# Colorectal Cancer in Young Adults: Improving Identification and Management of Familial Gastrointestinal Cancer Syndromes

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# Declaration of Originality

I hereby declare that this thesis contains work that has been performed by myself under the supervision of Professor Omar Faiz and Dr Andrew Latchford. Prof Faiz provided guidance with regards to the management of the HES and NCIN database, systematic review and meta-analysis. Dr Latchford provided guidance on genetic aspect of this thesis. Both supervisors were influential in editing of individual chapters and developing the structure of this thesis.

All data processing, statistical analysis and data interpretation, production of table/figures and body of thesis were performed by myself. Mr Samuel Adegbola acted as second reviewer for the systematic review and meta-analysis. All additional collaborations have been acknowledged and referenced accordingly.

# Declaration of copyrights

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# Thesis Abstract

This thesis evaluates colorectal cancer (CRC) outcomes in young adults and explores various approaches of improving identification and management of individuals with genetic familial gastrointestinal (GI) cancer syndromes such as Lynch syndrome (LS) and familial adenomatous polyposis (FAP). Several research methodologies were utilised to address various hypothesis.

Firstly, we evaluated differences in clinicopathological features between early onset CRC (adults less than 40 years of age) and late onset CRC and the prevalence of familial gastrointestinal (GI) cancer syndromes in the young adults with CRC. This thesis demonstrated that 28% of EOCRC had hereditary GI cancer syndromes. The rectum was the most common site of CRC and EOCRC tend to present with poor histological features and advanced disease. Although young age was not an independent prognostic factor, EOCRC had worse disease-free survival.

To improve management of individuals at risk of EOCRC, this thesis explored phenotypic and genotypic factors that can be optimised to improve diagnosis, surveillance and surgical

outcomes in LS and FAP. In FAP, we demonstrated that attenuated FAP is an obsolete term due to observed phenotypic and genotypic variability. We also found that the rate of adenoma of progression in the preoperative colorectum and postoperative rectal remnant was slow (12.5 and 5.5 polyps/year respectively). Therefore, tailored endoscopic surveillance and polypectomy (rectum) are appropriate surveillance strategies. Furthermore, surgical outcomes in individuals undergoing prophylactic surgery for can be improved by ileodistal anastomosis (IDSA), a modification of the conventional ileorectal anastomosis.

Finally, this thesis demonstrates that pre-operative screening for LS using mismatch repair immunohistochemistry (MMR IHC) testing on preoperative endoscopic biopsy and metastatic tissue is feasible. In the event of LS CRC, a systematic review and meta-analysis demonstrated that extended colectomy should be considered in young individuals with higher risk MMR pathogenic variant to reduce the risk of metachronous CRC.

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# Dedication

I dedicate this work to my amazing wife (Fionnuala) and children (Eoin and Aoife). Thank you for your love, support and understanding throughout this work.

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# Publications and Presentations

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1. Safety and efficacy of laparoscopic near-total colectomy and ileo-distal sigmoid anastomosis as a modification of total colectomy and ileorectal anastomosis for prophylactic surgery in patients with adenomatous polyposis syndromes: a comparative study.

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3. Laparoscopic near-total colectomy with ileo-distal sigmoid anastomosis in patients undergoing prophylactic colectomy for polyposis syndromes - a video vignette.

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   Anele CC, Adegbola SO, Askari A, Rajendran A, Clark SK, Latchford A, Faiz OD.

   Colorectal Dis. 2017 Jun;19(6):528-536.

5. Polyp Progression in Paediatric Patients with Familial Adenomatous Polyposis - A Single Centre Experience

   Anele Chukwuemeka C, Xiang Jinpo, Martin Isabel, Hawkins Menna, Clark Susan K, Faiz Omar D, Latchford Andrew, Hyer Warren

   Journal of Pediatric Gastroenterology and Nutrition: August 10, 2020 - doi: 10.1097/MPG.0000000000002845

6. Attenuated familial adenomatous polyposis (AFAP) - a phenotypic diagnosis but obsolete term?

   Anele Chukwuemeka C, Xiang Jinpo, Martin Isabel, Hawkins Menna, Clark Susan K, Faiz Omar D, Latchford Andrew

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## Presentations (Oral and Poster)

1. Surgical outcome after near-total colectomy with ileo-distal sigmoid anastomosis (IDSA) in patients with polyposis syndromes

   Association of Surgeons of Great Britain & Ireland (ACPGBI), Birmingham 2018

2. Molecular screening for Lynch syndrome in IBD colorectal cancer

   European Crohn's and Colitis organisation (ECCO) Vienna 2018

3. Colorectal cancer outcomes and survival in young vs elderly patients: a population-based study

   C Anele, C Arhi, G Worley, P Ayling, SK Clark, A Latchford, O Faiz

4. The impact of age on the clinicopathological characteristics and prognosis of colorectal cancer: a single center retrospective study

   C Anele, A Askari, L. Navaratne, K Patel, JT Jenkins, O Faiz, A Latchford

   International Society for Gastrointestinal Hereditary Tumours (InSiGHT), Auckland 2019

5. Polyp progression in paediatric patients with familial adenomatous polyposis syndrome - a single center experience

   CC Anele, J Xiang, M Hawkins, SK Clark, O Faiz, A Latchford, W Hyer

International Society for Gastrointestinal Hereditary Tumours (InSiGHT), Auckland 2019

6. Safety and efficacy of laparoscopic near-total colectomy and ileo-distal sigmoid anastomosis (NT-IDSA) as a modification of total colectomy and ileorectal anastomosis (TC-IRA) for prophylactic surgery in patients with adenomatous polyposis syndromes – a comparative study

   CC Anele, S Nachiappan, A Sinha, I Jenkins, S.K Clark, A Latchford, O Faiz

   International Society for Gastrointestinal Hereditary Tumours (InSiGHT), Auckland 2019

7. Molecular screening for Lynch syndrome in inflammatory bowel disease related colorectal cancer

   C Anele, I Al-Bakir, D Georgiou, M Moorghen, H Thomas, SK Clark, O Faiz, A Latchford.

   International Society for Gastrointestinal Hereditary Tumours (InSiGHT), Auckland 2019

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10. Risk of metachronous colorectal cancer following colectomy in Lynch syndrome: a systematic review and meta-analysis.
    Anele CC, Adegbola SO, Askari A, Rajendran A, Clark SK, Latchford A, Faiz OD.
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# Abbreviations

| | |
|---|---|
| 5-ASA | 5-Aminosalicyclic Acid |
| 5-FU | 5-Fluoruracil |
| ACPGBI | Association of Coloproctology Of Great Britain And Ireland |
| AFAP | Attenuated Familial Adenomatous Polyposis |
| (AICR | American Institute of Cancer research |
| AJCC | American Joint Committee on Cancer |
| ALM | Adenoma -Like Mass |
| *APC* | Adenomatous Polyposis Coli |
| APR | Abdominal perineal resection |
| ASA | American Society of Anaesthesiologists |
| AZA | Azathioprine |
| BCSP | Bowel Cancer Screening Program |
| BSG | British Society of Gastroenterologist |
| CAPP | Colorectal Adenoma/Carcinoma Prevention Programme |
| CEA | Carcinoembryonic antigen |
| CI | Confidence Interval |
| CIMP | CpG Island Methylator Phenotype |
| CIN | Chromosomal Instability |
| CME | Complete Mesocolic Excision |
| CNS | Central nervous system |
| COX | Cyclooxygenase |
| CRC | Colorectal Cancer |
| CT | Computer Tomography |
| CTC | Computer Tomography Colonography |
| CTVC | Computer Tomography Virtual Colonography |
| DALM | Dysplasia Associated Lesion Mass |
| DFS | Disease Free Survival |
| dMMR | Mismatch repair deficient |
| DNA | Deoxyribonucleic Acid |
| EB | EB |
| EMR | Endoscopic Mucosal Resection |
| EOCRC | Early Onset Colorectal Cancer |
| EPCAM | Epithelial cell adhesion molecule |
| ERP | Enhanced Recovery Programme |
| ESPGHAN | European Society for Paediatric Gastroenterology Hepatology and Nutrition |

| | |
|---|---|
| EXTC | Extended Colectomy |
| FAP | Familial Adenomatous Polyposis |
| FCC | Family Cancer Clinic |
| FDR | First Degree Relative |
| FH | Family History |
| FIT | Faecal Immunochemical Test |
| FOBT | Faecal Occult Blood Test |
| gFOBT | guaiac Faecal Occult Blood Test |
| GI | Gastrointestinal |
| HES | Hospital Episode Statistics |
| HGD | High Grade dysplasia |
| HNPCC | Hereditary Non-Polyposis Colorectal Cancer |
| HRA | Health Research Authority |
| IBD | Inflammatory Bowel Disease |
| ICD | International Classification for Disease |
| ICU | Intensive Care Unit |
| IDA | Iron Deficiency anaemia |
| IDSA | Ileo-distal Sigmoid anastomosis |
| IGF | Insulin growth Factor |
| IHC | Occult Blood Test |
| IHJT | IHC Immunohistochemistry |
| IPAA | Ileo Anal Pouch Anastomosis |
| IQR | Inter Quartile range |
| IRA | Ileo-Rectal Anastomosis |
| IRR | Incidence Rate Ratio |
| LOCRC | Late Onset Colorectal Cancer |
| LS | Lynch Syndrome |
| MAP | MUTYH-associated adenomatous polyposis |
| mCRC | Metachronous Colorectal Cancer |
| MDT | Multi-Disciplinary Meeting |
| MESH | Medical Subject Headings |
| MLH | MutL Homolog |
| MLPA | Multiplex ligation-dependent probe amplification |
| MMR | Mismatch Repair |
| MMRIHC | Mismatch Repair Immunohistochemistry |
| MRI | Magnetic Resonance Imaging |
| MSH | MutS homolog |
| MSI | Microsatellite Instability |
| MSS | Microsatellite Stable |
| NCIN | National Cancer Intelligence Network |

| | |
|---|---|
| NHS | National Health Service |
| NICE | National Institute for Health and Care Excellence |
| NOS | Newcastle-Ottawa Scale |
| NSAID | Non-steroidal Anti-inflammatory Drugs |
| ONS | Office for National Statistics |
| OS | Overall survival |
| PET | Positron Emission Tomography |
| PHE | Public Health England |
| PJS | Peutz-Jegher's Syndrome |
| pMMR | Mismatch repair proficient (normal) |
| PMS | PMS1 homolog |
| PPC | Pathology Polyp Count |
| PRISMA | Preferred Reporting Items for Systematic Reviews and Meta-analyses |
| RCT | Randomised Control Trial |
| REC, | Research Ethics Committee |
| RFA, | Radio Frequency Ablation |
| RPC | Restorative Proctocolectomy |
| RR | Relative Risk |
| SD | Standard Deviation |
| SR | Surgical resection |
| SEER | Surveillance, Epidemiology, and End Results |
| SEGC | Segmental Resection |
| SEMS | Self-Expanding Metal Stents |
| SPS | Serrated Polyposis Syndrome |
| SPSS | Statistical Package for Social Sciences |
| TAMIS | Transanal Minimally Invasive Surgery |
| TCF | T-cell factor |
| TEMS | Transanal Endoscopic Microsurgery |
| TILS | Tumour-Infiltrating Lymphocyte |
| TME | Total Mesorectal Excision |
| TNF | Tumour Necrosis Factor |
| TNM | Tumour Node Metastasis |
| TPC | Total Panproctocolectomy |
| TPN | Total Parenteral Nutrition |
| UC | Ulcerative Colitis |
| UICC | Union for International Cancer Control |
| UK | United Kingdom |
| WCRF | World Cancer Research Fund |
| WHO | World Health Organization |

# 1 Chapter 1- Introduction

Colorectal cancer (CRC) is the third most common cancer and a leading cause of cancer related death worldwide [1,2]. In the United Kingdom, CRC is the fourth most common cancer and accounts for 12% of all new cancer cases [3]. It is the second most common cause of cancer related death in the United kingdom with over 15,000 CRC related deaths per year reported in the last five years [3]. Geographical variation in the patterns and incidence of CRC have been reported across the world: lower rates observed in Africa and Asia and higher rates seen in Europe and America [4–6].

## 1.1 Incidence and global trend

Approximately 1.8 million cases of CRC are diagnosed annually worldwide. Colorectal cancer is traditionally believed to be a disease of the west with more than two-thirds of all cases occurring in countries with medium to high development index (HDI) [7,8]. However, recent data suggest CRC displays a highly heterogenous incidence and mortality globally [9]. In 2012, Arnol et al [10] evaluated global trends in the incidence and mortality of CRC from 37 countries using data from Cancer Incidence in Five Continents (CI5) volumes I–X and the World Health Organisation (WHO) mortality database. They identified an overall 10-fold increase in the incidence of CRC globally. On further analysis, the result concluded that CRC incidence and mortality appeared to be rising in countries with medium to low HDI such as Philippines, Brazil, Lithuania. Whereas in countries with high HDI such as Singapore, United Kingdom, Netherlands and Italy, although incident rate continues to increase, mortality rates appeared to be decreasing. Interestingly, some countries in this group appeared to display a stable

incident rate. Finally, a decrease in CRC incidence and mortality was observed in countries with very high HDI, such as United States, Austria, New Zealand and France.

The observed changes or increase in the incidence of CRC in developing countries or countries in rapid socioeconomic transition is thought to be due to the population adopting the so called 'Western lifestyle'. These include: engaging in high risk activities such as consumption of red and processed meat, increased alcohol consumption, sedentary lifestyle, obesity and smoking. The decline in countries with high or very high HDI is believed to be due to effective CRC screening programmes which results in early detection and removal of pre-malignant lesions or polyps. Consequently, effective CRC prevention programmes requires appropriate screening of at-risk individuals and management of modifiable risk factors.

## 1.2 Risk factors for colorectal cancer

The factors associated with an increased risk of developing CRC can be divided into two: modifiable and non-modifiable. Modifiable factors such as obesity, alcohol consumption, lack of physical activity and dietary patterns have been shown to be associated with an increased risk of developing in CRC [11,12]. In a systematic review performed by the World Cancer Research Fund (WCRF) and American Institute of Cancer research (AICR), obesity, low physical activity and poor diet (high intake of red and processed meat and low fibre) were all implicated as convincing risk factor for development of CRC [13]. Non-modifiable factors include: gender, age, family history, genetics and inflammatory bowel disease (IBD).

### 1.2.1 Obesity

Obesity or excess adipose tissue has been shown to be a risk factor for developing CRC with some studies demonstrating a stronger link to colon than rectal cancer [14,15]. The exact mechanism of association is uncertain; however, some authors have suggested that excess adipose tissue results in the elevation of circulating insulin and insulin growth factor (IGF)-1. This promotes carcinogenesis by facilitating cell proliferation and inhibiting apoptosis of colonocytes [16]. Obesity is usually measured either by Body Mass Index (BMI) which represents whole-body adiposity or waist circumference (WC) which measures abdominal fat distribution. Studies have suggested that BMI and WC have independent risk factors for CRC. For instance, a 10cm increase in WC is associated with a 2% increase in risk of CRC whereas a 5kg/m2 increase in BMI is associated with and 5% in the risk of CRC [13].

Furthermore, recent evidence suggests that distinguishing between adipose tissue (AT) surrounding subcutaneous tissues (SAT) from organ or visceral fat (VAT) is a better way of assessing the association between obesity and CRC. A meta-analysis by Keum et al demonstrated that a 25cm$^2$ increase in VAT was significantly associated with a 13% increase in the formation of advanced colorectal adenomas. The association remained strongly positive after adjustment for SAT, BMI and WC [17]. The authors concluded that VAT may be the underlying mediator for increased risk of CRC. This could also explain the gender variability observed between high BMI and risk of developing CRC. Men appear to have a higher distribution of VAT compared to women. Renehan et al [15] demonstrated that 5Kg/m2

increase in BMI was associated with a 24% increase in colon cancer in men compared to 9% in women.

### 1.2.2 Physical activity

The lack of physical activity or sedentary lifestyle has been linked with an increased risk of CRC [13,18,19]. There is currently no agreed definition on the level or type of physical activity an individual should undertake to reduce the risk of developing CRC. However, WCRF recommend 5 Metabolic Equivalent of Task (MET) hours per week reduces the risk of CRC by 2% [13]. Furthermore, a study by Keum et al [20] demonstrated that the reduction in risk of CRC is more pronounced in individuals engaging in aerobic exercise compare to resistance exercise or weight lifting. Physical activity predominantly reduces risk of CRC cancer because it: (1) reduces the percentage of VAT adipose tissue [20], (2) increases gut motility, (3) improves immune system and (4) stimulates production of metabolic hormones [8,20].

### 1.2.3 Diet

'Western style' diet such as diets high in red and processed meat, sugar and refined grains are associated with an increase in the risk of CRC. The meta-analysis by WCRF suggested that an intake of 50g/day of processed meat was associated with a 16% increase in the risk of CRC with the association stronger in colon cancer (23%) compared to rectum (8%) [13]. Whereas diet high in fibre, fruit and whole grain is associated with a decreased risk of developing CRC. Similarly, Garcia- Larsen et al [21] performed a systematic review of 28 studies comparing dietary patterns (Western style diet vs prudent or healthy diet) and the risk of CRC. They

found that Western style diet was associated with a 25% increase in the risk of CRC (RR 1.25; 95% CI 1.11, 1.40) compared to a 19% reduction in the healthy diet group CRC (RR 0.81; 95% CI 0.73, 0.91). Diets rich in insoluble fibres are thought to reduce the risk of CRC because they reduce colonic transit time thereby reducing the exposure of colorectal epithelium to carcinogens in faeces [22].

### 1.2.4 Smoking

Smoking has been strongly linked with an increased risk of developing various cancers including CRC. Specifically for CRC, the increased is estimated to be in the region of 16-50% and the association is thought to be dose and time dependent [13,23,24]. Huxley et al [24] demonstrated a 16% increase risk in smokers compared to non-smokers whereas [23]. Similarly, an even higher risk of greater than 50% in heavy smokers (60 pack years) compared to non-smokers was demonstrated by Liang et al. The more comprehensive meta-analyses by Johnson et al [25] showed with minimal heterogeneity that the risk of developing CRC is directly proportional to the number of pack years: 11% increase risk for 10 pack-years, 21% greater risk for 20 pack-years and 26% for 30 pack-years when compared to non-smokers.

Tobacco smoking is also thought to display a degree of heterogeneity with regards to anatomical site of CRC. In a European study of over half a million patients, the authors found that smoking was associated with a greater risk of rectal cancer, proximal colon cancer but not distal [26]. Tobacco contains harmful chemical that induce carcinogenesis. An epigenomic study by Zeilinger et al demonstrated high levels of deoxyribonucleic acid (DNA) methylation

patterns in smokers [27]. This methylation patterns induce changes in gene expression which leads to development and progression of CRC. The authors also found evidence to suggest that these changes are reversible in individuals who quit smoking; depending on the cessation time, the level of DNA methylation found in ex-smokers were almost similar to those in individuals who had never smoked.

