Novel Methods for the Detection, Risk Stratification and Management of Early Colorectal Cancer and Pre-Malignant Lesions

By

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Abstract

Colorectal cancer (CRC) is the 4th most common cancer in the United Kingdom (UK) with approximately 44,000 cases each year, and with 16,800 deaths, is the 2nd most common cause of cancer-related mortality. Outcome is directly linked to stage at diagnosis with 5-year survival estimated to be 90.9% for those with stage I disease, 84.3% for stage II, 65.0% for stage III and 10.5% for stage IV(1). Pathway to diagnosis in Scotland can be via symptomatic referral to an outpatient clinic, emergency symptomatic presentation or via the Scottish Bowel Screening Programme or surveillance colonoscopy. Screening in Scotland utilises a quantitative faecal immunochemical test (FIT) in individuals aged 50 to 74 years, followed by colonoscopy for those patients testing positive, at a faecal haemoglobin (f-Hb) threshold of 80 μg Hb/ g of faeces(2). Screening in this fashion increases the number of early-stage cancers diagnosed, reduces CRC-mortality and may reduce the incidence of CRC through the removal of precursor polyps(3-8). Unfortunately, screening only accounts for 19% of CRCs diagnosed in the West of Scotland(9). While the symptoms of CRC, including rectal bleeding, persistent change in bowel habit, abdominal pain and weight loss, are commonly present at the time of diagnosis, considerable symptomatic overlap exists with other significant bowel disease (advanced polyps and inflammatory bowel disease (IBD)) and functional bowel disorders. Therefore, the positive predictive value of such symptoms for CRC is low. Indeed, in a study of 384,510 colonoscopies performed in the UK, rectal bleeding and anaemia were associated with the highest adjusted positive predictive values (aPPV) for CRC (2.5% and 2.1% respectively), while all other symptoms were associated with a CRC aPPV of <1%(10). Conversely, FIT is a powerful objective biomarker of CRC-risk in symptomatic patients, with a linear relationship between f-Hb and CRC-risk observed(11). Consequently, in addition to its use in screening, FIT has now been widely embedded into symptomatic referral pathways as an effective means of triaging patients(11-22).

Chapter 1 provides a broad overview of the epidemiology, aetiology, signs and symptoms, investigation, staging and management of CRC.

In Chapter 2 the impact of integrating FIT into referral pathways from primary care to colorectal and gastroenterology was assessed in a cohort of 4968 symptomatic patients. Additionally, the association between CRC risk, symptoms, faecal haemoglobin (f-Hb) and anaemia were examined. GP referral and secondary care investigation patterns were indeed influenced by FIT, with a raised f-Hb correlating both with the decision to refer to secondary care and to perform colonoscopy. While rectal bleeding showed a positive correlation with CRC risk, no individual symptom independently predicted CRC on multivariate analysis. Conversely, both f-Hb and anaemia independently predicted CRC risk and represent valuable objective markers of risk in symptomatic patients. The combined absence of a raised f-Hb or anaemia effectively excluded CRC in 99.96% of cases.

In chapter 3 the results of a multicentred study of 5,761 patients investigating the prevalence of repeat FIT testing in primary care are presented. The study aimed to examine the relationship between serial f-Hb concentrations and CRC risk in symptomatic patients. Consecutive FIT tests submitted within 12 months of each other accounted for 9.1% of all FIT tests submitted to the laboratories of the Scottish health boards under investigation. CRC prevalence amongst patients with such serial FIT measurements was 0.7%, lower than the CRC rate observed in single FIT symptomatic cohorts. Patients with two f-Hb measurements <10μg/g had a significantly lower CRC risk (0.1%) than those with at least one f-Hb ≥10μg/g. As the number of FIT tests performed within a year rose, the likelihood of having a positive test rose, while the CRC rate fell. Performing two FITs within a year for patients with persistent symptoms therefore seems to be an effective safety netting practice, while performing more than two within this timeframe is unlikely to be beneficial.

In chapter 4, a cohort of 1,272 symptomatic patients who underwent FIT testing followed by colonoscopy was used to explore demographics and alternative lower gastrointestinal pathologies associated with a raised f-Hb. In addition to CRC, advanced adenomas, non-advanced polyps and IBD independently predicted a raised f-Hb, as did older age, deprivation, use of oral anticoagulants and self-reported rectal bleeding. Deprivation independently predicted a raised f-Hb in patients with no pathology found at colonoscopy.

In chapter 5 attention was turned to the bowel screening programme. A cohort was established of 770 patients who underwent potentially curative resection for CRC. Patients were grouped based on diagnosis via screening or symptomatic pathways and the impact of important covariables, comorbidity and the systemic inflammatory response (SIR), on outcome was assessed. Patients with screen-detected disease had tumours of an earlier stage, were significantly less comorbid as measured by the American Society of Anaesthesiologists (ASA) score and had a significantly lower SIR as compared to non-screen-detected patients. Despite this, after adjusting for numerous covariables, non-screen-detection and a raised SIR independently predicted poorer overall and cancer specific survival.

In chapter 6 a prospective observational study of the management and outcomes of 236 patients with T1 polyp CRCs is presented. Male sex, older age, distally located lesions and pedunculated morphology were more likely to be managed with polypectomy only, while proximally located lesions and larger polyp size were more likely to proceed directly to formal colorectal resection. Younger age, requirement for piecemeal polypectomy and an involved polypectomy margin were associated with a higher chance of progressing to formal colorectal resection after polypectomy. Poor differentiation independently predicted lymph node involvement, submucosal venous invasion (SMVI) and mucinous-subtype predicted recurrence and SMVI predicted CRC-specific survival. Although 64.4% of polypectomy only patients had margin involvement or other high-risk factors, zero

developed recurrence. Of 94 with polypectomy margin involvement, only 5 had confirmed residual tumour. Overall, lymph node metastases (7.1%), recurrence (4.2%) and cancer-specific mortality (3.0%) were rare. Surveillance following local excision of T1 CRC polyps may be safe for many patients.

Chapter 7 presents preliminary data from the Integrated Technologies for Improved Polyp Surveillance (INCISE) project; a large, retrospective, multi-partner collaborative aiming to use patient characteristics, digital pathology, immunohistochemistry (IHC), genomic and transcriptomic features of index polyp tissue to predict metachronous polyp risk and refine post-polypectomy surveillance. The INCISE cohort is formed of 2,643 patients who underwent polypectomy during screening colonoscopy followed by subsequent surveillance colonoscopy. In this particular sub-study, the most recent British Society of Gastroenterology post-polypectomy surveillance risk criteria (BSG-2020) were retrospectively applied to the cohort, such that 51.5% of patients would no longer qualify for surveillance. After a median 36 months, the metachronous advanced polyp/ CRC rate in BSG-2020 high risk patients was found to be only marginally greater than low risk individuals (16.3% vs 13.0%). Furthermore, while BSG 2020 risk stratification group was associated with a significant difference in overall metachronous lesion rate, it did not differentiate advanced and non-advanced metachronous lesions and was not significantly associated with late metachronous lesions detected after 2 years from index polypectomy, suggesting that current surveillance protocols would benefit from refinement.

In chapter 8 a review of established risk factors for metachronous polyp development is given, including patient demographics and comorbidities, chemopreventive medications and conventional polyp pathology. This precedes the results of a formal systematic review of all studies exploring genomics, transcriptomics, immunohistochemistry or features of the microbiome as novel biomarkers of metachronous polyp risk, following colorectal polypectomy. 4,165 paper titles, 303 abstracts and 215 full manuscripts were reviewed,

with 25 papers included in the final study. 49 mutations/ SNPs/ haplotypes in 23 genes/ chromosomal regions (KRAS, APC, EGFR, COX1/2, IL23R, DRD2, CYP2C9/24A1/7A1, UGT1A6, ODC, ALOX12/15, PGDH, SRC, IGSF5, KCNS3, EPHB1/ KY, FAM188b, 3p24.1, 9q33.2, 13q33.2) were found to predict metachronous adenoma / advanced adenoma risk, while the expression levels of 6 proteins correlated with metachronous adenoma (p53, β-catenin, COX2, Adnab-9, ALDH1A1) or sessile serrated polyp (ANXA10) risk.

Chapter 9 explored the utility of COX2 and p53 expression as potential biomarkers for predicting metachronous polyp or CRC risk in INCISE cohort patients. 1,236 of 2,643 INCISE patients had tissue retrieved for immunohistochemical assessment, with 859 of those randomised to the training cohort and 377 to the test cohort. Formalin-fixed, paraffin-embedded tissue blocks of index polyp tissue were used to construct tissue microarrays (TMA) with four cores available per patient. The cores were stained with COX2 and p53 antibody and cytoplasmic COX2 expression and nuclear p53 expression were quantified digitally using QuPath software. On univariate analysis high cytoplasmic COX2 expression ($p=0.019$) predicted shorter time to detection of any metachronous lesion, as did key demographics such as increasing age ($p=0.034$) and pathological parameters such as increased polyp number at index colonoscopy ($p<0.001$) and BSG 2020 high risk ($p<0.001$). While high cytoplasmic COX2 expression retained significance as independent predictor of shorter time to development of any metachronous lesion on multivariate analysis ($p=0.016$), the positive association between COX2 expression and metachronous polyp or CRC risk could not be replicated within test cohort patients and therefore does not appear to represent a useful biomarker for this purpose.

Chapter 10 presents the results of a smaller pilot study, examining β-catenin as a potential biomarker for metachronous polyp or CRC risk. The same TMA constructed for chapter 9 was stained with β-catenin antibody. Nuclear β-catenin expression was assessed using

QuPath software amongst 339 INCISE patients. Low β-catenin expression was found to predict a shorter time to detection of any metachronous polyp or CRC on both univariate and multivariate cox regression. Work is ongoing to score β-catenin expression in the whole cohort and validate these preliminary findings.

Finally, chapter 11 summarises the main findings of the thesis and lays out potential future work.

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Author's Declaration

The work presented in this thesis was undertaken while working as a clinical research fellow between August 2020 and August 2023 at the University of Glasgow Academic Unit of Surgery, Glasgow Royal Infirmary and Wolfson Wohl Cancer Research Centre. I declare that the work presented in this thesis was undertaken by myself, with the following exceptions:

- Mr Paul Burton identified patients with faecal immunochemical tests (FIT) submitted from primary care described in Chapter 2 and 4. I performed all subsequent data collection and analysis on the cohort.

- Mr Paul Burton identified patients with serial FIT tests submitted from primary care within NHS Greater Glasgow and Clyde. I performed all subsequent data collection for these patients. Dr Campbell MacLeod performed the equivalent data collection for NHS Highland and Dr Jayne Digby did so for NHS Tayside. I performed all subsequent analysis on the cohort.

- Mr Andrew McMahon compiled the prospective database of T1 polyp cancers described in chapter 6. I performed further data collection and all subsequent analysis.

- In Chapter 9 and 10, tissue microarrays (TMAs) were constructed by the Glasgow Tissue Research Facility (GTRF), led by Dr Jennifer Hay. P53 and COX2 immunohistochemical staining was outsourced to Dr Colin Nixon's laboratory. I performed scoring for COX2 and p53 on QuPath for batch 1 patients (n=339) and manually for 10% of patients. The full cohort (n=1,236) was additionally scored by Dr Chris Bigley (COX 2, p53), Dr Aula Ammar (p53) and Dr Assya Legrini (p53).

Publications

The work presented in this thesis has resulted in the following published papers:

- Johnstone MS, McMillan DC, Horgan PG, Mansouri D. The Relationship Between Co-morbidity, Screen-Detection and Outcome in Patients Undergoing Resection for Colorectal Cancer. World Journal of Surgery. 2021 Jul;45(7):2251-2260.

- Johnstone MS, Lynch G, Park J, McSorley S, Edwards J. Novel Methods of Risk Stratifying Patients for Metachronous, Pre-Malignant Colorectal Polyps: A Systematic Review. Critical Reviews in Oncology/ Hematology. 2021 Aug;164:103421

- Johnstone MS, Burton P, Kourounis G, Winter J, Crighton E, Mansouri D, Witherspoon P, Smith K, McSorley ST. Combining the quantitative faecal immunochemical test and full blood count reliably rules out colorectal cancer in a symptomatic patient referral pathway. International Journal of Colorectal Disease. 2022 Feb;37(2):457-466.

- Johnstone MS, Miller G, Pang G, Burton P, Kourounis G, Winter J, Crighton E, Mansouri D, Witherspoon P, Smith K, McSorley ST. Alternative diagnoses and demographics associated with a raised quantitative faecal immunochemical test in symptomatic patients. Annals of Clinical Biochemistry. 2022 Jul;59(4):277-287.

- Johnstone MS, MacLeod C, Digby J, Al-Azzawi Y, Pang G, Watson AJM, Strachan J, Mowat C, McSorley ST. Prevalence of repeat faecal immunochemical testing in symptomatic patients attending primary care. Colorectal Disease. 2022 Dec;24(12):1498-1504.

- Johnstone MS, Stoops Reiss, Lynch G, Hay J, Jawny J, Sloan W, Edwards J, McSorley ST. Risk stratification for the detection of metachronous polyps after bowel screening polypectomy: clinical outcomes form the Integrated Technologies for Improved Polyp Surveillance (INCISE) study cohort. BJS Open. 2023 May5; 7(3):zrad034.

- Johnstone MS, McSorley ST, McMahon AJ. Management of malignant T1 colorectal cancer polyps: results from a 10-year prospective observational study. Colorectal Disease. 2023 Oct;25(10):1960-1972.

- Johnstone MS, McSorley ST, McMillan DC, Horgan PG, Mansouri D. The relationship between systemic inflammatory response, screen detection and outcome in colorectal cancer. Colorectal Disease. 2024 Jan;26(1):81-94.

Presentations

The work presented in this thesis has resulted in the following presentations:

- Combining the quantitative faecal immunochemical test and full blood count reliably rules out colorectal cancer in a symptomatic patient referral pathway. American Society of Clinical Oncology, San Francisco, California, USA, January 2022 (poster, online).

- Prevalence of repeat FIT testing in symptomatic patients attending primary care. Moynihan Chirurgical Club Meeting, Glasgow, UK, January 2022 (poster). 1st place poster presentation.

- The Integrated Technologies for Improved Polyp Surveillance (INCISE) Study: Cohort Description, Factors Associated with Future Lesion Risk and Validation of the British Society of Gastroenterology 2020 Guidance. Moynihan Chirurgical Club Meeting, Glasgow, UK, October 2022 (poster).

- The Integrated Technologies for Improved Polyp Surveillance (INCISE) Study: Cohort Description, Factors Associated with Future Lesion Risk and Validation of the British Society of Gastroenterology 2020 Guidance. Early Detection of Cancer Conference, Portland, Oregon, USA, October 2022 (poster).

- Prevalence of Repeat FIT Testing in Symptomatic Patients Attending Primary Care. West of Scotland of Surgical Association Annual Meeting, Glasgow, UK, November 2022 (oral). 1st place oral presentation.

- Management of Malignant (T1) Colorectal Polyps, A Large Prospective Study. West of Scotland of Surgical Association Annual Meeting, Glasgow, UK, November 2022 (oral).

- Risk Stratification for the Detection of Metachronous Lesions After Screening Polypectomy. West of Scotland of Surgical Association Annual Meeting, Glasgow, UK, November 2022 (oral).

- Immunohistochemical Assessment of COX2, p53 and β-Catenin Expression to Predict Metachronous Lesion Development following Colorectal Polypectomy. American Society of Clinical Oncology, San Francisco, California, USA, January 2023 (poster).

- Management of Malignant (T1) Colorectal Polyps, A Large Prospective Study. Association of Coloproctology of Great Britain and Ireland Annual Meeting, Harrogate, UK, July 2025 (oral).

Dedication

To my wife Jen, who has provided constant support and encouragement throughout my career and is the most selfless, caring person I have ever met.

To my brother Craig, my Mum and Dad. I would not have succeeded along this career path without the constant support of my family.

To my daughters Penny and Mairi who always fill me with joy after a long day of work. May you stay forever young.

Definitions/Abbreviations

5-FU	Fluorouracil
ACE-I	Angiotensin converting enzyme inhibitor
ACPGBI	Association of Coloproctology of Great Britain and Ireland
ADR	Adenoma detection rate
AIDS	Acquired immunodeficiency syndrome
ANOVA	Analysis of variance
APC	Adenomatous polyposis coli
APR	Abdominoperineal resection
ARB	Angiotensin II receptor blocker
ASA	American Society of Anaesthesiologists physical status classification
BMI	Body mass index
BSG	British Society of Gastroenterology
CCE	Colon capsule endoscopy
CCF	Congestive cardiac failure
CEA	Carcinoembryonic antigen
CHI	Community health index
CI	Confidence interval
CIMP	CpG island methylator phenotype
COPD	Chronic obstructive pulmonary disease
COX1/2	Cyclooxygenase-1/2
CRC	Colorectal cancer
CRP	C reactive protein
CSS	Cancer specific survival
CT	Computed tomography
DFS	Disease free survival
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
EGFR	Epidermal growth factor receptor
EMR	Endoscopic mucosal resection
EMVI	Extramural venous invasion
ERAS	Enhanced recovery after surgery
FAP	Familial adenomatous polyposis
FDG-PET	Fluorodeoxyglucose-positron emission tomography
f-Hb	Faecal haemoglobin
FIT	Faecal immunochemical test
gFOBT	Guaiac faecal occult blood test
GG&C	Greater Glasgow and Clyde
GI	Gastrointestinal
GP	General practitioner
GWAS	Genome wide association study
Hb	Haemoglobin
HGD	High grade dysplasia
HIPEC	Hyperthermic intraperitoneal chemotherapy
HIV	Human immunodeficiency virus
HNPCC	Hereditary non-polyposis colorectal cancer
HR	Hazard ratio
IBD	Inflammatory bowel disease
ICCR	International collaboration on cancer reporting
IDA	Iron deficiency anaemia
IHC	Immunohistochemistry

INCISE	Integrated Technologies for Improved Polyp Surveillance
IQC	Internal quality control
IQR	Interquartile range
JAG	Joint Advisory Group on GI Endoscopy
LIMS	Laboratory information management system
LMR	Lymphocyte/ monocyte ratio
MCN	Managed clinical network
MCV	Mean corpuscular volume
MDT	Multidisciplinary team
MIS	Minimally invasive surgery
MMR	Mismatch repair
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MSI	Microsatellite instability
N/A	Not applicable
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NLR	Neutrophil/ lymphocyte ratio
NNS	Number needed to scope
NPV	Negative predictive value
NSAIDS	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
OS	Overall survival
PHE	Public Health England
PLR	Platelet/ lymphocyte ratio
PPI	Proton pump inhibitor
PPV	Positive predictive value
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PUD	Peptic ulcer disease
PVD	Peripheral vascular disease
RCT	Randomised control trial
RNA	Ribonucleic acid
RR	Risk ratio
SCC	Squamous cell carcinoma
SCNA	Somatic copy number alteration
SIGN	Scottish Intercollegiate Guideline Network
SII	Systemic immune-inflammation index
SIMD	Scottish index of multiple deprivation
SIR	Systemic inflammatory response
SMC	Scottish Medicines Consortium
SMLI	Submucosal lymphatic invasion
SMVI	Submucosal vascular invasion
SNP	Single nucleotide polymorphism
SPECC	Significant Polyp and Early Colorectal Cancer
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TAMIS	Transanal minimally invasive surgery
TEMS	Transanal endoscopic microsurgery
TMA	Tissue microarray
TME	Total mesorectal excision
TNT	Total neoadjuvant therapy
USoC	Urgent suspicion of cancer
VEGF	Vascular endothelial growth factor
WHO	World Health Organisation

1 Introduction

1.1 Epidemiology of Colorectal Cancer

1.1.1 Incidence

Colorectal cancer (CRC) is 4th most common cancer in the United Kingdom (UK) with approximately 44,000 cases each year, accounting for 11% of all new cancer diagnoses. CRC is slightly more common in males with 56% of cases occurring in men, and incidence is strongly linked to age with rates rising steeply from 50 years and peaking in those 85-89 years. Over the last decade CRC incidence rates have slowly declined with a 4% age-standardised decrease in females, a 10% age-standardised decrease in males and a 6% age-standardised decrease overall(1). This has been attributed to changes in lifestyle factors such as a reduction in smoking prevalence and to the introduction of the Bowel Screening Programme with an associated increase in the removal of premalignant polyps(23). However, these figures belie a 48% increase in CRC rates among those aged 25-49 years, a 6% decrease in those aged 60-74 years, with rates remaining stable in all other age groups(1). This dramatic increase in CRC observed in younger people, particularly of rectal and left-sided colonic cancers, is likely to be multifactorial but lifestyle factors including an increase in obesity in this group may contribute(24). Worldwide, CRC is the 3rd most common cancer with approximately 2 million new cases in 2020(23). The highest rates are currently seen in developed countries, however ongoing industrialisation in the developing world is predicted to contribute to an increase in worldwide incidence(24).

1.1.2 Mortality and survival

Approximately 16,800 people die of CRC each year in the UK, making it the 2nd most common cause of cancer death and accounting for 10% of all UK cancer deaths. Over the last decade age-standardised mortality rates have decreased by 11%. 10-year survival is 52.9% and 5-year survival 60.0% overall and these rates have more than doubled in the last

50 years in the UK. 5-year survival is estimated to be 90.9% for those with stage I disease at diagnosis, 84.3% for stage II, 65.0% for stage III and 10.5% for stage IV(1). Worldwide, CRC is the second most common cause of cancer death and was attributed to 1 million deaths in 2020(23).

1.2 Aetiology and Pathophysiology

The vast majority of colorectal malignancies are adenocarcinomas and are commonly referred to when discussing CRC. Rarer cancers to affect the colorectum include carcinoid tumours, gastrointestinal stromal cell tumours and lymphomas. CRC is usually sporadic and derived from a complex interaction of genetic predisposition and exposure to environmental factors. Additionally, a small proportion of cases arise secondary to inherited familial syndromes(25). On a molecular level CRC is now known to represent a heterogenous group of malignancies arising within the same organ with varied genetic and epigenetic aberrations underpinning their development(26).

1.2.1 Colorectal polyps with premalignant potential

It is widely accepted that CRCs originate from precursor lesions in the form of benign colorectal polyps(27). Colorectal polyps are small growths or aggregations of abnormal cells within the intestinal mucosa which protrude into the intestinal lumen. They may be pedunculated or sessile in shape(28). Histologically, colorectal polyps include adenomas, inflammatory polyps, hamartomatous polyps and serrated polyps (an umbrella term for hyperplastic polyps, sessile serrated lesions, traditional serrated adenomas and mixed polyps)(29, 30). However, only adenomas and serrated polyps (excluding diminutive (1-5mm) rectal hyperplastic polyps) have recognised malignant potential(28, 29, 31). The process begins with the development of an aberrant crypt within the colorectal epithelium which then develops into a benign polyp. Over time these benign polyps may become increasingly dysplastic and eventually malignant via two principal pathways: adenomas via the classic adenoma-carcinoma sequence and serrated polyps via the serrated polyp pathway(24). Premalignant polyps are common, affecting approximately 25-50% of all patients at screening age (50-74 years), but fortunately only a small proportion progress to malignancy(31). The process is thought to take 10-15 years(24). By removing benign dysplastic polyps endoscopically prior to malignant transformation, it should be possible to

reduce the incidence of CRC, a theory which is supported by data from bowel cancer screening populations(3).

1.2.1.1 Adenoma-carcinoma sequence

Adenomas are a common form of colorectal polyp. They are characterised by tubular histology with small, round atypical glands. As they grow, they may to a varying extent develop filamentous architecture, being classified as tubular, villous or tubulovillous(28). Adenomas are the most common CRC-precursor lesion with 60-90% of CRC cases thought to have initially developed within foci of an adenoma(24, 28, 32). The histological progression from normal colorectal epithelium to adenoma and then through increasing severity of dysplasia to carcinoma, along with the underpinning genetic and epigenetic changes is termed the adenoma carcinoma sequence and was first described by Fearon and Vogelstein in 1990(33). At a genetic level the pathway is characterised by early mutation of the adenomatous polyposis coli (APC) tumour suppressor gene followed by activation of the KRAS proto-oncogene and late loss of the TP53 tumour suppressor gene. Phenotypically there is chromosomal instability with changes in chromosome number and structure (24, 32).

1.2.1.2 Serrated pathway

In contrast to adenomas, serrated polyps are flat or carpet-like, typically found in the proximal colon and characterised by serrated or saw-toothed glands(28, 32). 15-35% of CRCs are thought to be derived from serrated polyps(24, 28, 32). At a molecular level this pathway is associated with BRAF mutations and epigenetic instability, characterised by CpG island methylation phenotype(24).

1.2.2 Inherited forms of colorectal cancer

Inherited familial syndromes account for a small proportion of CRC cases (2-5%)(25). However, much of our early understanding of the pathogenesis of CRC was derived from exploration of the genetic causes for these syndromes.

1.2.2.1 Familial adenomatous polyposis

Familial adenomatous polyposis (FAP) is a rare form of autosomal dominant inherited CRC and is caused by a germline mutation of the adenomatous polyposis coli (APC) gene. APC is a tumour suppressor gene with a key role in regulating Wnt signalling via degradation of β-catenin(34). FAP accounts for <1% of CRC cases in the UK(1). The condition is characterised by the development of hundreds of colorectal adenomas and close to a 100% risk of progression to CRC by the age of 40 years without prophylactic proctocolectomy. An attenuated form of FAP also exists and is characterised by <100 adenomas and an older age of CRC onset. Individuals with FAP also have an increased lifetime risk of extra-colonic malignancies including duodenal, ampullary, thyroid and gastric cancers, hepatoblastoma and desmoid tumours(34). Surveillance colonoscopy should be performed every 1-3 years for individuals with FAP from 12 to 14 years of age until time of prophylactic proctocolectomy(35).

1.2.2.2 Hereditary non-polyposis colorectal cancer (HNPCC)

HNPCC or Lynch syndrome is an autosomal dominantly inherited condition caused by a germline mutation in one of the mismatch-repair (MMR) genes (MLH1, MSH2, MSH6 or PMS2) or germline deletion in epithelial-cell adhesion molecule (EpCAM) (which leads to inactivation of MSH2)(35, 36). The MMR system corrects DNA base-pair mismatches generated during DNA replication and contributes to cell cycle arrest and apoptosis. Without an efficient MMR system there is a marked increase in the spontaneous mutation rate, which over time predisposes to cancer. Hypermutation may be noted through a large

increase in the frequency of insertion and deletion mutations in nucleotide repeat sequences (microsatellites), known as microsatellite instability (MSI)(36, 37). HNPCC is the most common of the inherited CRC syndromes accounting for 1-4% of CRC cases in the UK. Approximately 90% of men and 70% of women with Lynch syndrome will develop CRC by age 70(1). HNPCC's have a propensity to occur in the proximal colon and be poorly differentiated. There is a heightened risk of developing synchronous and metachronous CRCs. Lynch syndrome is also associated with extra-colonic malignancies including endometrial, small bowel, ureter and renal pelvis, gastric, ovarian and hepatobiliary cancers(36). Diagnosis of HNPCC can be difficult, particularly as sporadic CRCs may display MSI and have deficient MMR. Use of the Amsterdam II criteria(38) and revised Bethesda guidelines(39) which take into account family history, age at diagnosis, MSI and presence of extra-colonic Lynch-associated malignancies aid with the diagnosis and genetic testing for the presence of indicative germline mutations confirms(36). All patients diagnosed with CRC should now have testing for MMR deficiency/ MSI to guide the need for further testing and patients with confirmed HNPCC should be considered for subtotal colectomy given the high risk of developing metachronous disease. Patients diagnosed with Lynch syndrome prior to development of CRC should be considered for prophylaxis with aspirin and surveillance should be performed biennially from 25-35 years(35).

1.2.2.3 Other inherited forms of CRC

Other inherited forms of CRC include MUTYH-associated polyposis, an autosomal recessive condition caused by mutations in the MUTYH base-excision repair gene, Peutz-Jeghers syndrome, an autosomal dominant condition associated with hamartomatous polyposis and caused by mutations in the STK11 tumour suppressor gene, juvenile polyposis syndrome, an autosomal dominant condition caused by mutations in BMPR1A

or SMAD 4 and associated with hamartomas and the PTEN hamartoma tumour syndromes which include Cowden syndrome(35).

1.2.3 Molecular Pathology

The molecular changes underpinning Fearon and Vogelstein's original description of the adenoma-carcinoma sequence discussed in section 1.2.1.1, involve early mutation of the APC tumour suppressor gene, subsequent activation of the KRAS proto-oncogene and finally late loss of the TP53, a key tumour suppressor gene often termed the "guardian of the genome", known to play a key role in cell cycle regulation, apoptosis and DNA repair(24, 32, 40). Their model has now been modified and expanded upon, with CRC now recognised to be a heterogeneous disease(32). Several groups have sought to classify CRC according to gene-expression based signatures(41-46). In order to resolve inconsistencies between these publications, an international consortium of experts was formed in 2015 and proposed four definitive molecular subtypes: CMS1 (MSI Immune), CMS2 (Canonical), CMS3 (Metabolic) and CMS4 (Mesenchymal)(26, 47). Each subtype is characterised not only by a series of genomic, epigenetic and chromosomal changes but also by phenotypical characteristics such as varying stromal invasion and immune infiltrate and differing prognoses(26).

CMS1 (MSI Immune) CRC is characterised by microsatellite instability (MSI), CpG island methylation phenotype (CIMP) and BRAF mutational status(26). MSI is a type of genomic instability that arises when mutations occur throughout the genome in nucleotide repeat sequences, known as microsatellites. MSI results from dysfunction of the mismatch repair (MMR) system. The MMR system comprises at least seven different proteins (MLH1, MLH3, MSH2, MSH3, MSH6, PMS1 and PMS2) that act to recognise and repair mismatch errors in DNA replication. MMR dysfunction may arise due to inherited germline mutations in the MMR genes as in hereditary non-polyposis CRC (HNPCC or

Lynch syndrome), or MMR genes may be sporadically silenced via hyper-methylation of the MLH1 promoter region(40). CIMP is an epigenetic aberration characterised by hypermethylation of CpG islands within the promoter regions of certain tumour suppressor genes and DNA repair genes, inhibiting their transcription and functionally silencing these genes (26, 28, 40). CIMP is thought to be the main method by which the MLH1 promoter is hyper-methylated leading to sporadic MSI(26). BRAF is a serine/ threonine kinase that is a vital component of mitogen-activated protein kinase (MAPK) signalling. Activation of BRAF via the MAPK pathway results in cellular proliferation and inhibits apoptosis. A common BRAF mutation seen is a thymine to adenine transversion at nucleotide 1796 that results in the V600E substitution, leading to constitutive activation(40). Phenotypically CMS1 is associated with a high volume of local inflammatory infiltration with CD8+ cytotoxic T cells, CD4+ helper T cells and macrophages. CIMP tumours are more likely to be proximally located. CMS1 colorectal CRCs have a good overall prognosis compared to the other subtypes(26).

CMS2 (Canonical) CRC is characterised by somatic copy number alteration (SCNA) and dysregulated WNT and MAPK signalling. SCNAs are a measure of chromosomal instability and are characterised by changes to chromosome structure that result in loss or gain in sections of DNA. In CRC, gains are found in chromosome regions 20q, 13q, 8q and 7 and losses are found in 4, 8p, 18q and 17p. In CMS 2 these chromosomal alterations result in dysregulation of WNT and MAPK signalling pathways(26, 47). Sporadic mutations in the APC gene occur early in the development of adenomas and are present in the majority of CRC. Inherited APC mutations result in the autosomal dominant familial adenomatous polyposis (FAP) syndrome, associated with essentially a 100% risk of CRC without colectomy(40). APC is a tumour suppressor gene which downregulates β-catenin, a key protein in the pro-proliferative Wnt signalling pathway and plays a role in microtubule stabilisation for chromosome segregation during cell division(28, 40).

Following loss of APC, upregulation of nuclear β-catenin is seen during the progression to CRC. It may be upregulated by mutations in β-catenin itself or due to mutations in KRAS which promote the nuclear localisation of β-catenin(26). KRAS is a proto-oncogene which codes for a GTPase with a key role in extra to intra-cellular signal transduction. Its downstream mediators include MAPK. Increased KRAS expression is seen in dysplastic polyps and KRAS mutations are found in over 50% of CRC(40). Phenotypically CMS2 is characterised by a high proliferative rate, low immune infiltrate, low stromal invasion and a good prognosis(26).

CMS3 (Metabolic) CRC is characterised by the presence of KRAS mutations with low levels of CIMP or SCNA. As discussed previously, KRAS mutations lead to constitutive activation of MAPK signalling. CMS3 tumours are associated with a high level of metabolism, low stromal invasion, low immune infiltration and a poor prognosis(26).

CMS 4 (Mesenchymal), like CMS2, is characterised by a high number of SCNAs. In CMS4 this leads to dysregulation of TGF-β signalling(26). The TGF-β pathway is involved in the regulation of cellular proliferation, differentiation and apoptosis. Phenotypically CMS4 is characterised by a high level of stromal invasion, a low level of immune infiltrate and the poorest overall prognosis(26).

1.2.4 Risk Factors

The majority of CRCs are sporadic and arise following an accumulation of genetic and epigenetic changes as discussed above(32). Numerous risk factors have been identified that increase a person's likelihood of developing CRC, some modifiable and others non-modifiable.

1.2.4.1 Demographics

1.2.4.1.1 Age

There is a strong association between the risk of CRC and increasing age. Incidence is highest in those aged 85-89 years and 43% of CRCs are diagnosed in those over 75 years. This pattern is seen in most forms of cancer and can be explained by a tendency to accumulate genetic mutations over time either due to random errors in DNA replication and repair or due to exposure to carcinogenic risk factors(1).

1.2.4.1.2 Sex

CRC is marginally more common in males as compared to females in the UK. 23,900 males are diagnosed with CRC each year in the UK and 19,000 females. 1 in 15 males in the UK will be diagnosed with CRC during their lifetime while 1 in 18 females in the UK will. Age-standardised CRC mortality is higher in males as compared to females(1).

1.2.4.1.3 Deprivation

CRC incidence is 9% higher amongst males in the most deprived quintile as compared to those in the least deprived quintile in England. In females, incidence rates are similar between the most and least deprived quintiles(1). The same pattern has been observed in Scotland. Modifiable risk-factors for CRC such as smoking and poorer diet have been associated with deprivation in Scotland but the mechanism by which deprivation increases the incidence of CRC is likely to be complex and multifactorial(48). Age-standardised mortality rates are 30% higher for males in the most deprived areas as compared to least deprived. The association is weaker in females: 15% higher age-standardised mortality amongst the most deprived(1). One of likely numerous mechanisms by which deprivation impacts on outcomes in CRC is through poorer uptake of CRC screening and lower likelihood of progressing to colonoscopy following a positive FIT screening test(49).

1.2.4.2 Dietary and Lifestyle Factors

1.2.4.2.1 Processed and Red Meat

Processed meat is estimated to account for 13% of CRC cases in the UK(1, 50). Since 1982 the World Cancer Research Fund has conducted a continuously updated project examining how diet, physical activity and obesity affect the risk of developing multiple cancers(51). In a recently updated systematic review and meta-analysis of 111 cohort studies, a 12% increase in the risk of developing CRC was observed per 100 g of red and processed meat consumed per day (RR 1.12 (95% CI: 1.04-1.21))(52). Red meats contains haem which promotes the formation of carcinogenic N-nitroso compounds, particularly if nitrates or nitrites have been added as preservatives, and cytotoxic alkenals from fat peroxidation(52).

1.2.4.2.2 Fibre

An estimated 28% of CRC cases in the UK are attributed to eating too little fibre(1, 50). A systematic review and meta-analysis of 25 prospective studies found a pooled RR of 0.90 (95% CI: 0.86-0.94) per 10g/day of dietary fibre consumed. Cereal fibre and whole grains were found to be of particular benefit(53). Indeed, in the systematic review and meta-analysis of 111 cohort studies mentioned above, there was a 17% decrease in CRC risk for each 90g/day increase in whole grain intake (RR 0.83 (95% CI: 0.79-0.89))(52). Fibre increases stool bulk and reduces colonic transit time which may reduce exposure to carcinogens and bacterial fermentation of fibres to short chain fatty acids may have a protective effect against CRC(54).

1.2.4.2.3 Smoking

Smoking is the leading cause of cancer of any type worldwide and is thought to account for 7% of CRC cases in the UK(1, 50). A systematic review and meta-analysis of 106 observational studies found an adjusted pooled relative risk (RR) of 1.18 (95% CI: 1.11-1.25) for CRC in ever-smokers versus never-smokers. Additionally, a linear dose-response

effect was observed whereby those who smoked more cigarettes per day and for more years had an increasing risk of CRC; risk increased by 7.8% (95% CI: 5.7-10.0%) for every additional 10 cigarettes per day and by 4.4% (95% CI: 1.7%-7.2%) for every additional 10 pack years(55). The association between smoking and CRC is thought to be stronger in males and stronger for rectal as compared to colonic cancers(1).

1.2.4.2.4 Alcohol

Alcohol is thought to account for 6% of CRC cases in the UK(1, 50). A systematic review and meta-analysis of 61 observational studies found a RR of CRC of 1.21 (95% CI: 1.13-1.28) for moderate drinkers and 1.52 (95% CI: 1.27-1.81) for heavy drinkers(56). CRC risk increased by 7% per unit of alcohol consumed per day(1, 56). Numerous mechanisms for the positive association between alcohol and CRC have been proposed. Acetaldehyde, the primary metabolite of alcohol, is known to be carcinogenic. Alcohol interferes with retinoid metabolism which may disturb cellular growth, differentiation and apoptosis. Finally, alcohol acts as a solvent and may enhance the penetration of other carcinogens into cells(52).

1.2.4.2.5 Physical activity and obesity

A lack of physical activity accounts for 5% of CRC cases in the UK and being overweight or obese for 11%(1, 50). In a systematic review and meta-analysis of 38 observational studies, increased occupational (RR 0.74 (95% CI: 0.67-0.82)) and recreational (RR 0.80 (95% CI: 0.71-0.89)) physical activity decreased the risk of colon cancer. A smaller effect was seen for rectal cancer: occupational (RR 0.88 (95% CI: 0.79-0.98)) and recreational (RR 0.87 (95% CI: 0.75-1.01)). A systematic review and meta-analysis involving 47 studies, published as part of the World Cancer Research Fund continuous update project found a RR for CRC of 1.06 (95% CI: 1.04-1.07) per 5 kg/m^2 increase in body mass index (BMI)(57).

1.2.4.3 Medications

The concept of taking a medication to reduce the risk of a cancer, termed chemoprevention, has been extensively explored, particularly in CRC. Such studies are difficult to conduct due to the large sample size and follow-up required. Additionally, for a medication to be approved for use as a chemopreventive agent it would have to be well tolerated with minimal side effects and be cost effective(58). Of course, many of the medications studied may be taken by patients for alternative indications, with a reduction in CRC-risk representing a secondary effect.

1.2.4.3.1 Aspirin and NSAIDs

Perhaps the most studied CRC chemopreventive medication and the agent with the greatest evidence of efficacy is aspirin. Aspirin acts to irreversibly inhibit cyclooxygenase 1 (COX1) and cyclooxygenase 2 (COX2). Although the precise mechanism by which aspirin exerts the observed reduction in CRC-risk is unclear, it is known to downregulate inflammation, prostaglandin synthesis and platelet activation as well as having modulatory effects on Wnt-signalling, all processes which may contribute to colorectal carcinogenesis(58). A number of studies including randomised control trials (RCTs) have examined the effects of aspirin on CRC-risk. While many have shown a protective effect(59-61), others have failed to observe this(62, 63). One study of note combined data from two randomised control trials with over 20 years of follow-up. A total of 7588 patients were included with those allocated to treatment with aspirin taking doses between 300-1200mg daily. The pooled HR for CRC incidence for those randomised to aspirin was 0.74 (95% CI: 0.56-0.97; p=0.02) overall and 0.63 (95% CI: 0.47-0.85; p=0.002) for those allocated to 5 or more years of aspirin(59). Further studies have established aspirin's chemopreventive effects in patients with HNPCC(64) and the UKCAP trial observed a reduction in the risk of recurrent adenoma and advanced adenoma in those taking aspirin, known precursors of CRC(65).

Like aspirin, non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX 1 and 2, but in a competitive manner. A systematic review and meta-analysis of 23 observational studies involving over one million patients found a decreased risk of CRC in those taking regular NSAIDs (pooled OR 0.74 (95% CI: 0.67-0.81; $p<0.001$)), particularly at higher doses(66). Other studies have correlated NSAID-use with a decreased risk of adenomas(67) including in those with FAP(68). Both aspirin and NSAIDs are associated with a heightened risk of gastrointestinal bleeding(58) and the modest CRC-risk reduction must be weighed against this.

1.2.4.3.2 Metformin

Type II diabetes is a recognised risk factor for CRC and the chemopreventive effects of anti-diabetic medications including metformin have been studied. Metformin is a biguanide with potential antineoplastic mechanisms including activation of adenosine monophosphate-activated protein kinase (AMPK) which inhibits mammalian target of rapamycin (mTOR) and thus cellular proliferation, and inhibition of the cell cycle-regulator cyclin D1(58). One systematic review and meta-analysis of 9 observational studies involving diabetic patients showed a modest CRC risk reduction with metformin use (pooled adjusted OR 0.89 (95% CI: 0.80-0.99)(69). Additionally, a RCT showed a reduction in metachronous polyps in non-diabetics treated with metformin(70).

1.2.4.3.3 Statins

Statins are competitive HMG-coenzyme A reductase inhibitors which are used in the treatment of hypercholesterolaemia and cardiovascular disease prevention. HMG-coenzyme A reductase is the rate-limiting enzyme in mevalonate metabolism. Mevalonate and its metabolites are required for the activation of the Ras superfamily of GTPases with numerous downstream signal transduction pathways. Inhibition of mevalonate synthesis by statins has been shown to reduce cellular proliferation, induce apoptosis and may inhibit

metastatic transformation and angiogenesis(58, 71). While studies including a systematic review and meta-analysis of 22 case-control studies have shown a modest reduction in CRC risk with statin use (pooled OR 0.89 (95% CI: 0.82-0.97))(72), others have observed no significant effect including the West of Scotland Coronary Prevention Study which was a placebo-controlled RCT of statin use with 10 year follow-up (HR 0.82 (95% CI: 0.58-1.17; p=0.28))(73).

1.2.4.3.4 Calcium and Vitamin D

A systemic review and meta-analysis of 37 case control studies found a 6% decrease in CRC risk for every 300mg of calcium ingested (OR 0.94 (95% CI: 0.92-0.97)) and a 4% decrease in CRC risk for every 100 IU/day of vitamin D (OR 0.96 (95% CI: 0.93-0.98))(74).

1.2.4.4 Comorbidities

1.2.4.4.1 Inflammatory bowel disease (IBD)

The risk of developing CRC is 70% higher amongst patients with IBD (including ulcerative and Crohn's colitis) as compared to the general population according to a systematic review and meta-analysis. While high, this does represent a significant decline in CRC-risk in this group as compared to historically reported rates. The risk is highest in those with longest disease duration and the most extensive colitis(75). Another meta-analysis found that patients with IBD have poorer cancer specific survival (CSS) as compared to those without IBD. Sub-analysis revealed patients with IBD-associated CRC were more likely to be male, had higher rates of poor differentiation, mucinous or signet ring cell carcinoma, synchronous tumours, right-sided tumours and a higher likelihood of an R1 resection (an involved resection margin in which cancer cells are pathologically visualised within 1mm of, the edge of the resected specimen)(76, 77).

1.2.4.4.2 Diabetes mellitus

The risk of developing CRC is 22-30% higher amongst people with type II diabetes as compared with those without, according to multiple meta-analyses of cohort studies(1, 78-82). Additionally, patients with diabetes have a greater chance of dying of CRC(78, 80, 81). The mechanism by which diabetes increases CRC risk is likely to be multifactorial. Type II diabetes is more common amongst obese individuals, another recognised CRC risk factor and overlapping pathophysiology may exist. Type II diabetes is associated with peripheral insulin resistance and, in the early stages of the disease, compensatory hyperinsulinaemia. Insulin itself is known to stimulate mitosis and cellular proliferation, while downregulating the secretion of insulin-like growth factor binding proteins which increases the bioavailability of insulin-like growth factor 1(IGF-1). Both normal colorectal epithelial cells and cancer cells are known to express IGF-1 receptors and activation promotes proliferation and inhibits apoptosis(81).

1.2.4.5 Systemic Inflammation

It is now widely accepted that inflammation and cancer are inextricably linked. Early observations of increased inflammatory cells within the microenvironment of solid tumours, and an increased rate of cancer development within organs affected by chronic inflammatory conditions, laid foundations for the association(83), while a legion of data linking a raised systematic inflammatory response (SIR) and poorer outcomes in numerous cancers of varying advancement, has solidified the relationship(84). Tumour-promoting inflammation and the avoidance of immune destruction are now recognised as two of Hanahan and Weinberg's hallmarks of cancer(85, 86). In addition to the association between SIR and established cancer, there is also evidence that the presence of a raised SIR may predict the subsequent development of CRC. In the systematic review and meta-analysis of 14 case control studies investigating circulating markers of SIR and the risk of

colorectal adenoma, a positive association between circulating C-reactive protein (CRP) and the risk of advanced adenoma was found (OR 1.59 (95% CI: 1.09-2.32; I^2 = 44%, $_{heterogeneity}$ = 0.15)(87). Additionally, raised circulating markers of SIR have also been associated with an increased risk of developing CRC. One study estimated associations between pre-diagnostic SIR markers and cancer risk from 440,000 patients from the prospective UK Biobank cohort. The strongest association was found between the systemic immune-inflammation index (SII) and CRC risk. The hazard ratio for CRC per standard deviation increment in SII was 1.09 (95% CI: 1.02-1.16) for blood drawn five years prior to diagnosis and 1.50 (95% CI: 1.24-1.80) one month before diagnosis. Similar associations were observed for NLR (neutrophil lymphocyte ratio) and PLR (platelet lymphocyte ratio)(88). However, it is not clear whether SIR independently increases the risk of developing advanced adenoma and CRC, or whether confounding associations may play a role. Indeed, studies included in the meta-analysis above, which performed stratified analysis, identified heavy smoking and no aspirin use as potential confounding factors(87).

1.2.4.6 Family history

There are two broad categories of hereditary risk associated with CRC: a positive family history without a readily identifiable genetic aberration and a group of recognised inherited CRC syndromes (discussed in section 1.2.2). Approximately 20% of CRC cases in the UK can be attributed to hereditary factors outside of a formal hereditary syndrome(1). The risk associated with a positive family history increases with the number of affected relatives, the closer the degree of relative and the younger the age at diagnosis(24). A systematic review and meta-analysis of 59 studies showed the risk of CRC is more than doubled in those with one affected first degree relative (pooled RR 2.24 (95% CI: 2.06-2.43)) and close to four-fold in those with at least two affected first degree relatives (RR 3.97 (95% CI: 2.60-6.06))(89). While genome wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with CRC-susceptibility, a substantial

proportion of the observed risk associated with a positive family history remains unexplained(90).

1.3 Diagnosis

Patients are diagnosed with CRC via a variety of pathways: symptomatic patients may present to their general practitioner with suspicious symptoms and be referred to an outpatient clinic, symptomatic patients may present as an emergency with intestinal obstruction, peritonitis or bleeding, or asymptomatic patients may have CRC detected as part of the bowel screening programme or at a scheduled surveillance colonoscopy(25).

1.3.1 Symptoms and Signs

The National Institute for Health and Care Excellence (NICE) outline the symptoms which should trigger consideration of referral for suspected CRC in their Suspected cancer: recognition and referral NG12 guidance(91). The National Health Service (NHS) Scotland Suspected Cancer Guidelines outline a very similar set of symptoms(22). These include a persistent (>4 week) change in bowel habit, particularly diarrhoea, rectal bleeding without an obvious anal cause or any blood mixed with the stool, abdominal pain with weight loss, palpable abdominal or rectal masses and unexplained iron deficiency anaemia (IDA). Both guidelines advise tempering the presence of these symptoms with the patient's age. Other signs and symptoms which may be present include tenesmus with rectal cancer, rectal pain usually with advanced rectal cancers, severe abdominal pain if a cancer perforates causing peritonitis, abdominal pain and distension, vomiting and complete constipation if a patient develops intestinal obstruction and hepatomegaly and cachexia with metastatic disease Additionally, anaemia may be asymptomatic or associated with shortness of breath and fatigue(25).

While the above symptoms are commonly present when a patient is diagnosed with CRC, in isolation they are in fact poor predictors of cancer as similar symptoms are encountered with other significant bowel disease (advanced polyps or inflammatory bowel disease (IBD)) and functional bowel disorders(11). Indeed, after the introduction of the NG12

guidance, combined with a United Kingdom (UK)-based symptom public awareness campaign, the number of suspected CRC referrals increased, while the proportion diagnosed with CRC decreased and no change in stage at diagnosis was observed(92, 93). A systematic review and meta-analysis found a pooled positive predictive value (PPV) for CRC of 8.1% (95% CI: 6.0-10.8%) for presence of rectal bleeding alone in those aged ≥50 years, 3.3% (95% CI: 0.7-15.6%) for abdominal pain, and 9.7% (95% CI: 3.5-26.8%) for anaemia(94)

1.3.2 Diagnostic Investigations

1.3.2.1 FIT in symptomatic patients.

While the symptoms discussed above should act as triggers for consideration of referral for suspected CRC, the low PPV associated with symptoms alone, has driven the need for objective biomarkers of CRC-risk. The effectiveness of the faecal immunochemical test (FIT) for predicting the risk of CRC in symptomatic individuals has now been established(11-14, 16-21). One recent meta-analysis reported a pooled sensitivity of 87.2% (95% CI: 81.0-91.6%) and specificity of 84.4% (95% CI: 79.4-88.3%) for CRC detection at a f-Hb ≥10 μg/g threshold (86). Initially, NICE recommended the use of FIT only for patients with low-risk symptoms in the absence of rectal bleeding according to their DG30 guidance(95). However, FIT has proven utility for determining CRC risk in patients meeting both high risk (NG12) and low risk (DG30) symptoms(12, 93, 96) and in patients with and without rectal bleeding(97). In response, the most recent guidance from the Association of Coloproctology of Great Britain and Ireland (ACPGBI) and the British Society of Gastroenterology (BSG)(98), NICE(91) and NHS Scotland(22) have included the use of FIT for all patients with lower gastrointestinal symptoms. Consequently FIT has been widely integrated into all colorectal and gastroenterology referral pathways in most Scottish NHS health boards(15, 22).

1.3.2.2 Colonoscopy

Colonoscopy is a flexible endoscopic visualisation of the lumen of the colorectum following preparation of the bowel with osmotic laxatives and is considered the gold standard investigation with the highest sensitivity for the diagnosis of CRC. Colonoscopy allows for lesion visualisation and localisation, biopsy of lesions to obtain a tissue diagnosis, full colorectal examination to exclude synchronous cancers and for the removal of pre-malignant polyps (polypectomy) and indeed curative resection of small polyp CRCs(24, 25, 99). High quality colonoscopy is of the upmost importance to minimise the risk of missing pathology. The Joint Advisory Group on GI Endoscopy (JAG) advise the use of multiple colonoscopy quality indicators including a minimum caecal intubation rate of 90%, polyp detection rate of 15%, proportion of patients with adequate or better bowel preparation of 90%, rectal retroversion rate of 90% and withdrawal time of 6 minutes(100). Colonoscopy performance may be enhanced using narrow band imaging, the magnetic scope guide and most recently, pathology-detection software which utilises artificial intelligence(101). Colonoscopy is associated with a colonic perforation rate of up to 0.12%(25).

1.3.2.3 CT Colonography

Computed tomography (CT) colonography (also referred to as CT pneumocolon and virtual colonoscopy) is a radiological investigation with comparable sensitivity for CRC diagnosis to colonoscopy(102). Like colonoscopy osmotic bowel preparation is required and a rectal catheter is inserted to allow insufflation of the colorectum. However, if a colorectal lesion is detected, subsequent colonoscopy is required for polypectomy or tissue biopsy. As such CT colonography is primarily used as an adjunct to colonoscopy to complete assessment of the colon in cases of incomplete colonoscopy or in selected patients unsuitable for or unwilling to undergo colonoscopy.

1.3.2.4 Colon Capsule Endoscopy

Colon capsule endoscopy (CCE) is a novel, minimally invasive procedure which may be used as an alternative to colonoscopy. Patients require full bowel preparation with osmotic laxatives, equivalent to that required for colonoscopy or CT colonography. Additionally, a prokinetic agent such as metoclopramide is given after the capsule is swallowed to promote colonic motility and capsule excretion. The PillCam COLON 2 (Medtronic, UK) is currently used in Scotland and is equipped with two cameras, with images transmitted wirelessly to a data recorder worn on a belt as it passes through the gastrointestinal tract(103). The images are then manually reviewed by a physician to detect colorectal pathology. A recent systematic review and meta-analysis reported a mean sensitivity of 85% (95% CI: 73-92%) and specificity of 85% (95% CI: 70-93%) for CCE detection of polyps of any size, rising to 87% (95% CI: 83-90%) and 88% (75-95%) for polyps ≥10mm(104). Given the significant pressures on endoscopy services, CCE has been introduced to numerous Scottish health boards as an intermediary investigation for patients with colorectal symptoms and a moderately raised f-Hb. This aims to allow timely visualisation of the colorectum with only those patients found to have pathology requiring subsequent colonoscopy or flexible sigmoidoscopy for polypectomy or tumour biopsy for tissue diagnosis. Of course, the success of CCE relies upon complete examination of the colorectum and unfortunately rates as low as 54% have been reported, dependent on the regimen of bowel preparation and population undergoing examination(103, 105). With optimisation of bowel preparation and increased battery life of the CCE device it is likely that improved completion rates will be achieved in the future. Adverse events including bowel obstruction secondary to capsule retention have been reported in addition to acute kidney injuries associated with bowel preparation(103).

1.3.3 Bowel Screening Programme

The Scottish Bowel Screening Programme invites patients aged 50 to 74 years to undertake a quantitative faecal immunochemical test (FIT) followed by colonoscopy for those patients testing positive, at a threshold of 80 µg Hb/ g of faeces. Patients aged ≥75 years may participate on request(2). Numerous large, randomised control trials (RCTs) including the Minnesota Colon Cancer Control Study(4), Nottingham Bowel Cancer Screening Trial (5, 6), the Danish Fecal Occult Blood Testing Trial(7) and an RCT from Goteburg, Sweden(8) have shown that faecal occult blood-based screening increases the number of early-stage cancers diagnosed and reduces CRC-mortality. Additionally, a meta-analysis of such RCTs has suggested the incidence of CRC may be reduced through the removal of precursor polyps and that the requirement for more invasive surgical procedures may be reduced due to earlier diagnosis(3). It is generally accepted that the results of these early faecal occult blood-screening studies can be extrapolated to modern-day quantitative faecal immunochemical test (FIT)-based screening. Key performance indicators for high quality screening colonoscopy include achieving a minimum caecal intubation rate of 90%, polyp or adenoma detection rate of 15% and a withdrawal time of 6 minutes(100).

Alternative forms of bowel screening are utilised around the world, most notably colonoscopy-based screening. The Nordic-European Initiative on Colorectal Cancer (NordICC) trial was a large, multicentre, pragmatic randomised control trial which compared screening with a single colonoscopy to no screening and has cast doubt over the effectiveness of colonoscopy-based screening. 85,179 patients aged 55-64 with no previous exposure to screening were randomised in a 1:2 ratio to screening versus no screening. Intention-to-treat analysis observed a significant decrease in the risk of CRC at 10 years in the screening group (RR 0.82 (95% CI: 0.70-0.93)), but no significant difference in CRC-specific mortality at 10 years (RR 0.90 (95% CI: 0.64-1.16))(106). Criticism of the NordICC trial centred on lower-than-expected uptake (42.0%) and on questions over the

quality of colonoscopy; while the overall adenoma detection rate (ADR) was acceptable (30.7%), in subgroups ADR fell below recognised standards (14.4% in Swedish patients). Of course, it could be argued that the uptake of colonoscopy in this study simply represents the likely uptake should population level colonoscopy screening be introduced in Europe and highlights a significant limitation of screening with an invasive procedure in the absence of symptoms. In contrast, FIT seems to be an acceptable form of screening to patients with the most recently published FIT-uptake in Scotland being 66.7% and 75.0% colonoscopy-uptake following a positive FIT test(107).

1.3.4 Investigations for Pre-Operative Staging

1.3.4.1 CT Chest Abdomen and Pelvis

All patients diagnosed with CRC, regardless of primary tumour location and size, should undergo initial staging with CT imaging of the chest, abdomen and pelvis. CT determines the extent of local disease progression by identifying the depth of tumour invasion within the bowel wall, and by identifying any malignant appearing locoregional lymph nodes. Additionally, CT may establish the presence or absence of distant metastases. CT is performed with intravenous contrast unless contraindicated and oral contrast may be considered. This helps guide treatment planning and provides an estimate of prognosis at the time of diagnosis(25, 99).

1.3.4.2 MRI Pelvis

Magnetic resonance imaging (MRI) of the pelvis is required to complete the local staging of rectal cancer. MRI can accurately determine rectal T stage, involvement of the mesorectal fascia and hence circumferential resection margin, extramural venous involvement and can identify perirectal nodal involvement. This information is critical for determining which patients may benefit from neoadjuvant therapy prior to surgery(25, 99).

1.3.4.3 Additional Imaging

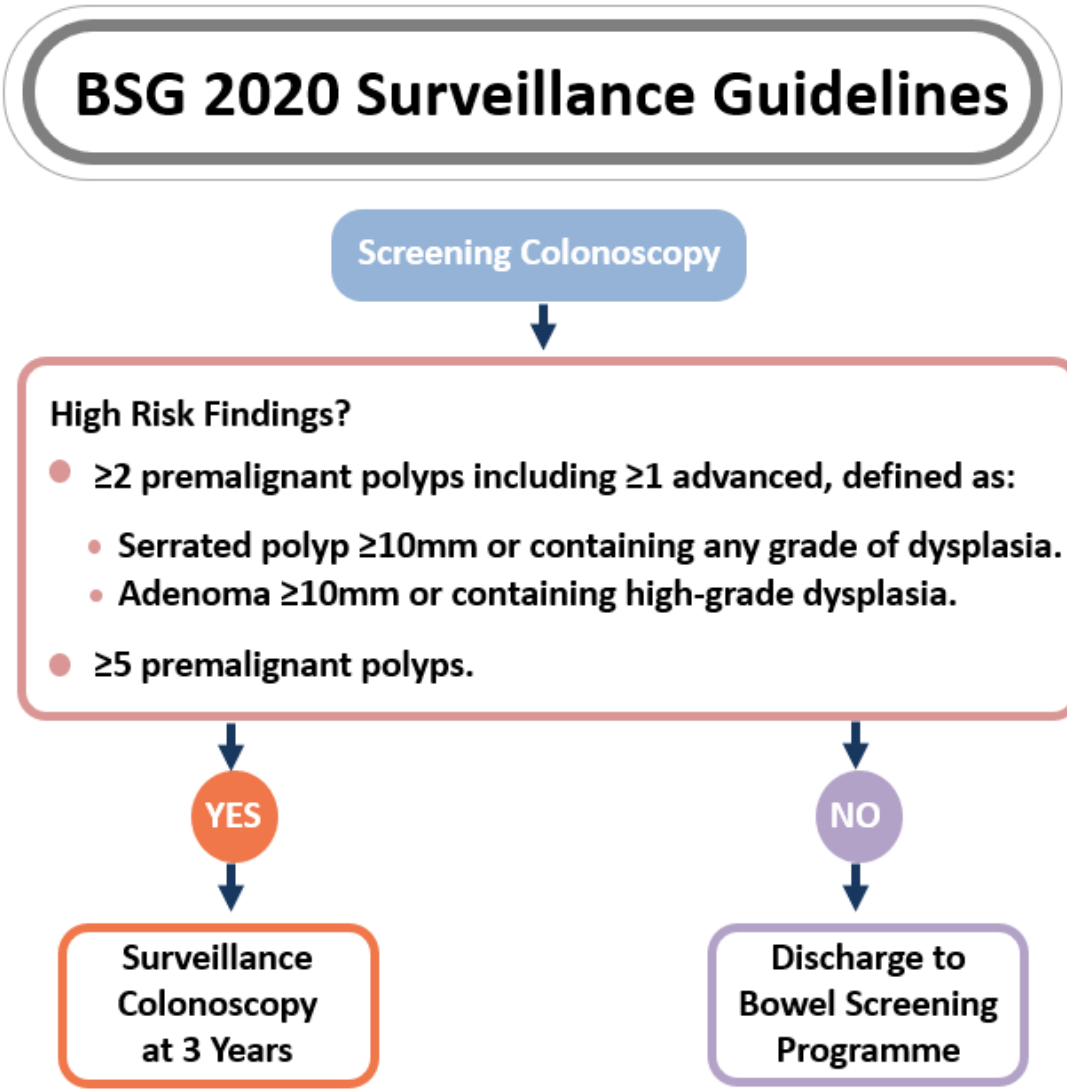
Additional imaging may be required to complete the staging of CRC, particularly if indeterminate lesions have been visualised on CT. For example, an MRI or ultrasound may better characterise liver lesions. Positron emission tomography (PET) utilises a radiotracer, most commonly fluorodeoxyglucose (FDG), to identify areas of abnormally increased metabolic activity in the body. By superimposing these functional images over standard CT scans, precise anatomical localisation of FDG-PET-avid lesions can be achieved. FDG-PET-CT may be considered for patients with known liver or lung metastases being considered for metastasectomy. If occult distant metastases are identified in such patients, extensive resection may not be appropriate. FDG-PET-CT may also play a role in patients with suspected disease recurrence due to a rising carcinoembryonic antigen (CEA) but without evidence of recurrence on conventional imaging(25, 99). Endoanal ultrasound may compliment MRI of the rectum for determining local depth of invasion, in particular for patients being considered for a local rectal excision(99).

1.3.5 Surveillance

The objective of a surveillance colonoscopy is to detect and if possible, remove metachronous colorectal polyps and early CRC from patients predicted to have a higher propensity for the development of such lesions. Currently, patients recommended to undergo surveillance colonoscopy are defined by the British Society of Gastroenterology (BSG)/ Association of Coloproctology of Great Britain and Ireland/Public Health England post-polypectomy and post-colorectal cancer resection surveillance guidelines (Figure 1.1)(31). Patients diagnosed with CRC are recommended to undergo surveillance colonoscopy 1 and 3 years after resection. Patients who have undergone polypectomy of premalignant lesions are risk stratified for metachronous polyp or CRC-risk based on polyp histology, grade of dysplasia, polyp size and polyp number. Those deemed high risk are invited for surveillance colonoscopy at 3 years(31). Surveillance colonoscopy accounts for

a large proportion of the colonoscopies performed in the UK; 100,000 of the 700,000 performed in England each year(108, 109).

1.3.5.1 Figure 1.1: British Society of Gastroenterology and Association of Coloproctology of Great Britain and Ireland post-polypectomy surveillance guidelines.



1.4 Management

1.4.1 MDT

The Scottish Intercollegiate Guideline Network (SIGN) recommend that all patients diagnosed with CRC be managed by a multidisciplinary team (MDT) consisting of surgeons, oncologists, pathologists, radiologists and specialist nurses. In addition to these core members of the MDT, input from palliative care specialists, general practitioners, clinical geneticists and gastroenterologists may be required for selected patients. Patients should be discussed at a CRC-specific MDT at each stage in their management including following diagnosis and before and after any surgical or oncological treatments(99). Indeed, there is evidence that a multidisciplinary approach to CRC management improves outcomes(110).

1.4.2 Neoadjuvant Therapy

Neoadjuvant chemoradiotherapy or radiotherapy should be offered to patients with T1-2 N1-2 M0 or T3-4 N0-2 M0 rectal cancer(111). Several RCTs have demonstrated that this is particularly important for reducing local recurrence(112-117), but also leads to a moderate improvement in overall(112, 116), disease-free (115, 118) and cancer specific survival(112, 113, 116), in such patients. In the United States, neoadjuvant therapy tends to take the form of long-course radiotherapy of 50.4 Gy delivered over 5 weeks with concurrent fluoropyrimidine-based chemotherapy. Outside of the US, many centres use short course neoadjuvant radiation in the form of 25 Gy of radiation delivered in 5 fractions over 1 week(25). An optimal, gold standard duration and type of radiotherapy or chemoradiotherapy has yet to be established and treatment varies between centres. Similar outcomes have been observed between long- and short course radiotherapy(119-122) and between chemoradiotherapy with or without prior induction chemotherapy(123, 124).

Total neoadjuvant therapy (TNT) is a treatment strategy that has emerged in recent years for patients with locally advanced rectal cancer. Patients are given multi-agent chemotherapy and chemoradiotherapy prior to planned surgery. Higher complete response rates including pathological response and sustained clinical response have been observed with TNT(125) and with close surveillance, surgery may not be required and organ preservation can be achieved(25).

Patients with T1-2 N0 rectal cancer may occasionally be offered neoadjuvant treatment if tumour location requires an abdominoperineal resection (APR) to increase the chance of sphincter preservation(25), or as part of a clinical trial such as the STAR-TREC trial which aims to compare conventional total mesorectal excision (TME) surgery to (chemo)radiotherapy followed by transanal excision or watchful waiting in early rectal cancer(126). Neoadjuvant therapy may be considered in patients with T4 colonic cancer(111). The FOxTROT study compared 6 weeks neoadjuvant and 18 weeks adjuvant oxaliplatin-fluoropyrimidine chemotherapy to 24 weeks adjuvant chemotherapy, in patients with T3-4 N0-2 M0 colon cancer. RAS-wildtype patients were also randomised to the addition of panitumumab. Marked T and N downstaging was observed with neoadjuvant therapy and R0 resection was achieved more often. Presence of residual or recurrent disease was significantly less at 2 years in neoadjuvant patients. Panitumumab did not enhance this benefit(127).

The use of immune check blockade agents in the form of the programmed cell death (PD-1) receptor blockers nivolumab and pembrolizumab, and the cytotoxic T cell associated protein 4 (CTLA-4) blocker ipilimumab, has been established as effective treatment for patients with metastatic defective mismatch repair (MMR) CRC(128-130). However, more recently immunotherapy has emerged as an effective neoadjuvant strategy also. Loss of effective MMR results in a high tumour mutational burden, phenotypically characterised by microsatellite instability (MSI) and an abundance of neoantigens. These neoantigens are

thought to illicit immune activation and the strong lymphocytic infiltrate seen in such tumours. However, in some cases T cells may be inactive due to binding of the PD-1 receptor to the PD-L1 tumour cell ligand with subsequent conversion of cytotoxic T cells to regulatory T cells. By blocking PD-1 with immunotherapy, cytotoxic T cells may be activated and initiate cancer cell destruction.(131). In the phase I/II NICHE study, patients with both MMR deficient and proficient early colonic cancer were treated with combined nivolumab and ipilimumab. All 20 patients with MMR deficient cancers had pathological response with a 95% major pathological response (≤10% viable tumour) and 60% complete pathological response rate. 4 of 15 patients with MMR proficient cancers showed a pathological response(132).

1.4.3 Surgery

1.4.3.1 Principles of Surgery

Surgical resection of the affected segment of colon or rectum accompanied by high ligation of the associated lymphovascular pedicle forms the mainstay of curative treatment for CRC. For the most part, surgery should be performed with the aim of achieving clear resection margins and cure. Palliative resections which may not improve survival may be justified to improve symptoms, for example to relieve large bowel obstruction. Conversely, where surgery is technically feasible, but the patient is very frail or comorbid, the risk of undergoing a major resection may outweigh the potential benefits. Surgical units that perform a high volume of CRC resections have improved outcomes. Minimally invasive surgery (MIS) using laparoscopic or robotic techniques are increasingly the mainstay of CRC surgical resection over open operations(25). The benefits of MIS include reduced post-operative pain and a shorter hospital stay and overall recovery, while recurrence and survival rates remain comparable to open surgery for both colonic(133-136) and rectal cancers(135-141). While several studies have shown similar oncological outcomes between laparoscopic and robotic rectal surgery(142-144), one more recent RCT did

observe reduced intra-operative complications, blood loss, need for open conversion or APR, circumferential resection margin positivity and post-operative complications with robotic as compared to laparoscopic rectal resection(145).

Patients who may require a stoma should be seen by a stoma nurse for counselling and marking prior to surgery wherever possible(25). This will help prepare the patient for life with a stoma in the post-operative period and ensure optimal siting for ease of stoma bag changing. Enhanced recovery after surgery (ERAS) principles should be applied to elective colorectal cancer resections to reduce overall complication risk and hospital length of stay(25, 146). These include optimal pain control with use of epidural or regional anaesthesia, a MIS-approach to surgery, avoidance of nasogastric tubes and drains and aggressive post-operative rehabilitation with early mobilisation, feeding and removal of urinary catheter(146).

1.4.3.2 Endoscopic Resection

There has been a recent paradigm shift, particularly in the treatment of rectal cancer, toward local excision of early CRCs to facilitate organ preservation. Malignant polyps, defined as a polyp which contains adenocarcinoma with invasion through the muscularis mucosae and into but not beyond the submucosa (T1-staged), are increasingly prevalent in the age of bowel screening, and endoscopic excision alone may be sufficient for many(147). If a polyp is suspected to harbour malignancy and it is felt appropriate and possible to excise it endoscopically with clear margins and ideally in an en bloc fashion, then this can be achieved in numerous ways. Snare polypectomy following submucosal injection to raise the lesion is the simplest technique and can be curative if the histology and margin are favourable. Endoscopic mucosal resection (EMR) may be required for larger or flatter lesions and again utilises submucosal injection to raise the lesion followed by snare excision with diathermy, preferably en bloc but can be completed piecemeal.

Endoscopic submucosal dissection is a more advanced endoscopic technique which may facilitate en bloc excision of larger malignant polyps. Following submucosal injection to lift the lesion, the mucosa is marked, incised and submucosal dissection is then performed with an endoscopic knife(147).

1.4.3.3 Surgery for Rectal Cancer

Rectal cancer may be classified as any CRC where the distal margin is below 15cm from the anal verge. Early rectal cancers (T1-2 N0 M0 staged) may be suitable for local excision(25, 111). Transanal excision and transanal endoscopic microsurgery (TEMS) have largely been replaced by transanal minimally invasive surgery (TAMIS), in which a single laparoscopic or robotic port is inserted into the rectum transanally and pneumorectum is established. Laparoscopic or robotic instruments can then be inserted and full thickness excision of the early rectal cancer with subsequent defect closure is possible(148).Such local excisions are associated with shorter operating time, less morbidity in terms of sexual and urinary dysfunction, shorter hospital stay and do not necessitate a stoma. However, with such procedures regional lymph nodes are not removed, risk of local and overall recurrence may be higher, and a more invasive formal resection may be required if histology is unfavourable(25, 111, 149).

Total mesorectal excision (TME) is the gold standard of rectal cancer surgery(25). TME allows for circumferential clearance of the tumour and is associated with reduced local recurrence and improved survival(99, 150). Definitive TME should be offered to patients with early rectal cancer (T1-2 N0 M0) with poor histology, patients with nodal involvement (T1-2 N1-2 M0) and to patients with more advanced rectal cancer (T3-4 N0-2 M0)(25, 111). The operation performed depends on the location of the tumour. Cancers in the upper third of the rectum may be managed with anterior resection with colorectal anastomosis, those in the middle or lower third require low anterior resection with coloanal

anastomosis, and those invading the pelvic floor, sphincter complex or anal canal require abdominoperineal resection (APR). Low anterior resections are associated with a higher risk of anastomotic leak and so a diverting loop ileostomy is often considered on a temporary basis to reduce the impact of a potential leak, while APR's necessitate permanent end colostomy(25). Patients with locally advanced or recurrent rectal cancers who require resection beyond a TME with potentially multi-visceral exenterative surgery should be referred to a unit that specialises in such resections(111).

1.4.3.4 Surgery for Colon Cancer

Surgical resection for colonic cancer involves segmental colectomy with en bloc excision of the vascular pedicle containing the regional lymph nodes(25). Procedures include right hemicolectomy, extended right hemicolectomy, left hemicolectomy and sigmoid colectomy, dependent on the tumour location. Resection and examination of a minimum of 12 lymph nodes is necessary for accurate staging(25).

1.4.4 Post-Operative Staging and Prognosis

An ability to accurately predict the risk of local and distant CRC recurrence following resection, allows patients to be properly informed and identifies those individuals who may benefit from adjuvant therapy.

1.4.4.1 Tumour Staging

Tumour stage is the single most important prognostic factor in CRC. Dukes' staging of CRC(151, 152) has now largely been superseded by the TNM classification, produced by the American Joint Committee on Cancer (AJCC), adopted by the Union for Internation Cancer Control (UICC) and is currently in its 8th edition (Table 1.1)(153). TNM staging exists for all solid tumours and describes the degree of local invasion of the primary tumour (T stage), locoregional lymph node involvement (N stage) and the presence or

absence of distant metastatic deposits (M stage). Prefixes and suffixes may be added to the TNM staging to provide additional information. The “c” prefix indicates clinical staging based on examination, endoscopy or surgical exploration without resection and radiological imaging, “p” prefix indicates pathological staging from a resected specimen, “y” prefix indicates previous neoadjuvant therapy, “r” prefix indicates recurrent disease, “a” prefix indicates staging based on an autopsy report and the “m" suffix indicates presence of multiple primary tumours. 5-year survival for patients with stage I CRC at diagnosis is 90.9% in England as compared to 84.3% for stage II disease, 65.0% for stage III and 10.5% for stage IV(1).

