonoscopy. Stop anticoagulants/antiplatelets prior endoscopy. If not safe to do so CTC.
2. Flexible sigmoidoscopy if bloods normal and FIT>4
3. Flexible sigmoidoscopy if bloods normal and FIT<4 and investigation still warranted after telephone consultation (or clinic)

Bowel Cancer Screening Programme:

Contact backlog patients who have returned FIT>120 in declining order and undertake colonoscopy – unless patient chooses CTC or declines.

Routine referrals, polyp follow up and CRC follow up:

Invite patients that are waiting to return a FIT kit to ensure patients with high risk of CRC not missed. FIT results to be used to guide prioritisation of backlog.

Appendix IV: Ethnicity Classification Groups

White

- English, Welsh, Scottish, Northern Irish or British
- Irish
- Gypsy or Irish Traveller
- Any other White background

Mixed or Multiple ethnic groups

- White and Black Caribbean
- White and Black African
- White and Asian
- Any other Mixed or Multiple ethnic background

Asian or Asian British

- Indian
- Pakistani
- Bangladeshi
- Chinese
- Any other Asian background

Black, African, Caribbean or Black British

- African
- Caribbean
- Any other Black, African or Caribbean background

Other ethnic group

- Arab

Any other ethnic group

Appendix V: Comparison of Base and Investigated Populations after Adjustment for Unknown Ethnicity

		Base population		Investigated population	
Variable	Categories	Totals	%	Totals	%
Ethnicity	White	753845	85.5	27277	89.3
	Asian	66220	7.5	1584	5.2
	Black	29565	3.4	801	2.6
	Mixed/Other	31750	3.6	876	2.9

		Base population		Investigated population	
Variable	Categories	Totals	%	Totals	%
Ethnicity	White	753845	75.7	27277	70.1
	Asian	66220	6.7	1584	4.1
	Black	29565	3	801	2.1
	Mixed/Other	31750	3.2	876	2.3
	Unknown	113935	11.4	8382	21.5

Appendix VI: Ethical Approval for Serial FIT Testing in Colorectal Cancer Study

Mr David Humes

E Floor West Block

les.nhs.uk Queens Medical Centre
Nottingham

NG7 2UH

Email: approvals@hra.nhs.uk
HCRW.approvals@wa

11 May 2020

Dear Mr Humes

HRA and Health and Care

Study title:	**Serial Faecal Immunochemical Testing in patients with Colorectal Cancer**
IRAS project ID:	**262746**
Protocol number:	**N/A**
REC reference:	**20/EM/0076**
Sponsor	**Nottingham University Hospitals NHS Trust**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?

HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?

HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?

Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **262746**. Please quote this on all correspondence.

Yours sincerely,

Alex Thorpe

Approvals Manager

Email: approvals@hra.nhs.uk

Copy to: *Ms Jennifer Boston, Nottingham University Hospitals' Trust, Sponsor's Representative*

List of Documents Reviewed

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
GP/consultant information sheets or letters [Gp letter - 262746 - v1.0 - 30-01-20]		30 January 2020
Instructions for use of medical device [Supplementary - FIT sample instructions in non-technical language]	1.0	21 April 2020
IRAS Application Form [IRAS_Form_12022020]		12 February 2020
IRAS Application Form XML file [IRAS_Form_12022020]		12 February 2020
IRAS Checklist XML [Checklist_06052020]		06 May 2020
Other [Summary of changes to PIS]	N/A	06 May 2020
Other [Ethics Board review - Table of revisions]	1.0	20 April 2020
Participant consent form [Consent Form Version 1.1]	1.1	20 April 2020
Participant information sheet (PIS) [Participant Information Sheet Version 1.1]	1.1	24 April 2020
Research protocol or project proposal [Protocol - 262746 - v1.0 - 3001-20]	1.0	12 February 2020
Summary CV for Chief Investigator (CI) [David Humes CV 10-09-19]		10 September 2019
Summary CV for student [James Bailey 2 page CV - 01-02-20]		01 February 2020
Summary CV for supervisor (student research) [David Humes CV]		10 September 2019

Appendix VII – Mason Medical Research Foundation Pump Priming Application and Approval

MASON MEDICAL RESEARCH FOUNDATION

APPLICATION FOR A MEDICAL RESEARCH GRANT

(A pdf of this form with signatures together with items 1(b) to 1(e) listed in the Rules of the Foundation to be emailed by 1 February 2021.)