### 1.2.5 Alcohol

The association between alcohol consumption and the risk of CRC has been established by various studies including the WCRF and AICR [13]. In a pooled analysis of 8 prospective studies from North America and Europe, Cho et al [28] found a 16% increase in the risk of CRC (1.16 (95% CI, 0.99 to 1.36)) in individuals who consumed 30-45g of alcohol per day and a 41% increase (RR 1.41 (CI, 1.16 to 1.72)) in those who consumed ≥ 45g per day when compared to non-drinkers. Similar correlations and dose-risk association have been reported by other pooled analysis and meta-analysis [29,30]. Furthermore, the association between alcohol consumption and site of cancer across the colon and rectum there have been conflicting. Some authors have reported a strong association between alcohol intake and colon cancer [31,32] whereas others have found an increased risk for rectal cancer [33,34]. Conversely, a meta-analysis by Fedirko et al[30], found no statistical significant difference between alcohol consumption and the site of CRC.

Th exact mechanism of alcohol induced colorectal carcinogenesis is unclear. One theory is that that alcohol reduces folate metabolism which is important for DNA synthesis and

methylation. Another is the role of the acetaldehyde, a metabolite of alcohol which has been described as a carcinogen by the International Agency for Research on Cancer (IARC) [35]. Alcohol is absorbed into circulation via the gastrointestinal tract where it is then metabolised by alcohol dehydrogenase into acetaldehyde. Acetaldehyde enters the intracellular matrix and causes DNA damage and colorectal carcinogenesis [36,37].

### 1.2.6 Gender

Several studies have demonstrated gender disparity in the risk and incidence of CRC. Compared to females, males appear to have a higher risk of CRC at any age. The reason behind this observed difference in unclear, however, multiple behavioural, environmental and social factors have been proposed. For instance, men are inherently more likely to be engage in high risk lifestyle activities such as smoking, excess alcohol consumption and poor diet [15,38,39]. Similarly, some authors have suggested that men have lower awareness of cancer. They are more likely to dismiss red flag colorectal symptoms and less likely to engage in and comply with CRC screening programmes [40,41]. In a systematic review to evaluate the differences in uptake of faecal immunochemical testing (FIT) between men and women, Clarke et al found that male uptake was significantly lower than female [odds ratio (OR), 0.84; 95% confidence interval (CI), 0.75–0.95; $P < 0.01$] [40].

With regards to environmental factors, males also appear to be more susceptible than females. In a study evaluating the risk of CRC amongst immigrants to Sweden compared to native Swedes, they found that risk of CRC in men were more likely to shift towards the host

country compared to women [42]. In men who immigrated to Sweden before the age of 30, CRC cancer rates decreased for those from higher risk countries and increased for those from low risk countries [42]. Other authors have suggested the observed gender difference in CRC risk could be explained by hormonal differences between the two groups. Higher levels of endogenous hormones particularly oestrogen have been shown to be associated with a decreased risk of developing CRC [43–46].

### 1.2.7 Genetics

Although the majority of CRC cases are thought to be sporadic or non-familial, familial colorectal cancer accounts for 10-15% of all CRC [47]. Features suggestive of genetic cancer predisposition syndromes include: young onset [48], presence of other cancers in a single patient or relative [49] and an autosomal dominant pattern of inheritance. Approximately 5-6% of all CRC cases are thought to be associated with a hereditary GI syndrome [47,50]. These syndromes can be divided into two groups; polyposis and non-polyposis phenotype. Polyposis phenotype include: familial adenomatous polyposis (FAP), attenuated familial adenomatous polyposis (AFAP), MUTYH-associated polyposis (MAP). Non-polyposis phenotype includes; Lynch syndrome (LS), juvenile polyposis syndrome, PTEN hamartomatous syndrome, serrated polyposis syndrome and familial colorectal cancer type x syndrome (FCC-X). Figure 1.1 shows the fractions of colon cancer cases that arise in various family risk setting [51]. Identification of these cancer syndromes through appropriate assessment of family history and molecular

testing is clinically relevant in the management of the proband and their first-degree relatives. Lynch syndrome, FAP and MAP are discussed in more details later in this chapter.

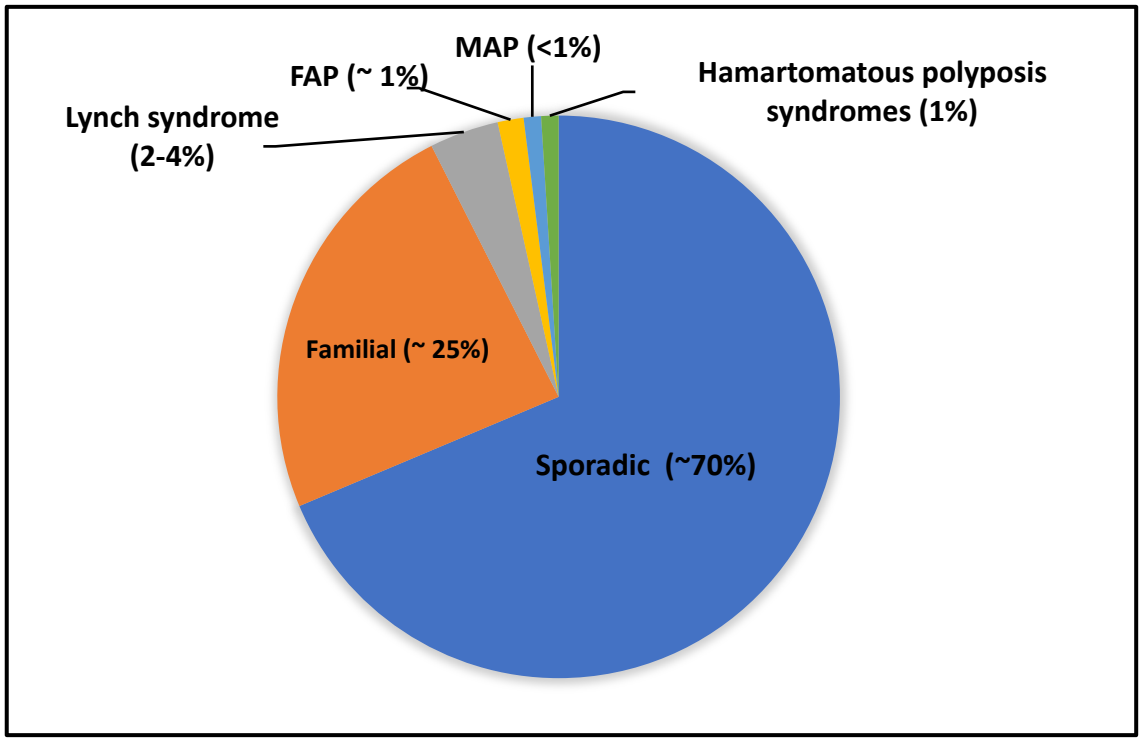



**Figure 1.1 Proportion of colorectal cancer associated with sporadic and hereditary factors**

### 1.2.8 Age

Colorectal cancer was historically thought to be a disease of older adults with over 90% of cases occurring in individuals over the age of 50 years [52,53]. In the United Kingdom, the incidence of CRC is highest in patients over the age of 75 years and between the ages of 65 and 74 in the United State of America (USA) [3,54]. The increased risk in the older age group is thought to be due to prolonged exposure to environmental carcinogens and prolonged cell proliferation results in likelihood of developing DNA replication errors or abnormalities.

In recent years, the incidence and mortality appears to declining in this age group due to uptake of bowel cancer screening programmes [55–57]. In recent years, CRC in younger population outside the screening age (termed Early onset colorectal cancer (EOCRC)) appears to be on the rise globally [52,58–62]. According to Bhandari et al [63], CRC is currently the second most common cancer among young men and women combined and a leading cause of cancer related mortality in young adults under the age of 50 in USA. Data from Surveillance, Epidemiology, and End Results (SEER) CRC registry estimates that based on current trends, by 2030, the incidence rate for colon and rectal cancer will increase by 90.0% and 124.2% for patients aged 20 to 34 years of age and by 27.7% and 46.0% for patients aged 35 to 49 years [64].

#### 1.2.8.1 *Age related differences in clinicopathological features*

Several factors behavioural, environmental and genetic factors have been postulated as contributing to the observed rise in EOCRC. They include: absence of routine screening, western-style diet and increased exposure to carcinogens [65], lack of clinician awareness resulting in dismissal of red flag colorectal symptoms in young adults [60] and an inherent reluctance of young adults to seek medical help [66] and genetic risk factors. Compared to Late onset CRC (LOCRC), management of EOCRC is thought to be distinct due to its pattern of presentation and the implications of treatment on fertility and familial link.

In addition, EOCRC tends to present at an advanced and more progressive disease. It is uncertain if this is because it is histologically a different and more rapidly progressive cancer or due to delayed progression. Published data comparing differences in clinicopathological features between EOCRC and LOCRC have reported conflicting findings. Some studies have suggested that young patients with CRC are more likely to present with poor histological and prognostic features than late onset CRC [61,62,67,68] whilst others have shown no difference between both cohorts [69–72].

The prevalence of predisposing genetic GI syndromes in EOCRC is thought to be higher than in general population. Studies suggest that familial history and genetic conditions accounts for approximately 30% of all EOCRCs [73,74]. Pearlman et al [74] found that in mutational analysis study of 450 cases of CRC under the age of 50 years, 16% had genetic mutation of which LS was the most common. In some cases of EOCRC, a genetic mutation can be identified despite the absence of a positive family history of CRC [75]. This has led to a call for further research into this topic. Using local and national databases, section I of this thesis aims to address some of the questions around EOCRC. We assess the prevalence of genetic or hereditary syndromes in individuals with EOCRC and evaluate the impact of age on clinicopathological characteristics and prognosis.

### 1.2.9 Inflammatory bowel disease

Inflammatory bowel disease (IBD) encompasses a spectrum of diseases that mainly affects the GI tract. The hallmark of which is dysregulated and uncontrolled immune mediated

inflammation of the GI tract. The aetiology of IBD is still unknown, however, susceptibility factors such as genetic, environment, gut microbiome and geographical factors have been reported [76]. Crohn's disease (CD) and ulcerative colitis (UC) are the two major types of IBD. When IBD is identified, both CD and UC may sometimes have overlapping clinical, endoscopic and histological features resulting in a diagnosis of another subtype of IBD termed indeterminate colitis. The incidence of IBD varies geographically with the highest incidence occurring in developed countries in North America and Europe. It is estimated that 2.5-3 million people are affected by IBD in Europe with current evidence suggesting an increasing trend [77].

Individuals with IBD are at increased risk of developing CRC due to the pro-neoplastic effect of chronic inflammation. The risk of CRC increases with the extent and duration of colitis and is decreased by exposure to anti-inflammatory medications such as steroids and 5-aminosalicylates [78]. In a meta-analysis of eight population-based cohort studies, Jess et al demonstrated a 2.4-fold increase in the risk of developing CRC in patients with ulcerative colitis (UC) during the first 14 years of follow-up [79]. The aim of endoscopic screening and surveillance is to identify early dysplasia and CRC and guide timing of surgery. High grade dysplasia (HGD), cancer are absolute indications for surgery in IBD [80].

#### 1.2.9.1 *Crohn's disease*

Crohn's disease (CD) is a chronic inflammatory disease than could affect the entire gastrointestinal tract from mouth to anus. It is characterised by deep transmural ulcerating

lesions which are usually discontinuous ('skip lesions') giving a cobblestone appearance. The median age of onset of CD is 30 years although a bimodal peak has been reported: highest between the ages of 20 and 30 and smaller around the age of 50 [81]. The prevalence of CD in in the United Kingdom varies from 85 to 144.8/100,000 persons [82,83].

Clinical presentation of CD is variable and largely depends on the site of the GI tract affected. They include: mouth ulcers, nausea and vomiting, diarrhoea, GI bleed, abdominal pain, malabsorption and weight loss and in severe cases small bowel strictures and fistula formation. If the lower GI tract is affected, CD can present as bloody diarrhoea, mucus per rectum and perianal abscesses and fistulas.

The diagnosis of CD is largely made on the basis of clinical, endoscopic, radiological and histological findings. Endoscopic assessment may identify areas of skip with varying degrees of inflammation. In some case of luminal CD, strictures or fistulation may be also be identified. Biopsy and histological assessment may reveal non-caseating granuloma, lymphocytic infiltration and crypt abscess. When endoscopic assessment is not possible, capsular endoscopy would also be performed. Radiological assessment using computed tomography enterography (CTE) and magnetic resonance enterography (MRE) could also be performed to assess and visualise intestinal wall and identify extraluminal complication of CD such as abscesses and fistulas.

Management of CD depends on the location and severity of the disease. Treatment is aimed at inducing and maintaining disease remission. Currently, this is achieved by used of immunosuppressive therapy such AZA (Azathioprine), 5-ASA (5-Aminosalicyclic Acid), 6-MP (6-Mercatopurine). Biological agents such as anti-tumour necrosis factor (TNF), anti-integrin, and IL-12 inhibitors are thought to be the most effective agents. Surgery is usually indicated in some situation including: bowel obstruction secondary to structuring disease, perianal fistulas or abscess and failure of medical therapy.

Crohn's disease is thought to be associated with a 2.4- 4.5 increased risk of developing CRC compared to healthy individuals [84,85]. The risk is thought to be mediated by the repeated inflammation of the bowel mucosa and is therefore highest in individuals with colonic Crohn's compared to small bowel Crohn's [84]. Generally, CD related CRC usually manifest in younger age group compared to sporadic CRC. Furthermore, the use of immunosuppressant in the management of CD has been shown to be associated with and increased risk of developing CRC [86].

#### 1.2.9.2 *Ulcerative colitis*

Compared to CD, ulcerative colitis (UC) is mainly confined to the colorectum. It is characterised by continuous mucosa inflammation which starts in rectum and extends proximally. The prevalence of UC in the United Kingdom is estimated at 243.4/100,000 [83]. Ulcerative colitis can affect individuals of any age. Individuals with UC typically present with lower GI related such as bloody diarrhoea, abdominal pain, weight loss and lethargy.

Diagnosis is made via endoscopic assessment of the colorectum which typically reveals continuous colorectal inflammation and histological assessment of colonic or rectal biopsies show evidence of chronic inflammation. Similar to CD, the goal of management of UC is to induce and maintain remission, reduce the risk of developing complications and CRC and improve quality of life. The choice of therapy is based on the severity of the disease starting with 5-ASA for mild to moderate disease and progressing to corticosteroids in moderate to severe disease or failed 5-ASA (5-Aminosalicyclic Acid) treatment. Steroid sparing agents (thiopurines and anti TNF agents) are reserved for severe disease. Surgery is indicated in patients with failed medical therapy, toxic megacolon or evidence of dysplasia or CRC. Unlike in CD, surgery in the form of subtotal colectomy or panproctocolectomy is curative in UC [87].

The association between UC and increased risk of developing CRC is more established than in CD. The cumulative risk of CRC in UC is estimated at 2% by 10 years, 8% by 20 years, and 18% by 30 years [88]. The risk of CRC is related to the extent of inflammation; patients with pancolitis have a higher risk of developing CRC. Similarly, patients with left side colitis have a higher risk compared to patients with proctitis. Colorectal cancer in UC develops via the dysplasia associated lesions or mass (DALM) or adenoma -like mass (ALM) [80].

## 1.3 Colorectal cancer carcinogenic pathways

Colorectal carcinogenesis results from 4 major stages: Initiation, Promotion, Progression and Metastasis. Initiation results from irreversible genetic alteration such as DNA alteration, deletion and simple mutations [89]. It is thought that this phenomenon occurs quite frequently

in humans, however, not all initiated cells progress to the subsequent stages or cancer. In the promotion stage, the initiated cells proliferate. This reversible stage does not involve changes in DNA but rather alterations in genomic expression resulting in abnormal tissue growth. The third irreversible stage of progression involves changes within the karyotype of cells, evolution of chromosomal abnormalities and development of malignant potential such as invasion and metastatic growth. Metastasis is defined by spread of cancer cells from primary organ to other tissues. In some cases, all four stages of carcinogenesis do not have to precede the development of CRC. In the presence of significant exposure to carcinogenic substances, the stages of initiation and promotion could be circumvented. Furthermore, it is impossible to estimate the interval between each stage of CRC carcinogenesis because it can be influenced by various environmental and genetic factors [90].

Colorectal cancer pathogenesis is characterised by genetic and epigenetic aberrancies that are acquired during life. Three distinct pathways of genomic instability have been well described in the literature. They include: (i) chromosomal instability (CIN), (ii) DNA mismatch repair (MMR) and microsatellite instability (MSI) and (iii) CpG island methylator phenotype (CIMP) and the "Serrated" Pathway.

### 1.3.1 Chromosomal instability (CIN) pathway

Chromosomal instability (CIN) is the most described CRC pathway and most common feature of neoplasm in humans. It is characterised by chromosomal abnormalities and widespread loss of heterozygosis [91]. It has been demonstrated that CRC tumorigenesis results from

mutation of DNA replication checkpoint regulators and tumour suppressor proteins [92]. Most notably is mutation in adenomatous polyposis coli (*APC*) gene located in the long (q) arm of chromosome 5 in band q22. 2 (5q22. 2). An early somatic mutation in *APC* gene is implicated in some sporadic CIN CRC whereas a germline or constitutional pathogenic variant is responsible for majority of cases of FAP.

Following the abovementioned CIN mutation, subsequent mutations are caused by activation of K-ras and inactivation of p53 genes [92,93]. The K-ras is a protooncogene that codes for GTPase protein involved in the extracellular signals. Mutation in the K-ras gene enables the cells to evade apoptosis thereby promoting uncontrolled proliferation. This facilitates adenoma growth and progression to carcinoma. The p53 gene is a tumour suppressor gene located on chromosome 17 (chromosome region 17p13). It is significantly involved in the control of cell cycle, DNA repair and apoptosis. Loss of function or inactivation of p53 usually occurs at the later stages of carcinogenesis and stimulates progression of adenoma to carcinoma [93] (Figure 1.2). Other pathways involved in CRC tumorigenesis include mutation in Smad2, Smad4, TGFBR and PIK3CA.