1.4.4.2 Table 1.1: TNM classification of colorectal tumours 8th edition.

Dukes Stage	TNM Stage	T Stage	N Stage	M Stage
	0	Tis: Carcinoma in situ: invasion of lamina propria.	N0: No regional lymph node metastatic disease.	M0: No distant metastatic disease.
A	I	T1: Tumour invades submucosa.	N0	M0
		T2: Tumour invades muscularis propria.	N0	M0
B	IIA	T3: Tumour invades subserosa or into non-peritonealised pericolic/perirectal tissues.	N0	M0
	IIB	T4a: Tumour perforates visceral peritoneum.	N0	M0
	IIC	T4b: Tumour directly invades other organs or structures.	N0	M0
C	IIIA	T1 or T2	N1a: Metastasis in 1 regional lymph node.	M0
		T1 or T2	N1b: Metastasis in 2 to 3 regional lymph nodes.	M0
		T1 or T2	N1c: Tumour deposit(s) i.e. satellites in the subserosa or in non-peritonealised pericolic or perirectal soft tissue without regional lymph node metastasis.	M0
		T1	N2a: Metastasis in 4-6 regional lymph nodes.	M0
	IIIB	T1 or T2	N2b: Metastasis in 7 or more regional lymph nodes.	M0
		T2 or T3	N2a	M0
		T3 or T4a	N1	M0
	IIIC	T3 or T4a	N2b	M0
		T4a	N2a	M0
		T4b	N1 or N2	M0
D	IVA	Any T	Any N	M1a: Metastasis confined to one organ (liver, lung, ovary, non-regional lymph node(s)) without peritoneal metastases.
	IVB	Any T	Any N	M1b: Metastasis in more than one organ.
	IVC	Any T	Any N	M1c: Metastasis in the peritoneum with or without other organ involvement.

1.4.4.3 Other Pathological Predictors of Prognosis

The International Collaboration on Cancer Reporting (ICCR) dataset for CRC, which has been adopted by the Royal College of Pathologists, specifies core (essential) and non-core (recommended) elements required to produce a high-quality CRC resection specimen report(154) (Table 1.2). This dataset contains pathological indicators of prognosis that may influence the need for adjuvant therapy.as well as findings that give an indication of the quality of the resection. The key pathological prognostic indicators T, N and M stage are of

course included, in addition to other valuable pathological findings that have been established as indicators of a poor outcome including the presence of tumour perforation(155), poorly differentiated or undifferentiated cancers(156), presence of lymphovascular invasion, particularly extramural venous invasion (EMVI)(157), perineural invasion(158), tumour budding(159), no or minimal tumour regression to neoadjuvant therapy(160) and R1 status(161). Ancillary studies include testing for mismatch repair (MMR) deficiency/ microsatellite instability (MSI), RAS and BRAF mutation status. Defective MMR may be sporadic or indicate Lynch syndrome and where appropriate a referral to clinical genetics may follow. Defective MMR correlates with an overall better prognosis, a poor response to 5-FU based chemotherapy and a better response to immunotherapy(154, 162). Patients with metastatic CRC being considered for anti-epidermal growth factor receptor (EGFR) therapy should have their RAS and BRAF mutation status checked to predict response to therapy(154). Indicators of resection adequacy include margin status, the plane of mesorectal, sphincter or mesocolic excision and lymph node yield. A median lymph node yield of at least 12 per resection should be targeted and a low lymph node yield is associated with a poorer prognosis(163).

1.4.4.4 Table 1.2: The International Collaboration on Cancer Reporting (ICCR) dataset for CRC.

Core Items	Non-Core Items

• Neoadjuvant therapy.
• Operative procedure.
• Tumour site.
• Tumour dimensions (maximum).
• Perforation.
• Relation of tumour to anterior peritoneal reflection.*
• Plane of mesorectal excision.*
  o Mesorectal fascia (complete TME).
  o Intramesorectal (near complete TME).
  o Muscularis propria (incomplete TME).
• Histological tumour type.
  o Adenocarcinoma most commonly.
  o Subtypes of adenocarcinoma e.g. mucinous, signet-ring cell or serrated.
  o Neuroendocrine neoplasms.
  o Undifferentiated carcinomas.
• Histological tumour grade.
  o 1 – well differentiated.
  o 2 – moderately differentiated.
  o 3 – poorly differentiated.
  o 4 – undifferentiated.
• Extent of invasion (T stage).
• Lymphatic and venous invasion.
• Perineural invasion.
• Lymph node status (N stage and lymph node yield).
• Tumour deposits/ satellites.
• Response to neoadjuvant therapy.
  o Tumour regression grade (TRG) 0 – no response.
  o TRG 1 – minor response.
  o TRG 2 – moderate response.
  o TRG 3 – near complete response.
  o TRG 4 – complete response.
• Margin status.
  o R0 – clear margins.
  o R1 - ≤1mm margins.
• Histologically confirmed distant metastases (M stage).
• Pathological staging (TMN stage).
	• Clinical information (e.g. polyposis syndrome, IBD).
	• Plane of sphincter excision.[#]
	  o Extralevator plane.
	  o Sphincteric plane.
	  o Intrasphincteric plane.
	• Plane of mesocolic excision.[¶]
	  o Mesocolic plane.
	  o Intramesocolic plane.
	  o Muscularis propria plane.
	• Measurement of invasion beyond muscularis propria.
	• Tumour budding.
	• Coexistent pathology (e.g. polyps, IBD, diverticular disease, sequelae of obstruction or neoadjuvant therapy).
	• Ancillary studies.

* Rectal cancer only
[#] APR only
[¶] Colon cancer only

1.4.5 Adjuvant Therapy

Following a CRC resection with curative intent, the administration of adjuvant systemic chemotherapy has made a significant contribution to the improved outcomes observed over the last 20 years. The rationale for its administration is based on the potential for occult metastatic disease (micrometastases) present at the time of resection that may subsequently lead to recurrence. The decision to administer chemotherapy should be made jointly by the patient and oncologist, should be ratified by the MDT and agent choice should consider risk factors for recurrence, performance status, contraindications and side effect profile(77).

Patients with stage III colonic cancer have been shown to have improved disease-dree survival (DFS) and overall survival (OS) with the administration of adjuvant fluoropyrimidine-based chemotherapy(164, 165). The X-ACT trial revealed that intravenous fluorouracil (5-FU) and the oral equivalent, capecitabine, may be equally effective(166) and many patients may find the oral route more convenient. The MOSAIC trial showed that the addition of oxaliplatin to 5-FU and folinic acid (FOLFOX) increased DFS(167) and the same has been show for the addition of oxaliplatin to capecitabine (XELOX)(168). These results have been extrapolated to rectal cancer such that SIGN, NICE and ACPGBI recommend that all patients with stage III CRC should be considered for 3-6 months of adjuvant FOLFOX or XELOX chemotherapy(77, 99, 111). The benefit of giving further chemotherapy to rectal cancer patients who received neoadjuvant chemoradiotherapy, particularly long course is debated(25).

The benefits of adjuvant chemotherapy in patients with stage II CRC are less certain. The QUASAR trial showed a modest improvement in survival in patients with stage II CRC given 5-FU and folinic acid(169). Subgroup analysis of stage II patients from the MOSAIC trial showed no statistically significant benefit in OS or DFS by adding oxaliplatin for

these patients(170) and it is not routinely used in stage II disease(77). Adjuvant therapy may be considered for patients with stage II disease and adverse histopathological features including T4 staging, obstructed tumours, poor differentiation, mucinous subtype, extramural vascular invasion (EMVI), fewer than 12 lymph nodes in the resection specimen and involved or close resection margins(25, 77, 99). Presence of microsatellite instability (MSI) in stage II colon cancers, particularly right-sided tumours, confers better DFS as compared to microsatellite stable tumours, and adjuvant chemotherapy does not appear to be beneficial in such patients(77).

1.4.6 Follow-up

Following diagnosis and potentially curative treatment for CRC, a dedicated and structured programme of follow-up is initiated. The purpose of this follow-up is to detect locally recurrent disease or metastatic disease progression, with the hope of providing salvage curative therapy or palliation in a timely manner. Additionally, patients are at an increased risk of developing metachronous premalignant polyps or CRCs in their remaining colorectum. Finally, follow-up provides an opportunity for ongoing psychological support for cancer survivors. Regular outpatient clinic appointments are scheduled with cancer nurse specialist presence. Serum carcinoembryonic antigen (CEA) levels are checked at least 6 monthly for 3 years, surveillance colonoscopy is performed 1 year post treatment and surveillance CT of the chest abdomen and pelvis is conducted at least twice in the first 3 years, generally on an annual basis. Follow-up may be terminated earlier in selected elderly or frail patients in agreement with the patient(31, 77, 111).

1.4.7 Treatment of Advanced CRC

1.4.7.1 Unresectable Primary CRC

Unresectable primary disease is more common in rectal than colonic cancer. As discussed previously, neoadjuvant chemoradiotherapy is offered to patients with locally advanced

rectal cancer and may downstage to allow TME. Chemotherapy and/ or radiotherapy for unresectable colonic cancer may improve symptoms and survival and may lead to downstaging and enable surgical intervention in the future(77). Placement of a colonic stent or a defunctioning colostomy or ileostomy can be performed to relieve pending intestinal obstruction.

1.4.7.2 Locally Recurrent CRC

Patients who develop local recurrence of CRC should be considered for salvage resection. This may involve referral to a unit that specialises in pelvic exenteration for recurrent rectal cancer. If a recurrent CRC is not deemed resectable, palliative chemotherapy or chemoradiotherapy may be given in the hope of achieving tumour shrinkage, allowing salvage surgery in the future(77, 147).

1.4.7.3 Resectable Metastatic Disease

There is a role for the resection of limited and technically operable liver or lung metastases. 5-year survival following such resection is reasonable at approximately 40%. However, such patients are highly selected and tend to be younger, have good performance status and have limited and slow growing metastases. No randomised control trial has been successfully completed comparing metastasectomy to systemic chemotherapy alone. The pulmonary metastasectomy versus continued active monitoring in colorectal cancer (PulMiCC) trial only managed to recruit 65 patients and the study was stopped early(171). It is unclear whether such procedures offer a true survival benefit, however, in the absence of evidence to the contrary, metastasectomy continues to be offered to favourable candidates.

In the EPOC trial patients with resectable colorectal liver metastases were randomised to receive FOLFOX chemotherapy before and after liver resection. The absolute increase in the rate of progression-free survival at 3 years was 7.3% (from 28.1% (95% CI: 21.3-35.5)

to 35.4% (95% CI: 28.1-42.7), HR 0.79 (95% CI: 0.62-1.02); p=0.058)), which was just short of significance(172). Practice varies but in general patients with high risk synchronous disease are offered neoadjuvant therapy prior to liver metastasectomy, while adjuvant therapy is offered to all(77).

Patients with peritoneal metastases and excellent performance status may be considered for referral to specialist units who offer cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC)(111).

1.4.7.4 Unresectable Metastatic Disease

Systemic chemotherapy with or without the addition of biological therapies forms the mainstay of treatment for patients with metastatic CRC. For the most part these are given with palliative intent, although if irresectable metastatic disease responds well and becomes technically resectable, potentially curative surgery may be offered at a later date.

Fluorouracil (5-FU) with folinic acid and oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) are the most commonly used chemotherapeutic regimes. Capecitabine can be substituted 5-FU/ folinic acid in combination with oxaliplatin (XELOX) with similar efficacy(25). A systematic review and meta-analyses found the combination of 5-FU, folinic acid, oxaliplatin and irinotecan (FOLFOXIRI) increased survival by 25% as compared to FOLFOX or FOLFIRI however there was also a significant increase in toxicity and such treatment should only be given to patients with good performance status.

Numerous biological therapies have been trialled for metastatic CRC. Many of these therapies take the form of monoclonal antibodies directed against pro-tumorigenic antigens. Cetuximab and panitumumab are epidermal growth factor receptor (EGFR) inhibitors which show efficacy in patients with RAS-wild-type disease. NICE has approved use of these agents for advanced CRC in combination with conventional

chemotherapy(77, 111). Aflibercept is an anti-vascular endothelial growth factor (VEGF) which inhibits angiogenesis. It has been approved in Scotland by the Scottish Medicine Consortium (SMC) but not by NICE and is therefore only currently available in Scotland, in combination with FOLFIRI for patients with advanced CRC(173). Encorafenib is a BRAF inhibitor that has shown efficacy for patients with CRC with the BRAF V600E mutation. It has been approved by NICE to be used in combination with cetuximab as a second line treatment for metastatic CRC. Regorafenib is a multi-kinase inhibitor which inhibits tyrosine kinases active in angiogenesis and tumour growth. It has been approved by NICE as a second line option in patients with metastatic CRC(111).

Immunotherapy is another form of biological therapy that has been established as effective treatment for patients with metastatic defective mismatch repair (MMR) CRC(128-130). In the CheckMate-142 trial patients, with metastatic MMR defective CRC were given a combination of nivolumab and ipilimumab. Progression-free and overall survival at 12 months were 71% and 85% respectively(129). In the KEYNOTE-177 trial, patients with metastatic MMR defective CRC were randomised in an open label fashion to pembrolizumab or standard chemotherapy. Median progression free survival in the pembrolizumab group was 16.5 months as compared to 8.2 months for the standard chemotherapy group (HR 0.60 (95% CI: 0.45-0.80; p=0.0002))(130). Pembrolizumab has been approved by NICE for first line treatment of metastatic CRC with confirmed MSI or MMR while nivolumab and ipilimumab can be given to such patients after conventional chemotherapy(111).

1.4.8 Palliation

Palliative care encompasses a holistic approach to the management of pain, psychological distress, social and spiritual support. Although typically associated with advanced cancer and end of life care, the expert assistance of palliative care consultants and specialist

nurses may be sought at various stages through a cancer journey, particularly in patients with difficult to manage symptoms or complex needs. Symptoms may arise in patients with CRC due to the disease itself or may be secondary to acute chemotherapeutic toxicity, typically nausea, vomiting and acute diarrhoea, or long-term sequelae of chemotherapy such as neuropathy or fatigue. In patients with CRC for which cure is not possible, advance care plans detailing preferred place of death (home, hospital, hospice) and decisions regarding refusal of treatments and do no attempt resuscitation orders can be beneficial. In the last days of life pharmacological management of pain, nausea, vomiting, respiratory secretions, breathlessness, anxiety and agitation is often required and anticipatory prescribing of these medications is recommended(77). Psychological and spiritual support are important in the final days of life and often extends to the family of the patient, grieving the loss of their loved one.

2 Combining the quantitative faecal immunochemical test and full blood count reliably rules out colorectal cancer in a symptomatic patient referral pathway.

2.1 Introduction

High risk lower gastrointestinal (GI) symptoms should trigger an urgent suspicion of colorectal cancer (CRC) referral, based on the National Institute for Health and Care Excellence (NICE) NG12(91) and NHS Scotland Suspected Cancer Guidelines(25). These symptoms include rectal bleeding with no obvious cause, a persistent (>4 week) change in bowel habit, particularly diarrhoea, palpable abdominal or rectal masses, abdominal pain with weight loss and unexplained iron deficiency anaemia (IDA), each of which may be tempered with the patient's age(25, 91). However, lower GI symptoms themselves are associated with low positive predictive value for CRC, with similar symptoms seen in both significant bowel disease (CRC, advanced polyps or inflammatory bowel disease (IBD)) and functional bowel disorders(11). Indeed, following introduction of the NICE NG12 guidance and a United Kingdom (UK)-based CRC symptom public awareness campaign, the number of suspected CRC referrals increased while the proportion found to have CRC decreased and there was no change in staging at diagnosis (92, 93). Therefore, objective biomarkers able to accurately predict CRC-risk in symptomatic individuals are desirable, allowing effective triage of patients for referral and definitive investigation with colonoscopy, cross-sectional imaging or capsule colon endoscopy (CCE). The Scottish Bowel Screening Programme is based on biennial quantitative faecal immunochemical testing (FIT) followed by colonoscopy for those testing positive with a faecal haemoglobin (f-Hb) threshold of 80 µg Hb/gram faeces(174). This approach to screening increases the number of early-stage cancers diagnosed, reduces cancer specific mortality(4, 6, 175, 176) and may reduce CRC incidence through removal of precursor polyps(176). More recently, the utility of FIT for CRC risk prediction in symptomatic patients has been proven (11-14,

16-21), with a recent meta-analysis reporting a pooled sensitivity of 87.2% (95% CI: 81.0-91.6%) and specificity of 84.4% (95% CI: 79.4-88.3%) for CRC detection at the f-Hb ≥10 μg/g threshold (14). In the initial National Institute for Health and Care Excellence (NICE) guidance regarding the use of FIT in symptomatic patients (DG30), it was advised that FIT only be used for patients with low-risk symptoms in the absence of rectal bleeding(95). However, FIT has proven utility for determining CRC risk in patients meeting both high risk (NG12) and low risk (DG30) symptoms(12, 93, 96) and in patients with and without rectal bleeding(97). In response, the most recent guidance from the Association of Coloproctology of Great Britain and Ireland (ACPGBI) and the British Society of Gastroenterology (BSG)(98), NICE(91) and NHS Scotland(22) have included the use of FIT for all patients with lower GI symptoms. Accordingly, FIT has now been widely integrated into all colorectal and gastroenterology referral pathways in most Scottish NHS health boards(15, 22).

Most studies that have examined the use of FIT in symptomatic patients have only included patients subsequently referred from primary care and definitively investigated. Conversely, the current study assessed the real-life impact of FIT-integration on general practitioner (GP) referral practice and colorectal and gastroenterology decision to investigate. Additionally, this study explored whether the combination of f-Hb and circulating haemoglobin (Hb) could enhance CRC risk prediction. This study therefore aimed to examine associations between CRC diagnosis, symptoms, f-Hb concentration and anaemia in patients both referred and not referred from primary care following the introduction of FIT into a symptomatic lower GI referral pathway.

2.2 Methods

2.2.1 Study Design, Setting and Participants

A multicentre, retrospective, observational study was conducted to include all patients ≥16 years old with a FIT submitted from primary care between August 2018 and January 2019 in the NHS Greater Glasgow and Clyde (NHS GG&C) health board. Of note, this was the period during which FIT was introduced to local referral pathways. The study was reported according to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines(174).

2.2.2 FIT Specimen Collection and Handling

FIT specimen collection kits were supplied to all GP practices as an adjunct to guide referral for patients with lower GI symptoms. Each kit contains a single FIT collection device (EXTEL HEMO-AUTO MC Collection Picker, Minaris Medical Co., Ltd, Tokyo, Japan, supplied by Alphas Labs Ltd, Eastleigh, Hants, UK), pictorial instructions and a return envelope. The collection device is a picker which obtains a consistent 2 mg sample and is inserted into a vial containing 2 ml of buffer. Patients being considered for symptomatic lower GI referral were asked to collect a single faecal sample and return to their GP practice as soon as possible. The samples were transported at ambient temperature via routine specimen collection services and stored at 4°C prior to analysis in a single centralised laboratory (Stobhill Hospital, Glasgow, UK).

2.2.3 FIT Analysis

Analysis was carried out on the HM-JACKarc system (Minaris Medical Co., Ltd) Monday to Friday so that most samples were analysed on day of receipt. The manufacturers limit of detection is 2 μg/g, limit of quantification 7 μg/g and upper measurement limit 400 μg/g.

Specimens with f-Hb concentrations above this limit were not diluted and re-analysed but simply reported as >400 µg/g.

2.2.4 FIT Result Quality Management

All biomedical science staff are Health Care and Professionals Council (HCPC) registered and undergo local competency assessment prior to using the HM-JACKarc analyser. There are two internal quality controls (IQCs): EXTEL HEMO AUTO HS Low IQC and EXTEL HEMO AUTO HS High IQC. West guard rule criteria are used for acceptance or rejection of analytical runs. The laboratory participates in appropriate external quality assessment (National External Quality Assessment Services (NEQAS)).

2.2.5 FIT Result Handling

FIT results were electronically transferred from the analyser into the Laboratory Information Management System (LIMS) and patient record as well as electronically reported to the requesting GP. A f-Hb ≥10 µg/g was defined as raised as per the NICE DG30 guidance(95) available at the time and subsequently recommended by the ACPGBI and BSG(98). GPs were asked to use the f-Hb measurement to guide the need for referral to specialist services. Patients with a f-Hb ≥10 µg/g qualified for urgent suspicion of cancer (USoC) referral. Of note, the referral guidance in place within NHS GG&C during the study period was such that patients with a rectal or abdominal mass, persistent (>4 weeks) rectal bleeding or diarrhoea, significant involuntary weight loss or new IDA with a f-Hb ≤10 µg/g were also triaged as USoC referrals, while patients with abdominal pain, intermittent rectal bleeding, other changes in bowel habit or anorectal symptoms with a f-Hb ≤10 µg/g qualified for routine clinic review.

2.2.6 Patient Identification and Data Collection

To identify study participants and capture all FIT samples submitted between August 2018 and January 2019, a search of the clinical biochemistry repository was conducted. These samples were then interrogated and where duplicate entries were identified the first valid sample was kept. Patients were excluded if they were <16 years old. To compile covariables and outcomes for each patient, multiple searches were then performed using the Community Health Index (CHI) number as the linkage variable. A search of SCI store (Scottish Care Information Store Version 8.5) allowed the identification of patient demographics and blood results. Post codes were used to determine each patient's Scottish Index of Multiple Deprivation (SIMD) score. SIMD is a measure of an area's deprivation according to income, employment, education, health, access to services, crime and housing(177). SCI Gateway (Scottish Care Information Gateway R 20.0) was searched to identify referral letters from primary care to general surgery or gastroenterology within 3 months prior or after FIT collection. These letters were manually screened to identify lower GI symptoms and coded as rectal bleeding, persistent diarrhoea, other change in bowel habit, weight loss, abdominal pain, anal pain, faecal soiling, rectal mass and abdominal mass. Referral letters were also used to identify patient co-morbidity. For the purposes of analysis asthma and chronic obstructive pulmonary disease (COPD) were grouped as "respiratory disease," ischaemic heart disease, cerebrovascular disease, peripheral vascular disease and hypertension were grouped as "cardiovascular disease" and previous diagnosis of Crohn's, ulcerative colitis or indeterminate colitis were grouped as "inflammatory bowel disease." Unisoft (Unisoft Medical Systems GI Reporting Tool) was used to identify all patients who underwent a colonoscopy following their FIT collection date. CRIS (Central Data Networks Radiology Information System) identified all patients who had a computed tomography (CT) colon, CT chest abdomen and pelvis or CT abdomen and pelvis as their only form of investigation following referral. To ensure no CRCs diagnosed out with the referral pathway under investigation were missed, the

managed clinical network (MCN) cancer registry was searched to identify all new diagnoses of CRC up to November 2020. Caldicott guardian approval was given by NHS GG&C to safeguard the record linkage with ethical approval waived for the purposes of service development.

2.2.7 Data Analysis and Statistical Methods

Patients were categorised into 3 groups: “Non-Referred” (FIT sample submitted from primary care but no accompanying referral letter to general surgery or gastroenterology) “Referred, No Colonoscopy” (FIT sample submitted with accompanying referral but colonoscopy not performed) and “Referred and Colonoscopy” (FIT sample submitted with accompanying referral and colonoscopy subsequently performed). Importantly, patients were only regarded as “referred” if a referral was made from primary care to general surgery or gastroenterology as part of the outpatient symptomatic lower GI referral pathway under investigation. Referrals out with this pathway to alternative specialities or emergency attendances were not regarded as “referred.” FIT results were grouped by f-Hb concentrations of <10 ug/g, 10-149 µg/g, 150-399 µg/g and ≥400 µg/g. Additionally, a final group consisted of those samples which could not be processed by the laboratory due to faecal contamination, expired collection device or insufficient patient identification and were not repeated. Patients were defined as anaemic (male <130 mg/L, female <120 mg/L) based on WHO guidelines(178) and iron deficient (ferritin <15 µg/L) based on BSG guidelines(179).

Covariables were compared using crosstabulation and the χ^2 or Fisher’s exact test. A value of $p<0.05$ was considered statistically significant. To identify covariables which independently predicted CRC-risk, univariate followed by multivariate binary logistic regression was performed. Selected covariables found to have a significant impact on CRC risk from the $\chi 2$ analysis were carried into the regression analysis. This allowed calculation

of odds ratios (ORs) and 95% confidence intervals (95% CI). Covariables significant on univariate analysis ($p<0.05$) were entered into a multivariate model using the backwards conditional method in which variables with a significance of $p>0.1$ were removed from the model in a stepwise fashion. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, United States of America (USA)).

2.3 Results

2.3.1 Cohort Description

4968 patients aged ≥16 years had a FIT sample submitted from primary care between August 2018 and January 2019 in NHS GG&C. The referral pathway, subsequent investigation and summarised f-Hb levels and CRC rate of each group can be seen in Figure 2.1. With a median 23-month (range 21-26) follow-up, 61 patients (1.2%) were diagnosed with CRC.

2.3.2 Referral and Subsequent Investigation Practice

A comparison between referred and non-referred patients and between those patients who did or did not subsequently undergo colonoscopy can be seen in Tables 2.1 and 2.2 respectively. Notably, patients who were referred were significantly older (median 60 versus 57 years, $p<0.001$), had significantly higher f-Hb (≥10 µg/g 37.9% versus 1.9%, $p<0.001$), were more likely to be anaemic (22.4% versus 17.6%, $p<0.001$) and had a greater proportion of IDA (6.8% versus 3.9%, $p<0.001$) as compared to non-referred patients. Patients selected for colonoscopy were significantly younger (median 60 versus 61 years, $p=0.02$), more likely to have reported PR bleeding (44.1% versus 27.3%, $p<0.001$) or persistent diarrhoea (27.4% versus 21.5%, $p=0.001$), had significantly higher f-Hb (≥10 µg/g 52.9% versus 19.8%, $p<0.001$) and more IDA (8.0% versus 5.4%, $p=0.018$).

47 patients had f-Hb ≥10µg/g but were not referred to the general surgery or gastroenterology service. The records of each of these patients were reviewed. 10 patients were deemed too frail for referral by their GP following positive FIT. 7 patients had a recent acute inpatient admission and investigation or decision not to investigate had been organised from that admission. 10 patients were already known to general surgery or gastroenterology and were regularly seen on an outpatient basis including patients

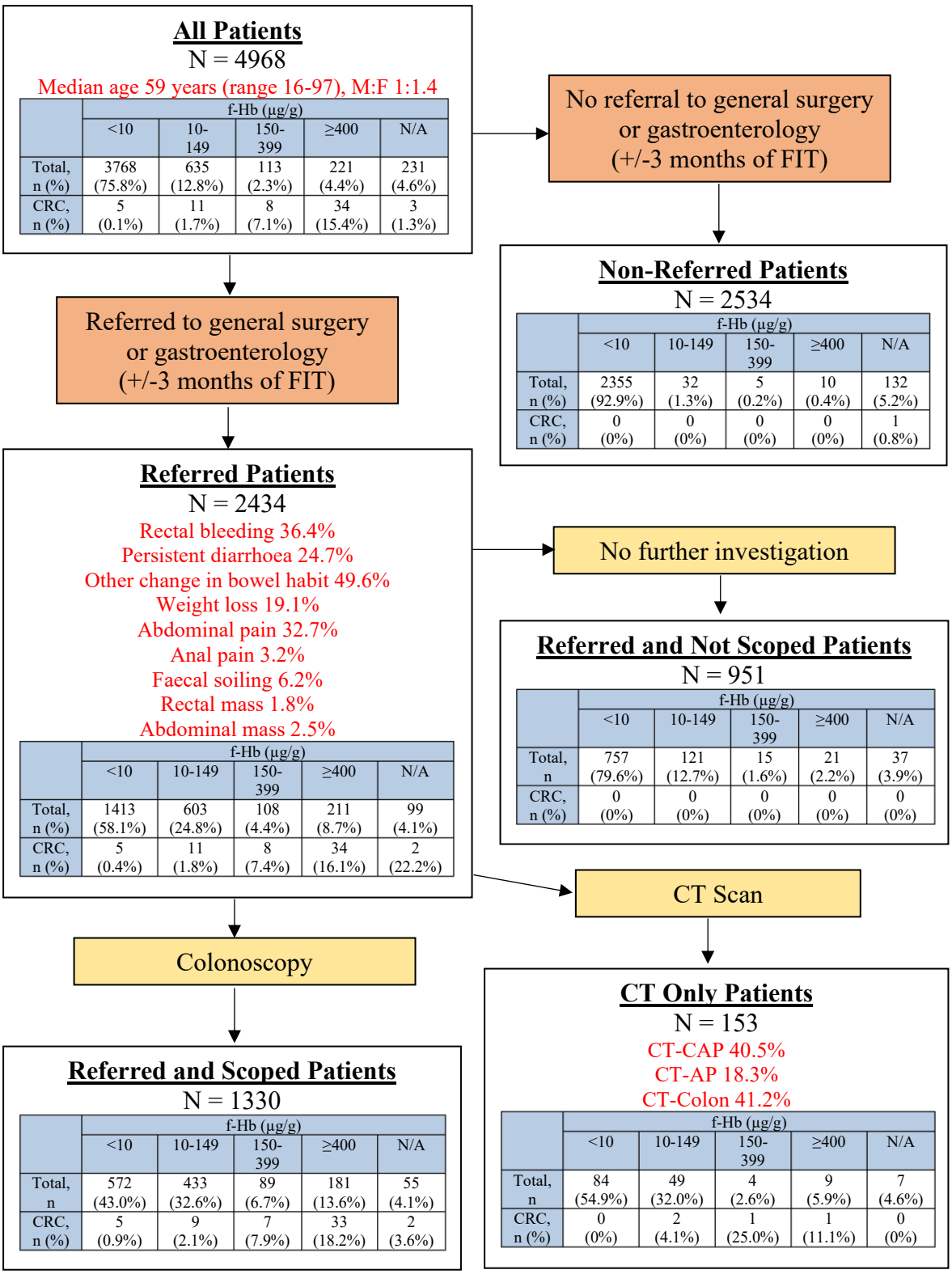
scheduled for surveillance colonoscopy. 3 patients were referred to care of the elderly rather than general surgery or gastroenterology. 17 patients had a positive FIT but no clear reason for no onward referral.

157 patients had f-Hb ≥10μg/g, were referred but did not undergo further investigation. Secondary care advised against further investigation due to frailty in 7 cases, recent colonoscopy or CT-colon in 15, or for other reasons in 12. 38 patients failed to attend clinic, while 27 did not attend further investigation appointments. 18 patients declined investigation, 2 patients did not tolerate bowel preparation, 5 patients died before reaching clinic or investigation and the reason for no investigation could not be determined in 33 cases.

2.3.3 CRC Cases

61 of 4968 (1.2%) were diagnosed with CRC. Of these, 56 (91.8%) belonged to the Referred and Colonoscopy group with the diagnosis confirmed at colonoscopy as a direct result of referral. 4 patients in the Referred, No Colonoscopy group were diagnosed with CRC. Of these, two were deemed too frail for colonoscopy and following referral underwent CT abdomen and pelvis which identified a CRC for which both had supportive management only. One patient underwent a CT colon following referral and proceeded straight to laparoscopic right hemicolectomy with tissue diagnosis only confirmed postoperatively. One patient was referred from primary care but prior to clinic review presented with small bowel obstruction secondary to a caecal cancer and underwent an emergency right hemicolectomy. Finally, one patient belonged to the Not Referred group. This patient's submitted FIT could not be processed by the laboratory and was not repeated. The patient was later admitted as an emergency with symptomatic anaemia and had a CRC diagnosed at inpatient colonoscopy.

2.3.4 Figure 2.1: Referral and investigation pathway, f-Hb levels and CRC cases.



2.3.5 Table 2.1: Comparison between referred and non-referred patients.

		Non-Referred	Referred	P
N		2534	2434	
Age	Median (range)	57 (16-97)	60 (16-95)	
	<50	841 (33.2%)	614 (25.2%)	
	50-74	1284 (50.7%)	1368 (56.2%)	
	≥75	409 (16.1%)	542 (18.6%)	<0.001
Sex	Male	1042 (41.1%)	1060 (43.5%)	
	Female	1492 (58.9%)	1374 (56.5%)	0.083
SIMD	1 (most deprived)	691 (27.3%)	795 (32.7%)	
	2	434 (17.1%)	434 (17.8%)	
	3	329 (13.0%)	292 (12.0%)	
	4	399 (15.7%)	367 (15.1%)	
	5 (least deprived)	681 (26.9%)	546 (22.4%)	<0.001
Medications	Aspirin	397 (15.7%)	539 (22.1%)	<0.001
	NSAIDs	267 (10.5%)	301 (12.4%)	0.043
	ACE Inhibitors	382 (15.1%)	470 (19.3%)	<0.001
	Statins	562 (22.2%)	688 (28.3%)	<0.001
	H2 Antagonists	72 (2.8%)	87 (3.6%)	0.142
	Metformin	72 (2.8%)	87 (3.6%)	0.142
	Oral Anticoagulants	73 (2.9%)	122 (5.0%)	<0.001
	Anti-spasmodics	685 (27.0%)	642 (26.4%)	0.601
f-Hb (µg/g)	<10	2355 (92.9%)	1413 (58.1%)	
	10-149	32 (1.3%)	603 (24.8%)	
	150-399	5 (0.2%)	108 (4.4%)	
	≥400	10 (0.4%)	211 (8.7%)	
	N/A	132 (5.2%)	99 (4.1%)	<0.001
Anaemia*	No	1708 (82.4%)	1676 (77.6%)	
	Yes	365 (17.6%)	483 (22.4%)	<0.001
Iron Deficiency Anaemia (Ferritin <15)#	No	1962 (96.1%)	1988 (93.2%)	
	Yes	80 (3.9%)	146 (6.8%)	<0.001
Anaemia and Mean Corpuscular Volume (MCV)£	Not Anaemic	1708 (82.4%)	1676 (77.6%)	
	Macrocytic Anaemia (MCV >100)	28 (1.4%)	27 (1.3%)	
	Normocytic Anaemia (MCV 80-100)	295 (14.2%)	370 (17.1%)	
	Microcytic Anaemia (MCV <80)	42 (2.0%)	86 (4.0%)	<0.001
CRC		1 (0.04%)	60 (2.5%)	<0.001

*Data missing for 736 (14.8%) patients.
Data missing for 792 (15.9%) patients.
£ Data missing for 736 (14.8%) patients.

2.3.6 Table 2.2: Comparison between Referred, No Colonoscopy and Referred and Colonoscopy patients.

		Referred, No Colonoscopy	Referred and Colonoscopy	P
N		1104	1330	
Age	Median (range)	61 (16-95)	60 (17-94)	
	<50	281 (25.5%)	333 (25.0%)	
	50-74	578 (52.4%)	790 (59.4%)	
	≥75	245 (22.2%)	207 (15.6%)	<0.001
Sex	Male	484 (43.8%)	576 (43.3%)	
	Female	620 (56.2%)	754 (56.7%)	0.792
SIMD	1 (most deprived)	349 (31.6%)	446 (33.5%)	
	2	197 (17.8%)	237 (17.8%)	
	3	120 (10.9%)	172 (12.9%)	
	4	175 (15.9%)	192 (14.4%)	
	5 (least deprived)	263 (23.8%)	283 (21.3%)	0.274
Co-morbidity*	Respiratory Disease	112 (17.5%)	165 (16.1%)	0.479
	Diabetes	80 (12.5%)	122 (11.9%)	0.741
	Cardiovascular Disease	106 (16.5%)	153 (15.0%)	0.391
	IBD	4 (0.6%)	2 (0.2%)	0.156
Medication	Aspirin	259 (23.5%)	280 (21.1%)	0.154
	NSAIDs	128 (11.6%)	173 (13.0%)	0.292
	ACE Inhibitors	202 (18.3%)	268 (20.2%)	0.249
	Statins	310 (28.1%)	378 (28.4%)	0.852
	H2 Antagonists	48 (4.3%)	39 (2.9%)	0.061
	Metformin	48 (4.3%)	39 (2.9%)	0.061
	Oral Anticoagulants	63 (5.7%)	59 (4.4%)	0.153
	Anti-spasmodics	317 (28.7%)	325 (24.4%)	0.017
Symptoms	Any Red Flag	993 (89.9%)	1201 (90.3%)	0.77
	Rectal Bleeding	301 (27.3%)	586 (44.1%)	<0.001
	Persistent Diarrhoea	237 (21.5%)	365 (27.4%)	0.001
	Other Change in Bowel Habit	567 (51.4%)	640 (48.1%)	0.112
	Weight Loss	237 (21.5%)	229 (17.2%)	0.008
	Abdominal Pain	395 (35.8%)	401 (30.2%)	0.003
	Anal Pain	44 (4.0%)	33 (2.5%)	0.035
	Faecal Soiling	65 (5.9%)	85 (6.4%)	0.607
	Rectal Mass	18 (1.6%)	26 (2.0%)	0.55
	Abdominal Mass	24 (2.2%)	36 (2.7%)	0.399
f-Hb (µg/g)	<10	841 (76.2%)	572 (43.0%)	
	10-149	170 (15.4%)	433 (32.6%)	
	150-399	19 (1.7%)	89 (6.7%)	
	≥400	30 (2.7%)	181 (13.6%)	
	N/A	44 (4.0%)	55 (4.1%)	<0.001
Anaemia#	No	750 (77.1%)	926 (78.1%)	
	Yes	223 (22.9%)	260 (21.9%)	0.58
Iron Deficiency Anaemia (Ferritin <15)¶	No	909 (94.6%)	1079 (92.0%)	
	Yes	52 (5.4%)	904 (8.0%)	0.018
Anaemia and MCV£	Not Anaemic	750 (76.9%)	926 (78.2%)	
	Macrocytic Anaemia (MCV >100)	30 (3.1%)	56 (4.7%)	
	Normocytic Anaemia (MCV 80-100)	183 (18.8%)	187 (15.8%)	
	Microcytic Anaemia (MCV <80)	12 (1.2%)	15 (1.3%)	0.088
CRC		4 (0.4%)	56 (4.2%)	<0.001

*Data missing for 771 (31.7%) patients.
#Data missing for 275 (11.3%) patients.
¶Data missing for 300 (12.3%) patients.
£Data missing for 275 (11.3%) patients.

2.3.7 Predictors of CRC

Table 2.3 compares those diagnosed with CRC and those who were not. Patients diagnosed with CRC were significantly older (median age 69 versus 59 years CRC and no CRC respectively, p=0.001), more likely to be male (55.7% versus 42.1%, p=0.033), have a history of IBD (2.1% versus 0.3%, p=0.04), have reported rectal bleeding (51.7% versus 36.1%, p=0.013) and significantly less likely to have reported abdominal pain (20.0% versus 33.0%, p=0.034). FIT predicted CRC (f-Hb <10 μg/g 8.2% versus 76.7% and f-Hb ≥400 μg/g 55.7% versus 3.8%, p<0.001), as did anaemia (45.9% versus 19.7%, p<0.001), IDA (26.2% versus 5.1%, p<0.001) and both normocytic (26.2% versus 15.6%, p<0.001) and microcytic anaemia (18.0% versus 2.8%, p<0.001). On multivariate binary logistic regression (Table 2.4), increasing age (50-74 years OR 2.749 (95% CI: 1.150-6.572; p=0.023) and ≥75 years OR 4.140 (95% CI: 1.610-10.641; p=0.003)), male sex (OR 1.817 (95% CI: 1.027-3.216; p=0.04)), f-Hb (10-149 μg/g OR 4.623 (95% CI: 1.587-13.465; p=0.005), 150-399 μg/g OR 19.690 (95% CI: 6.207-62.459; p<0.001) and ≥400 μg/g OR 54.256 (95% CI: 20.683-142.325; p<0.001)) and anaemia (OR 1.956 (1.071-3.574; p=0.029)) retained significance as independent predictors of CRC.

At a f-Hb threshold of 10 μg/g, sensitivity for colorectal cancer was 91.8%, specificity 80.4%, negative predictive value (NPV) 99.9% and positive predictive value (PPV) 5.5%. The number of colonoscopies (number needed to scope, NNS) that would have to be performed to diagnose one CRC at the 10 μg/g threshold, was 18.

2.3.8 Table 2.3: Comparison between patients diagnosed with CRC and those who were not.

		CRC		P
		Yes	No	
N		61	4907	
Age	Median (range)	69 (36-95)	59 (16-97)	
	<50	7 (11.5%)	1448 (29.5%)	
	50-74	30 (49.2%)	2622 (53.4%)	
	≥75	24 (39.3%)	837 (17.1%)	<0.001
Sex	Male	34 (55.7%)	2068 (42.1%)	
	Female	27 (44.3%)	2839 (57.9%)	0.033
Scottish Index of Multiple Deprivation	1 (most deprived)	16 (26.2%)	1470 (30.0%)	
	2	13 (21.3%)	855 (17.4%)	
	3	11 (18.0%)	610 (12.4%)	
	4	8 (13.1%)	758 (15.4%)	
	5 (least deprived)	13 (21.3%)	1214 (24.7%)	0.59
Co-morbidity*	Respiratory Disease	8 (17.0%)	270 (16.7%)	0.95
	Diabetes	6 (12.8%)	197 (12.2%)	0.902
	Cardiovascular Disease	9 (19.1%)	250 (15.4%)	0.489
	IBD	1 (2.1%)	5 (0.3%)	0.04
Symptoms#	Rectal Bleeding	31 (51.7%)	856 (36.1%)	0.013
	Persistent Diarrhoea	16 (26.7%)	586 (24.7%)	0.725
	Other Change in Bowel Habit	27 (45.0%)	1180 (49.7%)	0.472
	Weight Loss	12 (20.0%)	454 (19.1%)	0.865
	Abdominal Pain	12 (20.0%)	784 (33.0%)	0.034
	Anal Pain	0 (0%)	77 (3.2%)	0.156
	Faecal Soiling	4 (6.7%)	146 (6.1%)	0.869
	Rectal Mass	1 (1.7%)	43 (1.8%)	0.934
	Abdominal Mass	1 (1.7%)	59 (2.5%)	0.686
f-Hb (µg/g)	<10	5 (8.2%)	3763 (76.7%)	
	10-149	11 (18.0%)	624 (12.7%)	
	150-399	8 (13.1%)	105 (2.1%)	
	≥400	34 (55.7%)	187 (3.8%)	
	N/A	3 (4.9%)	228 (4.6%)	<0.001
Anaemia¶	No	33 (54.1%)	3351 (80.3%)	
	Yes	28 (45.9%)	820 (19.7%)	<0.001
Iron Deficiency Anaemia (Ferritin <15)Φ	No	45 (73.8%)	3905 (94.9%)	
	Yes	16 (26.2%)	210 (5.1%)	<0.001
Anaemia and MCV£	Not Anaemic	33 (54.1%)	3351 (80.3%)	
	Macrocytic Anaemia (MCV >100)	1 (1.6%)	54 (1.3%)	
	Normocytic Anaemia (MCV 80-100)	16 (26.2%)	649 (15.6%)	
	Microcytic Anaemia (MCV <80)	11 (18.0%)	117 (2.8%)	<0.001

*Data missing for 3302 (66.5%) patients.
#Data missing for 2534 (51.0%) patients.
¶Data missing for 736 (14.8%) patients.
Φ Data missing for 792 (15.9%) patients.
£Data missing for 736 (14.8%) patients.

2.3.9 Table 2.4: Univariate and multivariate binary logistic regression analysis of factors predicting CRC risk.

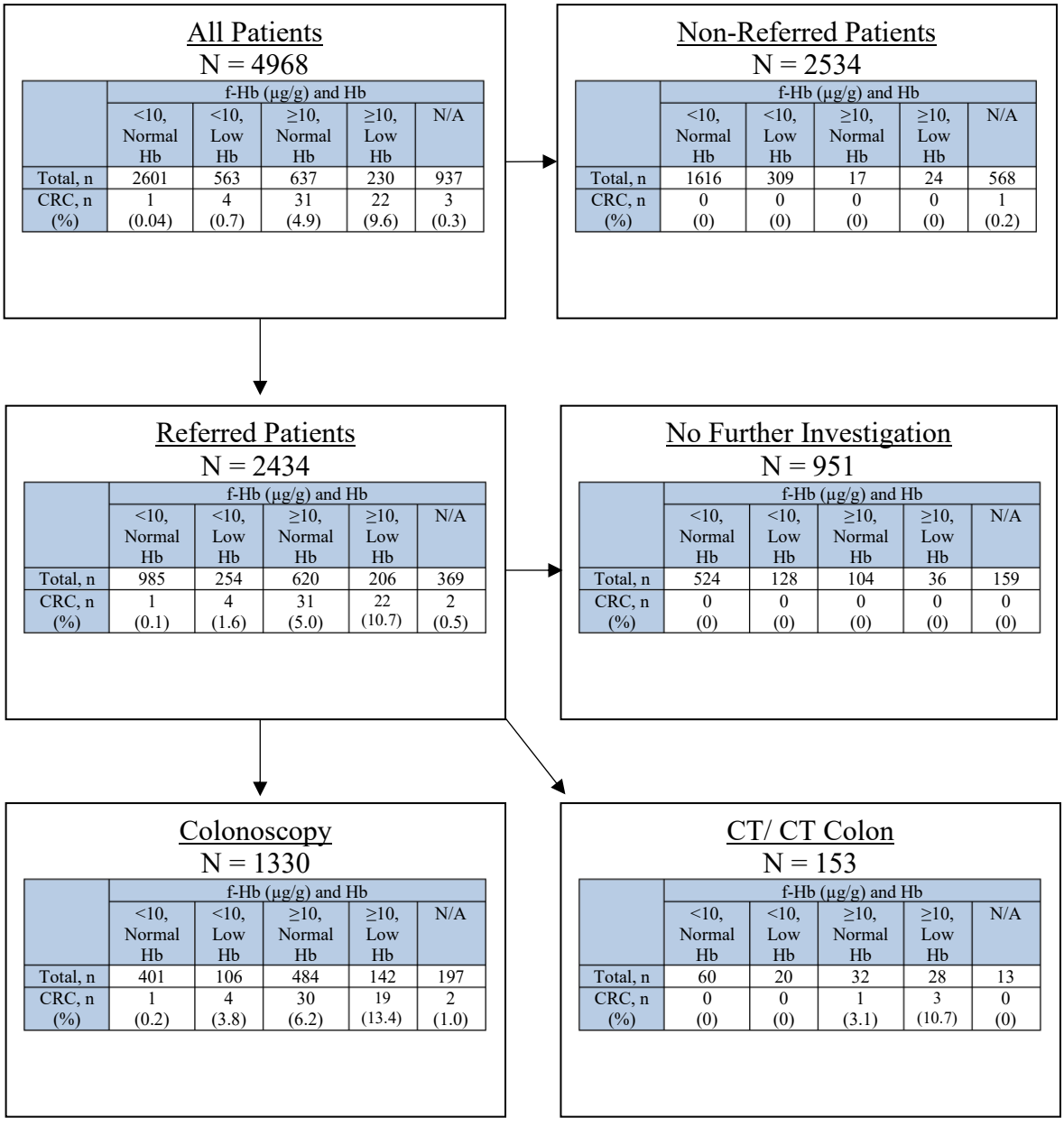
		Univariate			Multivariate		
		OR	95% C.I.	P	OR	95% C.I.	P
Age	<50	1.0			1.0		
	50-74	2.367	1.037-5.402	0.041	2.749	1.150-6.572	0.023
	≥75	5.931	2.545-13.825	<0.001	4.140	1.610-10.641	0.003
Sex	Female	1.0			1.0		
	Male	1.729	1.04-2.874	0.035	1.817	1.027-3.216	0.04
Rectal Bleeding	No	1.0			1.0		
	Yes	1.896	1.135-3.167	0.015	1.004	0.535-1.883	0.990
f-Hb (µg/g)	<10	1.0			1.0		
	10-149	13.267	4.594-38.313	<0.001	4.623	1.587-13.465	0.005
	150-399	57.341	18.448-178.229	<0.001	19.690	6.207-62.459	<0.001
	≥400	136.836	52.911-353.882	<0.001	54.256	20.683-142.325	<0.001
Anaemia	No	1.0			1.0		
	Yes	3.467	2.084-5.770	<0.001	1.956	1.071-3.574	0.029

2.3.10 Combination of f-Hb and anaemia to rule out CRC

There was a significant association between a raised f-Hb and anaemia: 563 of 3,164 (17.8%) patients with f-Hb <10 µg/g were anaemic as compared to 136 of 561 (24.2%) of those with f-Hb 10-149 µg/g, 32 of 104 (30.8%) f-Hb 150-399 µg/g and 62 of 202 (30.7%) f-Hb ≥400 µg/g ($p<0.001$). However, despite this relationship, f-Hb and anaemia were both found to be independent predictors of CRC and were therefore next combined. 4,031 of 4,968 (81.1%) patients in the study had both a valid FIT and circulating Hb. Combining FIT and Hb, 2,601 patients had a f-Hb <10 µg/g and were not anaemic, 563 f-Hb <10 µg/g but were anaemic, 637 f-Hb ≥10 µg/g but were not anaemic and 230 f-Hb ≥10 µg/g and anaemic. Figure 2.2 shows the investigation and referral pathway of all patients in the study using this combined FIT and anaemia measure and Table 2.5 shows a comparison between these four groups. 4 patients (0.7%) with f-Hb <10 µg/g but anaemic, 31 patients (4.9%) with f-Hb ≥10 µg/g but not anaemic and 22 patients (9.6%) with f-Hb ≥10 µg/g and anaemic were diagnosed with CRC. Only 1 patient (0.04%) with f-Hb <10 µg/g and not anaemic was diagnosed with CRC. Combining FIT at a f-Hb threshold of 10 µg/g with the presence or absence of anaemia resulted in a sensitivity for CRC of 98.28%, specificity

65.44%, NPV 99.96%, PPV 3.99% and NNS of 26. Finally, Table 2.6 shows a similar comparison between groups formed by combining FIT with IDA (ferritin<15).

2.3.11 Figure 2.2: Referral and investigation pathway, f-Hb and anaemia and CRC cases.



2.3.12 Table 2.5: Comparison by combined FIT and anaemia for all patients with both a valid FIT and full blood count.

		f-Hb <10 µg/g Not Anaemic	f-Hb <10 µg/g Anaemic	f-Hb ≥10 µg/g Not Anaemic	f-Hb ≥10 µg/g Anaemic	P
N		2601	563	637	230	
Age	Median (range)	57 (16-93)	69 (23-94)	60 (17-97)	75 (19-97)	<0.001
	<50	847 (32.6%)	78 (13.9%)	176 (27.6%)	30 (13.0%)	
	50-74	1454 (55.9%)	297 (52.8%)	343 (53.8%)	85 (37.0%)	
	≥75	300 (11.5%)	188 (33.4%)	118 (18.5%)	115 (50.0%)	
Sex	Male	1072 (41.2%)	225 (40.0%)	291 (45.7%)	99 (43.0%)	0.155
	Female	1529 (58.8%)	338 (60.0%)	346 (54.3%)	131 (57.0%)	
CRC		1 (0.04%)	4 (0.7%)	31 (4.9%)	22 (9.6%)	<0.001

2.3.13 Table 2.6: Comparison by combined FIT and iron deficiency anaemia with a valid FIT, full blood count and ferritin.

		f-HB <10 µg/g No IDA	f-Hb <10 µg/g IDA	f-Hb ≥10 µg/g No IDA	f-Hb ≥10 µg/g IDA	P
N		2987	152	793	64	
Age	Median (range)	59 (16-94)	56 (23-91)	63 (17-97)	64 (19-95)	<0.001
	<50	878 (29.5%)	44 (29.3%)	189 (23.9%)	16 (25.0%)	
	50-74	1648 (55.4%)	82 (54.7%)	395 (50.0%)	27 (42.2%)	
	≥75	450 (15.1%)	24 (16.0%)	206 (26.1%)	21 (32.8%)	
Sex	Male	1247 (41.9%)	38 (25.3%)	365 (46.2%)	21 (32.8%)	<0.001
	Female	1729 (58.1%)	112 (74.7%)	425 (53.8%)	43 (67.2%)	
CRC		3 (0.1%)	2 (1.3%)	40 (5.0%)	13 (20.3%)	<0.001

2.4 Discussion

This study provides a comprehensive description of the use of FIT in symptomatic patients during its initial period of use in NHS GG&C. This is one of few studies to include all patients with a FIT submitted from primary care regardless of onwards referral or decision to perform colonoscopy, reflecting real life practice. By using cancer registry data with long follow-up, it has been possible to capture all CRC cases rather than only those diagnosed following referral and colonoscopy. The results suggest that FIT is actively influencing GPs in their decision of whether to refer to colorectal and gastroenterology services and is influencing hospital doctors in their decision to perform colonoscopy. Additionally, these results add to the evidence that whilst symptoms should act as a trigger for assessment with FIT, they are poor predictors of the presence of CRC. In keeping with prior studies only the presence of rectal bleeding significantly correlates with malignancy(97). However, rectal bleeding did not remain an independent predictor of CRC on multivariate analysis. In contrast, f-Hb and the presence of anaemia were both independent predictors of CRC. Combining FIT at a f-Hb threshold of 10 μg/g with the absence of anaemia was able to effectively exclude CRC in 99.96% of cases, which should provide excellent reassurance to GPs and specialist practitioners. Patients with a f-Hb <10 μg/g and without anaemia represented 64.5% of patients. With appropriate safety netting in place these patients can be reassured.

There are a wide variety of sensitivities and specificities reported in the literature for CRC detection in symptomatic patients (85% to 100% and 56% to 91% respectively at ≥10 μg/g threshold) (12-14, 16-21). Several systematic review and meta-analyses have tried to amalgamate the available data(13, 14, 17, 18). In the most recent and largest by Pin Vieito et al(14) involving 15 studies and 48,872 patients undergoing FIT testing at the f-Hb ≥10 μg/g threshold, a pooled sensitivity of 87.2% (95% CI: 81.0-91.6%) and specificity of 84.4% (95% CI: 79.4-88.3%) for CRC detection was reported. Other studies have

compared the use of FIT in patients with high or low risk symptoms as per the NICE NG12 criteria (91) and NICE DG30 criteria (95) respectively. The NICE FIT study(93) reported on 9,822 patients referred to 50 English hospitals as urgent suspected CRC, who subsequently underwent colonoscopy. 7194 (73.2%) patients had NG12 high risk symptoms, 1,994 (20.3%) patients had DG30 low risk symptoms and 634 (6.5%) had other symptoms warranting urgent referral. At a f-Hb threshold of 10 μg/g, sensitivity and specificity for CRC for those with high-risk symptoms were 92.2% and 82.3% respectively. For those with low-risk symptoms sensitivity was 86.8% and specificity 88.4%. Higher sensitivities and lower specificities were reported for both groups using the HM-JACKarc limit of detection threshold (2 μg/g). The authors recommended use of this threshold to reduce missed CRC cases. Others have also suggested using the limit of quantification (7 μg/g)(20). Such strategies are associated with fewer false negative results but with a concomitant need for more invasive investigations. Furthermore, there have been concerns that the imprecision of f-Hb at such concentrations with current techniques may lead to spurious results(180).

Whilst FIT alone has been shown to be a very accurate predictor of CRC in symptomatic patients, there is a small rate of false negative results which could lead to missed CRC diagnoses. Prior studies have attempted to combine f-Hb with other risk factors including age and sex to improve the diagnostic utility of FIT, with mixed results(181). In the study by McSorley et al(11) which included 4,841 symptomatic patients from three Scottish health boards who underwent colonoscopy and had a FIT submitted from primary care, 14 (0.6%) patients with a normal f-Hb (<10μg/g) were diagnosed with CRC. 9 of these 14 patients (64.3%) were anaemic at the time of referral and it was suggested that anaemia may be helpful in reducing the false negative rate of FIT for CRC detection in symptomatic patients. In the present study, combining FIT with a f-Hb threshold of 10 μg/g and the presence or absence of anaemia increased sensitivity for CRC from 91.80% to

98.28% and NPV from 99.87% to 99.96%. The corresponding specificity and PPV decreased from 80.42% to 65.44% and 5.47% to 3.99% respectively, while the NNS to diagnose one CRC increased from 18 to 26. While a NNS acceptable to clinicians and patients has not been absolutely defined, NICE stipulate that tests resulting in a PPV for cancer ≥3% warrant urgent suspected cancer referral(91), which corresponds with a NNS of 33.

Other studies have considered combining FIT and anaemia including that by Chapman et al (182). Of 1,106 patients referred on an urgent 2-week suspected cancer pathway with accompanying FIT, a f-Hb threshold of >4 μg/g, gave a sensitivity and specificity for CRC of 97.5% and 64.5% respectively. By combining f-Hb >4 μg/g and/or the presence of anaemia, sensitivity rose to 100% and specificity dropped to 45.3%. However, patients with rectal bleeding and those referred out with a 2-week wait pathway were excluded. Bailey et al(183) reported on 13,361 FIT studies submitted from primary care as part of their suspected colorectal cancer referral pathway. Patients with f-Hb ≥10 μg/g met the threshold for urgent 2 week wait investigation. Of note, those with a f-Hb greater than 4 μg/g but less than 10 μg/g in the presence of anaemia, low ferritin or thrombocytosis were also eligible for urgent investigation. 10 patients (CRC rate 0.6%) with a f-Hb 4-9.9 μg/g were ultimately diagnosed with CRC. 5 of these 10 patients were anaemic and 6 had a low ferritin with 0 patients therefore not meeting urgent investigation criteria.

Anaemia in isolation, and in particular IDA, are well recognised to be associated with CRC and would usually prompt urgent referral(184). The overall rate of IDA in this cohort was relatively low at 5.4% for several reasons. Firstly, we have only included symptomatic patients, so no cases of asymptomatic IDA are represented. Secondly, we have chosen to present IDA as an objective parameter based on blood results, rather than as a reason for referral. It is very common to find that patients referred with “IDA” in fact have

normocytic anaemia and a normal ferritin. Finally, we have used a strict definition for IDA at ferritin <15 µg/L. There is wide variability in how iron deficiency is defined. NICE recommend a ferritin of <30 µg/L to confirm the diagnosis of IDA but do concede that the interpretation of a ferritin can be difficult as it may be raised in the presence of inflammation(185). Hamilton et al(186) who refined the risk of CRC associated with anaemia, used a ferritin <20 µg/L. The British Society of Gastroenterology guidelines state that a "serum ferritin <15 µg/L is highly specific for iron deficiency (specificity 0.99)" and we have followed this threshold. The existing evidence and the results of the present study suggest that circulating Hb, without ferritin or other measures of iron status could provide additional sensitivity to f-Hb for the detection of CRC in symptomatic patients. Of note, most patients with CRC who were anaemic had normocytic anaemia which has previously been established(187). Additionally, when FIT at a f-Hb threshold of 10 µg/g was combined with IDA (ferritin<15) in a similar manner to the combined FIT and anaemia measure, a less significant improvement in sensitivity was achieved (94.83%) (Table 2.6). Therefore, it seems that combining f-Hb with all anaemia is a simpler and superior measure.

This study has a number of strengths. It presents real-life practice in GG&C health board following introduction of FIT as a tool to guide referral to colorectal and gastroenterology services. The study reports not only on patients with FIT samples submitted as part of a referral but also on non-referred patients and has used this to establish that FIT is actively influencing referral and investigation decisions. While other studies have included FIT from referred and non-referred patients(16, 20, 183), a particular strength of the current study is the longer median follow up of 23 months, with linkage to cancer registry data to minimise the likelihood of missed cases. Additionally, the inclusion of patients with high and low risk symptoms and with and without rectal bleeding, reflects the most up to date evidence and real-life use of FIT. In the current study FIT has been effectively combined

with anaemia to form a highly effective way of excluding CRC. There are however limitations. The retrospective nature of the study meant that patient symptoms and co-morbidities were only available if the patient was referred to the colorectal or gastroenterology service as this information was obtained from referral letters. Although cancer registry linkage is robust it is possible that cases of CRC in those not further investigated were missed. As no statistical correction was made to the χ^2 analysis to account for the multiple comparisons made, an increased risk of type I errors may be anticipated. However, by assessing significant variables with multivariate binary logistic regression analysis, the impact of potential false positives is negated. Finally, the nature of the study meant that other significant bowel disease including advanced adenoma and inflammatory bowel disease were not included.

In conclusion, in NHS GG&C, GP referral pattern and secondary care investigation patterns were influenced by FIT. The addition of a normal circulating haemoglobin concentration to a f-Hb <10 µg/g was able to effectively exclude CRC in 99.96% of cases, providing excellent reassurance to GPs and to specialist practitioners who must prioritise access to endoscopy services, particularly in the context of the COVID pandemic recovery period. Patients with a f-Hb <10 µg/g and without anaemia represented 64.5% of patients. With appropriate safety netting in place these patients can be reassured.

3 Prevalence of repeat FIT testing in symptomatic patients attending primary care.

3.1 Introduction

The faecal immunochemical test (FIT) is an accurate method for predicting colorectal cancer (CRC) risk in symptomatic patients, prior to consideration of definitive investigation with colonoscopy, cross-sectional imaging or colon capsule endoscopy (CCE)(11-14, 16-21). Additionally, the sensitivity of this risk prediction can be enhanced by combining FIT with the presence or absence of anaemia(188). The National Institute for Health and Care Excellence (NICE) recommend FIT be used to guide referral for suspected CRC in patients with lower risk lower gastrointestinal (GI) symptoms(95) and more recent guidance from the Association of Coloproctology of Great Britain and Ireland (ACPGBI) and the British Society of Gastroenterology (BSG) has recommended the use of FIT in all patients with lower gastrointestinal (GI) symptoms(98). Consequently, FIT has now been integrated into referral pathways in twelve NHS health boards across Scotland as an adjunct to clinical acumen and full blood count(15, 25, 188). In chapter 2 it was shown that those with a faecal haemoglobin (f-Hb) <10 µg/g and without anaemia represented 64.5% of symptomatic patients and this combination could effectively exclude CRC in 99.96% of cases. It seems appropriate to recommend reassurance for these patients with safety netting in place where those with persistent symptoms be considered for referral. To date there is no specific guidance or evidence to support repeated FIT testing at a later date as a form of safety netting. Despite this it has been noted that laboratory databases contain a number of patients who have accrued multiple f-Hb results over time. This study therefore aimed to examine the prevalence of repeat FIT testing in primary care and the relationship between serial f-Hb concentrations and CRC risk in symptomatic patients.

3.2 Methods

3.2.1 Study Design, Setting and Participants

A multicentre, retrospective, observational study was conducted of symptomatic patients within three Scottish health boards: NHS Greater Glasgow and Clyde (GG&C) (1st f-Hb measurement collected between September 2018 and December 2020), NHS Tayside (December 2015 to December 2020) and NHS Highland (December 2018 to October 2021). Each health board utilises FIT in primary care for symptomatic lower GI referrals. Although no evidence exists on repeated or serial FIT testing, GPs have open access to FIT and use of FIT in this manner is at their discretion.

3.2.2 FIT Specimen Collection and Handling

FIT collection kits were supplied to GPs. Each contains a single FIT collection device (EXTEL HEMO-AUTO MC Collection Picker, Minaris Medical Co., Ltd, Tokyo, Japan, supplied by Alpha Labs Ltd, Eastleigh, Hants, UK), pictorial instructions and a return envelope. The collection device is a picker which obtains a consistent 2 mg sample and is inserted into a vial containing 2 ml of buffer. Patients being considered for symptomatic lower GI referral were asked to collect a single faecal sample and return to their GP practice as soon as possible. The samples were transported at ambient temperature via routine specimen collection services and stored at 4°C prior to analysis in centralised laboratories (Stobhill Hospital, Glasgow for NHS GG&C, Ninewells Hospital, Dundee for Tayside and Highlands).

3.2.3 FIT Analysis

The HM-JACKarc system (Minaris Medical Co., Ltd) was operated Monday to Friday so most samples were analysed on day of receipt. The manufacturers give a limit of detection of 2 μg/g, a limit of quantification of 7 μg/g and an upper measurement limit of 400 μg/g.

Specimens with f-Hb concentrations above this limit were not diluted and re-analysed but simply reported as >400 µg/g.

3.2.4 FIT Result Quality Management

All biomedical science staff in each laboratory are Health Care and Professionals Council (HCPC) registered and undergo local competency assessment prior to using the HM-JACKarc analyser. There are two internal quality controls (IQCs): EXTEL HEMO AUTO HS Low IQC and EXTEL HEMO AUTO HS High IQC. West guard rule criteria are used for acceptance or rejection of analytical runs. The laboratories participate in appropriate external quality assessment.

3.2.5 FIT Result Handling

FIT results are electronically transferred from the analyser into the Laboratory Information Management System (LIMS) and patient record as well as electronically reported to the requesting GP. FIT results ≥10 µg/g were defined as raised as per the NICE DG30 guidance(95) and GPs are asked to use the f-Hb measurement to guide the need for referral to specialist services.

3.2.6 Patient Identification and Data Collection

To identify study participants a search of the clinical biochemistry repository in each health board was conducted. Patients with two or more consecutive f-Hb measurements with an interval between samples of 1 week to 1 year were included. Patients were excluded if they were <16 years old, they had less than two valid f-Hb measurements, if they attended colonoscopy in between their two f-Hb dates or if they had a previous diagnosis of CRC. To obtain patient demographics and outcomes cross-referencing of the electronic patient record including referral letters, endoscopy, pathology and radiology reports was performed with the Community Health Index (CHI) number used as the

linkage variable. Demographics and bloods results were recorded at the date of the first f-Hb or as close as possible. To ensure no CRC diagnoses were missed, The Scottish Cancer Registry as well as regional cancer audit datasets were searched to identify all new diagnoses of CRC up to August 2021. This allowed identification of CRC cases diagnosed out with the referral pathways under examination by this study. Caldicott guardian approval was given by each health board to safeguard the record linkage with ethical approval waived for the purposes of service development. As the study was retrospective and observational and had no impact on patient care, consent was not obtained from each patient.

3.2.7 Data Analysis

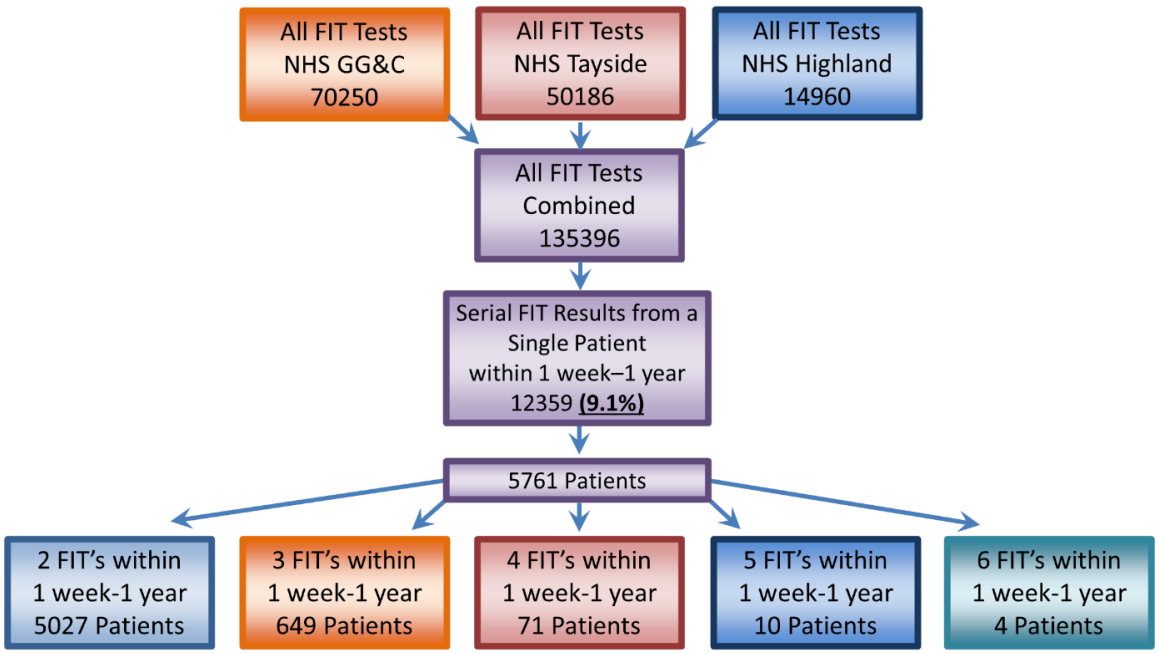
An elevated f-Hb was defined as ≥10 µg/g. Serial FIT measurements were categorised into four groups: two consecutive f-Hb results <10 µg/g, a f-Hb ≥10 µg/g followed by a f-Hb <10 µg/g, a f-Hb <10 µg/g followed by a f-Hb ≥10 µg/g and two consecutive f-Hb results ≥10 µg/g. The definition of anaemia on full blood count was based on WHO guidelines (male Hb <130 g/L, female Hb <120 g/L) (178). Patients diagnosed with CRC who had an initial f-Hb <10 µg/g were examined separately. Data analysis in GGC, Tayside and Highland were performed using SPSS software (SPSS Inc., Chicago, Illinois, USA). Amalgamation of the data was performed in Microsoft Excel (Microsoft, Microsoft Campus, Reading, UK). Categorical data was compared using crosstabulation and the χ^2 or Fisher's exact test. Continuous data was compared using analysis of variance (ANOVA). A value of $p<0.05$ was considered statistically significant.

3.3 Results

3.3.1 Cohort Description

Figure 3.1 shows the formation of the study cohort. Serial FIT results represented 9.1% of all valid FIT results. 5,761 patients had two or more consecutive f-Hb measurements and were included in the final analysis. The proportion of male to female patients was similar between all three Health Boards (p=0.289), while patients in NHS GG&C were significantly younger (p<0.001) and follow-up was significantly shorter in NHS Highland (p<0.001) (Table 3.1). 42 (0.7%) patients were found to have CRC.

3.3.2 Figure 3.1: Flowchart of cohort formation.



3.3.3 Table 3.1: Demographics, median interval between FIT tests and CRC incidence in symptomatic patients with more than one f-Hb result in a 12-month period.

		NHS Board			P
		NHS GG&C	NHS Tayside	NHS Highland	
Total		3018	1789	954	-
Sex	Male	1212 (40.2%)	781 (43.7%)	390 (40.9%)	
	Female	1806 (59.8%)	1008 (56.3%)	564 (59.1%)	0.289
Age (years)	Median (IQR)	63 (52-74)	69 (56-78)	69 (57-77)	<0.001
Interval between 1st and 2nd f-Hb (months)	Median (IQR)	5 (2-8)	6 (2-9)	5 (1-8)	<0.001
Follow up (months)	Median (IQR)	18 (12-23)	24 (36-43)	9 (5-15)	<0.001
CRC Cases		15 (0.5%)	19 (1.1%)	8 (0.8%)	0.162

3.3.4 Serial FIT

Comparing the first two consecutive valid FIT tests for all patients: 3,487 (60.5%) had two f-Hb results <10 µg/g of whom 3 patients (0.1%) were subsequently diagnosed with CRC (Table 3.2). By comparison, 626 (10.9%) had two f-Hb results ≥10 µg/g of whom 25 patients (4.0%) were subsequently diagnosed with CRC. Those patients with a f-Hb result ≥10 µg/g followed by a f-Hb result <10 µg/g and a f-Hb result <10 µg/g followed by a f-Hb result ≥10 µg/g also had an increased risk of CRC (0.4% and 1.4% respectively) (p<0.001).

3.3.5 Table 3.2: Comparison between serial FIT and CRC risk.

	Serial FIT					Total	p
	1st f-Hb	<10µg/g	≥10µg/g	<10µg/g	≥10µg/g		
	2nd f-Hb	<10 µg/g	<10µg/g	≥10µg/g	≥10µg/g		
All Patients		3487	944	704	626	5761	
CRC		3 (0.1%)	4 (0.4%)	10 (1.4%)	25 (4.0%)	42 (0.7%)	<0.001

3.3.6 Number of FIT Samples

Table 3.3 shows a comparison between number of f-Hb results within 12 months and CRC rate. The likelihood of at least one f-Hb ≥10µg/g rose from 40.4% with two samples to 100% with six samples, while the CRC rate fell from 0.8% with two samples to 0% with four or more samples.

3.3.7 Table 3.3: Comparison between number of FIT samples within 12 months and CRC rate.

	Number of FIT Tests within 12 Months					Total	p
	Two	Three	Four	Five	Six		
N	5027	649	71	10	4	5761	-
At least one f-Hb ≥10µg/g	2032 (40.4%)	290 (44.7%)	31 (43.7%)	6 (60.0%)	4 (100%)	2363 (41.0%)	0.121
CRC	40 (0.8%)	2 (0.3%)	0 (0%)	0 (0%)	0 (0%)	42 (0.7%)	0.362

3.3.8 CRC with first f-Hb <10 µg/g

The demographics and pathology of the 13 patients diagnosed with CRC whose first f-Hb <10 µg/g were reviewed (Table 3.4). 8 of 13 (61.5%) patients had tumours proximal to the splenic flexure. 10 of 13 (76.9%) had an anaemia on full blood count at the time of the 1st f-Hb.