Full name of applicant: (State Title i.e. Dr.) Mr JAMES BAILEY

Address: NOTTINGHAM COLORECTAL SERVICE, QMC CAMPUS, DERBY ROAD, NOTTINGHAM, NOTTS, NG7 2UH

Age 32

Qualifications BSc (Hons) BMBS PGDip MRCS

Present Post COLORECTAL RESEARCH FELLOW

Brief Curriculum Vitae of applicant (**max. 2 pages**). Any additional information regarding applicant which he or she considers relevant to application should be submitted. (**max. 2 pages**).

Clear Title of Project IDENTIFICATION OF KRAS/BRAF/PIK3CA MUTATIONS IN CIRCULATING TUMOUR DNA – IMPROVING THE SENSITIVITY OF FIT-STRATIFIED COLORECTAL CANCER DIAGNOSIS

Amount of grant required £ 6601 - 17

Organisation to whom the payment should be made together with bank details [NB the **precise name in whose favour it is to be paid**], if successful:

Organisation account bank name:
Organisation account name:
Organisation account number:
Organisation sort code:

I have read the rules of the Foundation and, if my application is successful, I agree to abide by them. I have obtained the approval of the local Ethical Committee (where relevant), and am **attaching evidence** of this.

(Signature)

The application should be submitted through the **Administration*** of the institution in which the applicant intends to undertake the work proposed.

I have read this application and confirm that it will be accommodated in this Institution and that, if the grant is awarded, the Institution will administer the grant.

(Signature)

(Office Held) Finance Officer - Pre Award Manager

*Finance Officer, Bursar, Registrar, Secretary etc.

Mr James A. Bailey BSc (Hons) BMBS PGDip MRCS

38 Nazareth Road, Nottingham, NG7 2TP

Email: james.bailey4@nhs.net Phone: +44 (0)7856917071

Academic Qualifications:

Aug 16	**Medical Education – Postgraduate Diploma (PGDip)** Cardiff University
May 15	**Member of the Royal College of Surgeons of England** Member by examination
Jul 13	**Bachelor of Medicine Bachelor of Surgery (BMBS)** University of Nottingham
Jul 09	**Biomedical Sciences with Molecular Biochemistry Bachelors of Science, First Class Honours** University of Wales Institute Cardiff

Employment History:

Aug 19-Present	Clinical Research Fellow in Colorectal Surgery (NUH NHS Trust)
Feb 19-Aug 19	ST4 Surgical Registrar – General/Transplant/Endocrine Surgery (NUH)
July 18-Feb 19	ST4 Surgical Registrar – General Surgery – Colorectal Surgery (CRH)
Feb 18-July 18	ST3 Surgical Registrar – General Surgery – HPB Surgery (Derby)
Aug 17-Feb 18	ST3 Surgical Registrar – General Surgery – Breast Surgery (NUH)
Apr 17-Aug 17	Core Surgical Trainee – General Surgery (CRH Foundation NHS Trust)
Dec 16-Apr 17	Core Surgical Trainee – General Surgery (NUH)
Aug 16-Dec 16	Core Surgical Trainee – General Surgery (ULH NHS Trust)
Jun 16-Aug 16	Major Trauma Fellow – Queens Medical Centre, Nottingham
Aug 15-May 16	Clinical Fellow in Hepato-Pancreatico-Biliary Surgery
Aug 14-Aug 15	Foundation Year 2 doctor (ULH NHS Trust)
Aug 13-Aug 14	Foundation Year 1 doctor (NUH)

Selected Presentations:

Jan 21	**FIT and blood tests for prioritisation of urgent colorectal cancer referrals in symptomatic patients** Online presentation, BSG Campus
Jul 20	**Post-faecal immunochemical test (FIT) colorectal cancer outcomes: evaluation of a symptomatic pathway at 2 years** Oral presentation, ACPGBI Annual Meeting

Selected Publications:

In press **FIT and blood tests for prioritisation of urgent colorectal cancer referrals in symptomatic patients: a two-year evaluation**
BJS Open. *BJS5-2020-07-0123*
Bailey JA, Weller J, Chapman CJ, Ford A, Hardy K, Oliver S, Morling JR, Simpson JA, Humes DJ, Banerjea A.

In press **Quantitative FIT stratification is superior to NICE referral criteria NG12 in a High-Risk Colorectal Cancer population**
Techniques in Coloproctology. *TCOL-D-20-00667*
Bailey JA, Ibrahim H, Bunce J, Chapman CJ, Morling JR, Simpson JA, Humes DJ, Banerjea A.

Jul 20 **Thrombocytosis helps to stratify risk of colorectal cancer in patients referred on a Two-Week Wait Pathway**
Int J Colorectal Dis. *35(7):1347-1350.*
Bailey JA, Hanbali N, Premji K, Bunce J, Simpson J, Humes DJ, Banerjea A.

Apr 20 **GP access to FIT increases the proportion of Colorectal Cancers detected on urgent pathways in symptomatic patients**
The Surgeon. https://doi.org/10.1016/j.surge.2020.03.002
Bailey JA, Khawaja A, Andrews H, Weller J, Chapman C, Morling J, Oliver S, Castle S, Simpson JA, Humes DJ, Banerjea A.

Current Research Interests:

I am currently undertaking a PhD with the University of Nottingham exploring the use of faecal immunochemical testing (FIT) in colorectal cancer (CRC). In my first 18 months I have published 4 peer-reviewed articles discussing the investigation of symptomatic patients in Nottingham University Hospitals. We showed the superior stratification value of FIT as compared to symptoms in a Two-Week-Wait pathway and confirmed the value of thrombocytosis in CRC detection, furthering previous departmental work on blood tests to stratify risk of CRC.

We demonstrated that incorporation of FIT into symptomatic pathways identifies more cancers but also increases the workload of diagnostic services, demanding novel solutions to improve sensitivity in clinical pathways. Circulating Tumour DNA (ctDNA) is emerging as a leading candidate to facilitate improvements in diagnostic performance alongside blood tests and FIT and will form an essential part of our research.

(Please contact for references)

Identification of KRAS/BRAF/PIK3CA mutations in Circulating Tumour DNA (ctDNA) – Improving the sensitivity of FIT-stratified Colorectal Cancer diagnosis

(i) Introduction

Colorectal cancer is a leading cause of cancer death in the UK and worldwide (1). Improved access to specialised care and screening services has contributed to a reduction in mortality in recent decades but challenges remain (2). Importantly, early CRC detection can significantly improve the cure rate (2). Traditional clinical diagnostic methods include colonoscopy, imaging, and tissue biopsy. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are used as serum tumour markers, but these two markers alone do not fully satisfy clinical needs due to their lack of sensitivity and specificity and are not used in the diagnostic setting (3). Faecal Immunochemical Testing (FIT) shows great promise stratifying risk of CRC in both screening and symptomatic patient populations by identifying microscopic quantities of haemoglobin from the colon (4, 5), but the optimal strategy for investigating patients with low or indeterminate faecal haemoglobin levels in the presence of ongoing symptoms is yet to be elucidated (5). Measurement of specific Volatile Organic Compounds (VOCs) in excreted biological materials and epigenetic changes in faecal-DNA have been shown to improve sensitivity for CRC detection but are reliant on technically complex and prohibitively expensive proprietary systems limiting their clinical suitability (6).