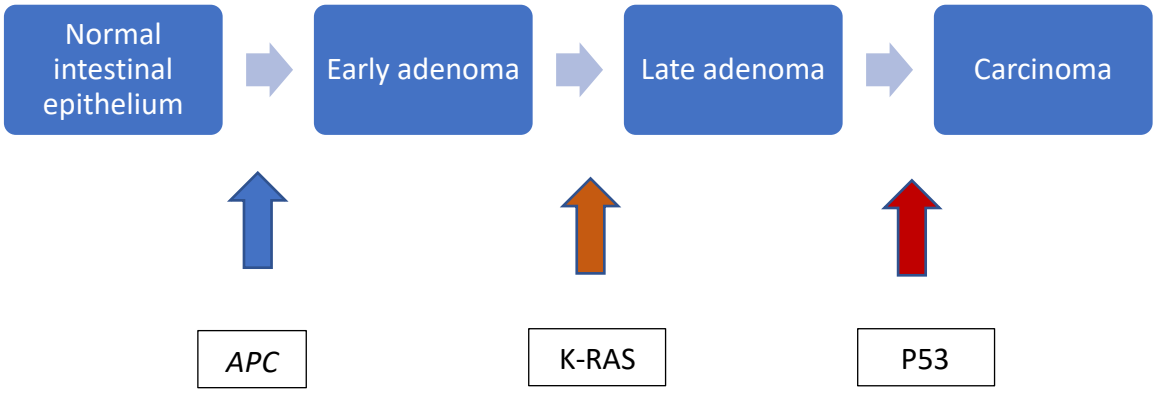



**Figure 1.2 Sequential genetic changes in colorectal tumorigenesis**

### 1.3.2 Microsatellite instability (MSI) and mismatch repair (MMR) pathway

Microsatellite instability (MSI) pathway is responsible for 15-20% of sporadic CRC and 95% of Lynch syndrome cases. It is characterised by inactivation of the DNA mismatch repair (MMR) system. During cell replication, DNA polymerase actively scans assembled nucleotides strands backwards (5'-3'direction) to identify and remove erroneous sections of replication. Unfortunately, this system is imperfect and prone to error of omission. Therefore, it is supplemented by the MMR system [94]. The MMR system is a 'spell-check' process responsible for identifying and fixing errors in the DNA replication process. A defective MMR system will leave the genome with small stretches of repetitive DNA strands containing replication errors.

These stretches of erroneous repetitive DNA strand or microsatellites are usually different from the parent cell and form the bases of microsatellite instability (MSI) [93]. In humans, there are four mismatch repair genes: MutL homologue 1 (MLH 1), MutS homologue 2 (MSH 2), MutS homologue 6 (MSH 6) and post meiotic segregation increased 2 (*PMS2*) [94].

Microsatellite instability high (MSI-H) or MMR deficient tumours are characteristically right sided, display a mucinous histology, and do not respond to certain chemotherapy agents particularly 5-Fluorouracil (5-FU) based agents [95]. They are also associated with better prognosis compared to sporadic or CIN tumours. Germline or constitutional pathogenic variant in one of the four mismatch repair genes is the hallmark of Lynch syndrome (LS); an autosomal dominant condition characterised by development of early onset CRC as well as other cancers. The risk of developing CRC is dependent of the MMR gene affected with the highest risk associated with a defective *MLH1* deficiency. Also, recent studies have shown that germline deletions of Epithelial Cell Adhesion Molecule (EpCAM) could cause LS in CRC tissues displaying *MSH2* deficiency [96].

Microsatellite instability high (MSI-H) tumours also occur in the sporadic CRC. The majority of the MMR deficient CRC occur due to epigenetic silencing of *MLH1* promoter gene expression by promotor hypermethylation. These MSI-H sporadic CRC also harbour *V600E* mutation of the BRAF oncogene which can be used to distinguish it from LS on molecular testing [97]. Microsatellite instability-H in sporadic CRC can also display features similar to CIMP pathway [92].

### 1.3.3 CpG island methylator phenotype (CIMP) and serrated pathway

CpG indicates Cytosine (C) followed by Guanine (G) which is bound by phosphodiester (p) bond. CpG island contains repeats of CG dinucleotide in a strand of DNA. These repeats usually exist in unmethylated state. In CpG island methylator phenotype (CIMP), aberrant hypermethylation of the promoter region of the gene occurs (attachment of methyl group to 5' position of cytosine) resulting in silencing of the tumour suppressor gene [93]. CIMP is found in approximately 20-30% of the CRC and be subclassified into CIMP high and CIMP low CRC [92]. CIMP-high CRC have also been shown to have BRAF oncogene mutation which is responsible for uncontrolled cell proliferation. BRAF mutation has also been implicated in molecular event in the serrated pathway.

Serrated pathway develops via sessile serrated adenoma. In this pathway, normal intestinal epithelial progress to hyperplastic polyp. This is thought to be mediated by mutation in the BRAF oncogene. CIMP then induces progression of hyperplastic polyp to sessile serrated adenoma and then to carcinoma. As a result, the majority of serrated CRC display high levels of CIMP positivity [98] (Figure 1.3).

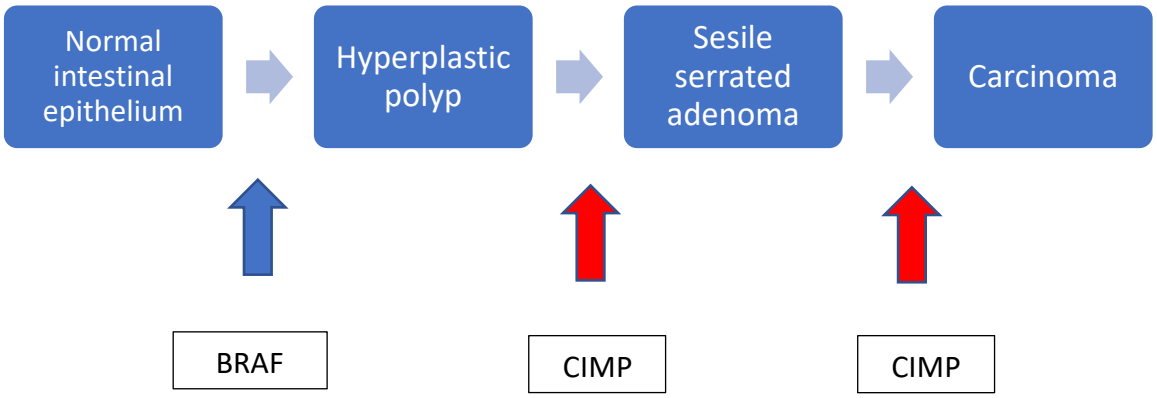



**Figure 1.3 Serrated pathway**

### 1.3.4 Inflammation pathway

Colorectal carcinogenesis from chronic inflammation or colitis usually develops from multiple flat dysplastic lesions to carcinoma. Unlike CIN tumours, the first molecular event is thought to be p53 mutation which facilities transition from normal mucosa to indefinite or low-grade dysplasia. This is then followed by *APC* mutation which leads to progression to carcinoma [78] (Figure 1.4).

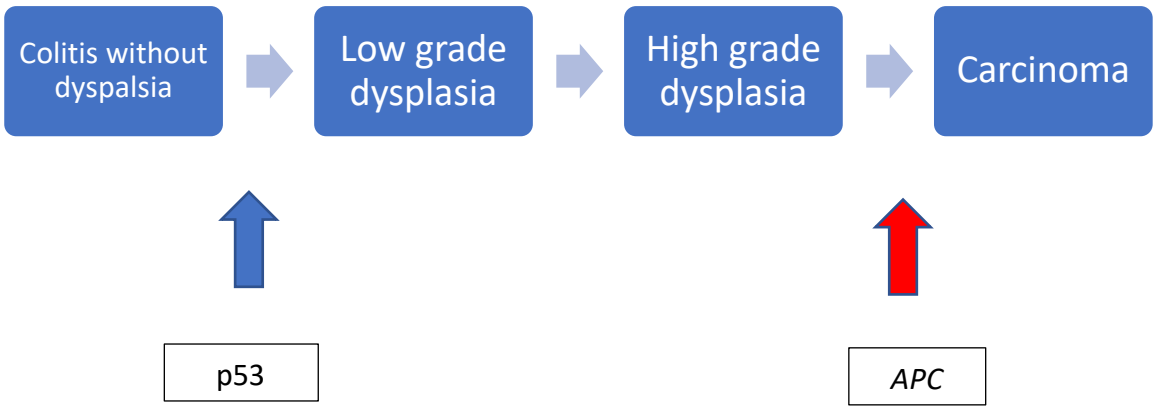



**Figure 1.4 Colitis associated colorectal cancer pathway**

## 1.4 Colorectal cancer presentation and diagnosis

### 1.4.1 Signs and symptoms

Clinical manifestations of CRC depend on the location of the tumour or cancer in the colorectum. A persistent change in bowel habit and alternating stool consistency between diarrhoea and constipation are common presenting symptoms [99]. Both right and left sided colon cancers present with occult bleeding whereas rectal lesion usually causes bright red bleeding and the feeling of incomplete evacuation. Although rectal bleeding itself is not discriminatory for rectal cancer, a combination with the other red flag colorectal symptoms should increase the index of suspicion and warrant investigation [100]. Other symptoms of CRC include: anaemia secondary to blood loss, fatigue, abdominal discomfort and unexplained weight loss. In up to 20% of patients, CRC is diagnosed at an acute phase with patients

presenting with obstructive symptoms [101]. In 25% of individuals with colon cancer and 18% of patients with rectal cancer, metastasis is present at the time of diagnosis [102].

### 1.4.2 Screening in general population

Characteristically, sporadic colorectal cancer is preceded by endoscopically detectable precancerous lesions or adenomas [103,104]. Identification and where possible removal of these lesions at an early stage could halt the malignant process. Consequently, various countries have introduced bowel cancer screening programs which have been shown to be effective in reducing the incidence of CRC and improving prognosis in patients within the screening age [55–57]. Current screening methods are designed to either detect trace amount of blood or by direct visualization of the colorectum either endoscopically or radiologically. Faecal test such as faecal occult blood test (FOBT) and faecal immunochemical test (FIT) detect faecal haemoglobin which results from abnormal increase in gastrointestinal blood loss from vascularized polyps or adenoma [105]. Several studies have demonstrated improved CRC survival with population screening using gFOBT [106,107]. A Cochrane review of randomised control trials (RCT) demonstrated that screening using FOBT resulted in a 16% reduction in the relative risk (RR) of CRC mortality (RR 0.84, 95% confidence interval [CI] 0.78-0.90) and a 25% risk reduction (RR 0.75, 95% CI 0.66-0.84) in those who participated in the screening program. In the United Kingdom, the FOBT test or FIT test is offered to individuals between the ages of 60- 74 years. Individuals over the age of 74 years can request the test every 2 years. An unexplained presence of blood in the faces will prompt further investigations in the form of direct visualization of the colorectum. Data from Bowel Cancer Screening Program

(BCSP) in England demonstrated uptake of 56% with 98% of individuals with positive test undergoing colonoscopies as their first investigation [108]. The FIT test is the newer and improved version of the gFOBT. It works by using antibodies against the globlin component of haemoglobin which is more specific to human blood and therefore does not cross-react with dietary meats [105]. The FIT test is a simpler and easier to collect and analyse because it only requires one faecal sample for analysis compared to the three required in gFOBT. Furthermore, the FIT test has been shown to have better sensitivity and specificity for detecting adenoma and CRC than gFOBT [109,110].

Direct visualization of the colorectum can be achieved by invasive (colonoscopy, flexible sigmoidoscopy, double contrast enema) or non-invasive (e.g. CT colonography or CT abdomen and pelvis) techniques. Colonoscopy is the best investigative tool for CRC diagnosis in individuals with positive FOBT or FIT test. A full colonoscopy up to the ileocaecal valve is the gold standard for investigating and diagnosing colorectal lesion. Colonoscopy is both a diagnostic and therapeutic procedure because it allows for lesion localisation and in some cases removal of precancerous adenomatous polyps or adenoma. The risk of complications such as bowel perforation following screening colonoscopies is less than 2 per 1,000 endoscopies and the risk or death is estimated at about one in 125,000 cases [105]. These risk increases with age, comorbidities and when therapeutic procedures such as polypectomies or Endoscopic Mucosal Resection (EMR) are undertaken. The uptake of screening colonoscopy is generally poor [111] because it exposes otherwise asymptomatic individuals to

unnecessary risk. Nonetheless, it is the preferred screening tool for high risk individuals with gastrointestinal genetic syndromes and Inflammatory bowel disease.

Flexible sigmoidoscopy is safer and faster alternative to colonoscopy. It is performed without sedation and does not require bowel preparation. The procedure visualizes the left side of the colon up to the splenic version where the majority of CRC occur. Although flexible sigmoidoscopy is less invasive and therefore likely to have higher uptake than colonoscopy, a completion colonoscopy is often required when cancer or high-risk adenomas are detected on flexible sigmoidoscopy. In a UK study, 5% of individuals who underwent a flexible sigmoidoscopy were referred for completion colonoscopy because high risk adenomas were detected [112]. A study by Atkin [112] et al demonstrated that once-only flexible sigmoidoscopy resulted in a 33% reduction in the incidence of CRC, 50% reduction is incidence of distal cancer and 43% reduction in CRC mortality. As a result, in 2011, the United kingdom National Screening Committee approved the addition of a single flexible sigmoidoscopy screening at age of 55 years to the BCSP [113].

In patients with poor performance status, colonoscopy might be considered inappropriate therefore other less invasive investigative radiological modalities such as such as Computer Tomography (CT) or CTVC may be warranted to investigate the colorectum. CT colonography or virtual colonoscopy (CTVC) has been shown to have an excellent sensitivity for larger polyps (lesions > 10mm in size), however, this is not the case in lesions than 6 mm [114]. Furthermore, the incidence of complications from CTC compared to colonoscopy is low and the theoretical

radiation risk associated with CTC is thought to be offset by the benefits of cancer screening and prevention [105,115]. Computer colonography also provides the opportunity to evaluate extra colonic organs and can therefore be useful in detecting metastasis or extra colonic cancers simultaneously. Although CTC or CTVC provide a safe and alternative screening technique when colonoscopy is contraindicated, they do not offer the opportunity for tissue sampling.

The appropriate investigation and timing of investigation is critical to ensure accurate diagnosis, management and surveillance of CRC. Studies suggest that improvements in diagnostic and investigative modalities have led to a 20% decrease in CRC related mortality. The National Institute for Clinical Excellence (NICE) produced guidelines on the appropriate investigative methods produced diagnostic guidelines for investigating CRC.

## 1.5 Tumour Staging

Staging of CRC is important to inform treatment planning, prognosis and surveillance. Its main function is to determine local, regional and distant spread or metastasis. This requires endoscopic, histologically and radiological assessment. Computed Tomography (CT) usually of chest, abdomen and pelvis is the most commonly used imaging modality for detection of distant metastasis and staging of CRC. Magnetic Resonance imaging (MRI) can also be used to ascertain the disease staging particularly in preoperative staging of rectal cancer. It evaluates local invasion, mesorectal fascia involvement and circumferential resection margins. Positron Emission Tomography (PET) can be used to evaluate ascertaining disease

spread. Information gathered from these investigations are combined to define an overall disease stage. The staging systems utilised in clinical practise include: Dukes staging, TNM (Tumour, Node and metastasis) and the UICC (Union for International Cancer Control) systems.

### 1.5.1 Dukes' staging

Duke staging is a CRC staging system first described in 1932 by Cuthbert Dukes a histopathologist from St Mark's hospital. It was originally published for staging of rectal cancer and did not include distant metastasis. Dukes' staging was initially based on the resection of tumour and measurement of depth of invasion within the mucosa and bowel wall (A-C). It has since been adapted and modified to include non-resectable tumour and distant metastasis.

| | | |
|---|---|---|
| **Stage** | **A** | Tumour confined to bowel mucosa |
| **Stage** | **B1** | Tumour invaded the muscular propria |
| | **B2** | Tumour invaded muscular propria and serosa (full thickness) |
| **Stage** | **C1** | Tumour spread to 1-4 regional lymph nodes |
| | **C2** | Tumour spread to >4 regional lymph nodes |
| **Stage** | **D** | Distant metastasis (liver, lung, bones) |

### 1.5.2 Tumour, Node, Metastases (TNM) staging

Duke staging has largely been replaced by TNM staging. This staging system is classified according to local invasion (T stage), lymph node involvement (N stage) and presence of distant metastasis.

**Tumour**

Tis- Carcinoma in situ

T1- Tumour invades the submucosa

T2- Tumour invades the muscularis propria

T3- Tumour invades through the muscularis propria into the peri-colorectal tissues

T4- Tumour invades the visceral peritoneum and surrounding organs or structures

**Node**

N0- No regional lymph node metastasis

N2- metastasis to 1-3 lymph nodes

N3 – metastasis to 4 or more lymph nodes

**Metastasis**

M1 - Metastasis to one or more distant sites or organs or peritoneal metastasis

### 1.5.3 Union for International Cancer Control (UICC) staging

The UICC staging is a more standardised staging system linked to the TNM staging[116]. It consists of 5 stages (0-IV) as described in Table 1.1.

**Table 1.1 Union for International Cancer Control (UICC) staging of colorectal cancer**

| UICC stage | T | N | M |
|---|---|---|---|
| **0** | Tis | N0 | M0 |
| **I** | T1 | N0 | M0 |
| **I** | T2 | N0 | M0 |
| **IIA** | T3 | N0 | M0 |
| **IIB** | T4 | N0 | M0 |
| **IIIA** | T1-T2 | N1 | M0 |
| **IIIB** | T3-T4 | N1 | M0 |
| **IIIC** | Any T | N2 | M0 |
| **IV** | Any T | Any N | M1 |

## 1.6 Management and treatment

The primary aim of management of colorectal cancer is surgical resection of the cancer or high-grade dysplastic lesion. This can be supplemented with local or systemic adjuvant or neoadjuvant treatment. In the modern era, management of patients with CRC is now achieved via a multidisciplinary approach comprising of colorectal surgeons, gastroenterologist, histopathologists, radiologist, oncologist and nurse specialist. The

decision on type of treatment depends on various factors including patients age and comorbidities, location of cancer (colon or rectum), type of CRC, stage of cancer, mode of presentation (emergency or elective) and patient's preference.

### 1.6.1 Management of colon cancer

The management of primary colon cancer without metastatic disease is achieved via segmental resection of the part of the colon containing the cancer and its surrounding mesentery. Complete mesocolic (CME) involves the ligation of the vascular pedicle (arteries and veins) associated with that region of the colon. The type of segmental resection is determined by the location of the cancer and include: right hemicolectomy, transverse colectomy, extended right hemicolectomy and left hemi colectomy. In some cases, such as in the presence of synchronous colon cancer, extensive resection in the form of subtotal or total colectomy is necessitated.

Currently, the majority of colectomy or surgery for CRC are performed via minimally invasive approach such as laparoscopic or robotic surgery. Studies have demonstrated that compared to open surgery, minimal invasive surgery is associated with less post-operative pain, better cosmesis and shorter length of stay when. Similar oncological and survival outcomes and have been reported between laparoscopic and open surgery [117–119].