3.3.9 Table 3.4: Demographics and pathology of 13 patients diagnosed with CRC whose first f-Hb <10 µg/g.

Serial FIT (µg/g)		Age (years)	Sex	Symptoms	Hb (mg/L)	CRC size (mm)	Primary CRC site	TNM stage
1st f-Hb	2nd f-Hb							
<10	≥10	79	F	Weight loss, anaemia	107	100	Distal transverse	cT4b cN2 cM0
<10	≥10	85	M	Weight loss, anaemia	121	No size	Sigmoid	cT4b cN1 cM0
<10	≥10	51	F	PR bleeding and abdominal pain	147	50	Sigmoid	pT3 pN2a
<10	≥10	82	F	Weight loss, PR bleeding, abdominal pain, anaemia	117	33	Caecum	pT3 pN0
<10	≥10	64	F	Abdominal pain, anaemia	76	50	Ascending colon	pT4b pN1a
<10	≥10	56	F	Weight loss, anaemia	80	No size	Caecum	cTx cN2 cM1.
<10	<10	70	F	Abdominal pain	126	No size	Appendix	cTx cN2 cM1.
<10	<10	67	F	Anaemia	116	40	Transverse	ypT4b ypN0
<10	<10	63	M	Abdominal pain, weight loss, anaemia	115	13	Caecum	cTx cN2 cM1.
<10	≥10	87	M	Anaemia	101	22	Sigmoid	pT1 pN1
<10	≥10	75	M	Anaemia	126	17	Rectum	pT1 pN0
<10	≥10	78	M	Anaemia	95	52	Ascending colon	pT3 pN0
<10	≥10	87	F	Altered bowel habit	129	2	Distal Sigmoid	pT1

3.4 Discussion

To date, evidence-based practice guidance has focused on the utility of a single FIT test when patients present to primary care with new lower GI symptoms. Reassessment of those with a normal f-Hb <10 μg/g but with persistent symptoms is recommended as a safety-netting mechanism. However, no specific advice is given on further FIT testing in the absence of published data. This is the first study to report the prevalence of serial FIT testing in a symptomatic population and has filled an important gap in the current literature by examining the incidence rate of CRC by serial f-Hb results. In the current study, patients with two consecutive f-Hb's <10 μg/g had a very low CRC risk of 0.1%. In addition, only 0.4% of patients with a f-Hb ≥10 μg/g followed by f-Hb <10 μg/g were found to have CRC, although the reason for the second FIT rather than colonoscopy, cross-sectional imaging or CCE following the first FIT result was not known. Perhaps such patients were deemed very low risk for CRC and the FIT was repeated to ensure it was not persistently elevated. These results should provide reassurance to GPs and secondary care practitioners who triage patients for referral and investigation. In these patients, further investigation should be determined by the reason for referral and with the aim of symptom improvement, rather than to exclude CRC. In contrast, two consecutive f-Hb's ≥10 μg/g or a f-Hb <10 μg/g followed by f-Hb ≥10 μg/g were associated with a significantly higher risk of CRC (4.0% and 1.4% respectively) and these patients should be prioritised for referral and urgent colonoscopy/ imaging/ CCE.

Additionally, it has been shown that as the number of FIT tests performed over a 12-month period increases, the likelihood of having at least one f-Hb ≥10 μg/g increases, and conversely the CRC rate fell. Combined with the findings above, this would suggest firstly that patients with a single raised f-Hb ≥10 μg/g should be referred and definitively investigated. Secondly, while repeating FIT testing once within a 12-month period for patients with persistent or recurrent symptoms provides an additional layer of safety

netting, more frequent repeated f-Hb measurements is unhelpful and could lead to unnecessary invasive investigation. A single f-Hb costs the NHS less than £10. If a serial FIT strategy of performing a second FIT within 12 months for those patients with persistent symptoms whose initial f-Hb result was <10 μg/g were to be adopted, the potential cost saving in terms of avoiding unnecessary referral and further, far more expensive diagnostic tests, could be significant.

Interestingly, the overall CRC rate in this cohort of patients (42 of 5,761, 0.7%) was lower than that observed in previous studies with similar cohorts of patients with single FIT measurement (1.1-1.8%)(11, 15, 20, 188, 189). It may be that patients with persistent unexplained and functional lower GI symptoms are more likely to re-present and undergo serial FIT testing, and that the cohort presented within this study is likely to be different to those described in previous studies of the use of FIT within symptomatic referral pathways.

The demographics and pathology of the 13 patients diagnosed with CRC whose first f-Hb <10 μg/g was presented above. It was interesting to note that 8 of these patients had tumours proximal to the splenic flexure. FIT has previously been shown to be less sensitive for the detection of such tumours(11). 10 of these 13 patients were anaemic at the time of 1st FIT. In chapter 2 it was confirmed that combining a single f-Hb ≥10 μg/g with presence of anaemia, two objective indicators of CRC risk, was able to reduce the false negative rate for CRC from 5.2% to 1.7%(188). The false negative rate of FIT for CRC is generally reported as 5-10%(11, 20, 190). In the current study, combining serial FIT with anaemia reduced the false negative rate for CRC from 7.1% to 2.4%.

While no studies have examined the utility of serial f-Hb measurements over time for CRC detection, a small number of studies have investigated whether multiple FIT samples taken at the same time may improve diagnostic accuracy. Auge et al(191) measured f-Hb levels from two consecutive bowel motions in 208 symptomatic patients undergoing

colonoscopy. They examined diagnostic yield for advanced colorectal neoplasia (ACRN) using the first of two f-Hb levels ("FIT/1") as compared to the maximum f-Hb level measured over two samples ("FIT/max"). With a cut off of 10 µg/g, FIT/1 sensitivity and specificity for ACRN was 34.5% and 87.2% respectively. Similar results could be obtained for FIT/max using a higher f-Hb cut off of 20 µg/g (sensitivity 34.5% and specificity 85.6%). In a similar study by Matter et al(192), 280 patients were randomised to a single FIT or two FIT samples in consecutive days prior to undergoing planned colonoscopy. A f-Hb threshold of ≥10 µg Hb/g faeces was used, and patients randomised to two FIT samples who recorded one positive sample and one negative sample were defined as positive. One FIT sample had a sensitivity of 83.3% (95% CI: 36.5%-99.1%) and specificity of 86.9% (95% CI: 77.3-92.9%) for CRC detection compared to sensitivity of 75% (95% CI: 35.6-95.5%) and specificity of 92.9% (95% CI: 82.2-97.7%) for two FIT samples. There was no significant benefit of two FIT over one FIT sampling.

In the studies by Miller et al(193) and Maeda et al(194), they discuss their Covid-19-adapted CRC referral pathway, which utilised two FITs in quick succession combined with a CT with oral contrast. A high f-Hb threshold of 80 µg/g was used. 422 patients were included. The overall CRC detection rate of 3.1% during utilisation of the pathway was similar to that in the period prior to the pandemic (3.3%)(193). Subsequent analysis revealed that if double FIT testing was used alone at a 10 µg/g threshold, the risk of missing a CRC would be 15.5%(194). All of the studies discussed here have examined double FIT measurement within a short time period rather than serial FIT as was examined in the current study.

This study has a number of strengths. It is the first study to report the prevalence of serial FIT tests in the symptomatic population and examine the incidence rate of CRC by serial f-Hb result. The study was multicentred and reflects real-life practice in health boards across Scotland following introduction of FIT as a tool to guide referral to colorectal and

gastroenterology services. Patients with both high and low risk symptoms and with and without rectal bleeding were included, reflecting the most up to date evidence(98) and clinical use of FIT(15, 22, 188), rather than the current NICE guidance on FIT use in symptomatic patients(95). Potential sources of bias have been carefully considered. As study participants were identified by interrogating each health board's clinical biochemistry repository, with results automatically uploaded to electronic patient records, a very low number of missed patients would be anticipated. Use of cancer registry data has ensured a low rate of missed CRCs. The current study does however have limitations. It is retrospective and observational in nature and hence there is wide variability in the interval between FIT samples. Sample size and event rate was such that a formal analysis of optimum interval between FIT samples was not possible. Nor was it possible to analyse whether the magnitude of change in the f-Hb was informative, instead being scrutinised as a binary raised (f-Hb ≥10 μg/g) versus normal (f-Hb <10 μg/g) value. Only patients with serial FIT testing were included in this study. Patients with persistent symptoms but without repeat FIT measurement were not captured by this study and the results cannot necessarily be extrapolated to this group. However, based on the findings, the recommendation that GPs should consider repeat FIT testing in all patients with persistent symptoms would be maintained. Access to primary care records were not available to determine the reasons why patients were subjected to repeat FIT tests but it has been assumed this was for persistent or recurrent symptoms. It is possible that a small proportion of patients had FIT testing performed in the absence of symptoms, against NICE and local recommendations, for example in patients found incidentally to be anaemic or in patients with a strong family history of CRC. It was not possible to determine why a proportion of patients with a first f-Hb ≥10 μg/g had a second FIT rather than colonoscopy/ imaging/ CCE, nor why the patients with anaemia detected at the time of a first f-Hb <10 μg/g were not referred for investigation at that time. Finally, the

diagnostic accuracy of serial FIT for other significant bowel disease including advanced polyps and inflammatory bowel disease was out with the scope of this study.

In conclusion, this is the first study to examine prevalence of serial FIT measurements in symptomatic patients and the associated rate of CRC. Serial f-Hb results account for almost one tenth of all test results and this cohort of patients had a lower prevalence of CRC overall compared to those cohorts described in previous studies(11, 20, 188, 195). Those patients with two consecutive f-Hb results <10 μg/g in a 12-month period have a very low CRC risk of 0.1%. In contrast, patients with at least one f-Hb result ≥10 μg/g had a higher CRC risk and should be prioritised for investigation. Performing two FIT tests within 12 months for patients with persistent symptoms adds an additional layer of safety netting, while performing three or more within the same time is unlikely to be beneficial. Further studies, with additional patient numbers should be conducted to validate our findings. Additionally, a formal cost-utility analysis of a serial FIT strategy, would help confirm the potential financial benefits.

4 Alternative diagnoses and demographics associated with a raised quantitative faecal immunochemical test in symptomatic patients.

4.1 Introduction

The faecal immunochemical test (FIT) accurately predicts colorectal cancer (CRC) risk in symptomatic patients and can be used to triage patients for referral and definitive investigation(11-14, 16-21, 188). In chapter 2 it was shown that the sensitivity of this risk prediction can be enhanced by combining faecal haemoglobin (f-Hb) with the presence or absence of anaemia from a circulating haemoglobin (Hb)(188), and in chapter 3 it was shown that repeated FIT testing once within a year is an effective safety netting measure for those with persistent or recurrent lower gastrointestinal (GI) symptoms(196). However, most patients with a raised f-Hb will not have CRC. In chapter 2, 1,200 of 4,968 symptomatic patients with a FIT submitted from primary care had a f-Hb ≥10 μg Hb/ g faeces and only 56 (4.7%) were diagnosed with CRC(188). It is therefore important to identify alternative pathologies associated with a raised f-Hb and factors associated with false positivity. A raised f-Hb in symptomatic patients has been correlated with advanced adenomas and inflammatory bowel disease(197-200). Indeed, there is evidence that f-Hb can be used as a marker of disease activity in ulcerative colitis(201-205) and colonic Crohn's(206) as an adjunct to faecal calprotectin. Additionally, higher FIT positivity in the context of bowel cancer screening has been independently associated with older age, male sex, deprivation, aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), oral anticoagulants, proton pump inhibitors (PPIs), antibiotics and smoking(207-210) and false positivity has been related to younger age, female sex, smoking, high BMI, successive screening, aspirin, NSAIDs, PPIs, antibiotics, laxatives, non-advanced adenomas, diverticular disease, haemorrhoids, anal fissures and peptic ulceration(207, 210-216).

To date, no studies have examined demographics which independently predict a raised f-Hb in symptomatic patients and very few have explored non-cancer diagnoses which correlate with f-Hb. This study aimed to establish demographics and alternative pathologies associated with a raised f-Hb in a cohort of symptomatic patients.

4.2 Methods

4.2.1 Study Design, Setting and Participants

A multicentre, retrospective, observational study was conducted to include all patients ≥16 years old with a FIT submitted from primary care between August 2018 and January 2019 in NHS Greater Glasgow and Clyde (NHS GG&C). The formation of this cohort, FIT specimen collection, handling and analysis, result handling and quality management and identification of demographics, blood results, symptoms and comorbidities, were previously described in chapter 2(188).

4.2.2 Identification of Alternative Pathology

Each colonoscopy report and any accompanying pathology records were screened manually to identify lower GI diagnoses and coded as CRC, advanced adenoma(s), any advanced polyp(s), non-advanced polyp(s), inflammatory bowel disease (IBD), other inflammation (infective colitis, collagenous colitis, lymphocytic colitis, inflammatory polyps), diverticulosis, haemorrhoids, angiodysplasia/ telangiectasia, radiation proctitis, other malignancy (anal squamous cell carcinoma and rectal lymphoma), melanosis coli, anal fissure or fistula, rectal prolapse, fibroepithelial anal polyp and lipoma. Of note, if multiple pathologies were identified at colonoscopy, the patient would be coded as having each pathology identified. Advanced adenomas were defined as those ≥10 mm or with the presence of high-grade dysplasia. Advanced polyps were defined as advanced adenomas or advanced serrated polyps ≥10 mm or with the presence of any grade of dysplasia as per The British Society of Gastroenterology/ Association of Coloproctology of Great Britain and Ireland surveillance guidelines(31).

4.2.3 Data Analysis and Statistical Methods

For the purposes of analysis, patients were divided into those with significant bowel disease (CRC, advanced adenoma, advanced polyp, ≥5 non-advanced polyps or IBD), other bowel disease (any other positive finding at colonoscopy) and no pathology (entirely normal colonoscopy). Covariables were compared using crosstabulation and the χ^2 test or Fisher's exact test. A value of $p< 0.05$ was considered statistically significant. To identify covariables which independently predicted a raised f-Hb, univariate followed by multivariate binary logistic regression was performed. Covariables of interest from the χ^2 analysis were carried into the regression analysis. Variables found to be significant on χ^2 analysis but where there were insufficient numbers for regression analysis were excluded. For the purposes of regression analysis FIT was converted to a binary variable: normal (f-Hb <10 μg/g) versus raised (f-Hb ≥10 μg/g). This allowed calculation of odds ratios (ORs) and 95% confidence intervals (95% CI). Covariables significant on univariate analysis ($p<0.05$) were entered into a multivariate model using the backwards conditional method in which variables with a significance of $p<0.1$ were removed in a stepwise fashion. The same process was then performed in turn only for those patients with significant bowel disease, other bowel disease and no pathology. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, USA).

4.3 Results

4.3.1 Cohort Description

4,968 patients had a FIT sample submitted from primary care between August 2018 and January 2019 in NHS GG&C. Of these, 2,434 patients were subsequently referred to general surgery or gastroenterology and 1327 of those underwent colonoscopy. Of those who underwent colonoscopy 572 (43.1%) had f-Hb <10 μg/g and 700 (52.8%) f-Hb ≥10 μg/g, with 430 (32.4%) between 10 and 149 μg/g, 89 (6.7%) between 150 and 399 μg/g and 181 (13.6%) ≥400 μg/g. 55 (4.1%) samples could not be processed by the laboratory due to faecal contamination, expired collection device or insufficient patient identification, and were not repeated. These patients were excluded from the final analysis leaving a total of 1,272 patients who underwent colonoscopy and had a valid FIT. Median age of these 1,272 patients was 60 years (range 17-94), with 558 (43.9%) male and 714 (56.1%) females. 561 (44.1%) patients reported rectal bleeding, 348 (27.4%) persistent diarrhoea, 602 (47.3%) other change in bowel habit, 214 (16.8%) weight loss, 383 (30.1%) abdominal pain, 33 (2.6%) anal pain, 77 (6.1%) faecal soiling, 25 (2.0%) rectal mass and 31 (2.4%) abdominal mass.

4.3.2 Comparison of Demographics by f-Hb Concentration

Table 4.1 shows a comparison of demographics by f-Hb concentration. Having a raised f-Hb was associated with either being below (<50 years) or above (≥75 years) the Scottish Bowel Screening Programme age (50-74 years) ($p<0.001$). There was no association between f-Hb and sex ($p=0.08$). Deprivation was associated with a higher f-Hb ($p=0.004$). No individual co-morbidity was associated with a raised f-Hb, however the presence of diabetes ($p=0.015$) or hypertension ($p=0.004$) seemed to be mildly protective. Patients on oral anticoagulants or PPIs were significantly more likely to have a raised f-Hb ($p=0.017$) or raised f-Hb between 10-399 μg/g ($p=0.007$) respectively. Patients self-reporting rectal

bleeding were more likely to have a raised f-Hb (p<0.001), while a history of persistent diarrhoea (p<0.001), other change in bowel habit (p=0.004) or faecal soiling (p<0.001) were associated with a lower f-Hb.

4.3.3 Table 4.1: Comparison of demographics by f-Hb concentration.

			f-Hb (µg Hb/g faeces)				P
		All	<10	10-149	150-399	≥400	
N		1272	572	430	89	181	
Age (years)	Median (range)	60 (17-94)	58 (17-88)	63 (19-92)	56 (19-94)	56 (17-90)	<0.001
	<50	318 (25.0%)	142 (24.8%)	82 (19.1%)	32 (36.0%)	62 (34.3%)	
	50-74	759 (59.7%)	370 (64.7%)	259 (60.2%)	44 (49.4%)	86 (47.5%)	
	≥75	195 (15.3%)	60 (10.5%)	89 (20.7%)	13 (14.6%)	33 (18.2%)	
Sex	Male	558 (43.9%)	235 (41.1%)	188 (43.7%)	48 (53.9%)	87 (48.1%)	0.080
	Female	714 (56.1%)	337 (58.9%)	242 (56.3%)	41 (46.1%)	94 (51.9%)	
Scottish Index of Multiple Deprivation	Non-deprived (SIMD 3-5)	627 (49.3%)	314 (54.9%)	193 (44.9%)	37 (41.6%)	83 (45.9%)	0.004
	Deprived (SIMD 1-2)	645 (50.7%)	258 (45.1%)	237 (55.1%)	52 (58.4%)	98 (54.1%)	
Co-morbidity	Asthma	163 (12.8%)	71 (12.4%)	59 (13.7%)	14 (15.7%)	19 (10.5%)	0.584
	COPD	76 (6.0%)	31 (5.4%)	38 (8.8%)	5 (5.6%)	2 (1.1%)	0.003
	Diabetes	157 (12.3%)	82 (14.3%)	57 (13.3%)	6 (6.7%)	12 (6.6%)	0.015
	Hypertension	109 (8.6%)	65 (11.4%)	33 (7.7%)	4 (4.5%)	7 (3.9%)	0.004
	IHD	150 (11.8%)	60 (10.5%)	61 (14.2%)	9 (10.1%)	20 (11.0%)	0.303
	Cerebrovascular Disease	42 (3.3%)	27 (4.7%)	9 (2.1%)	2 (2.2%)	4 (2.2%)	0.087
	PVD	9 (0.7%)	4 (0.7%)	2 (0.5%)	2 (2.2%)	1 (0.6%)	0.331
	IBD (prior diagnosis)	6 (0.5%)	3 (0.5%)	2 (0.5%)	0 (0.0%)	1 (0.6%)	0.923
	CRC (prior diagnosis)	8 (0.6%)	3 (0.5%)	5 (1.2%)	0 (0.0%)	0 (0.0%)	0.287
Medication	Aspirin	268 (21.1%)	116 (20.3%)	100 (23.3%)	18 (20.2%)	34 (18.8%)	0.561
	NSAIDs	166 (13.1%)	77 (13.5%)	49 (11.4%)	14 (15.7%)	26 (14.4%)	0.580
	Clopidogrel/ Ticagrelor	58 (4.6%)	26 (4.5%)	18 (4.2%)	5 (5.6%)	9 (5.0%)	0.932
	Oral Anticoagulants	69 (5.4%)	20 (3.5%)	26 (6.0%)	9 (10.1%)	14 (7.7%)	0.017
	ACE Inhibitors	259 (20.4%)	124 (21.7%)	86 (20.0%)	14 (15.7%)	35 (19.3%)	0.585
	Statins	364 (28.6%)	150 (26.2%)	134 (31.2%)	28 (31.5%)	52 (28.7%)	0.345
	PPI	606 (47.6%)	257 (44.9%)	228 (53.0%)	48 (53.9%)	73 (40.3%)	0.007
	H2 Antagonists	39 (3.1%)	16 (2.8%)	14 (3.3%)	5 (5.6%)	4 (2.2%)	0.460
	Metformin	76 (6.0%)	39 (6.8%)	26 (6.1%)	6 (6.7%)	5 (2.8%)	0.249
	Anti-spasmodics	315 (24.8%)	156 (27.3%)	98 (22.8%)	21 (23.6%)	40 (22.1%)	0.310
Symptoms	Rectal Bleeding	561 (44.1%)	221 (38.6%)	160 (37.2%)	51 (57.3%)	129 (71.3%)	<0.001
	Persistent Diarrhoea	348 (27.4%)	195 (34.1%)	88 (20.5%)	21 (23.6%)	44 (24.3%)	<0.001
	Other Change in Bowel Habit	602 (47.3%)	284 (49.7%)	213 (49.5%)	42 (47.2%)	63 (34.8%)	0.004
	Weight Loss	214 (16.8%)	108 (18.9%)	73 (17.0%)	11 (12.4%)	22 (12.2%)	0.120
	Abdominal Pain	383 (30.1%)	189 (33.0%)	123 (28.6%)	27 (30.3%)	44 (24.3%)	0.127
	Anal Pain	33 (2.6%)	14 (2.4%)	9 (2.1%)	2 (2.2%)	8 (4.4%)	0.406
	Faecal Soiling	77 (6.1%)	51 (8.9%)	17 (4.0%)	6 (6.7%)	3 (1.7%)	<0.001
	Rectal Mass	25 (2.0%)	17 (3.0%)	7 (1.6%)	0 (0.0%)	1 (0.6%)	0.074
	Abdominal Mass	31 (2.4%)	12 (2.1%)	12 (2.8%)	3 (3.4%)	4 (2.2%)	0.833
Anaemia	Anaemia*	246 (21.8%)	106 (20.9%)	74 (19.6%)	22 (26.8%)	44 (26.8%)	0.173
	Iron Deficiency Anaemia#	91 (8.1%)	46 (9.2%)	21 (5.6%)	4 (4.9%)	20 (12.3%)	0.032

*Missing data for 141 (11.1%) patients.
#Data missing for 153 (12.0%) patients.

4.3.4 CRC Cases

With a median 23-month (range 21-25) follow-up, 54 patients were diagnosed with CRC. 5 (9.3%) had a f-Hb <10 μg/g, 9 (16.7%) between 10 and 149 μg/g, 7 (13.0%) between 150 and 399 μg/g and 33 (61.1%) ≥400 μg/g.

4.3.5 Alternative Pathology Associated with a Raised f-Hb

Table 4.2 shows a comparison of colonoscopic/ pathology findings by f-Hb concentration. As well as being strongly associated with CRC ($p<0.001$), a raised f-Hb also correlated with the risk of advanced adenoma ($p<0.001$), any advanced polyp ($p<0.001$), non-advanced polyps ($p<0.001$), IBD ($p<0.001$) and other malignancy (anal squamous cell carcinoma (SCC) or rectal lymphoma, $p<0.001$). There was also a correlation with diverticulosis ($p<0.001$) although this was predominantly associated with a mildly raised f-Hb (10-149 μg/g). Raised f-Hb was associated with having any pathology found at colonoscopy ($p<0.001$), although of interest, 142 (20.3%) patients with a raised f-Hb had a completely normal colonoscopy, including 28 (15.5%) with a f-Hb ≥400 μg/g.

4.3.6 Table 4.2: Comparison of pathological findings by f-Hb concentration.

		f-Hb (µg Hb/g faeces)				P
	All	<10	10-149	150-399	≥400	
N	1272	572	430	89	181	
Significant Bowel Disease	223 (17.5%)	36 (6.3%)	71 (16.5%)	34 (38.2%)	82 (45.3%)	<0.001
Colorectal Cancer	54 (4.2%)	5 (0.9%)	9 (2.1%)	7 (7.9%)	33 (18.2%)	<0.001
Advanced Adenoma	93 (7.3%)	11 (1.9%)	42 (9.8%)	14 (15.7%)	26 (14.4%)	<0.001
Any Advanced Polyp	99 (7.8%)	15 (2.6%)	43 (10.0%)	15 (16.9%)	26 (14.4%)	<0.001
≥5 Polyps	33 (2.6%)	6 (1.0%)	18 (4.2%)	5 (5.6%)	4 (2.2%)	0.005
IBD	66 (5.2%)	12 (2.1%)	14 (3.3%)	11 (12.4%)	29 (16.0%)	<0.001
Other Pathology	682 (53.6%)	311 (54.4%)	261 (60.7%)	39 (43.8%)	71 (39.2%)	<0.001
Non-Advanced Polyp	271 (21.3%)	104 (18.2%)	122 (28.4%)	16 (18.0%)	29 (16.0%)	<0.001
Other Inflammation	41 (3.2%)	20 (3.5%)	11 (2.6%)	4 (4.5%)	6 (3.3%)	0.750
Diverticulosis	408 (32.1%)	172 (30.1%)	169 (39.3%)	22 (24.7%)	45 (24.9%)	<0.001
Haemorrhoids	197 (15.5%)	100 (17.5%)	56 (13.0%)	14 (15.7%)	27 (14.9%)	0.286
Angiodysplasia/ Telangiectasia	10 (0.8%)	3 (0.5%)	5 (1.2%)	0 (0.0%)	2 (1.1%)	0.527
Radiation Proctitis	12 (0.9%)	1 (0.2%)	8 (1.9%)	1 (1.1%)	2 (1.1%)	0.056
Anal SCC or Rectal Lymphoma	4 (0.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (2.2%)	<0.001
Melanosis Coli	11 (0.9%)	4 (0.7%)	5 (1.2%)	1 (1.1%)	1 (0.6%)	0.825
Anal Fissure/ Fistula	8 (0.6%)	4 (0.7%)	1 (0.2%)	0 (0.0%)	3 (1.7%)	0.191
Rectal Prolapse	3 (0.2%)	0 (0.0%)	2 (0.5%)	1 (1.1%)	0 (0.0%)	0.126
Fibroepithelial Anal Polyp	14 (1.1%)	3 (0.5%)	7 (1.6%)	3 (3.4%)	1 (0.6%)	0.056
Lipoma	4 (0.3%)	3 (0.5%)	1 (0.2%)	0 (0.0%)	0 (0.0%)	0.626
No Pathology	367 (28.9%)	225 (39.3%)	98 (22.8%)	16 (18.0%)	28 (15.5%)	<0.001

4.3.7 Raised f-Hb - Binary Logistic Regression

Twelve variables were chosen for binary logistic regression: age, sex, SIMD, oral anticoagulants, PPI, rectal bleeding, CRC, advanced adenoma, any advanced polyp, any non-advanced polyp, IBD and diverticulosis (Table 4.3). While anal squamous cell carcinoma or rectal lymphoma was found to be significant in χ^2 analysis, the absolute number of cases was very small (n=4) and this could not be included in regression analysis. On univariate analysis older age (≥75 years: OR 1.82 (95% CI: 1.25-2.64; p=0.002)), deprivation (SIMD 1-2: OR 1.51 (95% CI: 1.21-1.88; p<0.001)), oral anticoagulants (OR 1.82 (95% CI: 1.02-3.27; p=0.045)), rectal bleeding (OR 1.50 (95%

CI: 1.20-1.88; p<0.001)), CRC (OR 8.54 (95% CI: 3.38-21.57; p<0.001)), advanced adenoma (OR 6.68 (95% CI: 3.57-12.83; p<0.001)), any advanced polyp (OR 5.06 (95% CI: 2.89-8.88; p<0.001)), non-advanced polyps (OR 1.41 (95% CI: 1.07-1.86; p=0.014)) and IBD (OR 3.90 (95% CI: 2.07-7.37; p<0.001)) correlated with a raised f-Hb. On multivariate analysis older age (≥75 years: OR 1.52 (95% CI: 1.00-2.32; p=0.050)), deprivation (SIMD 1-2: OR 1.54 (95% CI: 1.21-1.94; p<0.001)), oral anticoagulants (OR 1.78 (95% CI: 1.01-3.15; p=0.046)), rectal bleeding (OR 1.47 (95% CI: 1.15-1.88; p=0.002)), CRC (OR 9.27 (95% CI: 3.61-23.83; p<0.001)), advanced adenoma (OR 7.52 (95% CI: 3.90-14.49; p<0.001)), non-advanced polyps (OR 1.78 (95% CI: 1.33-2.38; p<0.001)) and IBD (OR 4.19 (95% CI: 2.17-8.07; p<0.001)) retained significance as independent predictors of a raised f-Hb.

4.3.8 Table 4.3: Univariate and multivariate binary logistic regression of factors associated with f-Hb≥10 µg/g.

		Univariate			Multivariate		
		OR	95% C.I.	P	OR	95% C.I.	P
Age (years)	<50	1.0			1.0		
	50-74	0.85	0.65-1.10	0.220	0.82	0.61-1.09	0.163
	≥75	1.82	1.25-2.64	0.002	1.52	1.00-2.32	0.050
Sex	Male	1.0					
	Female	0.81	0.65-1.02	0.071			
Scottish Index of Multiple Deprivation	Non-Deprived (SIMD 3-5)	1.0			1.0		
	Deprived (SIMD 1-2)	1.51	1.21-1.88	<0.001	1.54	1.21-1.94	<0.001
Oral Anticoagulants	No	1.0			1.0		
	Yes	1.82	1.02-3.27	0.045	1.78	1.01-3.15	0.046
PPI	No	1.0					
	Yes	1.22	0.98-1.52	0.080			
Rectal Bleeding	No	1.0			1.0		
	Yes	1.50	1.20-1.88	<0.001	1.47	1.15-1.88	0.002
CRC	No	1.0			1.0		
	Yes	8.54	3.38-21.57	<0.001	9.27	3.61-23.83	<0.001
Advanced Adenoma	No	1.0			1.0		
	Yes	6.77	3.57-12.83	<0.001	7.52	3.90-14.49	<0.001
Any Advanced Polyp	No	1.0			–		
	Yes	5.06	2.89-8.88	<0.001	–	–	0.484
Any Non-Advanced Polyp	No	1.0			1.0		
	Yes	1.41	1.07-1.86	0.014	1.78	1.33-2.38	<0.001
Inflammatory Bowel Disease	No	1.0			1.0		
	Yes	3.90	2.07-7.37	<0.001	4.19	2.17-8.07	<0.001
Diverticulosis	No	1.0					
	Yes	1.18	0.93-1.50	0.166			

4.3.9 SBD, Other Pathology, No Pathology

Next, patients were divided into those with significant bowel disease, other pathology and no pathology. A comparison of these three groups by f-Hb concentration can be seen in Table 4.2. 223 patients were found to have CRC, advanced adenoma, advanced polyps, ≥5 polyps or IBD (significant bowel disease). 36 (16.1%) had f-Hb <10 μg/g, 71 (31.8%) 10-149 μg/g, 34 (15.2%) 150-399 μg/g and 82 (36.8%) ≥400 μg/g. 682 patients were found to have other bowel disease. 311 (45.6%) had f-Hb <10 μg/g, 261 (38.3%) 10-149 μg/g, 39 (5.7%) 150-399 μg/g and 71 (10.4%) ≥400 μg/g. 367 had no pathology found at colonoscopy. 225 (61.3%) had f-Hb <10 μg/g, 98 (26.7%) 10-149 μg/g, 16 (4.4%) 150-399 μg/g and 28 (7.6%) ≥400 μg/g. There was a highly significant association between f-Hb concentration and increasing 'severity' of colonoscopic findings from no pathology to other pathology to significant bowel disease ($p<0.001$).

4.3.10 Demographics Associated with Raised f-Hb in those with SBD, Other Pathology, No Pathology - Binary Logistic Regression

Six demographics were chosen for binary logistic regression: age, sex, SIMD, oral anticoagulants, PPI and rectal bleeding (Table 4.4). For those patients with significant bowel disease, only rectal bleeding (OR 3.63 (95% CI: 1.66-7.97; p=0.001)) correlated with a raised f-Hb on univariate analysis. Multivariate analysis was therefore not performed. For those patients with other bowel disease, only PPI use (OR 1.60 (95% CI: 1.15-2.11; p=0.004)) correlated with a raised f-Hb on univariate analysis. Again, multivariate analysis could not be performed. For those with no pathology, bowel screening age (50-74 years) (OR 0.55 (95% CI: 0.36-0.86; p=0.009)) predicted lower risk of a raised f-Hb and deprivation (SIMD 1-2: OR 2.12 (95% CI: 1.38-3.25; p=0.001)) predicted a higher risk of raised f-Hb. On multivariate analysis, bowel screening age (OR 0.56 (95% CI: 0.36-0.89; p=0.013)) and deprivation (SIMD 1-2: OR 2.13 (95% CI: 1.38-

3.29; p=0.001)) retained significance as independent predictors of lower and higher risk of a raised f-Hb, respectively.

4.3.11 Table 4.4: Univariate and multivariate binary logistic regression of factors associated with f-Hb≥10µg/g by significant bowel disease, other pathology and no pathology groups.

			Univariate			Multivariate		
			OR	95% C.I.	P	OR	95% C.I.	P
Significant Bowel Disease (n=223)	Age (years)	<50	1.0					
		50-74	0.42	0.15-1.17	0.097			
		≥75	1.16	0.32-4.29	0.821			
	Sex	Male	1.0					
		Female	0.94	0.46-1.93	0.865			
	Scottish Index of Multiple Deprivation	Non-Deprived (SIMD 3-5)	1.0					
		Deprived (SIMD 1-2)	1.17	0.58-2.40	0.659			
	Oral Anti-coagulant	No	1.0					
		Yes	3.28	0.42-25.51	0.257			
	PPI	No	1.0					
		Yes	0.90	0.43-1.87	0.774			
	Rectal Bleeding	No	1.0					
		Yes	3.63	1.66-7.97	0.001			
Other Bowel Disease (n=682)	Age (years)	<50	1.0					
		50-74	1.02	0.69-1.50	0.937			
		≥75	1.43	0.87-2.36	0.164			
	Sex	Male	1.0					
		Female	0.96	0.71-1.29	0.768			
	Scottish Index of Multiple Deprivation	Non-Deprived (SIMD 3-5)	1.0					
		Deprived (SIMD 1-2)	1.32	0.98-1.78	0.072			
	Oral Anti-coagulant	No	1.0					
		Yes	1.60	0.82-3.12	0.169			
	PPI	No	1.0					
		Yes	1.60	1.15-2.11	0.004			
	Rectal Bleeding	No	1.0					
		Yes	1.22	0.90-1.65	0.205			
No Pathology (n=367)	Age (years)	<50	1.0			1.0		
		50-74	0.55	0.36-0.86	0.009	0.56	0.36-0.89	0.013
		≥75	1.57	0.62-3.96	0.343	1.71	0.66-4.39	0.268
	Sex	Male	1.0					
		Female	0.88	0.57-1.37	0.578			
	Scottish Index of Multiple Deprivation	Non-Deprived (SIMD 3-5)	1.0			1.0		
		Deprived (SIMD 1-2)	2.12	1.38-3.25	0.001	2.13	1.38-3.29	0.001
	Oral Anti-coagulant	No	1.0					
		Yes	2.28	0.71-7.33	0.166			
	PPI	No	1.0					
		Yes	1.25	0.82-1.91	0.293			
	Rectal Bleeding	No	1.0					
		Yes	1.33	0.87-2.03	0.194			

4.4 Discussion

To date, no studies have explored demographics independently associated with a raised f-Hb in symptomatic patients. In screening participants, a higher f-Hb independently correlates with older age, male sex, deprivation, smoking and use of aspirin, NSAIDs, oral anticoagulants, PPIs and antibiotics(207-210). In this study it has been shown higher f-Hb concentrations are seen in older symptomatic patients (≥75 years) but also in younger patients (<50 years). This may be related to the impact of bowel cancer screening, with those aged 50-74 years with a raised f-Hb being more likely to be investigated via the screening pathway in Scotland. On multivariate analysis, older age independently predicted a raised f-Hb (p=0.050). While in the current study males did constitute a greater proportion of those with a raised f-Hb (males accounted for 43.9% of all participants, 48.1% of those with f-Hb ≥400 μg/g and 53.9% f-Hb 150-399 μg/g), this did not reach statistical significance (p=0.080). In agreement with studies investigating screening participants, this study has shown deprivation (p=0.004) and oral anticoagulants (p=0.017) to be associated with higher f-Hb, and these retained significance on multivariate analysis (p<0.001 and p=0.046). Patients on PPIs were more likely to have a raised f-Hb, but only between 10 and 399 μg/g (p=0.007). No associations between NSAIDs or aspirin and f-Hb were detected.

Several studies have investigated the use of FIT for the diagnosis of significant bowel disease in symptomatic patients. McDonald et al(198) reported on 280 patients referred from primary care with lower GI symptoms. They found that those with significant bowel disease had a median f-Hb of 15 μg/g which was significantly higher than those without (p<0.0001). Additionally, patients with low-risk adenoma had a raised median f-Hb of 13 μg/g. In a similar study by Godber et al(199) of 484 symptomatic patients, 45 had significant bowel disease, 196 low risk adenoma, hyperplastic polyps, diverticular disease

or haemorrhoids and 243 patients had normal examinations. Median f-Hb for each group was 113 μg/g, 3 μg/g and 2 μg/g respectively ($p<0.0001$).

This study has confirmed that in addition to CRC ($p<0.001$), advanced adenoma ($p<0.001$), non-advanced polyps ($p<0.001$) and IBD ($p<0.001$) are all diagnoses independently associated with a raised f-Hb. Additionally, diverticulosis was found to correlate with a mildly raised f-Hb (10-149 μg/g, $p<0.001$) and a notable association between a raised f-Hb and other lower GI malignancies (anal SCC or rectal lymphoma) was observed (all 4 cases f-Hb ≥400 μg/g, $p<0.001$). Interestingly while any advanced polyp (advanced adenoma or advanced sessile serrated polyp) predicted increased f-Hb on χ^2 analysis ($p<0.001$) and univariate binary logistic regression ($p<0.001$), this did not retain significance on multivariate analysis. This most likely reflects the low number of advanced sessile serrated polyps in this study (n=6) but may also relate to previous evidence suggesting that FIT is less sensitive for the detection of sessile serrated polyps as compared to adenomas, which may in part be explained by their frequent proximal colonic location(217, 218).

Several studies have previously examined factors correlating with FIT false positivity in screening participants. In the study by Ibanez-Sanz et al(207) 89,199 bowel screening FITs from 46,783 patients were reviewed. False positivity was defined as f-Hb ≥20 μg/g without intermediate/ high-risk polyps or CRC. Independent predictors of false positivity were younger age (OR 1.28 (95% CI: 1.12-1.46; p=0.0002)), female sex (OR 2.31 (95% CI: 2.03-2.64; p<0.0001)), successive screening round (OR 1.53 (95% CI: 1.35-1.74; p<0.0001)), aspirin (OR 1.30 (95% CI: 1.04-1.64; p=0.02)), NSAID (OR 1.48 (95% CI: 1.23-1.78; p<0.0001)), PPI (OR 1.39 (95% CI: 1.18-1.65; p=0.0001)), antibiotics (OR 1.32 (95% CI: 1.03-1.71; p=0.03)) and laxative (OR 2.26 (95% CI: 1.06-4.80; p=0.03)) use. Further studies have related false positivity in screening participants to both older age(214) and younger age(207, 210), female(207, 210, 211, 216) and male sex(214), smoking(214), high BMI(214), successive screening(207, 211), the use of aspirin(207), NSAIDs(207),

PPIs(207, 211, 215), antibiotics(207) and laxatives(207), non-advanced adenomas(212), diverticular disease(212) and anal pathology including haemorrhoids and anal fissures(211, 212, 214). De Klerk et al(213) performed a systematic review and meta-analysis of such studies and found younger age, female sex, NSAIDs, PPIs, anal fissures and peptic ulceration to be predictors of FIT false positivity in screener participants.

In the current study it has been established that deprivation is independently associated with a raised f-Hb in the absence of pathology at colonoscopy (p=0.001). Mansouri et al(49) found deprived individuals less likely to have CRC identified as a result of a positive FIT, within the Scottish Bowel Screening Programme. It is interesting that this association with deprivation is shared by screening and symptomatic patients. In the review by Barnett et al(219) they hypothesise that an elevated systemic inflammatory response (SIR) may explain the higher f-Hb concentrations observed in the absence of colorectal pathology, in screening participants with chronic conditions (ischaemic heart disease, cerebrovascular disease, diabetes, hypertension) and on certain medications (PPIs, anticoagulants). Numerous studies have observed a positive correlation between deprivation and surrogates for a raised systemic inflammatory response including an elevated CRP(220-227), fibrinogen(220, 222, 224, 226), IL-6(221, 222, 224, 225, 227), IL-18(222), TNF-α(222), white blood cell count(226) and a low albumin(223). Indeed, two large systematic reviews and meta-analyses involving 96,746(228) and 111,156(229) patients found deprivation to be associated with an elevated CRP and both CRP and IL-6 respectively. The mechanistic link between deprivation and a raised SIR is not fully understood but is likely multifactorial. Deprived individuals are known to be more comorbid including higher rates of cardiovascular disease, obesity and metabolic syndrome. Deprivation has been correlated with a preponderance for higher risk behaviours associated with a raised SIR including smoking, high alcohol intake, poor diet and lack of physical activity. Chronic psychosocial stress associated with factors such as

financial insecurity and exposure to crime may lead to an elevated SIR through activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Finally, deprivation has been associated with the methylation of certain genes involved in inflammation; perhaps early life adversity leads to a pro-inflammatory epigenetic modulation of the immune system (220-222, 225, 226, 229). Perhaps a heightened SIR is one confounding variable which may link deprivation, co-morbidity and a raised f-Hb in the absence of colorectal pathology.

This study has a number of strengths. It is the first to perform multivariate analysis to establish independent predictors of a raised f-Hb in patients with lower GI symptoms. While this question has been applied to screening participants, it cannot be assumed that the same associations will be seen in symptomatic patients and indeed several similarities and differences have been established. The study reflects real-life practice in the GG&C: patients with both high and low risk symptoms and with and without rectal bleeding were included, reflecting the most up to date evidence(12, 93, 97, 98, 230) and clinical use of FIT(15, 25, 188). The current study does however have limitations. It is retrospective in nature and with the current sample size it was difficult to establish clear associations between FIT and rarer diagnoses such as angiodysplasia, radiation proctitis, anal SCC, rectal prolapse and sessile serrated adenomas. Additionally, although f-Hb was found to be predictive of a number of diagnoses, calculating the diagnostic accuracy of FIT for each of these conditions was out with the scope of this study. As no statistical correction was made to the χ^2 analysis to account for the multiple comparisons made, an increased risk of type I errors may be anticipated. However, by assessing significant variables with multivariate binary logistic regression analysis, the impact of potential false positives is negated. Finally, while bowel screening age and deprivation independently predicted a lower and higher risk of f-Hb ≥10μg/g respectively in those without pathology, it is unclear how this may be interpreted in the clinical setting. In the long-term it would be optimal to identify

those patients with a raised f-Hb who have a low chance of significant pathology in order to avoid unnecessary invasive investigation. Perhaps a more tailored approach could be adopted whereby varying f-Hb thresholds could be applied depending on age, deprivation and the presence or absence of other biomarkers of CRC-risk such as anaemia. The current study could be repeated on a larger scale to allow more intricate analysis of multiple f-Hb thresholds, rather than analysing FIT as a binary variable (f-Hb ≥10 μg/g raised/ <10 μg/g normal), as was conducted here.

In conclusion, demographics including older age, deprivation and the use of oral anticoagulants has been independently associated with a raised f-Hb in patients with lower GI symptoms. In addition to CRC, advanced adenoma, non-advanced polyps, IBD, diverticulosis and anal SCC/ rectal lymphoma are associated with a raised f-Hb. Deprivation is independently associated with a raised f-Hb in the absence of pathology. This should be considered when utilising FIT as part of a symptomatic referral pathway. Further work is required to establish why deprived patients are more likely to exhibit a raised f-Hb without pathology.

5 The relationship between co-morbidity, systemic inflammatory response, screen-detection and outcome in patients undergoing resection for colorectal cancer.

5.1 Introduction

The Scottish Bowel Screening Programme invites patients aged 50 to 74 years to undertake a quantitative faecal immunochemical test (FIT) followed by colonoscopy for those patients testing positive, at a threshold of 80 µg Hb/ g of faeces(2). There is good evidence to suggest that this approach to screening increases the number of early-stage cancers diagnosed and reduces cancer specific mortality(3, 4, 6, 175). Additionally, some evidence suggests the incidence of colorectal cancer (CRC) may be reduced through the removal of precursor polyps and that the requirement for more invasive surgical procedures may be reduced due to earlier diagnosis(3).

Chapter 2, 3 and 4 explored the utility of FIT for the diagnosis of CRC in symptomatic patients. FIT was shown to be a sensitive tool for CRC-risk prediction in such patients; a sensitivity that could be enhanced by combining faecal haemoglobin (f-Hb) with the presence or absence of anaemia from circulating haemoglobin (Hb)(188), or by repeating the FIT test once within a year for those with persistent or recurrent symptoms(196). However, a level of complexity to its use has been revealed, with both patient factors including older age, deprivation and use of oral anticoagulants, and alternative diagnoses to CRC including advanced adenoma, non-advanced polyps and inflammatory bowel disease (IBD) being independent predictors of a raised f-Hb. Similar complexities exist with the use of FIT for CRC screening. For example, a raised f-Hb independently correlates with older age, male sex, deprivation, smoking and use of aspirin, NSAIDs, oral anticoagulants, PPIs and antibiotics in screening participants(207-210). Additionally, patients ultimately diagnosed with CRC through screening are more likely to be male, younger and less socioeconomically deprived as compared to those diagnosed via symptomatic pathways,

and in those undergoing resection, to have lower T staging, less venous invasion, less peritoneal involvement and less margin involvement(49, 174, 231). It is important to continue the search for inherent differences between screen-detected and non-screen-detected CRC in terms of patient and tumour factors. Co-morbidity is an important host factor that, to date has not been studied in detail within the context of CRC screening outcomes. It has previously been shown that patients with screen-detected disease have a lower burden of co-morbidity due to their demographic profile and that this may influence post-operative outcome(232). However, the effect of comorbidity on long term outcome is unclear. Additionally, the presence of a raised systemic inflammatory response (SIR), which is known to be associated with adverse outcomes after a diagnosis of CRC, may act as a confounder. The aim of the present study was to assess the relationship between co-morbidity, systemic inflammatory response, screen-detection and overall survival in patients with CRC.

5.2 Methods

5.2.1 Study Design, Setting and Participants

A retrospective observational cohort study was conducted. The cohort was formed of all patients invited to participate in the first complete round of the Scottish Bowel Screening Programme in NHS Greater Glasgow and Clyde (NHS GG&C) between April 2009 and March 2011, regardless of screening participation. As per the screening protocol in Scotland, this involves those aged 50-74 years. Patients were only included if they were diagnosed with a CRC and underwent resection with curative intent within 2 years of their screening invitation. Patients were classified as those diagnosed with CRC directly through Scottish Bowel Screening Programme participation (screen-detected patients) or via symptomatic pathways (non-screen-detected patients). In Scotland, colonoscopy is only routinely performed in asymptomatic individuals within the Scottish Bowel Screening Programme and so all non-screen-detected patients were scoped via symptomatic referral pathways. Approval for this study was given by the Caldicott Guardian. Ethical approval and individual patient consent were waived as the study was entirely retrospective, observational and anonymised. The results have been reported according to STROBE guidelines(174).

5.2.2 Variables and Data Sources

Details of patients who were invited to screening during the study period were extracted from a prospectively maintained database. To identify patients who were invited to screening but were diagnosed with CRC via symptomatic referral pathways, the West of Scotland Colorectal Cancer Managed Clinical Network (MCN) dataset and the Scottish Cancer Registry (SMR06) datasets were cross-referenced. Baseline demographics, co-morbidity, body mass index (BMI), American Society of Anaesthesiology grade (ASA), preoperative blood results and survival were obtained on a case-by-case basis from NHS

electronic patient and theatre records. Patients were excluded from the final analysis if their records were absent from the NHS electronic portal system, with 770 patients in total; 331 screen-detected and 439 non-screen-detected.

Co-morbidity was objectively quantified using ASA and two validated co-morbidity scoring systems: the Lee Index and the Charlson Index. The American Society of Anaesthesiology grade is the gold standard system for assessing a patient's pre-operative physical status and medical co-morbidities and ranges from I for a normal healthy patient to V for a moribund patient not expected to survive with or without surgery. For the purposes of the analysis an ASA grade of I-II was classified as low and III-V high. The Lee Index is a co-morbidity score which was developed to predict the risk of cardiac complications among patients undergoing non-cardiac surgery. It is based on 6 variables: a history of coronary artery disease, congestive heart failure, cerebrovascular disease, diabetes mellitus requiring insulin therapy, chronic kidney disease (defined as a pre-operative serum creatinine >2mg/dl) and whether the patient is due to undergo high risk surgery (defined as intraperitoneal, intrathoracic or suprainguinal vascular surgery)(233). As patients were only included in this study if they had undergone a colorectal resection, all patients scored at least 1 and a high Lee Index was defined as ≥2. The Charlson Index was developed to objectively quantify co-morbidity and associated mortality risk for the specific purpose of use in longitudinal studies. It is based on a history of myocardial infarction, congestive cardiac failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic lung disease, connective tissue disease, peptic ulcer, diabetes mellitus (with or without end-organ damage), chronic kidney disease, hemiplegia, leukaemia, lymphoma, solid tumours (either localised or metastatic), liver disease (mild or moderate to severe) and acquired immunodeficiency syndrome (AIDS) (234). A high Charlson Index was defined as a score ≥3.

The presence of a SIR was quantified using the previously validated neutrophil/ lymphocyte ratio (NLR), lymphocyte/ monocyte ratio (LMR) and platelet/ lymphocyte ratio (PLR). These scores are derived from circulating neutrophil, lymphocyte, monocyte and platelet counts, taken from a preoperative full blood count. In each case the ratios were calculated by dividing the former by the latter. A greater systemic inflammatory response is associated with a higher NLR or PLR and a lower LMR. Thresholds were derived from previously published data: low NLR <3, moderate NLR 3-5, high NLR >5; low LMR <2.4, high LMR ≥2.4; low PLR ≤150, high PLR >150(235).

Deprivation was quantified using the Scottish Index of Multiple Deprivation (SIMD), derived from each patient's post code. SIMD is a measure of an area's deprivation based on income, employment, education, health, access to services, crime and housing(177).

5.2.3 Data Analysis and Statistical Methods

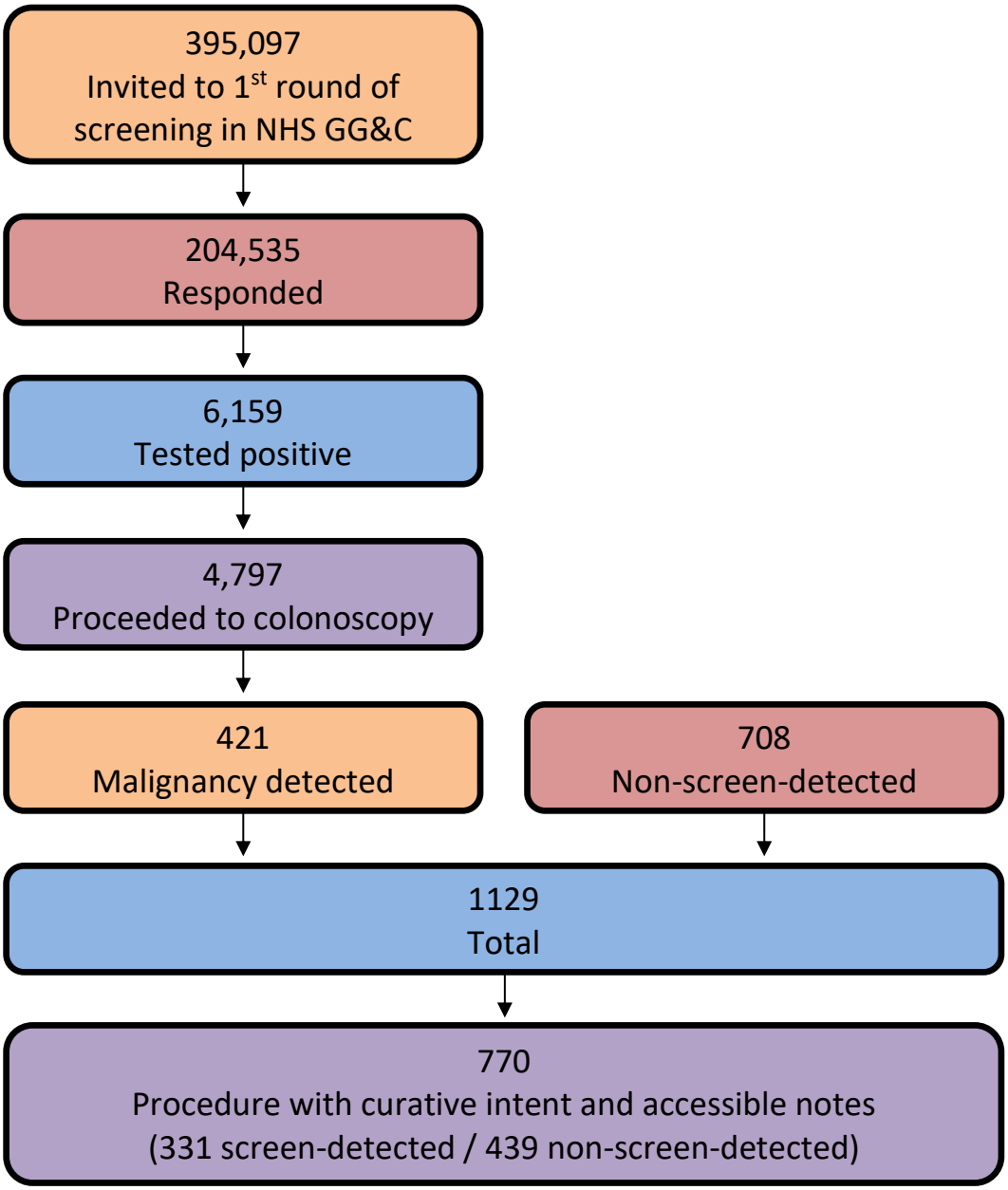
Covariables were compared using the χ^2 test. A value of $p<0.05$ was considered statistically significant. Overall survival (OS) and cancer specific survival (CSS) were analysed using Cox Regression, with survival measured from the date of resection to event or date of censor (4th of May 2016). Post-operative deaths within 30 days of surgery were excluded from cancer-specific survival analysis. All covariables found to be statistically significant ($p<0.05$) predictors of survival on univariate analysis were carried forward to a multivariate survival analysis. In order to reduce the impact of collinearity between explanatory variables, a stepwise backward method was used to produce a final model of variables with a significant independent impact on survival, where variables were removed from the model when the corresponding p value was >0.05. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, USA).

5.3 Results

5.3.1 Participants

Of all 395,097 patients invited to participate in the first complete round of screening in NHS GG&C, 204,535 (52%) responded of which 6,159 (3%) tested positive. Of those testing positive, 4,797 (78%) proceeded to colonoscopy and 421 (9%) of those patients were found to have CRC. There were 708 patients with non-screen-detected CRCs diagnosed in NHS GG&C during the same time period of which 468 (65%) of these were non-responders to the screening programme, 182 (25%) were interval cancers (within two years of a negative screening test), 43 (6%) were individuals who chose not to attend colonoscopy and 15 (2%) had no malignancy detected at index screening colonoscopy. 393 of 421 (93%) screen-detected patients proceeded to a resection with curative intent as compared to 479 of 708 (68%) patients with non-screen detected disease ($p<0.001$) and was predominantly explained by lower staging at diagnosis: 28 of 421 (7%) screen-detected patients had metastases at diagnosis (stage IV) as compared to 192 of 708 (27%) ($p<0.001$). Of the 1,129 total (421 screen-detected and 708 non-screen-detected), 770 patients underwent a surgical resection with curative intent and had complete NHS electronic portal records and were included in the final analysis (331 screen-detected and 439 non-screen-detected disease) (Figure 5.1).

5.3.2 Figure 5.1: Flowchart of patient identification.



5.3.3 Demographics

Of all 770 patients included in the study, the median age was 67 years, 456 (59%) were males and 247 (37%) had rectal cancer. TNM distribution was: stage I 234 (30%), II 262 (34%), III 236 (31%), IV 38 (5%). A comparison of demographics between screen-detected and non-screen-detected patients can be seen in Table 5.1. Patients with screen-detected disease were significantly more likely to be male (64.4% vs 55.4%; p=0.012), have an earlier TNM stage (p=0.001), have colonic tumours (73.7% vs 63.4%; p=0.002) and had a lower rate of emergency presentations (0.6% vs 17.1%; p<0.001). 2 (0.6%) screen-detected patients required emergency operations. The first was admitted for elective laparoscopic right hemicolectomy following positive screening, but on admission had clinical and radiological evidence of obstruction and perforation necessitating laparotomy and the second attended for colonoscopy following a positive screening test and was clinically and radiologically obstructed and was taken for an emergency subtotal colectomy.

5.3.4 Table 5.1: Baseline demographics and comparison of patients with screen-detected and non-screen-detected colorectal cancer.

	All patients n(%)	Screen-detected n(%)	Non-screen-detected n(%)	p-value
Age				
≤62	254 (33.0%)	101 (30.5%)	153 (34.9%)	
63-70	256 (33.2%)	120 (36.3%)	136 (31.0%)	
≥71	260 (33.8%)	110 (33.2%)	150 (34.2%)	0.259
Sex				
Male	456 (59.2%)	213 (64.4%)	243 (55.4%)	
Female	314 (40.8%)	118 (35.6%)	196 (44.6%)	0.012
SIMD				
1 (most deprived)	254 (33.1%)	100 (30.3%)	154 (35.2%)	
2	141 (18.4%)	54 (16.4%)	87 (19.9%)	
3	129 (16.8%)	61 (18.5%)	68 (15.5%)	
4	107 (13.9%)	51 (15.5%)	56 (12.8%)	
5 (least deprived)	137 (17.8%)	64 (19.4%)	73 (16.7%)	0.255
Presentation				
Elective	693 (90.0%)	329 (99.4%)	364 (82.9%)	
Emergency	77 (10.0%)	2 (0.6%)	75 (17.1%)	<0.001
Tumour Site				
Colon	521 (67.8%)	244 (73.7%)	277 (63.4%)	
Rectum	247 (36.6%)	87 (26.3%)	160 (36.6%)	0.002
TNM Stage				
I	234 (30.4%)	129 (39.0%)	105 (23.9%)	
II	262 (34.0%)	91 (27.5%)	171 (39.0%)	
III	236 (30.6%)	101 (30.5%)	135 (30.8%)	
IV	38 (4.9%)	10 (3.0%)	28 (6.4%)	0.001
ASA*				
Low (<3)	439 (66.8%)	195 (72.8%)	244 (62.7%)	
High (≥3)	218 (33.2%)	73 (27.2%)	145 (37.3%)	0.007
Lee Index				
Low (<2)	620 (80.5%)	277 (83.7%)	343 (78.1%)	
High (≥2)	150 (19.5%)	54 (16.3%)	96 (21.9%)	0.054
Charlson Index				
Low (<3)	577 (74.9%)	256 (77.3%)	321 (73.1%)	
High (≥3)	193 (25.1%)	75 (22.7%)	118 (26.9%)	0.181
NLR[#]				
Low (<3)	433 (56.9%)	216 (66.3%)	217 (49.9%)	
Moderate (3-5)	218 (28.6%)	85 (26.1%)	133 (30.6%)	
High (>5)	110 (14.5%)	25 (7.7%)	85 (19.5%)	<0.001
LMR[#]				
High (≥2.4)	473 (62.2%)	232 (71.2%)	241 (55.4%)	
Low (<2.4)	288 (37.8%)	94 (28.8%)	194 (44.6%)	<0.001
PLR[#]				
Low (≤150)	326 (42.8%)	172 (52.8%)	154 (35.4%)	
High (>150)	435 (57.2%)	154 (47.2%)	281 (64.6%)	<0.001

* Data missing for 113 (14.7%) patients.

[#] Data missing for 9 (1.2%) patients.

5.3.5 Co-Morbidity

Examining co-morbidity indices, screen-detected patients were less likely to have a high ASA score (≥3) as compared to non-screen-detected patients (27.2% vs 37.3%; p=0.007). There was no difference in the proportion of patients with a high Lee Index (≥2) (16.3% vs 21.9%; p = 0.054), nor high Charlson Index (≥3) (22.7% vs 26.9%; p=0.181).

5.3.6 Systemic Inflammatory Response

Screen-detected patients were less likely to have evidence of a high SIR as compared to non-screen-detected patients as measured by a high NLR (>5) (7.7% vs 19.5%; p<0.001), moderate NLR (3-5) (26.1% vs 30.6%; p<0.001), low LMR (<2.4) (28.8% vs 44.6%; p<0.001) and a high PLR (>150) (47.2% vs 64.6%; p<0.001).

5.3.7 Survival

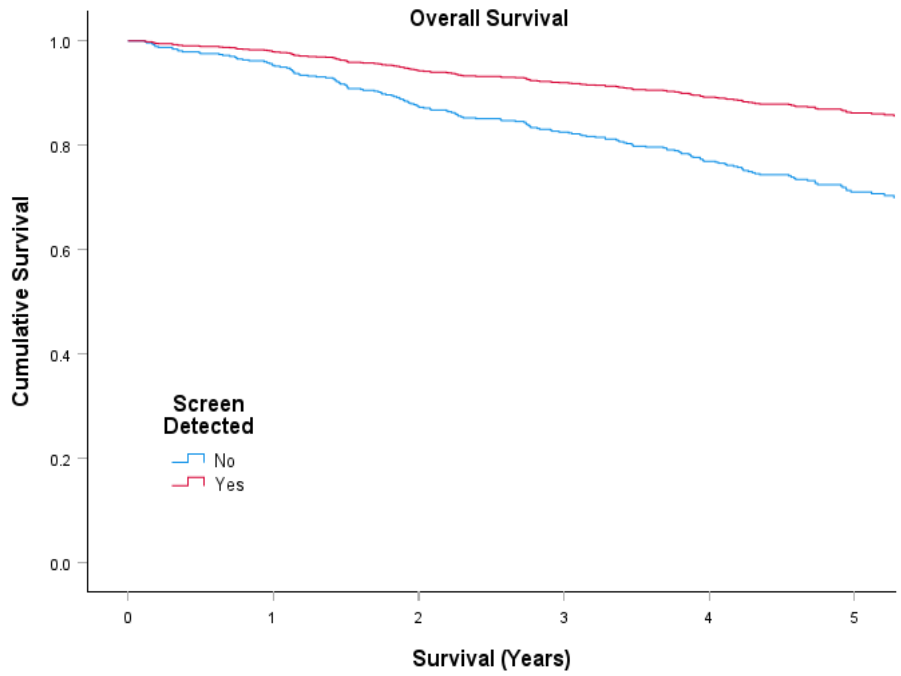
With a median follow-up of 63 months (range 33-83 months), 188 (24%) patients died of which 106 (56%) patients died of CRC. 8 (1%) died within 30 days of their operation (4 screen-detected, 4 non-screen-detected). 5-year overall survival (OS) and cancer specific survival (CSS) was 77% (168 deaths, 361 patients reaching 5 year follow-up) and 85% (100 deaths, 361 patients reaching 5 year follow-up) respectively.

On univariate analysis, non-screen-detection (HR 2.300 (1.664-3.181; p<0.001)) (Figure 5.2), emergency presentation (HR 3.409 (2.395-4.854; p<0.001)), advanced TNM stage (p<0.001) (Figure 5.3), high ASA (HR 1.826 (1.330-2.508; p<0.001)) (Figure 5.4), high Charlson Index (HR 1.756 (1.290-2.392; p<0.001)) (Figure 5.5), moderate NLR (HR 1.588 (1.128-2.235; p=0.008)), high NLR (HR 2.382 (1.626-3.491; p<0.001)) (Figure 5.6), low LMR (HR 2.038 (1.514-2.742; p<0.001)) and high PLR (HR 1.827 (1.326-2.519; p<0.001)) were all associated with poorer OS. Non-screen-detection (HR 2.763 (1.776-4.298; p<0.001)), emergency presentation (HR 5.141 (3.388-7.801; p<0.001)), advanced

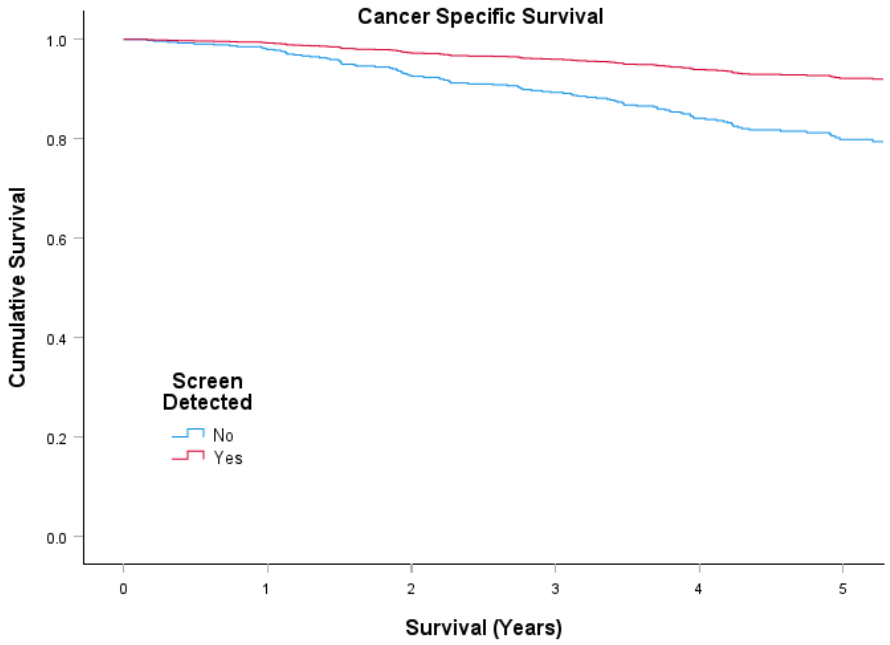
TNM stage (p<0.001), high NLR (HR 2.368 (1.448-3.875; p<0.001)), low LMR (HR 1.969 (1.340-2.893; p<0.001)) and high PLR (HR 2.110 (1.374-3.240; p<0.001)) were also associated with poorer CSS. Table 5.2 and 5.3 display the outcomes of both univariate and multivariate survival analysis for OS and CSS respectively.

On multivariate analysis non-screen-detection (HR 1.670 (1.133-2.463; p=0.001)), emergency presentation (HR 2.065 (1.359-3.136; p<0.001)), advanced TNM stage (p<0.001), high Charlson Index (HR 1.612 (1.140-2.280; p=0.007)) and low LMR (HR 1.544 (1.107-2.154; p=0.011)) retained significance as independent predictors of OS. Non-screen-detection (HR 1.847 (1.144-2.983; p=0.012)), emergency presentation (HR 2.399 (1.507-3.820; p<0.001)), advanced TNM stage (p<0.001) and high PLR (HR 1.578 (1.018-2.444; p=0.041)) retained significance as independent predictors of CSS.