"Circulating tumour DNA (ctDNA) obtained from a "liquid biopsy" blood test has emerged as a promising diagnostic tool for CRC (7). ctDNA arises from apoptotic or necrotic tumour cells, active tumour cells and circulating tumour cells (CTCs). Typically, ctDNA PCR aims to identify mutant-band alleles associated with cancer which are disparate in their length and composition to wild-type alleles (8) in a process which may be more sensitive at detecting malignancy than conventional clinical and radiological approaches (9).

Improvements in polymerase chain reaction (PCR) methodology now permit the exploration of cell-free and ctDNA as a biomarker for CRC. HOT_ARMS is a highly optimised method utilising the Amplification Refractory Mutation System (ARMS) to create an ultra-sensitive real-time PCR based single nucleotide mutation detection system specifically designed for cell-free DNA which can scale across the whole genome with detection as low as 1 mutant copy.

Local lab data using standardised samples found KRAS/BRAF/PIK3CA mutations associated with CRC using HOT_ARMS from plasma samples in two-thirds of the total patients harbouring mutations in their FFPE tumour block, representing a significant advancement in accuracy (9, 10). An opportunity exists to explore the relationship between the detectability of CRC using HOT_ARMS PCR in a clinical setting.

(ii) Hypothesis/Research Question

- Is ctDNA present in patients diagnosed with CRC?
- What is the added value of ctDNA in CRC in a FIT-stratified population?

(iii) Experimental Plan

The proposed pilot study is an observational prospective cohort study. Patients with a diagnosis of colorectal adenocarcinoma at Nottingham University Hospitals between May 2021 and August 2022 will be invited to participate. Patients will be consented in accordance with Good Clinical Practice (GCP) guidelines with Research Ethics Committee (REC) approval.

Participants will be identified and offered to participate in this study alongside "Serial FIT in CRC" (NCT04242901), a clinical research project which is actively recruiting participants. An amendment to NCT04242901 has been reviewed locally and submitted for REC approval to include a liquid-biopsy for identification of KRAS/BRAF/PIK3CA mutations in ctDNA. 180 participants with confirmed CRC will be recruited for this study over an 18-month period: a feasible target based on 20 participants recruited to NCT04242901 in a 7-week period. Liquid biopsy blood samples will be drawn at the same time as venepuncture as part of usual routine care. The sample will be sent to an on-site UKAS accredited medical laboratory for DNA extraction and optimised PCR analysis. Liquid biopsy results will be compared with corresponding formalin-fixed paraffin-embedded (FFPE) tumour biopsies to identify hotspot mutations in PIK3CA, BRAF and KRAS genes indicating presence of CRC. Liquid biopsy and tumour biopsy results will be analysed with FIT results and blood test results (FBC, CEA) collected as part of routine care to identify whether there is added value to detecting ctDNA for diagnosis of CRC. The diagnostic performance of HOT_ARMS PCR in addition to Quantitative FIT will be evaluated.

(iv) Expected Outcome and Justification

The liquid biopsy methodology described is ultrasensitive, cheap, reliable and scalable unlike anything else currently available. This proposal represents the first study to evaluate the clinical validity and potential impact of HOT_ARMS PCR on CRC diagnosis. As a pioneering centre researching the broader utilisation of FIT for CRC, Nottingham has the advantage of being able to compare ctDNA results with FIT results. This may reveal a subset of patients with "FIT-negative" CRC that can be detected by liquid biopsy – which would be revolutionary in diagnosis of the disease.

(v) Amount for which Grant application is being made

Total amount requested including VAT – £6601.17 (£36.67 per participant)

- Liquid biopsy ctDNA extraction (£12.50 excluding VAT)
- FFPE ctDNA extraction (£6 excluding VAT)
- KRAS/BRAF/PIK3CA target PCR Assays (£12.17 excluding VAT)

Appendix References

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Mason Medical Research Trust – Grant

131anice.botting@rsmuk.com

<131anice.botting@rsmuk.com>

Mon 29/03/2021 12:28

To: BAILEY, James (HEALTH EDUCATION EAST MIDLANDS) <james.bailey4@nhs.net>

Dear James

I am writing to advise you that you were awarded £6,601.17 at the recent panel meeting.