Colectomy for stage I colon cancer is usually curative and adjuvant treatment or chemotherapy is not needed. For stage II disease, the evidence for adjuvant chemotherapy is

poor. There are no RCTs comparing adjuvant chemotherapy to no treatments in patients with stage II colon cancer. In a single centre study, Lin et al [120] demonstrated no significant difference in survival in adjuvant chemotherapy group compared to the non-adjuvant group in patients with stage II colon cancer. However, in the subgroup analysis of patients with high-risk factors, there was a significant 3-year disease free survival benefit (96.4% versus 84.7%, p=0.045) and 5-year overall survival benefit (100% versus 86.4%, p=0.015) in favour of adjuvant chemotherapy. As a result, some institutions recommend adjuvant chemotherapy be considered in patients with stage II colon and high risk pathological features such as pT4 tumours, poorly differentiated tumours, extramural vascular invasion and perineural invasion [121]. The decision to commence adjuvant treatment should be made on an individual bases with benefits and risk of toxicity from chemotherapy agents discussed with patients. In contrast, the benefits of adjuvant chemotherapy in patients with stage III colon cancer is well established and it is therefore recommended.

### 1.6.2 Management of rectal cancer

Treatment of rectal cancer depends on factors such stage of disease, site of tumour (distance from anal verge) and circumferential margin. In addition to CT staging, preoperative MRI and endoscopic ultrasound is performed to determine lymph node staging, mesorectal fascia involvement, circumferential resection margin, need for neoadjuvant chemoradiotherapy and suitability for endoscopic or local excision. Some stage I or early rectal cancer are amenable to local excision by means of procedures such as endoscopic submucosal dissection (ESD) or transanal endoscopic micro surgery (TEMS) or transanal minimally invasive surgery

(TAMIS) [122,123]. Although local excision is considered sufficient in management of these cancers, the risk of local recurrence and need for subsequent segmental resection remains a concern. Several factors such as polyp classification (Kikuchi, Haggit and Ueno) have been proposed to predict risk of recurrence, however there is poor evidence to support these. The most reliable predictor is the completeness of excision and resection margin. Nonetheless, these group of patients would require stringent regular endoscopic surveillance.

Surgery remains the mainstay of treatment for early rectal cancer (stage I & II) with low risk of recurrence. The type of operation depends on the location of the tumour; Anterior resection by total mesorectal excision is the accepted standard of resection for most rectal cancers. Abdominal perineal resection (APR) is reserved for low rectal cancers approaching the anal margins. In patients with moderate to high risk of local recurrence operable rectal cancers, neoadjuvant therapy such as preoperative radiotherapy or Short-Course Preoperative Radiotherapy (SCPRT) is recommended. Neoadjuvant chemoradiotherapy may also be offered to patients with locally advanced rectal cancer to allow for tumour response and shrinkage prior to surgery. Similar to colon cancer, adjuvant chemotherapy should be considered in individuals with high grade stage II and all stage III rectal cancer. The choice of chemotherapy agent depends on both patient and tumour factors. For example, although 5-fluorouracil (5-FU) is one of the most commonly used neoadjuvant chemotherapy agent in rectal cancer, it is thought to be less effective in mismatch repair (MMR) deficient tumour such as in Lynch syndrome [124,125]. As a result, some authors have recommended preoperative MMR testing prior to commencing neoadjuvant treatment.

### 1.6.3 Management of metastatic (stage IV disease) colorectal cancer

It is thought that approximately 25% of patients with colorectal cancer have metastatic disease at the time of diagnosis [102]. Historically, these patients are not considered for surgical resection, as the risk of the surgery outweighs the benefit. In recent years, the approach to management of these group of patients has evolved due to advances in systemic chemotherapy agents. The options include: resection of the primary tumour for symptomatic relief or to prevent future complications such as obstruction and perforation. This can be supplemented with adjuvant chemotherapy if metastasis is not amenable to surgery. The other approach is curative intent in which both resection of the primary cancer and metastatic deposit (liver, lungs) are undertaken either. Management of these patients is complex; although the aim of treatment is to improve the overall survival, consideration should be giving to other factors such as quality of life. Consequently, these cases are usually managed by a specialist multidisciplinary team.

In cases where the tumour is deemed inoperable either due to the metastatic nature of the disease or patient related factors which precludes surgery, the primary aim of treatment is therefore symptomatic relief. Self-expanding metal stents (SEMS) may be used for endoscopic decompression to prevent the risk of obstruction and perforation. The palliative chemotherapy and supportive care may also be beneficial in this group of patients.

## 1.7 Survival

There has been significant improvement in CRC survival in recent years. This is partly due to early detection via screening programs and better treatment. Survival is largely stage dependent with a five-year survival for stage one and two disease reported at about 83% to 64% respectively. Whereas five-year survival for stages III & IV are 38% and 20% respectively. Tumour histological characteristics also impact on prognosis of CRC. They include tumour differentiation (well, moderate and poorly), mucinous or non-mucinous and lymphocytic infiltration. For instance, it is thought that the presence of infiltrating lymphocyte in LS-related CRC confers a survival advantage when compared with stage-adjusted sporadic CRC [126,127].

## 1.8 Follow-up and Disease monitoring

Follow-up after curative treatment of correct cancer is to identify local recurrence, metachronous CRC or metastasis at an early stage to offer a chance of second curative treatment. Laubert et al [128] demonstrated improved survival with intensive surveillance after curative surgery compared to minimal or no surveillance. In their study, the 5-year survival rates were 79% (intensive), 76% (minimal) and 54% (none) (OR 1.480, (95% CI 1.135-1.929); p<0.0001). Initial follow-up in the immediate post-operative period is also recommended to assess post-operative recovery, discuss histological findings and need for adjuvant treatment. Currently in the UK, follow-up entails regular (6 monthly) serum carcinoembryonic antigen (CEA) levels, CT scan of chest abdomen and pelvis at intervals and colonoscopy at one and 5 years after surgery.

## 1.9 Hereditary gastrointestinal cancer syndromes

### 1.9.1 Familial adenomatous polyposis

Familial adenomatous polyposis (FAP) is an autosomal dominant condition that predisposes to colorectal cancer. FAP is caused by a constitutional pathogenic variant in the adenomatous polyposis coli *(APC)* gene [129]. The *APC* gene is a tumour suppressor gene located on chromosome 5q21-22 [130]. It consists of 8535 bp spanning 21 exons and encodes 2843 amino acids proteins[131]. The *APC* gene is involved in the *APC*/β-catenin/Tcf pathway and its main function is to downregulate intracellular β-catenin levels via the "Wnt" signalling pathway. The normal *APC* gene forms a protein complex with GSK-3β and axin, which binds and degrades β-catenin. Inactivation of the *APC* gene results in a failure to degrade β-catenin, which results in an increased signalling of WNT pathway. The increased cytoplasmic β-catenin translocates into the cellular nucleus where it binds to DNA binding proteins of the T-cell factor (TCF) thereby stimulating DNA transcription and increased cellular proliferation and differentiation [92,132].

A constitutional pathogenic variant in the *APC* gene has been identified in over 80% of families with FAP. Inactivation of *APC* gene occurs when both alleles are damaged. Majority of patients with FAP inherit a germline pathogenic variant in one allele usually via frameshift or nonsense mutations which leads to synthesis of a truncated protein with abnormal function [133,134]. According to Knudsen two-hit hypothesis, a further somatic *APC* mutation at the locus (loss of heterozygosity) is required for colorectal tumorigenesis to occur [133,135,136].

Patients with FAP characteristically develop hundreds to thousands of adenomatous colorectal polyps by adolescence or the third decade of life [137]. The penetrance of colonic adenomatous polyposis achieves close to 100% penetrance and progression to colorectal cancer is almost inevitable if left untreated [138]. The risk of colorectal cancer is considered to be related to the number of colorectal polyps [139]. Extracolonic manifestations such as upper gastrointestinal tract polyps, desmoid tumours, osteomas and congenital hypertrophy of retina pigment epithelium are also common in FAP. There is distinct variation in the genotype-phenotype manifestations of the disease based on the location of constitutional pathogenic variant on the *APC* gene [140].

#### 1.9.1.1 *Genotype – phenotype correlation in familial adenomatous polyposis*

Since the detection of the *APC* gene, several studies have demonstrated a correlation between the site of constitutional pathogenic variant on the *APC* gene and clinical manifestations and severity of disease [136,141]. The most common site is between codon 1250-1595 on exon 15 of the *APC* gene. This corresponds to the mutation cluster region (MCR) and is associated with severe polyposis phenotype [142]. Patients with constitutional pathogenic variant between codon 1303-1309 have been shown to have very severe phenotype (colonic polyp count of over 1000) and early onset of colorectal adenomas [143]. Constitutional pathogenic variant 5' of codon 233, 3' of 1595 and the alternative spliced region of exon 9 (codon 312-412) are associated with an attenuated phenotype with fewer number of adenomas and a later onset of adenoma development [144–148]. Figure 1.5 illustrates the *APC* gene and common genotypic-phenotypic correlation in FAP.

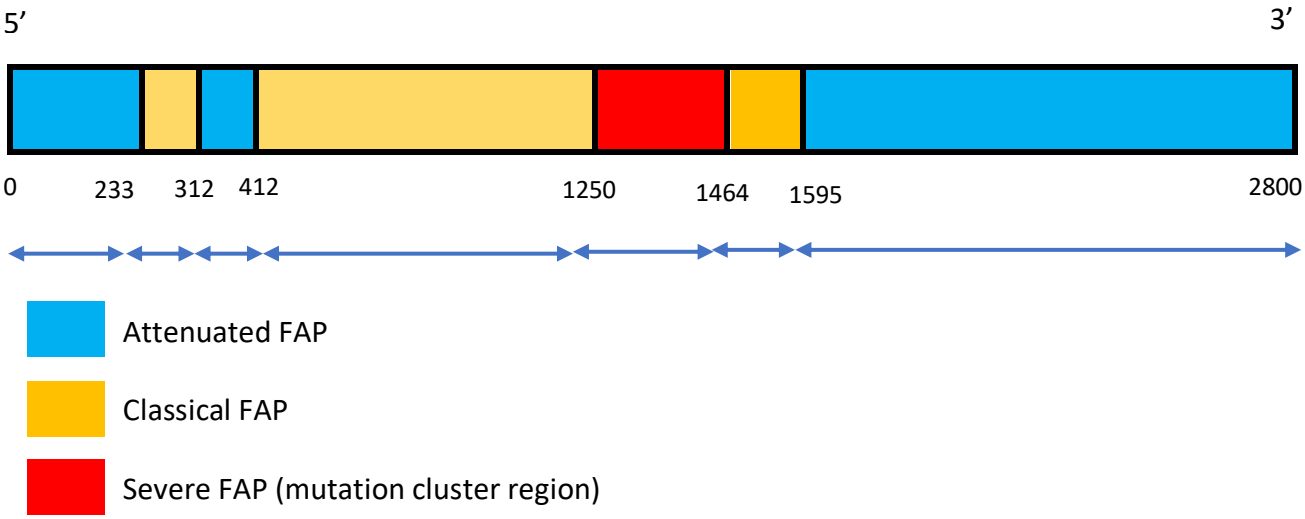



**Figure 1.5 APC genotype-Phenotype in Familial adenomatous polyposis**

### 1.9.1.2 *Attenuated Familial adenomatous polyposis (AFAP)*

Attenuated Familial adenomatous polyposis (AFAP) is a variant of FAP characterised by a milder course of disease. Features of AFAP include: less than 100 colorectal adenomas, later onset of colorectal adenomas, bowel symptoms and colorectal cancer and a milder expression of extra colonic features. Attenuated FAP has been linked to constitutional pathogenic variant 5' of codon 233, 3' of 1595 and the alternative spliced region of exon 9 of the *APC* gene [147,149]. In a study by Friedl et al, the average age of onset of colorectal symptoms in individuals with constitutional pathogenic variant in attenuated region was found to be 52 years compared to 30 years in those in the classical region of *APC* gene [150]. Similarly, a study of large kindreds with AFAP reported that the average age at CRC diagnosis was 58 years

(range 29-81) and the cumulative risk of CRC by the age of 80 years was estimated at 69% [151]. Consequently, some authors and institutions recommend delaying onset of screening and frequency of colonoscopy surveillance in these patients. However, large phenotypic variabilities have been observed amongst patients with identical constitutional pathogenic variant.

#### 1.9.1.3 *Intrafamilial + familial variability in AFAP*

Although AFAP has been linked to constitutional pathogenic variant in three regions of the *APC* gene described above, significant genotype-phenotype variations have also been reported [144,150]. Soravia et al [144] demonstrated significant variabilities in the number of colorectal adenomas in patients with mutation at the 5' region. Some individuals in their cohort exhibited colonic phenotypes similar to classical FAP. Similarly, intrafamilial phenotypic variability has also been observed in patients with constitutional pathogenic variant in alternatively spliced region of exon 9 [146,152]. The risk of CRC in individuals with pathogenic variants in the AFAP region also appears to be variable. Although the emergence of adenomas is delayed by up to 10-20 years in AFAP, several studies have reported CRC in AFAP families even in the presence of few adenomas [144,151,153] and in individuals as young as 24 years of age [154]. Furthermore, extra colonic manifestations such as duodenal adenomas have also been reported in some patients with AFAP [144,155,156]. There is currently no evidence to suggest correlation between site of constitutional pathogenic variant and presence of severity of upper GI disease [150]. Finally, constitutional pathogenic variant 3' of 1595 are also associated with an increased risk of developing desmoid disease [150,157]. It is uncertain if this

influences the timing and type of surgery. Evidently, AFAP is a poorly understood entity with some authors suggesting it is a variation of FAP rather than a separate disease. Also, the lack of precise definition of the age cut-off at which adenoma count is made to determine the presence of attenuated colonic phenotype poses a diagnostic and surveillance challenge. Chapter 5 of this thesis evaluates phenotypic and genotypic correlation in patients with presumed AFAP and also assesses familial variability.

#### 1.9.1.4 *Screening and genetic testing for FAP*

There are sparse data to suggest the number of adenomas that should prompt genetic testing. Studies have suggested using cut off of 20 colorectal adenomas as a trigger for performing genetic testing for polyposis syndrome such as FAP. In a across sectional study of 8676 unrelated individuals with multiple adenomas evaluated for the presence of pathogenic variants, 82% of individuals with >1000 polyps, 63% of individuals with 100 to 999 polyps, 17% of individuals with 20 to 99 polyps, and 9% of individuals with 10 to 19 adenomas demonstrated pathogenic variants in *APC* or *MUTYH* gene [158].

The agreed upon clinical diagnosis of FAP is the presence of >100 adenomatous polyp in the colorectum. Screening and testing for FAP requires a combination of clinical, genetic, endoscopic and histopathological assessment. An individual found to have adenomatous colonic adenomas on endoscopic assessment for lower GI symptoms such as rectal bleeding should be referred to a specialist polyposis registry and geneticist. A detailed family history is obtained and genetic testing offered if appropriate. The absence of family history of CRC or

FAP in a patient with new diagnosis of polyposis does not exclude a possible diagnosis of FAP. In a study by Aretz et al, 15% of patients with FAP had de novo pathogenic variant of [159]. A diagnosis of FAP is usually confirmed if a pathogenic variant in *APC* gene is identified at genetic testing. The index patient's first-degree relatives are then offered predictive testing. In the absence of pathogenic variant, the index patient should be treated as having a polyposis phenotype and first degree relative are offered colonoscopy instead.

In at risk individuals with known family history of FAP, the current guideline is to offer genetic testing to children at the age of 12-14 years. Colonic adenomas generally manifest in early teens and CRC before the age of 20 years is extremely rare [160]. A delay in genetic testing ensures that children are able to comprehend and consent for the test thereby reducing the impact of a genetic diagnosis on the psychological and social development of the child [161]. However, some authors have suggested there is no disadvantage to early genetic testing [162,163]. Kattentidt-Mouravieva et al reported no negative mental or physical disadvantage to parents when genetic testing was done before the age of 10 years [162]. Similarly, Michie et al found children did not show any significant distress within the first year following predictive genetic testing [163]. In some cases, earlier testing might be necessary on clinical grounds. For example, children with severe phenotype and genotype (pathogenic variant in codon 1309) may present symptomatically at a younger age [160]. Similarly, parents might with known *APC* pathogenic variant might request earlier testing to alleviate the anxiety associated with uncertainty of diagnosis and to inform the onset and frequency of colonoscopy surveillance.

Nevertheless, it is highly recommended that genetic testing should be supplemented with appropriate genetic counselling [164].

#### 1.9.1.5 *Lower gastrointestinal surveillance in FAP*

Surveillance and management of individuals with FAP have been shown to be more effective when carried out in a centralised well-established family cancer registry. A systematic review of multiple single centre studies demonstrated a reduction in the incidence and mortality of CRC in FAP patients managed in a designated registry screening program [165]. Individuals with or predicted to have FAP should undergo regular colonoscopy surveillance of the colorectum. The role of surveillance is to assess adenoma size, enumeration and distribution to help inform the timing and choice of prophylactic surgery. Studies have demonstrated better outcome when surveillance is commenced before onset of colorectal symptoms [166]. However, the age at which surveillance should commenced or whether genetic testing should precede endoscopic assessment remains contentious. Recent international guidelines recommend starting surveillance at the age of 12-14 after genetic testing has been performed [167,168]. However, early screening maybe be warranted in symptomatic patients.

Once adenoma has been identified, guidelines recommend annual surveillance colonoscopies until colectomy is performed. However, studies on the natural history of polyp progression have shown no evidence of accelerated carcinogenesis in FAP [169]. As a result, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) now recommend individualising the frequency of surveillance based on patient's colorectal

phenotype and genotype [168]. Chapter six of this thesis hopes to provide further evidence to support individualisation of surveillance protocol by assessing the rate of polyp progression in children with FAP under surveillance.

#### 1.9.1.6 *Surgery in FAP*

Colorectal cancer is inevitable in individuals with FAP unless colectomy is performed. The role of surgery is to decrease or eliminate the risk of CRC with minimal disruption to the psychological, social and educational development and quality of life of the individual. Therefore, the timing and choice of surgery is crucial. The evolution of surgery in FAP is such that the choice of surgery is determined by factors such as genotype, phenotype, adenoma enumeration, adenoma distribution, rectal adenoma count and risk of desmoid disease. The choice of surgery includes total colectomy and ileorectal anastomosis (TC-IRA) or restorative proctocolectomy (RPC) and ileo-anal pouch anastomosis (IPAA). On rare occasions, panproctocolectomy with end ileostomy is performed when mesenteric desmoid precludes RPC.