5.3.8 Figure 5.2: Relationship between screen detection and OS and CSS.

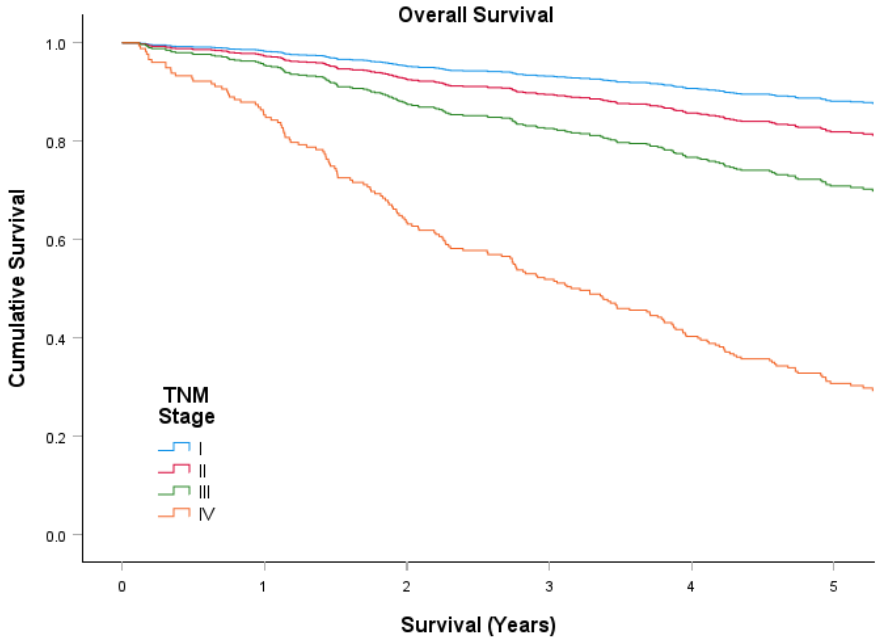


NSD	439	414	380	329	220	93
SD	331	321	309	302	259	162

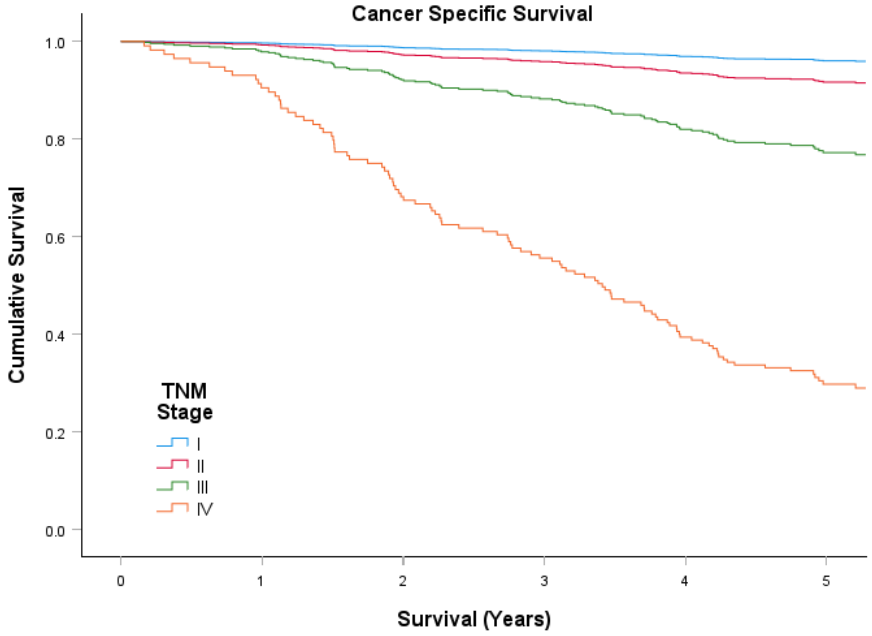


NSD	430	407	376	327	217	92
SD	324	319	307	301	257	159

5.3.9 Figure 5.3: Relationship between TNM stage and OS and CSS.

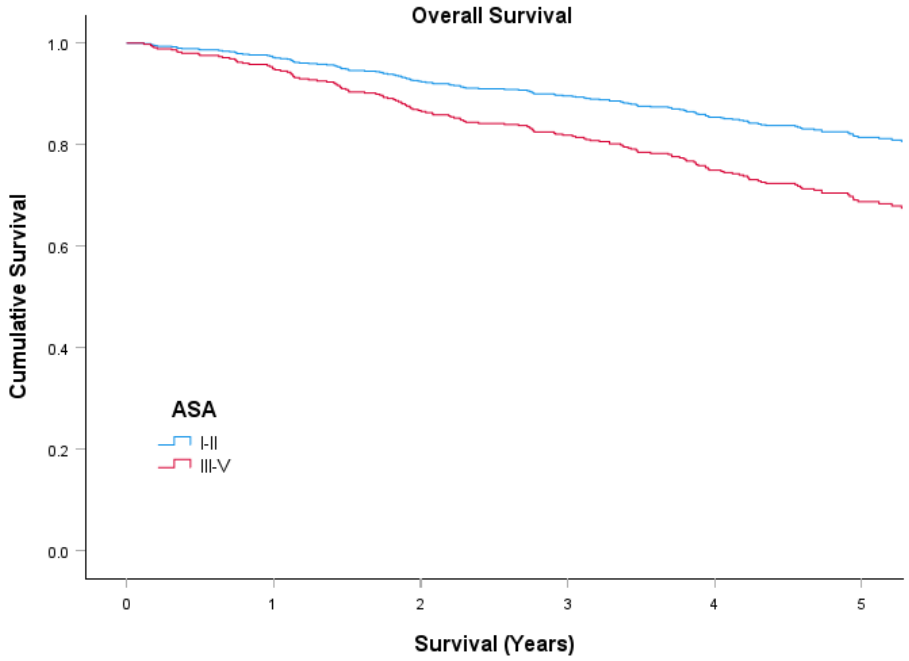


Stage I	234	228	224	212	168	90
Stage II	262	250	238	219	164	89
Stage III	236	225	202	180	136	69
Stage IV	38	32	25	20	12	7

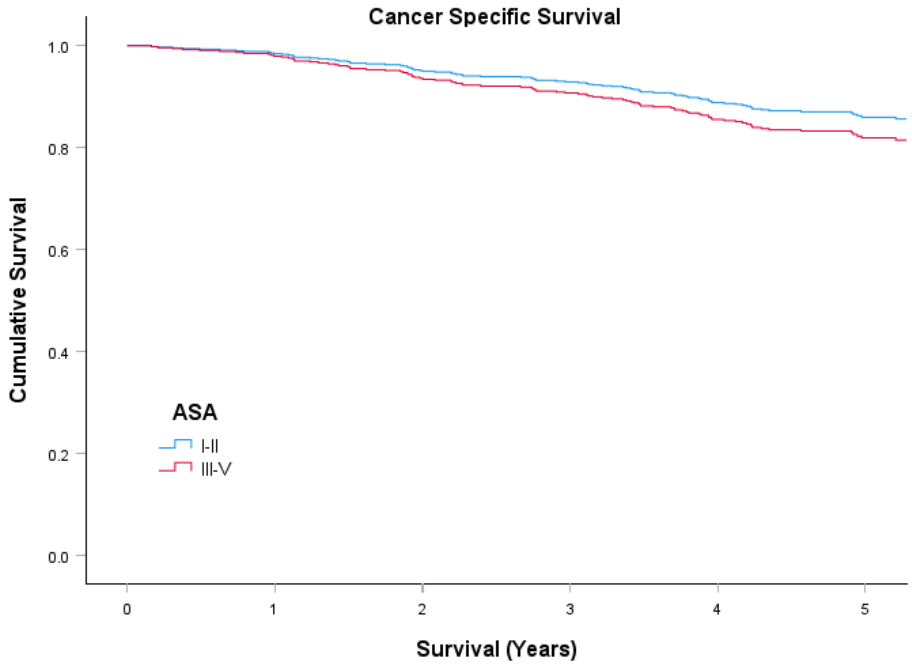


Stage I	230	227	222	211	166	88
Stage II	255	248	237	217	163	87
Stage III	232	220	200	180	134	69
Stage IV	38	32	25	20	11	7

5.3.10 Figure 5.4: Relationship between ASA and OS and CSS.

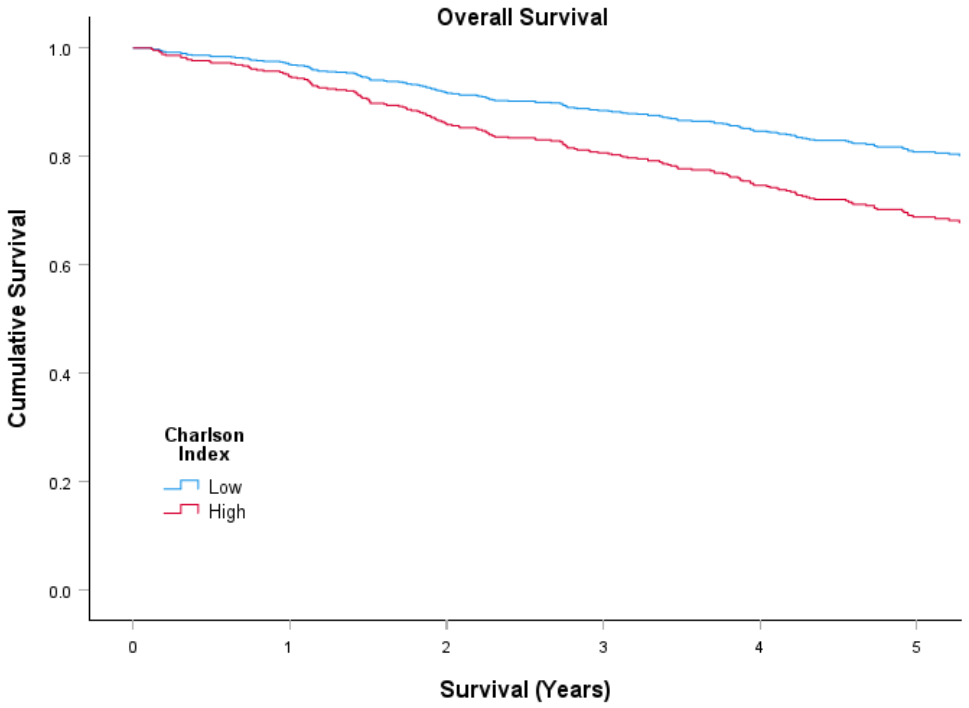


I-II	439	425	405	369	266	129
III-V	218	202	184	169	124	60

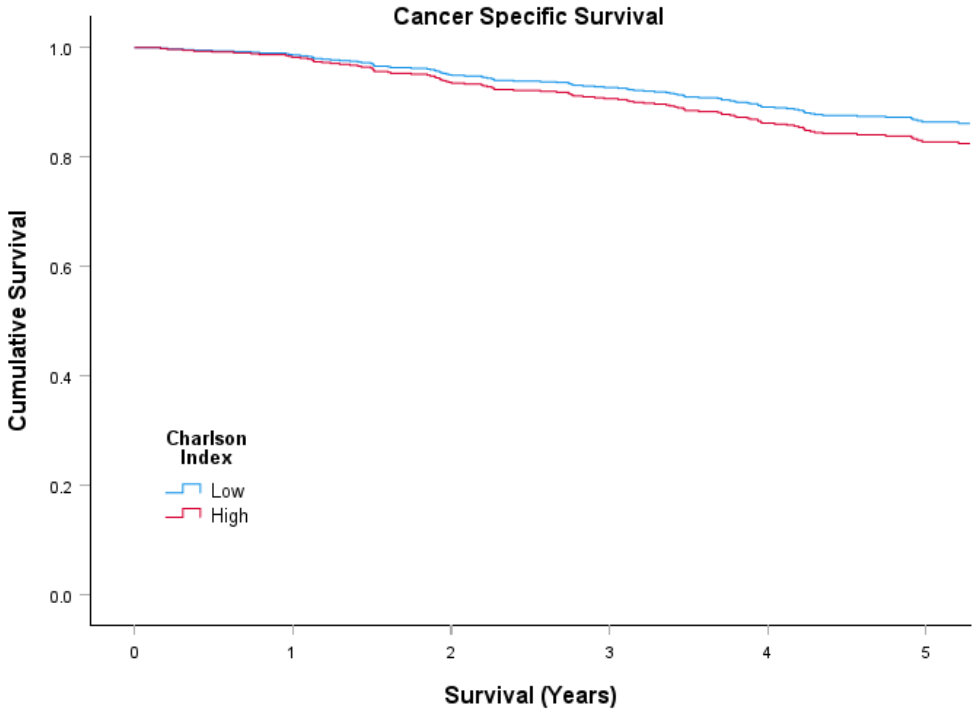


I-II	433	423	403	368	264	128
III-V	211	197	182	166	121	58

5.3.11 Figure 5.5: Relationship between Charlson Index and OS and CSS.

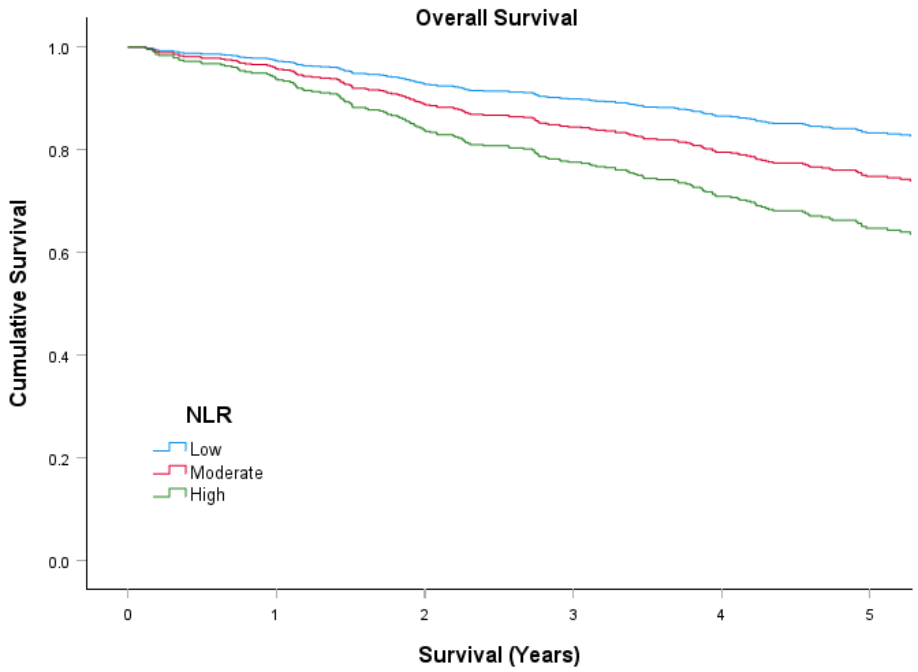


Low	577	563	533	488	369	200
High	193	172	156	143	110	55

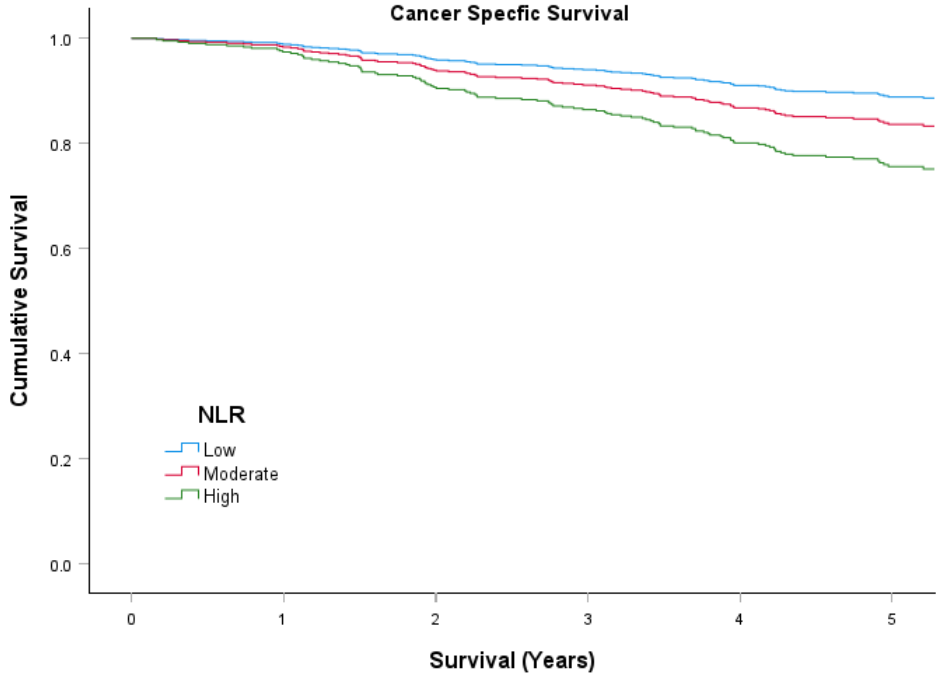


Low	571	558	529	486	367	197
High	183	169	154	142	107	54

5.3.12 Figure 5.6: Relationship between NLR and OS and CSS.

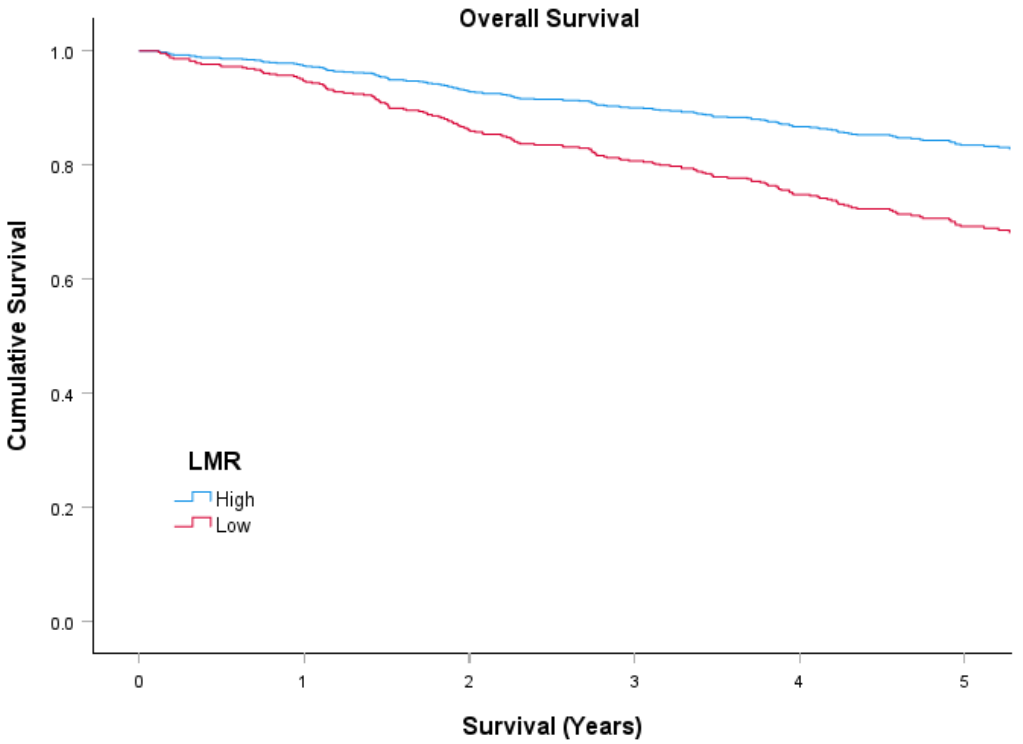


Low	430	419	404	371	284	114
Moderate	255	248	237	217	163	87
High	232	220	200	180	134	69

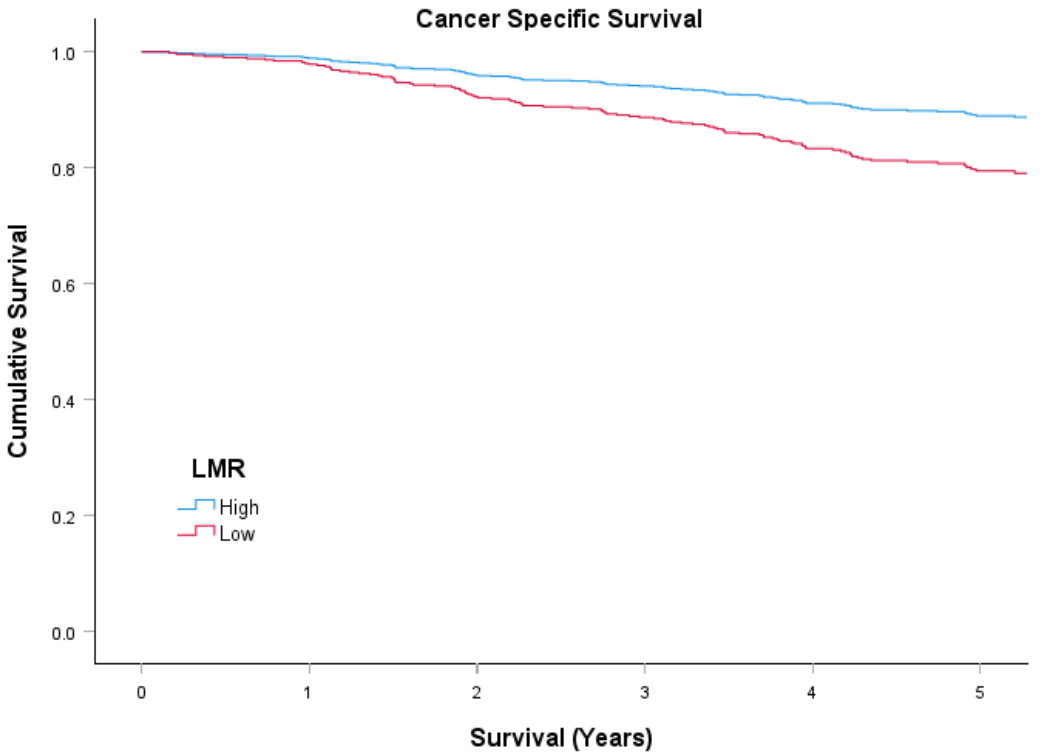


Low	427	417	400	370	282	110
Moderate	212	201	187	171	130	52
High	106	100	89	80	57	20

5.3.13 Figure 5.7: Relationship between LMR and OS and CSS.

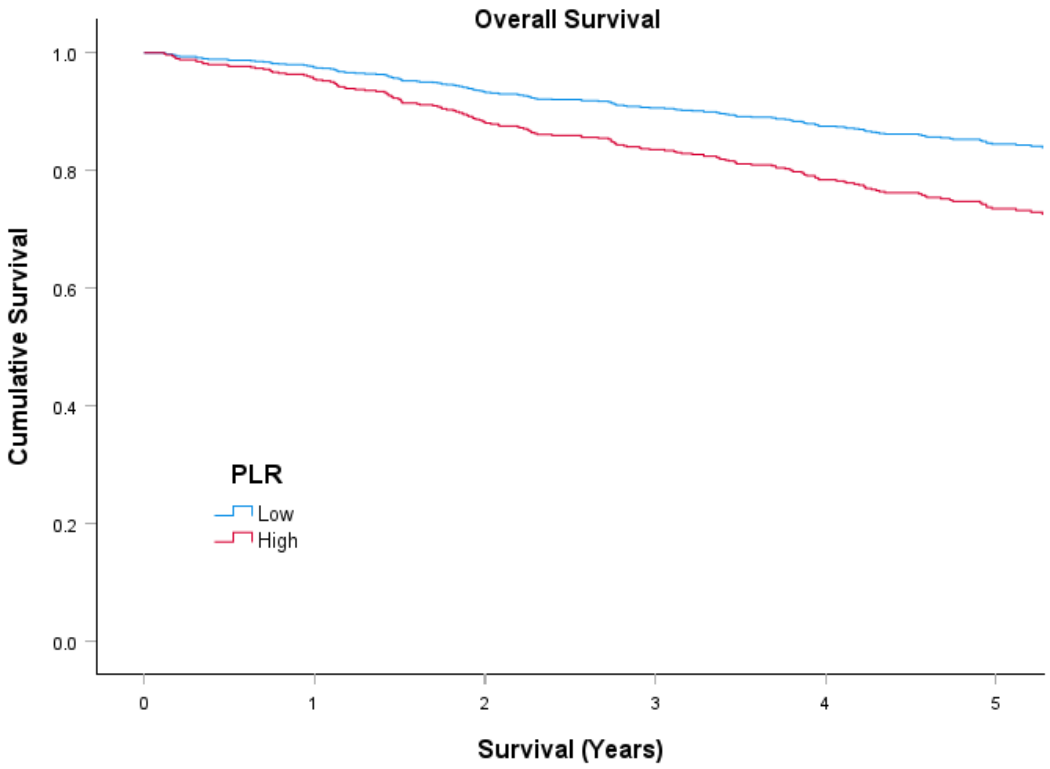


High	470	460	446	410	315	128
Low	283	266	235	213	158	62

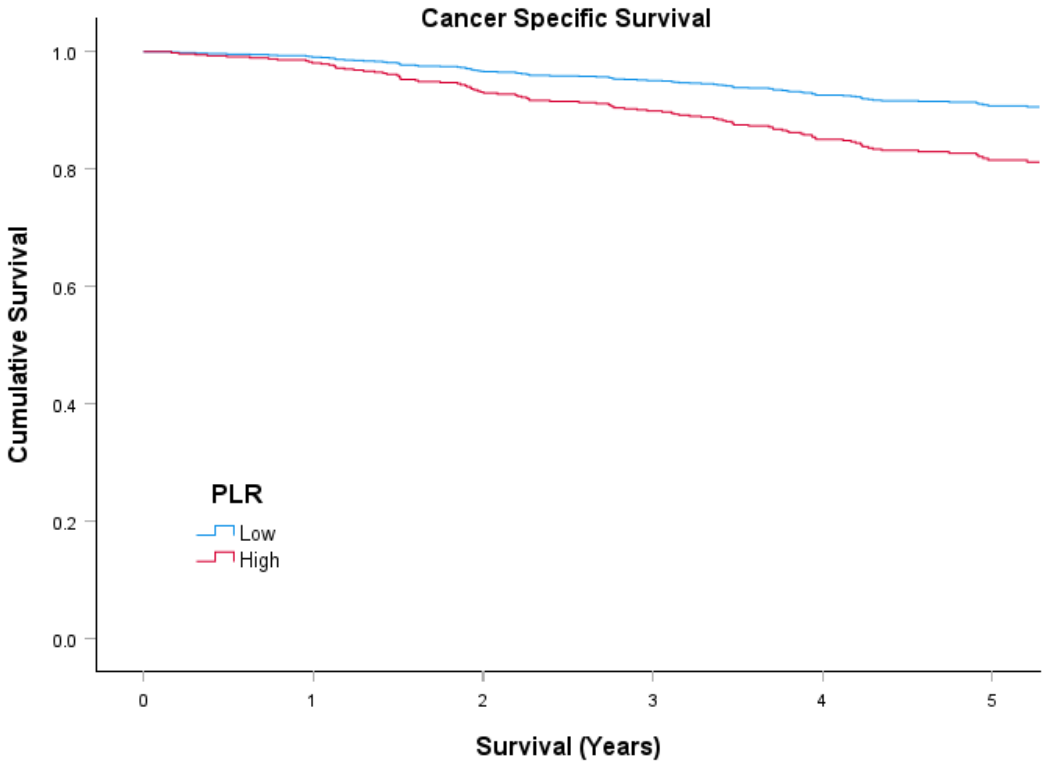


High	467	458	442	408	312	124
Low	278	260	233	212	156	59

5.3.14 Figure 5.8: Relationship between PLR and OS and CSS.



Low	322	315	302	280	216	128
High	431	411	379	343	256	101



Low	320	313	299	279	214	86
High	425	405	376	341	254	97

5.3.15 Table 5.2: Factors associated with overall survival in patients with colorectal cancer undergoing resection with a curative intent.

	Univariate			**Multivariate**		
	H.R.	95% C.I.	p-value	H.R.	95% C.I.	p-value
Age						
<62	1.0					
63-70	1.001	0.685-1.463	0.995			
≥71	1.342	0.940-1.916	0.105			
Sex						
Male	1.0					
Female	0.996	0.738-1.344	0.978			
Screen Detected						
Yes	1.0			1.0		
No	2.300	1.664-3.181	<0.001	1.670	1.133-2.463	0.01
SIMD						
Non-deprived	1.0					
Deprived	1.257	0.936-1.689	0.128			
Presentation						
Elective	1.0			1.0		
Emergency	3.409	2.395-4.854	<0.001	2.065	1.359-3.136	<0.001
Tumour Site						
Colon	1.0					
Rectum	1.029	0.748-1.415	0.860			
TNM Stage						
I	1.0			1.0		
II	1.583	1.005-2.494	0.048	1.162	0.695-1.942	0.567
III	2.729	1.782-4.179	<0.001	2.370	1.473-3.811	<0.001
IV	9.360	5.579-15.702	<0.001	7.297	4.106-12.967	<0.001
ASA						
Low	1.0			1.0		
High	1.826	1.330-2.508	<0.001	1.271	0.888-1.819	0.190
Lee Index						
Low	1.0					
High	1.331	0.941-1.884	0.106			
Charlson Index						
Low	1.0			1.0		
High	1.756	1.290-2.392	<0.001	1.612	1.140-2.280	0.007
NLR						
Low	1.0			1.0		
Moderate	1.588	1.128-2.235	0.008	0.943	0.605-1.469	0.796
High	2.382	1.626-3.491	<0.001	0.601	0.339-1.064	0.081
LMR						
High	1.0			1.0		
Low	2.038	1.514-2.742	<0.001	1.544	1.107-2.154	0.011
PLR						
Low	1.0			1.0		
High	1.827	1.326-2.519	<0.001	1.346	0.921-1.968	0.125

5.3.16 Table 5.3: Factors associated with cancer specific survival in patients with colorectal cancer undergoing resection with a curative intent.

	Univariate			**Multivariate**		
	H.R.	95% C.I.	p-value	H.R.	95% C.I.	p-value
Age						
<62	1.0					
63-70	0.741	0.461-1.189	0.214			
≥71	0.875	0.556-1.377	0.563			
Sex						
Male	1.0					
Female	1.036	0.702-1.528	0.859			
Screen Detected						
Yes	1.0			1.0		
No	2.763	1.776-4.298	<0.001	1.847	1.144-2.983	0.012
SIMD						
Non-deprived	1.0					
Deprived	1.020	0.695-1.495	0.920			
Presentation						
Elective	1.0			1.0		
Emergency	5.141	3.388-7.801	<0.001	2.399	1.507-3.820	<0.001
Tumour Site						
Colon	1.0					
Rectum	1.208	0.787-1.853	0.388			
TNM Stage						
I	1.0			1.0		
II	2.153	0.980-4.730	0.056	1.533	0.689-3.410	0.295
III	6.405	3.149-13.027	<0.001	4.884	2.374-10.049	<0.001
IV	30.064	14.054-64.313	<0.001	19.917	9.099-43.594	<0.001
ASA						
Low	1.0					
High	1.321	0.868-2.009	0.194			
Lee Index						
Low	1.0					
High	1.245	0.784-1.977	0.354			
Charlson Index						
Low	1.0					
High	1.293	0.843-1.983	0.240			
NLR						
Low	1.0			1.0		
Moderate	1.513	0.969-2.361	0.068	0.853	0.487-1.494	0.579
High	2.368	1.448-3.875	<0.001	0.664	0.340-1.298	0.231
LMR						
High	1.0			1.0		
Low	1.969	1.340-2.893	<0.001	1.304	0.855-1.987	0.218
PLR						
Low	1.0			1.0		
High	2.110	1.374-3.240	<0.001	1.578	1.018-2.444	0.041

5.4 Discussion

The present study provides a comprehensive analysis of outcome in patients diagnosed with CRC following an invite to participate in the first round of the Scottish Bowel Screening Programme in our geographical area. It has identified that patients with screen-detected disease have tumours of an earlier TNM stage, have lower ASA scores, one measure of co-morbidity, and have a lower SIR as measured by NLR, LMR and PLR. Additionally, a raised SIR as measured by LMR has been associated with poorer OS, and a raised SIR as measured by PLR with poorer CSS, independent of screening status.

It has been well established that patients with screen-detected CRC have improved outcomes as compared to their non-screen-detected counterparts(3, 4, 6, 49, 174, 175, 231). Earlier stage of presentation is certainly a key determinant of these improved outcomes. Indeed, in the current study it was confirmed that patients with screen-detected disease have significantly lower TNM staging and less emergency operations than those with non-screen-detected disease. Additionally, previous work has shown that screen-detected patients undergoing resection have less venous invasion and less peritoneal and margin involvement(49, 231). However, there are a number of inherent differences between screen-detected and non-screen-detected patients which may also contribute to improved outcome. Lower uptake of bowel screening has been shown to be associated with younger age, male sex and socioeconomic deprivation(49). In agreement with previous work(49, 174, 231, 236), screen-detected patients in this study were more likely to be male, less likely to have rectal cancers and there was a non-significant trend towards lower socioeconomic deprivation. Comorbidity and systemic inflammation are host factors that, to date, have not been compared in detail between screen-detected and non-screen-detected patients.

Three, previously validated measures of comorbidity were used in the current study: ASA, Lee and Charlson indices. Patients with screen-detected disease were significantly less co-morbid as measured by the ASA only. While a lower proportion of screen-detected patients had a high Lee Index co-morbidity score, this did not reach statistical significance (16.3% screen-detected vs 21.9% non-screen-detected; p=0.054). The reason behind this disparity in ASA scores is likely multifactorial and may reflect either the underlying difference in co-morbidity between those that choose to participate in the screening programme, or the morbidity associated with presenting with more advanced disease.

The impact of co-morbidity on bowel cancer screening uptake has been previously studied. A cross-sectional study which focussed on the Barcelona population-based colorectal cancer screening programme included 36,208 patients from 10 primary care centres with 17,404 (48%) of those participating in screening. Non-participants were significantly more likely to be male, socioeconomically deprived, smokers, have high risk alcohol intake, be obese or be in the highest co-morbidity group. Having three or more dominant chronic diseases was associated with lower participation in the screening programme (incidence rate ratio IRR 0.76, 95% CI 0.65-0.89; p=0.001)(237). In addition, there is evidence that co-morbidity may be associated with non-participation in breast and cervical cancer screening programmes(238). It is therefore conceivable that significant co-morbidity could act as a barrier to participating in the Scottish Bowel Screening Programme.

One previous study has examined the impact of screen-detection and co-morbidity on postoperative morbidity in patients undergoing resection for CRC. In this retrospective study from Spain of just under 200 patients, there were no significant differences between the screen-detected and non-screen-detected groups in terms of ASA or Charlson Index, however the percentage of patients with low ASA scores (I or II) was greater in the screen-detected group(236).

A plethora of evidence has linked poorer prognosis in CRC with the presence of a raised SIR. A heightened SIR is associated with adverse prognostic features including higher TNM staging(239, 240), poorly differentiated tumours(235, 239, 240), the presence of venous invasion(235, 239), perineural invasion(241), peritoneal involvement(235, 239), margin involvement(235, 239), emergency presentation(240) and tumour perforation(235, 239). Furthermore, a raised SIR has been shown to independently predict OS and CSS in patients with both primary resectable(187, 235, 239-247) and metastatic CRC(248-252), including in large systematic review and meta-analyses(253-257).

The current study has, for the first time, compared SIR between screen-detected and non-screen-detected CRC patients and examined its impact on outcome. A broad panel of validated markers of SIR (NLR, LMR and PLR) were used and indeed, all three markers confirmed significantly less systemic inflammation amongst screen-detected patients. Additionally, on multivariate survival analysis, LMR was able to independently predict OS and PLR was able to predict CSS. Simultaneously, screen-detection retained significance as an independent predictor of both OS and CSS. We can therefore conclude that screen-detected patients have less systemic inflammation and that, along with other screen-detected benefits including earlier staging at diagnosis, less deprivation and lower comorbidity, this may be one factor which contributes to the improved outcomes seen within this group. However, while there is a relationship between screen-detection and a lower systemic inflammatory response, it is important to note that both represent independent and valuable prognostic markers. Therefore, measures of the SIR remain valid predictors of survival in screen-detected patients as well as non-screen-detected patients. Additionally, further work is required to refine the inherent differences between screen-detected and non-screen-detected patients, both in terms of host and tumour factors.

The present study has a number of strengths. A comprehensive cohort of both screen-detected and non-screen-detected CRC patients diagnosed during the same period has been

formed. Access to Scottish Bowel Screening Programme data allowed the identification of all screen-detected patients, while the use of cancer registries ensured capture of non-screen-detected patients diagnosed via symptomatic pathways at the same time. Extensive, manual review of case notes has allowed a high level of detail regarding comorbid disease. This is the first study to compare the SIR between screen-detected and non-screen detected patients. By performing multivariate survival analysis with a long median follow-up of 63 months and with an extensive list of covariables, we have been able to establish the impact of SIR on outcomes in the Scottish Bowel Screening Programme. Limitations of the study include its retrospective nature such that ASA was missing for 14.7% of patients and pre-operative blood count for the purposes of calculating NLR, LMR and PLR was missing for 1.2% of patients. The modified Glasgow Prognostic Score is another widely validated measure of systemic inflammatory response that utilises C reactive protein (CRP) and albumin levels, a positive and a negative acute phase reactant protein respectively. Unfortunately, we were unable to include this measure due lack of data. As no statistical correction was made to the χ^2 analysis to account for the multiple comparisons made, an increased risk of type I errors may be anticipated. However, by assessing significant variables with multivariate cox regression survival analysis, the impact of potential false positives is negated. Additionally, while we have tried to account for potential confounding by performing multivariate analysis, the included list of covariables is not exhaustive and missing information, notably smoking status, has not been accounted for. Finally, the effect of lead-time bias, where earlier detection artificially lengthens a patient's survival following a cancer diagnosis, has not been taken into account. However, adjusting for this confounder within the context of a retrospective cohort study is complex and out with the scope of the present study.

In conclusion, patients with screen-detected disease have tumours of an earlier stage, have lower ASA scores and are less likely to have evidence of a SIR than their non-screen-

detected counterparts. Despite this, after adjusting for a broad range of covariables, both non-screen-detection and a raised SIR as measured by LMR and PLR, retained significance as independent predictors of poorer OS and CSS, respectively. Further work is required to refine the inherent differences between screen-detected and non-screen-detected patients with regards to the SIR.

6 Management of malignant colorectal polyps and T1 colorectal cancers: a 10-year, prospective observational study.

6.1 Introduction

In chapters 2 and 3 it was shown that the faecal immunochemical test (FIT) can aid with colorectal cancer (CRC) detection in symptomatic patients(188, 196). Indeed, in chapter 4, a raised faecal haemoglobin (f-Hb) was also found to correlate with premalignant colorectal polyps(258). As our ability to predict CRC risk in symptomatic patients improves and with the introduction of Bowel Screening, there is likely to be an increase in the number of early-stage CRCs diagnosed (259). In chapter 5 and 6, the impact of the Bowel Screening Programme was emphasised, with approximately 40% of screen-detected patients undergoing resection having stage I disease as compared to 24% of those with non-screen-disease(260, 261). A proportion of these early CRCs include those termed malignant polyps. A malignant polyp is one which contains adenocarcinoma with evidence of invasion through the muscularis mucosae and into but not beyond the submucosa (T1 staged)(147, 262). These account for 10% of all screen-detected CRCs(259) and with advancing endoscopic technology, many of these can be resected at colonoscopy(147, 263). This has created a new management dilemma: the recurrence risk associated with leaving residual malignant cells within the bowel wall or regional lymph nodes, must be weighed against the morbidity associated with progressing to formal colorectal resection(147).

The evidence on which the management of these patients is based is limited, retrospective and heterogenous in nature and no randomised control trials exist. Overall, malignant polyps are associated with a low risk of lymph node metastasis, disease recurrence and cancer-specific mortality(147, 263). Therefore, large studies are required to identify factors associated with heightened risk. The most widely reported risk factors include submucosal

venous invasion (SMVI), submucosal lymphatic invasion (SMLI) (147, 264, 265), poor differentiation(147, 266, 267), positive endoscopic polypectomy resection margin (≤1mm clearance from malignant cells)(147, 264, 268) and mucinous-subtype CRCs(269). Others include submucosal tumour depth >1000μm(265), presence of tumour budding(265, 270) and a high Haggitt(147, 271) or Kikuchi level(147, 272). The latter two are classification systems used to describe the degree of invasion arising from a malignant polyp. Haggitt level applies to pedunculated malignant polyps only: in level 1 carcinoma invades into the submucosa but is limited to the head of polyp, in level 2 carcinoma invades to the neck (junction between the head and stalk), level 3 carcinoma invades the stalk and level 4 carcinoma invades the submucosa of the bowel wall below the stalk but remains above the muscularis propria(147, 271). Conversely, Kikuchi level applies to sessile malignant polyps: in Sm1 carcinoma invades the upper third of the submucosa, Sm2 carcinoma invades the middle third of the submucosa and Sm3 carcinoma invades the lower third of the submucosa(147, 272). Notably, even with the presence of high-risk features, the chance of residual cancer being found at the polypectomy resection site or in locoregional lymph nodes at formal resection, is low(273). Therefore, it is important to thoroughly discuss operative morbidity, possibility of a permanent stoma and sexual/ urinary dysfunction even where high risk features are present, to ensure an informed decision is made.

While large retrospective studies have identified risk factors associated with an increased risk of lymph node metastases or disease recurrence, there is a distinct paucity of prospective data. The aim of the current study was therefore to describe the management and outcome of patients with T1 polyp CRCs in a large, tertiary teaching hospital, collected over a 10-year period and validate previously identified risk factors for lymph node metastases, recurrence and cancer-specific survival (CSS) in this prospective cohort.

6.2 Methods

6.2.1 Study Design, Setting and Participants

A prospective observational study was conducted. All patients diagnosed with T1 CRC between March 2007 and March 2017 at the Glasgow Royal Infirmary were prospectively entered into the study, with the finalised histopathological staging used to define T1 tumours. Patients were identified from the local cancer registry to ensure no missed cases. Caldicott guardian approval was given by NHS Greater Glasgow & Clyde to safeguard the data with ethical approval waived for the purposes of service development and results reported according to STROBE guidelines(274).

6.2.2 Variables and Data Sources

To obtain patient demographics and outcomes cross-referencing of the NHS Clinical Portal was performed with the community health index number used as the linkage variable. This allowed access to clinic letters, colonoscopy reports, operation notes and pathology records. Variables collected included age at time of primary procedure, sex, tumour location, polyp morphology (pedunculated or sessile), whether polypectomy was performed, whether this was whole or piecemeal and whether a definitive procedure was performed (formal colorectal resection or rectal local excision). Presence of recognised risk factors for residual disease or recurrence were documented: SMVI, SMLI, poor differentiation, mucinous-subtype, submucosal depth >1,000μm, Haggitt level, Kikuchi level and a positive endoscopic resection margin (≤1mm clearance from malignant cells). Outcomes recorded were presence of lymph node involvement (where a formal resection was performed), disease recurrence and cancer-specific mortality.

6.2.3 Data Analysis and Statistical Methods

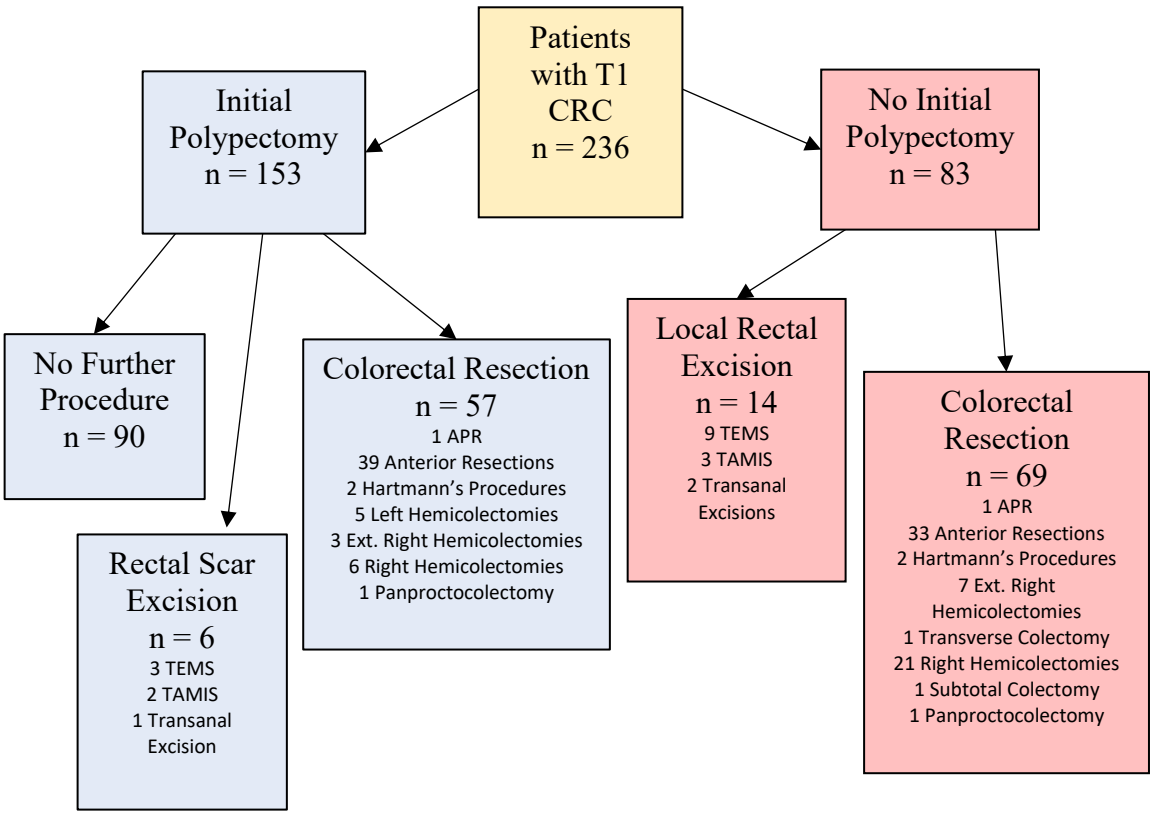
For the purposes of analysis patients were divided firstly by whether polypectomy was performed and secondly by method of definitive management: no further procedure, rectal local excision (trans-anal endoscopic microsurgery (TEMS), trans-anal minimally invasive surgery (TAMIS) or trans-anal excision) or formal colorectal resection. This produced five treatment groups for comparison. Covariables were compared using crosstabulation and the χ^2 test for linear trend. A value of $p<0.05$ was considered statistically significant. To identify variables which independently predicted lymph node metastases binary logistic regression was performed, allowing calculation of odds ratios (ORs) and 95% confidence intervals (95% CIs). To identify variables which independently predicted disease recurrence and CSS, cox regression analysis was used with resultant hazard ratios (HRs) and 95% CIs presented. In all cases, covariables $p<0.1$ on univariate analysis were entered into a multivariate model using the backwards conditional method in which variables with a significance of $p>0.1$ were removed from the model in a stepwise fashion. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, USA).

6.3 Results

6.3.1 Participants and Outcomes

Between March 2007 and March 2017, 236 patients were diagnosed with a T1 CRC at the Glasgow Royal Infirmary. 5 patients had two synchronous T1 CRCs and 1 patient had three. Median age was 68 years (interquartile range (IQR) 61-75) and 103 (43.6%) were female. 113 (47.9%) were screen-detected while 123 (52.1%) were diagnosed via symptomatic or surveillance pathways. Figure 6.1 shows the management pathway of all patients, including division into our five predefined management groups. A comparison of demographics, pathology and outcomes between the groups can be seen in Table 6.1. Overall, 9 of 126 (7.1%) patients who underwent resection had lymph node involvement. With a median follow-up of 7.4 years (IQR 5.0-9.9 years), 10 of 236 (4.2%) patients developed recurrent disease and 7 (3.0%) died of CRC.

6.3.2 Figure 6.1: Management pathway of all 236 patients with T1 colorectal cancer.



6.3.3 Table 6.1: Comparison of demographics, pathological characteristics and recognised risk factors for residual/ recurrent disease between all five treatment groups.

	All	Group I: Polypectomy Only	Group II: Excision of Rectal Scar after Polypectomy	Group III: Polypectomy then Colorectal Resection	Group IV: Rectal Excision Only	Group V: Colorectal Resection Only	P (Group I vs. Group III vs. Group V comparison)
Total	236	90	6	57	14	69	
Sex							
Male	133 (56%)	60 (67%)	4 (67%)	28 (49%)	8 (57%)	33 (48%)	
Female	103 (44%)	30 (33%)	2 (33%)	29 (51%)	6 (43%)	36 (52%)	0.023
Age (years) Median (range)	68 (27-93)	71 (46-93)	65 (57-88)	63 (27-79)	73 (56-80)	69 (32-83)	<0.001
Location							
Rectum	73 (31%)	23 (26%)	6 (100%)	13 (23%)	14 (100%)	17 (25%)	
Sigmoid Colon	109 (46%)	60 (67%)	0 (0%)	29 (51%)	0 (0%)	20 (29%)	
Proximal	54 (23%)	7 (8%)	0 (0%)	15 (26%)	0 (0%)	32 (46%)	<0.001
Diagnosis							
Screen Detected	113 (48%)	45 (50%)	4 (67%)	29 (50%)	5 (36%)	30 (43%)	
Symptomatic	123 (52%)	45 (50%)	2 (33%)	28 (49%)	9 (64%)	39 (57%)	0.639
Morphology							
Pedunculated	70 (30%)	45 (50%)	0 (0%)	16 (28%)	1 (7%)	8 (12%)	
Sessile	166 (70%)	45 (50%)	6 (100%)	41 (72%)	13 (93%)	61 (88%)	<0.001
Polypectomy							
Whole	115 (75%)	76 (84%)	4 (67%)	35 (61%)	NA	NA	
Piecemeal	38 (25%)	14 (16%)	2 (33%)	22 (39%)	NA	NA	0.002
Polyp Size (mm) Median (range)	20 (5-75)	16 (5-42)	14 (8-21)	17 (5-50)	35 (20-70)	25 (5-75)	<0.001
SMVI							
Present	57 (24%)	13 (14%)	3 (50%)	11 (19%)	8 (57%)	22 (32%)	
Absent	149 (63%)	61 (68%)	3 (50%)	34 (60%)	5 (36%)	46 (67%)	
Not Reported	30 (13%)	16 (18%)	0 (0%)	12 (21%)	1 (7%)	1 (1%)	0.124
SMLI							
Present	23 (12%)	10 (11%)	0 (0%)	6 (11%)	3 (21%)	4 (6%)	
Absent	169 (72%)	63 (70%)	6 (100%)	33 (58%)	10 (71%)	57 (83%)	
Not Reported	44 (19%)	17 (19%)	0 (0%)	18 (32%)	1 (7%)	8 (12%)	0.305
Differentiation							
Poor	10 (4%)	3 (3%)	0 (0%)	4 (7%)	0 (0%)	3 (4%)	
Moderate	186 (79%)	70 (78%)	6 (100%)	46 (81%)	10 (71%)	54 (78%)	
Well	20 (9%)	7 (8%)	0 (0%)	1 (2%)	2 (14%)	10 (15%)	
Not Reported	20 (9%)	10 (11%)	0 (0%)	6 (11%)	2 (14%)	2 (3 %)	0.561
Mucinous							
Yes	11 (5%)	4 (4%)	0 (0%)	2 (4%)	1 (7%)	4 (6%)	
No	225 (95%)	86 (96%)	6 (100%)	55 (97%)	13 (93%)	65 (94%)	0.826
Submucosal							
Depth	24 (10%)	13 (14%)	0 (0%)	7 (12%)	3 (21%)	1 (1%)	
>1mm	9 (4%)	5 (6%)	0 (0%)	0 (0%)	2 (14%)	2 (3%)	
≤1mm	203 (86%)	72 (80%)	6 (100%)	50 (88%)	9 (64%)	66 (96%)	NA
Not Reported							
Haggitt Level							
4	4 (2%)	1 (1%)	0 (0%)	0 (0%)	0 (0%)	3 (4%)	
3	14 (6%)	4 (4%)	0 (0%)	7 (12%)	1 (7%)	2 (3%)	
2	12 (5%)	7 (8%)	0 (0%)	4 (7%)	0 (0%)	1 (1%)	
1	9 (4%)	6 (7%)	0 (0%)	0 (0%)	0 (0%)	3 (4%)	
Not Reported	197 (84%)	72 (80%)	6 (100%)	46 (81%)	13 (93%)	60 (87%)	NA
Kikuchi Level							
3	14 (6%)	0 (0%)	0 (0%)	4 (7%)	4 (29%)	6 (9%)	
2	8 (3%)	1 (1%)	0 (0%)	2 (4%)	4 (29%)	2 (3%)	
1	8 (3%)	4 (4%)	1 (17%)	0 (0%)	2 (14%)	1 (1%)	
Not Reported	206 (87%)	85 (94%)	5 (83%)	51 (90%)	4 (29%)	60 (87%)	NA
Polyp Margin							
≤1mm	94 (61%)	38 (42%)	6 (100%)	50 (88%)	NA	NA	
>1mm	59 (39%)	52 (58%)	0 (0%)	7 (12%)	NA	NA	<0.001
≥1 Risk Factor Exc. Margin Involvement	111 (47%)	38 (42%)	3 (50%)	25 (44%)	12 (86%)	33 (48%)	NA

≥1 Risk Factor Inc. Margin Involvement	164 (69%)	58 (64%)	6 (100%)	55 (97%)	NA	NA	NA
Residual Disease at Polypectomy Site	1 (0.5%)	NA	0 (0%)	1 (2%)	NA	NA	NA
EMVI	2 (1%)	NA	NA	1 (2%)	NA	1 (1%)	NA
Lymph Node Involvement	9 (4%)	NA	NA	3 (5%)	NA	6 (9%)	NA
Median Follow-up (Years)	7.2	7.2	6.0	7.7	6.7	7.6	NA
Recurrence	10 (4%)	0 (0%)	0 (0%)	4 (7%)	3 (21%)	3 (4%)	NA
CRC Death	7 (3%)	0 (0%)	0 (0%)	4 (7%)	1 (7%)	2 (3%)	NA

6.3.4 Group I - Polypectomy Only

90 patients were managed with polypectomy only. 38 of 90 (42.2%) had ≥1 risk factor excluding a positive polypectomy resection margin and 58 (64.4%) had ≥1 risk factor of any type. The reason for not proceeding to resection in these 58 patients was: 32 (55.2%) unfit for resection, 9 (15.5%) MDT decision, 6 (10.3%) patient choice, 2 underwent chemoradiotherapy and 1 radiotherapy instead to avoid abdominoperineal resection of the rectum (APR) and 8 (13.8%) unclear. Of 38 patients with an involved polypectomy resection margin, 31 (81.6%) had a colonoscopy/ sigmoidoscopy site check within 6 months. Long-term follow-up varied but most had colonoscopy, CT and clinic review. With a median follow-up of 7.2 years, 0 patients developed recurrent disease nor died of CRC.

6.3.5 Group II – Polypectomy followed by Excision of Rectal Scar

6 patients proceeded from rectal polypectomy to local rectal excision. All 6 polyps were sessile. All 6 had a positive polypectomy resection margin. 3 had additional risk factors. 0 patients had residual disease found within their local rectal excision specimen. With a median follow-up of 6.0 years, 0 patients developed recurrent disease nor died of CRC.

6.3.6 Group III – Polypectomy followed by Formal Colorectal Resection

57 patients proceeded from polypectomy to formal surgical resection. 25 of 57 (43.9%) patients had ≥1 risk factor excluding a positive polypectomy margin and 55 (96.5%) had ≥1 risk factor of any type. Following pathological examination, 5 of 57 (9%) resection specimens were found to have residual disease: 1 (1.8%) small focus at polypectomy site, 1 (1.8%) case of extramural venous invasion (EMVI) and 3 (5.3%) patients had lymph node involvement (T1N1). All 5 had an involved polypectomy margin and 3 had another risk factor. With a median follow-up of 7.7 years, 4 (7.0%) patients developed disseminated metastatic disease. None of these 4 patients had residual tumour in their resection specimens, including no nodal disease. Median survival of these 4 patients was 7.9 years and only 1 patient died before 5 years giving a 5-year CSS for this group of 98.2%.

6.3.7 Group IV – Rectal Excision Only

14 patients underwent rectal local excision alone. 12 (85.7%) had ≥1 risk factor. 1 patient received chemoradiotherapy and 2 radiotherapy alone. 0 patients had residual disease found within their local rectal excision specimen. With a median follow-up of 6.7 years, 3 (21.4%) developed recurrent disease. 1 patient died at 6 years, 1 was lost to follow-up at 3.5 years and the final patient is alive at 10 years with a 5-year CSS for this group of 100%.

6.3.8 Group V – Formal Colorectal Resection Only

69 patients proceeded directly to formal colorectal resection. 33 of 69 (47.8%) had ≥1 risk factor. The reason for no initial polypectomy in these patients were: 20 had lesions too large for endoscopic excision (≥30mm), 7 lesions would not raise on submucosal injection, 4 had other technical reasons making complete endoscopic resection impossible (excessive looping, lesion on a poorly accessible fold, incomplete colonoscopy but large polyp found

on CT colon), 3 patients declined attempted endoscopic/ local resection, 5 patients were over-staged by imaging (MRI or endoanal ultrasound), 7 had other reasons for resection (polyposis, inflammatory bowel disease, colovesical fistula), 14 had resection based on MDT recommendation and 8 unknown. With a median follow-up of 7.6 years, 3 (4.3%) patients developed recurrent disease and 2 (2.9%) died from recurrent CRC, both after 5 years, giving a 5-year CSS of 100%.

6.3.9 Group Comparisons

A formal comparison was made between the three main groups: polypectomy only (group I), polypectomy followed by formal colorectal resection (group III) and formal colorectal resection only (group V) (Table 6.1). Undergoing polypectomy only was associated with male sex ($p=0.028$), older age ($p<0.001$) and pedunculated polyps ($p<0.001$). Proceeding from polypectomy to formal colorectal resection was associated with younger age ($p<0.001$), piecemeal polypectomy ($p=0.002$) and involved polypectomy resection margin ($p<0.001$). Finally, proceeding directly to formal colorectal resection was associated with proximal lesions ($p<0.001$) and larger polyps (<0.001). In terms of recognised risk factors, there was no significant difference between the groups in SMVI ($p=0.124$), SMLI ($p=0.305$), poor differentiation ($p=0.561$) or mucinous-subtype ($p=0.826$). Of note, as submucosal depth >1,000μm, Haggitt and Kikuchi level were under-reported, these were not included in the formal comparison.

6.3.10 Lymph Node Metastases – Binary Logistic Regression

On univariate analysis only poor differentiation significantly predicted lymph node metastases (OR 7.000 (95% CI: 1.118-43.840; $p=0.038$)) (Table 6.2). Polyp size ≥20mm did not reach significance but as $p<0.1$ was carried forward to multivariate analysis. On multivariate analysis only poor differentiation independently predicted lymph node metastases (OR 7.86 (95% CI: 1.117-55.328; $p=0.038$)).

6.3.11 Table 6.2: Binary logistic regression analysis of factors associated with risk of lymph node metastases.

		Lymph Node Mets		Univariate			Multivariate		
		No	Yes	OR	95% CI	P	OR	95% CI	P
Sex	Male	55 (90%)	6 (10%)	1.0					
	Female	62 (95%)	3 (5%)	0.444	0.106-1.858	0.266			
Age (Years)	Median (Range)	67 (27-83)	67 (32-79)	0.974	0.917-1.034	0.384			
Location	Rectum	27 (90%)	3 (10%)	1.0					
	Sigmoid	44 (90%)	5 (10%)	1.023	0.226-4.627	0.977			
	Proximal	46 (98%)	1 (2%)	0.196	0.019-1.976	0.167			
Morphology	Pedunculated	23 (96%)	1 (4%)	1.0					
	Sessile	94 (92%)	8 (8%)	1.957	0.233-16.443	0.536			
Polyp Size	<20mm	54 (98%)	1 (2%)	1.0			1.0		
	≥20mm	63 (89%)	8 (11%)	6.857	0.831-56.582	0.074	6.502	0.741-57.055	0.091
SMVI	No	77 (96%)	3 (4%)	1.0					
	Yes	29 (88%)	4 (12%)	3.540	0.746-16.791	0.111			
SMLI	No	83 (92%)	7 (8%)	1.0					
	Yes	10 (100%)	0 (0%)	--	--	0.999			
Poor Differentiation	No	105 (95%)	6 (5%)	1.0			1.0		
	Yes	5 (71%)	2 (29%)	7.000	1.118-43.84	0.038	7.860	1.117-55.328	0.038
Mucinous	No	111 (93%)	9 (8%)	1.0					
	Yes	6 (100%)	0 (0%)	--	--	0.999			

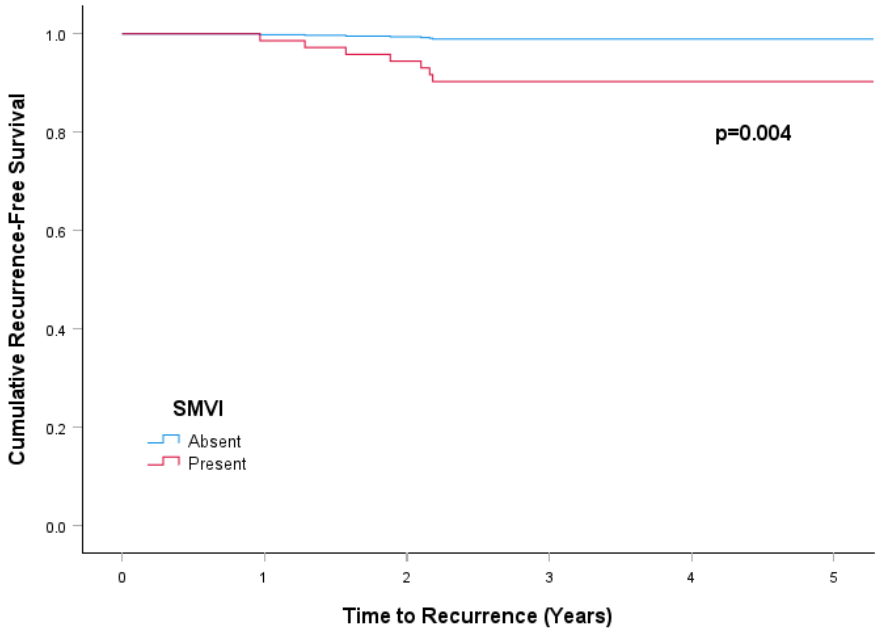
6.3.12 Disease Recurrence – Cox Regression

On univariate analysis SMVI predicted time to disease recurrence (HR 9.570 (95% CI: 1.986-46.113; p=0.005)) as did mucinous-subtype (HR 5.611 (95% CI: 1.189-26.471; p=0.029)) (Table 6.3). On multivariate analysis SMVI (HR 10.154 (95% CI: 2.087-49.396; p=0.004)) (Figure 6.2) and mucinous-subtype (HR 7.779 (95% CI: 1.566-38.625; p=0.012)) (Figure 6.3) retained significance as independent predictors of time to disease recurrence.

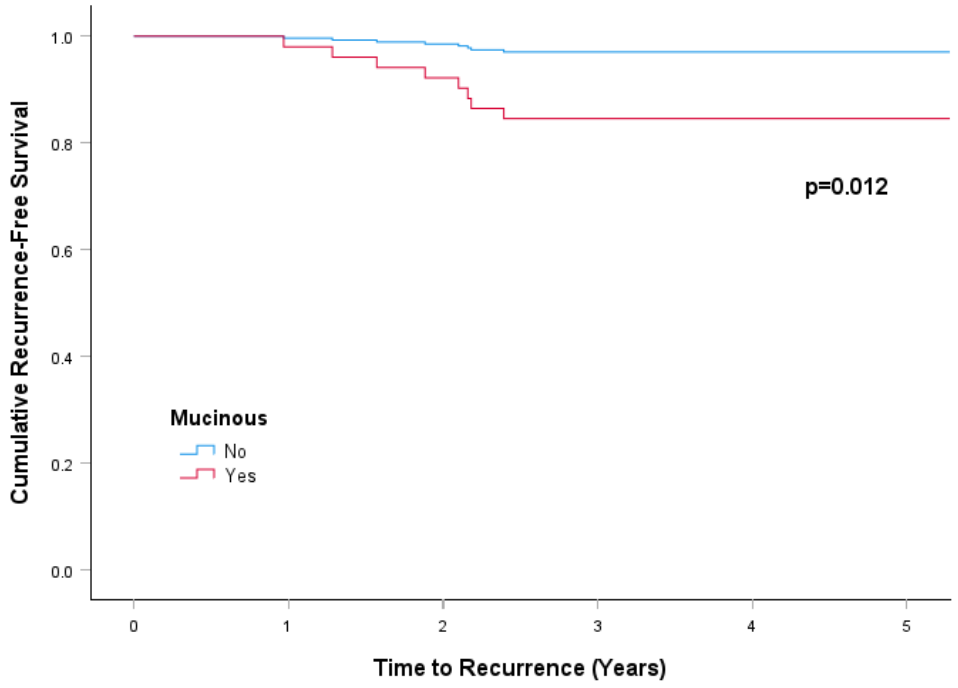
6.3.13 Table 6.3: Cox regression analysis of factors associated with time to disease recurrence.

		Recurrence		Univariate			Multivariate		
		No	Yes	HR	95% CI	P	HR	95% CI	P
Sex	Male	128 (96%)	5 (4%)	1.0					
	Female	98 (95%)	5 (5%)	1.236	0.358-4.272	0.738			
Age (Years)	Median (Range)	68 (27-93)	67 (52-78)	1.005	0.945-1.069	0.882			
Location	Rectum	68 (93%)	5 (7%)	1.0					
	Sigmoid	106 (97%)	3 (3%)	0.389	0.093-1.627	0.196			
	Proximal	52 (96%)	2 (4%)	0.518	0.100-2.668	0.431			
Morphology	Pedunculated	68 (97%)	2 (3%)	1.0					
	Sessile	158 (95%)	8 (5%)	1.711	0.363-8.060	0.497			
Polyp Size	<20mm	113 (98%)	2 (2%)	1.0			1.0		
	≥20mm	113 (93%)	8 (7%)	3.912	0.831-18.422	0.084	2.976	0.611-14.494	0.177
SMVI	No	147 (99%)	2 (1%)	1.0			1.0		
	Yes	50 (88%)	7 (12%)	9.570	1.986-46.113	0.005	10.154	2.087-49.396	0.004
SMLI	No	161 (95%)	8 (5%)	1.0					
	Yes	22 (96%)	1 (4%)	0.998	0.125-7.990	0.999			
Poor Differentiation	No	196 (95%)	10 (5%)	1.0					
	Yes	10 (100%)	0 (0%)	--	--	0.633			
Mucinous	No	217 (96%)	8 (4%)	1.0			1.0		
	Yes	9 (82%)	2 (18%)	5.611	1.189-26.471	0.029	7.779	1.566-38.625	0.012
Margin ≤1mm	No	59 (100%)	0 (0%)	1.0					
	Yes	90 (96%)	4 (4%)	--	--	0.373			
Excision	Local	107 (97%)	3 (3%)	1.0					
	Resection	119 (94%)	7 (6%)	1.915	0.495-7.407	0.347			

6.3.14 Figure 6.2: Relationship between SMVI and time to disease recurrence.



6.3.15 Figure 6.3: Relationship between mucinous-subtype and time to disease recurrence.



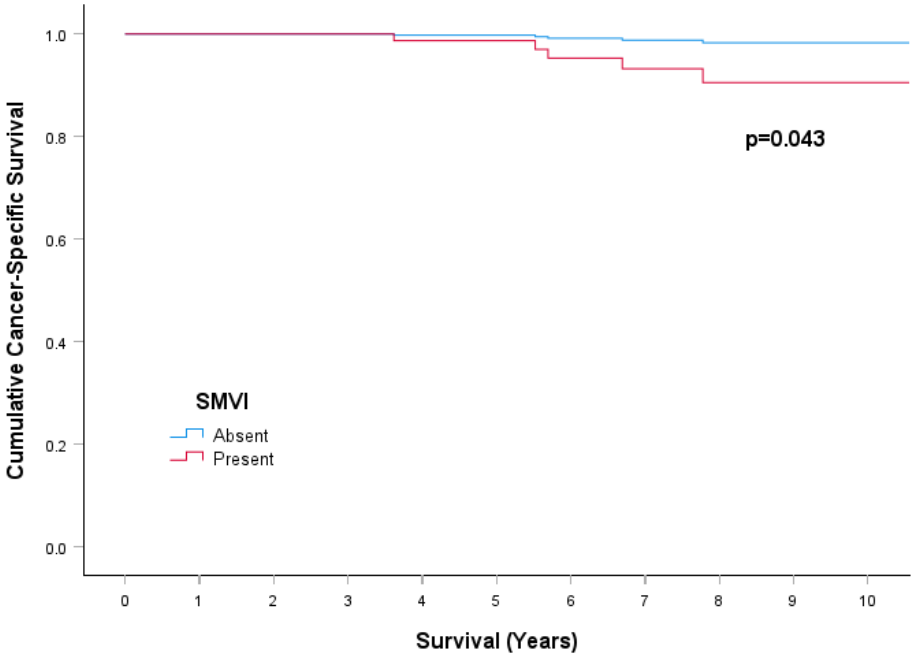
6.3.16 Cancer-Specific Survival – Cox Regression

On univariate analysis SMVI predicted CSS (HR 5.792 (95% CI: 1.056-31.754; p=0.043)) (Table 6.4) (Figure 6.4). As no other factors were predictive, multivariate analysis was not performed.

6.3.17 Table 6.4: Cox regression analysis of factors associated with cancer specific survival.

		CRC Death		Univariate			Multivariate		
		No	Yes	HR	95% CI	P	HR	95% CI	P
Sex	Male	130 (98%)	3 (2%)	1.0					
	Female	99 (96%)	4 (4%)	1.380	0.307-6.201	0.675			
Age (Years)	Median (Range)	68 (27-93)	65 (52-78)	0.993	0.921-1.071	0.857			
Location	Rectum	71 (97%)	2 (3%)	1.0					
	Sigmoid	106 (97%)	3 (3%)	1.011	0.169-6.055	0.990			
	Proximal	52 (96%)	2 (4%)	1.318	0.186-9.363	0.782			
Morphology	Pedunculated	68 (97%)	2 (3%)	1.0					
	Sessile	161 (97%)	5 (3%)	1.117	0.216-5.784	0.895			
Polyp Size	<20mm	113 (98%)	2 (2%)	1.0					
	≥20mm	116 (96%)	5 (4%)	2.586	0.500-13.362	0.257			
SMVI	No	147 (99%)	2 (1%)	1.0					
	Yes	53 (93%)	4 (7%)	5.792	1.056-31.754	0.043			
SMLI	No	164 (97%)	5 (3%)	1.0					
	Yes	22 (96%)	1 (4%)	1.468	0.171-12.628	0.727			
Poor Differentiation	No	199 (97%)	7 (3%)	1.0					
	Yes	10 (100%)	0 (0%)	--	--	0.690			
Mucinous	No	219 (97%)	6 (3%)	1.0					
	Yes	10 (91%)	1 (9%)	4.438	0.532-37.040	0.169			
Margin ≤1mm	No	59 (100%)	0 (0%)	1.0					
	Yes	90 (96%)	4 (4%)	--	--	0.387			
Excision	Local	109 (99%)	1 (1%)	1.0					
	Resection	120 (95%)	6 (5%)	4.547	0.547-37.775	0.161			

6.3.18 Figure 6.4: Relationship between SMVI and cancer-specific survival.



6.4 Discussion

This study describes the management and outcomes of 236 T1 CRC patients prospectively compiled over 10 years. Management varied, with 38.1% having polypectomy alone, 2.5% proceeding from polypectomy to rectal scar excision, 24.2% polypectomy followed by formal colorectal resection and 5.9% and 35.2% local rectal excision or segmental resection as first line treatment respectively, following lesion biopsy. Overall, outcomes were excellent with low rates of lymph node involvement (7.1%), disease recurrence (4.2%) and cancer-related mortality (3.0%). CSS was 99.6% overall at 5 years and 97.4% at 10 years.

There are a number of histopathological risk factors recognised to be associated with increased likelihood of locoregional lymph node involvement and recurrence in T1 CRCs, including intramural lymphovascular invasion(147, 264, 265), poor differentiation(147, 266, 267) and invasive characteristics such as depth of tumour within the submucosa defined using the Haggitt(147, 271) or Kikuchi(147, 272) systems dependent on lesion morphology. In addition, technical factors such as the presence of viable tumour at the lateral or deep excision margins have been reported to be associated with local recurrence(147, 264). In the current, prospective study, SMVI has emerged as a particularly important factor to consider, correlating with disease recurrence and cancer-specific mortality. Additionally, mucinous-subtype independently predicted recurrence, while poor differentiation independently predicted lymph node metastases. Conversely, the importance of polypectomy resection margin involvement has been brought into question. 94 of 153 (61.4%) patients initially managed with polypectomy had a positive margin. 38 belonged to group I (polypectomy only) with none developing recurrent disease, 6 belonged to group II (polypectomy followed by local excision of rectal scar) with no residual disease nor recurrence and 50 belonged to group III (polypectomy followed by surgical resection) with 1 found to have EMVI, 1 having residual malignant cells at

polypectomy site and 3 having involved lymph nodes). Thus only 5 of 94 (5.3%) with a positive polypectomy resection margin had evidence of locoregional residual tumour and only 4 of 94 (4.3%) developed long-term recurrence.

In recent history, presence of high-risk features prompted consideration for formal segmental resection using traditional surgical oncologic principles including ensuring clear longitudinal and circumferential margins, with high vascular ties to include locoregional lymph nodes. However, recent paradigm shifts, particularly in the treatment of rectal cancer, are increasingly leading clinicians and patients toward the addition of systemic anticancer therapies, radiation, or even moving to active surveillance strategies in place of radical resection in select cases(275). The excellent long-term outcomes demonstrated in the current study among those undergoing local excision alone would appear to support conservative management strategies. As residual disease was rare in those with an apparently involved polypectomy resection margin, endoscopic surveillance and site check for early luminal recurrence seems a notably acceptable management option for such patients, in the absence of other risk factors. Furthermore, all 6 patients who proceeded from polypectomy to rectal scar excision had a positive polypectomy resection margin, but none were found to have evidence of local residual disease. MRI surveillance may be more difficult after such a rectal excision and perhaps this approach should be avoided, instead opting for surveillance or formal resection.

Given the low likelihood of locoregional disease, disease recurrence and CRC-related death in patients with T1 CRCs, overtreatment is a concern. Formal segmental resection carries the risk of perioperative morbidity, mortality or reduction in quality of life. In the large systematic review and meta-analysis by Yeh et al(276) of 19,979 patients with T1 CRC, no significant difference was found between those undergoing endoscopic resection only and those proceeding directly to formal resection in recurrence-free survival (96.0% versus 96.7%, HR 1.28 (95% CI: 0.87-1.88)), CSS (94.8% versus 96.5%, HR 1.09 (95%

CI: 0.67-1.78)), nor overall survival (79.6% versus 82.1%, HR 1.10 (95% CI: 0.84-1.45)). However, formal resection was associated with a significantly higher rate of procedure-related adverse events (10.9% versus 2.3%; $p<0.001$). Despite this, adopting an active surveillance strategy with frequent endoscopy and imaging over a number of years comes with its own concerns including patient acceptability, morbidity or psychological stress and the potential for under-staging in selected individuals with resultant local or distant recurrence. It is worth noting that 4 of 57 (7.0%) patients who proceeded from polypectomy to formal colorectal resection in this study developed disseminated malignancy while having no residual malignant cells found at the polypectomy site nor lymph node involvement. This highlights the unpredictable biology of a proportion of these early CRCs. The identification of novel factors which may enable risk stratification with greater accuracy would aid in the decision-making and indeed, certain molecular signatures have been identified which correlate with risk of distant metastases(277).

Given the complexity of the decision-making, it seems prudent that such cases are discussed at specialist CRC multi-disciplinary team (MDT) meetings, and if possible, one focused on advanced polyps. Indeed, such an approach has been advocated by the Significant Polyp and Early Colorectal Cancer (SPECC) programme group(278). The role of these MDTs should be to determine if endoscopic or local resection is technically possible and to estimate the associated risk of recurrence, with the ultimate management of that risk left to the patient in informed discussion with the surgeon. Such strategies may reduce the rate of segmental resection while ensuring no significant increase in local and distant disease recurrence or cancer-specific mortality. For patients who do not undergo bowel resection we would recommend the following surveillance protocol. For T1 rectal cancer, which was macroscopically but not microscopically completely removed, we recommend a flexible sigmoidoscopy (to confirm absence of residual macroscopic tumour) and MRI scan (to look for mesorectal nodes) within 6 weeks and then 6 monthly for 2

years. If T1 rectal cancer is microscopically completely excised, 6-week flexible sigmoidoscopy is not necessary. If there are no risk factors for recurrence, follow-up MRI and flexible sigmoidoscopy are probably not necessary. For T1 colon cancer, which was macroscopically completely removed but had a microscopically involved margin, we recommend a flexible sigmoidoscopy/ colonoscopy (to confirm absence of residual macroscopic tumour) within 6 weeks. As recommended by the British Society of Gastroenterology and Association of Coloproctology of Great Britain and Ireland post-polypectomy and post-colorectal cancer resection surveillance guidelines(31), all CRC patients should have colonoscopy at 1 and 3 years. All patients additionally should have surveillance for metastatic disease by CT chest, abdomen and pelvis at 1, 2 and 3 years and annual carcinoembryonic antigen (CEA) check. If a patient is found to have luminal evidence of residual or recurrent disease, or if there is a suspicion of lymphadenopathy at MRI or CT surveillance, this prompts an immediate consideration for formal resection.

This study of patients undergoing treatment for T1 CRC is unique in its prospective nature with patients entered sequentially over 10 years and has a protracted follow-up. However, it must be noted that this is a purely observational study with no allocation of patients to a particular management pathway. Differences in characteristics and outcomes of the patients belonging to each management pathway have been reported, but the study did not seek to establish superiority of any pathway. There is inherent selection and reporting bias to a study of this type. Treatment decisions were made by a specialist colorectal oncology MDT, complemented by informed patient choice. As these decisions are complex, it is not possible to gauge what influence histological risk factors, patient age and comorbidity, potential for operative morbidity, tumour location and patient choice had on treatment allocation in each case. With a lack of standardised protocols or randomisation of treatment there is likely to be allocation bias. However, the results represent heterogeneous real-world practice. Many of the key risk factors including SMVI, SMLI, submucosal depth and

in particular Haggitt and Kikuchi levels were underreported. A key recommendation of this study is for universal reporting of these risk factors for T1 polyp CRCs. As no statistical correction was made to the χ^2 analysis to account for the multiple comparisons made, an increased risk of type I errors may be anticipated. However, by assessing significant variables with multivariate binary logistic regression/ cox regression, the impact of potential false positives is negated. While the study size is large for a prospective cohort of this type, it is smaller than previously published retrospective studies. With a low number of events with regards to lymph node involvement, recurrence and cancer-specific mortality, the study may be underpowered to detect significance in all risk factors assessed. However, our findings are largely concordant with larger retrospective studies, and we have filled an important gap in the literature in terms of prospective data with long follow-up. It is important that as we adopt more conservative management approaches to the management of T1 CRC polyps, that ongoing data collection and analysis is performed in a similar fashion to the current study to validate our findings and ensure no negative impact on outcomes.

In conclusion, despite 64.4% of those undergoing polypectomy alone having ≥1 recognised risk factor, there were no recurrences. Furthermore, only 5.3% of patients with a positive polypectomy margin had evidence of residual disease. Therefore, it seems feasible that those with a positive margin or single risk factor be offered endoscopic surveillance. Further studies are required to confirm these findings. This study reinforces the importance of reporting SMVI, SMLI, submucosal depth, Haggitt and Kikuchi levels for all T1 CRC polyps, and the findings highlight the need for discussion at sub-speciality MDTs to reduce unnecessary segmental resections and related morbidity, while ensuring effective surveillance and early salvage for those who recur. Patients should be offered a choice following an informed discussion.

7 Risk stratification for the detection of metachronous polyps after bowel screening polypectomy: clinical outcomes from the Integrated Technologies for Improved Polyp Surveillance Study (INCISE) cohort.

7.1 Introduction

Colorectal cancer (CRC) is known to develop from precursor lesions in the form of benign colorectal polyps(27). There are two main types of colorectal polyps with recognised malignant potential: adenomas and serrated polyps (28, 29, 31). Over an estimated 7 to 15 years a small proportion of these benign polyps become increasingly dysplastic and eventually malignant via two principal pathways: adenomas via the classic adenoma-carcinoma (~70%) sequence and sessile serrated polyps via the serrated polyp pathway (~30%) (28, 30). In chapters 2 and 3 it was shown that the faecal immunochemical test (FIT) can aid with CRC detection in symptomatic patients(188, 196). Additionally, in chapter 4, data was presented showing that a raised faecal haemoglobin (f-Hb) also correlates with premalignant colorectal polyps in symptomatic patients(258). Patients found to have a raised f-Hb as part of the Scottish Bowel Screening Programme are also more likely to have premalignant polyps found at colonoscopy and indeed, one aim of screening is to identify and remove premalignant polyps endoscopically prior to malignant transformation, thus reducing CRC incidence(3, 30).