The money will be paid into the University of Nottingham's bank account as stated on your application form.

Kind regards

Janice

Janice Botting Assistant

RSM UK Tax and Accounting Limited
Portland, 25 High Street, Crawley, West Sussex, RH10 1BG
T: +44 845 057 0700 | **DL:** +44 1293 591869 | **W:** www.rsmuk.com

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Appendix VIII – NIHR RFPB Approval

Thursday 21 July 2022

Dear Mr Humes,

Research for Patient Benefit (RfPB) Programme: NIHR204124 – Faecal Immunochemical Test (FIT) for Surveillance of Colorectal Cancer Study (FITS Study)

I am pleased to inform you that the Committee has recommended your application, submitted for consideration in Competition 46, for funding. The Department of Health and Social Care, in their capacity as the National Institute for Health and Care Research (NIHR), has confirmed their intention to award funding upon acceptance of the terms and conditions set out in the Standard Research Contract and pending agreement to the suggested amendments recommended by the Committee, as detailed below under the 'Committee feedback' heading.

The Standard Research Contract, between Contractors and the Secretary of State for Health for all initiatives can be found on the NIHR website.

Next Steps

The NIHR is committed to the rapid initiation of research following the decision to fund to benefit patients as soon as possible. Therefore, we expect funded researchers to be working towards gaining the necessary contractual agreements and governance approvals required to start the project between **01 November 2022** and **30 April 2023** or by a date mutually agreed by both parties on acceptance of the award.

The NIHR acknowledges the risk to organisations around committing resources to research before a contract is in place; however, it is rare to not reach contractual terms unless the circumstance of the research team changes. The NIHR, therefore, encourages organisations to commit staff to setting up projects at as early an opportunity as possible in order to expedite the formal commencement of research. It is acknowledged that there can be unforeseen delays in starting up a research project, but in order to help reduce these it is your responsibility to work closely with your organisation's R&D department or equivalent as well as other colleagues / departments involved in the administration and management of the research, and to start these discussions at the earliest opportunity.

To ensure that the project starts within the agreed timeframe with all the required agreements and approvals in place, appropriate staff (such as project and/or study managers) need to be in post as early as possible after receiving this letter of intent. These staff costs will ultimately be covered through the research funding award, but you are encouraged to meet them from Research Capability Funding (RCF) prior to the research contract being agreed.

To support the often-iterative process towards agreement of the contract, we have set out the guiding timeframes for the submission of responses or information for each step towards the agreement of the Standard Research Contract as well as the anticipated start date. However, we are aware that meeting these deadlines might be difficult for some research institutions over the summer holiday period and so we would allow some flexibility.

- Confirmation of acceptance of funding – no later than **04 August 2022**
- Responses to Committee feedback and queries – no later than **04 August 2022**
- Responses to Finance, Contracting and IP queries – no later than **04 August 2022**
- Contract signature – no later than **21 December 2022**
- Contracted commencement start – between **01 November 2022** and **30 April 2023** or by a date mutually agreed by both parties on acceptance of the award
- Submission of draft collaboration agreements and/or subcontracts (where applicable) – 6 months from the start of the project, or a date mutually agreed on acceptance of the award

On receipt of information as set out above, the NIHR through the Central Commissioning Facility is committed to responding to your submission of information within two weeks or we will update you on progress.

Please take the time to carefully read the enclosures to this letter which details the feedback on your application, your contact, Dr Phoebe Walsh within the Central Commissioning Facility who will be working with you on the contract, the processes to be undertaken during the next steps, as well as additional information relating to your award. Please note that all the responses to the queries listed below need to be sent directly to **Dr Phoebe Walsh** at **phoebe.walsh@nihr.ac.uk**.

Yours sincerely,

Mr Ben Morgan

Assistant Director, NIHR Research for Patient Benefit Programme

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