Studies have shown that the risk of rectal cancer and secondary proctectomy can be reduced by adopting a selective approach [170,171]. Sinha et al [170] analysed 427 patients with FAP who had undergone TC-IRA. They demonstrated that 50% of these patients still had their rectum at the age of 60 years. The risk of secondary proctectomy was independently associated with (i) pathogenic variant in MCR region (codon 1250 to 1464), (ii) 500 or more colonic polyps, (iii) twenty or more rectal polyps and (iv) age less than 25 years at primary surgery. Currently,

in centres that adopt the selective approach, young patients with milder phenotype and genotype are offered TC-IRA. Restorative panproctocolectomy is now reserved for individuals with severe disease. Nonetheless, some authors and institutions still advocate RPC in all cases of FAP as the risk of developing rectal cancer following TC-IRA is considered unacceptably high [172].

There are no randomised control trials comparing postoperative outcomes between TC-IRA and RPC. A meta-analysis demonstrated some merits of each procedure [173]. Total colectomy and ileorectal anastomosis is a more straightforward procedure especially when performed laparoscopically. It is associated with fewer postoperative morbidity and better bowel function and quality of life. Restorative panproctocolectomy and IPAA requires pelvic dissection and is therefore associated with risk of erectile dysfunction, reduction in female fecundity and higher peri-operative morbidity [173–175]. Although TC-IRA has been shown to have fewer post-operative complication rates compared to IPAA, the anastomotic leaks rates and subsequent reoperation rates have been problematic. In one study, the authors have reported a 11.6% 30-day re-operation rate following prophylactic TC-IRA colectomy for FAP [173]. This level of risk is considered too high for young individuals. Chapter 7 of this thesis demonstrates the outcome of a modified anastomotic technique aimed at reducing anastomotic leak rates and surgical outcomes in this cohort.

Following surgery, regular endoscopic surveillance of the rectum (TC-IRA) and pouch body and anal transition zone (RPC) is required in all patients. Some centres advocate yearly

surveillance whereas others recommend biannually. During surveillance, polyp enumeration and size are assessed. With modern advances in endoscopic techniques, polyp burden can now be managed endoscopically by means of multiple polypectomies. The role of endoscopy in management of rectum in following TC-IRA in FAP has not been described in the literature. Chapter 8 of this thesis evaluates the natural history and progression of adenomas in rectum and the role of modern endoscopic interventions in managing the rectum and reducing the risk of rectal failure.

#### 1.9.1.7 *Extra colonic manifestations of FAP*

Individuals with FAP are also at risk of developing gastric and duodenal adenoma. Studies have shown that duodenal adenomas occur in over 50% of individuals with FAP [176]. The frequency of surveillance and the severity of duodenal disease is guided by the Spigelman classification [177]. This system grades the duodenal disease based on adenoma number, size, histology and presence of dysplasia. Stage I (1-4 points) indicates mild duodenal disease and are manged with regular endoscopic surveillance every 3-5 years. Whereas stage III-IV indicate severe disease. Individuals with stage III disease undergo 1-2 yearly endoscopic examination which might include therapeutic endoscopic interventions such as snare excision, thermal ablation and argon plasma coagulation [167]. Grove et al [178] reported that individuals with stage IV disease have a 36% chance of developing invasive carcinoma within ten years. These group of patients should be identified early and referred for consideration of surgery.

Other extra colonic manifestation of FAP include desmoid disease, gall balder carcinoma, pancreatic and thyroid cancer, adrenal adenoma and hepatoblastoma.

### 1.9.2 Lynch syndrome (LS)

Lynch syndrome (LS) is a dominantly inherited cancer predisposition syndrome. It is the most common cause of inherited colorectal cancer (CRC), accounting for 2-4% of CRC cases [47,179]. It is caused by a defect in one of the MMR genes (*MLH1*, *MSH2*, *MSH6* or *PMS2* genes) or the epi-mutation in *EPCAM,* which leads to silencing of *MSH2* [180]. Germline or constitutional pathogenic variant in the MMR genes lead to the inability to repair certain DNA replication errors. The resulting effect is a predisposition to early onset of various cancers especially CRC [180,181]. The condition is characterised by a lifetime risk of CRC of between 27% and 45% by the age of 70 years [182,183]. There is also an increased risk of developing cancer in other sites including: endometrium, ovaries, stomach, brain (glioblastomas) and urinary tract [182,183].

#### 1.9.2.1 *Screening and genetic testing*

Identification of individuals with LS is imperative as it influences management of the patient and their first degree relative. In 1989, the International Collaborative Group on hereditary non-polyposis colorectal cancer (HNPCC) devised the Amsterdam criteria to identify individuals at risk of having LS. The original Amsterdam criteria was based entirely on strong family history of CRC at a young age (Table 1.2). These criteria were later revised in 1999 to include extracolonic tumours (Table 1.3). The development of MSI and MMR IHC testing led

to the development of Bethesda and subsequently revised Bethesda criteria [184] to identify those individuals who require further testing for LS (Table 1.4). Unlike the Amsterdam criteria where individuals have to fulfil all criteria, only one Bethesda criteria is required to trigger molecular testing. Individuals who fulfil the Bethesda criteria have their CRC tissue undergo molecular testing to screen for LS by either MSI studies or MMR immunohistochemistry (MMR IHC) [184]. The revised Bethesda criteria has been shown to be the most sensitive criteria in identifying mutation carriers, unfortunately it is also the least specific [184,185].

| **Table 1.2 Amsterdam I criteria** |
| --- |
| 1. At least 3 relatives with CRC<br>2. At least 1 case in a first degree relative<br>3. At least 2 successive generations affected<br>4. At least 1 tumour should be diagnosed before the age of 50 years<br>5. FAP should be excluded |

| **Table 1.3 Amsterdam II** criteria |
| --- |
| 1. At least 3 separate relatives with CRC or LS associated cancer; one relative must be first degree relative of the other two<br>2. At least two successive generation affected<br>3. At least one tumour should be diagnosed before age of 50 years<br>4. FAP excluded in all cases<br>5. Tumours pathologically verified |

| **Table 1.4 The Revised Bethesda Guidelines for testing colorectal tumours** [184] |
| --- |
| 1. Colorectal cancer diagnosed in individual < 50 years of age<br>2. Presence of synchronous, metachronous CRC, or other LS-associated tumours, regardless of age<br>3. Colorectal cancer with the MSI-H histology diagnosed in a patient < 60 years of age<br>4. Colorectal cancer or LS associated tumour diagnosed in >1 FDR, with one of the cancers being diagnosed < 50 years of age<br>5. Colorectal cancer or LS-related tumours diagnosed in two or more FDR- or SDR relatives w, regardless of age. |

The current standard for diagnosing LS is through identification of constitutional variant in 1 of the 4 MMR genes and deletion of the *EPCAM* gene via molecular testing (sequencing and multiplex ligation-dependent probe amplification (MLPA)). However, it is not feasible to perform this test on all CRC cancer. Therefore, the use of MMR IHC to detect MMR deficiency in tumours provides a screening method for identifying patients who require constitutional mutational analysis. A loss of one or more of the MMR proteins is considered MMR deficient (dMMR). A loss of staining in *MLH1* can also occur in sporadic cancer, hence further testing (BRAF V600E or *MLH1* hypermethylation) is needed. Colorectal tumours that show pathogenic variant in BRAF or methylated *MLH1* promoter are likely to be sporadic tumours. In 2017, National institute for Health and Care Excellence (NICE) recommended universal MMR IHC testing for all CRC cases in the united kingdom [186].

Mismatch repair IHC are predominantly performed on resected CRC specimen. However, in patients who undergo neoadjuvant treatment for rectal cancer with complete tumour response, the preoperative biopsy could be the only tissue available for MMR IHC testing. Furthermore, for those individuals who present with an advanced colorectal cancer, biopsy tissue may be the only confirmation of a diagnosis of cancer and the only tissue upon which MMR studies can be performed. There are scant data to evaluating the reliability of performing MMR IHC on endoscopic biopsies and non-colorectal cancer tissue (metastatic tissues). Similarly, the reliability of MMR IHC in CRC tissue following chemoradiotherapy is uncertain. The current assumption is that it is unaffected by chemoradiotherapy. Chapter 9 of this thesis evaluates the reliability of MMR IHC on non-resected CRC tissues.

#### 1.9.2.2 *Mismatch repair gene and colorectal phenotype*

Phenotypic expression in LS is variable and dependent on the mismatch repair gene with constitutional pathogenic variant. The lifetime CRC risk and age of cancer diagnosis varies depending on the MMR gene affected. Constitutional pathogenic variant in *MLH1* and *MSH2* confers the highest cancer risk and are associated with earlier onset CRC [180,183]. Individuals with *MSH6* and *PMS2* constitutional pathogenic variant have lower penetrance and display variable disease expression. Pathogenic variant in *MSH6* gene has been shown to confer lower risk of CRC but highest risk of endometrial cancer whilst individuals with pathogenic variant in *PMS2* typically develop CRC at a later age and in some cases have no history of CRC [187]. Lynch syndrome patients with *EPCAM* deletion have phenotypes similar to *MSH2* [188]. Table 1.5 summarises the CRC phenotypic variability based on MMR gene.

**Table 1.5 Mutated mismatch repair gene and cumulative colorectal cancer incidences by age of 40 years and 70 years** [189]

| MMR gene | Cumulative colorectal cancer incidence at age of 40 (years) | Cumulative colorectal cancer incidence at age of 70 (years) |
|---|---|---|
| *MLH1* | 14% | 46% |
| *MSH2* | 9% | 35% |
| *MSH6* | 0 | 20% |
| *PMS2* | 0 | 10% |

#### 1.9.2.3 *Lower gastrointestinal surveillance for Lynch syndrome*

A key feature of LS is the development of colorectal cancer via accelerated adenoma-carcinoma sequence [190,191]. Jass and Stewart proposed that adenomas in LS do not occur in large numbers, rather they develop in young patients and rapidly progress to carcinoma at a rate faster than in sporadic tumours [191]. Consequently, the purpose of lower GI endoscopic surveillance is to identify and remove these premalignant polyps or adenomas. Several retrospective and prospective studies have demonstrated a reduction in risk of LS-CRC in patients undergoing regular colonoscopy surveillance [47,166,192–196]. Jarveen et al [192] demonstrated that the risk of CRC in MMR mutation positive patients under surveillance was 18% compared to 41% in individuals not under surveillance. The authors concluded the observed difference was as a result of identification and removal of adenomas in 30% of the cohort under surveillance. In addition, some studies have demonstrated an association between frequency of colonoscopy surveillance and the magnitude of reduction of risk of CRC [194,197]. In the Vansen study, surveillance interval of 1-2 years was associated with a lower risk of developing CRC compared to 2-3 yearly [197]. Several institutions including the British Society of Gastroenterology (BSG) and the Association of Coloproctology of Great Britain and Ireland (ACPGBI) now recommend biennial colonoscopy in patients with LS [198]. Surveillance should commence at the age of 25 and continue to the age of 70-75 years until the risk of complications from colonoscopy outweighs the benefit due to co-morbidities. It is also recommended that testing and surveillance should be carried out in a regional genetic centre or institution.

Despite the accepted surveillance frequency, interval CRC continue to occur in LS patients under surveillance. This could not be entirely explained by non-compliance or quality of endoscopy. Currently, it is suspected that not all CRC in LS develop via visible adenoma precursor, rather some CRC can develop from microscopic MMR-deficient crypts which are invisible at colonoscopy [189,199]. The need for frequent surveillance and risk of interval CRC has led to some institution recommending extensive surgery in LS-CRC to reduce the risk of metachronous CRC (mCRC).

#### 1.9.2.4 *Surgery in Lynch syndrome*

Traditionally, oncological resection of sporadic CRC involves resection of the segment with CRC. In colon cancer this includes: right hemicolectomy, extended right hemicolectomy, left hemicolectomy or sigmoid colectomy. And in rectal cancer, anterior resection of abdominal perineal resection can be performed. Following primary surgery, colonoscopy surveillance is undertaken to prevent recurrence. However, in patients with LS, extended colectomy (EXTC) such as subtotal colectomy or total colectomy and Ileorectal anastomosis (TC-IRA) have been recommended due an increased risk of developing mCRC [200,201]. In the De Vos tot Nederveen Cappel study [193], the 10-year risk of developing mCRC following segmental colectomy (SC) was 16% compared to 3% following extended colectomy. Similarly, a retrospective study by Kalady et al [202] of LS patients with rectal cancer found that 15% develop metachronous colon cancer at a median of 6 years (range 3.5-16) after proctectomy. Despite this demonstrable risk of mCRC, the choice of surgery has to be balanced against the perioperative morbidity and poor functional outcomes associated with EXTC compared to SC particularly in older

patients [203,204]. Using mathematical models, some authors have demonstrated an age dependent benefit in terms of increase in life expectancy following subtotal colectomy in LS CRC [205,206]. De Vos tot Nederveen Cappel reported life expectancy gained following subtotal colectomy compared with segmental hemicolectomy at ages 27, 47, and 67 was 2.3, 1, and 0.3 years respectively [206]. Findings from these retrospective studies has led to recent guidelines recommending extended resection in some young patients with LS CRC especially when compliance with colonoscopy surveillance is problematic [207,208]. In chapter 10, we perform a systematic review and meta-analyses of published studies to assess the risk of mCRC following colectomy. In addition to reducing the risk of mCRC, hysterectomy and salpingo-oophorectomy should be considered in post-menopausal women at the time of cancer resection [208].

### 1.9.3 MUTYH (MYH)-associated polyposis (MAP)

MUTYH (MYH)-associated polyposis (MAP) is an autosomal recessively inherited polyposis syndrome caused by a biallelic mutation in MUTY gene [158]. Human MutY homologue (MUTYH) gene is a member of base-excision repair gene located on chromosome 1. It is responsible for DNA oxidative damage repair process and a deactivation of this gene result in CG-AT transversion in multiple genes [209,210]. Consequently, constitutional mutation of MUTYH gene results in increased risk of developing colorectal adenoma formation and thus CRC. The increase in CRC risk in individuals with biallelic mutation is well established, however, It is uncertain if monoallelic carriers have an increased risk of CRC compared to the general population [211,212]. Biallelic MYH mutation is thought to occur in less than 1% of the population

[211,213]. In a Finnish study of 1,042 individuals with CRC, biallelic MUTYH mutation was identified in 0.4% of the cohort [213].

#### 1.9.3.1 *Screening and Surveillance*

Individuals with MAP are thought to display phenotype similar to AFAP. MAP is generally identified in individuals with 20-99 colorectal adenoma count [143,154,214]. The polyp type is commonly adenomatous although hyperplastic and serrated polyps have been known to occur. Although some authors have suggested accelerated adenoma-carcinoma sequence in MAP [215], CRC is uncommon before the age of 30 years. The average age of MAP related CRC is thought to be around 47 years (range 29-72) and it has a preponderance for the proximal colon[215–217]. As a result, guidelines recommend adopting screening programmes similar to AFAP [208]. Biallelic MYH mutation carriers should undergo yearly colonoscopy screening commencing at the age of 18-20. Also, since both parents of affected individuals have to be heterozygous carriers, genetic testing of siblings is also recommended. Currently, there is no evidence to suggest increased surveillance in monoallelic carriers.

#### 1.9.3.2 *Surgery in MAP*

In the event of colon cancer in patients with known MAP, extensive resection in the form of TC-IRA should be offered followed by regular endoscopic surveillance of the rectum [208,218].

## 1.10 Chemoprevention

The role of chemoprevention in management of CRC has been extensively investigated in the literature. Data from a 20-year observational study demonstrated a decreased risk of developing CRC in individuals taking regular aspirin for several years [219,220]. The mechanism of action of aspirin and non-steroidal anti-inflammatory (NSAID) agent is thought to stem from their ability to inhibit cyclo-oxygenase (COX) enzyme. Cyclo-oxygenase enzyme is responsible for the production of prostaglandins and it exists in two isoforms; cyclo-oxygenase (COX 1) 1 and cyclo-oxygenase 2 (Cox 2). The Cox-1 isoform is expressed in most tissues and is responsible for gastric mucosal protection and platelet aggregation. Consequently, inhibition of COX-1 results in the classical gastrointestinal (GI) side effects (gastric ulceration and bleeding) associated with NSAID or aspirin use which includes [221]. Whereas COX-2 is expressed in tissues involved in inflammation and is upregulated in colorectal adenoma and adenocarcinomas [222]. Therefore Cox-2 specific agents such as Celecoxib and Sulidinac are thought to inhibit development of colorectal adenoma, induce apoptosis and prevent CRC without the gastrointestinal side effect associated with Cox 1 agents [223].

In FAP, several RCTs and observational studies have evaluated the role of COX-2 agents in reduction of adenoma account and size. Four randomised trials have evaluated the role of Sulindac of which three reported positive findings [224–227]. A reduction in polyp size and number was also observed with celecoxib [228]. Importantly, none of these trials demonstrated

complete regression in polyp formation, hence, these agents should be considered as substitute for colectomy.

With regards to LS and chemoprevention, the Colorectal Adenoma/Carcinoma Prevention Programme 2 (CAPP2) trial was set up to evaluate the role of aspirin in the prevention of colonic adenoma and CRC in individuals. The initial finding was ineffective, however, after a longer follow-up, 600mg of Aspirin per day was found to significantly reduce the incidence of CRC [229]. The CAPP 3 trial looking at different doses of aspirin in chemoprevention in LS is currently ongoing.

## 1.11 Hypothesis and objectives

### 1.11.1 Thesis hypothesis

This thesis set out to test the following hypothesis:

1. Early onset CRC is associated with worse histological features and poor prognosis compared to late onset CRC.

2. Attenuated familial adenomatous polyposis (AFAP) is a variable disease and can display genotypic and phenotypic variability

3. Endoscopic surveillance and prophylactic surgery in FAP can be tailored based on the individual's colorectal phenotype

4. Diagnosis and identification of individuals with LS can be optimised by performing MMR IHC on non-resected CRC tissue and risk of metachronous CRC following colectomy for LS CRC is reduced by extended colectomy

### 1.11.2 Thesis objectives

The primary objectives of this thesis are:

1. Improve diagnosis and management of early onset CRC by evaluating the prevalence of hereditary GI cancer syndromes in individuals with EOCRC and assessing age associated differences in clinicopathological features and survival

2. Optimise identification, endoscopic surveillance and surgical management of individuals with Lynch syndrome and familial adenomatous polyposis syndrome (FAP).

## 1.12 Thesis structure

This thesis has been structured into four main sections that aim to address the thesis objectives. They include:

### 1.12.1 Section I- Incidence of CRC in young patients and evaluation of age-related difference in clinicopathological features and survival.