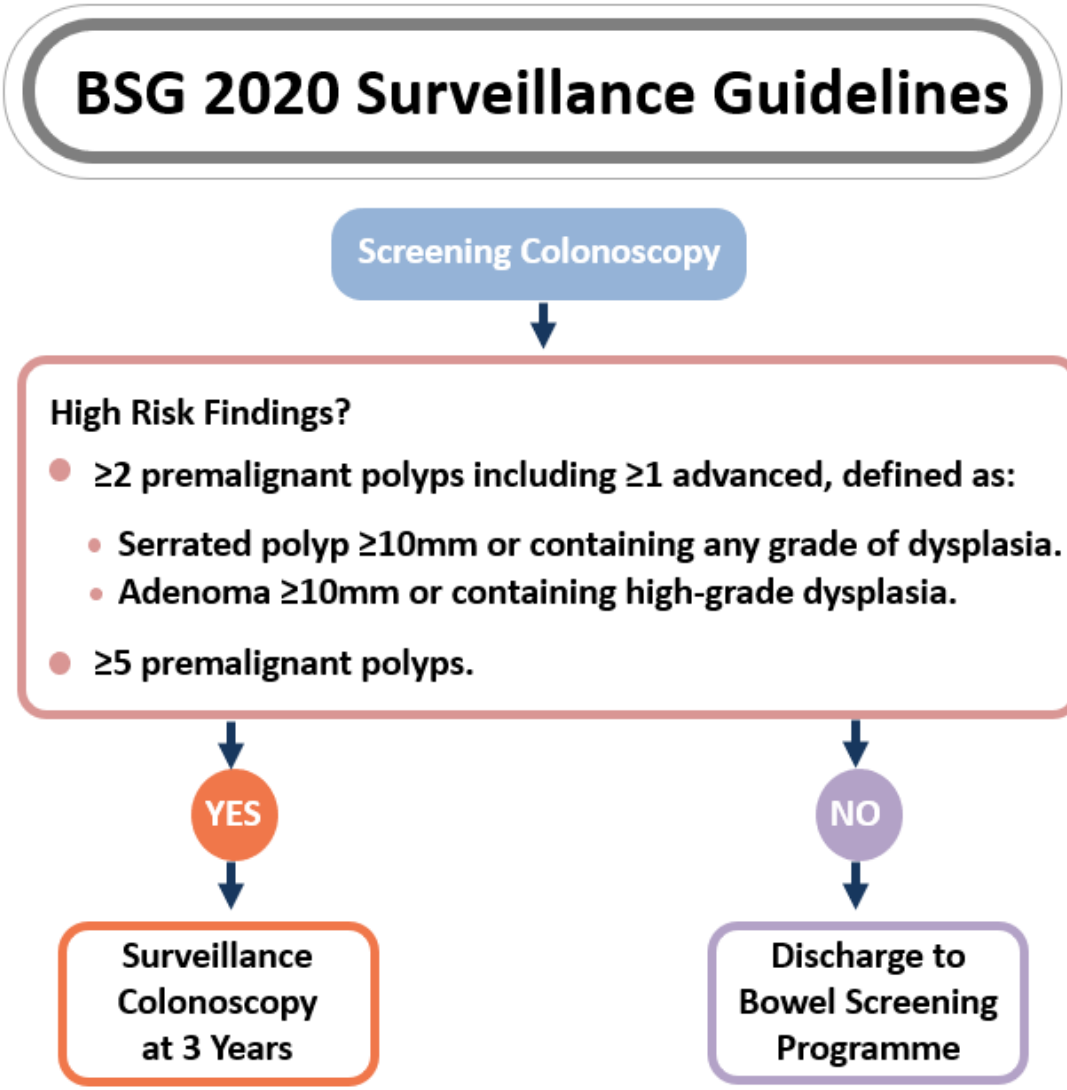
Premalignant polyps are common, occurring in 25-50% of all patients at screening age (50-74 years)(31). Whether detected via symptomatic or screening pathways, once a premalignant polyp is removed via polypectomy it is estimated that 20-50% of patients will develop further, metachronous polyps(279) and a proportion are at higher long-term risk of developing CRC(280). Therefore, in addition to ongoing participation in the Scottish Bowel Screening Programme, scheduled surveillance colonoscopy after polypectomy is widely recommended(31). However, as a large proportion of patients will

never develop metachronous polyps and as few will progress to malignancy it would be inefficient and unnecessary to offer surveillance colonoscopy to all. Additionally, given that colonoscopy is an invasive procedure with a low but significant rate of recognised complications, subjecting patients with a very low risk of developing further pathology could not be justified. Instead, patients are stratified for metachronous polyp and CRC risk based on polyp histology, grade of dysplasia, polyp size and polyp number, according to the British Society of Gastroenterology (BSG) /Association of Coloproctology of Great Britain and Ireland (ACPGBI)/Public Health England (PHE) post-polypectomy surveillance 2020 guidelines (Figure 7.1)(31), henceforth referred to as the BSG 2020 guidance. On this basis, patients are divided into high- and low-risk groups: high-risk patients are invited to surveillance colonoscopy at 3 years and low-risk patients are discharged to the screening programme. Although the number of patients qualifying for surveillance are reduced using these conventional risk measures, surveillance colonoscopy still accounts for 100,000 of the 700,000 colonoscopies performed in England each year(108). A more accurate risk stratification would allow for more efficient NHS resource allocation.

The Integrated Technologies for Improved Polyp Surveillance (INCISE) project is a large, retrospective, multi-partner collaborative study which aims to use patient characteristics, digital pathology, immunohistochemistry (IHC), genomic and transcriptomic features of index polyp tissue to predict metachronous polyp risk and refine current surveillance protocols(281). It is hoped this may relieve pressure on endoscopy services and avoid unnecessary invasive investigations in low-risk patients. The aim of the present study was to retrospectively apply the BSG 2020 guidelines to INCISE cohort patients, whose surveillance strategy following screening polypectomy was determined using previous, less conservative guidance(282, 283). By comparing metachronous polyp/ CRC rate by BSG

2020 high- and low-risk features in these patients, this study aimed to establish the baseline efficiency of current risk stratification.

7.1.1 Figure 7.1: British Society of Gastroenterology and Association of Coloproctology of Great Britain and Ireland post-polypectomy surveillance guidelines



7.2 Methods

7.2.1 Study Design and Participants

A retrospective, multicentred observational cohort study was conducted. The INCISE cohort was formed to include all patients who underwent polypectomy at screening colonoscopy in NHS Greater Glasgow & Clyde (GG&C) between May 2009 and December 2016. During the study period the Scottish Bowel Screening Programme was based on biannual guaiac faecal occult blood test (gFOBT), followed by invitation to colonoscopy in those with a positive stool test(284). Patients were only included in the current study if they had a histologically confirmed premalignant polyp (adenoma or serrated polyps, excluding diminutive rectal hyperplastic polyps <5mm), at their index screening colonoscopy. Patients must have undergone a further colonoscopy 6 months to 6 years from their index colonoscopy, to allow identification of those patients who went on to develop metachronous polyps or CRC. Patients were excluded if they were found to have CRC at their index screening colonoscopy, had a previous histological diagnosis of CRC, had a diagnosis of inflammatory bowel disease, had a known inherited polyposis or CRC syndrome, or did not have a surveillance colonoscopy within the above date ranges. Each patient was assigned a unique INCISE number and the entire, anonymised database was stored on the NHS Safe Haven platform (Safe Haven, NHS Scotland) to ensure compliance with data protection and patient confidentiality. Ethics approval was obtained for the INCISE project (GSH/20/CO/002) and the outcomes were reported according to STROBE guidelines(274).

7.2.2 Variables and Data Sources

Patient demographics, comorbidities, and medications were extracted by searching local electronic case notes with the unique Scottish community health index (CHI) number used as the linkage variable. Demographics collected included age, sex and deprivation.

Deprivation was quantified using the Scottish Index of Multiple Deprivation (SIMD) score(177). Comorbidities were recorded individually and used to calculate the Charlson comorbidity index(234). The local pathology database was used to determine the number of index polypectomy specimens and the histological subtype (adenoma versus serrated polyp), location (rectum, left-sided and right-sided colonic), size, morphology (presence or absence of villous architecture) and degree of dysplasia (high- or low-grade) of the most advanced index polyp. The BSG 2020 guidelines(31) were used to define those patients with a non-advanced index polyp (adenoma <10mm and not containing high-grade dysplasia (HGD) or serrated polyps <10mm and not containing dysplasia), those with an advanced index polyp (adenoma ≥10mm or containing HGD or a serrated polyp ≥10mm or containing any grade of dysplasia) and those deemed high-risk of developing metachronous polyps (Figure 7.1). To define outcomes for each patient, the electronic endoscopy reporting software (Unisoft Medical Systems GI Reporting Software) and electronic pathology database (TelePath) were used to determine the presence or absence of metachronous lesions at surveillance colonoscopy.

7.2.3 Outcomes of Interest

The primary study outcome was the detection of metachronous lesions (no metachronous lesions versus non-advanced lesions versus advanced lesions detected at surveillance colonoscopy). Non-advanced lesions were defined as non-advanced polyps and advanced lesions were defined as advanced polyps (as defined above) or CRC. The secondary study outcome was the detection of metachronous lesions by timing (no metachronous lesions versus early versus late metachronous lesion detection). Early metachronous lesions were defined as those detected <2 years from index polypectomy and late >2 years from index polypectomy.

7.2.4 Statistics

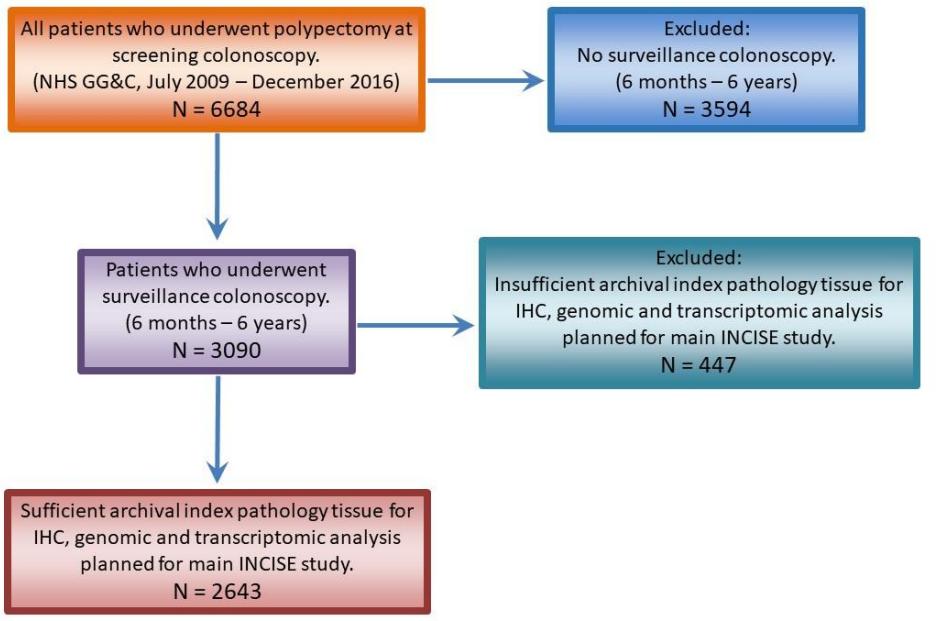
Demographics including age, sex, screening cycle, deprivation, comorbidities, medications and index polyp characteristics and location, including BSG 2020 risk categories were compared for the primary and secondary outcomes of interest using crosstabulation and the χ^2 test for categorical variables and Kruskal Wallis one-way ANOVA for continuous data. A value of $p<0.05$ was considered statistically significant. Multivariate polynomial regression was used to identify independent predictors of advanced metachronous lesion development both <2 years and >2 years from index colonoscopy.

7.3 Results

7.3.1 Study Population

Figure 7.2 shows the INCISE cohort patient selection. Of 6,684 patients who underwent polypectomy at screening colonoscopy during the study period, 3,090 underwent surveillance colonoscopy (6 months to 6 years from index) and 2643 patients were included in the final analysis. All patients in this study underwent surveillance colonoscopy, based on the guidance in use at the time(282, 283). However, applying the most recent BSG 2020 guidelines(31) to this cohort of patients, 1360 (51.5%) patients would be low-risk and would no longer qualify for surveillance. Median age was 63 years (range 50-83 years), with a male/female ratio of 2.2:1. Overall, 32.8% had a single index polyp, 54.5% had 2-4 polyps and 12.6% had 5 or more polyps. 1,730 (65.5%) patients had a polyp ≥10mm found at index scope, 285 (10.8%) had a polyp containing HGD and 1,038 (39.3%) had a polyp with villous morphology. In total, 1,757 (66.5%) patients had an advanced polyp found at index colonoscopy of which 1,693 (96.4%) were advanced adenoma and 64 (3.6%) were advanced sessile polyps.

7.3.2 Figure 7.2: Flowchart showing formation of the INCISE cohort.



NHS GG&C National Health Service Greater Glasgow and Clyde, IHC immunohistochemistry, INCISE INtegrated TeChnologies for Improved Polyp SurveillancE

7.3.3 Outcomes of Surveillance

Median time to surveillance colonoscopy was 36 months (range 6-83). At surveillance colonoscopy, 1,205 (45.6%) patients had no metachronous lesion found, while 1,438 (54.4%) were found to have any metachronous lesion. 1,051 (39.8%) patients had non-advanced polyps, 363 (13.7%) had advanced polyps and 24 (0.9%) patients were found to have CRC. 655 (45.5%) of these 1,438 patients had their lesions identified early within 2 years of index colonoscopy and 783 (54.5%) were found late after 2 years.

7.3.4 Variables Associated with Metachronous Lesion Risk

Table 7.1 shows a comparison of patient demographics and index pathology characteristics between those found to have no metachronous lesions, non-advanced metachronous polyps and those with advanced polyps or CRC (primary study outcome). Patients with advanced lesions at follow-up were older (no metachronous lesion versus non-advanced lesion versus advanced lesion: median age 63, 63 and 65 years respectively; p=0.008). Patients with either a non-advanced or advanced metachronous lesion were more likely to be male (no metachronous lesion versus non-advanced lesion versus advanced lesion: male 63.9%, 73.8% and 71.8% respectively; p<0.001), to have undergone their index screening colonoscopy in the later years of the study (p=0.037), to have congestive heart failure (p=0.037), take aspirin (p=0.035) or a statin (p=0.004). Having an increased number of index polyps was associated with a higher risk of either non-advanced or advanced metachronous lesions (no metachronous lesion versus non-advanced lesion versus advanced lesion: 5+ polyps 7.1%, 16.7% and 18.9% respectively; p<0.001). As compared to having an index polyp in the rectum, right-sided colonic index polyps were associated with a higher rate of metachronous lesions and left-sided index polyps were associated with a lower risk (p=0.001). The BSG 2020 guideline risk stratification of the index scope was significantly associated with metachronous lesion likelihood but did not differentiate those with metachronous non-advanced and advanced lesions (no metachronous lesion

versus non-advanced lesion versus advanced lesion: BSG 2020 high-risk 41.7%, 54.2% and 54.3% respectively; $p<0.001$).

Next the same comparison of demographics and index pathology characteristics was made between those found to have no metachronous lesion, an early metachronous lesion (<2 years from index colonoscopy) and a late metachronous lesion (>2 years from index colonoscopy) (Table 7.2). Patients who developed early metachronous lesions were significantly older (no metachronous lesion versus early lesion versus late lesion: median age 63, 65 and 63 years respectively; $p<0.001$) and were more likely to be taking angiotensin receptor blockers ($p=0.001$), aspirin ($p=0.023$) or a statin ($p=0.002$). Patients with either an early or late metachronous lesion were more likely to be male (no metachronous lesion versus early lesion versus late lesion: male 63.9%, 74.2% and 72.5%; respectively $p<0.001$) and to have congestive heart failure ($p=0.015$). Having an index advanced polyp was associated with a higher risk of early but not late metachronous lesions (no metachronous lesion versus early lesion versus late lesion: advanced index polyp 67.1%, 69.6% and 62.8% respectively; $p=0.02$). Having an increased number of index polyps was associated with early or late metachronous lesions (no metachronous lesion versus early lesion versus late lesion: 5+ polyps 7.1%, 25.2% and 10.7% respectively; $p<0.001$). Index villous lesions were associated with early but not late metachronous lesions ($p=0.006$). Right-sided index lesions were associated with a higher risk of both early and late metachronous lesions ($p<0.001$). BSG 2020 high risk features were associated with a higher rate of both early and late metachronous lesions, but there was a stronger association with early lesions (no metachronous lesion versus early lesion versus late lesion: BSG 2020 high-risk groups 41.7%, 64.1% and 46.0% respectively; $p<0.001$).

7.3.5 Table 7.1: Factors associated with metachronous polyp or colorectal cancer after polypectomy at index screening colonoscopy, grouped by advancement.

Variable			Development of metachronous polyp or CRC during follow-up			p
		All	No	Non-advanced Polyp	Advanced Polyp or CRC	
		2643	1205	1051	387	
Demographics (n=2643)						
Age (years)	Median (IQR)	63 (57-69)	63 (57-69)	63 (59-69)	65 (59-69)	0.008
Sex, n (%)	Male	1824 (69.0)	770 (63.9)	776 (73.8)	278 (71.8)	<0.001
Screening cycle, n (%)	’09-’11	824 (31.2)	406 (33.7)	305 (29.0)	113 (29.2)	0.037
	’11-‘13	848 (32.1)	398 (33.0)	328 (31.2)	122 (31.5)	
	’13-‘15	628 (23.8)	267 (22.2)	265 (25.2)	96 (24.8)	
	’15-‘17	343 (13.0)	134 (11.1)	153 (14.6)	56 (14.5)	
SIMD quintile ’09, n (%) (n=2375)	1	785 (33.1)	352 (32.7)	322 (33.9)	111 (31.6)	0.288
	2	417 (17.6)	177 (16.5)	176 (18.4)	65 (18.5)	
	3	390 (16.4)	180 (16.7)	156 (16.4)	54 (15.4)	
	4	328 (13.8)	137 (12.7)	133 (14.0)	58 (16.5)	
	5	455 (19.2)	229 (21.3)	163 (17.2)	63 (17.9)	
Co-morbidity (n=2643)						
MI, n (%)	Yes	143 (5.4)	59 (4.9)	69 (6.6)	15 (3.9)	0.076
CCF, n(%)	Yes	50 (1.9)	16 (1.3)	21 (2.0)	13 (3.4)	0.037
PVD, n (%)	Yes	61 (2.3)	24 (2.0)	26 (2.5)	11 (2.8)	0.562
CVA, n (%)	Yes	83 (3.1)	36 (3.0)	30 (2.9)	17 (4.4)	0.306
Dementia, n (%)	Yes	1 (0.04)	0 (0)	1 (0.1)	0 (0)	0.469
COPD, n (%)	Yes	183 (6.9)	83 (6.9)	76 (7.2)	24 (6.2)	0.791
Rheumatic disease, n (%)	Yes	34 (1.3)	18 (1.5)	13 (1.2)	3 (0.8)	0.542
PUD, n (%)	Yes	86 (3.3)	43 (3.6)	32 (3.0)	11 (2.8)	0.693
Mild liver disease, n (%)	Yes	42 (1.6)	21 (1.7)	16 (1.5)	5 (1.3)	0.806
Moderate / severe liver disease, n (%)	Yes	18 (0.7)	9 (0.7)	6 (0.6)	3 (0.8)	0.854
DM uncomplicated, n (%)	Yes	126 (4.8)	56 (4.6)	47 (4.5)	23 (5.9)	0.492
DM complicated, n (%)	Yes	10 (0.4)	4 (0.3)	5 (0.5)	1 (0.3)	0.786
Hemi/paraplegia, n (%)	Yes	10 (0.4)	4 (0.3)	4 (0.4)	2 (1)	0.876
Renal disease, n (%)	Yes	33 (1.2)	14 (1.2)	12 (1.1)	7 (2)	0.561
Any malignancy, n (%)	Yes	177 (6.7)	70 (5.8)	82 (7.8)	25 (6)	0.165
Metastatic malignancy, n (%)	Yes	8 (0.3)	2 (0.2)	5 (0.5)	1 (0.3)	0.404
HIV/AIDS, n (%)	Yes	0 (0)	0 (0)	0 (0)	0 (0)	---
Charlson Index (0-33)	Median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0.237
Medication (n=2472)						
ACE-I, n (%)	Yes	673 (27.2)	293 (25.9)	284 (29.0)	96 (26.7)	0.283
ARB, n (%)	Yes	276 (11.2)	115 (10.2)	112 (11.4)	49 (13.6)	0.186
Aspirin, n (%)	Yes	795 (32.2)	334 (29.5)	335 (34.1)	126 (35.0)	0.035
Statin, n (%)	Yes	1120 (45.3)	472 (41.7)	470 (47.9)	178 (49.4)	0.004
Steroid, n (%)	Yes	331 (13.4)	157 (13.9)	127 (12.9)	47 (13.1)	0.804
NSAIDs, n (%)	Yes	1037 (41.9)	485 (42.9)	419 (42.7)	133 (36.9)	0.114
Immunosuppressants, n (%)	Yes	62 (2.5)	34 (3.0)	17 (1.7)	11 (3.1)	0.135
Metformin, n (%)	Yes	218 (8.8)	91 (8.0)	88 (9.0)	39 (10.8)	0.274
Pathology (n=2643)						
Index polyp advanced*, n (%)	Yes	1757 (67.5)	809 (67.1)	683 (65.0)	265 (68.5)	0.372
Index Polyp number, n (%)	1	868 (32.8)	482 (40.0)	273 (26.0)	113 (29.2)	
	2-4	1441 (54.5)	638 (52.9)	602 (57.3)	201 (51.9)	

	5+	334 (12.6)	85 (7.1)	176 (16.7)	73 (18.9)	<0.001
Index polyp villous*, n (%)	Yes	1038 (39.3)	485 (40.2)	388 (36.9)	165 (40.6)	0.092
Index polyp type*, n (%)	Adenoma	2503 (94.7)	1142 (94.8)	993 (94.5)	368 (95.1)	0.891
	Serrated Polyps	140 (5.3)	63 (5.2)	58 (5.5)	19 (4.9)	
Index polyp HGD*, n (%)	Yes	285 (10.8)	124 (10.3)	114 (10.8)	47 (12.1)	0.590
Index polyp size*, n (%) (mm)	<10	913 (34.5)	408 (33.9)	375 (35.7)	130 (33.6)	0.605
	≥10	1730 (65.5)	797 (66.1)	676 (64.3)	257 (66.4)	
Index polyp location*, n (%) (n=2639)	Rectum	345 (13.1)	158 (13.1)	129 (12.3)	58 (15.0)	0.001
	Left colon	1595 (60.3)	769 (63.8)	618 (58.8)	208 (53.7)	
	Right colon	697 (26.4)	275 (22.8)	302 (28.7)	120 (31.0)	
BSG 2020 risk index procedure, n (%)	Low	1360 (51.5)	702 (58.3)	481 (45.8)	177 (45.7)	<0.001
	High	1283 (48.5)	503 (41.7)	570 (54.2)	210 (54.3)	

*Applies to the most advanced polyp if multiple removed at index procedure

7.3.6 Table 7.2: Factors associated with metachronous polyp or colorectal cancer after polypectomy at index screening colonoscopy, grouped by time of detection.

Variable			Development of metachronous polyp or CRC during follow-up			P
		All	No	Yes <2yrs	Yes >2yrs	
		2643	1205	655	783	
Demographics (n=2643)						
Age (years)	Median (IQR)	63 (57-69)	63 (57-69)	65 (59-71)	63 (58-69)	<0.001
Sex, n (%)	Male	1824 (69.0)	770 (63.9)	486 (74.2)	568 (72.5)	<0.001
Screening cycle, n (%)	'09-'11	824 (31.2)	406 (33.7)	198 (30.2)	220 (28.1)	<0.001
	'11-'13	848 (32.1)	398 (33.0)	168 (25.6)	282 (36.0)	
	'13-'15	628 (23.8)	267 (22.2)	163 (24.9)	198 (25.3)	
	'15-'17	343 (13.0)	134 (11.1)	126 (19.2)	83 (10.6)	
SIMD quintile '09, n (%) (n=2375)	1	785 (33.1)	352 (32.7)	200 (34.3)	233 (32.5)	0.185
	2	417 (17.6)	177 (16.5)	105 (18.0)	135 (18.8)	
	3	390 (16.4)	180 (16.7)	101 (17.3)	109 (15.2)	
	4	328 (13.8)	137 (12.7)	87 (14.9)	104 (14.5)	
	5	455 (19.2)	229 (21.3)	90 (15.4)	136 (19.0)	
Co-morbidity (n=2643)						
MI, n (%)	Yes	143 (5.4)	59 (4.9)	46 (7.0)	38 (4.9)	0.109
CCF, n(%)	Yes	50 (1.9)	16 (1.3)	21 (3.2)	13 (1.7)	0.015
PVD, n (%)	Yes	61 (2.3)	24 (2.0)	16 (2.4)	21 (2.7)	0.585
CVA, n (%)	Yes	83 (3.1)	36 (3.0)	24 (3.7)	23 (2.9)	0.674
Dementia, n (%)	Yes	1 (0.04)	0 (0)	0 (0)	1 (0.1)	0.305
COPD, n (%)	Yes	183 (6.9)	83 (6.9)	50 (7.6)	50 (6.4)	0.648
Rheumatic disease, n (%)	Yes	34 (1.3)	18 (1.5)	9 (1.4)	7 (0.9)	0.497
PUD, n (%)	Yes	86 (3.3)	43 (3.6)	22 (3.4)	21 (2.7)	0.545
Mild liver disease, n (%)	Yes	42 (1.6)	21 (1.7)	6 (0.9)	15 (1.9)	0.271
Moderate / severe liver disease, n (%)	Yes	18 (1.6)	9 (0.7)	5 (0.8)	4 (0.5)	0.787
DM uncomplicated, n (%)	Yes	126 (4.8)	56 (4.6)	37 (5.6)	33 (4.2)	0.430
DM complicated, n (%)	Yes	10 (0.4)	4 (0.3)	3 (1)	3 (0.4)	0.914
Hemi/paraplegia, n (%)	Yes	10 (0.4)	4 (0.3)	2 (0.3)	4 (0.5)	0.769
Renal disease, n (%)	Yes	33 (1.2)	14 (1.2)	9 (1.4)	10 (1.3)	0.922
Any malignancy, n (%)	Yes	177 (6.7)	70 (5.8)	51 (7.8)	56 (7.2)	0.221
Metastatic malignancy, n (%)	Yes	8 (0.3)	2 (0.2)	4 (0.6)	2 (0.3)	0.239
HIV/AIDS, n (%)	Yes	0 (0)	0 (0)	0 (0)	0 (0)	---

Charlson Index (0-33)	Median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0.079
Medication (n=2472)						
ACE-I, n (%)	Yes	673 (27.2)	293 (25.9)	169 (28.0)	211 (28.6)	0.387
ARB, n (%)	Yes	276 (11.2)	115 (10.2)	93 (15.4)	68 (9.2)	0.001
Aspirin, n (%)	Yes	795 (32.2)	334 (29.5)	216 (35.8)	245 (33.2)	0.023
Statin, n (%)	Yes	1120 (45.3)	472 (41.7)	303 (50.2)	345 (46.8)	0.002
Steroid, n (%)	Yes	331 (13.4)	157 (13.9)	80 (13.2)	94 (12.8)	0.778
NSAIDs, n (%)	Yes	1037 (41.9)	485 (42.9)	233 (38.6)	319 (43.3)	0.152
Immunosuppressants, n (%)	Yes	62 (2.5)	34 (3.0)	11 (1.8)	17 (2.3)	0.296
Metformin, n (%)	Yes	218 (8.8)	91 (8.0)	68 (11.3)	59 (8.0)	0.052
Pathology (n=2643)						
Index polyp advanced*, n (%)	Yes	1757 (67.5)	809 (67.1)	456 (69.6)	492 (62.8)	0.020
Index Polyp number, n (%)	1	868 (32.8)	482 (40.0)	129 (19.7)	257 (32.8)	<0.001
	2-4	1441 (54.5)	638 (52.9)	361 (55.1)	442 (56.4)	
	5+	334 (12.6)	85 (7.1)	165 (25.2)	84 (10.7)	
Index polyp villous*, n (%)	Yes	1038 (39.3)	485 (40.2)	280 (42.7)	273 (34.9)	0.006
Index polyp type*, n (%)	Adenoma	2503 (94.7)	1142 (94.8)	627 (95.7)	734 (93.7)	0.244
	Serrated Polyps	140 (5.3)	63 (5.2)	28 (4.3)	49 (6.3)	
Index polyp HGD*, n (%)	Yes	285 (10.8)	124 (10.3)	84 (12.8)	77 (9.8)	0.144
Index polyp size* (mm)	<10	913 (34.5)	408 (33.9)	210 (32.1)	295 (37.7)	0.066
	≥10	1730 (65.5)	797 (66.1)	445 (67.9)	488 (62.3)	
Index polyp location* (n=2639)	Rectum	345 (13.1)	158 (13.1)	93 (14.2)	94 (12.0)	<0.001
	Left colon	1595 (60.3)	769 (63.8)	346 (52.8)	480 (61.3)	
	Right colon	697 (26.4)	275 (22.8)	214 (32.7)	208 (26.6)	
BSG 2020 risk index procedure	Low	1360 (51.5)	702 (58.3)	235 (35.9)	423 (54.0)	<0.001
	High	1283 (48.5)	503 (41.7)	420 (64.1)	360 (46.0)	

*Applies to the most advanced polyp if multiple removed at index procedure.

7.3.7 Multivariate Polynomial Regression

Based on the initial univariable comparisons, the following variables were taken forward to multivariate polynomial regression analysis: age, sex, aspirin, statin, index advanced polyp, polyp number, polyp location, villous features and BSG 2020 risk score. Index polyp number (OR 1.154 (95% CI: 1.065-1.251; p<0.001)) and index villous features (OR 1.486 (95% CI: 1.050-2.103; p=0.025)) were independently associated with the detection of advanced metachronous lesions within 2 years of index polypectomy. No variable was independently associated with advanced lesions after 2 years (Table 7.3).

7.3.8 Table 7.3: Multivariate multinomial logistic regression of factors relating to time to advanced metachronous lesion development after index polypectomy.

		Advanced Polyp or CRC ≤2 Years			Advanced Polyp or CRC >2yr		
		OR	95% CI	P	OR	95% CI	P
Age	Years	1.011	0.987-1.036	0.369	1.007	0.985-1.028	0.551
Sex	Female	0.903	0.626-1.303	0.587	0.853	0.614-1.185	0.344
Aspirin	Yes	1.132	0.760-1.687	0.543	0.987	0.670-1.455	0.948
Statin	Yes	1.378	0.935-2.033	0.105	0.925	0.644-1.327	0.671
Index Advanced	Yes	1.214	0.747-1.972	0.433	1.027	0.678-1.555	0.900
Index Polyp Number	Number	1.154	1.065-1.251	<0.001	1.021	0.925-1.128	0.676
Index Location	Rectum	1.0			1.0		
	Left	0.763	0.462-1.259	0.290	0.881	0.565-1.374	0.577
	Right	1.606	0.946-2.728	0.079	0.912	0.552-1.506	0.718
Index Villous Features	Yes	1.486	1.050-2.103	0.025	1.032	0.749-1.423	0.847
Index BSG-2020	High	1.428	0.876-2.328	0.153	0.857	0.549-1.338	0.498

7.3.9 BSG-2020 High- Versus Low-Risk Patients.

Finally, a comparison was made in outcome between patients who would be deemed low-risk (n=1,360) and high-risk (n=1,283) based on their index screening colonoscopy findings, according to the current BSG 2020 guidelines(31) (Table 7.4). There was a higher rate of both non-advanced metachronous polyps (44.4% versus 35.4%) and advanced metachronous polyps (15.7% versus 11.8%) amongst the BSG 2020 high-risk patients as compared to low-risk ($p<0.001$), but a similar rate of CRC (0.6% versus 1.2%). The group who would be high-risk per BSG 2020 criteria contained 503 (39.2%) patients with no metachronous lesions, while the proportion with advanced polyps or CRC in the group who would be low-risk was 13.0% (n=177). Comparable differences were observed when examining early metachronous lesions only (detected <2 years from index colonoscopy): non-advanced polyps (23.9% versus 12.6%), advanced metachronous polyps (8.7% versus 4.3%) and CRC (0.2% versus 0.3%) ($p<0.001$). However, if you examine only those lesions detected >2 years from index colonoscopy, no significant differences are observed: non-advanced polyps (20.6% versus 22.7%), advanced metachronous polyps (7.1% versus 7.5%) and CRC (0.4% versus 0.9%) ($p=0.140$).

7.3.10 Table 7.4: Multi-layered factors associated with Index British Society of Gastroenterologists' 2020 risk category.

			Index BSG 2020 Risk Group		
			Low	High	P
Total			1360	1283	
Metachronous Polyp/CRC	No Metachronous Lesion		702 (51.6%)	503 (39.2%)	<0.001
	All	Non-Advanced Polyp	481 (35.4%)	570 (44.4%)	
		Advanced Polyp	161 (11.8%)	202 (15.7%)	
		CRC	16 (1.2%)	8 (0.6%)	
	Early (<2 Years)	Non-Advanced Polyp	172 (12.6%)	306 (23.9%)	<0.001
		Advanced Polyp	59 (4.3%)	111 (8.7%)	
		CRC	4 (0.3%)	3 (0.2%)	
	Late (>2 years)	Non-Advanced Polyp	309 (22.7%)	264 (20.6%)	0.140
		Advanced	102 (7.5%)	91 (7.1%)	
		CRC	12 (0.9%)	5 (0.4%)	

7.4 Discussion

This study examined the impact of applying the current BSG 2020 surveillance guidelines to a large retrospective cohort of patients whose surveillance strategy following screening polypectomy was determined by previous guidance(282, 283). It was observed that the overall rate of metachronous advanced polyps or CRC was relatively low (14.6%) and nearly half (45.6%) had no metachronous lesion. When those patients who would still be recommended surveillance based on current BSG guidance were selected, the rate of advanced metachronous polyps or CRC remained low (16.3%) and the rate of no metachronous lesion remained high (39.2%). The rate of advanced metachronous polyps or CRC in the low-risk BSG 2020 patients was only marginally lower (13.0%) and perhaps suggests that current protocols would benefit from refinement. Furthermore, while BSG 2020 risk stratification group was associated with a significant difference in overall metachronous lesion rate, it did not differentiate advanced and non-advanced metachronous lesions and was not significantly associated with late metachronous lesions detected after 2 years from index polypectomy.

The BSG 2020 guidelines include a comprehensive literature review on which their recommendations are based(31). Evidence is presented of a heightened risk of advanced adenoma/ neoplasia at surveillance colonoscopy, with index findings of HGD(285-290), increased polyp number(285-289, 291-295) and larger index polyps (predominantly ≥20mm)(285-289, 291, 294, 296, 297). However, other studies which failed to find significant associations between metachronous advanced adenoma/ neoplasia and index HGD(291, 293, 294, 297-299), polyp number(299-303) or index polyp size at a lower threshold of ≥10mm are also highlighted(286). In the current study, the presence of HGD or index polyp size ≥10mm did not correlate with advanced nor non-advanced metachronous lesions. Increased index polyp number predicted both advanced and non-advanced lesions and predicted developing any metachronous lesion less than 2 years from

index colonoscopy. Additionally, index polyp number was an independent predictor of early advanced metachronous lesions on multivariate polynomial regression. This may reflect a strong patient propensity to developing multiple colorectal polyps and/ or incomplete polyp clearance at index colonoscopy. A further factor found to independently predict early advanced metachronous lesions was villous index morphology. While BSG 2020 highlighted numerous studies associating villous morphology with metachronous advanced lesion risk(285-287, 289, 291, 293, 294, 297, 299, 300, 304), it has historically not been included in UK-based risk stratification due to concerns over heterogeneity in pathological reporting and the additional surveillance workload that inclusion may produce(31). However, this study suggests it may be a more useful basic pathological variable than others currently in use.

Other studies have examined the efficacy of BSG 2020 risk. In a retrospective study of 21318 patients who underwent polypectomy, CRC incidence in BSG 2020 low-risk patients was significantly lower than the general population without surveillance, suggesting that benefit from polyp clearance has already been derived and no further surveillance is required. CRC incidence in BSG 2020 high-risk patients was significantly higher than the general population without surveillance, and incidence was similar to the general population with surveillance, perhaps suggesting benefit(305). In contrast, this smaller but more recent study, suggests that many BSG 2020 high-risk patients do not develop metachronous lesions and the rate of advanced metachronous lesions is only marginally higher than low-risk patients. Additionally, although BSG 2020 high-risk was associated with metachronous advanced lesions, when adjusting for potential confounders with multivariate analysis, it was not an independent predictor of metachronous advanced lesions detected <2 years or >2 years.

The current study suggests that risk stratification may benefit from refinement. The INCISE collaborative intends to evaluate the addition of a panel of novel risk factors for

metachronous lesion development to the BSG 2020 risk score. Factors such as patient characteristics, protein expression, genomic and transcriptomic features of index polyp tissue will be used, with the hope of increasing the positive yield of surveillance colonoscopy and reducing unnecessary invasive investigation for those at lower risk. Novel risk factors will be integrated using machine learning to produce a risk stratification tool that can be delivered to clinicians.

The current study has a number of strengths. It is large and multicentred in nature. As patients were identified by interrogating both endoscopy- and pathology-based reporting software, a very low number of missed eligible patients would be anticipated during the study period. A broad range of demographics, comorbidities, medications and index pathological characteristics were screened for impact on metachronous lesion risk. The majority of significant factors were carried forward to multivariate analysis to account for confounding and there was sufficiently long follow-up. There are however limitations. It is retrospective and observational in nature and hence there was variability in patients' surveillance interval. While every effort was made to ensure follow-up colonoscopies were appropriate in each case, for example excluding those performed for polypectomy site checks, the exact indication was not always recorded and a proportion may have been performed due to further screening positivity or for symptomatic reasons, rather than representing true surveillance colonoscopies. Only those patients who participated in bowel screening, had a positive screening test and proceeded to colonoscopy could be included. The mean screening uptake rate in NHS GG&C during the study period was 51.7%, test positivity rate 2.7% and rate of patients with a positive screening test proceeding to colonoscopy 76.2%(306). There is potential for selection bias at each stage in this process whereby those who do not proceed are not represented. To date those patients who underwent polypectomy but were not invited/ did not return for surveillance were not included, however a linked study examining such patients is underway. The

surveillance protocols that were applied to the patients of this study are now historical and in general terms less conservative than current BSG 2020 guidance. Therefore, it may be anticipated that the burden of surveillance colonoscopy and rate of negative investigations has decreased. However, by using this patient group we were able to create a cohort of BSG 2020 high- and low-risk patients, who all underwent surveillance colonoscopy to allow for outcome analysis. As no statistical correction was made to the χ^2 analysis to account for the multiple comparisons made, an increased risk of type I errors may be anticipated. However, by assessing significant variables with multivariate polynomial regression analysis, the impact of potential false positives is negated. Finally, in terms of CRC there were a low number of events (n=24). It has been highlighted previously that the finding of advanced metachronous polyps is only a surrogate marker for CRC-risk(307) and it is difficult to make firm conclusions about risk stratification of CRC.

In conclusion, this study has shown that BSG 2020 high-risk features are associated with metachronous lesion detection, particularly those detected less than 2 years from index screening polypectomy. However, BSG 2020 risk grouping did not differentiate metachronous advanced and non-advanced lesions and was less discriminatory in lesions detected beyond 2 years. Additionally, the proportion of patients with no metachronous lesions in the BSG 2020 high-risk group (503 of 1283, 39.2%) and the proportion with advanced polyps or CRC in the BSG 2020 low-risk group (177 of 1360, 13.0%) were relatively high. This suggests that post-polypectomy surveillance may benefit from refinement and the INCISE project aims to do this by applying novel techniques to index pathology tissue and integrating relevant outputs to produce a valuable risk stratification tool than can be delivered to clinicians and patients.

8 Novel methods of risk stratifying patients for metachronous, pre-malignant colorectal polyps: a systematic review.

8.1 Introduction

In chapter 7, the current British Society of Gastroenterology (BSG) /Association of Coloproctology of Great Britain and Ireland/Public Health England post-polypectomy surveillance 2020 guidelines (BSG 2020), were applied to a large cohort of patients who underwent surveillance following screening polypectomy. BSG 2020 high-risk was found to be predictive of metachronous lesion development, including both advanced and non-advanced lesions, and early (<2 years) and late (>2 years) lesions. However, BSG 2020 did not differentiate metachronous advanced and non-advanced lesions well, was less predictive of late lesions and was not an independent predictor of early or late advanced metachronous polyps on multivariate analysis. Additionally, the rate of advanced metachronous lesions was only marginally higher in BSG 2020 high-risk patients as compared to low-risk (16.3% vs. 13.0%). These findings indicate that current protocols would benefit from further refinement to predict those patients at highest risk of developing metachronous lesions and therefore most likely to derive benefit from invasive surveillance colonoscopy.

As stated previously the Integrated Technologies for Improved Polyp Surveillance (INCISE) project aims to combine patient characteristics with tissue analysis of polyps, including digital pathology, immunohistochemistry (IHC), genomic and transcriptomics, integrated using machine learning, to better predict future polyp risk and improve current surveillance protocols. One of the first steps of this project was to establish whether other studies had previously been conducted, which may inform this work. This chapter will begin by giving a review of established risk factors for metachronous polyp development including patient factors such as basic demographics, comorbidities, use of

chemopreventive medications and other modifiable risk factors, and discuss conventional pathological polyp characteristics, some of which are included in the BSG 2020 guidance(31). Following this will be a systematic review produced on behalf of the INCISE collaborative, which aimed to identify all studies exploring genomics, transcriptomics, immunohistochemistry or features of the microbiome as novel biomarkers of metachronous polyp risk, following colorectal polypectomy.

8.1.1 Established Risk Factors for Metachronous Polyp Development

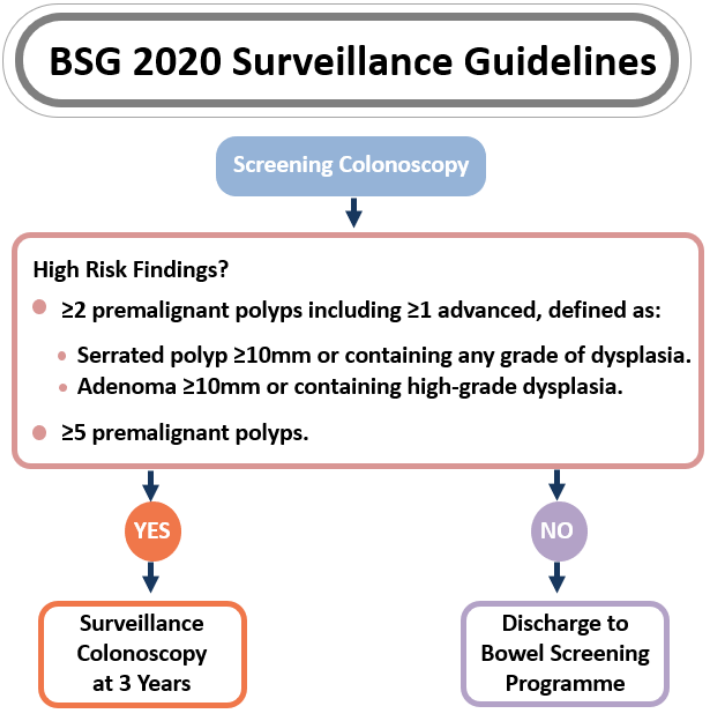
8.1.1.1 Polyp Pathology

In Scotland and throughout the United Kingdom, requirement for post-polypectomy surveillance is determined by the current iteration of the BSG 2020 guidance, first released in November 2019 (Figure 8.1)(31). This guidance firstly takes account of polyp histology, with only polyps with proven premalignant potential considered: adenomas and serrated polyps (an umbrella term for hyperplastic polyps, sessile serrated lesions, traditional serrated adenomas and mixed polyps)(29-31). Secondly, degree of dysplasia is considered(31). The presence of high-grade dysplasia within an index polyp has been widely reported to be associated with risk of metachronous advanced adenoma development (adenoma ≥10mm or containing high-grade dysplasia)(286-290) and future colorectal cancer (CRC)-risk(287, 289, 308) with reported incidences of 19-28%(286, 287) and 3%(287) respectively. Thirdly, polyp size is considered(31). Not only is polyp size directly related to the chance that an index polyp will harbour high-grade dysplasia or malignancy(309), but also correlates with the future risk of developing advanced adenoma(286-289), advanced neoplasia (advanced adenoma or CRC)(291, 294, 297) and CRC(287, 289). This heightened risk is most likely associated with adenomas ≥10mm and certainly with those ≥20mm(31). Finally, the multiplicity of polyps is considered(31). There is consistent evidence that an increasing number of adenomas found at index

colonoscopy leads to a heightened risk of metachronous advanced adenomas(286-289, 308, 310, 311) and advanced neoplasia(291, 294, 295, 298). While some studies have found an association with future CRC risk(289, 312) others found no statistically significant increased risk(287, 313).

The presence of tubulovillous or villous histology in an adenoma has been shown to be associated with an increased future risk of advanced adenoma(286, 287, 289, 300), advanced neoplasia(291, 294, 298, 299) and CRC(287, 289, 308, 310, 313). Despite this, it is not included in the risk stratification algorithm, with the BSG/ACPGBI guidelines citing a large degree of inter-observer disagreement over villous pathology and the additional surveillance workload that would be generated by its inclusion(31). Interestingly, in Chapter 7 it was shown that presence of villous histology was an independent predictor of early (< 2 years) advanced metachronous lesions in the INCISE cohort, while other conventional pathological parameters were not independently predictive(314).

8.1.1.2 Figure 8.1: British Society of Gastroenterology and Association of Coloproctology of Great Britain and Ireland post-polypectomy surveillance guidelines



8.1.1.3 Patient Factors

A number of patient factors including increased age, male sex, high body mass index (BMI), smoking status and hypertension have been linked with a higher likelihood of developing metachronous colorectal polyps. A systematic review and meta-analysis found seven studies assessing the impact of age on metachronous adenoma risk with a total of 22,547 patients. 35% of patients ≥60 years developed metachronous adenoma on follow-up as compared to 29% of those <60 years (OR 1.56, 95% CI: 1.13-2.14, p<0.01). The same meta-analysis identified twelve studies assessing the impact of sex with a total of 31,277 patients. 43% of males vs 32% of females developed metachronous adenoma (OR 1.58, 95% CI: 1.42-1.76, p<0.001). Finally, four studies assessing the impact of BMI were identified with 13,606 patients. 48% of patients with a BMI ≥25 developed metachronous adenoma compared to 42% of those with a BMI <25 (OR 1.35, 95% CI: 1.14-1.58, p<0.001)(315). Smoking has been linked with an increased rate of metachronous adenoma(316, 317) however there is no clear association with the development of metachronous advanced adenoma or advanced neoplasia(291, 297, 303). There is some evidence that hypertension increases the risk of metachronous adenoma (316).

Diet has been extensively investigated. Studies worth noting are the Polyp Prevention Trial and the Wheat Bran Fibre trial. The Polyp Prevention Trial was a multi-centre clinical trial in which 2,079 patients with colorectal adenoma were randomised to a high-fibre, high-fruit and -vegetable and low-fat diet versus no change to diet. 1,905 of those patients were followed up to 4 years and 801 to 8 years. At both 4-year and 8-year follow-up, the intervention diet had no impact on the chance of developing metachronous adenoma (RR 1.00, 95% CI: 0.90-1.12 and RR 0.98, 95% CI: 0.88-1.09 respectively)(318, 319). However, in a side study it was observed that patients who were most strongly compliant with the intervention diet did achieve a significant reduction in the risk of metachronous adenoma compared to the control group (OR 0.65, 95% CI: 0.47-0.92)(320). In the Wheat-

Bran Fibre trial 1,429 patients with colorectal adenoma were randomised to high- or low-dietary wheat bran fibre. 1,303 completed the study and on multivariate adjusted analysis they found no significant difference in the risk of metachronous adenoma (OR 0.88, 95% CI: 0.7-1.11, p=0.28)(321). Another study by Cottet et al(322) found that women had a significantly reduced risk of metachronous adenoma if they consumed a 'Mediterranean' diet characterised by a high consumption of olive oil, fruit, vegetables, fish and lean meat.

Chronic inflammation has been established as a risk factor for colorectal carcinogenesis(323). The presence of a systemic inflammatory response (SIR) has been investigated as a possible risk factor for the development of metachronous adenoma, however the evidence is mixed. Bobe et al(324) measured serum levels of the pro-inflammatory cytokine IL-6 at baseline, year 1 and year 3 in 872 patients from the intervention arm of the Polyp Prevention Trial as described above. They found that a decrease in IL-6 levels during the trial resulted in a significantly lower risk of metachronous high-risk adenoma (OR 0.44, 95% CI: 0.23-0.84). In another study, also by Bobe et al(323), they measured serum concentrations of IL-1β, IL-2, IL-8, IL-10, IL-12p70, granulocyte macrophage colony stimulating factor, interferon-γ and TNF-α in the same group of patients. A decrease in IL-2 concentrations during the trial was associated with increased risk of metachronous adenoma (OR 1.68, 95% CI: 1.13-2.49), whereas a decrease in IL-1β or IL-10 reduced the risk of metachronous advanced adenoma (OR 0.37, 95% CI: 0.15-0.94 and OR 0.39, 95% CI: 0.15-0.98 respectively). These findings were in agreement with previous studies which had found increased IL-6 and IL-10 and decreased IL-2 concentrations to be associated with an increased risk of colorectal adenoma or CRC and increased IL-1β expression within colorectal adenoma and adenocarcinoma tissue(323, 324). C-reactive protein (CRP) has also been used as a marker of a systemic inflammatory response. In the study by Crockett et al(325) they measured serum CRP 1 year after index polypectomy in 689 patients participating in a calcium supplementation polyp prevention

trial. They found no association between CRP and the risk of metachronous adenomas, serrated polyps or advanced lesions. Other inflammatory markers and mediators that have been investigated and have shown no relationship with metachronous adenomas include lipoxin A4 and resolvin D1(326).

8.1.1.4 Chemopreventive Medications

A variety of medications have been studied as potential chemopreventive agents against the development of metachronous colorectal polyps. Perhaps the most studied is aspirin. Long-term aspirin use has been shown to reduce the risk of CRC, postulated via COX1/2 inhibition(327). A number of studies have found aspirin effective for reducing metachronous polyp risk(65, 328, 329), while others have found no significant benefit(330, 331). Several systematic reviews and meta-analyses have now attempted to amalgamate the available evidence. Gao et al's(327) meta-analysis included 3 randomised control trials (RCTs) and 2,175 patients. One RCT followed patients up with colonoscopy to 3 years and two RCTs to 1 year and the dose of aspirin ranged from 81 to 325mg. On meta-analysis aspirin was overall found to significantly reduce the rate of metachronous adenoma (RR 0.836, 95% CI: 0.746-0.937, p=0.002). Significance was retained regardless of aspirin dose. Cole et al(332) had similar findings in their systematic review and meta-analysis of four RCTs involving 2698 patients. Again, aspirin dosing ranged from 81 to 325mg and median follow-up was 33 months. Overall, aspirin significantly reduced the rate of metachronous adenoma (RR 0.83, 95% CI: 0.72-0.96). The greatest reduction in risk occurred during the first year (RR 0.62, 95% CI: 0.72-0.96) and aspirin had no effect beyond 38 months (RR 0.99, 95% CI: 0.78-1.26). Similarly, in the meta-analysis by Zhao et al(333), aspirin conferred a significant benefit at one year (RR 0.73, 95% CI: 0.55-0.98, p=0.039) but not beyond one year (RR 0.84, 95% CI: 0.72-0.98, p=0.484).

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have also been shown to have anti-neoplastic effects on the colorectum and like aspirin, exert their effects via COX inhibition(334). Several RCTs have reported a lower metachronous adenoma and advanced adenoma risk in patients taking NSAIDs(335-337). However, others have suggested that while an early benefit is observed, withdrawal of NSAIDs may conversely increase metachronous adenoma risk in those patients previously on active treatment(338). In the systematic review by Veettill et al(339) they studied the effects of both aspirin and NSAIDs. On meta-analysis low dose aspirin (80-160mg) or NSAID-use significantly reduced metachronous adenoma or advanced adenoma risk. However, for NSAIDs this effect did not persist and they observed an increased risk of metachronous adenomas 2 years after withdrawal of active treatment. Additionally, cardiovascular and gastrointestinal adverse events were reported to be higher in patients taking NSAIDs as compared to placebo in the majority of studies(335, 336, 338).

It has been suggested that calcium intake may be protective against CRC. Calcium is known to bind bile acids in the bowel lumen thereby inhibiting their known proliferative and carcinogenic effects. Additionally, calcium has been shown to have direct antiproliferative effects on epithelial cells of the colorectum, promoting differentiation and apoptosis(340, 341). The role of calcium supplementation for the prevention of metachronous colorectal adenoma has been extensively studied. While a few studies have found no significant impact of calcium supplementation(342-344), multiple RCTs(345-348) and systematic reviews and meta-analyses(340, 341, 349-352) have shown calcium supplementation to be effective in preventing metachronous colorectal adenoma. Vitamin D has also been explored as a possible chemopreventive agent. Low serum levels of 25-hydroxy-vitamin D and low dietary intake of vitamin D have both been associated with a higher risk of colorectal adenoma and CRC(353, 354). Vitamin D in its active form (1, 25-hydroxy-vitamin D) acts on the vitamin D receptor, a transcription factor which has been

shown to inhibit colorectal epithelial cell proliferation and induce differentiation and apoptosis(354). Despite this, the majority of studies have shown no significant decrease in the rate of metachronous adenoma with vitamin D supplementation(343, 344, 353, 354), with only one showing a significant benefit(348).

It has been suggested that a diet rich in folic acid or the use of supplementary folic acid may reduce the risk of colorectal adenoma and CRC, however trial evidence has been inconsistent(355, 356). Folate plays a role in DNA synthesis and methylation and it is thought it may reduce the risk of CRC by reducing aberrations in DNA methylation and by contributing towards DNA repair(65, 356). Both RCTs(65, 357) and a systematic review and meta-analysis(355) have found no significant impact of supplemental folic acid on the rate of metachronous adenoma. One RCT found no overall benefit, but did observe a significant reduction in metachronous adenoma among patients known to be folate deficient prior to being randomised to take folate supplements(356). One RCT found a significantly reduced rate of metachronous adenoma using high dose (5mg/day) folic acid supplements(358).

Other potential chemopreventive medications studied include metformin and ursodeoxycholic acid. In the study by Higurashi et al(70) they randomised non-diabetic patients with a history of colorectal polyps to 250 mg of metformin or placebo. Metformin significantly reduced the rate of any metachronous polyp (RR 0.67, 95% CI: 0.47-0.97, p=0.034) and the rate of metachronous adenoma (RR 0.60, 95% CI: 0.39-0.92, p=0.016). In the randomised control trial by Alberts et al(359) they found that ursodeoxycholic acid did not significantly reduce the rate of metachronous adenoma but did significantly reduce the rate of metachronous adenoma with the presence of high-grade dysplasia.

8.2 Methods

A systematic review was performed to identify all studies which used genomics, transcriptomics, immunohistochemistry or features of the microbiome as novel biomarkers of metachronous polyp risk following colorectal polypectomy. The study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. Studies were included that used the development of any metachronous polyps or advanced metachronous polyps only, as their primary outcome measure. Studies which included the development of CRC as a secondary outcome measure were also evaluated.

A search for other systematic reviews was performed first. One narrative review that addressed polyp characteristics, patient factors, chemopreventive medications, diet and gene polymorphism was identified (279). No study was identified which addressed the wide range of novel methods of risk stratification as was intended to be explored in the current study.

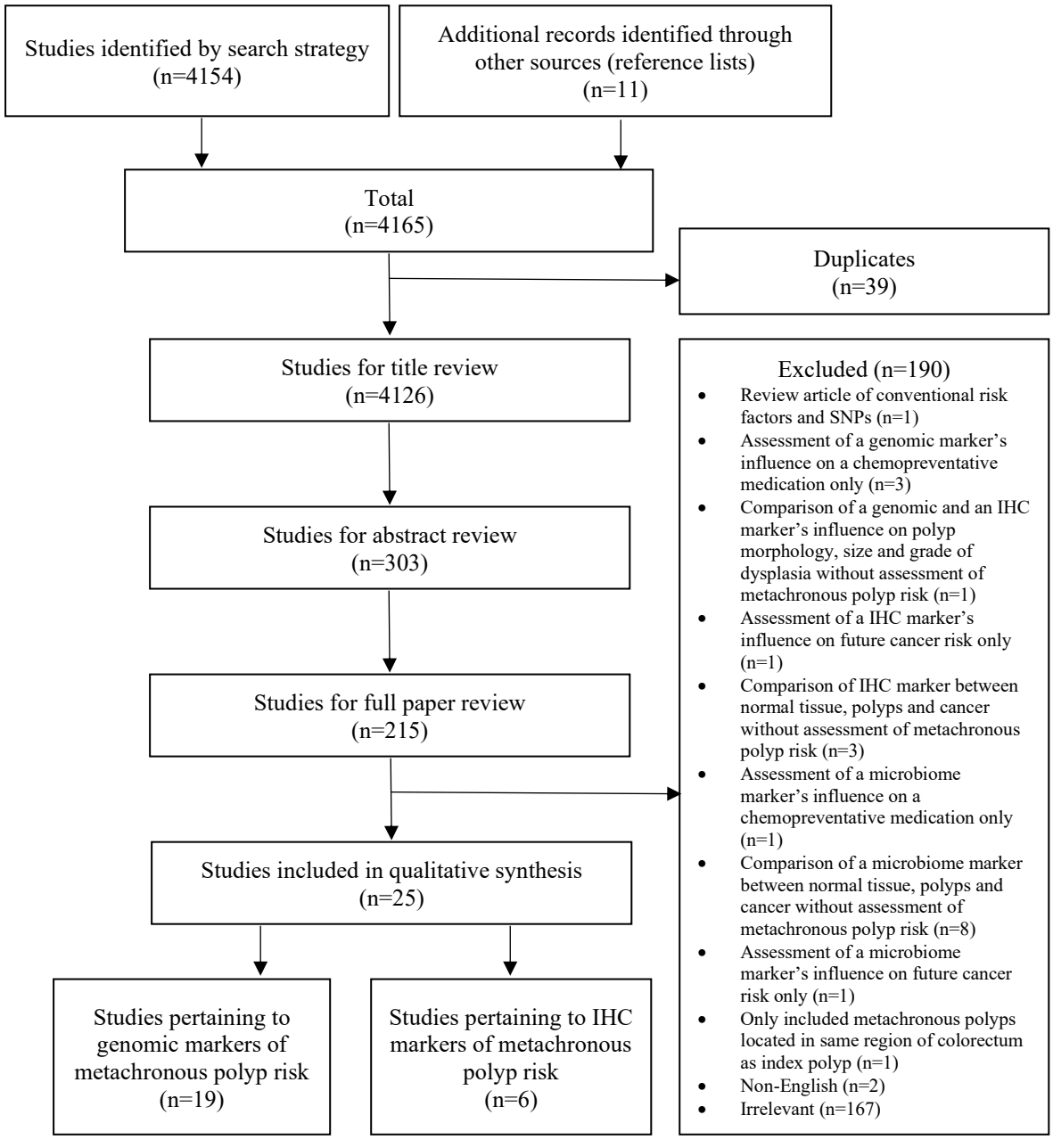
Next a systematic literature review was performed of PubMed from inception until August 2020 inclusive, using the following MeSH terms: “colorectal”, “polyp”, “adenoma” “metachronous”, “recurrence”, “future risk”, “mutation”, “genetics”, “genome”, “mRNA”, “transcriptome”, “expression”, “immunohistochemistry”, “IHC” and “microbiome.” Observational studies, randomised control trials and systematic review and meta-analyses were included that used genomics, transcriptomics, immunohistochemistry or microbiome as novel markers of metachronous colorectal polyp risk. Narrative reviews, animal studies, conference abstracts, non-English studies and those not addressing our primary outcomes of interest were excluded. Study titles were screened for relevance followed by a review of selected abstracts and full texts by the lead author and ratified by a second. Reference lists

from identified studies were also searched for other eligible studies. It was not possible to perform a formal meta-analysis due to a paucity of available comparable data.

8.4 Results

A total of 4165 papers were identified using the systematic search protocol. Each title was reviewed followed by abstract review of 303 papers and full paper review of 215 (Figure 8.2). 25 papers met the inclusion criteria, with 19 pertaining to genomic markers (Table 8.1) and 6 pertaining to IHC markers (Table 8.2) of metachronous polyp risk. No papers were identified that used transcriptome or microbiome as novel markers of metachronous polyp risk.

8.4.1 Figure 8.2: PRISMA Flow Chart



8.4.2 Table 8.1: Genomic markers of metachronous polyp risk.

Gene/ Chromosome Region	Mutation or SNP	Paper	Findings
KRAS	Somatic mutations in Exon 2 (codon 12 and 13)	Juarez	**Increased risk of metachronous advanced polyps (OR 2.27, 95% CI: 1.15-4.46, p=0.018).** **Increased risk of metachronous advanced adenoma (OR 2.23, 95% CI: 1.02-4.85, p=0.044).**
	Somatic mutations in Exon 1	Nusko	**On univariate analysis KRAS mutation increased risk of metachronous adenoma >5mm (OR 4.00, 95% CI: 1.18-13.6, p=0.0265)** however on multivariate analysis KRAS mutant status did not retain significance (OR 3.92, 95% CI: 0.82-18.72, p=0.0871).
	Somatic mutation, not specified	Benamouzig	No significant impact on metachronous adenoma risk (metachronous adenoma rate in patients with KRAS mutation vs wild type = 37.78% vs 33.33%, p>0.005).
BRAF	Somatic V600E mutation	Juarez	No impact on risk of metachronous advanced polyps (OR 1.08, 95% CI: 0.43-2.71, p=0.9). No impact on risk of metachronous advanced adenoma (OR 0.99, 95% CI: 0.31-3.12, p=1.0).
APC	rs2229992 (C486T)	Egan	Neither CT genotype (OR 1.12, 95% CI: 0.87-1.45) nor TT genotype (OR 0.72, 95% CI: 0.51-1.02) had an impact on metachronous adenoma risk as compared to CC wildtype.
	rs42427 (A1678G)	Egan	Neither AG genotype (OR 1.05, 95% CI: 0.82-1.35) nor GG genotype (OR 0.78, 95% CI: 0.55-1.12) had an impact on metachronous adenoma risk as compared to AA wildtype.
	rs459552 (A1822T)	Egan	Neither AT genotype (OR 1.16, 95% CI: 0.91-1.48) nor TT genotype (OR 1.21, 95% CI: 0.74-1.99) had an impact on metachronous adenoma risk as compared to AA wildtype.
	rs465899 (T1960C)	Egan	Neither TC genotype (OR 1.07, 95% CI: 0.83-1.38) nor CC genotype (OR 0.78, 95% CI: 0.55-1.12) had an impact on metachronous adenoma risk as compared to TT wildtype.
	rs2229995 (C2502T)	Egan	CT genotype (OR 1.13, 95% CI: 0.62-2.06) had no impact on metachronous adenoma risk as compared to CC wildtype. 0 patients in the study had the TT genotype to allow for comparison.
	TGACC Haplotype (a)	Egan	**TGACC haplotype significantly reduced risk of metachronous adenoma (OR 0.73, 95% CI: 0.57-0.94).** **TGACC haplotype significantly reduced risk of metachronous advanced adenoma (OR 0.63, 95% CI: 0.42-0.94).**
	TA Haplotype (b)	Egan	**TA haplotype significantly reduced risk of metachronous adenoma (OR 0.73, 95% CI: 0.59-0.91).** No impact on risk of metachronous advanced adenoma (OR 0.76, 95% CI: 0.54-1.07).
EGFR	rs7801956 (G>A, intron 4)	Kraus	**Increased risk of metachronous adenoma (HR 2.65, 95% CI: 1.03-6.86, p=0.04).**
COX1	rs3842787 (C50T)	Hubner	Neither CT genotype (OR 1.01, 95% CI: 0.66-1.54) nor TT genotype (OR 0.91, 95% CI: 0.14-6.07) had an impact on metachronous neoplasia risk as compared to CC wildtype.
	rs10306110 (A>G, near gene-5')	Kraus	**Increased risk of metachronous adenoma (HR 15.46, 95% CI: 1.58-151.67, p=0.02).**
	rs10306122 (T>C, intron)	Kraus	**Increased risk of metachronous adenoma (HR 15.58, 95% CI: 1.59-152.67, p=0.02).**
	rs10306164 (G>T, intron)	Kraus	**Increased risk of metachronous adenoma (HR 2.53, 95% CI: 1.02-6.29, p=0.05).**
	rs1236913 (C>T, W8R)	Kraus	**Increased risk of metachronous adenoma (HR 3.78, 95% CI: 1.32-10.80, p=0.01).**
	rs1330344 (G>A, near gene-5')	Kraus	**Increased risk of metachronous adenoma (HR 2.75, 95% CI: 1.12-6.75, p=0.03).**
	rs3119773 (T>C, intron)	Kraus	**Increased risk of metachronous adenoma (HR 4.58, 95% CI: 1.69-12.44, p<0.01).**
COX2	rs20417 (G765C)	Hubner	Neither GC genotype (OR 0.96, 95% CI: 0.70-1.30) nor CC genotype (OR 1.32, 95% CI: 0.66-2.62) had an impact on metachronous neoplasia risk as compared to GG wildtype.
		Barry	Neither GC genotype (RR 1.11, 95% CI: 0.94-1.31) nor CC genotype (RR 0.97, 95% CI: 0.64-1.46) had an impact on metachronous neoplasia risk as compared to GG wildtype.
	rs2745557 (C>T, intron 1)	Barry	Neither CT genotype (RR 0.99, 95% CI: 0.85-1.16) nor TT genotype (RR 0.90, 95% CI: 0.58-1.42) had an impact on metachronous neoplasia risk as compared to CC wildtype.
	rs5277 (G>C, exon 3)	Barry	While GC genotype had no impact on metachronous adenoma risk (OR 1.05, 95% CI: 0.89-1.24), **CC genotype significantly increased metachronous adenoma risk (RR 1.49, 95% CI: 1.00-2.23) as compared to GG wildtype.**
	rs20432 (T>G, intron 5)	Barry	Neither TG genotype (RR 1.09, 95% CI: 0.93-1.29) nor GG genotype (RR 1.21, 95% CI: 0.85-1.72) had an impact on metachronous adenoma risk as compared to TT wildtype.

	rs4648310 (A>G, near gene-3')	Barry	**AG genotype increased risk of metachronous adenoma (RR 1.35, 95% CI: 1.03-1.77) as compared to AA wildtype.**
	rs4648268 (G>A, intron)	Kraus	**Increased risk of metachronous adenoma (HR 3.73, 95% CI: 1.26-11.11, p=0.02).**
	rs5275 (T>C, exon 10-3' UTR)	Barry	Neither TC genotype (RR 0.96, 95% CI: 0.82-1.12) nor CC genotype (RR 1.04, 95% CI: 0.84-1.29) had an impact on metachronous neoplasia risk as compared to TT wildtype.
		Kraus	No significant impact on metachronous adenoma risk (HR 3.09, 95% CI: 0.88-10.82, p=0.08).
	rs689469 (G>A, 3' UTR)	Kraus	**Increased risk of metachronous adenoma (HR 2.65, 95% CI: 1.03-6.86, p=0.04).**
IL-1β	rs16944 (C511T)	Bobe	Neither CT genotype (OR 0.90, 95% CI: 0.66-1.24) nor TT genotype (OR 0.83, 95% CI: 0.49-1.41) had an impact on metachronous adenoma risk as compared to CC wildtype.
		Sansbury	Neither CT genotype (OR 0.92, 95% CI: 0.74-1.15) nor TT genotype (OR 0.91, 95% CI: 0.64- 1.29) had an impact on metachronous adenoma risk as compared to CC wildtype.
IL-6	rs1800795 (G174C)	Bobe	Neither GC genotype (OR 1.25, 95% CI: 0.89-1.75) nor CC genotype (OR 1.19, 95% CI: 0.74-1.91) had an impact on metachronous adenoma risk as compared to GG wildtype.
		Sansbury	Neither GC genotype (OR 1.25, 95% CI: 0.99-1.57) nor CC genotype (OR 0.85, 95% CI: 0.61-1.19) had an impact on metachronous adenoma risk as compared to GG wildtype.
IL-8	rs4073 (T251A)	Bobe	Neither AT genotype (OR 1.16, 95% CI: 0.80-1.68) nor AA genotype (OR 1.03, 95% CI: 0.66-1.60) had an impact on metachronous adenoma risk as compared to TT wildtype.
		Sansbury	Neither AT genotype (OR 1.18, 95% CI: 0.92-1.52) nor AA genotype (OR 1.05, 95% CI: 0.77-1.42) had an impact on metachronous adenoma risk as compared to TT wildtype.
IL-10	rs1800872 (C592A)	Hubner	Neither CA genotype (OR 1.11, 95% CI: 0.83-1.47) nor AA genotype (OR 1.24, 95% CI: 0.74-2.07) had an impact on metachronous neoplasia risk as compared to CC wildtype.
		Bobe	Neither CA genotype (OR 0.92, 95% CI: 0.67-1.26) nor AA genotype (OR 1.45, 95% CI: 0.83-2.53) had an impact on metachronous adenoma risk as compared to CC wildtype.
	rs1800896 (G1082A)	Bobe	Neither AG genotype (OR 0.93, 95% CI: 0.66-1.31) nor GG genotype (OR 1.06, 95% CI: 0.68-1.63) had an impact on metachronous adenoma risk as compared to AA wildtype.
		Sansbury	Neither AG genotype (OR 0.98, 95% CI: 0.75-1.27) nor GG genotype (OR 1.01, 95% CI: 0.75-1.36) had an impact on metachronous adenoma risk as compared to AA wildtype.
	rs1800871 (C819T)	Bobe	Neither CT genotype (OR 0.90, 95% CI: 0.66-1.23) nor TT genotype (OR 1.35, 95% CI: 0.79-2.33) had an impact on metachronous adenoma risk as compared to CC wildtype.
		Sansbury	Neither CT genotype (OR 1.05, 95% CI: 0.85-1.31) nor TT genotype (OR 1.13, 95% CI: 0.72-1.76) had an impact on metachronous adenoma risk as compared to CC wildtype.
IL23R	rs10889675 (C>A, intron)	Kraus	**Increased risk of metachronous adenoma (HR 3.08, 95% CI: 1.05-9.04, p=0.04).**
	rs6683455 (T>C, near gene-5')	Kraus	**Increased risk of metachronous adenoma (HR 2.51, 95% CI: 1.06-5.96, p=0.04).**
	rs7518660 (G>A, intron)	Kraus	No significant impact on metachronous adenoma risk (HR 1.34, 95% CI: 0.54-3.32, p=0.53).
DRD2	rs1799732 (141 C>del)	Murphy	While TT genotype had no impact on metachronous adenoma risk (OR 1.25, 95% CI: 0.57-2.75), **CT genotype significantly increased metachronous adenoma risk (OR 1.30, 95% CI: 1.01-1.69) as compared to CC wildtype.**
	rs6277 (C957T)	Murphy	Neither CT genotype (OR 1.00, 95% CI: 0.77-1.29) nor TT genotype (OR 0.94, 95% CI: 0.69-1.26) had an impact on metachronous adenoma risk as compared to CC wildtype.
	rs1800497 (A>G, TaqIA)	Murphy	Neither CT genotype (OR 1.00, 95% CI: 0.80-1.26) nor TT genotype (OR 0.98, 95% CI: 0.59-1.65) had an impact on metachronous adenoma risk as compared to CC wildtype. While CT genotype had no impact on metachronous advanced adenoma risk (OR 1.09, 95% CI: 0.69-1.72), **TT genotype significantly increased metachronous advanced adenoma risk (OR 2.40, 95% CI: 1.11-5.20) as compared to CC wildtype.**
CYP2C9	rs1799853 (C430T, CYP2C9*2)	Barry2	No significant impact on metachronous adenoma risk (RR 1.09, 95% CI: 0.91-1.31, p=0.33).
	rs1057910 (A1075C, CYP2C9*3)	Barry2	**Increased risk of metachronous adenoma (RR 1.47, 95% CI: 1.19-1.83, p<0.001).** **Increased risk of metachronous advanced lesions or multiple adenoma (RR 1.79, 95% CI: 1.16-2.75, p=0.008).**
	rs1799853 (C430T, CYP2C9*2) or	Hubner2	Presence of any variant CYP2C9, had no impact on risk of metachronous colorectal neoplasia (RR 1.09, 95% CI: 0.82-1.44) as compared to patients with homozygous wild-type genotype.