*Chapter 3 - Colorectal cancer outcomes and survival in young vs elderly patients: population-based study:* National data from the Hospital episode Statistics (HES) and National Cancer Institute Network (NCIN) will be used to evaluate incidence of CRC young patients and evaluate age-related differences in clinicopathological features and survival

*Chapter 4: The association of age on the clinicopathological characteristics and prognosis of colorectal: UK single center retrospective study:* a prospectively maintained 10-year hospital database will be used to determine the incidence of CRC in young patients, and evaluate association between age and clinicopathological features and prognosis. This chapter also identifies the predisposing hereditary gastrointestinal syndrome (LS and FAP) in EOCRC.

### 1.12.2 Section II- Improving surveillance and management of familial adenomatous polyposis

*Chapter 5- Attenuated familial adenomatous polyposis (AFAP) - a phenotypic diagnosis but obsolete term?* Attenuated FAP is a poorly understood disease entity due to its wide genotypic and phenotypic variability. The lack of consensus on the diagnostic criteria creates a management conundrum. Most published studies have been on individual family groups or kindred. Using data from a large prospectively maintained polyposis registry, this chapter evaluates phenotypic and genotypic variabilities in individuals with presumed AFAP and also assesses familial variability.

*Chapter 6- Polyp progression in paediatric patients with familial adenomatous polyposis - a single centre experience:* Using data from prospectively maintained polyposis registry, this chapter aims to describe the natural history of polyposis in the colorectum by evaluating adenoma progression in children with FAP. It also assesses factors influencing choice and timing of prophylactic colectomy.

*Chapter 7: Safety and efficacy of laparoscopic near-total colectomy and ileo-distal sigmoid anastomosis as a modification of total colectomy and ileorectal anastomosis for prophylactic surgery in patients with adenomatous polyposis syndromes: a comparative study:* Laparoscopic total colectomy and ileorectal anastomosis (TC-IRA) is the choice of surgery in some individuals with adenomatous polyposis syndromes. However, TC-IRA is associated with high risk of anastomotic leak rate which is deemed unacceptable for prophylactic surgery in

otherwise healthy young individuals. This chapter compares surgical outcome between a modified anastomotic technique (NT-IDSA) and conventional TC-IRA.

*Chapter 8- Regular endoscopic surveillance and polypectomy is effective in managing rectal adenoma progression following colectomy and ileorectal anastomosis in patients with familial adenomatous polyposis:* This chapter evaluates adenoma progression in the rectum in patients who have undergone TC-IRA with emphasis on the role of therapeutic endoscopic procedures in the rectum. It also describes factors influencing progression to secondary proctectomy.

### 1.12.3 Section III- Improving identification and management of Lynch syndrome

Chapter 9: Concordance of Mismatch repair Immunohistochemistry (MMR IHC) between biopsy and resected CRC tissues (Laboratory study). Mismatch repair Immunohistochemistry (MMR IHC) is predominantly performed on resected CRC specimens as molecular screening for Lynch syndrome (LS). There are scant data to evaluate the reliability of performing MMR IHC on endoscopic biopsies or metastatic tissues. Also, the reliability of MMR IHC on resected rectal cancer cases that have undergone neo-adjuvant chemoradiotherapy is uncertain. This laboratory study aims to evaluate concordance of MMR IHC between non-resected tissues and match resected colorectal tissue.

*Chapter 10: Risk of metachronous colorectal cancer following colectomy in lynch syndrome: a systematic review and meta-analysis.* Several retrospective studies have reported SC is

associated with an increased risk of mCRC compared to EXTC in patients with LS-CRC. This chapter is a systematic review and meta-analysis of existing literature.

### 1.12.4 Section IV: Thesis discussion and Future work

*Chapter 11: Thesis Discussion and future work:* Each chapter contains a discussion section. In addition, chapter 11 summarises the findings of the entire thesis in relation to its aims and objectives. This chapter also describes scope for future research based on the findings and limitations of this thesis.

# 2 Chapter 2 – Methods

Various research methodologies were utilized in addressing the aim and objectives of this thesis. They include:

1. Retrospective analysis of single institution (local) databases
2. Analysis of prospectively maintained St Mark's Polyposis registry database
3. National dataset (Hospital episode statistics) and National cancer institute network (NCIN) database
4. Systematic review and meta-analysis of published literature
5. Laboratory study- mismatch immunohistochemistry of CRC tissue samples

## 2.1 Ethical Approval

All required ethical approvals from Health Research Authority (HRA) were sought for local and national studies involving patient data and laboratory studies:

- HES and NCIN data: 244473/18/LO/0948
- Local St Mark's Data: 240103; 18/YH/0287
- Attenuated Familial adenomatous polyposis- Attenuated familial adenomatous polyposis – clinical outcomes and assessment of familial variability: 253340; 18/NW/0664
- Laboratory study: 207917: 16/LO/1857

All studies in this thesis were approved by the research and development department of London Northwest University Hospital NHS trust. Data were anonymised and all research

documents and files were stored securely in NHS computers and only accessible by authorised personnel. The studies involving patient data complied with General Data Protection Regulation 2018. Ethical approval was not required for the systematic review and meta-analysis.

## 2.2 Datasets

### 2.2.1 National Cancer Intelligence Network (NCIN), Hospital episode statistics (HES) and Office of National statistics (ONS)

Trends in incidence of CRC can be studied using large population databases. A good resource of such data in England is the National Cancer Intelligence Network (NCIN) and Hospital Episode Statistics (HES). These databases contain data collated from individual trusts in England. These population-based registries provide data on patient demographics (age at diagnosis, sex, and ethnicity), tumour location, cancer numbers, tumour size, histology type, tumour grade, Tumour, Node, Metastasis (TNM) stage and number of lymph nodes evaluated. The databases are also linked with Office of National Statistic (ONS) which provides date of death for all patients. The combined data is obtained from Public Health England (PHE) and approval was sought prior to accessing data.

#### 2.2.1.1 *Diagnosis coding*

Hospital episode statistics (HES) database primarily contains coding for diagnosis and procedure. The diagnosis code is determined using the World Health Organisation (WHO) International Classification for Disease version 10 (ICD-10). For CRC, the diagnostic code

depends on the location of the cancer in the colorectum. The following diagnostic codes were used in this thesis:

C.18 Malignant neoplasm of the colon

C18.0 Caecum

C18.1: Appendix

C18.2: Ascending colon

C18.3: Hepatic flexure

C18.4: Transverse colon

C18.5: Splenic flexure

C18.6: Descending colon

C18.7: Sigmoid colon

C18.8: Overlapping lesion of colon

C18.9: Colon unspecified

C19: Malignant neoplasm of rectosigmoid junction

C20: malignant neoplasm of the rectum

All cases of appendiceal cancer and concurrent Inflammatory bowel disease diagnosis (K50-K51) were excluded from the dataset.

### 2.2.2 Local datasets

St Mark's Hospital is a high-volume tertiary centre for various colorectal conditions and familial adenomatous syndromes such as FAP, MAP and LS. Therefore, it is a brilliant resource

for collecting data for research and academic purposes. Variables such as patient demographic, mode of presentation (elective or emergency), operative procedure, histopathological data such as site, UICC stage and history of CRC predisposing inflammatory or genetic conditions can be collected. Eligible patients were identified from coding department, multidisciplinary team meetings and various genetic and family registry such as The Polyposis Registry and Family Cancer Clinic (FCC).

### 2.2.3 St Mark's Polyposis Registry

The St Marks Polyposis registry is the oldest and one of the largest polyposis registries in the world. The registry was established in 1924 by Dr Cuthbert Dukes and HJR Bussey to record the details of patients with multiple colorectal polyps most of whom had a family history. Data is collected prospectively and historically data are continually updated with new and updated information. The database initially contained information on patients with FAP, however, it was expanded to include the other adenomatous polyposis syndromes such as Peutz Jeghers syndrome (PJS), juvenile polyposis syndrome (JPS), MYH associated polyposis (MAP) and serrated polyposis syndrome (SPS). The database contains data on patient demographics, family history, gene affected, endoscopic surveillance, surgery, operative notes, histopathological data (pathology polyp count) and other relevant information. The St Mark's Polyposis registry therefore provides a rich resource for research studies on polyposis syndromes.

## 2.3 Definitions

### 2.3.1 Early onset colorectal cancer (EORC)

There is currently no consensus on the definition of "young onset" CRC. As a result, a variety of age ranges from 35 to 50 have been used in the literature. O'Connell et al [61] performed a systematic review to assess the characteristics, management and outcome of CRC in young population below the bowel cancer screening age. In this study of 6425 patients identified from 55 articles, they found 67% (n=37) of the studies defined "Early or young onset CRC" as patients under the age of 40 years. Subsequent retrospective studies have also used similar age cut-off [62,230,231]. As a result, the age cut-off of 40 years or younger was chosen to represent EOCRC in this thesis.

### 2.3.2 Estimating endoscopic and pathological adenoma count

Individuals with adenomatous polyposis syndromes registered at St Mark's Polyposis Registry undergo regular endoscopic surveillance. This is usually performed by experienced gastroenterologist or paediatric gastroenterologist. The endoscopic colorectal polyp count (EPC) is calculated by counting the number of individual polyps or adenomas on withdrawal, or if adenomas are too numerous to count individually, it is estimated as previously described by Crabtree et al [232]. Total colorectal polyp count is estimated by calculating the number of adenomas in a given area and then correcting for the total colorectal mucosal area. For consistency, similar methods were used to estimate pathology polyp count (PPC). Adenoma size was estimated relative to the size of open biopsy forceps as previously described in published literature [233,234].

### 2.3.3 Colorectal Surgery and complications

In this thesis, post-operative morbidity following colorectal surgery were graded using the Clavien-Dindo classification [235]. Clavien-Dindo is a well-recognised classification and has been validated in different surgical specialities. It consists of 5 main grades (Table 2.1)

**Table 2.1 Clavien-Dindo classification of surgical complications [235]**

| Grade | Definition |
|---|---|
| Grade I | Any deviation from the normal postoperative course without the need for pharmacological, surgical, radiological and endoscopic intervention<br><br>Allowed therapeutic regimens include: antiemetics, antipyretics, analgesia, diuretics, electrolytes and physiotherapy. Also includes wound infection opened at bedside |
| Grade II | Complications requiring pharmacological treatments other than those allowed for Grade I complications; this includes blood transfusion and total parenteral nutrition (TPN) |
| Grade III | Complications requiring surgical, endoscopic or radiological intervention |
| IIIa | Intervention not under general anaesthetic |
| IIIb | intervention under general anaesthetic |
| Grade IV | Life-threatening complications; this includes CNS complications (e.g. brain haemorrhage, ischaemic stroke, subarachnoid haemorrhage) which require intensive care, but excludes transient ischaemic attacks (TIAs) |
| IVa | Single organ dysfunction (including dialysis) |
| IVb | Multiorgan dysfunction |
| Grade V | Death of a patient |

## 2.4 Methodological and statistical support

Both supervisors overseeing this thesis are experienced researchers and have published extensively on the topics relevant to this thesis. Professor Omar Faiz has a wealth of experience in management of large national databases such as HES and NCIN and has supervised multiple thesis in this topic. Dr Andrew Latchford is a in expert on hereditary gastrointestinal cancer syndromes. Both were involved in the in development of methodology and structure of this thesis. When required, we sought help from a medical statistician for complex statistical analysis.

# SECTION I: INCIDENCE OF COLORECTAL CANCER IN YOUNG PATIENTS AND EVALUATION OF CLINICOPATHOLOGICAL DIFFERENCES AND SURVIVAL – NATIONAL AND LOCAL

## 3 Chapter 3 -Colorectal cancer outcomes and survival in young vs elderly patients: a population-based study

### 3.1 Study abstract

**Background:** Several population studies have reported an increase in the incidence of colorectal cancer (CRC) in young adults below the screening age. This group of patients are also thought to have poor histological features and prognosis. We aimed to evaluate differences in clinicopathological features and survival in EOCRC versus older LOCRC.

**Method**: All patients over the age of 18 diagnosed with CRC between 1997-2012 were identified from the National Cancer Intelligence Network (NCIN) linked with Hospital episode statistics (HES) database. Patients were stratified into three age groups: (1) 18-40 years, (2) 41-60 years and (3) > 60 years. Clinicopathological features were evaluated and compared between the groups. Overall survival (OS) curves were constructed using the Kaplan–Meier method, and multivariate analysis was performed to evaluate the independent prognostic factors.

**Results:** A total of 391,976 CRC patients were included: 5307 (1.4%) in group 1, 65,538 (16.7%) in group 2, and 321,086 (81.9%) in group 3. Rectal cancer was the most common location of cancer in all 3 groups (Group 1— 44.7%, Group 2—48.8 %, Group 3—39.7%). Young CRC patients presented with a higher incidence of poorly differentiated tumours (Group 1— 24.7 %, Group 2—16 %, Group 3—15.9 %, p= 0.0001) and more advanced (UICC stage 3&4) disease (Group 1—60.5 %, Group 2—55 %, Group 3—49 %, p = 0.001). The 5-year OS rates for patients in groups 1, 2, and 3 were 62%, 60.1%, and 41% respectively (p<0.001). Multivariate analysis revealed older age (> 40 years) was an independent predictor of poor prognosis (HR, 1.2; 95% CI, 1.13–1.27; p < 0.001) in group 2 and (HR, 2.3; 95% CI, 2.17–2.44; p < 0.001) in the group 3.

**Conclusion:** Our data suggest that although young patients aged 18-40 years with CRC present with poorer pathological features and more advanced disease, they do not have worse prognosis. The overall survival should be interpreted with caution because the population data did not exclude patients with familial gastrointestinal cancer syndrome such as Lynch syndrome.

## 3.3 Introduction

Historically , CRC was thought to be a disease that mainly affected the elderly with incidence highest in patients over the age of 65 [3,54]. Whilst the incidence of LOCRC appears to be decreasing due to bowel cancer screening programs [55–57], the proportion of EOCRC (individuals outside the screening ag)e has steadily increased [52,58–62]. Screening is not routinely performed in individuals with EOCRC, therefore diagnosis of CRC is usually made when patients present with red flag colorectal symptoms such as rectal bleeding, weight loss and in some cases as surgical emergency with obstructive symptoms or bowel perforation. Recent population studies predict a greater than 90% increase in rate of EOCRC by the year 2030 [236]. This has led to a campaign to increase clinician and patient awareness of the risk of CRC in young adults.

Several studies have suggested that young patients with CRC have different clinicopathological features and are more likely to have aggressive and rapid progressive tumours compared to LOCRC [61,62,67,68]. Others have found no difference between the two age groups [70,237]. Similar controversies exist with regards to prognosis and survival [60,238–240]. Several factors have been identified as contributing to the observed age-related differences in incidence and prognosis in published literature. They include: absence of routine screening, genetic risk, environmental and life style factors such as western-style diet and increased exposure to carcinogens [65], lack of clinician awareness leading to dismissal of red flag colorectal symptoms (change in bowel habit, per rectal bleeding or altered bowel habit) in

young adults [60]. Also, young adults are inherently less likely to seek medical help compared to older adults [66].

The majority of the published data are from single centre studies with a small sample size which are inherently prone to institutional and referral bias. Population based study from the United States using the Surveillance, Epidemiology, and End Result (SEER) database demonstrated that although young patient with CRC present with advanced disease, they achieve better overall and disease-specific survival [241]. There is sparse literature describing age-related differences in patients with CRC from the United Kingdom. This chapter aims to evaluate the incidence of EOCRC and compare age-associated differences in clinicopathological features and survival using local and national database.

## 3.4 Method

### 3.4.1 Ethical approval

Ethical approval for this study was obtained from Health Research Authority (HRA) and committee (Research ethics committee reference number 18/LO/0948) and Research and Development Department of London North West University Healthcare NHS trust.

### 3.4.2 Study population

National Cancer Registry (NCR) is a national administrative database that encompasses all hospital admissions in England (HES) of patients diagnosed with cancer. We performed a retrospective cohort study of patients over the age of 18 years diagnosed with primary

colorectal cancer between 1997 to 2012 using International Classification of Diseases for oncology (ICD-10). The HES database collects demographic information (age, sex) and clinical information such as primary tumour site, tumour histology, histology, disease stage and date of death (obtained through the Office for National Statistics (ONS) and linked directly to the database). Tumour location codes were stratified into the following groups: right colon (C18.0, C18.2–C18.4), left colon (C18.5–C18.7), large intestine NOS (C18.8–C18.9) and rectum (C19.9 and C20). Appendiceal malignancies were excluded from the analysis as they were considered distinct from CRC. Patient were also excluded if they had a diagnosis of Inflammatory bowel disease (IBD).

Early onset CRC was defined as patients diagnosed at the age of 40 or younger. The age at CRC diagnosis was stratified into three age groups: group 1 (18-40 years), Group 2 (41-60), Group 3 (>60 years old). Tumour location was stratified right colon (caecum, ascending colon, hepatic flexure, and transverse colon), the left colon (splenic flexure, descending colon, and sigmoid colon), and rectum (rectosigmoid junction and rectum). Histology type was described as adenocarcinoma (well, moderate, poorly) or unknown. Tumour grade was classified as: G1-well differentiated, G2-moderately differentiated, G2-poorly differentiated and G4-undifferentiated and stage were described according to Union for International Cancer Control – UICC stage manual. Overall survival was calculated from the time of CRC diagnosis to date of death of the patient due to any cause.

### 3.4.3 Statistical analysis

Continuous data were expressed as mean ± standard deviation or median and interquartile range. The Chi-square test was used to assess differences between the groups in terms of baseline characteristic and clinicopathological features. A *P* value of less than 0.05 was considered statistically significant. Survival curves were generated using Kaplan-Meier (KM) curve and differences between the curves were analysed using the log-rank test. Univariate and multivariable Cox proportional hazard regression models were built for analysis of each characteristic on survival. The data were summarized with hazard ratio (HR) and their 95% confidence interval (CI). A *p* value of less than 0.10 in the univariable analyses were considered statistically significant and were further evaluated in the multivariable analysis. The youngest cohort was used as reference. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) IBM version 24.0.

## 3.5 Results

### 3.5.1 Patient characteristics

During the study period, a total of 391,976 patients were diagnosed with CRC of which 177, 431 (45%) were females. The median age at CRC diagnosis was 72 (range 25-80) and rectal cancer was the most common site of cancer in the entire cohort. The demographics and characteristics are summarised in Table 3.1.