	rs1057910 (A1075C, CYP2C9*3)	Barry2	**Presence of any variant CYP2C9, increased the risk of metachronous adenoma (RR 1.29, 95% CI: 1.09-1.51, p=0.002) as compared to patients with homozygous wild-type genotype. Presence of any variant CYP2C9, increased risk of metachronous advanced lesions or multiple adenoma (RR 1.64, 95% CI: 1.18-2.28, p=0.003).**
CYP24A1	rs6013905 (T>C)	Hibler	Neither TC genotype (OR 0.97, 95% CI: 0.76-1.25) nor CC genotype (OR 1.01, 95% CI: 0.49-2.07) had an impact on metachronous adenoma risk as compared to TT wildtype.
	rs2585428 (G>A)	Hibler	Neither AG genotype (OR 0.85, 95% CI: 0.66-1.10) nor AA genotype (OR 0.86, 95% CI: 0.63-1.18) had an impact on metachronous adenoma risk as compared to GG wildtype.
	rs2296241 (A>G)	Hibler	Neither AG genotype (OR 1.01, 95% CI: 0.77-1.32) nor GG genotype (OR 1.22, 95% CI: 0.90-1.66) had an impact on metachronous adenoma risk as compared to AA wildtype.
	rs2762939 (G>C)	Hibler	Neither CG genotype (OR 1.11, 95% CI: 0.88-1.40) nor CC genotype (OR 0.59, 95% CI: 0.36-0.97) had an impact on metachronous adenoma risk as compared to GG wildtype.
	rs35051736 (G>A)	Hibler	GA genotype had no impact on risk of metachronous adenoma (OR 1.98, 95% CI: 0.70-5.62) as compared to GG wildtype.
	rs6022999 (A>G)	Hibler	Neither AG genotype (OR 0.98, 95% CI: 0.77-1.25) nor GG genotype (OR 0.70, 95% CI: 0.43-1.13) had an impact on metachronous adenoma risk as compared to AA wildtype.
	rs4809958 (T>G)	Hibler	Neither GT genotype (OR 0.95, 95% CI: 0.74-1.21) nor GG genotype (OR 0.98, 95% CI: 0.49-1.96) had an impact on metachronous adenoma risk as compared to TT wildtype.
	rs276942 (A>G)	Hibler	Neither AG genotype (OR 1.19, 95% CI: 0.83-1.71) nor GG genotype (OR 0.20, 95% CI: 0.02-1.66) had an impact on metachronous adenoma risk as compared to AA wildtype.
	rs927650 (C>T)	Hibler	While TC genotype had no impact on metachronous adenoma risk (OR 1.30, 95% CI: 0.99-1.70), **TT genotype significantly increased metachronous adenoma risk (RR 1.38, 95% CI: 1.01-1.89) as compared to CC wildtype.**
	rs6013897 (T>A)	Hibler	Neither AT genotype (OR 0.90, 95% CI: 0.66-1.22) nor AA genotype (OR 0.85, 95% CI: 0.51-1.39) had an impact on metachronous adenoma risk as compared to TT wildtype.
	rs4809960 (T>C)	Hibler	Neither TC genotype (OR 1.04, 95% CI: 0.82-1.32) nor CC genotype (OR 1.05, 95% CI: 0.67-1.66) had an impact on metachronous adenoma risk as compared to TT wildtype.
CYP27B1	rs4646536 (T>C)	Hibler	Neither CT genotype (OR 0.89, 95% CI: 0.70-1.13) nor CC genotype (OR 1.04, 95% CI: 0.71-1.53) had an impact on metachronous adenoma risk as compared to TT wildtype.
CYP7A1	rs10957057 (C>T)	Wertheim	Neither CT genotype (OR 0.89, 95% CI: 0.52-1.50) nor TT genotype (OR 0.52, 95% CI: 0.10-2.81) had an impact on metachronous adenoma risk as compared to CC wildtype.
	rs8192879 (C>T)	Wertheim	Neither CT genotype (OR 1.47, 95% CI: 0.90-2.40) nor TT genotype (OR 1.47, 95% CI: 0.76-2.84) had an impact on metachronous adenoma risk as compared to CC wildtype.
	rs8192877 (A>G)	Wertheim	Neither AG genotype (OR 1.47, 95% CI: 0.88-2.43) nor GG genotype (OR 1.03, 95% CI: 0.22-4.86) had an impact on metachronous adenoma risk as compared to AA wildtype.
	rs11786580 (C>T)	Wertheim	Neither CT genotype (OR 1.13, 95% CI: 0.71-1.82) nor TT genotype (OR 1.19, 95% CI: 0.44-3.20) had an impact on metachronous adenoma risk as compared to CC wildtype.
	rs8192871 (A>G)	Wertheim	While AG genotype had no impact on metachronous adenoma risk (OR 0.78, 95% CI: 0.49-1.26), **GG genotype significantly reduced metachronous adenoma risk (RR 0.41, 95% CI: 0.19-0.89) as compared to AA wildtype.**
	rs13251096 (G>A)	Wertheim	Neither GA genotype (OR 0.70, 95% CI: 0.43-1.13) nor AA genotype (OR 0.54, 95% CI: 0.28-1.05) had an impact on metachronous adenoma risk as compared to GG wildtype.
	CCGTAG Haplotype (c)	Wertheim	**CCGTAG haplotype significantly increased risk of metachronous adenoma (OR 1.89, 95% CI: 1.00-3.57) as compared to common CTACAG haplotype.**
UGT1A6	rs2070959 (T181A) or rs1105879 (R184S)	Hubner2	**Presence of any variant UGT1A6 allele, had a significantly reduced risk of metachronous colorectal neoplasia (RR 0.68, 95% CI: 0.52-0.89) as compared to patients with homozygous wild-type genotype.**
ODC	rs2302615 (G316A)	Hubner3	Neither GA genotype (RR 0.92, 95% CI: 0.70-1.21) nor AA genotype (RR 0.43, 95% CI: 0.16-1.15) had an impact on metachronous adenoma risk as compared to GG wildtype.
		Martinez	While GA genotype had no impact on metachronous adenoma risk (OR 0.96, 95% CI: 0.68-1.34), **AA genotype significantly reduced metachronous adenoma risk (OR 0.48, 95% CI: 0.24-0.99) as compared to GG wildtype.**
		Barry3	Neither GA genotype (RR 1.03, 95% CI: 0.89-1.20) nor AA genotype (RR 0.98, 95% CI: 0.73-1.32) had an impact on metachronous adenoma risk as compared to GG wildtype.
MTHFR	rs1801133 (C677T)	Levine	Neither CT genotype (RR 0.97, 95% CI: 0.83-1.14) nor TT genotype (RR 0.89, 95% CI: 0.68-1.16) had an impact on metachronous adenoma risk as compared to CC wildtype.

	rs11801131 (A1298C)	Levine	Neither AC genotype (RR 1.14, 95% CI: 0.98-1.33) nor CC genotype (RR 1.14, 95% CI: 0.87-1.50) had an impact on metachronous adenoma risk as compared to AA wildtype.
ALOX12	rs11078659 (G>A, intron)	Kraus	No significant impact on metachronous adenoma risk (HR 2.33, 95% CI: 0.70-7.81, p=0.17).
	rs2073438 (G>A, intron)	Kraus	**Increased risk of metachronous adenoma (HR 2.38, 95% CI: 1.00-5.68, p=0.05).**
	rs2292350 (G>A, intron)	Kraus	**Decreased risk of metachronous adenoma (HR 0.41, 95% CI: 0.17-0.98, p=0.05).**
ALOX15	rs4796535 (A>G, intron)	Kraus	**Increased risk of metachronous adenoma (HR 3.56, 95% CI: 1.33-9.56, p=0.01).**
PGDH	rs7349744 (G>A, intron)	Kraus	No significant impact on metachronous adenoma risk (HR 0.72, 95% CI: 0.30-1.72, p=0.45).
	rs1365613 (A>G, intron)	Kraus	No significant impact on metachronous adenoma risk (HR 0.72, 95% CI: 0.29-1.79+, p=0.48).
	rs45567139 (C>A, intron)	Kraus	**Increased risk of metachronous adenoma (HR 12.44, 95% CI: 2.16-71.55, p<0.01).**
PLA2A	rs9657930 (A>C, intron)	Kraus	No significant impact on metachronous adenoma risk (HR 3.98, 95% CI: 0.84-18.74, p=0.08).
SRC	rs6063022 (C>T, near gene-5')	Kraus	**Increased risk of metachronous adenoma (HR 3.38, 95% CI: 1.35-8.50, p<0.01).**
GPX1	rs1050450 (C>T, P200L)	Kraus	No significant impact on metachronous adenoma risk (HR 0.46, 95% CI: 0.19-1.09, p=0.08).
IGSF5	rs2837156	Wang	**Increased risk of advanced metachronous adenoma (OR 2.22, 95% CI: 1.62-3.03, $p=3.2x10^{-7}$).**
	rs7278863	Wang	**Increased risk of advanced metachronous adenoma (OR 2.48, 95% CI: 1.80-3.42, $p=1.4x10^{-8}$).**
	rs2837237	Wang	**Increased risk of advanced metachronous adenoma (OR 2.48, 95% CI: 1.82-3.38, $p=3.6x10^{-9}$).**
	rs2837241	Wang	**Increased risk of advanced metachronous adenoma (OR 2.48, 95% CI: 1.82-3.38, $p=3.7x10^{-9}$).**
	rs2837254	Wang	**Increased risk of advanced metachronous adenoma (OR 2.55, 95% CI: 1.86-3.51, $p=2.9x10^{-9}$).**
	rs741864	Wang	**Increased risk of advanced metachronous adenoma (OR 2.48, 95% CI: 1.80-3.41, $p=1.1x10^{-8}$).**
3p24.1	rs1381392	Wang	**Increased risk of advanced metachronous adenoma (OR 2.01, 95% CI: 1.52-2.65, $p=7.4x10^{-7}$).**
	rs17651822	Wang	**Increased risk of advanced metachronous adenoma (OR 2.16, 95% CI: 1.61-2.91, $p=2.1x10^{-7}$).**
KCNS3	rs11886781	Wang	**Increased risk of advanced metachronous adenoma (OR 2.25, 95% CI: 1.56-3.25, $p=9.7x10^{-6}$).**
EPHB1, KY	rs13085889	Wang	**Increased risk of advanced metachronous adenoma (OR 1.77, 95% CI: 1.37-2.29, $p=8.8x10^{-6}$).**
PLXNA4	rs1424593	Wang	**Decreased risk of advanced metachronous adenoma (OR 0.56, 95% CI: 0.44-0.73, $p=9.1x10^{-6}$).**
	rs1364512	Wang	**Decreased risk of advanced metachronous adenoma (OR 0.56, 95% CI: 0.42-0.71, $p=8.6x10^{-6}$).**
	rs7778725	Wang	**Decreased risk of advanced metachronous adenoma (OR 0.55, 95% CI: 0.42-0.71, $p=4.0x10^{-6}$).**
9q33.2	rs16909065	Wang	**Increased risk of advanced metachronous adenoma (OR 2.59, 95% CI: 1.71-3.93, $p=3.6x10^{-6}$).**
	rs16909036	Wang	**Increased risk of advanced metachronous adenoma (OR 2.59, 95% CI: 1.71-3.93, $p=3.7x10^{-6}$).**
13q33.2	rs1535989	Wang	**Increased risk of advanced metachronous adenoma (OR 2.09, 95% CI: 1.50-2.91, $p=8.9x10^{-6}$).** **Increased risk of colorectal cancer (OR 1.12, 95% CI: 1.019-1.23, p=0.019).**
	rs17654765	Wang	**Increased risk of advanced metachronous adenoma (OR 2.14, 95% CI: 1.53-2.98, $p=4.7x10^{-6}$).**
	rs9582985	Wang	**Increased risk of advanced metachronous adenoma (OR 2.05, 95% CI: 1.48-2.83, $p=9.3x10^{-6}$).**
FAM188b	rs17781398	Wang	**Decreased risk of advanced metachronous adenoma (OR 0.19, 95% CI: 0.08-0.43, $p=9.0x10^{-6}$).**

8.4.3 Table 8.2: IHC markers of metachronous polyp risk.

Protein	Paper	Findings
p53	Brand	**Nuclear p53 expression was associated with a significantly increased risk of metachronous adenoma (72.3% of patients who developed metachronous adenoma had positive p53 nuclear staining vs 20.5% of patients without metachronous adenoma, OR 10.15, p=0.001).**
	Sheikh	**p53 expression was associated with a significantly increased risk of metachronous adenoma (83.3% of patients who developed metachronous adenoma stained positive for p53 vs 50.0% of those without metachronous adenoma, p=0.025).**
	Vernillo	No association between p53 expression and metachronous adenoma risk (p>0.05).
	Benamouzig	No association between p53 expression and metachronous adenoma risk (62.0% of patients who developed metachronous adenoma stained positive for p53 vs 51.0% of those without metachronous adenoma, p>0.05).
β-catenin	Brand	**Nuclear β-catenin expression was associated with significantly increased risk of metachronous adenoma (OR 3.49, p=0.002).**
COX2	Brand	**COX2 expression was associated with significantly increased risk of metachronous adenoma (OR 3.53, p=0.001).**
	Benamouzig	**Deep stromal COX2 expression was associated with a significantly increased risk of metachronous adenoma (OR 2.78, 95% CI: 1.18 to 6.25, p=0.02).**
Ki-67	Vernillo	No association between Ki-67 expression and metachronous adenoma risk (p>0.05).
Adnab-9	Sheikh	**Adnab-9 expression was associated with a significantly increased risk of metachronous adenoma (76.4% of patients who developed metachronous adenoma stained positive for Adnab-9 vs 38.8% of those without metachronous adenoma, p=0.024).**
Cyclin D1	Benamouzig	No association between cyclin D1 expression and metachronous adenoma risk (67.0% of patients who developed metachronous adenoma stained positive for cyclin D1 vs 50.0% of those without metachronous adenoma p>0.05).
Annexin A10 (ANXA10)	Macaron	**ANXA10 expression was associated with a significantly increased risk of metachronous sessile serrated polyps (HR 2.7, p=0.048).** There was no association between ANXA10 expression and metachronous adenomas (p=0.52).
Aldehyde Dehydrogenase Isoform 1A1 (ALDH1A1)	Bartley	**ALDH1A1 expression was associated with a significantly increased risk of metachronous adenomas (mean ALDH1A1 labelling index 22.5% for patients who developed metachronous adenoma vs 15.0% for those without metachronous adenoma, p=0.03).**
Combination of β-catenin, COX2 and p53.	Brand	**Positivity for ≥1 of these markers was associated with a significantly increased risk of metachronous adenoma as compared to triple negativity (OR 13.54, p<0.001)**

8.4.4 Genomic Markers of Metachronous Polyp Risk

19 papers were identified that addressed risk stratification for metachronous polyps using genetic markers. A small proportion of these were somatic mutations identified through analysis of index polyp tissue while the majority were germline single nucleotide polymorphisms and haplotypes (Table 8.1).

8.4.4.1 **Key Proto-Oncogenes and Tumour Suppressor Genes – KRAS, BRAF, APC, EGFR.**

Juárez et al(360) investigated whether the KRAS or BRAF mutation status of index polypectomy specimens could determine the risk of developing metachronous advanced neoplasia at surveillance colonoscopy. In this retrospective study, 995 polyps from 308 patients were sequenced for KRAS mutations at exon 2 and for BRAF mutations at codon 600 (V600E) and divided into three groups: at least one KRAS mutated polyp (22.8%), at least one BRAF mutated polyp (14.9%) and wild type (62.3%). Patients with both KRAS and BRAF mutant status were excluded. On multivariate analysis KRAS mutation was associated with the development of metachronous advanced polyps (OR 2.27, 95% CI: 1.15-4.46, p=0.018) and more specifically with the development of metachronous advanced adenomas (OR 2.23, 95% CI: 1.02-4.85, p=0.044). BRAF mutation status had no impact on the development of metachronous advanced polyps. In Nusko et al(304)'s study of 54 patients, KRAS mutant index adenoma status did not impact overall metachronous adenomas rate. However, having a KRAS mutated index adenoma (OR 4.00, 95% CI: 1.18-13.6, p=0.0265) or an index adenoma ≥20mm (p=0.0259) were predictors of developing metachronous adenomas >5mm. On multivariate logistic regression KRAS mutation did not retain significance as an independent predictor of metachronous adenoma >5mm (OR 3.92, 95% CI: 0.82-18.72, p=0.0871), while adenoma ≥20mm did (p=0.0084). Benamouzig et al(361) found no significant difference in metachronous adenoma rate between KRAS mutated and wild type polyp in their study of 104 adenomas (37.78% vs 33.33%, p>0.05).

Egan et al(362) investigated the impact of 5 SNPs in the APC tumour suppressor gene (rs2229992, rs42427, rs459552, rs465899 and rs2229995) in their study of 1399 patients. No individual SNP had a significant impact, however a haplotype consisting of all five SNPs (TGACC for rs222992, rs42427, rs459552, rs465899 and rs2229995 respectively)

significantly reduced the metachronous adenoma rate as compared to the common haplotype (CAATC) (OR 0.73, 95% CI: 0.57-0.94). A truncated TA haplotype (TA rs2229992 and rs459552) was also associated with a reduced metachronous adenoma rate compared to the common CA haplotype (OR 0.73, 95% CI: 0.59–0.91). For the risk of metachronous advanced adenoma, the TGACC but not the truncated TA haplotype retained significance (OR 0.63, 95% CI: 0.42–0.94 and OR 0.76, 95% CI: 0.54-1.07).

Kraus et al(363) genotyped a number of target genes including EGFR in their study of 117 patients taken from a NSAID chemoprevention trial. Among patients on placebo they found that rs7801956 SNP in the EGFR gene significantly increased the risk of metachronous adenoma (HR 2.65, 95% CI: 1.03-6.86, p=0.04).

8.4.4.2 COX

Cyclooxygenase 1 (COX1) and cyclooxygenase 2 (COX2) catalyse prostaglandin synthesis and play a key role in inflammation (364, 365). COX2 activation stimulates cell proliferation and angiogenesis and inhibits apoptosis. COX2 expression is elevated in ~50% of colorectal adenomas and >85% of CRCs and increased expression is associated with poorer survival in CRC (364). Both COX1 and COX2 are inhibited by aspirin and NSAIDs, which have been associated with a reduced metachronous adenoma rate and reduced incidence of colorectal cancer(365). Hubner et al(365) investigated the COX 1 rs3842787 SNP and the COX2 rs20417 SNP in 546 patients from the UK Colorectal Adenoma Prevention trial. In the parent study patients were randomised to aspirin, folate, both or placebo and scoped at 3 years. Neither SNP of interest had an impact on metachronous adenoma rate or influenced the observed benefit of taking aspirin. While in a similar study by Barry et al(364) the COX2 rs20417 SNP also had no impact on metachronous adenoma rate, CC genotype for rs5277 (RR 1.49, 95% CI: 1.00-2.23) and AG genotype for the rs4648310 (RR 1.35, 95% CI: 1.03-1.77) SNPs in the COX 2 gene

significantly increased metachronous adenoma risk as compared to GG and AA wildtypes respectively. Kraus et al(363), mentioned previously, assessed SNPs in both COX1 and COX2. Examining only the patients on placebo in this NSAID chemoprevention trial, they found that the rs10306110, rs10306122, rs10306164, rs1236913, rs1330344 and rs3119773 SNPs in COX1 and rs4648268 and rs689469 SNPs in the COX2 gene significantly increased metachronous adenoma rate.

8.4.4.3 Interleukins

Chronic inflammation, under the influence of both pro- and anti-inflammatory cytokines, is known to drive colorectal carcinogenesis(366). Bobe et al(366) explored the impact of SNPs in the promoter regions of IL-1β, IL-6, IL-8 and IL-10 on the rate of metachronous adenoma. 808 patients were recruited from the intervention arm of the Polyp Prevention Trial, an RCT investigating a low-fat, high-fibre, -fruit and -vegetable diet. SNPs investigated were IL-1β (rs16944), IL-6 (rs1800795), IL-8 (rs4073), and IL-10 (rs1800872, rs1800871 and rs1800896). No SNP in isolation could predict metachronous adenoma risk. In Hubner et al's(365) study mentioned previously they examined the IL-10 rs1800872 SNP and found no impact on metachronous adenoma rate. Sansbury et al(367) conducted a study exploring a similar set of SNPs to Bobe et al (IL-1β rs16944, IL-6 rs1800795, IL-8 rs4073 and IL-10 rs1800896 and rs1800871) in 1,723 patients. In agreement with Bobe et al, no individual SNP impacted metachronous adenoma rate. Kraus et al(363) mentioned previously, found two SNPs in the IL23R gene (rs10889675 and rs6683455) significantly increased the risk of developing metachronous adenoma (HR 3.08, 95% CI: 1.05-9.04, p=0.04 and HR 2.51, 95% CI: 1.06-5.96, p=0.04 respectively).

8.4.4.4 Dopamine

Murphy et al(368) noted that three SNPs in the dopamine D2 receptor (DRD2) have previously been associated with CRC risk: rs1799732, rs6277 and rs1800497.

Additionally, they linked the rs1799732 CT genotype to increased metachronous adenoma rate (OR 1.30, 95% CI: 1.01-1.69) and the rs1800497 TT genotype to increased advanced adenoma rate (OR 2.4, 95% CI: 1.11-5.20).

8.4.4.5 Cytochrome P450

The Cytochrome P450 enzyme family catalyse the metabolism of a variety of endogenous and exogenous compounds. CYP2C9 is involved in the metabolism of 10-30% of commonly used medications and gene polymorphisms have been associated with colorectal adenoma and CRC-risk and may modify the protective effects of aspirin and NSAIDs(369). Barry et al(369) screened 928 patients participating in an aspirin and folate chemoprevention trial for the rs1799853 and rs1057910 SNPs in the CYP2C9 gene. While the rs1799853 SNP had no impact (RR 1.09, 95% CI: 0.91-1.31), the rs1057910 variant allele increased the risk of metachronous adenoma (RR 1.47, 95% CI 1.19-1.83). Likewise, the risk for metachronous advanced lesions or multiple (≥3) adenomas was not impacted by the rs1799853 SNP (RR 1.29, 95% CI: 0.89-1.85) but was increased by the rs1057910 SNP (RR=1.79, 95% CI=1.16–2.75). Presence of any variant CYP2C9, increased the risk of metachronous adenoma (RR 1.29, 95% CI: 1.09-1.51, p=0.002) and the risk of metachronous advanced lesions or multiple adenoma (RR 1.64, 95% CI: 1.18-2.28, p=0.003). Hubner et al(370) genotyped the same CYP2C9 SNPs in 546 patients, but found no significant interaction with metachronous adenoma rate.

Other cytochrome P450 genes investigated include CYP24A1 and CYP27B1, involved in the synthesis and metabolism of vitamin D. Vitamin D deficiency has been associated with colorectal adenoma and CRC(371). Hibler et al(371) investigated 11 SNPs in CYP24A1 and 1 SNP in the CYP27B1 gene (Table 8.1) in 1,188 patients. A TT genotype in the rs927650 SNP of the CYP24A1 gene was associated with increased metachronous

adenoma risk as compared to CC wildtype (OR 1.38, 95% CI: 1.01-1.89). No other SNP had a significant impact.

Finally, cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in the conversion of cholesterol to bile acids. While bile acids such as chenodeoxycholic acid and deoxycholic acid have been implicated in colorectal carcinogenesis, ursodeoxycholic acid (UDCA) has been shown to be protective(372). Wertheim et al(372) examined six SNPs in the CYP7A1 gene (Table 8.1) among 703 patients from a UDCA randomised chemoprevention trial. Of the six SNPs, the GG genotype for rs8192871 was the only to significantly impact metachronous adenoma risk (OR 0.41, 95% CI: 0.19-0.89 as compared to AA wildtype). Additionally, they found the CCGTAG haplotype (rs10957057, rs8192879, rs8192877, rs11786580, rs8192871 and rs13251096) increased metachronous adenoma risk (OR 1.89, 95% CI: 1.00-3.57) as compared to the common CTACAG haplotype.

8.4.4.6 UGT1A6

UDP-glucuronosyltransferase isoenzyme 1A6 (UGT1A6) is an enzyme involved in the metabolism of aspirin. Two SNPs in the UGT1A6 gene (rs2070959 and rs1105879) are known to be associated with lower enzyme activity and variant alleles may enhance the chemopreventive effects of aspirin (373). Hubner et al(370) found the presence of either SNP significantly reduced the risk of metachronous colorectal neoplasia (RR 0.68, 95% CI: 0.52-0.89).

8.4.4.7 ODC

Polyamines are a group of cations implicated in carcinogenesis and ornithine decarboxylase (ODC) is the rate limiting enzyme in polyamine synthesis. The ODC gene is a target of the MYC transcription factor which is overexpressed following loss of APC

early in colorectal carcinogenesis. Aspirin is known to induce polyamine catabolism and may be a chemopreventive mechanism (374). Hubner et al(374) genotyped 546 patients from the UKCAP aspirin and folic acid chemoprevention trial for the rs2302615 SNP in the ODC gene. Neither GA genotype (RR 0.92, 95% CI: 0.70-1.21) nor AA genotype (RR 0.43, 95% CI: 0.16-1.15) had an impact on metachronous adenoma risk as compared to GG wildtype. In a similar study by Martinez et al(375) involving 688 patients, GA genotype had no impact however AA genotype significantly reduced the risk of metachronous adenoma (OR 0.48, 95% CI: 0.24-0.99, p=0.05). Finally in the study by Barry et al(376) involving 973 patients, neither variant homozygotes (AA) nor heterozygotes (GA) had an altered risk of metachronous adenoma as compared to wild type (GG). Carrying at least one A allele was shown to significantly reduce the risk of metachronous adenoma in patients randomised to aspirin in this study.

8.4.4.8 MTHFR

Folate deficiency has been linked with colorectal neoplasia. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism and polymorphisms in the MTHFR gene have previously been associated with reduced CRC-risk in folate deficient individuals(377). Levine et al(377), investigated two SNPs in the MTHFR gene (rs1801133 and rs11801131) known to be associated with reduced enzyme activity. Neither SNP was associated with metachronous adenoma risk. Folate supplementation did not affect this outcome.

8.4.4.9 Other

Kraus et al(363) screened a large numbers of genes for SNPs impacting metachronous adenoma risk (Table 8.1). The rs2073438 SNP in the ALOX12 gene, rs4796535 in ALOX15, rs45567139 in PGDH and rs6063022 in SRC all significantly increased the risk of metachronous adenoma. The rs2292350 SNP in the ALOX12 gene significantly

decreased metachronous adenoma risk. These genes are involved in arachidonate and leukotriene synthesis, regulation of prostaglandin synthesis and EGFR signalling(363).

8.4.4.10 Genome Wide Association Study (GWAS)

One GWAS was identified which aimed to identify SNPs associated with metachronous advanced adenoma. Wang et al(378) created a discovery set of 1,406 patients from the Adenoma Prevention with Celecoxib trial and a validation set of 4175 familial colorectal adenoma or CRC cases and 5,036 controls from the CORGI, Scotland, VQ58 and Australia GWAS consortia. 19 SNPs with moderate to strong association with metachronous advanced adenoma risk were identified (OR≥2) (Table 8.1). In the validation phase, the rs1535989 SNP was additionally associated with CRC development (OR 1.12, 95% CI: 1.019-1.23, p=0.019).

8.4.5 Immunohistochemistry/ Protein Expression Markers of Metachronous Polyp Risk

6 studies were identified that used immunohistochemistry (IHC) to assess target protein expression as potential markers of metachronous polyp risk (Table 8.2).

8.4.5.1 P53

Mutations in the p53 tumour suppressor gene are a late event in colorectal carcinogenesis and generally result in overexpression of the gene product(40, 379, 380). Brand et al(380) studied the expression of p53 of index polypectomy specimens from 109 patients from the German 5-ASA Polyp Prevention Study, a randomised, placebo-controlled mesalazine adenoma chemoprevention trial. They reported that nuclear expression of p53 was significantly associated with metachronous adenoma risk at 3 years (72.3% vs 20.5% of patients with and without metachronous adenoma had positive p53 nuclear staining, OR

10.15, p=0.001). Sheikh et al(381) conducted a similar study only including patients with index adenomas with high-grade dysplasia. 83.3% of patients who developed metachronous adenoma showed p53 positivity in their index polyp as compared to 50.0% of patients with no metachronous adenoma (p=0.025). In contrast, studies by Vernillo et al(382) and Benamouzig et al(361) found no association between p53 expression and metachronous polyp risk.

8.4.5.2 β-Catenin

β-catenin is a key protein in the pro-proliferative Wnt signalling pathway(40). Increased expression of β-catenin is present in the majority of colorectal adenomas and nearly all CRCs(380). Brand et al(380) described above, found nuclear β-catenin expression to be associated with metachronous adenoma risk at 3 years (OR 3.49, p=0.002).

8.4.5.3 COX2

Benamouzig et al(361) assessed COX2 expression in 219 index adenomas from 136 patients participating in a double-blind aspirin chemoprevention RCT. While strong overall COX2 expression had no significant association with the risk of metachronous adenoma (42.0% vs 45.0% of patients with and without metachronous adenomas had strong overall COX2 expression, p>0.05), strong deep stromal COX2 expression was able to predict metachronous adenoma (42.0% vs 25.0% of patients with and without metachronous adenoma had strong deep stromal COX2 expression, p=0.04). On multivariate analysis deep stromal COX2 expression was an independent predictor of metachronous adenoma (OR 2.78, 95% CI: 1.18 to 6.25, p=0.02). Brand et al(380) described previously, also found COX2 positivity to be associated with metachronous adenoma risk at 3 years (OR 3.53, p=0.001).

8.4.5.4 Ki67

Ki67 is a nuclear protein only present in cycling cells and whose expression is used as a marker of cellular proliferation. Increased Ki67 expression has been associated with colorectal adenomas with high grade dysplasia(382). Vernillo et al(382) found no association between Ki67 expression and risk of metachronous polyps in their study of 78 adenoma from 51 patients.

8.4.5.5 Adnab-9

Adnab-9 is a monoclonal antibody developed to react to an adenoma-associated antigen expressed early in the adenoma-carcinoma sequence. It stains at-risk, dysplastic, non-invasive colorectal epithelium but not invasive tumour tissue and has been shown to correlate with future CRC risk(381). In Sheikh et al's (381) study, as described previously, including patients with index adenomas with high grade dysplasia only, 76.4% of patients who developed metachronous adenoma stained positive for Adnab-9 at IHC as compared to 38.8% of patients without metachronous adenoma ($p=0.024$).

8.4.5.6 Cyclin D1

Cyclin D1 is a proto-oncogene with important roles in regulating cell cycle progression(383). It is activated by β-catenin, a key component of the Wnt-signalling pathway known to be upregulated in colorectal carcinogenesis(361) and increased cyclin D1 expression has been associated with more advanced colorectal malignancies and reduced overall survival(384). In the study by Benamouzig et al(361) they found 66.7% of patients with high cyclin D1 expression developed metachronous adenoma as compared to 50.8% of patients with low cyclin D1 expression but this did not reach statistical significance.

8.4.5.7 Annexin A10

Annexin A10 (ANXA10) is a calcium and phospholipid binding protein with roles in growth regulation, cell division, apoptosis and differentiation. High ANXA10 expression has been shown to occur more frequently in sessile serrated polyps as compared to hyperplastic polyps and may differentiate these effectively. It is more highly expressed by serrated colonic carcinomas as opposed to conventional colon cancers(385). Macaron et al(385) used IHC to assess the expression of ANXA10 in 179 patients with either a sessile serrated or hyperplastic polyp. Patients with high levels of ANXA10 expression within their index polyp had an increased risk of metachronous sessile serrated polyp at follow-up colonoscopy (HR 2.7, p=0.048), particularly in the proximal colon (HR 4.0, p=0.02). The rate of metachronous adenomatous polyp was similar between the groups (18.8% vs 19.4%, p=0.52).

8.4.5.8 ALDH1A1

A number of solid tumours have been shown to possess cells with stem cell-like properties including ability to self-renew and multipotency. These stem-like cells are believed to possess tumour initiation and maintenance capabilities and have been reported to be present in premalignant adenomas. Aldehyde dehydrogenase isoform 1A1 (ALDH1A1) is a well-recognised biomarker for the presence of stem-like cells(386). Bartley et al(386) performed IHC using ALDH1A1 antibody on index polyps taken from placebo-arm patients from two polyp prevention trials. 20 polyps from 20 patients were used to form an exploratory set and 89 polyps from 76 patients known to be high risk for metachronous adenomas acted as a validation set. In both sets, patients who developed metachronous adenoma had a significantly higher expression of ALDH1A1 compared to those without metachronous adenomas (mean ALDH1A1 labelling index 22.5% vs 15.0%, p=0.03 for the validation set).

8.4.5.9 Combination of IHC Markers.

As discussed previously, Brand et al(380) found the expression of β-catenin, COX2 and p53 to all individually be associated with the risk of metachronous adenomas. They therefore combined these three markers to explore whether collectively they represent a more powerful predictor. Of the 109 study participants, 26 (23.9%) patient's adenomas were triple-negative, while 83 (76.1%) patient's adenomas were positive for at least one marker. Only 3 of 26 (11.5%) triple-negative patients developed metachronous adenomas while 53 of 83 (63.8%) patients with at least one positive marker did. This translated into a negative predictive value of 88.5% and a sensitivity of 94.6% for freedom from metachronous adenoma and an OR 13.54 for metachronous adenoma.

8.5 Discussion

This study provides a comprehensive literature review for novel markers of metachronous polyp risk. 19 papers exploring 94 individual mutations, SNPs or haplotypes as predictors of future polyp risk in 33 different genes or non-coding chromosomal regions were identified. Six papers were found that attempted to predict metachronous polyp risk using IHC to measure the expression levels of eight different target proteins and one combination of target proteins. While the results are promising, no clear definitive marker of future polyp risk has been identified.

Genomic markers are the most studied. It is important to note that only three studies directly assessed the genomics of index polyp tissue (304, 360, 361), while most studies used the presence of germline SNPs, as determined from blood samples, to assess the influence of target genes on metachronous polyp risk. While several positive genetic markers were identified, most individual mutations or SNPs were not able to significantly predict future polyp risk. Additionally, it was not uncommon for one study to find a mutation or SNP to be an accurate predictor, while a second study has disputed this positive finding.

Perhaps one of the most intriguing papers is that by Juárez et al(360) which examined the KRAS and BRAF mutation status of index polyps. KRAS and BRAF are key proto-oncogenes, frequently activated in colorectal carcinogenesis. While BRAF had no impact, KRAS mutant status was significantly associated with the development of metachronous advanced polyps and advanced adenomas. Of note patients with index polyps with both KRAS and BRAF mutations were excluded, and it is not clear how this may influence risk. KRAS mutant status is already routinely tested in patients with metastatic CRC being considered for treatment with the anti-EGFR monoclonal antibody, cetuximab. This testing may feasibly be applied to polypectomy specimens to refine risk stratification for

colonoscopic surveillance. It should be noted that two smaller studies did not find KRAS mutant status to correlate with metachronous adenoma risk [49, 50]. Other positive genomic markers of note included the TGACC (rs222992, rs42427, rs459552, rs465899 and rs2229995) and TA (rs2229992 and rs459552) haplotypes in the APC gene which significantly reduced metachronous adenoma rate (362), as well as numerous SNPs in the COX1 and COX2 genes which increased this risk (Table 8.1) (363, 364).

A number of immunohistochemical markers of metachronous polyp risk have also been identified including the key tumour suppressor gene p53 (380, 381), β-catenin (380), Adnab-9 (381) and ALDH1A1 (386). The important role of COX2 has been reconfirmed at the protein expression level (361, 380) and ANXA10 has been identified as a marker specific to metachronous sessile serrated polyp risk (385). The study by Brand et al(380) is perhaps the most interesting IHC-based paper in that it combined the expression of β-catenin, COX2 and p53 into a single powerful predictor. Only 11.5% of patients who were triple-negative for these markers developed metachronous adenomas compared to 63.8% of patients positive for at least one. It seems likely that a wide panel of markers may have to be combined in this manner to accurately predict metachronous polyp risk.

No studies were identified which examined the microbiome in the context of metachronous polyp risk. There is a rapidly expanding literature relating the colonic microbiome to polyps and CRC in the contexts of either detection and diagnosis(387), or in carcinogenesis pathways(388). Indeed, there are studies which link specific species of bacteria such as Fusobacterium nucleatum to serrated and traditional adenomas, increasing dysplasia and early cancer(389). Furthermore, it may be that different types of polyp are associated with different microbiomic landscapes(390). Although there remains debate as to whether such dysbiosis is causal, or is an epiphenomenon of colonic tumorigenesis, there is evidence that altering the microbiome can have effects on established CRC in animal models(391).

Therefore, further of investigation of the role of the microbiome in metachronous polyp risk would seem important.

Likewise, no papers were identified that used transcriptomics to predict future polyp risk. Studies have however compared polyp subtypes and advancement in the context of their transcriptome. Druliner et al(392) used RNA sequencing to compare polyps with or without an adjacent synchronous CRC (cancer adjacent polyp (CAP) and cancer free polyp (CFP) respectively). CAPs showed significantly higher levels of CXCL5, GREM1, IGF2, CTGF and PLAU expression, as compared to CFPs. Several of these genes are known to play a role in colorectal carcinogenesis. Chang et al(393) performed RNA sequencing on 301 adenomas and 88 serrated polyps to establish whether the CRC CMS classification could be applied to premalignant polyps. They found that adenomas predominantly displayed a CMS2-like phenotype with WNT and MYC activation, while hyperplastic and serrated polyps most commonly displayed CMS1-like phenotype with strong immune activation. Both of these studies give us important insights into the transcriptomic landscapes of premalignant colorectal polyps, however the application of transcriptomics to assess future polyp risk represents a significant gap in the literature.

While no other systematic review was identified which addressed the wide range of novel methods of risk stratification as was explored in the current study, a narrative review by Hao et al did examine gene polymorphism studies as well as conventional influencers of metachronous polyp risk such as polyp characteristics, patient factors, chemopreventive medications and diet (279). In agreement with the present study they found the rs5277 CC and rs4648310 AG genotypes in the COX2 gene, the rs927650 TT or TC genotype in the CYP24A1 gene and the presence of any variant allele (rs1057910 or rs1799853) in the CYP2C9 gene, to increase the risk of metachronous adenoma compared to wildtype. Additionally, they found the rs1799732 CT or rs1800497 TT genotypes in the DRD2 gene increased the risk of metachronous advanced adenoma. They concluded that most

individual gene polymorphisms did not alter metachronous polyp risk independently but may alter this risk when combined with an intervention; dietary changes being the focus of that review.

The main limitation of the current systematic review is the generally small sample size of each identified study, with 20 of 25 included studies involving less than 1,000 patients and all six IHC-based studies involving less than 200 patients. Additionally, 19 of the 25 studies were published prior to 2010. It is possible to argue that the results may not be entirely applicable to current practice given advancements in colonoscopic technology, the introduction of bowel screening programmes, altered surveillance guidelines and the more recent recognition of sessile serrated polyps as a distinct malignant precursor. Indeed, the vast majority of included papers focussed on metachronous adenoma rate only. Additionally, there was heterogeneity in length of follow up and most of the patients in the genomic studies were recruited from RCTs examining a chemopreventive medication or dietary change which may limit generalisability.

The strengths of the current study include the systematic nature of the literature review with two authors participating in the title, abstract and full paper appraisal. Additionally, this is the first paper to systematically review for studies that have used genomics, transcriptomics, immunohistochemistry and microbiome as novel techniques of metachronous polyp risk stratification. It has identified a gap in the literature in terms of a definitive multi-modal novel metachronous polyp risk prediction tool.

In conclusion, a variety of genomic and immunohistochemical markers which significantly correlated with metachronous polyp risk have been identified within the literature. It seems likely that future research will have to amalgamate a panel of novel markers in order to develop a score able to accurately determine future polyp risk. The INCISE project is a large, retrospective, multi-partner collaborative project which aims to use patient

characteristics, digital pathology, immunohistochemistry, genomic and transcriptomic features, merged using machine learning, to predict future polyp risk(281). It is hoped that the technology used in this study could be applied in clinical practice to markedly refine surveillance protocols. This may reduce the number of unnecessary, invasive colonoscopies performed, reduce the burden on stretched endoscopy services and simultaneously increase the detection yield for high-risk metachronous polyps.

9 Expression of COX2 and p53 to predict metachronous lesion development following polypectomy: an Integrated Technologies for Improved Polyp Surveillance (INCISE) project study.

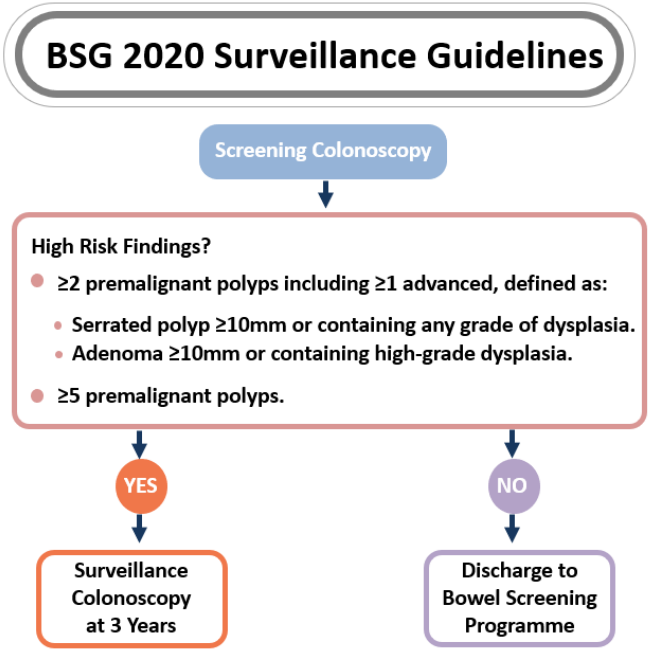
9.1 Introduction

The presence of high-grade dysplasia(286-290, 308), larger polyp size(286-289) and an increasing number of polyps found at index colonoscopy(286-289, 308, 310-312) are well-established indicators of heightened risk of developing metachronous advanced polyps or colorectal cancer (CRC), following polypectomy. These indices are therefore currently used to select which patients to offer surveillance colonoscopy, according to the British Society of Gastroenterology 2020 guidelines (BSG 2020) (Figure 9.1). However, in chapter 7, the accuracy of BSG 2020 was called into question. In this retrospective study of 2,643 patients undergoing post-polypectomy surveillance prior to the introduction of the current guidance, retrospective application of the BSG 2020 criteria meant 1,360 (51.5%) would now be deemed low risk and would no longer qualify for surveillance colonoscopy. 210 of 1,283 (16.3%) BSG 2020 high-risk patients and 177 of 1,360 (13.0%) BSG 2020 low-risk patients were found to have advanced metachronous polyps or CRC at surveillance colonoscopy(314). Clearly, this misclassification is problematic and suggests a proportion of BSG 2020 low-risk patients are being under-surveyed while the majority of both high- and low-risk patients may be undergoing unnecessary invasive investigation. The more accurate our ability to risk stratify patients for metachronous lesion development, the better the risk/ benefit profile is for our patients and the more efficiently we can allocate National Health Service (NHS) resources.

The Integrated Technologies for Improved Polyp Surveillance (INCISE) project is a large, retrospective, collaborative study which aims to use patient characteristics, digital pathology, immunohistochemistry (IHC), genomic and transcriptomic features of index

polyp tissue to predict metachronous polyp risk and refine BSG 2020 surveillance protocols(281). In chapter 8 the results of a systematic literature review for novel biomarkers of metachronous polyp development were presented(394). A myriad of gene mutations, single nucleotide polymorphisms (SNPs) or haplotypes were identified as useful markers, in addition to a smaller number of immunohistochemical (IHC) markers. Perhaps one of the most interesting IHC-based papers was that by Brand et al(380) in which the expression of β-catenin, COX2 and p53 were combined into a single powerful predictor: only 11.5% of patients who were triple-negative for these markers developed metachronous adenomas compared to 63.8% of patients positive for at least one. This chapter aimed to assess COX2 and p53 expression as potential biomarkers to predict metachronous polyp or CRC risk in INCISE cohort patients. Chapter 10 will focus on β-catenin.

9.1.1 Figure 9.1: British Society of Gastroenterology and Association of Coloproctology of Great Britain and Ireland post-polypectomy surveillance guidelines



9.2 Methods

9.2.1 Patients

The INCISE cohort has previously been described in chapter 7(314). Briefly, all patients undergoing polypectomy at screening colonoscopy in NHS Greater Glasgow and Clyde (GG&C) between April 2009 and December 2016 were identified. Patients were only included if they had reached the lower age for invitation to the Scottish Bowel Screening Programme (≥50 years), had a positive faecal occult blood, proceeded to screening colonoscopy, had a premalignant polyp excised (adenoma or serrated polyps (excluding diminutive rectal hyperplastic polyps <5mm)) and underwent a further colonoscopy within 6 months to 6 years of their index scope to allow identification of metachronous polyps or CRC. The earliest polypectomy was chosen as the index for each patient. Patients were excluded if they were found to have CRC at their index colonoscopy, had a previous histological diagnosis of CRC or inflammatory bowel disease or a known inherited polyposis or CRC syndrome. Additionally, patients were excluded if they had insufficient index pathology tissue available for genomic, transcriptomic and immunohistochemical analysis. The full INCISE cohort comprises of 2,643 patients. A subset of 1,236 patients had tissue retrieved for immunohistochemical assessment and formed the cohort for the current study. Ethical approval was obtained for the INCISE project (GSH/20/CO/002). The methods and results were reported according to Strobe guidelines.

9.2.2 Clinicopathological Variables

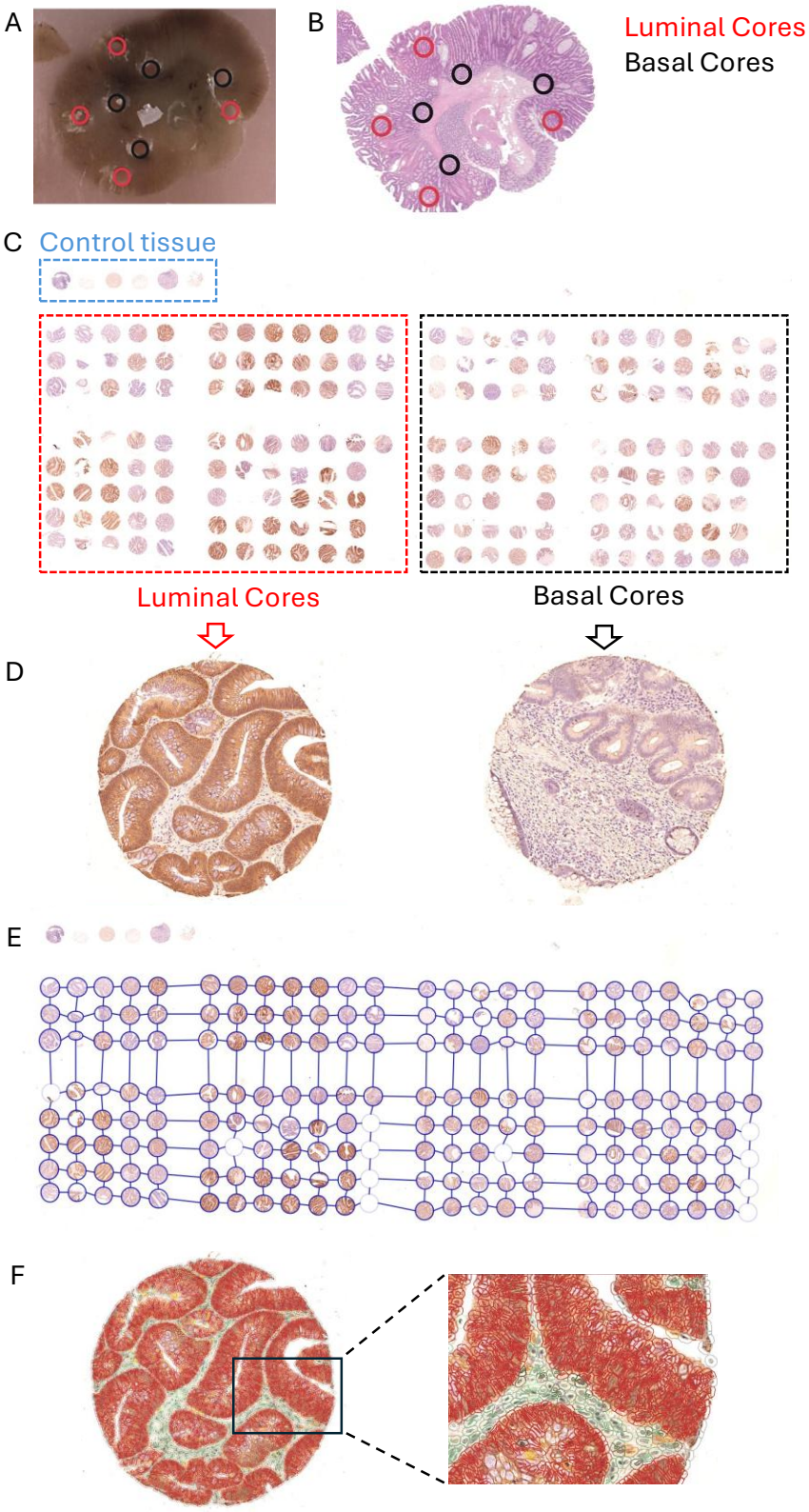
Electronic case notes were used to obtain patient demographics. Endoscopy and pathology reports were used to identify index pathology details including number of index polyps excised and, for the most advanced index polyp, histological subtype (adenoma vs serrated polyp), location, size, morphology (presence or absence of villous architecture) and degree

of dysplasia (high- or low-grade). Advanced polyps were defined as adenoma ≥10mm or containing high-grade dysplasia (HGD) or a serrated polyp ≥10mm or containing any grade of dysplasia (31). All patients in this study underwent surveillance colonoscopy based on the guidance in use at the time(282, 283), however, the most recent BSG 2020 guidelines(31) were retrospectively applied to each patient thus categorising them as high-risk (qualifying for surveillance colonoscopy in present day practice) or low-risk (no longer qualifying for surveillance) (Figure 9.1). Endoscopy and pathology reports were also used to determine the presence or absence of metachronous lesions (non-advanced polyps, advanced polyps or CRC) at surveillance colonoscopy, the study outcome variable.

9.2.3 Immunohistochemistry Staining

Cytoplasmic COX2 and nuclear p53 expression were assessed using immunohistochemistry. Formalin-fixed, paraffin-embedded tissue blocks were retrieved from the archive for the most advanced index polyp for each patient and processed in a centralised laboratory (Queen Elizabeth University Hospital, Glasgow, UK). Tissue microarrays (TMA) were constructed with four 0.6mm polyp cores available per patient in case of heterogeneity in expression: two cores were taken from the luminal surface of the polyp and two were taken from the basal portion in each case (Figure 9.2). Positive controls of tonsil, colon, liver, prostate, spleen, lung, breast and skin tissue were included in each TMA. Additionally, colorectal tissue was used for the negative control and isotype control for each stain.

9.2.4 Figure 9.2: TMA construction and QuPath immunohistochemical scoring.



A: Gross polyp block. Red circles indicate cores taken from luminal edge of epithelium and black circles indicate basal edge. **B**: Haematoxylin and eosin (H&E) stained polyp. **C**: TMA slide with control tissue, luminal and basal cores indicated. **D**: Example luminal and basal core. **E**: Dearrayed TMA. **F**: TMA core following cell detection and annotation.

9.2.4.1 COX2

The TMA's were stained with COX2 antibody (D5H5, Cell Signalling, Danvers, MA, USA) at a concentration of 1:300. Antigen retrieval was with high pH TRS. Rabbit EnVision secondary antibody was used along with liquid DAB chromogen, using the Agilent Autostainer Link 48 (Colin Nixon, Histology Service, Beatson Institute for Cancer Research, UK).

9.2.4.2 P53

The TMA's were stained in p53 antibody (M70001, Agilent, Santa Clara, CA, USA) at a concentration of 1:1000. Antigen retrieval was with high pH TRS. Rabbit EnVision secondary antibody was used along with liquid DAB chromogen, using the Agilent Autostainer Link 48 (Colin Nixon, Histology Service, Beatson Institute for Cancer Research, UK).

9.2.5 Immunohistochemistry Scoring

Stained TMA's were scanned with a high-resolution digital scanner (Hamamatsu NanoZoomer, Hamamatsu, Welwyn Garden City, UK) and could be accessed and viewed on an encrypted server (NanoZoomer Digital Pathology). For the purposes of digital scoring these TMA slides were exported into QuPath (Version 0.2.0-M4, Quantitative Pathology & Bioimage Analysis). The software TMA Dearrayer function was used to create a TMA grid which could be manually manipulated if any cores were off centre. Stain vectors were estimated followed by cell detection. The epithelium and lamina propria were then manually annotated in a small proportion of every single core to train the software to recognise each tissue type. Additional annotations were added where required to ignore artefact and white space. A random trees detection classifier was created to quantify the expression of each biomarker using 22 features for COX2 and 41 features for p53 including nucleus and cell area, perimeter, circularity and eccentricity, nuclear: cell

ratio and nucleus, cell and cytoplasm optical density. Three intensity thresholds were selected which divided staining into negative, weak, moderate and strong. After building the classifier for each TMA the auto-update feature was turned on and each core was checked for errors and the classifier re-validated in real time (Figure 9.2). Once the classifier was finalised, a weighted histoscore (H-score, calculated as: (% of negative stained tumour cells × 0) + (% of weakly stained tumour cells × 1) + (% of moderately stained tumour cells × 2) + (% of strongly stained tumour cells × 3) to give a range from 0 to 300)(395) could be exported for each core, cellular compartment (cytoplasm or nuclear) and epithelial edge (luminal or basal). One author (MJ) scored all batch 1 patients (n=339) according to the above methodology, while other authors scored the full cohort (n=1236) for COX2 (CB) and p53 (CB, AA and AL). 10% of all cores were also scored manually to ensure accuracy of the automated scoring.

9.2.6 Statistical Analysis

Intraclass correlation coefficients were calculated between the primary author (MJ) and all other authors and between digital and manual scoring to ensure objectivity and consistency with a threshold of above 0.75 considered acceptable(396). The entire INCISE cohort was previously randomised into training (discovery) and test (validation) cohorts, to allow internal validation of any biomarkers of metachronous polyp risk that may arise from INCISE project studies. This was performed as a 70:30% training: test split and was weighted to ensure an equal distribution of sex and outcome (future polyp or CRC). After staining and scoring of all TMAs, all statistical analyses were initially only performed on training cohort patients and only where significant observations were made were these repeated on test patients for validation.

Average COX2 expression for each patient was determined by calculating the mean H-score across the cores available. These were imported into R studio (Version 2022.02.2,

Boston, MA, USA) and using maximally selected rank statistics with the presence or absence of any metachronous lesion as the outcome of interest, a cut point was generated to define high and low expression.

In current clinical practice when pathologists examine tumoural p53 expression using IHC, zero p53 expression and very high p53 expression are regarded as potentially mutant, with tissue subsequently sent for mutational analysis to confirm this at the DNA level. Therefore, for p53 a decision was made to select the core with the highest H score for each patient, rather than calculating mean expression across the cores, which may dilute those with very high p53 expression. Maximum p53 scores were then dichotomised using an H score of 200 as the cut off for high and low expression. Binary scores for each biomarker were then transferred to SPSS (IBM SPSS Statistics, version 28.0) where all further analysis was conducted.

Covariables were compared using crosstabulation and the χ^2 or Fisher's exact test for categorical variables and the Mann Whitney U Test for continuous data. A value of $p<0.05$ was considered statistically significant. To identify covariables which independently predicted time to metachronous lesion detection, univariate followed by multivariate cox regression analysis was performed. This allowed calculation of hazard ratios (HRs) and 95% confidence intervals (95% CI). Covariables significant on univariate analysis ($P<0.05$) were entered into a multivariate model using the backwards conditional method in which variables with a significance of $p<0.1$ were removed from the model in a stepwise fashion. 1-survival curves were created.

9.3 Results

9.3.1 Patients, Clinicopathological Variables and Outcomes

Table 9.1 shows the demographics, pathology and outcomes of all 1,236 patients, as well as a comparison between training (n=859) and test (n=377) patients. With a median time to surveillance colonoscopy of 3 years (IQR 1.4–3.7 years), 673 (54.4%) patients were found to have any metachronous polyp or CRC and 185 (15.0%) had an advanced polyp or CRC. Training and test patients were generally similar, however the training cohort was marginally older (median 63 vs 61 years, p=0.013), had a higher proportion of index rectal polyps (14.8 vs 10.1%) and less index right colonic polyps (12.7 vs 17.2%, p=0.017), as compared to the test cohort. There was no significant difference between training and test cohorts in terms of outcome (38.5 vs 41.6% metachronous non-advanced polyp, 15.1 vs 14.6% metachronous advanced polyp or CRC, p=0.585).

9.3.2 Table 9.1: Comparison of demographics, pathology and outcomes between training and test patients.

		All	Training	Test	P
N		1236	859	377	
Age (years)	Median (IQR)	63 (57-69)	63 (57-69)	61 (55-69)	0.013
Sex	Male	879 (71.1%)	604 (70.3%)	275 (72.9%)	
	Female	357 (28.9%)	255 (29.7%)	102 (27.1%)	0.348
Index Polyp Advanced		22 (1.8%)	15 (1.7%)	7 (1.9%)	
	Yes	1214 (98.2%)	844 (98.3%)	370 (98.1%)	0.892
Index Polyp Number	1	395 (32.0%)	285 (33.2%)	110 (29.2%)	
	2-4	675 (54.6%)	469 (54.6%)	206 (54.6%)	
	5+	166 (13.4%)	105 (12.2%)	61 (16.2%)	0.113
Index Polyp Villous	No	556 (45.0%)	396 (46.1%)	160 (42.4%)	
	Yes	680 (55.0%)	463 (53.9%)	217 (57.6%)	0.234
Index Polyp Type	Adenoma	1210 (97.9%)	845 (98.4%)	365 (96.8%)	
	Serrated Polyps	26 (2.1%)	14 (1.6%)	12 (3.2%)	0.080
Index HGD	No	1045 (84.5%)	725 (84.4%)	320 (84.9)	
	Yes	191 (15.5%)	134 (15.6%)	57 (15.1%)	0.830
Index Polyp Size (mm)	<10	24 (1.9%)	17 (2.0%)	7 (1.9%)	
	≥10	1212 (98.1%)	842 (98.0%)	370 (98.1%)	0.886
Index Polyp Location	Rectum	165 (13.3%)	127 (14.8%)	38 (10.1%)	
	Left Colon	897 (72.6%)	623 (72.5%)	274 (72.7%)	
	Right Colon	174 (14.1%)	109 (12.7%)	65 (17.2%)	0.017
BSG 2020 Risk Index Procedure	Low	411 (33.3%)	296 (34.5%)	115 (30.5%)	
	High	825 (66.7%)	563 (65.5%)	262 (69.5%)	0.174
Metachronous Lesion	No	563 (45.6%)	398 (46.3%)	165 (43.85)	
	Non-advanced Polyp	488 (39.5%)	331 (38.5%)	157 (41.6%)	
	Advanced Polyp or CRC	185 (15.0%)	130 (15.1%)	55 (14.6%)	0.585

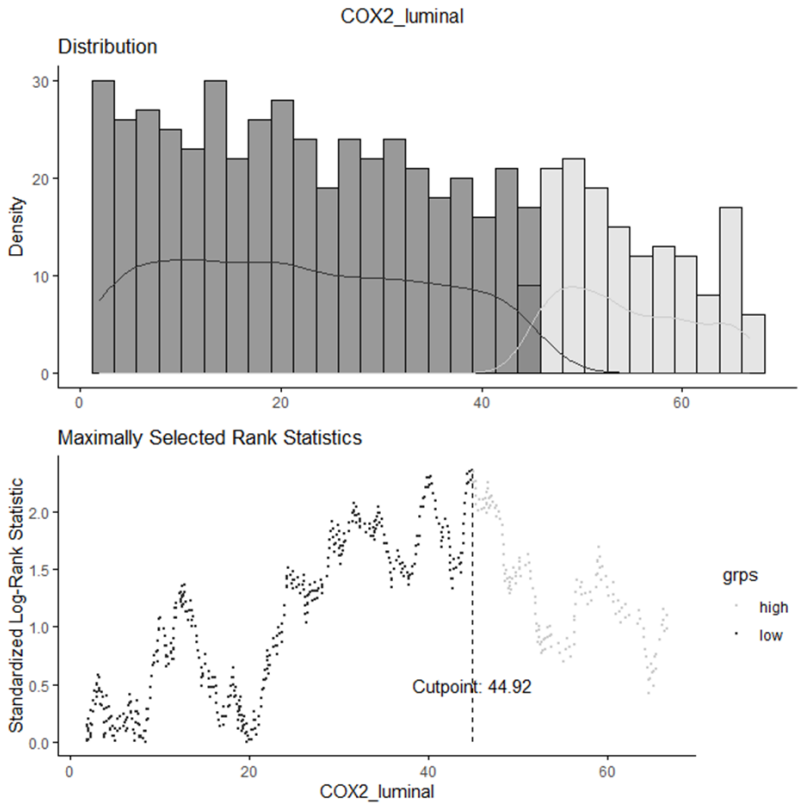
9.3.3 COX2

COX2 expression was observed in the cytoplasm of cells within the epithelial layer of index polyps, both in cores taken from the luminal and basal edge of the epithelium. However, luminal epithelial staining showed greater uniformity and was felt to be more representative of the degree of COX2 expression and was carried forward for analysis. Intraclass correlation coefficient between both scorers for COX2 luminal epithelial cytoplasmic expression was 0.918 (95% CI: 0.898-0.934) and between digital and manual scoring was 0.963 (95% CI: 0.950-0.973). Amongst 859 training patients, 108 (12.6%)

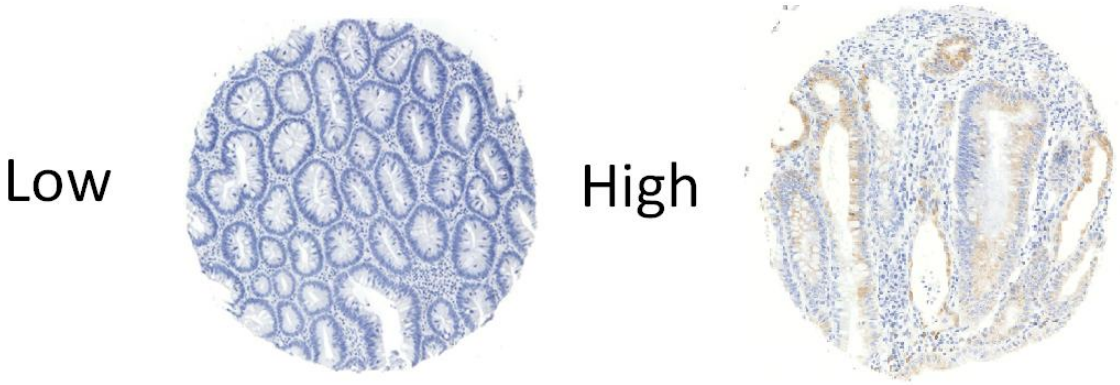
patients had missing TMA cores or cores of insufficient quality to assess COX2 expression, leaving 751 (87.4%) patients for analysis.

For each patient an average epithelial COX2 cytoplasmic expression was calculated by taking a mean H-score from the two luminal epithelial cores. Median average luminal epithelial H-score for the 751 valid training patients was 29.38 (range 0-119.48, IQR 10.81-49.08). These scores were imported into R studio and using maximally selected rank statistics with the presence or absence of any metachronous lesion as the outcome of interest, a cut point was generated to define high (H score ≥44.92) and low COX2 expression (<44.92) (Figure 9.3). Examples of high and low COX2 expression are shown in Figure 9.4. It should be noted that COX2 expression was generally low throughout the TMA cohort, and the term "high COX2 expression" should be interpreted in this context simply as meaning expression in the upper half of the dichotomised score. Amongst training patients, there were no significant differences between patients with index polyps exhibiting high and low COX2 expression in terms of patient demographics nor index polyp pathological characteristics. However, high COX2 expression did correlate with a higher rate of metachronous lesion development (p=0.006) (Table 9.2).

9.3.4 Figure 9.3: Maximally selected rank statistics used to define high and low COX2 expression.



9.3.5 Figure 9.4: TMA cores displaying low and high expression of COX2.



9.3.6 Table 9.2: Comparison of demographics, pathology and outcomes by expression of each biomarker in INCISE training cohort.

		COX2		P	p53		P
		Low	High		Low	High	
N		463	288		762	17	
Age (years)	Median (IQR)	63(59-69)	65 (57-69)	0.402	63 (57-69)	67 (58-68)	0.314
Sex	Male	325 (70.2%)	207 (71.9%)		543 (71.3%)	14 (82.4%)	0.421
	Female	138 (29.8%)	81 (28.1%)	0.622	219 (28.7%)	3 (17.6%)	
Index Polyp Advanced	No	5 (1.1%)	8 (2.8%)		15 (2.0%)	0 (0%)	
	Yes	458 (98.9%)	280 (97.2%)	0.092	747 (98.0%)	17 (100%)	1.0
Index Polyp Number	1	157 (33.9%)	97 (33.7%)		252 (33.1%)	7 (41.2%)	
	2-4	246 (53.1%)	162 (56.3%)		416 (54.6%)	8 (47.1%)	
	5+	60 (13.0%)	29 (10.1%)	0.456	94 (12.3%)	2 (11.8%)	0.776
Index Polyp Villous	No	208 (44.9%)	133 (46.2%)		350 (45.9%)	5 (29.4%)	
	Yes	255 (55.1%)	155 (53.8%)	0.737	412 (54.1%)	12 (70.6%)	0.176
Index Polyp Type	Adenoma	456 (98.5%)	286 (99.3%)		752 (98.7%)	17 (100%)	
	Serrated Polyps	7 (1.5%)	2 (0.7%)	0.494	10 (1.3%)	0 (0%)	1.0
Index HGD	No	395 (85.3%)	236 (81.9%)		648 (85.0%)	5 (29.4%)	
	Yes	68 (14.7%)	52 (18.1%)	0.221	114 (15.0%)	12 (70.6%)	<0.001
Index Polyp Size (mm)	<10	7 (1.5%)	8 (2.8%)		17 (2.2%)	0 (0%)	
	≥10	456 (98.5%)	280 (97.2%)	0.228	745 (97.8%)	17 (100%)	1.0
Index Polyp Location	Rectum	55 (11.9%)	51 (17.7%)		109 (14.3%)	3 (17.6%)	
	Left Colon	347 (74.9%)	205 (71.2%)		558 (73.2%)	13 (76.5%)	
	Right Colon	61 (13.2%)	32 (11.1%)	0.073	95 (12.5%)	1 (5.9%)	0.691
BSG 2020 Risk Index Procedure	Low	163 (35.2%)	100 (34.7%)	0.893	263 (34.5%)	7 (41.2%)	0.568
	High	300 (64.8%)	188 (65.3%)		499 (65.5%)	10 (58.8%)	
Metachronous Polyp or CRC	No	228 (49.2%)	112 (38.9%)		341 (44.8%)	6 (35.3%)	
	Yes	235 (50.8%)	176 (61.1%)	0.006	421 (55.2%)	11 (64.7%)	0.438
Metachronous Advanced Lesion	No	396 (85.5%)	242 (84.0%)		645 (84.6%)	12 (70.6%)	
	Yes	67 (14.5%)	46 (16.0%)	0.576	117 (15.4%)	5 (29.4%)	0.165

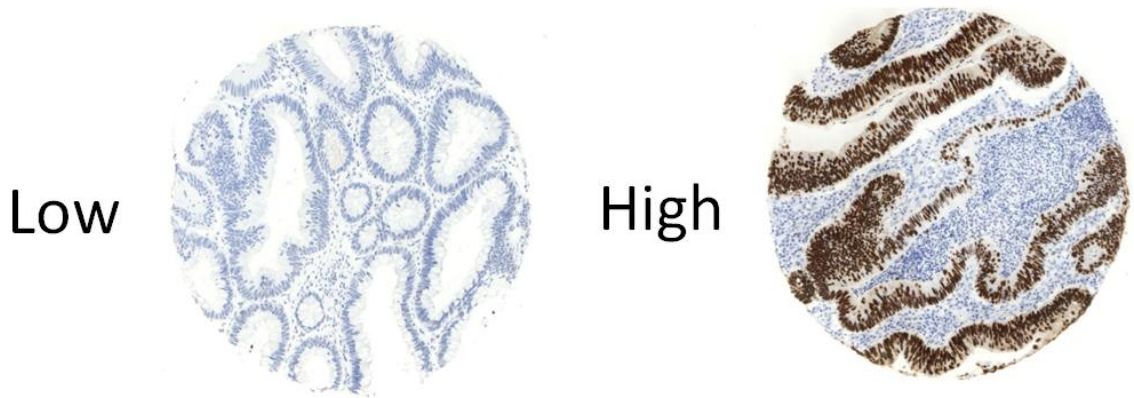
9.3.7 P53

P53 expression was observed in the cytoplasm and nucleus of cells within the epithelial layer of index polyps, both in cores taken from the luminal and basal edge of the epithelium. The quality and uniformity of the staining was similar in luminal and basal cores and so all four cores available for each patient could be used for analysis. As p53 is primarily a nuclear transcription factor, nuclear expression was carried forward for analysis. Intraclass correlation coefficient between both scorers for p53 epithelial nuclear expression was 0.753 (95% CI: 0.702-0.797) and between digital and manual scoring was 0.972 (95% CI: 0.961-0.979). Amongst 859 training patients, 80 (9.3%) patients had

missing TMA cores or cores of insufficient quality to assess p53 expression, leaving 779 (90.7%) patients for analysis.

The maximum p53 H-score from the four cores available for each patient was recorded. Median maximum H-score for the 779 valid training patients was 8.64 (range 0-273.61, IQR 3.52-8.64). These scores were dichotomised into high (H-score score >200) and low (≤200) p53 expression. Examples of high and low p53 expression are shown in Figure 9.5. Amongst training patients, there were no significant differences between patients with index polyps exhibiting high and low p53 expression in terms of patient demographics nor index polyp pathological characteristics, except for the presence of HGD which significantly correlated with high p53 expression ($p<0.001$). P53 expression did not significantly correlate with the rate of metachronous lesion development ($p=0.284$) but there was a non-significant trend towards a higher rate of advanced metachronous lesions amongst those with high p53 expression (Table 9.2).

9.3.8 Figure 9.5: TMA cores displaying low and high expression of p53.



9.3.9 Predictors of Metachronous Polyp or CRC Development

Table 9.3 shows a comparison of patient demographics, index polyp pathological characteristics and COX2 and P53 expression, between those patients who did or did not develop metachronous lesions. Male sex (p=0.003), increasing index polyp number (p<0.001), right or rectal index polyp (p=0.004), BSG 2020 high risk (p<0.001) and high COX2 expression (p=0.006) correlated with metachronous polyp or CRC development. Increasing index polyp number (p=0.03) and right or rectal index polyp (p=0.004) correlated with metachronous advanced polyp or CRC.

Next, univariate and multivariate cox regression was performed to identify independent predictors of metachronous polyp or CRC risk (Table 9.4). On univariate analysis increasing age (p=0.034), male sex (p<0.001), increased polyp number at index colonoscopy (p<0.001), right (p<0.001) or rectal index polyp (p=0.014), BSG 2020 high risk (p<0.001) and high cytoplasmic COX2 expression (median time to development of any metachronous lesion or censor 3.9 years vs 5.6 years; HR 1.264 (95% CI: 1.039-1.537; p=0.019)) (Figure 9.6) were predictive of a shorter time to detection of any metachronous lesion. On multivariate analysis male sex (p<0.002), increased polyp number at index colonoscopy (p<0.003), right (p=0.001) or rectal index polyp (p=0.018) and high cytoplasmic COX2 expression (HR 1.273 (95% CI: 1.046-1.550; p=0.016)) retained significance as independent predictors of shorter time to development of any metachronous lesion.

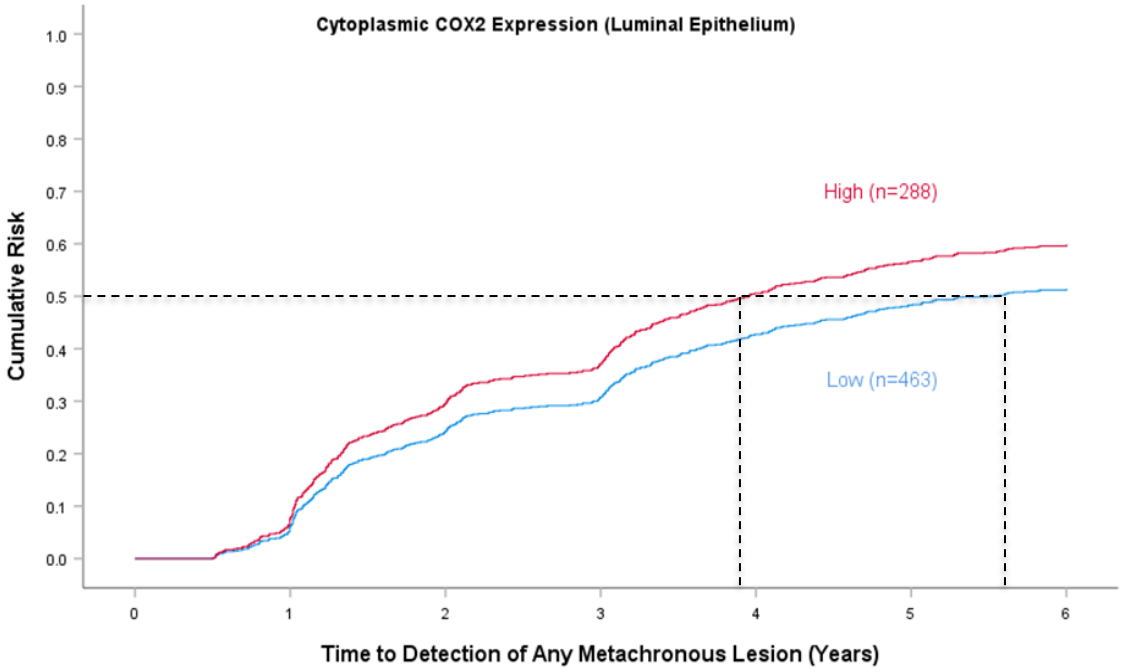
9.3.10 Table 9.3: Comparison of outcome by demographics, pathology and expression of each biomarker in INCISE training cohort.

		All	Metachronous Polyp or CRC			Metachronous Advanced Lesion		
			No	Yes	P	No	Yes	P
N		859	398	461		729	130	
Age (years)	Median (IQR)	63 (57-69)	63 (57-69)	64 (59-69)	0.094	63 (57-69)	65 (57-71)	0.096
Sex	Male	604 (70.3%)	260 (65.3%)	344 (74.6%)	0.003	511 (70.1%)	93 (71.5%)	0.74
	Female	255 (29.7%)	138 (34.7%)	117 (25.4%)		218 (29.9%)	37 (28.5%)	
Index Polyp Advanced	No	15 (1.7%)	7 (1.8%)	8 (1.7%)	0.979	13 (1.8%)	2 (1.5%)	1.0
	Yes	844 (98.3%)	391 (98.2%)	453 (98.3%)		716 (98.2%)	123 (98.5%)	
Index Polyp Number	1	285 (33.2%)	162 (40.7%)	123 (26.7%)	<0.001	246 (33.7%)	39 (30.0%)	0.03
	2-4	469 (54.6%)	207 (52.0%)	262 (56.8%)		403 (55.3%)	66 (50.8%)	
	5+	105 (12.2%)	29 (7.3%)	76 (16.5%)		80 (11.0%)	25 (19.2%)	
Index Polyp Villous	No	396 (46.1%)	180 (45.2%)	216 (46.9%)	0.633	338 (46.4%)	58 (44.6%)	0.712
	Yes	463 (53.9%)	218 (54.8%)	245 (53.1%)		391 (53.6%)	72 (55.4%)	
Index Polyp Type	Adenoma	845 (98.4%)	393 (98.7%)	452 (98.0%)	0.422	718 (98.5%)	127 (97.7%)	0.508
	Serrated Polyps	14 (1.6%)	9 (2.0%)	9 (2.0%)		11 (1.5%)	3 (2.3%)	
Index HGD	No	725 (84.4%)	340 (85.4%)	385 (83.5%)	0.441	616 (84.5%)	109 (83.8%)	0.85
	Yes	134 (15.6%)	58 (14.6%)	76 (16.5%)		113 (15.5%)	21 (16.2%)	
Index Polyp Size (mm)	<10	17 (2.0%)	8 (2.0%)	9 (2.0%)	0.952	14 (1.9%)	3 (2.3%)	0.732
	≥10	842 (98.0%)	390 (98.0%)	452 (98.0%)		715 (98.1%)	127 (97.7%)	
Index Polyp Location	Left Colon	623 (72.5%)	310 (77.9%)	313 (67.9%)	0.004	544 (74.6%)	79 (60.8%)	0.004
	Right Colon	109 (12.7%)	38 (9.5%)	71 (15.4%)		84 (11.5%)	25 (19.2%)	
	Rectum	127 (14.8%)	50 (12.6%)	77 (16.7%)		101 (13.9%)	26 (20.0%)	
BSG 2020 Risk Index Procedure	Low	296 (34.5%)	167 (42.0%)	129 (28.0%)	<0.001	257 (35.3%)	39 (30.0%)	0.246
	High	563 (65.5%)	231 (58.0%)	332 (72.0%)		472 (64.7%)	91 (70.0%)	
COX2 Expression	Low	463 (61.7%)	228 (67.1%)	235 (57.2%)	0.006	396 (62.1%)	67 (59.3%)	0.576
	High	288 (38.3%)	112 (32.9%)	176 (42.8%)		242 (37.9%)	46 (40.7%)	
P53 Expression	Low	671 (86.1%)	306 (88.2%)	365 (84.5%)	0.138	645 (98.2%)	117 (95.9%)	0.165
	High	108 (13.9%)	41 (11.8%)	67 (15.5%)		12 (1.8%)	58 (4.1%)	

9.3.11 Table 9.4: Univariate and multivariate cox regression analysis of factors associated with time to metachronous polyp or CRC development in INCISE training cohort.

		Univariate			Multivariate		
		HR	95% CI	P	HR	95% CI	P
Age (years)		1.014	1.001-1.028	0.034	1.008	0.994-1.023	0.251
Sex	Male	1.0			1.0		
	Female	0.698	0.566-0.861	<0.001	0.703	0.560-0.882	0.002
Index Polyp Advanced	No	1.0					
	Yes	0.973	0.483-1.957	0.938			
Index Polyp Number	1	1.0			1.0		
	2-4	1.509	1.218-1.870	<0.001	1.408	1.123-1.766	0.003
	5+	2.666	2.000-3.552	<0.001	2.612	1.922-3.549	<0.001
Index Polyp Villous	No	1.0					
	Yes	0.976	0.813-1.172	0.793			
Index Polyp Type	Adenoma	1.0					
	Serrated Polyps	1.228	0.635-2.375	0.542			
HGD	No	1.0					
	Yes	1.137	0.889-1.454	0.307			
Index Polyp Size (mm)	<10	1.0					
	≥10	0.950	0.491-1.838	0.88			
Index Polyp Location	Left Colon	1.0			1.0		
	Right Colon	1.587	1.227-2.054	<0.001	1.569	1.193-2.063	0.001
	Rectum	1.369	1.067-1.757	0.014	1.386	1.057-1.817	0.018
BSG 2020 Risk Index Procedure	Low	1.0			1.0		
	High	1.651	1.347-2.023	<0.001	0.921	0.378-2.243	0.857
COX Expression	Low	1.0			1.0		
	High	1.264	1.039-1.537	0.019	1.273	1.046-1.550	0.016
P53 Expression	Low	1.0					
	High	1.426	0.784-2.595	0.245			

9.3.12 Figure 9.6: Relationship between cytoplasmic COX2 expression and time to metachronous polyp or CRC development.



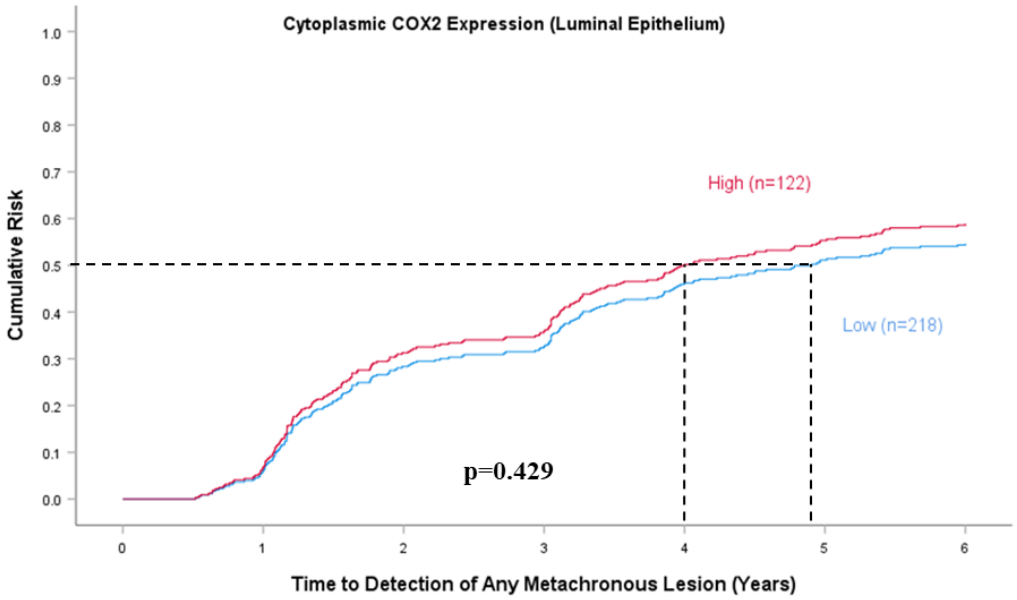
9.3.13 Test Cohort Validation

Cytoplasmic COX2 expression was next assessed as a predictor of metachronous polyp or CRC risk amongst INCISE test cohort patients. Of 377 test patients, 37 (9.8%) had missing TMA cores or cores of insufficient quality to assess COX2 expression, leaving 340 (90.2%) patients for analysis. Median average luminal epithelial H-score for the 340 valid test patients was 28.25 (range 0-95.19, IQR 10.27-46.84), similar to training patients. On χ^2 analysis there was no significant correlation between COX2 expression and risk of metachronous polyp or CRC (p=0.309) (Table 9.5). Likewise, on univariate cox regression analysis, high cytoplasmic COX2 expression was not predictive of a shorter time to detection of metachronous polyp or CRC (median time to development of any metachronous lesion or censor 4.0 years vs 4.9 years; HR 1.125 (95% CI: 0.840-1.506; p=0.429)) (Figure 9.7).

9.3.14 Table 9.5: Comparison of outcome by COX2 expression in INCISE test cohort patients.

		All	Metachronous Polyp or CRC			Metachronous Advanced Lesion		
			No	Yes	P	No	Yes	P
N		340	149	191				
COX2 Expression	Low	218 (64.1%)	100 (67.1%)	118 (61.8%)		188 (64.2%)	30 (63.8%)	
	High	122 (35.9%)	49 (32.9%)	73 (38.2%)	0.309	105 (35.8%)	17 (36.2%)	0.965

9.3.15 Figure 9.7: Relationship between cytoplasmic COX2 expression and time to metachronous polyp or CRC development in test cohort patients.



9.4 Discussion

In chapter 8, a systematic literature review identified COX2 and p53 as biomarkers which may predict the risk of developing metachronous colorectal polyps(394). The current chapter attempted to use the expression of each of these proteins, assessed with immunohistochemistry and digital pathology analysis, to predict the risk of future polyp development amongst INCISE cohort patients. In the training cohort, increased cytoplasmic COX2 expression did correlate with a higher rate of metachronous polyp or CRC development (p=0.006) and on univariate cox regression analysis was predictive of a shorter time to detection of any metachronous lesion (median time to development of any metachronous lesion or censor 3.9 years vs 5.6 years; HR 1.264 (95% CI: 1.039-1.537; p=0.019)) (Figure 9.6). Indeed, COX2 retained significance as an independent predictor of shorter time to detection of metachronous polyp or CRC on multivariate analysis (HR 1.273 (95% CI: 1.046-1.550; p=0.016)) (Table 9.4). No such correlation was observed for nuclear p53. However, when attempting to validate COX2 as a useful biomarker in the test cohort, the significant association with metachronous polyp risk did not persist on χ^2 analysis (p=0.309) (Table 9.5), nor on univariate cox regression analysis (HR 1.125 (95% CI: 0.840-1.506; p=0.429)) (Figure 9.7).

As discussed in Chapter 8, other authors have assessed the utility COX2 and p53 expression as potential markers of metachronous polyp risk, with mixed findings. Brand et al(380) assessed COX2, p53 and β-catenin expression of index polypectomy specimens from 109 patients from the German 5-ASA Polyp Prevention Study, a randomised, placebo-controlled mesalazine adenoma chemoprevention trial. Adenomas were classified as COX2 positive if >10% of epithelial adenomatous cells exhibited COX2 expression, as p53 positive if >40% of nuclei displayed p53 expression and as β-catenin positive if >5% of nuclei displayed β-catenin expression. COX2 (OR 3.53, p=0.001), p53 (OR 10.15, p=0.001) and β-catenin (OR 3.49, p=0.002) positivity were associated with metachronous

adenoma risk at 3 years. There are several differences between Brand et al's study and the current paper. The current study assessed protein expression digitally rather than manually and using the weighted histoscore, a more quantitative method of calculating protein expression as compared to percentage cellular positivity. The current study also had a significantly larger number of index polyp tissue samples to assess and utilised a test cohort to validate positive findings.

In the study by Benamouzig et al(361) 219 index adenomas from 136 patients participating in a double-blind aspirin chemoprevention RCT were assessed for COX2 and p53 expression. COX2 expression was estimated for 3 different compartments of cells: epithelial, superior stromal (luminal surface) and deep stromal cells (within the body of the adenoma). While strong overall COX2 expression had no significant association with the risk of metachronous adenoma (42.0% vs 45.0% of patients with and without metachronous adenomas had strong overall COX2 expression, $p>0.05$), strong deep stromal COX2 expression was able to predict metachronous adenoma (42.0% vs 25.0% of patients with and without metachronous adenoma had strong deep stromal COX2 expression, $p=0.04$). On multivariate analysis deep stromal COX2 expression was an independent predictor of metachronous adenoma (OR 2.78 (95% CI: 1.18-6.25; $p=0.02$)). In the current study only epithelial COX2 expression was assessed and following attempted validation within the test cohort, was not found to be predictive of metachronous lesion risk. Benamouzig's positive findings have not be validated within a test cohort of patients to date. In agreement with the current study, Benamouzig found no association between p53 expression and metachronous adenoma risk (62.0% of patients who developed metachronous adenoma stained positive for p53 vs 51.0% of those without metachronous adenoma, $p>0.05$).

Sheikh et al(381) assessed p53 expression in index adenomas with high-grade dysplasia from 42 patients. 83.3% of patients who developed metachronous adenoma showed p53

positivity in their index polyp as compared to 50.0% of patients with no metachronous adenoma (p=0.025). Finally, in the study by Vernillo et al(382) of 78 adenomas from 51 patients, no association was found between p53 expression and metachronous adenoma risk (p>0.05), in concordance with the current study.

The current study has a number of strengths. Firstly, in terms of sample size, the INCISE study represents possibly the largest premalignant colorectal tissue cohort in existence. Secondly, the accuracy of immunohistochemical scoring was ensured by using a combination of manual and digital scoring, as well as multiple scorers for each biomarker, with intraclass correlation coefficients calculated. Finally, the use of a training and test cohort ensures that positive findings are internally validated. The study does however have limitations. Firstly, there is an over representation of large and therefore advanced polyps within the TMA cohort. While polyps ≥10mm represented 98.1% of polyps within this study, only 65.5% of patients within the entire INCISE cohort had polyps ≥10mm. Likewise advanced polyps represented 98.2% of patients in this study as compared to 66.5% of the full cohort. This is because patients were more likely to be selected for the TMA tissue cohort if they had sufficient polyp tissue for analysis. Secondly, the intraclass correlation coefficient between both scorers for p53 nuclear expression was a little lower than is desirable at 0.753 (95% CI: 0.702-0.797), while being within the acceptable range. Finally, as no statistical correction was made to the χ^2 analysis to account for the multiple comparisons made, an increased risk of type I errors may be anticipated. However, by assessing significant variables with multivariate cox regression survival analysis, the impact of potential false positives is negated.

In conclusion, the current study has shown that no association between nuclear p53 expression within index polyp tissue and the rate of metachronous polyps or CRC. Additionally, while cytoplasmic COX2 expression did correlate metachronous polyp or CRC risk within the training cohort, this could not be replicated amongst test patients.

Further work is required to determine what role if any, COX2 may have in predicting metachronous polyp risk. However, based on the current findings it would not be possible to conclude that it is a useful biomarker for this purpose. Further interrogation of the INCISE cohort for alternative biomarkers which may enhance the prediction of future polyp or CRC risk continues.

10 Expression of β-catenin to predict metachronous lesion development following polypectomy: an Integrated Technologies for Improved Polyp Surveillance (INCISE) project pilot study.

10.1 Introduction

In chapter 9, COX2 and p53 were explored as potential biomarkers for metachronous polyp or colorectal cancer (CRC) risk following polypectomy at screening colonoscopy. COX2 showed initial promise in the training cohort, with increased cytoplasmic expression correlating with a higher rate of metachronous polyp or CRC development and with a shorter time to detection of any metachronous lesion on univariate and multivariate cox regression analysis. However, the significant observations made in the training cohort could not be replicated within the test cohort and neither COX2 nor p53 were ultimately found to be useful biomarkers in this context.

Another protein which was identified as a candidate immunohistochemical biomarker from the systematic review presented in chapter 8, was β-catenin. β-catenin is a key protein in the pro-proliferative Wnt signalling pathway and increased expression of β-catenin is present in the majority of colorectal adenomas and nearly all CRCs (40, 380) (Figure 10.1). This is a pathway of particular interest given the emergence of Wnt signalling inhibitors(397). This chapter presents the results of a pilot study, involving a small subset of Integrated Technologies for Improved Polyp Surveillance (INCISE) cohort patients, which aimed to assess the utility of β-catenin expression as a marker for metachronous lesion risk.

10.1.1 Figure 10.1: Wnt signalling pathway.

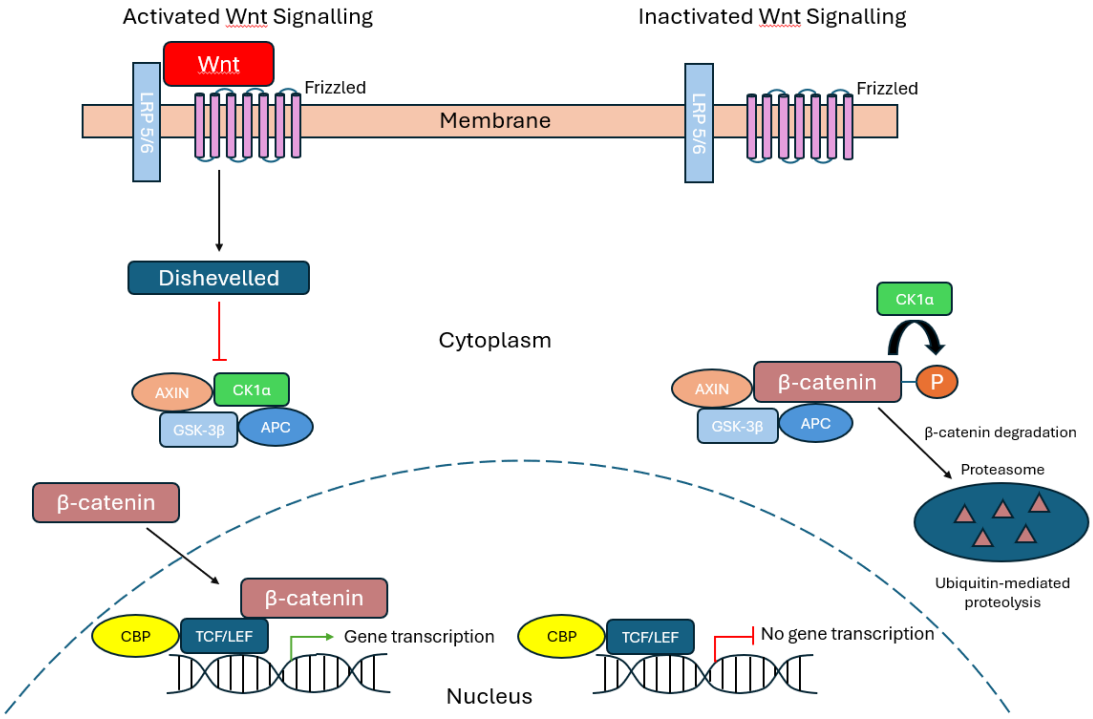


Figure adapted from Zhang et al(398).

10.2 Methods

10.2.1 Patients

The INCISE cohort was described in chapter 7 and 9 and comprises patients undergoing polypectomy at screening colonoscopy, who underwent a further colonoscopy 6 months to 6 years later. The full INCISE cohort comprises of 2,643 patients, while 12,36 patients had tissue retrieved for immunohistochemical assessment. The focus of the current pilot study was a subset of 339 patients first entered in the INCISE cohort, referred to as batch 1 patients.

10.2.2 Clinicopathological Variables

As in chapter 9, demographics, basic pathological information from the index colonoscopy, BSG 2020 risk status and presence of absence of metachronous polyp or CRC at surveillance colonoscopy, was available for each patient.

10.2.3 Immunohistochemistry Staining

β-catenin expression was assessed using immunohistochemistry. As in chapter 9, tissue microarrays (TMA) were constructed from formalin-fixed, paraffin-embedded whole tissue blocks. Four 0.6mm polyp cores available per patient; two cores from the luminal surface of the polyp and two from the basal portion. Positive, negative and isotype controls were available. While the staining of COX2 and P53 were outsourced to another laboratory, β-catenin staining was performed in house. Batch 1 patients (n=339), the focus of the current chapter, were stained prior to the full cohort (n=1236) to allow optimisation of the protocol. Both manual and automated staining were performed.

For manual staining, TMA's were first baked for 60 minutes at 60°C followed by dewaxing in Histoclear (HS-202 National Diagnostics, Nottingham, UK) and rehydration through graded alcohol (2 minutes each 100%, 100%, 90% and 70% ethanol) and finally

rinsed in water for 10 minutes. Pressurised, heat-induced antigen retrieval was performed in citrate buffer at pH6 in a microwave. Endogenous peroxidase activity was blocked with 3% H_2O_2 and non-specific binding was blocked with 1% goat serum **(**Vector Laboratories, Upper Heyford, UK). All TMA cores were incubated overnight at 4°C in 1:600 β-catenin antibodies (Dako monoclonal mouse, anti-human, clone β-catenin-1, reference M3539, lot 11158895, Dako North America Inc., California, USA). Control tissue was incubated under the same conditions in no antibody (negative control) and IgG1k (isotype control). ImmPRESS (Vector Laboratories) secondary anti-mouse/ rabbit antibody incubation was performed for 30 minutes at room temperature before incubating with ImPACT DAB (Vector Laboratories) as chromogen. The TMA's were counterstained with haematoxylin, dipped in acid alcohol, washed in water (2 minutes), Scott's tap water (2 minutes), further water (2 minutes) then dehydrated through graded alcohol (2 minutes each 70%, 90%, 100% and 100% ethanol) and mounted with Pertex (Cat. SEA-0100-00A, CellPath, Newton, UK) and coverslips.

For autostaining the TMA's were stained using the same β-catenin antibody at 1:1000 concentration based on optimisation runs at various concentrations (1:300, 1:600, 1:1000, 1:1500, 1:2000 and 1:3000) on the Leica Bond Rx autostainer (Leica Microsystems Ltd, Milton Keynes, UK). Negative and isotype controls were again prepared and the antigen retrieval step used pH 6 buffer for 20 minutes. After autostaining was complete the slides were dehydrated through graded alcohol (2 minutes each 70%, 90%, 100% and 100% ethanol) and mounted with Pertex and coverslips. The TMAs stained with the autostainer were deemed to be of similar quality to the manual staining and were used for the analysis described here.

10.2.4 Immunohistochemistry Scoring

Stained TMA's were digitally scanned before being transferred to QuPath for analysis. The TMA was dearrayed, stain vectors estimated and cell detection performed. The epithelium and lamina propria were manually annotated in a small proportion of every single core to train the software to recognise each tissue type. Artefact and white space were annotated to ensure they were ignored. A random trees detection classifier was created to quantify the expression of β-catenin using 41 features including nucleus and cell area, perimeter, circularity and eccentricity, nuclear: cell ratio and nucleus, cell and cytoplasm optical density. Three intensity thresholds were selected which divided staining into negative, weak, moderate and strong. After building the classifier for each TMA the auto-update feature was turned on and each core was checked for errors and the classifier re-validated in real time. Once the classifier was finalised, weighted histoscores (H-scores) for each cellular compartment (cytoplasm or nuclear) and epithelial edge (luminal or basal) could be exported. 10% of all cores were additionally scored manually.

10.2.5 Statistical Analysis

Intraclass correlation coefficients were calculated between automated and manual scores. For analysis, average β-catenin expression for each patient was determined by calculating the mean H-score across the cores available. These were imported into R studio (Version 2022.02.2, Boston, MA, USA) and using maximally selected rank statistics with the presence or absence of any metachronous lesion as the outcome of interest, a cut point was generated to define high and low expression.

Covariables were compared using crosstabulation and the χ^2 or Fisher's exact test for categorical variables and the Mann Whitney U Test for continuous data. A value of $p<0.05$ was considered statistically significant. To identify covariables which independently predicted time to metachronous lesion detection, univariate followed by multivariate cox

regression 1-survival analysis was performed. This allowed calculation of hazard ratios (HRs) and 95% confidence intervals (95% CI). Covariables significant on univariate analysis ($p<0.05$) were entered into a multivariate model using the backwards conditional method in which variables with a significance of $p<0.1$ were removed from the model in a stepwise fashion. 1-survival curves were created.

10.3 Results

10.3.1 Patients, Clinicopathological Variables and Outcomes

Table 10.1 shows the demographics, pathology and outcomes of all 339 patients. With a median time to surveillance colonoscopy of 3 years (IQR 1.6–3.8 years), 164 (48.4%) patients were found to have any metachronous polyp or CRC and 44 (13.0%) had an advanced polyp or CRC.

10.3.2 Table 10.1: Demographics, pathology and outcomes of all patients.

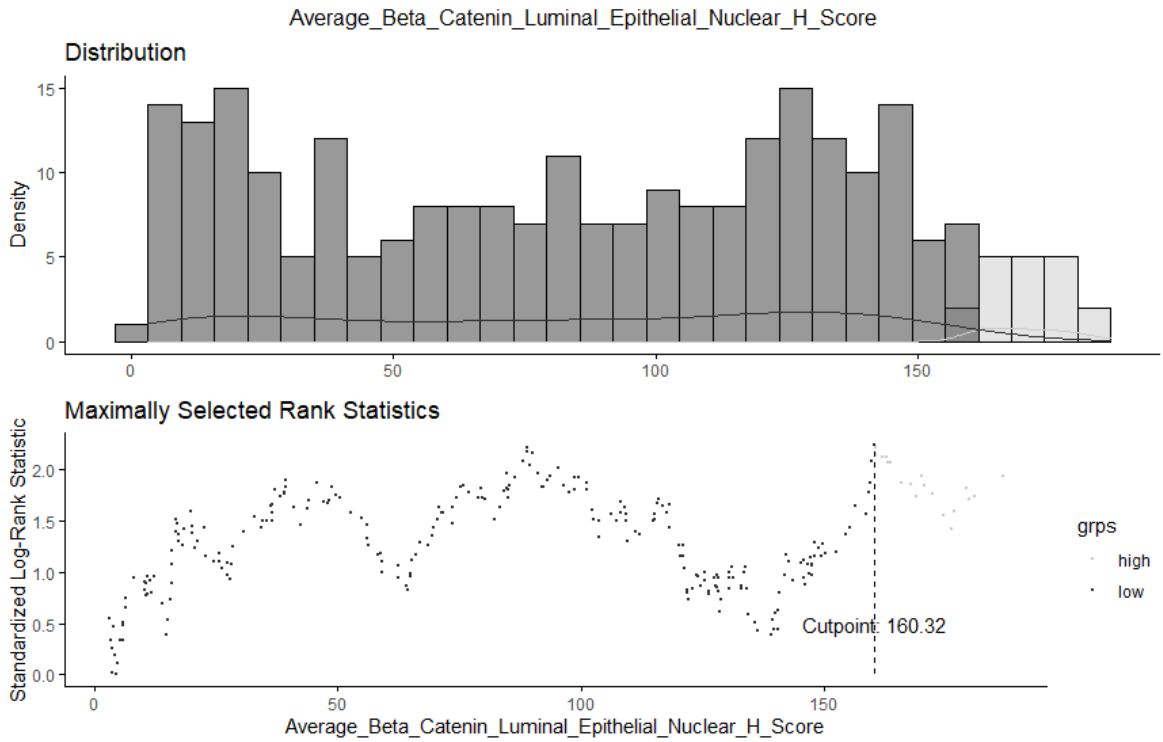
Total	N	339
Age (years)	Median (IQR)	63 (57-69)
Sex	Male	249 (73.5%)
	Female	90 (26.5%)
Index Polyp Advanced	No	5 (1.5%)
	Yes	334 (98.5%)
Index Polyp Number	1	109 (32.2%)
	2-4	159 (46.9%)
	5+	71 (20.9%)
Index Polyp Villous	No	134 (39.5%)
	Yes	205 (60.5%)
Index Polyp Type	Adenoma	335 (98.8%)
	Serrated Polyps	4 (1.2%)
Index HGD	No	284 (83.8%)
	Yes	55 (16.2%)
Index Polyp Size (mm)	<10	5 (1.5%)
	≥10	334 (98.5%)
Index Polyp Location	Rectum	28 (8.3%)
	Left Colon	281 (82.9%)
	Right Colon	30 (8.8%)
BSG 2020 Risk Index Procedure	Low	119 (35.1%)
	High	220 (64.9%)
Metachronous Polyp or CRC	No	175 (51.6%)
	Yes	164 (48.4%)
Metachronous Advanced Lesion	No	295 (87.0%)
	Yes	44 (13.0%)

10.3.3 β-catenin Expression

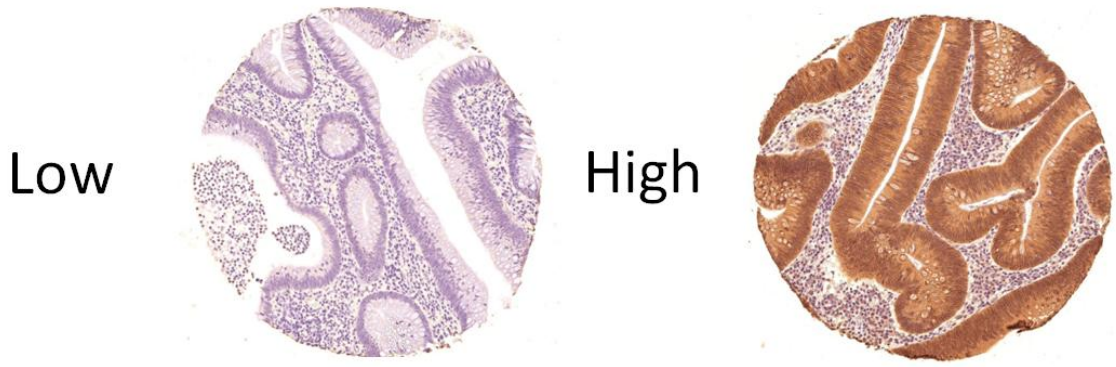
β-catenin expression was observed in the cytoplasm, nucleus and membranes of cells within the epithelial layer of index polyps, both in cores taken from the luminal and basal edge of the epithelium. Staining within cores taken from the luminal edge was of higher quality and used for analysis. A decision was made to focus on nuclear β-catenin staining for the current study as nuclear localisation of β-catenin is indicative of Wnt pathway activation. Conversely, cytoplasmic β-catenin expression could theoretically be visualised with β-catenin in its inactive form, incorporated into the degradation complex prior to proteolysis (Figure 10.1). Intraclass correlation co-efficient between automated and manual scoring was 0.944 (95% CI: 0.924-0.959). Amongst 339 patients, 19 (5.6%) patients had missing TMA cores or cores of insufficient quality to assess β-catenin expression, leaving 320 (94.4%) patients for analysis.

For each patient an average epithelial β-catenin nuclear expression was calculated by taking a mean H score from the two luminal cores available. Median average luminal epithelial H score for the 320 valid patients was 90.73 (range 0-289.85, IQR 26.63-141.60). These scores were imported into R studio and using maximally selected rank statistics with the presence or absence of any metachronous lesion as the outcome of interest, a cut point was generated to define high (H score ≥160.32) and low β-catenin expression (<160.32) (Figure 10.2). Examples of high and low β-catenin expression are shown in Figure 10.3. There were no significant differences between patients with index polyps exhibiting high and low nuclear β-catenin expression in terms of patient demographics nor index polyp pathological characteristics. However, low nuclear β-catenin expression did correlate with a higher rate of metachronous lesion development (p=0.011) (Table 10.2).

10.3.4 Figure 10.2: Maximally selected rank statistics used to define high and low β-catenin expression.



10.3.5 Figure 10.3: TMA cores displaying low and high expression β-catenin expression.



10.3.6 Table 10.2: Comparison of demographics, pathology and outcomes by expression of β-catenin in INCISE training cohort.

		All	β-catenin		P
			Low	High	
N		339	268	52	
Age (years)	Median (IQR)	63 (57-69)	63 (59-69)	63 (57-69)	0.765
Sex	Male	249 (73.5%)	194 (72.4%)	41 (78.8%)	0.335
	Female	90 (26.5%)	74 (27.6%)	11 (21.2%)	
Index Polyp Advanced	No	5 (1.5%)	4 (1.5%)	1 (1.9%)	0.59
	Yes	334 (98.5%)	264 (98.5%)	51 (98.1%)	
Index Polyp Number	1	109 (32.2%)	82 (30.6%)	18 (34.6%)	0.822
	2-4	159 (46.9%)	130 (48.5%)	23 (44.2%)	
	5+	71 (20.9%)	56 (20.9%)	11 (21.2%)	
Index Polyp Villous	No	134 (39.5%)	100 (37.3%)	25 (48.1)	0.145
	Yes	205 (60.5%)	168 (62.7%)	27 (51.9%)	
Index Polyp Type	Adenoma	335 (98.8%)	264 (98.5%)	52 (100%)	1.00
	Serrated Polyps	4 (1.2%)	4 (1.5%)	0 (0%)	
Index HGD	No	284 (83.8%)	221 (82.5%)	46 (88.5%)	0.287
	Yes	55 (16.2%)	47 (17.5%)	6 (11.5%)	
Index Polyp Size (mm)	<10	5 (1.5%)	4 (1.5%)	1 (1.9%)	0.59
	≥10	334 (98.5%)	264 (98.5%)	51 (98.1%)	
Index Polyp Location	Rectum	28 (8.3%)	25 (9.3%)	2 (3.8%)	0.393
	Left Colon	281 (82.9%)	219 (81.7%)	46 (88.5%)	
	Right Colon	30 (8.8%)	24 (9.0%)	4 (7.7%)	
BSG 2020 Risk Index Procedure	Low	119 (35.1%)	90 (33.6%)	20 (38.5%)	0.498
	High	220 (64.9%)	178 (66.4%)	32 (61.5%)	
Metachronous Polyp or CRC	No	175 (51.6%)	129 (48.1%)	35 (67.3%)	0.011
	Yes	164 (48.4%)	139 (51.9%)	17 (32.7%)	
Metachronous Advanced Lesion	No	295 (87.0%)	231 (86.2%)	46 (88.5%)	0.661
	Yes	44 (13.0%)	37 (13.8%)	6 (11.5%)	

10.3.7 Predictors of Metachronous Polyp or CRC Development

Table 10.3 shows a comparison of patient demographics, index polyp pathological characteristics and β-catenin expression, between those patients who did or did not develop metachronous lesions. Increasing index polyp number ($p<0.001$), BSG 2020 high risk ($p<0.001$) and low β-catenin expression ($p=0.011$) correlated with metachronous polyp or CRC development. Presence of HGD ($p=0.033$) correlated with metachronous advanced polyp or CRC.

Next, univariate and multivariate cox regression was performed to identify independent predictors of metachronous polyp or CRC risk (Table 10.4). On univariate analysis increased polyp number at index colonoscopy (p=0.001), right-sided index polyp (p=0.007), BSG 2020 high risk (p<0.001) and low nuclear β-catenin expression (HR 1.914 (95% CI: 1.156-3.168; p=0.012) (Figure 10.4) were predictive of a shorter time to detection of any metachronous lesion. On multivariate analysis increased polyp number at index colonoscopy (p=0.002) and low nuclear β-catenin expression (HR 1.933 (95% CI: 1.274-2.932; p=0.002)) retained significance as independent predictors of shorter time to development of any metachronous lesion.

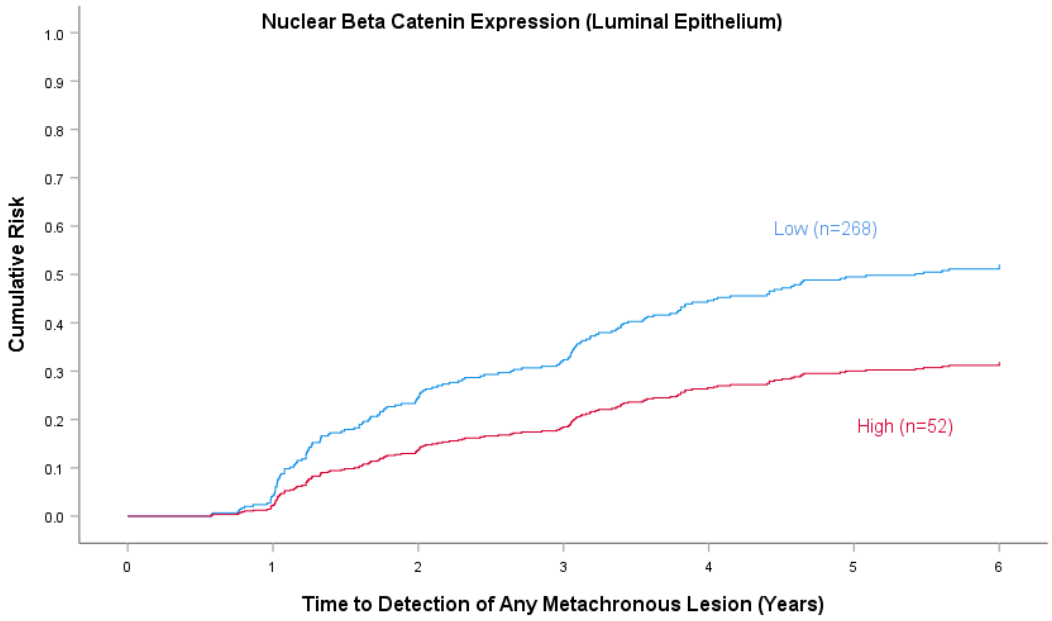
10.3.8 Table 10.3: Comparison of outcome by demographics, pathology and expression of β-catenin.

		All	Metachronous Polyp or CRC			Metachronous Advanced Lesion		
			No	Yes	P	No	Yes	P
N		339	175	164		295	44	
Age (years)	Median (IQR)	63 (57-69)	63 (57-69)	63 (59-69)	0.219	63 (57-69)	65 (59-71)	0.183
Sex	Male	249 (73.5%)	124 (70.9%)	125 (76.2%)		220 (74.6%)	29 (65.9%)	
	Female	90 (26.5%)	51 (29.1%)	39 (23.8%)	0.264	75 (25.4%)	15 (34.1%)	0.225
Index Polyp Advanced	No	5 (1.5%)	3 (1.7%)	2 (1.2%)	1.00	5 (1.7%)	0 (0%)	1.00
	Yes	334 (98.5%)	172 (98.3%)	162 (98.8%)		290 (98.3%)	44 (100%)	
Index Polyp Number	1	109 (32.2%)	76 (43.4%)	33 (20.1%)		100 (33.9%)	9 (20.5%)	
	2-4	159 (46.9%)	78 (44.6%)	81 (49.4%)		136 (46.1%)	23 (52.3%)	
	5+	71 (20.9%)	21 (12.0%)	50 (30.5%)	<0.001	59 (20.0%)	12 (27.3%)	0.18
Index Polyp Villous	No	134 (39.5%)	61 (34.9%)	73 (44.5%)	0.069	117 (39.7%)	17 (38.6%)	0.897
	Yes	205 (60.5%)	114 (65.1%)	91 (55.5%)		178 (60.3%)	27 (61.4%)	
Index Polyp Type	Adenoma	335 (98.8%)	173 (98.9%)	162 (98.8%)		291 (98.6%)	44 (100%)	
	Serrated Polyps	4 (1.2%)	2 (1.1%)	2 (1.2%)	1.00	4 (1.4%)	0 (0%)	1.00
Index HGD	No	284 (83.8%)	145 (82.9%)	139 (84.8%)	0.636	252 (85.4%)	32 (72.7%)	0.033
	Yes	55 (16.2%)	30 (17.1%)	25 (15.2%)		43 (14.6%)	12 (27.3%)	
Index Polyp Size (mm)	<10	5 (1.5%)	3 (1.7%)	2 (1.2%)		5 (1.7%)	0 (0%)	
	≥10	334 (98.5%)	172 (98.3%)	162 (98.8%)	1.00	290 (98.3%)	44 (100%)	1.00
Index Polyp Location	Rectum	28 (8.3%)	16 (9.1%)	12 (7.3%)		22 (7.5%)	6 (13.6%)	
	Left Colon	281 (82.9%)	149 (85.1%)	132 (80.5%)		249 (84.4%)	32 (72.7%)	
	Right Colon	30 (8.8%)	10 (5.7%)	20 (12.2%)	0.101	24 (8.1%)	6 (13.6%)	0.157
BSG 2020 Risk Index Procedure	Low	119 (35.1%)	81 (46.3%)	38 (23.2%)		109 (36.9%)	10 (22.7%)	
	High	220 (64.9%)	94 (53.7%)	126 (76.8%)	<0.001	186 (63.1%)	34 (77.3%)	0.065
β-catenin Expression	Low	268 (79.1%)	129 (78.7%)	139 (89.1%)		231 (83.4%)	37 (86.0%)	
	High	52 (15.3%)	35 (21.3%)	17 (10.9%)	0.011	46 (16.6%)	6 (14.0%)	0.661

10.3.9 Table 10.4: Univariate and multivariate cox regression analysis of factors associated with time to metachronous polyp or CRC development.

		Univariate			Multivariate		
		HR	95% CI	P	HR	95% CI	P
Age (years)	Median (Range)	1.013	0.991-1.035	0.264			
Sex	Male	1.0					
	Female	0.768	0.536-1.101	0.151			
Index Polyp Advanced	No	1.0					
	Yes	1.178	0.292-4.751	0.818			
Index Polyp Number	1	1.0			1.0		
	2-4	2.007	1.339-3.010	0.001	1.933	1.274-2.932	0.002
	5+	3.789	2.436-5.895	<0.001	3.892	2.465-6.145	<0.001
Index Polyp Villous	No	1.0					
	Yes	0.777	0.571-1.057	0.108			
Index Polyp Type	Adenoma	1.0					
	Serrated Polyps	1.267	0.314-5.111	0.74			
HGD	No	1.0					
	Yes	0.891	0.582-1.365	0.597			
Index Polyp Size (mm)	<10	1.0					
	≥10	1.178	0.292-4.751	0.818			
Index Polyp Location	Left Colon	1.0			1.0		
	Right Colon	1.910	1.192-3.060	0.007	1.413	0.863-2.314	0.169
	Rectum	0.820	0.454-1.480	0.82	0.678	0.365-1.257	0.217
BSG 2020 Risk Index Procedure	Low	1.0			1.0		
	High	2.305	1.603-3.315	<0.001	0.974	0.393-2.415	0.955
β-catenin Expression	High	1.0			1.0		
	Low	1.914	1.156-3.168	0.012	1.933	1.274-2.932	0.002

10.3.10 Figure 10.4: Relationship between nuclear β-catenin expression and time to metachronous polyp or CRC development.



10.4 Discussion

In the current study, low expression of β-catenin correlated with a higher risk of developing metachronous polyps or CRC and indeed was an independent predictor of such on multivariate cox regression analysis. Number of polyps at index colonoscopy was also an independent predictor, while BSG 2020 risk score was significant on univariate but not multivariate analysis. The observations surrounding β-catenin are surprising and somewhat counterintuitive. β-catenin is a key protein in the pro-proliferative Wnt signalling pathway and increased expression is observed in most colorectal adenomas and nearly all CRCs (40, 380). One might expect that polyps exhibiting high rather than low β-catenin expression may be associated with more advanced biology and perhaps that patients with such polyps would have a higher propensity to metachronous lesion development. Indeed, in the study by Brand et al(380) β-catenin expression was assessed in 109 index polypectomy specimens from patients from the German 5-ASA Polyp Prevention Study, a randomised, placebo-controlled mesalazine adenoma chemoprevention trial (380)**.** Adenomas were classified as β-catenin positive if >5% of nuclei displayed β-catenin expression. β-catenin positivity was associated with metachronous adenoma risk at 3 years (OR 3.49, p=0.002). As highlighted in chapter 9, the study by Brand et al assessed protein expression manually rather than digitally and using percentage cellular positivity rather than the weighted histoscore used here. The current study was also larger in sample size.

It should be stressed that this chapter represents data from the pilot/ optimisation phase of the study. While the entire tissue cohort has been satisfactorily stained for β-catenin expression and digitally scanned, scoring is ongoing for the remainer of the cohort. Examining the preliminary findings of this study in a larger cohort of patients, divided into training and test cohorts will provide scientific rigor to any conclusions that may be made. Despite this, the sample size is still larger than many studies published to date and

the use of digital expression scoring with manual scoring as a quality control adds to the reliability.

In conclusion, the current study has shown that low nuclear β-catenin expression within index polyp tissue excised during screening colonoscopy, correlates with a higher rate of metachronous polyp or CRC development. Furthermore, low nuclear β-catenin expression independently predicted shorter time to metachronous polyp or CRC development on cox regression 1-survival analysis. The results of this study will now be taken forward into the whole INCISE tissue cohort and if significant observations remain, must be validated in a test cohort.

11 Conclusions

The thesis presented here is a culmination of 3 years of research carried out at the University of Glasgow Academic Unit of Surgery, Glasgow Royal Infirmary and Wolfson Wohl Cancer Research Centre. The theme of the thesis was early colorectal cancer (CRC) and premalignant polyp detection and management. CRC is amongst the most common cancers in the UK and is the 2nd most common cause of cancer related mortality. Early detection is critical to improving outcomes, with excellent prognosis seen with stage I disease (90.9% 5-year survival), but much poor outcomes with stage IV disease (10.5% 5-year survival)(1).

The first phase of this thesis (chapter 2, 3 and 4) focused on the use of the faecal immunochemical test (FIT) within the symptomatic population. While symptoms including rectal bleeding and persistent change in bowel habit are commonly present at the time of CRC diagnosis, symptoms in isolation are associated with a low positive predictive value for CRC due to significant overlap with inflammatory bowel disease (IBD) and functional bowel disorders(11). Indeed, the work presented here showed that no individual symptom was able to independently predict CRC on multivariate analysis. Conversely, faecal haemoglobin (f-Hb) as measured by a FIT test and the presence of anaemia both independently predicted CRC risk and represent valuable objective markers in symptomatic patients. Indeed, combining absence of a raised f-Hb or anaemia is a powerful tool, able to effectively exclude CRC in 99.96% of cases. Furthermore, it was shown that repeating a FIT test once within 12 months for patients with persistent or recurrent symptoms is a valuable method for safety netting with patients with two f-Hb measurements <10µg/g having a very low CRC risk (0.1%). Conversely, performing serial FIT tests more often than this was shown to be unhelpful with the chance of false positivity becoming unacceptably high.

At the time of commencing the research presented here, routine FIT testing in symptomatic patients was in its infancy, with limited data published on its utility in such patients and a lack of a national consensus on how to implement symptomatic FIT testing. FIT is now a well-established part of colorectal and gastroenterology referral pathways and published versions of the research presented here have been referenced in National Institute for Health and Care Excellence (NICE) clinical guidelines(188, 196, 399), as well as influencing Scottish Government policy with anaemia having a more prominent role in triaging patients for investigation and, for the first time, repeat FIT measurements being recommended for patients with persistent or recurrent symptoms(22). It is hoped that these minor refinements to how FIT is used in symptomatic patients will ensure patients with the highest probability of having CRC are assessed early and endoscopy waiting lists can be kept at a manageable level while ensuring a low miss rate. The work of this thesis also showed that, in addition to CRC, advanced adenomas, non-advanced polyps and IBD independently predict a raised f-Hb, as do the use of oral anticoagulants and demographics including older age and deprivation. The role of deprivation is of particular interest and independently predicted a raised f-Hb in patients with no pathology found at colonoscopy. Future work aims to explore why deprivation may be associated with FIT false positivity.

Since the completion of this thesis a number of important studies have been published which may influence how FIT is used in symptomatic patients in the future. The COLOFIT project aimed to optimise the use of FIT in symptomatic patients by developing a prediction model for CRC-risk encompassing f-Hb, patient demographics and commonly available blood tests. Patients who had a FIT submitted from primary care were included with division into derivation (n=34,435) and validation (n=37,216) cohorts. Additionally, an external validation cohort (n=30,291) was identified. Using cox proportional hazards survival analysis within the derivation cohort, a final model was created which included f-Hb, age, sex, mean corpuscular volume (MCV) and platelet count, with excellent

discrimination for CRC risk (c statistic 0.937 (95% CI: 0.916-0.957)). The model was found to perform well within the internal and external validation cohorts and across different age ranges and ethnicities. Extrapolating the true and false positive and negative rates from the validation cohort to 100,000 FITs, the model was able to reduce the number of unnecessary colonoscopies by 40% (n= 4716 (95% CI: 4257-5177)) as compared to using FIT alone with a f-Hb threshold of ≥10 µg/g, with no statistically significant concomitant increase in the number of missed cancers (n=9 (95% CI: -3 – 29))(400). In the study by Digby et al(401), CRC prevalence in symptomatic patients was explored across a range of f-Hb measurements, stratified by age and the presence or absence of iron deficiency anaemia (IDA), amongst 34,647 FIT tests submitted from primary care. At each f-Hb threshold, positive predictive value (PPV) for CRC increased with increasing age. NICE currently stipulate that tests resulting in a PPV for cancer ≥3% warrant urgent suspected cancer referral(91). Interestingly this threshold wasn't reached until a f-Hb range of 20-39.9 µg/g was observed in those >85 years. The additional layer of IDA-presence (as defined by low Hb and low MCV) caused a downshift, with the 3% threshold first reached with a f-Hb of 10-19.9 µg/g in patients aged >70 years. Both of these studies have established that combining FIT with other objective markers of CRC-risk, including Hb/MCV/ IDA, may enhance the accuracy of CRC-risk prediction in symptomatic patients, similar to the inclusion of anaemia seen in chapter 2. Furthermore, it would seem probable that in the future a more tailored approach to the use of FIT in CRC-risk prediction may be adopted, such that the threshold for FIT positivity may be adjusted based on demographics including age and the presence or absence of other biomarkers of cancer risk. Deprivation may be one such demographic given the observation in Chapter 4 that deprivation independently predicts a raised f-Hb in patients with no pathology found at colonoscopy.

Another study of note was that by Gerrard et al(402) in which the use of two FIT tests in quick succession was explored as a potential safety netting measure. Two sequential prospective cohorts of patients with lower GI symptoms were studied: the first (n=2,260) undertook a single FIT test followed by colonoscopy or CT colon while the second (n=3,426) were sent two FIT tests of which 2,637 completed both and proceed to definitive investigation. At a threshold of 10 μg/g there was a non-significant increase in sensitivity for CRC detection from 84.1% (95% CI: 73.3-91.8) with single FIT to 96.6% (95% CI: 90.4-99.3) with double FIT, with an associated significant drop in potentially missed CRC cases (p=0.009). Number needed to scope (NNS) to diagnose one CRC at the 10 μg/g threshold was 10 in both cohorts. In Chapter 2 a NNS of 18 was observed with use of single FIT at the 10 μg/g threshold, but it should be noted that this study included all patients with a FIT submitted regardless of symptom profile as compared to only patients with high-risk symptoms in Gerrard et al's study. Of 11 patients diagnosed with CRC who would have been missed using the single FIT strategy of the first cohort, 8 were anaemic and 8 had right-sided tumours, similar to observations made in Chapter 3 of this thesis.

In chapter 5 attention was turned to the Scottish bowel screening programme, in particular, factors that may influence outcome amongst participating patients. As may be expected, patients with screen-detected disease had tumours of an earlier stage. However, screen-detected patients were also significantly less comorbid as measured by the American Society of Anaesthesiologists (ASA) score and had a significantly lower SIR as compared to non-screen-detected patients. Comorbidity and SIR may represent confounders that account for a proportion of the improved outcomes observed amongst screen-detected patients. After adjusting for numerous covariables, screen-detection still independently predicted improved overall and cancer specific survival, as did a lower SIR.

The focus of chapter 6 was the management of T1 CRC polyps. Until recently, the presence of any adverse pathological feature in such a tumour would have mandated

formal colorectal resection. However, with the introduction of TAMIS and ESD, as well as the adoption of watch and wait strategies in selected rectal cancers, there is now an acceptance that organ preservation can be safe. We have confirmed that rates of lymph node metastases (7.1%), recurrence (4.2%) and cancer-specific mortality (3.0%) are low amongst patients such patients and that pathological findings including poor differentiation, submucosal venous invasion (SMVI) and mucinous-subtype are important prognostic factors. However, novel observations were that despite 64.4% of polypectomy-only patients having margin involvement or other high-risk factors, zero developed recurrence and only 5 of 94 patients with polypectomy margin involvement had confirmed residual tumour at resection. These findings undermine the importance of an involved polypectomy margin and suggest that surveillance following local excision of T1 CRC polyps may be safe for many patients.

The final section of the thesis (chapter 7, 8, 9 and 10) reported on studies from the Integrated Technologies for Improved Polyp Surveillance (INCISE) project which aims to refine post-polypectomy surveillance. The effectiveness of the British Society of Gastroenterology post-polypectomy guidance (BSG 2020) has been called into question with the rate of metachronous advanced polyp or CRC amongst BSG 2020 high risk INCISE patients only being marginally higher than low risk (16.3% vs 13.0%). Results of a systematic review were presented which identified mutations, single nucleotide polymorphisms, haplotypes and expression levels of several proteins in index polyp tissue which may help predict which patients will development metachronous polyps and benefit from surveillance colonoscopy. Three of the proteins identified in this review, COX2, p53 and β-catenin, were examined as potential biomarkers of metachronous polyp or CRC risk by assessing expression levels in index polyps excised from INCISE patients. COX2 and p53 were not proven to be of use, while β-catenin showed promise, albeit only within a pilot study on a small number of patients. Future work plans to complete the assessment of

β-catenin within the full cohort and to explore a broad range of other biomarkers with the hope that a panel of these can be brought together to produce a risk stratification tool for future polyp and CRC risk. It is hoped that accurate risk stratification will relieve pressure on endoscopy services, avoid unnecessary invasive investigations in low-risk patients and prevent CRC by identifying high-risk patients who are likely to require future polypectomy.

List of References

1. Cancer Research UK. Bowel Cancer Statistics. 2025. [cited 23/07/2025. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer].
2. Cancer Research UK. Bowel Cancer Screening. 2025. [cited 23/07/2025. Available from: https://www.cancerresearchuk.org/health-professional/screening/bowel-cancer-screening].
3. Hewitson P, Glasziou P, Irwig L, Towler B, Watson E. Screening for colorectal cancer using the faecal occult blood test, Hemoccult. Cochrane Database of Systematic Reviews. 2007;2007(1):Cd001216.
4. Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, et al. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. New England Journal of Medicine. 1993;328(19):1365-71.
5. Robinson MH, Hardcastle JD, Moss SM, Amar SS, Chamberlain JO, Armitage NC, et al. The risks of screening: data from the Nottingham randomised controlled trial of faecal occult blood screening for colorectal cancer. Gut. 1999;45(4):588-92.
6. Hardcastle JD, Chamberlain JO, Robinson MH, Moss SM, Amar SS, Balfour TW, et al. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. The Lancet. 1996;348(9040):1472-7.
7. Kronborg O, Fenger C, Olsen J, Bech K, Søndergaard O. Repeated screening for colorectal cancer with fecal occult blood test. A prospective randomized study at Funen, Denmark. Scandinavian Journal of Gastroenterology. 1989;24(5):599-606.
8. Kewenter J, Brevinge H, Engarås B, Haglind E, Ahrén C. Results of screening, rescreening, and follow-up in a prospective randomized study for detection of colorectal cancer by fecal occult blood testing. Results for 68,308 subjects. Scandinavian Journal of Gastroenterology. 1994;29(5):468-73.
9. Golder AM, Mshihadani A, McMillan DC, Horgan PG, Roxburgh CS, Mansouri D. Route to diagnosis of colorectal cancer and association with survival within the context of a bowel screening programme. Public Health. 2022;211:53-61.
10. Beaton D, Sharp L, Lu L, Trudgill N, Thoufeeq M, Nicholson B, et al. Diagnostic yield from symptomatic lower gastrointestinal endoscopy in the UK: A British Society of Gastroenterology analysis using data from the National Endoscopy Database. Alimentary Pharmacology and Therapeutics. 2024;59(12):1589-603.
11. McSorley ST, Digby J, Clyde D, Cruickshank N, Burton P, Barker L, et al. Yield of colorectal cancer at colonoscopy according to faecal haemoglobin concentration in symptomatic patients referred from primary care. Colorectal Disease. 2021;23(7):1615-21.
12. Farrugia A, Widlak M, Evans C, Smith SC, Arasaradnam R. Faecal immunochemical testing (FIT) in symptomatic patients: what are we missing? Frontline Gastroenterology. 2020;11(1):28-33.
13. Pin Vieito N, Zarraquiños S, Cubiella J. High-risk symptoms and quantitative faecal immunochemical test accuracy: Systematic review and meta-analysis. World Journal of Gastroenterology. 2019;25(19):2383-401.
14. Pin-Vieito N, Tejido-Sandoval C, de Vicente-Bielza N, Sánchez-Gómez C, Cubiella J. Faecal immunochemical tests safely enhance rational use of resources during the assessment of suspected symptomatic colorectal cancer in primary care: systematic review and meta-analysis. Gut. 2022;71(5):950-60.
15. Mowat C, Digby J, Strachan JA, McCann R, Hall C, Heather D, et al. Impact of introducing a faecal immunochemical test (FIT) for haemoglobin into primary care on the outcome of patients with new bowel symptoms: a prospective cohort study. BMJ Open Gastroenterology. 2019;6(1):e000293.
16. Mowat C, Digby J, Strachan JA, McCann RK, Carey FA, Fraser CG, et al. Faecal haemoglobin concentration thresholds for reassurance and urgent investigation for colorectal cancer based on a faecal immunochemical test in symptomatic patients in primary care. Annals of Clinical Biochemistry. 2021:4563220985547.

17. Westwood M, Corro Ramos I, Lang S, Luyendijk M, Zaim R, Stirk L, et al. Faecal immunochemical tests to triage patients with lower abdominal symptoms for suspected colorectal cancer referrals in primary care: a systematic review and cost-effectiveness analysis. Health Technology Assessment. 2017;21(33):1-234.
18. Westwood M, Lang S, Armstrong N, van Turenhout S, Cubiella J, Stirk L, et al. Faecal immunochemical tests (FIT) can help to rule out colorectal cancer in patients presenting in primary care with lower abdominal symptoms: a systematic review conducted to inform new NICE DG30 diagnostic guidance. BMC Medicine. 2017;15(1):189.
19. Khan AA, Klimovskij M, Harshen R. Accuracy of faecal immunochemical testing in patients with symptomatic colorectal cancer. BJS Open. 2020;4(6):1180-8.
20. Nicholson BD, James T, Paddon M, Justice S, Oke JL, East JE, et al. Faecal immunochemical testing for adults with symptoms of colorectal cancer attending English primary care: a retrospective cohort study of 14 487 consecutive test requests. Alimentary Pharmacology and Therapeutics. 2020;52(6):1031-41.
21. Ayling RM, Machesney M. Service evaluation of faecal immunochemical testing introduced for use in North East London for patients at low risk of colorectal cancer. Journal of Clinical Pathology. 2021;74(3):163-6.
22. Scottish Referral Guidelines for Suspected Cancer: Lower Gastrointestinal Cancer. 2023. [cited 26/01/2025. Available from: https://www.cancerreferral.scot.nhs.uk/lower-gastrointestinal-cancer/?alttemplate=Guideline].
23. Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, et al. Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. Gut. 2023;72(2):338-44.
24. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. The Lancet. 2019;394(10207):1467-80.
25. BMJ Best Practice: Colorectal cancer. 2025. [cited 26/01/2025. Available from: https://bestpractice.bmj.com/topics/en-gb/258].
26. Roseweir AK, McMillan DC, Horgan PG, Edwards J. Colorectal cancer subtypes: Translation to routine clinical pathology. Cancer Treatment Reviews. 2017;57:1-7.
27. Pickhardt PJ, Pooler BD, Kim DH, Hassan C, Matkowskyj KA, Halberg RB. The Natural History of Colorectal Polyps: Overview of Predictive Static and Dynamic Features. Gastroenterology Clinics of North America. 2018;47(3):515-36.
28. Simon K. Colorectal cancer development and advances in screening. Clinical Interventions in Aging. 2016;11:967-76.
29. Bonnington SN, Rutter MD. Surveillance of colonic polyps: Are we getting it right? World Journal of Gastroenterology. 2016;22(6):1925-34.
30. McFerran E, O'Mahony JF, Fallis R, McVicar D, Zauber AG, Kee F. Evaluation of the Effectiveness and Cost-Effectiveness of Personalized Surveillance After Colorectal Adenomatous Polypectomy. Epidemiologic Reviews. 2017;39(1):148-60.
31. Rutter MD, East J, Rees CJ, Cripps N, Docherty J, Dolwani S, et al. British Society of Gastroenterology/Association of Coloproctology of Great Britain and Ireland/Public Health England post-polypectomy and post-colorectal cancer resection surveillance guidelines. Gut. 2020;69(2):201-23.
32. Brenner H, Kloor M, Pox CP. Colorectal cancer. The Lancet. 2014;383(9927):1490-502.
33. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;61(5):759-67.
34. BMJ Best Practice: Familial adenomatous polyposis syndromes. 2025. [cited 23/07/2025. Available from: https://bestpractice.bmj.com/topics/en-gb/652?locale=ja].
35. Monahan KJ, Bradshaw N, Dolwani S, Desouza B, Dunlop MG, East JE, et al. Guidelines for the management of hereditary colorectal cancer from the British Society of Gastroenterology (BSG)/Association of Coloproctology of Great Britain and Ireland (ACPGBI)/United Kingdom Cancer Genetics Group (UKCGG). Gut. 2020;69(3):411-44.
36. Sinicrope FA. Lynch Syndrome-Associated Colorectal Cancer. New England Journal of Medicine. 2018;379(8):764-73.

37. Li G-M. Mechanisms and functions of DNA mismatch repair. Cell Research. 2008;18(1):85-98.
38. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology. 1999;116(6):1453-6.
39. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. Journal of the National Cancer Institute. 2004;96(4):261-8.
40. Harrison S, Benziger H. The molecular biology of colorectal carcinoma and its implications: a review. Surgeon. 2011;9(4):200-10.
41. Roepman P, Schlicker A, Tabernero J, Majewski I, Tian S, Moreno V, et al. Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition. International Journal of Cancer. 2014;134(3):552-62.
42. Budinska E, Popovici V, Tejpar S, D'Ario G, Lapique N, Sikora KO, et al. Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. Journal of Pathology. 2013;231(1):63-76.
43. Schlicker A, Beran G, Chresta CM, McWalter G, Pritchard A, Weston S, et al. Subtypes of primary colorectal tumors correlate with response to targeted treatment in colorectal cell lines. BMC Medical Genomics. 2012;5:66.
44. Sadanandam A, Lyssiotis CA, Homicsko K, Collisson EA, Gibb WJ, Wullschleger S, et al. A colorectal cancer classification system that associates cellular phenotype and responses to therapy. Nature Medicine. 2013;19(5):619-25.
45. De Sousa EMF, Wang X, Jansen M, Fessler E, Trinh A, de Rooij LP, et al. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. Nature Medicine. 2013;19(5):614-8.
46. Marisa L, de Reyniès A, Duval A, Selves J, Gaub MP, Vescovo L, et al. Gene expression classification of colon cancer into molecular subtypes: characterization, validation, and prognostic value. PLoS Medicine. 2013;10(5):e1001453.
47. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nature Medicine. 2015;21(11):1350-6.
48. Oliphant R, Brewster DH, Morrison DS. The changing association between socioeconomic circumstances and the incidence of colorectal cancer: a population-based study. British Journal of Cancer. 2011;104(11):1791-6.
49. Mansouri D, McMillan DC, Grant Y, Crighton EM, Horgan PG. The impact of age, sex and socioeconomic deprivation on outcomes in a colorectal cancer screening programme. PloS One. 2013;8(6):e66063-e.
50. Brown KF, Rumgay H, Dunlop C, Ryan M, Quartly F, Cox A, et al. The fraction of cancer attributable to modifiable risk factors in England, Wales, Scotland, Northern Ireland, and the United Kingdom in 2015. British Journal of Cancer. 2018;118(8):1130-41.
51. World Cancer Research Fund International: Who we are & what we do. 2025. [cited 10/02/2024. Available from: https://www.wcrf.org/latest/about-us/who-we-are-what-we-do/].
52. Vieira AR, Abar L, Chan DSM, Vingeliene S, Polemiti E, Stevens C, et al. Foods and beverages and colorectal cancer risk: a systematic review and meta-analysis of cohort studies, an update of the evidence of the WCRF-AICR Continuous Update Project. Annals of Oncology. 2017;28(8):1788-802.
53. Aune D, Chan DS, Lau R, Vieira R, Greenwood DC, Kampman E, et al. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. BMJ. 2011;343:d6617.
54. Lipkin M, Reddy B, Newmark H, Lamprecht SA. Dietary factors in human colorectal cancer. Annual Review of Nutrition. 1999;19:545-86.
55. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and Colorectal Cancer: A Meta-analysis. JAMA. 2008;300(23):2765-78.
56. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, et al. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. Annals of Oncology. 2011;22(9):1958-72.

57. Abar L, Vieira AR, Aune D, Sobiecki JG, Vingeliene S, Polemiti E, et al. Height and body fatness and colorectal cancer risk: an update of the WCRF-AICR systematic review of published prospective studies. European Journal of Nutrition. 2018;57(5):1701-20.
58. Katona BW, Weiss JM. Chemoprevention of Colorectal Cancer. Gastroenterology. 2020;158(2):368-88.
59. Flossmann E, Rothwell PM. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. The Lancet. 2007;369(9573):1603-13.
60. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. The Lancet. 2010;376(9754):1741-50.
61. Cook NR, Lee IM, Zhang SM, Moorthy MV, Buring JE. Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. Annals of Internal Medicine. 2013;159(2):77-85.
62. McNeil JJ, Nelson MR, Woods RL, Lockery JE, Wolfe R, Reid CM, et al. Effect of Aspirin on All-Cause Mortality in the Healthy Elderly. New England Journal of Medicine. 2018;379(16):1519-28.
63. Gann PH, Manson JE, Glynn RJ, Buring JE, Hennekens CH. Low-dose aspirin and incidence of colorectal tumors in a randomized trial. Journal of the National Cancer Institute. 1993;85(15):1220-4.
64. Burn J, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. The Lancet. 2011;378(9809):2081-7.
65. Logan RF, Grainge MJ, Shepherd VC, Armitage NC, Muir KR. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. Gastroenterology. 2008;134(1):29-38.
66. Tomić T, Domínguez-López S, Barrios-Rodríguez R. Non-aspirin non-steroidal anti-inflammatory drugs in prevention of colorectal cancer in people aged 40 or older: A systematic review and meta-analysis. Cancer Epidemiology. 2019;58:52-62.
67. Arber N, Eagle CJ, Spicak J, Rácz I, Dite P, Hajer J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. New England Journal of Medicine. 2006;355(9):885-95.
68. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. New England Journal of Medicine. 2000;342(26):1946-52.
69. Singh S, Singh H, Singh PP, Murad MH, Limburg PJ. Antidiabetic medications and the risk of colorectal cancer in patients with diabetes mellitus: a systematic review and meta-analysis. Cancer Epidemiology, Biomarkers and Prevention. 2013;22(12):2258-68.
70. Higurashi T, Hosono K, Takahashi H, Komiya Y, Umezawa S, Sakai E, et al. Metformin for chemoprevention of metachronous colorectal adenoma or polyps in post-polypectomy patients without diabetes: a multicentre double-blind, placebo-controlled, randomised phase 3 trial. The Lancet Oncology. 2016;17(4):475-83.
71. Park JH, McMillan DC, Horgan PG, Roxburgh CS. The impact of anti-inflammatory agents on the outcome of patients with colorectal cancer. Cancer Treatment Reviews. 2014;40(1):68-77.
72. Taylor ML, Wells BJ, Smolak MJ. Statins and cancer: a meta-analysis of case-control studies. European Journal of Cancer Prevention. 2008;17(3):259-68.
73. Ford I, Murray H, Packard CJ, Shepherd J, Macfarlane PW, Cobbe SM. Long-Term Follow-up of the West of Scotland Coronary Prevention Study. New England Journal of Medicine. 2007;357(15):1477-86.
74. Lopez-Caleya JF, Ortega-Valín L, Fernández-Villa T, Delgado-Rodríguez M, Martín-Sánchez V, Molina AJ. The role of calcium and vitamin D dietary intake on risk of colorectal cancer: systematic review and meta-analysis of case-control studies. Cancer Causes and Control. 2022;33(2):167-82.
75. Lutgens MW, van Oijen MG, van der Heijden GJ, Vleggaar FP, Siersema PD, Oldenburg B. Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. Inflammatory Bowel Diseases. 2013;19(4):789-99.

76. Lu C, Schardey J, Zhang T, Crispin A, Wirth U, Karcz KW, et al. Survival Outcomes and Clinicopathological Features in Inflammatory Bowel Disease-associated Colorectal Cancer: A Systematic Review and Meta-analysis. Annals of Surgery. 2022;276(5):e319-e30.
77. Gollins S, Moran B, Adams R, Cunningham C, Bach S, Myint AS, et al. Association of Coloproctology of Great Britain & Ireland (ACPGBI): Guidelines for the Management of Cancer of the Colon, Rectum and Anus (2017) – Multidisciplinary Management. Colorectal Disease. 2017;19(S1):37-66.
78. Jiang Y, Ben Q, Shen H, Lu W, Zhang Y, Zhu J. Diabetes mellitus and incidence and mortality of colorectal cancer: a systematic review and meta-analysis of cohort studies. European Journal of Epidemiology. 2011;26(11):863-76.
79. Krämer HU, Schöttker B, Raum E, Brenner H. Type 2 diabetes mellitus and colorectal cancer: meta-analysis on sex-specific differences. European Journal of Cancer. 2012;48(9):1269-82.
80. Larsson SC, Orsini N, Wolk A. Diabetes mellitus and risk of colorectal cancer: a meta-analysis. Journal of the National Cancer Institute. 2005;97(22):1679-87.
81. Luo W, Cao Y, Liao C, Gao F. Diabetes mellitus and the incidence and mortality of colorectal cancer: a meta-analysis of 24 cohort studies. Colorectal Disease. 2012;14(11):1307-12.
82. Wu L, Yu C, Jiang H, Tang J, Huang HL, Gao J, et al. Diabetes mellitus and the occurrence of colorectal cancer: an updated meta-analysis of cohort studies. Diabetes Technology & Therapeutics. 2013;15(5):419-27.
83. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454(7203):436-44.
84. McMillan DC. The systemic inflammation-based Glasgow Prognostic Score: A decade of experience in patients with cancer. Cancer Treatment Reviews. 2013;39(5):534-40.
85. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
86. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discovery. 2022;12(1):31-46.
87. Godos J, Biondi A, Galvano F, Basile F, Sciacca S, Giovannucci EL, et al. Markers of systemic inflammation and colorectal adenoma risk: Meta-analysis of observational studies. World Journal of Gastroenterology. 2017;23(10):1909-19.
88. Nøst TH, Alcala K, Urbarova I, Byrne KS, Guida F, Sandanger TM, et al. Systemic inflammation markers and cancer incidence in the UK Biobank. European Journal of Epidemiology. 2021;36(8):841-8.
89. Butterworth AS, Higgins JP, Pharoah P. Relative and absolute risk of colorectal cancer for individuals with a family history: a meta-analysis. European Journal of Cancer. 2006;42(2):216-27.
90. Jiao S, Peters U, Berndt S, Brenner H, Butterbach K, Caan BJ, et al. Estimating the heritability of colorectal cancer. Human Molecular Genetics. 2014;23(14):3898-905.
91. National Insitute for Health and Care Excellence. Suspected cancer: recognition and referral. NG12. 2015. [cited 09/04/2021. Available from: https://www.nice.org.uk/guidance/ng12/chapter/1-Recommendations-organised-by-site-of-cancer#lower-gastrointestinal-tract-cancers].
92. Peacock O, Clayton S, Atkinson F, Tierney GM, Lund JN. 'Be Clear on Cancer': the impact of the UK National Bowel Cancer Awareness Campaign. Colorectal Disease. 2013;15(8):963-7.
93. D'Souza N, Delisle TG, Chen M, Benton SC, Abulafi M, Warren O, et al. Faecal immunochemical testing in symptomatic patients to prioritize investigation: diagnostic accuracy from NICE FIT Study. British Journal of Surgery. 2021.
94. Astin M, Griffin T, Neal RD, Rose P, Hamilton W. The diagnostic value of symptoms for colorectal cancer in primary care: a systematic review. British Journal of General Practice. 2011;61(586):e231-43.
95. National Institute for Health and Care Excellence. Quantitative faecal immunochemical tests to guide referral for colorectal cancer in primary care. DG30. 2017. [cited 01/04/2021. Available from: https://www.nice.org.uk/guidance/dg30/chapter/1-Recommendations].
96. Turvill J, Turnock D, Cottingham D, Haritakis M, Jeffery L, Girdwood A, et al. The Fast Track FIT study: diagnostic accuracy of faecal immunochemical test for haemoglobin in patients with suspected colorectal cancer. British Journal of General Practice. 2021:BJGP.2020.1098.