### 3.5.2 Demographical and clinicopathological differences between the age groups

There were 5307 (1.4%) in group 1, 65,538 (16.7%) in group 2, and 321,086 (81.9%) in group 3 (Table 3.1). The mean age at diagnosis in groups 1, 2, and 3 were 34.4 ± 5.2 years, 54.0 ± 5.0 years and 75.2±8.1years respectively. There were statistically significant differences in clinicopathological features between the three groups. With regards to tumour location, rectal cancer was more common in group 1 (44.7%) and group 2 (48.8%) compared to group 3 (39.7%). Individuals in Group 1 had worst histological features (poorly and undifferentiated tumours) compared to the other two groups (25.1% in group 1, 16.2% in group 2 and 16.1% in group: $P<0.001$). Group 1 were also more likely to present with advanced disease at the time of diagnosis (UICC stage III and IV) (60.5% in group 1, 40.7% in group 2 and 49% in group: P<0.001) (Table 3.2).

| Table 3.1 Demographic and clinicopathological | | |
|---|---|---|
| **Characteristics** | **Category** | **CRC patients N=391,976** |
| Age | Median (IQR) | 72 (25-80) |
| | Mean | 71.1 (±)11.8 |
| | Group 1 (18-40 years) | 5307 (1.4) |
| | Group 2 (41-60 years) | 65,538 (16.7) |
| | Group 3 (>60 years) | 321,086 (81.9) |
| Sex | Male | 214,545 (54.7) |
| | Female | 177,431 (45.3) |
| Tumour location | Right colon | 107,369 (27.4) |
| | Left colon | 99.944 (25.5) |
| | Rectum/Rectosigmoid | 145,759 (37.2) |
| | Large intestine, NOS | 38,904 (9.9) |
| Tumour differentiation | Well | 22,333 (5.7) |
| | Moderately | 224,181 (57.2) |
| | Poorly | 47, 169 (12.0) |
| | Undifferentiated | 644 (0.2) |
| | Unknown | 97,649 (24.49) |
| UICC | I | 39,670 (10.1) |
| | II | 101,253 (25.8) |
| | III | 98,815 (25.2) |
| | IV | 43,681 (11.1) |
| | Missing | 108,557 (27.7) |

**Table 3.2 Demographics and clinicopathological characteristics of colorectal cancer patients**

| Variable | | Whole cohort | Group 1<br>18-40 years | Group 2<br>41-60 years | Group 3<br>>60 years | P value |
|---|---|---|---|---|---|---|
| Cases, n (%) | | 391,976 | 5307 (1.4) | 65,583 (16.7) | 321,086 (81.9) | |
| Mean age (yr., ±SD) | | | 34.4 ± 5.2 | 54.0 (5.029) | 75.2 (±8.1) | |
| Sex | Male | 214,545 (54.7) | 2716 (51.2) | 37,894 (57.8) | 173,935 (54.7) | P<0.001 |
| | Female | 177,431 (45.3) | 2591 (48.8) | 27,689 (42.2) | 147,151 (45.3) | |
| Tumour location | Right | 107,369 | 1386 (29) | 13,776 (22.9) | 92,207 (32.0) | P<0.001 |
| | Left | 99,944 | 1257 (26.3) | 17,033 (28.3) | 81,654 (28.3) | |
| | Rectum | 145,759 | 2137 (44.7) | 29,320 (48.8) | 114,302 (39.7) | |
| | Colon unspecified | 38,904 | 527 | 5454 | 32923 | |
| Histology type | Well | 22,333 | 286 (6.9) | 3897 (7.4) | 18,150 (7.6) | P<0.001 |
| | Moderately | 224,181 | 2826 (68) | 40323 (76.4) | 181,032 (76.3) | |
| | Poorly | 47,169 | 1025 (24.7) | 8452 (16) | 37,692 (15.9) | |
| | undifferentiated | 644 | 16 (0.4) | 96 (0.2) | 532 (0.2) | |
| | Unknowns | 97,649 | 1154 | 12,815 | 83,7680 | |
| UICC Stage | 1 | 39,670 | 451 (11.2) | 6900 (13.6) | 32,319 (14.1) | P<0.001 |
| | 2 | 101,253 | 1136 (28.3) | 15775 (31.2) | 84,342 (36.9) | |
| | 3 | 98,815 | 1720 (42.9) | 19782 (39.1) | 77,313 (33.8) | |
| | 4 | 43,681 | 707 (17.6) | 8094 (16) | 34,880 (15.2) | |
| | Unknowns/ missing | 108,557 | 1293 | 15,032 | 92,232 | |

### 3.5.4 Overall survival and differences between age groups

The 5-year overall survival rates for patients in groups 1, 2, and 3 were 62%, 60.1% and 41% respectively (p<0.001). Figure 3.1 shows the Kaplan–Meier survival curves for the three groups. Univariate analysis demonstrated that age, gender, tumour location, tumour histology and UICC stage were statistically significant (p<0.05) prognostic factors for OS (Table 3.3). In multivariate Cox proportional hazard regression, most of these factors remained independent prognostic factors. Older age (> 40 years) was an independent predictor of poor prognosis: Group 2 (HR, 1.2; 95% CI, 1.13–1.27; p < 0.001) and Group 3 (HR, 2.3; 95% CI, 2.17–2.44; p < 0.001).

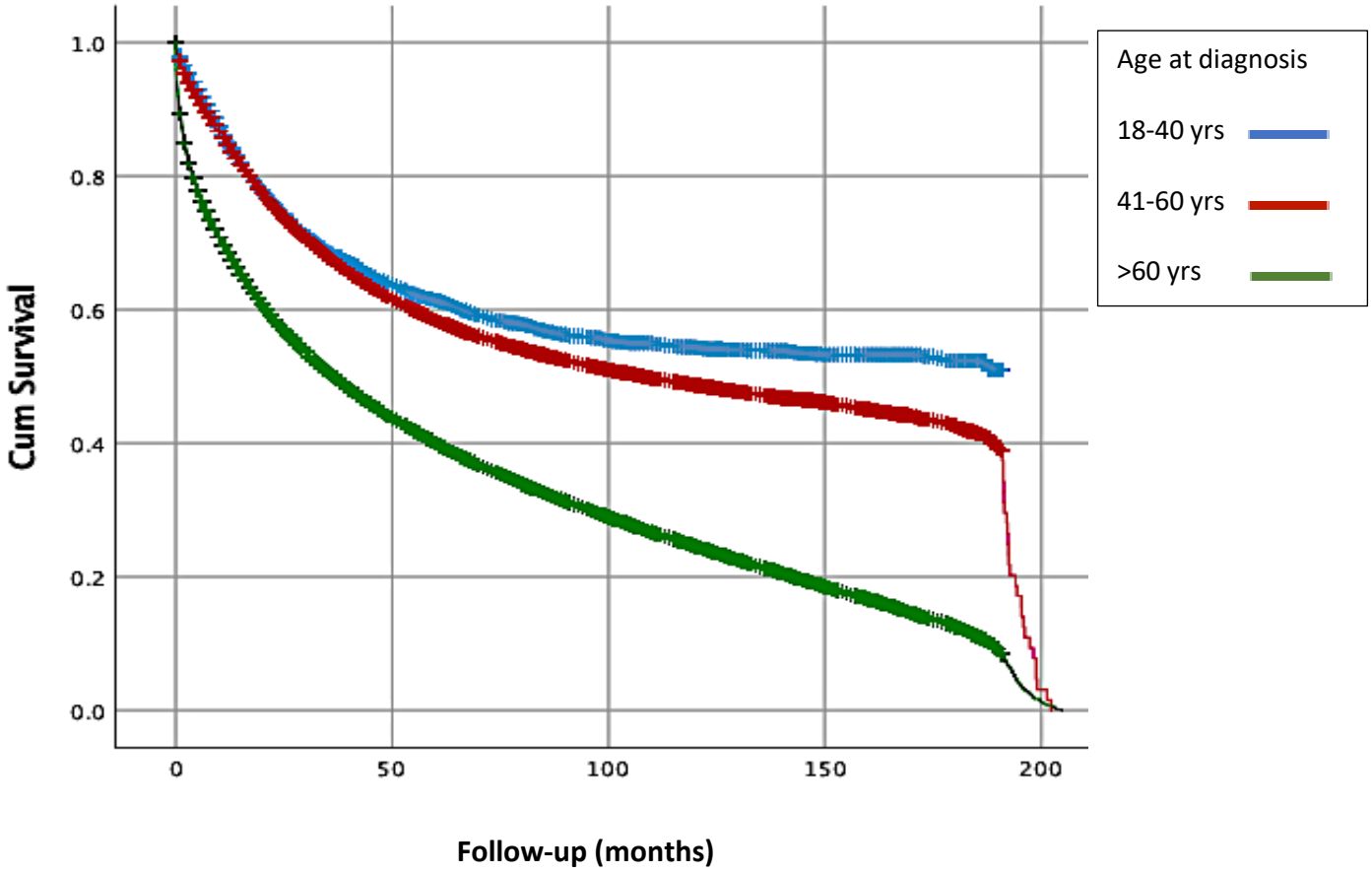



**Figure 3.1 Colorectal cancer overall survival (OS) stratified by age (Kaplan-Meier) and life table age**

**Table 3.3 Univariate and multivariate analyses (Cox proportional hazards model) of patient and factors influencing cancer-overall survival**

| Variable | Category | Univariable HR (95% CI) | p-value | Multivariable HR (95% CI) | P-value |
|---|---|---|---|---|---|
| Age | 18 – 40 | 1 | <0.001 | 1 | <0.001 |
| | 41 – 60 | 1.14 (1.09,1.19) | | 1.20 (1.13,1.27) | |
| | > 60 | 2.24 (2.14,2.34) | | 2.30 (2,17,2.44) | |
| Sex | Male | 1 | 0.007 | 1 | <0.001 |
| | Female | 0.99 (0.98-1.00) | | 0.90 (0.89,0.91) | |
| Tumour Location | Right | 1 | <0.001 | 1 | <0.001 |
| | Left | 0.83 (0.82-0.84) | | 0.91 (0.91, 93) | |
| | Rectum | 0.87 (0.86-0.877) | | 0.89 (0.88,0.90) | |
| Tumour histology | Well | 1 | <0.001 | 1 | <0.001 |
| | Moderately | 1.18 (1.16-1.21) | | 1.06 (1.04,1.09) | |
| | Poorly | 1.88 (1.84,1.92) | | 1.50 (1.46,1.54) | |
| | Undifferentiated | 2.70 (2.47-2.95) | | 1.69 (1.51,1.88) | |
| UICC stage | 1 | 1 | <0.001 | 1 | <0.001 |
| | 2 | 1.49 (1.46-1.52) | | 1.41(1.38,1.44) | |
| | 3 | 2.51 (2.46-2.56) | | 2.44(2.39,2.49) | |
| | 4 | 11.0 (10.77-11.23) | | 9.13(8.92,9.37) | |

## 3.6 Discussion

The incidence and mortality from EOCRC appears to be rising globally [58,60,64], this has led to an increase in public health campaigns to raise awareness. The reported incidence of EOCRC ranges from 0.45- 36% with an average of 6% after adjusting for outliers [61]. In this study, I evaluated age associated differences in clinicopathological characteristics and prognosis in patients with CRC. Using national population database, I found that the incidence of CRC in adults less than 40, 41-60 and > 60 years were 1.4%, 16.7% and 81.9% respectively. Furthermore, the results demonstrated that although patients aged 18-40 years with CRC are more likely to present with poorer histological features and more advanced disease, they do not have worse prognosis. The lack of consensus on the definition of "young onset CRC" makes it somewhat difficult to make meaningful comparison between different population studies. Some researchers have used various age cut-offs including: 30 years [242,243] or 45 years [244] or < 50 years [60,245]. However, the majority of studies have used less than 40 years as the preferred definition [52,75,237,239,246–248]. In the comprehensive systematic review by O'Connell et al [61], they found that 37 out of 55 (67%) studies included defined "young onset" as patients under the age of 40 years. In this study, the incident of CRC in adults less than 40, 41-60 and > 60 years were 1.4%, 16.7% and 81.9% respectively. A population study from the SEER database using the same age cut-off demonstrated similar findings. In the Wang et al study [249], the incidence of CRC in patients under that age of 40 years (EOCRC) was 2.4% and these patients were found to have better overall survival despite presenting with aggressive pathological features and more advanced disease.

Similar to the findings reported by O'Connell et al [61] and Wang et al [241], CRC was more common in males across all three groups. The reason for this observed gender difference is uncertain, however, some researchers have suggested that men are more likely to engage in high risk activities (smoking and alcohol consumption) and are less complaint with surveillance [15,39]. In terms of tumour location, rectal cancer was the most common site of CRC in all three groups. In this study, over 45% of individuals in group 1 and 2 presented with rectal cancer compared to 38% in group 3 (Table 3.2). Left sided tumours accounted for 70% of all the CRC cases in the patients under the age of 60 years (group 1 and 2). These findings are consistent with previously published studies [61,64,249,250]. Some authors believe that this predilection for left side could give insight into the aetiology and behaviour of EOCRC. For instance, although evidence for this is poor, left-sided cancers (distal colon and rectum) are thought to be associated with high intake of so-called westernized diet which contains high proportion of alcohol, red and processed meat and low levels of fruit and vegetables [34,251,252].

With regards to histopathological features, this population study demonstrates that patients under the age of 40 years old were significantly more likely to present with poor histological features such as poorly and undifferentiated tumours and advanced disease compared to the other two groups (stage III and IV) $P<0.001$. This is consistent with findings from other studies [61,241]. Despite the observed poor prognostic features, individuals in group 1 were found to have the best overall survival (Figure 3.1). The univariate and multivariate analysis further demonstrated that increasing age was a negative prognostic factor for overall survival. These findings can be explained by the fact that young patients are more likely to have fewer

comorbidities whereas individuals in group 3 are more likely to die from other causes related to old age such as cardiopulmonary disease and frailty. Cancer related mortality or disease-free survival would be a better assessment of prognosis ,however, this data was not available from the NCIN and HES database. Chapter 4 of this thesis assesses disease free-survival between the three groups.

## 3.7 Study limitations

Although this is a population base study which therefore reduces a degree of institutional bias, I acknowledge several limitations. Firstly, the limitations associated with use of administrative data such as HES and NCR database has been well documented. Some major flaws include: the lack of control over how data is coded, the accuracy of coding and paucity of data particularly with respect to tumour staging which would have undoubtedly affected our survival analysis. In addition, although individuals with IBD were excluded, the data included patients with hereditary gastrointestinal cancer syndromes such as FAP and LS. It is currently estimated that approximately 30% of individuals with EOCRC have a positive family history or history of hereditary conditions with LS being the most common [73,74]. This would have overestimated the incidence of CRC particularly in group 1. Furthermore, there is evidence to suggest that individuals with MSI or MMR deficient CRC as seen in LS have better prognosis when compared stage for stage with sporadic CRC [253]. Inclusion of these patients would have affected our survival analysis. Finally, this data did not include all the important histological and anatomical features such as lymphovascular invasion, history of synchronous

or metachronous tumours and treatment modalities such as a neoadjuvant and adjuvant treatment.

## 3.8 Conclusion

In conclusion, our data suggest that although young patients aged 18-40 years with CRC present with poorer pathological features and more advanced disease, they do not have worse prognosis. The overall survival should be interpreted with caution because the population data did not evaluate important cofounding factors such as the presence of known familial gastrointestinal cancer syndrome and cancer therapy such as adjuvant and neo adjuvant treatment

# 4 Chapter 4: The association of age on the clinicopathological characteristics and prognosis of colorectal: UK single center retrospective study

## 4.1 Study abstract

**Introduction**: Colorectal cancer (CRC) in patients under 40 years old is uncommon and its association with high frequency of hereditary gastrointestinal syndromes and poor histological features. This study aimed to determine the frequency of hereditary gastrointestinal syndromes in individuals with early onset CRC (EOCRC) and evaluate age related differences in clinicopathological features and prognosis in patients diagnosed with CRC.

**Method**: A single center retrospective review of all patients diagnosed with CRC between 2004 and 2013 was performed. Patients were stratified into three age groups: (1) 18-40 years, (2) 41-60 years and (3) > 60 years. Clinicopathological characteristics and outcomes were compared between the three groups.

**Results:** Overall, 1,328 patients were included of which 57.2% were male. Of the 56 patients initially identified with EOCRC, 16 (29%) had hereditary gastrointestinal syndrome. There were 28 (2.1%) patients in group 1, 287 (21.6%) in group 2 and 1,013 (76.3%) in group 3. Group 1 had the highest proportion of rectal tumours (57.1% in group 1, 50.2% in group 2 and 31.9% in group 3; $p<0.001$). Tumour histology and disease stage were comparable between the groups. Group 1 had significantly worse disease-free survival (DFS) compared to the older two groups (44%, 78%, 77% $p=0.022$). Multivariate analysis demonstrated that age was not an independent prognostic factor whereas stage 3 disease (HR 4.42; 95% confidence interval

(CI): 2.81-6.94, p<0.001) and neo-adjuvant chemotherapy (HR 1.65; 95% CI: 1.06-2.58, p=0.026) were associated with increased risk of recurrence.

**Conclusion:** Hereditary gastrointestinal syndromes account for 28% of individuals with EOCRC. Patients under 40 years old are more likely to present with rectal cancer and have comparable histological features compared to the older groups. Despite higher rates of adjuvant and neo-adjuvant treatment, the young group were found to have worse disease-free survival.

## 4.3 Introduction

The recent increase in the incidence of EOCRC worldwide is thought to be due to modifiable risk factors (environmental and diet), however, the impact of genetic predisposing conditions cannot be ignored. The hallmark of familial GI cancer syndromes is development of cancer at a young age. The prevalence of familial or hereditary GI cancer syndromes in the general population is thought to be in the region of 2-5% [50], however, a higher frequency has been reported in individuals with EOCRC [48,48,253–255]. In the Pearlman study, of the 450 patients with CRC under the age of 50 years, genetic mutation was identified in 16% of cases and the majority had LS [74].

There have been demonstrable clinicopathological and survival differences between hereditary and sporadic CRC. For instance, individuals with known hereditary cancer syndromes are more likely to be diagnosed earlier due to active surveillance measures. Individuals with LS are more likely to present with right sided CRC compared to sporadic which has a preponderance for the left colon and rectum. Furthermore, LS related-CRC is thought to have better prognosis compared to sporadic and some cancer therapeutics agents appear to be less effective in LS [256,257]. In chapter 3, we could not exclude individuals with these genetic syndromes because there are currently no ICD codes for these conditions. This is likely to have overestimated the true incidence of sporadic EOCRC and also lead to bias in survival analysis due to their inherently different tumour biology. In this chapter, we sought to evaluate the frequency of hereditary gastrointestinal syndromes in individuals with EOCRC

and further evaluate the age associated differences in clinicopathological features and survival in individuals with sporadic CRC.

## 4.4 Methods

### 4.4.1 Ethical approval

Ethical approval for this study was obtained from Health Research Authority (HRA) and committee (Research ethics committee reference number 240103; 18/YH/0287) and Research and Development Department of London North West University Healthcare NHS trust.