97. Hicks G, D'Souza N, Georgiou Delisle T, Chen M, Benton SC, Abulafi M, et al. Using the faecal immunochemical test in patients with rectal bleeding: evidence from the NICE FIT study. Colorectal Disease. 2021;23(7):1630-8.
98. Monahan KJ, Davies MM, Abulafi M, Banerjea A, Nicholson BD, Arasaradnam R, et al. Faecal immunochemical testing (FIT) in patients with signs or symptoms of suspected colorectal cancer (CRC): a joint guideline from the Association of Coloproctology of Great Britain and Ireland (ACPGBI) and the British Society of Gastroenterology (BSG). Gut. 2022;71(10):1939-62.
99. Scottish Intercollegiate Guidelines Network (SIGN). Diagnosis and management of colorectal cancer. 2016. [cited 12/12/2024. Available from: https://www.sign.ac.uk/our-guidelines/diagnosis-and-management-of-colorectal-cancer/].
100. JAG accreditation programme: Guide to meeting the quality and safety standards. 2022. [cited 11/10/2024. Available from: https://www.thejag.org.uk/JAGguidance].
101. Deliwala SS, Hamid K, Barbarawi M, Lakshman H, Zayed Y, Kandel P, et al. Artificial intelligence (AI) real-time detection vs. routine colonoscopy for colorectal neoplasia: a meta-analysis and trial sequential analysis. International Journal of Colorectal Disease. 2021;36(11):2291-303.
102. Pickhardt PJ, Hassan C, Halligan S, Marmo R. Colorectal Cancer: CT Colonography and Colonoscopy for Detection—Systematic Review and Meta-Analysis. Radiology. 2011;259(2):393-405.
103. MacLeod C, Hudson J, Brogan M, Cotton S, Treweek S, MacLennan G, et al. ScotCap - A large observational cohort study. Colorectal Disease. 2022;24(4):411-21.
104. Kjølhede T, Ølholm AM, Kaalby L, Kidholm K, Qvist N, Baatrup G. Diagnostic accuracy of capsule endoscopy compared with colonoscopy for polyp detection: systematic review and meta-analyses. Endoscopy. 2020;53(07):713-21.
105. Kroijer R, Dyrvig AK, Kobaek-Larsen M, Støvring JO, Qvist N, Baatrup G. Booster medication to achieve capsule excretion in colon capsule endoscopy: a randomized controlled trial of three regimens. Endoscopy International Open. 2018;6(11):E1363-e8.
106. Bretthauer M, Løberg M, Wieszczy P, Kalager M, Emilsson L, Garborg K, et al. Effect of Colonoscopy Screening on Risks of Colorectal Cancer and Related Death. New England Journal of Medicine. 2022;387(17):1547-56.
107. Public Health Scotland. Scottish bowel screening programme statistics. 2023. [cited 27/05/2024. Available from: https://publichealthscotland.scot/publications/scottish-bowel-screening-programme-statistics/scottish-bowel-screening-programme-statistics-for-the-period-of-invitations-from-may-2020-to-april-2022/].
108. Richards M. Report of The Independent Review of Adult Screening Programmes in England. 2019. [cited 09/05/2023. Available from: https://www.england.nhs.uk/wp-content/uploads/2019/02/report-of-the-independent-review-of-adult-screening-programme-in-england.pdf].
109. Richards PSM. Report of The Independent Review of Adult Screening Programmes in England. October 2019.
110. MacDermid E, Hooton G, MacDonald M, McKay G, Grose D, Mohammed N, et al. Improving patient survival with the colorectal cancer multi-disciplinary team. Colorectal Disease. 2009;11(3):291-5.
111. National Institute for Health and Care Excellence. Colorectal cancer (NG151). 2021. [cited 27/05/2024. Available from: https://www.nice.org.uk/guidance/ng151].
112. Folkesson J, Birgisson H, Pahlman L, Cedermark B, Glimelius B, Gunnarsson U. Swedish Rectal Cancer Trial: long lasting benefits from radiotherapy on survival and local recurrence rate. Journal of Clinical Oncology. 2005;23(24):5644-50.
113. van Gijn W, Marijnen CA, Nagtegaal ID, Kranenbarg EM, Putter H, Wiggers T, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. The Lancet Oncology. 2011;12(6):575-82.
114. Sauer R, Liersch T, Merkel S, Fietkau R, Hohenberger W, Hess C, et al. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: results of the German

CAO/ARO/AIO-94 randomized phase III trial after a median follow-up of 11 years. Journal of Clinical Oncology. 2012;30(16):1926-33.
115. Sebag-Montefiore D, Stephens RJ, Steele R, Monson J, Grieve R, Khanna S, et al. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. The Lancet. 2009;373(9666):811-20.
116. Cedermark B, Dahlberg M, Glimelius B, Påhlman L, Rutqvist LE, Wilking N. Improved survival with preoperative radiotherapy in resectable rectal cancer. New England Journal of Medicine. 1997;336(14):980-7.
117. Peeters KC, Marijnen CA, Nagtegaal ID, Kranenbarg EK, Putter H, Wiggers T, et al. The TME trial after a median follow-up of 6 years: increased local control but no survival benefit in irradiated patients with resectable rectal carcinoma. Annals of Surgery. 2007;246(5):693-701.
118. Roh MS, Colangelo LH, O'Connell MJ, Yothers G, Deutsch M, Allegra CJ, et al. Preoperative multimodality therapy improves disease-free survival in patients with carcinoma of the rectum: NSABP R-03. Journal of Clinical Oncology. 2009;27(31):5124-30.
119. Erlandsson J, Holm T, Pettersson D, Berglund Å, Cedermark B, Radu C, et al. Optimal fractionation of preoperative radiotherapy and timing to surgery for rectal cancer (Stockholm III): a multicentre, randomised, non-blinded, phase 3, non-inferiority trial. The Lancet Oncology. 2017;18(3):336-46.
120. Eitta MA, El-Wahidi GF, Fouda MA, El-Hak NG, Abo El-Naga EM. Preoperative radiotherapy in resectable rectal cancer: a prospective randomized study of two different approaches. Journal of the Egyptian National Cancer Institute. 2010;22(3):155-64.
121. Bujko K, Nowacki MP, Nasierowska-Guttmejer A, Michalski W, Bebenek M, Kryj M. Long-term results of a randomized trial comparing preoperative short-course radiotherapy with preoperative conventionally fractionated chemoradiation for rectal cancer. British Journal of Surgery. 2006;93(10):1215-23.
122. Ngan SY, Burmeister B, Fisher RJ, Solomon M, Goldstein D, Joseph D, et al. Randomized trial of short-course radiotherapy versus long-course chemoradiation comparing rates of local recurrence in patients with T3 rectal cancer: Trans-Tasman Radiation Oncology Group trial 01.04. Journal of Clinical Oncology. 2012;30(31):3827-33.
123. Fernandez-Martos C, Garcia-Albeniz X, Pericay C, Maurel J, Aparicio J, Montagut C, et al. Chemoradiation, surgery and adjuvant chemotherapy versus induction chemotherapy followed by chemoradiation and surgery: long-term results of the Spanish GCR-3 phase II randomized trial. Annals of Oncology. 2015;26(8):1722-8.
124. Maréchal R, Vos B, Polus M, Delaunoit T, Peeters M, Demetter P, et al. Short course chemotherapy followed by concomitant chemoradiotherapy and surgery in locally advanced rectal cancer: a randomized multicentric phase II study. Annals of Oncology. 2012;23(6):1525-30.
125. Cercek A, Roxburgh CSD, Strombom P, Smith JJ, Temple LKF, Nash GM, et al. Adoption of Total Neoadjuvant Therapy for Locally Advanced Rectal Cancer. JAMA Oncology. 2018;4(6):e180071-e.
126. Bach SP. STAR-TREC: An International Three-arm Multicentre, Partially Randomised Controlled Trial Incorporating an External Pilot. Clinical Oncology (Royal College of Radiologists). 2023;35(2):e107-e9.
127. Morton D, Seymour M, Magill L, Handley K, Glasbey J, Glimelius B, et al. Preoperative Chemotherapy for Operable Colon Cancer: Mature Results of an International Randomized Controlled Trial. Journal of Clinical Oncology. 2023;41(8):1541-52.
128. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. New England Journal of Medicine. 2015;372(26):2509-20.
129. Overman MJ, Lonardi S, Wong KYM, Lenz HJ, Gelsomino F, Aglietta M, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. Journal of Clinical Oncology. 2018;36(8):773-9.
130. André T, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, et al. Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. New England Journal of Medicine. 2020;383(23):2207-18.

131. Kanani A, Veen T, Søreide K. Neoadjuvant immunotherapy in primary and metastatic colorectal cancer. British Journal of Surgery. 2021;108(12):1417-25.
132. Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. Nature Medicine. 2020;26(4):566-76.
133. Nelson H, Sargent DJ, Wieand HS, Fleshman J, Anvari M, Stryker SJ, et al. A comparison of laparoscopically assisted and open colectomy for colon cancer. New England Journal of Medicine. 2004;350(20):2050-9.
134. Buunen M, Veldkamp R, Hop WC, Kuhry E, Jeekel J, Haglind E, et al. Survival after laparoscopic surgery versus open surgery for colon cancer: long-term outcome of a randomised clinical trial. The Lancet Oncology. 2009;10(1):44-52.
135. Green BL, Marshall HC, Collinson F, Quirke P, Guillou P, Jayne DG, et al. Long-term follow-up of the Medical Research Council CLASICC trial of conventional versus laparoscopically assisted resection in colorectal cancer. British Journal of Surgery. 2013;100(1):75-82.
136. Kennedy RH, Francis EA, Wharton R, Blazeby JM, Quirke P, West NP, et al. Multicenter randomized controlled trial of conventional versus laparoscopic surgery for colorectal cancer within an enhanced recovery programme: EnROL. Journal of Clinical Oncology. 2014;32(17):1804-11.
137. Anderson C, Uman G, Pigazzi A. Oncologic outcomes of laparoscopic surgery for rectal cancer: a systematic review and meta-analysis of the literature. European Journal of Surgical Oncology. 2008;34(10):1135-42.
138. Ng SS, Lee JF, Yiu RY, Li JC, Hon SS, Mak TW, et al. Long-term oncologic outcomes of laparoscopic versus open surgery for rectal cancer: a pooled analysis of 3 randomized controlled trials. Annals of Surgery. 2014;259(1):139-47.
139. Vennix S, Pelzers L, Bouvy N, Beets GL, Pierie JP, Wiggers T, et al. Laparoscopic versus open total mesorectal excision for rectal cancer. Cochrane Database of Systematic Reviews. 2014;2014(4):Cd005200.
140. Bonjer HJ, Deijen CL, Abis GA, Cuesta MA, van der Pas MH, de Lange-de Klerk ES, et al. A randomized trial of laparoscopic versus open surgery for rectal cancer. New England Journal of Medicine. 2015;372(14):1324-32.
141. Jeong SY, Park JW, Nam BH, Kim S, Kang SB, Lim SB, et al. Open versus laparoscopic surgery for mid-rectal or low-rectal cancer after neoadjuvant chemoradiotherapy (COREAN trial): survival outcomes of an open-label, non-inferiority, randomised controlled trial. The Lancet Oncology. 2014;15(7):767-74.
142. Prete FP, Pezzolla A, Prete F, Testini M, Marzaioli R, Patriti A, et al. Robotic Versus Laparoscopic Minimally Invasive Surgery for Rectal Cancer: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Annals of Surgery. 2018;267(6):1034-46.
143. Holmer C, Kreis ME. Systematic review of robotic low anterior resection for rectal cancer. Surgical Endoscopy. 2018;32(2):569-81.
144. Lee SH, Kim DH, Lim SW. Robotic versus laparoscopic intersphincteric resection for low rectal cancer: a systematic review and meta-analysis. International Journal of Colorectal Disease. 2018;33(12):1741-53.
145. Feng Q, Yuan W, Li T, Tang B, Jia B, Zhou Y, et al. Robotic versus laparoscopic surgery for middle and low rectal cancer (REAL): short-term outcomes of a multicentre randomised controlled trial. The Lancet Gastroenterology and Hepatology. 2022;7(11):991-1004.
146. Spanjersberg WR, Reurings J, Keus F, van Laarhoven CJ. Fast track surgery versus conventional recovery strategies for colorectal surgery. Cochrane Database of Systematic Reviews. 2011(2):Cd007635.
147. Williams JG, Pullan RD, Hill J, Horgan PG, Salmo E, Buchanan GN, et al. Management of the malignant colorectal polyp: ACPGBI position statement. Colorectal Disease. 2013;15 Suppl 2:1-38.
148. deBeche-Adams T, Nassif G. Transanal Minimally Invasive Surgery. Clinics in Colon and Rectal Surgery. 2015;28(3):176-80.

149. Sajid MS, Farag S, Leung P, Sains P, Miles WF, Baig MK. Systematic review and meta-analysis of published trials comparing the effectiveness of transanal endoscopic microsurgery and radical resection in the management of early rectal cancer. Colorectal Disease. 2014;16(1):2-14.
150. Martling AL, Holm T, Rutqvist LE, Moran BJ, Heald RJ, Cedemark B. Effect of a surgical training programme on outcome of rectal cancer in the County of Stockholm. Stockholm Colorectal Cancer Study Group, Basingstoke Bowel Cancer Research Project. The Lancet. 2000;356(9224):93-6.
151. Dukes CE, Bussey HJ. The spread of rectal cancer and its effect on prognosis. British Journal of Cancer. 1958;12(3):309-20.
152. Turnbull RB, Jr., Kyle K, Watson FR, Spratt J. Cancer of the colon: the influence of the no-touch isolation technic on survival rates. Annals of Surgery. 1967;166(3):420-7.
153. Brierley J.D. GMK, Wittekind C. TNM Classification of Malignant Tumours (8th Edition): Oxford, UK: Wiley-Blackwell; 2017.
154. Loughrey MB, Webster F, Arends MJ, Brown I, Burgart LJ, Cunningham C, et al. Dataset for Pathology Reporting of Colorectal Cancer: Recommendations From the International Collaboration on Cancer Reporting (ICCR). Annals of Surgery. 2022;275(3):e549-e61.
155. Petersen VC, Baxter KJ, Love SB, Shepherd NA. Identification of objective pathological prognostic determinants and models of prognosis in Dukes' B colon cancer. Gut. 2002;51(1):65-9.
156. Weiser MR, Gönen M, Chou JF, Kattan MW, Schrag D. Predicting survival after curative colectomy for cancer: individualizing colon cancer staging. Journal of Clinical Oncology. 2011;29(36):4796-802.
157. Freedman LS, Macaskill P, Smith AN. Multivariate analysis of prognostic factors for operable rectal cancer. The Lancet. 1984;2(8405):733-6.
158. Knijn N, Mogk SC, Teerenstra S, Simmer F, Nagtegaal ID. Perineural Invasion is a Strong Prognostic Factor in Colorectal Cancer: A Systematic Review. American Journal of Surgical Pathology. 2016;40(1):103-12.
159. van Wyk HC, Park J, Roxburgh C, Horgan P, Foulis A, McMillan DC. The role of tumour budding in predicting survival in patients with primary operable colorectal cancer: a systematic review. Cancer Treatment Reviews. 2015;41(2):151-9.
160. Lu X, Qi R, Xu Y, Wang X, Cai Y, Wang C. Tumor regression grade in locally advanced rectal cancer after neoadjuvant chemoradiotherapy: influencing factors and prognostic significance. International Journal of Clinical and Experimental Pathology. 2023;16(6):124-32.
161. Nagtegaal ID, Quirke P. What is the role for the circumferential margin in the modern treatment of rectal cancer? Journal of Clinical Oncology. 2008;26(2):303-12.
162. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357(6349):409-13.
163. Chang GJ, Rodriguez-Bigas MA, Skibber JM, Moyer VA. Lymph node evaluation and survival after curative resection of colon cancer: systematic review. Journal of the National Cancer Institute. 2007;99(6):433-41.
164. Gill S, Loprinzi CL, Sargent DJ, Thomé SD, Alberts SR, Haller DG, et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? Journal of Clinical Oncology. 2004;22(10):1797-806.
165. Figueredo A, Fine S, Maroun J, Walker-Dilks C, Wong S. Adjuvant therapy for stage III colon cancer after complete resection. Provincial Gastrointestinal Disease Site Group. Cancer Prevention and Control. 1997;1(4):304-19.
166. Twelves C, Scheithauer W, McKendrick J, Seitz JF, Van Hazel G, Wong A, et al. Capecitabine versus 5-fluorouracil/folinic acid as adjuvant therapy for stage III colon cancer: final results from the X-ACT trial with analysis by age and preliminary evidence of a pharmacodynamic marker of efficacy. Annals of Oncology. 2012;23(5):1190-7.
167. André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. New England Journal of Medicine. 2004;350(23):2343-51.
168. Haller DG, Tabernero J, Maroun J, de Braud F, Price T, Van Cutsem E, et al. Capecitabine plus oxaliplatin compared with fluorouracil and folinic acid as adjuvant therapy for stage III colon cancer. Journal of Clinical Oncology. 2011;29(11):1465-71.

169. Gray R, Barnwell J, McConkey C, Hills RK, Williams NS, Kerr DJ. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. The Lancet. 2007;370(9604):2020-9.
170. Tournigand C, André T, Bonnetain F, Chibaudel B, Lledo G, Hickish T, et al. Adjuvant therapy with fluorouracil and oxaliplatin in stage II and elderly patients (between ages 70 and 75 years) with colon cancer: subgroup analyses of the Multicenter International Study of Oxaliplatin, Fluorouracil, and Leucovorin in the Adjuvant Treatment of Colon Cancer trial. Journal of Clinical Oncology. 2012;30(27):3353-60.
171. Treasure T, Farewell V, Macbeth F, Monson K, Williams NR, Brew-Graves C, et al. Pulmonary Metastasectomy versus Continued Active Monitoring in Colorectal Cancer (PulMiCC): a multicentre randomised clinical trial. Trials. 2019;20(1):718.
172. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, et al. Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. The Lancet. 2008;371(9617):1007-16.
173. Scottish Medicines Consoritum. Aflibercept. 2014. [cited 26/01/2025. Available from: https://scottishmedicines.org.uk/medicines-advice/aflibercept-zaltrap-resubmission-87813/].
174. Morris EJ, Whitehouse LE, Farrell T, Nickerson C, Thomas JD, Quirke P, et al. A retrospective observational study examining the characteristics and outcomes of tumours diagnosed within and without of the English NHS Bowel Cancer Screening Programme. British Journal of Cancer. 2012;107(5):757-64.
175. Kronborg O, Fenger C, Olsen J, Jørgensen OD, Søndergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. The Lancet. 1996;348(9040):1467-71.
176. Hewitson P, Glasziou P, Watson E, Towler B, Irwig L. Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update. American Journal of Gastroenterology. 2008;103(6):1541-9.
177. Scottish Government. Scottish Index of Multiple Deprivation. 2020. [cited 08/11/2021. Available from: https://www.gov.scot/collections/scottish-index-of-multiple-deprivation-2020/].
178. World Health Organisation. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011. [cited 21/07/2025. Available from: https://www.who.int/publications/i/item/WHO-NMH-NHD-MNM-11.1].
179. British Society of Gastroenterology: Guidelines for the Management of Iron Deficiency Anaemia in Adults. 2021. [cited 10/11/2021. Available from: https://www.bsg.org.uk/clinical-resource/guidelines-for-the-management-of-iron-deficiency-anaemia/].
180. Fraser CG. Assuring the quality of examinations using faecal immunochemical tests for haemoglobin (FIT). Clinical Chemistry and Laboratory Medicine (CCLM). 2021;59(2):245-7.
181. Digby J, Strachan JA, Mowat C, Steele RJC, Fraser CG. Appraisal of the faecal haemoglobin, age and sex test (FAST) score in assessment of patients with lower bowel symptoms: an observational study. BMC Gastroenterology. 2019;19(1):213.
182. Chapman C, Bunce J, Oliver S, Ng O, Tangri A, Rogers R, et al. Service evaluation of faecal immunochemical testing and anaemia for risk stratification in the 2-week-wait pathway for colorectal cancer. BJS Open. 2019;3(3):395-402.
183. Bailey JA, Weller J, Chapman CJ, Ford A, Hardy K, Oliver S, et al. Faecal immunochemical testing and blood tests for prioritization of urgent colorectal cancer referrals in symptomatic patients: a 2-year evaluation. BJS Open. 2021;5(2).
184. Hippisley-Cox J, Coupland C. Identifying patients with suspected colorectal cancer in primary care: derivation and validation of an algorithm. British Journal of General Practice. 2012;62(594):e29-37.
185. National Institute for Health and Care Excellence. Anaemia - iron deficiency. 2015. [cited 26/01/2025. Available from: https://cks.nice.org.uk/topics/anaemia-iron-deficiency/].
186. Hamilton W, Lancashire R, Sharp D, Peters TJ, Cheng KK, Marshall T. The importance of anaemia in diagnosing colorectal cancer: a case-control study using electronic primary care records. British Journal of Cancer. 2008;98(2):323-7.

187. McSorley ST, Johnstone M, Steele CW, Roxburgh CSD, Horgan PG, McMillan DC, et al. Normocytic anaemia is associated with systemic inflammation and poorer survival in patients with colorectal cancer treated with curative intent. International Journal of Colorectal Disease. 2019;34(3):401-8.
188. Johnstone MS, Burton P, Kourounis G, Winter J, Crighton E, Mansouri D, et al. Combining the quantitative faecal immunochemical test and full blood count reliably rules out colorectal cancer in a symptomatic patient referral pathway. International Journal of Colorectal Disease. 2022;37(2):457-66.
189. Chapman C, Thomas C, Morling J, Tangri A, Oliver S, Simpson JA, et al. Early clinical outcomes of a rapid colorectal cancer diagnosis pathway using faecal immunochemical testing in Nottingham. Colorectal Disease. 2020;22(6):679-88.
190. D'Souza N, Georgiou Delisle T, Chen M, Benton S, Abulafi M. Faecal immunochemical test is superior to symptoms in predicting pathology in patients with suspected colorectal cancer symptoms referred on a 2WW pathway: a diagnostic accuracy study. Gut. 2021;70(6):1130-8.
191. Auge JM, Fraser CG, Rodriguez C, Roset A, Lopez-Ceron M, Grau J, et al. Clinical utility of one versus two faecal immunochemical test samples in the detection of advanced colorectal neoplasia in symptomatic patients. Clinical Chemistry and Laboratory Medicine. 2016;54(1):125-32.
192. Mattar R, Marques SB, Minata MK, Silva-Etto J, Sakai P, de Moura EGH. Diagnostic accuracy of one sample or two samples quantitative fecal immunochemical tests for intestinal neoplasia detection. Arquivos de Gastroenterologia. 2020;57(3):316-22.
193. Miller J, Maeda Y, Au S, Gunn F, Porteous L, Pattenden R, et al. Short-term outcomes of a COVID-adapted triage pathway for colorectal cancer detection. 2021;23(7):1639-48.
194. Maeda Y, Gray E, Figueroa JD, Hall PS, Weller D, Dunlop MG, et al. Risk of missing colorectal cancer with a COVID-adapted diagnostic pathway using quantitative faecal immunochemical testing. BJS Open. 2021;5(4).
195. Chapman C, Thomas C, Morling J, Tangri A, Oliver S, Simpson JA, et al. Early clinical outcomes of a rapid colorectal cancer diagnosis pathway using faecal immunochemical testing in Nottingham. Colorectal Dis. 2020;22(6):679-88.
196. Johnstone MS, MacLeod C, Digby J, Al-Azzawi Y, Pang G, Watson AJM, et al. Prevalence of repeat faecal immunochemical testing in symptomatic patients attending primary care. Colorectal Disease. 2022;24(12):1498-504.
197. Digby J, Strachan JA, McCann R, Steele RJ, Fraser CG, Mowat C. Measurement of faecal haemoglobin with a faecal immunochemical test can assist in defining which patients attending primary care with rectal bleeding require urgent referral. Annals of Clinical Biochemistry. 2020;57(4):325-7.
198. McDonald PJ, Digby J, Innes C, Strachan JA, Carey FA, Steele RJC, et al. Low faecal haemoglobin concentration potentially rules out significant colorectal disease. Colorectal Disease. 2013;15(3):e151-e9.
199. Godber IM, Todd LM, Fraser CG, MacDonald LR, Younes HB. Use of a faecal immunochemical test for haemoglobin can aid in the investigation of patients with lower abdominal symptoms. Clinical Chemistry and Laboratory Medicine. 2016;54(4):595-602.
200. Juul JS, Hornung N, Andersen B, Laurberg S, Olesen F, Vedsted P. The value of using the faecal immunochemical test in general practice on patients presenting with non-alarm symptoms of colorectal cancer. British Journal of Cancer. 2018;119(4):471-9.
201. Nakarai A, Hiraoka S, Takahashi S, Inaba T, Higashi R, Mizuno M, et al. Simultaneous Measurements of Faecal Calprotectin and the Faecal Immunochemical Test in Quiescent Ulcerative Colitis Patients Can Stratify Risk of Relapse. Journal of Crohn's and Colitis. 2017;12(1):71-6.
202. Shi HY, Chan FKL, Chan AWH, Higashimori A, Kyaw M, Ching JYL, et al. Accuracy of Faecal Immunochemical Test to Predict Endoscopic and Histological Healing in Ulcerative Colitis: A Prospective Study Based on Validated Histological Scores. Journal of Crohn's & Colitis. 2017;11(9):1071-7.
203. Kim DJ, Jeoun YM, Lee DW, Koo JS, Lee SW. Usefulness of fecal immunochemical test and fecal calprotectin for detection of active ulcerative colitis. Intest Res. 2018;16(4):563-70.

204. Dai C, Jiang M, Sun MJ, Cao Q. Fecal immunochemical test for predicting mucosal healing in ulcerative colitis patients: A systematic review and meta-analysis. Journal of Gastroenterology and Hepatology. 2018;33(5):990-7.
205. Takashima S, Kato J, Hiraoka S, Nakarai A, Takei D, Inokuchi T, et al. Evaluation of Mucosal Healing in Ulcerative Colitis by Fecal Calprotectin Vs. Fecal Immunochemical Test. American Journal of Gastroenterology. 2015;110(6):873-80.
206. Inokuchi T, Kato J, Hiraoka S, Takashima S, Nakarai A, Takei D, et al. Fecal Immunochemical Test Versus Fecal Calprotectin for Prediction of Mucosal Healing in Crohn's Disease. Inflammatory Bowel Diseases. 2016;22(5):1078-85.
207. Ibáñez-Sanz G, Milà N, de la Peña-Negro LC, Garcia M, Vidal C, Rodríguez-Alonso L, et al. Proton-pump inhibitors are associated with a high false-positivity rate in faecal immunochemical testing. Journal of Gastroenterology. 2021;56(1):42-53.
208. Clark GRC, Strachan JA, McPherson A, Digby J, Mowat C, Steele RJC, et al. Faecal haemoglobin distributions by sex, age, deprivation and geographical region: consequences for colorectal cancer screening strategies. Clinical Chemistry and Laboratory Medicine. 2020;58(12):2073-80.
209. Digby J, McDonald PJ, Strachan JA, Libby G, Steele RJ, Fraser CG. Deprivation and faecal haemoglobin: implications for bowel cancer screening. Journal of Medical Screening. 2014;21(2):95-7.
210. Symonds EL, Osborne JM, Cole SR, Bampton PA, Fraser RJ, Young GP. Factors affecting faecal immunochemical test positive rates: demographic, pathological, behavioural and environmental variables. Journal of Medical Screening. 2015;22(4):187-93.
211. Ibáñez-Sanz G, Garcia M, Rodríguez-Moranta F, Binefa G, Gómez-Matas J, Domènech X, et al. Prescription drugs associated with false-positive results when using faecal immunochemical tests for colorectal cancer screening. Digestive and Liver Disease. 2016;48(10):1249-54.
212. Kim NH, Park JH, Park DI, Sohn CI, Choi K, Jung YS. Are Hemorrhoids Associated with False-Positive Fecal Immunochemical Test Results? Yonsei Medical Journal. 2017;58(1):150-7.
213. de Klerk CM, Vendrig LM, Bossuyt PM, Dekker E. Participant-Related Risk Factors for False-Positive and False-Negative Fecal Immunochemical Tests in Colorectal Cancer Screening: Systematic Review and Meta-Analysis. The American Journal of Gastroenterology. 2018;113(12):1778-87.
214. Amitay EL, Cuk K, Niedermaier T, Weigl K, Brenner H. Factors associated with false-positive fecal immunochemical tests in a large German colorectal cancer screening study. International Journal of Cancer. 2019;144(10):2419-27.
215. Rodriguez-Alonso L, Rodriguez-Moranta F, Arajol C, Gilabert P, Serra K, Martin A, et al. Proton pump inhibitors reduce the accuracy of faecal immunochemical test for detecting advanced colorectal neoplasia in symptomatic patients. PloS One. 2018;13(8):e0203359.
216. Domper Arnal MJ, García Mateo S, Hermoso-Durán S, Abad D, Carrera-Lasfuentes P, Velazquez-Campoy A, et al. False-positive fecal immunochemical test results in colorectal cancer screening and gastrointestinal drug use. International Journal of Colorectal Disease. 2021;36(9):1861-9.
217. Carot L, Castells A, Hernández C, Alvarez-Urturi C, Balaguer F, Lanas A, et al. Detection of serrated lesions in proximal colon by simulated sigmoidoscopy vs faecal immunochemical testing in a multicentre, pragmatic, randomised controlled trial. United European Gastroenterology Journal. 2018;6(10):1527-37.
218. Chang LC, Shun CT, Hsu WF, Tu CH, Tsai PY, Lin BR, et al. Fecal Immunochemical Test Detects Sessile Serrated Adenomas and Polyps With a Low Level of Sensitivity. Clinical Gastroenterology and Hepatology. 2017;15(6):872-9.e1.
219. Barnett KN, Clark GRC, Steele RJC, Fraser CG. Faecal Haemoglobin Estimated by Faecal Immunochemical Tests—An Indicator of Systemic Inflammation with Real Clinical Potential. Diagnostics. 2021;11(11):2093.
220. Tabassum F, Kumari M, Rumley A, Lowe G, Power C, Strachan DP. Effects of Socioeconomic Position on Inflammatory and Hemostatic Markers: A Life-Course Analysis in the 1958 British Birth Cohort. American Journal of Epidemiology. 2008;167(11):1332-41.

221. Petersen KL, Marsland AL, Flory J, Votruba-Drzal E, Muldoon MF, Manuck SB. Community socioeconomic status is associated with circulating interleukin-6 and C-reactive protein. Psychosomatic Medicine. 2008;70(6):646-52.
222. Welsh P, Woodward M, Rumley A, Lowe G. Associations of plasma pro-inflammatory cytokines, fibrinogen, viscosity and C-reactive protein with cardiovascular risk factors and social deprivation: the fourth Glasgow MONICA study. British Journal of Haematology. 2008;141(6):852-61.
223. Al Murri AM, Doughty JC, Lannigan A, Wilson C, McArdle CS, McMillan DC. The relationship between deprivation, tumour stage and the systemic inflammatory response in patients with primary operable breast cancer. British Journal of Cancer. 2004;91(6):1063-5.
224. Nazmi A, Diez Roux A, Ranjit N, Seeman TE, Jenny NS. Cross-sectional and longitudinal associations of neighborhood characteristics with inflammatory markers: findings from the multi-ethnic study of atherosclerosis. Health Place. 2010;16(6):1104-12.
225. Lin Y-H, Jen M-H, Chien K-L. Association between life-course socioeconomic position and inflammatory biomarkers in older age: a nationally representative cohort study in Taiwan. BMC Geriatrics. 2017;17(1):201.
226. Pollitt RA, Kaufman JS, Rose KM, Diez-Roux AV, Zeng D, Heiss G. Early-life and adult socioeconomic status and inflammatory risk markers in adulthood. European Journal of Epidemiology. 2007;22(1):55-66.
227. Gimeno D, Brunner EJ, Lowe GDO, Rumley A, Marmot MG, Ferrie JE. Adult socioeconomic position, C-reactive protein and interleukin-6 in the Whitehall II prospective study. European Journal of Epidemiology. 2007;22(10):675-83.
228. Nazmi A, Victora CG. Socioeconomic and racial/ethnic differentials of C-reactive protein levels: a systematic review of population-based studies. BMC Public Health. 2007;7(1):212.
229. Muscatell KA, Brosso SN, Humphreys KL. Socioeconomic status and inflammation: a meta-analysis. Molecular Psychiatry. 2020;25(9):2189-99.
230. Pin-Vieito N, Puga M, Fernández-de-Castro D, Cubiella J. Faecal immunochemical test outside colorectal cancer screening? World Journal of Gastroenterology. 2021;27(38):6415-29.
231. Mansouri D, McMillan DC, McIlveen E, Crighton EM, Morrison DS, Horgan PG. A comparison of tumour and host prognostic factors in screen-detected vs nonscreen-detected colorectal cancer: a contemporaneous study. Colorectal Disease. 2016;18(10):967-75.
232. de Neree Tot Babberich MPM, Vermeer NCA, Wouters M, van Grevenstein WMU, Peeters K, Dekker E, et al. Postoperative Outcomes of Screen-Detected vs Non-Screen-Detected Colorectal Cancer in the Netherlands. JAMA Surgery. 2018;153(12):e183567.
233. Lee TH, Marcantonio ER, Mangione CM, Thomas EJ, Polanczyk CA, Cook EF, et al. Derivation and prospective validation of a simple index for prediction of cardiac risk of major noncardiac surgery. Circulation. 1999;100(10):1043-9.
234. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. Journal of Chronic Diseases. 1987;40(5):373-83.
235. Dolan RD, McSorley ST, Park JH, Watt DG, Roxburgh CS, Horgan PG, et al. The prognostic value of systemic inflammation in patients undergoing surgery for colon cancer: comparison of composite ratios and cumulative scores. British Journal of Cancer. 2018;119(1):40-51.
236. Sebastian E, Courtier R, Macià F, Grande L, Pera M. The impact of screening on short-term outcome after surgery for colorectal cancer. Revista Española de Enfermedades Digestivas. 2017;109(7):485-90.
237. Guiriguet C, Pera G, Castells A, Toran P, Grau J, Rivero I, et al. Impact of comorbid conditions on participation in an organised colorectal cancer screening programme: a cross-sectional study. BMC Cancer. 2017;17(1):524.
238. Diaz A, Kang J, Moore SP, Baade P, Langbecker D, Condon JR, et al. Association between comorbidity and participation in breast and cervical cancer screening: A systematic review and meta-analysis. Cancer Epidemiology. 2017;47:7-19.
239. Park JH, Watt DG, Roxburgh CS, Horgan PG, McMillan DC. Colorectal Cancer, Systemic Inflammation, and Outcome: Staging the Tumor and Staging the Host. Annals of Surgery. 2016;263(2):326-36.

240. Park JH, Fuglestad AJ, Køstner AH, Oliwa A, Graham J, Horgan PG, et al. Systemic Inflammation and Outcome in 2295 Patients with Stage I-III Colorectal Cancer from Scotland and Norway: First Results from the ScotScan Colorectal Cancer Group. Annals of Surgical Oncology. 2020;27(8):2784-94.
241. Jakubowska K, Koda M, Grudzińska M, Kańczuga-Koda L, Famulski W. Monocyte-to-lymphocyte ratio as a prognostic factor in peripheral whole blood samples of colorectal cancer patients. World Journal of Gastroenterology. 2020;26(31):4639-55.
242. McSorley ST, Black DH, Horgan PG, McMillan DC. The relationship between tumour stage, systemic inflammation, body composition and survival in patients with colorectal cancer. Clinical Nutrition. 2018;37(4):1279-85.
243. Dolan RD, Almasaudi AS, Dieu LB, Horgan PG, McSorley ST, McMillan DC. The relationship between computed tomography-derived body composition, systemic inflammatory response, and survival in patients undergoing surgery for colorectal cancer. Journal of Cachexia, Sarcopenia and Muscle. 2019;10(1):111-22.
244. Mazaki J, Katsumata K, Kasahara K, Tago T, Wada T, Kuwabara H, et al. Neutrophil-to-lymphocyte ratio is a prognostic factor for colon cancer: a propensity score analysis. BMC Cancer. 2020;20(1):922-.
245. Chan JC, Chan DL, Diakos CI, Engel A, Pavlakis N, Gill A, et al. The Lymphocyte-to-Monocyte Ratio is a Superior Predictor of Overall Survival in Comparison to Established Biomarkers of Resectable Colorectal Cancer. Annals of Surgery. 2017;265(3):539-46.
246. Golder AM, McMillan DC, Park JH, Mansouri D, Horgan PG, Roxburgh CS. The prognostic value of combined measures of the systemic inflammatory response in patients with colon cancer: an analysis of 1700 patients. British Journal of Cancer. 2021;124(11):1828-35.
247. Chen JH, Zhai ET, Yuan YJ, Wu KM, Xu JB, Peng JJ, et al. Systemic immune-inflammation index for predicting prognosis of colorectal cancer. World Journal of Gastroenterology. 2017;23(34):6261-72.
248. Køstner AH, Kersten C, Löwenmark T, Ydsten KA, Peltonen R, Isoniemi H, et al. The prognostic role of systemic inflammation in patients undergoing resection of colorectal liver metastases: C-reactive protein (CRP) is a strong negative prognostic biomarker. Journal of Surgical Oncology. 2016;114(7):895-9.
249. Li H, Liu Q, Liang S, Yao P, Lv J, Wang G, et al. Circulating tumor cells and neutrophil-lymphocyte ratio are predictive markers for metastatic colorectal cancer patients. Translational Cancer Research. 2021;10(1):288-97.
250. Verter E, Berger Y, Perl G, Peretz I, Tovar A, Morgenstern S, et al. Neutrophil-to-Lymphocyte Ratio Predicts Recurrence Pattern in Patients with Resectable Colorectal Liver Metastases. Annals of Surgical Oncology. 2021;28(8):4320-9.
251. Shibutani M, Maeda K, Nagahara H, Ohtani H, Sakurai K, Yamazoe S, et al. Prognostic significance of the lymphocyte-to-monocyte ratio in patients with metastatic colorectal cancer. World Journal of Gastroenterology. 2015;21(34):9966-73.
252. Gui W, Wang X, Luo Y, Wang J. Platelet to lymphocyte ratio as a prognostic factor in patients with advanced colorectal cancer undergoing palliative treatment. Annals of Palliative Medicine. 2020;9(5):3271-7.
253. Tan D, Fu Y, Su Q, Wang H. Prognostic role of platelet-lymphocyte ratio in colorectal cancer: A systematic review and meta-analysis. Medicine (Baltimore). 2016;95(24):e3837.
254. Wang J, Li J, Wei S, Xu J, Jiang X, Yang L. The Ratio of Platelets to Lymphocytes Predicts the Prognosis of Metastatic Colorectal Cancer: A Review and Meta-Analysis. Gastroenterology Research and Practice. 2021;2021:9699499.
255. Zhang J, Zhang H-Y, Li J, Shao X-Y, Zhang C-X. The elevated NLR, PLR and PLT may predict the prognosis of patients with colorectal cancer: a systematic review and meta-analysis. Oncotarget. 2017;8(40):68837-46.
256. Rossi S, Basso M, Strippoli A, Schinzari G, D'Argento E, Larocca M, et al. Are Markers of Systemic Inflammation Good Prognostic Indicators in Colorectal Cancer? Clinical Colorectal Cancer. 2017;16(4):264-74.

257. Yamamoto T, Kawada K, Obama K. Inflammation-Related Biomarkers for the Prediction of Prognosis in Colorectal Cancer Patients. International Journal of Molecular Sciences. 2021;22(15):8002.
258. Johnstone MS, Miller G, Pang G, Burton P, Kourounis G, Winter J, et al. Alternative diagnoses and demographics associated with a raised quantitative faecal immunochemical test in symptomatic patients. Annals of Clinical Biochemistry. 2022;59(4):277-87.
259. Logan RFA, Patnick J, Nickerson C, Coleman L, Rutter MD, von Wagner C. Outcomes of the Bowel Cancer Screening Programme (BCSP) in England after the first 1 million tests. Gut. 2012;61(10):1439.
260. Johnstone MS, McMillan DC, Horgan PG, Mansouri D. The Relationship Between Co-morbidity, Screen-Detection and Outcome in Patients Undergoing Resection for Colorectal Cancer. World Journal of Surgery. 2021;45(7):2251-60.
261. Johnstone MS, McSorley ST, McMillan DC, Horgan PG, Mansouri D. The relationship between systemic inflammatory response, screen detection and outcome in colorectal cancer. Colorectal Disease. 2024;26(1):81-94.
262. Kuo E, Wang K, Liu X. A Focused Review on Advances in Risk Stratification of Malignant Polyps. Gastroenterology Research. 2020;13(5):163-83.
263. Antonelli G, Vanella G, Orlando D, Angeletti S, Di Giulio E. Recurrence and cancer-specific mortality after endoscopic resection of low- and high-risk pT1 colorectal cancers: a meta-analysis. Gastrointestinal Endoscopy. 2019;90(4):559-69.e3.
264. Hassan C, Zullo A, Risio M, Rossini FP, Morini S. Histologic risk factors and clinical outcome in colorectal malignant polyp: a pooled-data analysis. Diseases of the Colon and Rectum. 2005;48(8):1588-96.
265. Kitajima K, Fujimori T, Fujii S, Takeda J, Ohkura Y, Kawamata H, et al. Correlations between lymph node metastasis and depth of submucosal invasion in submucosal invasive colorectal carcinoma: a Japanese collaborative study. Journal of Gastroenterology. 2004;39(6):534-43.
266. Netzer P, Forster C, Biral R, Ruchti C, Neuweiler J, Stauffer E, et al. Risk factor assessment of endoscopically removed malignant colorectal polyps. Gut. 1998;43(5):669-74.
267. Seitz U, Bohnacker S, Seewald S, Thonke F, Brand B, Bräiutigam T, et al. Is endoscopic polypectomy an adequate therapy for malignant colorectal adenomas? Presentation of 114 patients and review of the literature. Diseases of the Colon and Rectum. 2004;47(11):1789-96; discussion 96-7.
268. Cooper HS, Deppisch LM, Gourley WK, Kahn EI, Lev R, Manley PN, et al. Endoscopically removed malignant colorectal polyps: Clinicopathologic correlations. Gastroenterology. 1995;108(6):1657-65.
269. Ueno H, Mochizuki H, Hashiguchi Y, Shimazaki H, Aida S, Hase K, et al. Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterology. 2004;127(2):385-94.
270. Kaneko I, Tanaka S, Oka S, Kawamura T, Hiyama T, Ito M, et al. Lymphatic vessel density at the site of deepest penetration as a predictor of lymph node metastasis in submucosal colorectal cancer. Diseases of the Colon and Rectum. 2007;50(1):13-21.
271. Haggitt RC, Glotzbach RE, Soffer EE, Wruble LD. Prognostic factors in colorectal carcinomas arising in adenomas: implications for lesions removed by endoscopic polypectomy. Gastroenterology. 1985;89(2):328-36.
272. Kudo S. Endoscopic mucosal resection of flat and depressed types of early colorectal cancer. Endoscopy. 1993;25(7):455-61.
273. Gill MD, Rutter MD, Holtham SJ. Management and short-term outcome of malignant colorectal polyps in the north of England(1). Colorectal Disease. 2013;15(2):169-76.
274. Strengthening the reporting of observational studies in epidemiology. 2025. [cited 23/07/2025. Available from: https://www.strobe-statement.org/].
275. Smith JJ, Strombom P, Chow OS, Roxburgh CS, Lynn P, Eaton A, et al. Assessment of a Watch-and-Wait Strategy for Rectal Cancer in Patients With a Complete Response After Neoadjuvant Therapy. JAMA Oncology. 2019;5(4):e185896.

276. Yeh JH, Tseng CH, Huang RY, Lin CW, Lee CT, Hsiao PJ, et al. Long-term Outcomes of Primary Endoscopic Resection vs Surgery for T1 Colorectal Cancer: A Systematic Review and Meta-analysis. Clinical Gastroenterology and Hepatology. 2020;18(12):2813-23.e5.
277. Flanagan DJ, Amirkhah R, Vincent DF, Gundaz N, Gentaz P, Cammareri P, et al. Epithelial TGFβ engages growth-factor signalling to circumvent apoptosis and drive intestinal tumourigenesis with aggressive features. Nature Communications. 2022;13(1):7551.
278. Dolwani S. Significant polyp and early colorectal cancer - decision-making and treatment planning in regional networks and multidisciplinary teams. Colorectal Disease. 2019;21 Suppl 1:16-8.
279. Hao Y, Wang Y, Qi M, He X, Zhu Y, Hong J. Risk Factors for Recurrent Colorectal Polyps. Gut Liver. 2020;14(4):399-411.
280. Løberg M, Kalager M, Holme Ø, Hoff G, Adami HO, Bretthauer M. Long-term colorectal-cancer mortality after adenoma removal. New England Journal of Medicine. 2014;371(9):799-807.
281. Integrated Technologies for Improved Polyp Surveillance - About INCISE. 2020. [cited 15/02/2021. Available from: https://www.gla.ac.uk/research/az/incise/aboutincise/].
282. Atkin WS, Saunders BP. Surveillance guidelines after removal of colorectal adenomatous polyps. Gut. 2002;51 Suppl 5(Suppl 5):V6-9.
283. Cairns SR, Scholefield JH, Steele RJ, Dunlop MG, Thomas HJ, Evans GD, et al. Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). Gut. 2010;59(5):666-89.
284. Fraser CG, Digby J, McDonald PJ, Strachan JA, Carey FA, Steele RJ. Experience with a two-tier reflex gFOBT/FIT strategy in a national bowel screening programme. Journal of Medical Screening. 2012;19(1):8-13.
285. Atkin W, Wooldrage K, Brenner A, Martin J, Shah U, Perera S, et al. Adenoma surveillance and colorectal cancer incidence: a retrospective, multicentre, cohort study. The Lancet Oncology. 2017;18(6):823-34.
286. Huang Y, Gong W, Su B, Zhi F, Liu S, Bai Y, et al. Recurrence and surveillance of colorectal adenoma after polypectomy in a southern Chinese population. Journal of Gastroenterology. 2010;45(8):838-45.
287. Atkin W, Brenner A, Martin J, Wooldrage K, Shah U, Lucas F, et al. The clinical effectiveness of different surveillance strategies to prevent colorectal cancer in people with intermediate-grade colorectal adenomas: a retrospective cohort analysis, and psychological and economic evaluations. Health Technology Assessment. 2017;21(25):1-536.
288. Facciorusso A, Di Maso M, Serviddio G, Vendemiale G, Spada C, Costamagna G, et al. Factors Associated With Recurrence of Advanced Colorectal Adenoma After Endoscopic Resection. Clinical Gastroenterology and Hepatology. 2016;14(8):1148-54.e4.
289. Fairley KJ, Li J, Komar M, Steigerwalt N, Erlich P. Predicting the risk of recurrent adenoma and incident colorectal cancer based on findings of the baseline colonoscopy. Clinical and Translational Gastroenterology. 2014;5(12):e64.
290. van Enckevort CC, de Graaf AP, Hollema H, Sluiter WJ, Kleibeuker JH, Koornstra JJ. Predictors of colorectal neoplasia after polypectomy: based on initial and consecutive findings. Netherlands Journal of Medicine. 2014;72(3):139-45.
291. Martínez ME, Baron JA, Lieberman DA, Schatzkin A, Lanza E, Winawer SJ, et al. A pooled analysis of advanced colorectal neoplasia diagnoses after colonoscopic polypectomy. Gastroenterology. 2009;136(3):832-41.
292. Huang Y, Li X, Wang Z, Su B. Five-year risk of colorectal neoplasia after normal baseline colonoscopy in asymptomatic Chinese Mongolian over 50 years of age. International Journal of Colorectal Disease. 2012;27(12):1651-6.
293. Cubiella J, Carballo F, Portillo I, Quevedo JC, Salas D, Binefa G, et al. Incidence of advanced neoplasia during surveillance in high-and intermediate-risk groups of the European colorectal cancer screening guidelines. Endoscopy. 2016;48(11):995-1002.
294. van Heijningen EM, Lansdorp-Vogelaar I, Kuipers EJ, Dekker E, Lesterhuis W, Ter Borg F, et al. Features of adenoma and colonoscopy associated with recurrent colorectal neoplasia based on a large community-based study. Gastroenterology. 2013;144(7):1410-8.

295. Vemulapalli KC, Rex DK. Risk of advanced lesions at first follow-up colonoscopy in high-risk groups as defined by the United Kingdom post-polypectomy surveillance guideline: data from a single U.S. center. Gastrointestinal Endoscopy. 2014;80(2):299-306.
296. Facciorusso A, Di Maso M, Serviddio G, Vendemiale G, Muscatiello N. Development and validation of a risk score for advanced colorectal adenoma recurrence after endoscopic resection. World Journal of Gastroenterology. 2016;22(26):6049.
297. Park SK, Kim NH, Jung YS, Kim WH, Eun CS, Ko BM, et al. Risk of developing advanced colorectal neoplasia after removing high-risk adenoma detected at index colonoscopy in young patients: A KASID study. Journal of Gastroenterology and Hepatology. 2016;31(1):138-44.
298. Cubiella J, Carballo F, Portillo I, Cruzado Quevedo J, Salas D, Binefa G, et al. Incidence of advanced neoplasia during surveillance in high- and intermediate-risk groups of the European colorectal cancer screening guidelines. Endoscopy. 2016;48(11):995-1002.
299. Lee TJ, Nickerson C, Goddard AF, Rees CJ, McNally RJ, Rutter MD. Outcome of 12-month surveillance colonoscopy in high-risk patients in the National Health Service Bowel Cancer Screening Programme. Colorectal Disease. 2013;15(8):e435-42.
300. Laiyemo AO, Murphy G, Albert PS, Sansbury LB, Wang Z, Cross AJ, et al. Postpolypectomy colonoscopy surveillance guidelines: predictive accuracy for advanced adenoma at 4 years. Annals of Internal Medicine. 2008;148(6):419-26.
301. Solakoğlu T, Köseoğlu H, SARI SÖ, Akin FE, Bolat AD, Yürekli ÖT, et al. Role of baseline adenoma characteristics for adenoma recurrencein patients with high-risk adenoma. Turkish Journal of Medical Sciences. 2017;47(5):1416-24.
302. Jung YS, Park DI, Kim WH, Eun CS, Park S-K, Ko BM, et al. Risk of advanced colorectal neoplasia according to the number of high-risk findings at index colonoscopy: a Korean Association for the Study of Intestinal Disease (KASID) study. Digestive Diseases and Sciences. 2016;61(6):1661-8.
303. Lee JL, Cha JM, Lee HM, Jeon JW, Kwak MS, Yoon JY, et al. Determining the optimal surveillance interval after a colonoscopic polypectomy for the Korean population? Intestinal Research. 2017;15(1):109-17.
304. Nusko G, Sachse R, Mansmann U, Wittekind C, Hahn EG. K-RAS-2 gene mutations as predictors of metachronous colorectal adenomas. Scandinavian Journal of Gastroenterology. 1997;32(10):1035-41.
305. Cross AJ, Robbins EC, Pack K, Stenson I, Patel B, Rutter MD, et al. Colorectal cancer risk following polypectomy in a multicentre, retrospective, cohort study: an evaluation of the 2020 UK post-polypectomy surveillance guidelines. Gut. 2021;70(12):2307-20.
306. NHS National Services Scotland. National Statistics: Scottish Bowel Screening Programme Statistics. 2019. [cited 01/11/2022. Available from: https://www.isdscotland.org/Health-Topics/Cancer/Publications/index.asp#597].
307. Lieberman D. Colon-polyp surveillance--do patients benefit? New England Journal of Medicine. 2014;371(9):860-1.
308. Huang Y, Gong W, Su B, Zhi F, Liu S, Jiang B. Risk and cause of interval colorectal cancer after colonoscopic polypectomy. Digestion. 2012;86(2):148-54.
309. Pickhardt PJ, Hain KS, Kim DH, Hassan C. Low rates of cancer or high-grade dysplasia in colorectal polyps collected from computed tomography colonography screening. Clinical Gastroenterology and Hepatology. 2010;8(7):610-5.
310. Laish I, Seregeev I, Naftali T, Konikoff FM. Surveillance after positive colonoscopy based on adenoma characteristics. Digestive and Liver Disease. 2017;49(10):1115-20.
311. Jang HW, Park SJ, Hong SP, Cheon JH, Kim WH, Kim TI. Risk Factors for Recurrent High-Risk Polyps after the Removal of High-Risk Polyps at Initial Colonoscopy. Yonsei Medical Journal. 2015;56(6):1559-65.
312. Cottet V, Jooste V, Fournel I, Bouvier AM, Faivre J, Bonithon-Kopp C. Long-term risk of colorectal cancer after adenoma removal: a population-based cohort study. Gut. 2012;61(8):1180-6.
313. Coleman HG, Loughrey MB, Murray LJ, Johnston BT, Gavin AT, Shrubsole MJ, et al. Colorectal Cancer Risk Following Adenoma Removal: A Large Prospective Population-Based Cohort Study. Cancer Epidemiology, Biomarkers and Prevention. 2015;24(9):1373-80.

314. Johnstone MS, Stoops R, Lynch G, Hay J, Jawny J, Sloan W, et al. Risk stratification for the detection of metachronous polyps after bowel screening polypectomy: clinical outcomes from the Integrated Technologies for Improved Polyp Surveillance (INCISE) study cohort. BJS Open. 2023;7(3).
315. Di Rollo DG. An investigation into the role of adenoma and host-specific factors on the incidence and recurrence of colorectal neoplasia. MD thesis, University of Glasgow. 2020. [Available from: http://theses.gla.ac.uk/81657/].
316. Lin CC, Huang KW, Luo JC, Wang YW, Hou MC, Lin HC, et al. Hypertension is an important predictor of recurrent colorectal adenoma after screening colonoscopy with adenoma polypectomy. Journal of the Chinese Medical Association. 2014;77(10):508-12.
317. Harrington LX, Wei JW, Suriawinata AA, Mackenzie TA, Hassanpour S. Predicting colorectal polyp recurrence using time-to-event analysis of medical records. AMIA Joint Summits on Translational Science Proceedings 2020;2020:211-20.
318. Lanza E, Yu B, Murphy G, Albert PS, Caan B, Marshall JR, et al. The polyp prevention trial continued follow-up study: no effect of a low-fat, high-fiber, high-fruit, and -vegetable diet on adenoma recurrence eight years after randomization. Cancer Epidemiology, Biomarkers and Prevention. 2007;16(9):1745-52.
319. Schatzkin A, Lanza E, Corle D, Lance P, Iber F, Caan B, et al. Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. Polyp Prevention Trial Study Group. New England Journal of Medicine. 2000;342(16):1149-55.
320. Sansbury LB, Wanke K, Albert PS, Kahle L, Schatzkin A, Lanza E. The effect of strict adherence to a high-fiber, high-fruit and -vegetable, and low-fat eating pattern on adenoma recurrence. American Journal of Epidemiology. 2009;170(5):576-84.
321. Alberts DS, Martínez ME, Roe DJ, Guillén-Rodríguez JM, Marshall JR, van Leeuwen JB, et al. Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. Phoenix Colon Cancer Prevention Physicians' Network. New England Journal of Medicine. 2000;342(16):1156-62.
322. Cottet V, Bonithon-Kopp C, Kronborg O, Santos L, Andreatta R, Boutron-Ruault MC, et al. Dietary patterns and the risk of colorectal adenoma recurrence in a European intervention trial. European Journal of Cancer Prevention. 2005;14(1):21-9.
323. Bobe G, Murphy G, Albert PS, Sansbury LB, Lanza E, Schatzkin A, et al. Serum cytokine concentrations, flavonol intake and colorectal adenoma recurrence in the Polyp Prevention Trial. British Journal of Cancer. 2010;103(9):1453-61.
324. Bobe G, Albert PS, Sansbury LB, Lanza E, Schatzkin A, Colburn NH, et al. Interleukin-6 as a potential indicator for prevention of high-risk adenoma recurrence by dietary flavonols in the polyp prevention trial. Cancer Prevention Research (Philadelphia, Pa). 2010;3(6):764-75.
325. Crockett SD, Mott LA, Barry EL, Figueiredo JC, Burke CA, Baxter GJ, et al. C-reactive protein and risk of colorectal adenomas or serrated polyps: a prospective study. Cancer Prevention Research (Philadelphia, Pa). 2014;7(11):1122-7.
326. Fedirko V, McKeown-Eyssen G, Serhan CN, Barry EL, Sandler RS, Figueiredo JC, et al. Plasma lipoxin A(4) and resolvin D1 are not associated with reduced adenoma risk in a randomized trial of aspirin to prevent colon adenomas. Molecular Carcinogenesis. 2017;56(8):1977-83.
327. Gao F, Liao C, Liu L, Tan A, Cao Y, Mo Z. The effect of aspirin in the recurrence of colorectal adenomas: a meta-analysis of randomized controlled trials. Colorectal Disease. 2009;11(9):893-901.
328. Ishikawa H, Mutoh M, Suzuki S, Tokudome S, Saida Y, Abe T, et al. The preventive effects of low-dose enteric-coated aspirin tablets on the development of colorectal tumours in Asian patients: a randomised trial. Gut. 2014;63(11):1755-9.
329. Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. New England Journal of Medicine. 2003;348(10):883-90.
330. Hull MA, Sprange K, Hepburn T, Tan W, Shafayat A, Rees CJ, et al. Eicosapentaenoic acid and aspirin, alone and in combination, for the prevention of colorectal adenomas (seAFOod Polyp

Prevention trial): a multicentre, randomised, double-blind, placebo-controlled, 2 × 2 factorial trial. The Lancet. 2018;392(10164):2583-94.
331. Viel JF, Studer JM, Ottignon Y, Hirsch JP. Predictors of colorectal polyp recurrence after the first polypectomy in private practice settings: a cohort study. PloS One. 2012;7(12):e50990.
332. Cole BF, Logan RF, Halabi S, Benamouzig R, Sandler RS, Grainge MJ, et al. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. Journal of the National Cancer Institute. 2009;101(4):256-66.
333. Zhao TY, Tu J, Wang Y, Cheng DW, Gao XK, Luo H, et al. The Efficacy of Aspirin in Preventing the Recurrence of Colorectal Adenoma: a Renewed Meta-Analysis of Randomized Trials. Asian Pacific Journal of Cancer Prevention. 2016;17(5):2711-7.
334. Wang Y, Zhang FC, Wang YJ. The efficacy and safety of non-steroidal anti-inflammatory drugs in preventing the recurrence of colorectal adenoma: a meta-analysis and systematic review of randomized trials. Colorectal Disease. 2015;17(3):188-96.
335. Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Breazna A, Kim K, et al. Five-year efficacy and safety analysis of the Adenoma Prevention with Celecoxib Trial. Cancer Prevention Research (Philadelphia, Pa). 2009;2(4):310-21.
336. Baron JA, Sandler RS, Bresalier RS, Quan H, Riddell R, Lanas A, et al. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterology. 2006;131(6):1674-82.
337. Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, et al. Celecoxib for the prevention of sporadic colorectal adenomas. New England Journal of Medicine. 2006;355(9):873-84.
338. Arber N, Spicak J, Rácz I, Zavoral M, Breazna A, Gerletti P, et al. Five-year analysis of the prevention of colorectal sporadic adenomatous polyps trial. American Journal of Gastroenterology. 2011;106(6):1135-46.
339. Veettil SK, Lim KG, Ching SM, Saokaew S, Phisalprapa P, Chaiyakunapruk N. Effects of aspirin and non-aspirin nonsteroidal anti-inflammatory drugs on the incidence of recurrent colorectal adenomas: a systematic review with meta-analysis and trial sequential analysis of randomized clinical trials. BMC Cancer. 2017;17(1):763-.
340. Bonovas S, Fiorino G, Lytras T, Malesci A, Danese S. Calcium supplementation for the prevention of colorectal adenomas: A systematic review and meta-analysis of randomized controlled trials. World Journal of Gastroenterology. 2016;22(18):4594-603.
341. Shaukat A, Scouras N, Schünemann HJ. Role of supplemental calcium in the recurrence of colorectal adenomas: a metaanalysis of randomized controlled trials. American Journal of Gastroenterology. 2005;100(2):390-4.
342. Bonithon-Kopp C, Kronborg O, Giacosa A, Räth U, Faivre J. Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. European Cancer Prevention Organisation Study Group. The Lancet. 2000;356(9238):1300-6.
343. Baron JA, Barry EL, Mott LA, Rees JR, Sandler RS, Snover DC, et al. A Trial of Calcium and Vitamin D for the Prevention of Colorectal Adenomas. New England Journal of Medicine. 2015;373(16):1519-30.
344. Calderwood AH, Baron JA, Mott LA, Ahnen DJ, Bostick RM, Figueiredo JC, et al. No Evidence for Posttreatment Effects of Vitamin D and Calcium Supplementation on Risk of Colorectal Adenomas in a Randomized Trial. Cancer Prevention Research (Philadelphia, Pa). 2019;12(5):295-304.
345. Grau MV, Baron JA, Sandler RS, Wallace K, Haile RW, Church TR, et al. Prolonged effect of calcium supplementation on risk of colorectal adenomas in a randomized trial. Journal of the National Cancer Institute. 2007;99(2):129-36.
346. Baron JA, Beach M, Mandel JS, van Stolk RU, Haile RW, Sandler RS, et al. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. New England Journal of Medicine. 1999;340(2):101-7.
347. Baron JA, Beach M, Mandel JS, van Stolk RU, Haile RW, Sandler RS, et al. Calcium supplements and colorectal adenomas. Polyp Prevention Study Group. Annals of the New York Academy of Sciences. 1999;889:138-45.

348. Hartman TJ, Albert PS, Snyder K, Slattery ML, Caan B, Paskett E, et al. The association of calcium and vitamin D with risk of colorectal adenomas. Journal of Nutrition. 2005;135(2):252-9.
349. Weingarten MA, Zalmanovici A, Yaphe J. Dietary calcium supplementation for preventing colorectal cancer and adenomatous polyps. Cochrane Database of Systematic Reviews. 2004(1):Cd003548.
350. Keum N, Lee DH, Greenwood DC, Zhang X, Giovannucci EL. Calcium intake and colorectal adenoma risk: dose-response meta-analysis of prospective observational studies. International Journal of Cancer. 2015;136(7):1680-7.
351. Weingarten MA, Zalmanovici A, Yaphe J. Dietary calcium supplementation for preventing colorectal cancer and adenomatous polyps. Cochrane Database of Systematic Reviews. 2008(1):Cd003548.
352. Veettil SK, Ching SM, Lim KG, Saokaew S, Phisalprapa P, Chaiyakunapruk N. Effects of calcium on the incidence of recurrent colorectal adenomas: A systematic review with meta-analysis and trial sequential analysis of randomized controlled trials. Medicine (Baltimore). 2017;96(32):e7661.
353. Jacobs ET, Alberts DS, Benuzillo J, Hollis BW, Thompson PA, Martínez ME. Serum 25(OH)D levels, dietary intake of vitamin D, and colorectal adenoma recurrence. Journal of Steroid Biochemistry and Molecular Biology. 2007;103(3-5):752-6.
354. Wei MY, Garland CF, Gorham ED, Mohr SB, Giovannucci E. Vitamin D and prevention of colorectal adenoma: a meta-analysis. Cancer Epidemiology, Biomarkers and Prevention. 2008;17(11):2958-69.
355. Ibrahim EM, Zekri JM. Folic acid supplementation for the prevention of recurrence of colorectal adenomas: metaanalysis of interventional trials. Medical Oncology. 2010;27(3):915-8.
356. Wu K, Platz EA, Willett WC, Fuchs CS, Selhub J, Rosner BA, et al. A randomized trial on folic acid supplementation and risk of recurrent colorectal adenoma. American Journal of Clinical Nutrition. 2009;90(6):1623-31.
357. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA. 2007;297(21):2351-9.
358. Jaszewski R, Misra S, Tobi M, Ullah N, Naumoff JA, Kucuk O, et al. Folic acid supplementation inhibits recurrence of colorectal adenomas: a randomized chemoprevention trial. World Journal of Gastroenterology. 2008;14(28):4492-8.
359. Alberts DS, Martínez ME, Hess LM, Einspahr JG, Green SB, Bhattacharyya AK, et al. Phase III trial of ursodeoxycholic acid to prevent colorectal adenoma recurrence. Journal of the National Cancer Institute. 2005;97(11):846-53.
360. Juárez M, Egoavil C, Rodríguez-Soler M, Hernández-Illán E, Guarinos C, García-Martínez A, et al. KRAS and BRAF somatic mutations in colonic polyps and the risk of metachronous neoplasia. PloS One. 2017;12(9):e0184937.
361. Benamouzig R, Uzzan B, Martin A, Deyra J, Little J, Girard B, et al. Cyclooxygenase-2 expression and recurrence of colorectal adenomas: effect of aspirin chemoprevention. Gut. 2010;59(5):622-9.
362. Egan JB, Jacobs ET, Martínez ME, Gerner EW, Jurutka PW, Thompson PA. Presence of a TA haplotype in the APC gene containing the common 1822 polymorphism and colorectal adenoma. Cancer Research. 2008;68(14):6006-13.
363. Kraus S, Hummler S, Toriola AT, Poole EM, Scherer D, Kotzmann J, et al. Impact of genetic polymorphisms on adenoma recurrence and toxicity in a COX2 inhibitor (celecoxib) trial: results from a pilot study. Pharmacogenetics and Genomics. 2013;23(8):428-37.
364. Barry EL, Sansbury LB, Grau MV, Ali IU, Tsang S, Munroe DJ, et al. Cyclooxygenase-2 polymorphisms, aspirin treatment, and risk for colorectal adenoma recurrence--data from a randomized clinical trial. Cancer Epidemiology, Biomarkers and Prevention. 2009;18(10):2726-33.
365. Hubner RA, Muir KR, Liu J-F, Logan RFA, Grainge MJ, Houlston RS, et al. Polymorphisms in PTGS1, PTGS2 and IL-10 do not influence colorectal adenoma recurrence in the context of a randomized aspirin intervention trial. International Journal of Cancer. 2007;121(9):2001-4.
366. Bobe G, Murphy G, Albert PS, Sansbury LB, Young MR, Lanza E, et al. Do interleukin polymorphisms play a role in the prevention of colorectal adenoma recurrence by dietary flavonols? European Journal of Cancer Prevention. 2011;20(2):86-95.

367. Sansbury LB, Bergen AW, Wanke KL, Yu B, Caporaso NE, Chatterjee N, et al. Inflammatory cytokine gene polymorphisms, nonsteroidal anti-inflammatory drug use, and risk of adenoma polyp recurrence in the polyp prevention trial. Cancer Epidemiology, Biomarkers and Prevention. 2006;15(3):494-501.
368. Murphy G, Cross AJ, Sansbury LS, Bergen A, Laiyemo AO, Albert PS, et al. Dopamine D2 receptor polymorphisms and adenoma recurrence in the Polyp Prevention Trial. International Journal of Cancer. 2009;124(9):2148-51.
369. Barry EL, Poole EM, Baron JA, Makar KW, Mott LA, Sandler RS, et al. CYP2C9 variants increase risk of colorectal adenoma recurrence and modify associations with smoking but not aspirin treatment. Cancer Causes and Control. 2013;24(1):47-54.
370. Hubner RA, Muir KR, Liu J-F, Logan RFA, Grainge M, Armitage N, et al. Genetic Variants of UGT1A6 Influence Risk of Colorectal Adenoma Recurrence. Clinical Cancer Research. 2006;12(21):6585-9.
371. Hibler EA, Klimentidis YC, Jurutka PW, Kohler LN, Lance P, Roe DJ, et al. CYP24A1 and CYP27B1 Polymorphisms, Concentrations of Vitamin D Metabolites, and Odds of Colorectal Adenoma Recurrence. Nutrition and Cancer. 2015;67(7):1131-41.
372. Wertheim BC, Smith JW, Fang C, Alberts DS, Lance P, Thompson PA. Risk modification of colorectal adenoma by CYP7A1 polymorphisms and the role of bile acid metabolism in carcinogenesis. Cancer Prevention Research (Philadelphia, Pa). 2012;5(2):197-204.
373. Chan AT, Hsu M, Zauber AG, Hawk ET, Bertagnolli MM. The influence of UGT1A6 variants and aspirin use in a randomized trial of celecoxib for prevention of colorectal adenoma. Cancer Prevention Research (Philadelphia, Pa). 2012;5(1):61-72.
374. Hubner RA, Muir KR, Liu JF, Logan RF, Grainge MJ, Houlston RS. Ornithine decarboxylase G316A genotype is prognostic for colorectal adenoma recurrence and predicts efficacy of aspirin chemoprevention. Clinical Cancer Research. 2008;14(8):2303-9.
375. Martinez ME, O'Brien TG, Fultz KE, Babbar N, Yerushalmi H, Qu N, et al. Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(13):7859-64.
376. Barry ELR, Baron JA, Bhat S, Grau MV, Burke CA, Sandler RS, et al. Ornithine Decarboxylase Polymorphism Modification of Response to Aspirin Treatment for Colorectal Adenoma Prevention. JNCI: Journal of the National Cancer Institute. 2006;98(20):1494-500.
377. Levine AJ, Wallace K, Tsang S, Haile RW, Saibil F, Ahnen D, et al. MTHFR genotype and colorectal adenoma recurrence: data from a double-blind placebo-controlled clinical trial. Cancer Epidemiology, Biomarkers and Prevention. 2008;17(9):2409-15.
378. Wang J, Carvajal-Carmona LG, Chu JH, Zauber AG, Kubo M, Matsuda K, et al. Germline variants and advanced colorectal adenomas: adenoma prevention with celecoxib trial genome-wide association study. Clinical Cancer Research. 2013;19(23):6430-7.
379. Einspahr JG, Martinez ME, Jiang R, Hsu CH, Rashid A, Bhattacharrya AK, et al. Associations of Ki-ras proto-oncogene mutation and p53 gene overexpression in sporadic colorectal adenomas with demographic and clinicopathologic characteristics. Cancer Epidemiology, Biomarkers and Prevention. 2006;15(8):1443-50.
380. Brand L, Munding J, Pox CP, Ziebarth W, Reiser M, Hüppe D, et al. ß-Catenin, Cox-2 and p53 immunostaining in colorectal adenomas to predict recurrence after endoscopic polypectomy. International Journal of Colorectal Disease. 2013;28(8):1091-8.
381. Sheikh RA, Min BH, Yasmeen S, Teplitz R, Tesluk H, Ruebner BH, et al. Correlation of Ki-67, p53, and Adnab-9 Immunohistochemical Staining and Ploidy with Clinical and Histopathologic Features of Severely Dysplastic Colorectal Adenomas. Digestive Diseases and Sciences. 2003;48(1):223-9.
382. Vernillo R, Lorenzi B, Banducci T, Minacci C, Vindigni C, Lucenti Fei A, et al. Immunohistochemical expression of p53 and Ki67 in colorectal adenomas and prediction of malignancy and development of new polyps. International Journal of Biological Markers. 2008;23(2):89-95.
383. Alao JP. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. Molecular Cancer. 2007;6:24-.

384. Bahnassy AA, Zekri A-RN, El-Houssini S, El-Shehaby AMR, Mahmoud MR, Abdallah S, et al. Cyclin A and cyclin D1 as significant prognostic markers in colorectal cancer patients. BMC Gastroenterology. 2004;4(1):22.
385. Macaron C, Lopez R, Pai RK, Burke CA. Expression of Annexin A10 in Serrated Polyps Predicts the Development of Metachronous Serrated Polyps. Clinical and Translational Gastroenterology. 2016;7(12):e205-e.
386. Bartley AN, Parikh N, Hsu C-H, Roe DJ, Buckmeier JA, Corley L, et al. Colorectal adenoma stem-like cell populations: associations with adenoma characteristics and metachronous colorectal neoplasia. Cancer Prevention Research (Philadelphia, Pa). 2013;6(11):1162-70.
387. Villéger R, Lopès A, Veziant J, Gagnière J, Barnich N, Billard E, et al. Microbial markers in colorectal cancer detection and/or prognosis. World Journal of Gastroenterology. 2018;24(22):2327-47.
388. Pleguezuelos-Manzano C, Puschhof J, Rosendahl Huber A, van Hoeck A, Wood HM, Nomburg J, et al. Mutational signature in colorectal cancer caused by genotoxic pks(+) E. coli. Nature. 2020;580(7802):269-73.
389. Lee SA, Liu F, Riordan SM, Lee CS, Zhang L. Global Investigations of Fusobacterium nucleatum in Human Colorectal Cancer. Frontiers in Oncology. 2019;9(566).
390. Rezasoltani S, Asadzadeh Aghdaei H, Dabiri H, Akhavan Sepahi A, Modarressi MH, Nazemalhosseini Mojarad E. The association between fecal microbiota and different types of colorectal polyp as precursors of colorectal cancer. Microbial Pathogenesis. 2018;124:244-9.
391. Bullman S, Pedamallu CS, Sicinska E, Clancy TE, Zhang X, Cai D, et al. Analysis of Fusobacterium persistence and antibiotic response in colorectal cancer. Science. 2017;358(6369):1443-8.
392. Druliner BR, Wang P, Bae T, Baheti S, Slettedahl S, Mahoney D, et al. Molecular characterization of colorectal adenomas with and without malignancy reveals distinguishing genome, transcriptome and methylome alterations. Scientific Reports. 2018;8(1):3161.
393. Chang K, Willis JA, Reumers J, Taggart MW, San Lucas FA, Thirumurthi S, et al. Colorectal premalignancy is associated with consensus molecular subtypes 1 and 2. Annals of oncology : official journal of the European Society for Medical Oncology. 2018;29(10):2061-7.
394. Johnstone MS, Lynch G, Park J, McSorley S, Edwards J. Novel Methods of Risk Stratifying Patients for Metachronous, Pre-Malignant Colorectal Polyps: A Systematic Review. Critical Reviews in Oncology/Hematology. 2021;164:103421.
395. Kirkegaard T, Edwards J, Tovey S, McGlynn LM, Krishna SN, Mukherjee R, et al. Observer variation in immunohistochemical analysis of protein expression, time for a change? Histopathology. 2006;48(7):787-94.
396. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. Journal of Chiropractic Medicine. 2016;15(2):155-63.
397. Pećina-Šlaus N, Aničić S, Bukovac A, Kafka A. Wnt Signaling Inhibitors and Their Promising Role in Tumor Treatment. International Journal of Molecular Sciences. 2023;24(7).
398. Zhang Y, Wang X. Targeting the Wnt/β-catenin signaling pathway in cancer. Journal of Hematology & Oncology. 2020;13(1):165.
399. National Institute for Health and Care Excellence. Quantitative faecal immunochemical testing to guide colorectal cancer pathway referral in primary care. DG56. 2023. [cited 30/01/2025. Available from: https://www.nice.org.uk/guidance/dg56].
400. Crooks CJ, West J, Jones J, Hamilton W, Bailey SER, Abel G, et al. COLOFIT: Development and Internal-External Validation of Models Using Age, Sex, Faecal Immunochemical and Blood Tests to Optimise Diagnosis of Colorectal Cancer in Symptomatic Patients. Alimentary Pharmacology and Therapeutics. 2025;61(5):852-64.
401. Digby J, Nobes J, Strachan JA, McCann R, Hall C, Fraser CG, et al. Combining faecal haemoglobin, iron deficiency anaemia status and age can improve colorectal cancer risk prediction in patients attending primary care with bowel symptoms: a retrospective observational study. Gut. 2025.
402. Gerrard AD, Maeda Y, Miller J, Gunn F, Theodoratou E, Noble C, et al. Double faecal immunochemical testing in patients with symptoms suspicious of colorectal cancer. British Journal of Surgery. 2023;110(4):471-80.