### 4.4.2 Study population

We performed a retrospective review of all patients diagnosed with CRC at our institution between 2004 and 2013. The inclusion criteria include: (1) patients over the age of 18 years (2) patients with histologically confirmed CRC (3) Patients who underwent operation including palliative surgery for primary CRC tumour. Patients with inflammatory bowel disease (IBD), recurrent CRC, genetic predispositions (e.g. familial adenomatous polyposis (FAP), MutYH associated polyposis (MAP), Lynch syndrome (LS)) and serrated polyposis and patients referred from other institutions with complex recurrent CRC were identified and excluded from the analysis. Data were collected from a variety of sources including: electronic and medical records, endoscopic, radiological and histopathology reports. Demographic data collected included: gender, age at diagnosis, mode of presentation (emergency or elective) and history of inflammatory bowel disease. Oncological and surgical data collected include:

location of CRC, tumour stage (TNM and Union for International Cancer Control – UICC [116]) and tumour differentiation. Similar to Chapter 3, tumour locations were stratified into 3 groups: right (caecum, ascending colon, hepatic flexure and transverse colon), left (splenic flexure, descending and sigmoid colon) and rectum. Histology type was described as adenocarcinoma (well, moderate, poorly) or mucinous.

Similar to the national study, we stratified the patient cohort into three age groups: (1) 18-40 years, (2) 41-60 years and (3) > 60 years. Overall survival was calculated from the time of CRC diagnosis to date of death of the patient due to any cause. Clinic follow-up or date of investigations were used to derive censoring date for overall survival (OS). Disease free survival (DFS) was estimated for patients with stage 1 to 3 disease. Recurrence was defined as local or distant metastases occurring after curative resection as proven by CT, Positron Emission Tomography (PET), MRI or colonoscopy.

### 4.4.3 Statistical analysis

Patient demographics, clinicopathological characteristics and genetic variables were analysed. Descriptive statistics such as mean ± standard deviation or median interquartile range were used for continuous variables and numbers and frequencies for categorical variables. The Chi-square test was used to assess differences between the groups. A *p* value of less than 0.05 was considered statistically significant. Survival probability was estimated using Kaplan-Meier (KM) method and differences between the curves were analysed using the log-rank test. Uni and multivariable Cox proportional hazard regression models were built

for analysis of each characteristic on survival. The data were summarized with hazard ratio (HR) and their 95% confidence interval (CI). *P* values of less than 0.10 in the univariable analyses were considered statistically significant and were further evaluated in the multivariable analysis. The youngest cohort was used as reference. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) IBM version 24.0.

## 4.5 Results

### 4.5.1 Patient characteristics

A total of 1,474 patients were diagnosed with CRC at our institution during the study period. We excluded patients with: recurrent colorectal cancer or complex cancers referred from other institutions (n=17), IBD related CRC (n=79), LS (n= 23), FAP (n=17), MAP (n=7), serrated polyposis (n=8), juvenile polyposis syndrome (n=1) and Cowden's syndrome (n=1). Of the 56 patients initially identified in the less than 40 years group or EOCRC, 16 (28%) had predisposing genetic GI syndromes and are described in Table 4.1. Overall, 1,328 patients met our inclusion criteria of which 57.2% were male and the median age at CRC diagnosis was 70 [IQR 61-78] years. There were 28 (2.1%) patients in group 1, 287 (21.6%) in group 2 and 1013 (76.3%) in group 3. The median age at CRC diagnosis in the three groups were 35 [IQR 32-38] years, 55 [IQR 50-58] years and 74 [IQR 69-80] years respectively. There was no statistically significant difference in terms of gender distribution between the three groups (p=0.796). Patient demographics are described in Table 4.2.

| Table 4.1 Prevalence of hereditary gastrointestinal syndrome in the Early onset colorectal cancer | |
|---|---|
| Hereditary syndrome | Number (%) |
| LS | 7 |
| FAP | 7 |
| MAP | 2 |

LS Lynch syndrome; FAP Familial adenomatous polyposis, MAP MutYH polyposis

### 4.5.2 Clinicopathological characteristics

In total, 97.8% of the tumours were adenocarcinoma and the rectum was the most common site of tumour occurrence (36.4%). Tumour location was significantly different between the three groups, with the 18-40 years group having a higher proportion of rectal tumours than the other two groups (57.1% in group 1, 50.2% in group 2 and 31.9% in group 3; *P* <0.001). The 18-40 years' group were more likely to present with stage III or IV disease (67.8% in group 1, 46% in group 2 and 46.2% in group: *P*=0.213) although this was not statistically significant. Patients in group 1 were more likely to receive adjuvant chemotherapy compared to the other two groups (59.3% in group 1, 39.8% in group 2, 25.4% in group 3, *P*<0.001). Similar findings were observed in terms of neo-adjuvant chemoradiotherapy (Table 4.2**).**

**Table 4.2 Demographics and clinicopathological characteristics of colorectal cancer patients**

| Variable | | Whole cohort | Group 1 18-40 years | Group 2 41-60 years | Group 3 >60 years | P value |
|---|---|---|---|---|---|---|
| Cases, n (%) | | 1328 | 28 (2.1) | 287 (21.6) | 1013 (76.3) | |
| Age (y, median IQR) | | 70 (61-78) | 35 (32-38) | 55 (50-58) | 74 (69-80) | |
| Sex | Male | 760 (57.2) | 17 (60.7) | 169 (58.9) | 574 (56.7) | 0.743 |
| | Female | 568 (42.8) | 11 (39.3) | 118 (41.1) | 439 (43.3) | |
| Tumour location | Right | 465 (35.0) | 4 (14.3) | 63 (22.0) | 398 (39.3%) | <0.001 |
| | Left | 380 (28.6) | 8 (28.6) | 80 (29.7) | 292 (28.8) | |
| | Rectum | 483 (36.4) | 16 (57.1) | 144 (50.2) | 323 (31.9) | |
| Histology type | Well | 38 (2.9) | 0 (0) | 9 (3.2) | 39 (2.9) | 0.058 |
| | Moderately | 1063 (82.1) | 21 (75) | 235 (84.2) | 807 (81.7) | |
| | Poorly | 166 (12.8) | 4 (14.3) | 31 (11.1) | 131 (13.3) | |
| | Mucinous | 28 (2.2) | 3 (10.7) | 4 (1.4) | 21 (2.1) | |
| | Missing | 33 | 0 | 8 | 25 | |
| T | 1 | 143 (10.8) | 1 (3.6) | 43 (15.0) | 99 (9.8) | 0.062 |
| | 2 | 206 (15.5) | 6 (21.4) | 39 (13.6) | 161 (15.9) | |
| | 3 | 674 (50.8) | 14 (50.0) | 152 (53.0) | 508 (50.1) | |
| | 4 | 305 (23.0) | 7 (25.0) | 53 (18.5) | 245 (24.2) | |
| N | 0 | 741 (55.8) | 9 (32.1) | 162 (56.4) | 570 (56.3) | 0.036 |
| | 1 | 324 (24.4) | 8 (28.6) | 63 (22.0) | 253 (25.0) | |
| | 2 | 263 (19.8) | 11 (39.3) | 62 (21.6) | 190 (18.7) | |
| M | 0 | 1161 (87.4) | 25 (89.3) | 249 (86.8) | 887 (87.6) | 0.895 |
| | 1 | 167 (12.6) | 3 (10.7) | 38 (13.2) | 126 (12.4) | |
| UICC Stage | 1 | 280 (21.1) | 5 (17.9) | 65 (22.6) | 210 (20.7) | 0.213 |
| | 2 | 429 (32.3) | 4 (14.3) | 90 (31.4) | 335 (33.1) | |
| | 3 | 452 (34.0) | 16 (57.1) | 94 (32.8) | 342 (33.8) | |
| | 4 | 167 (12.6) | 3 (10.7) | 38 (13.2) | 126 (12.4) | |
| Resection margin | R0 | 1311 (98.7) | 26 (92.9) | 284 (99.0) | 1001 (98.8) | 0.042 |
| | R1/R2 | 17 (1.3) | 2 (7.1) | 3 (1.0) | 12 (1.2) | |
| Mode of admission | Elective | 1117 (84.3) | 25 (89.3) | 253 (88.2) | 839 (82.8) | 0.070 |
| | Emergency | 211 (15.7) | 3 (10.3) | 34 (11.8) | 174 (17.2) | |
| Neoadjuvant chemoradiotherapy | Yes | 104 (7.8) | 6 (21.4) | 51 (18.0) | 47 (4.7) | <0.001 |
| | No | 1214 (92.2) | 22 (78.6) | 232 (82.0) | 960 (93.3) | |
| | Unknown | 10 | 0 | 4 | 6 | |
| Adjuvant chemotherapy | Yes | 367 (29.2) | 16 (59.3) | 106 (39.8) | 245 (25.4) | <0.001 |
| | No | 892 (70.8) | 11 (40.7) | 160 (60.2) | 721 (74.6) | |
| | Unknown | 69 | 1 | 21 | 47 | |

### 4.5.3 Adjuvant and neoadjuvant therapy stratified by age

Chemotherapy use in UICC stage II and III decreased with increasing age. Patients with stage II disease over the age of 60 years received chemotherapy in only 11.7% of cases compared to 30.7% in the 41-60 age-group and 50.0% in the <40 years group (*P*<0.001). Similar results were observed in stage III disease (Table 4.3**).** Neoadjuvant treatment for rectal cancer stage III was higher in the younger age groups (45.5% vs 43.8 vs 11.6% *P*<0.001;Table 4.3).

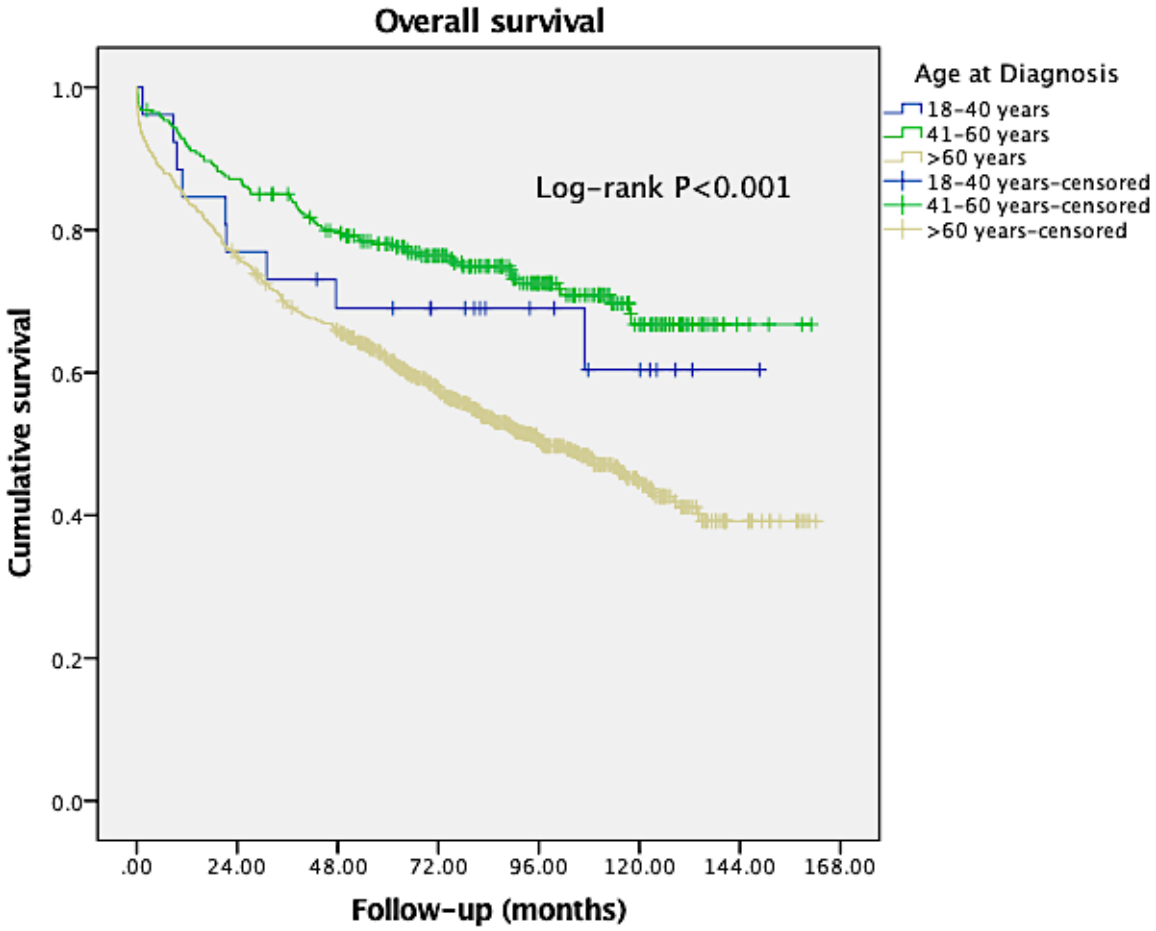
**Table 4.3 Chemotherapy and neoadjuvant therapy stratified by age**

| | 18-40 years | 41-60 years | >60 years | *P* value |
|---|---|---|---|---|
| Chemotherapy, % | | | | |
| Stage II | 50 | 30.7 | 11.7 | <0.001 |
| Stage III | 80 | 69.8 | 44.9 | <0.001 |
| | | | | |
| Neoadjuvant treatment, % | | | | |
| Stage II | 0 | 38.5 | 13.4 | 0.005 |
| Stage III | 45.5 | 34.8 | 11.6 | <0.001 |

### 4.5.4 Overall survival

The median follow-up was 71 (IQR 30-97) months. The >60-year group had significantly worse 3-year overall survival (OS) than the 18-40-year and 41-60-year groups (73%, 81%, 68% respectively; *P*<0.001). This remained true for 5-year overall survival (69%, 77%, 60% *P*<0.001). Figure 4.1 shows the Kaplan–Meier survival curves for the three groups. Univariate analysis demonstrated that tumour location, tumour histology, UICC stage, resection margin and mode of admission were statistically significant (p<0.05) prognostic factors for OS (Table 4.3). Right sided tumours, poorly differentiated tumours UICC stage 2-4, R1 and R2 resection

margins and emergency admission were all associated with poor OS (p<0.05). In the multivariable analysis, these factors with the exception of tumour location and differentiation remained independent prognostic factors for OS in (Table 4.4).




| Year | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| **18-40** | 26 | 22 | 20 | 19 | 17 | 9 |
| **41-60** | 280 | 257 | 242 | 233 | 209 | 107 |
| **>60** | 999 | 841 | 758 | 687 | 625 | 335 |

**Numbers at risk**

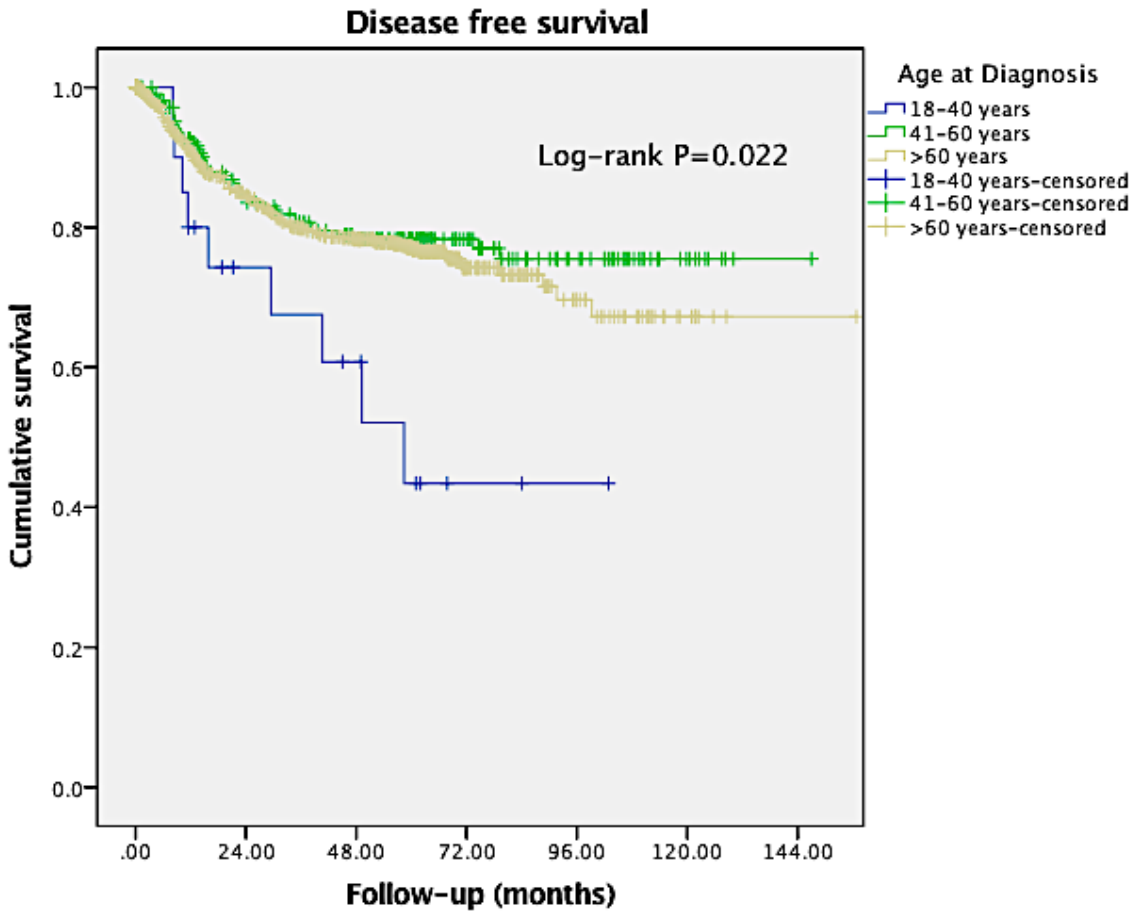
**Figure 4.1 Colorectal cancer overall survival (OS) stratified by age (Kaplan-Meier) and life table age**

**Table 4.4 Univariate and multivariate analyses (Cox proportional hazards model) of patient and factors influencing overall survival**

| Variable | Category | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| | | HR (95% CI) | P-value | HR (95% CI) | P-value |
| Age | 18 – 40 | 1 | | 1 | |
| | 41 – 60 | 0.75 (0.38, 1.49) | 0.412 | 0.80 (0.40, 1.61) | 0.535 |
| | > 60 | 1.57 (0.81, 3.03) | 0.182 | 1.68 (0.86, 3.27) | 0.128 |
| Gender | Male | 1 | | - | |
| | Female | 1.00 (0.85, 1.19) | 0.934 | | |
| Tumour Location | Right | 1 | | 1 | |
| | Left | 0.78 (0.64, 0.96) | 0.017 | 0.96 (0.78-1.19) | 0.962 |
| | Rectum | 0.75 (0.62, 0.91) | 0.004 | 1.09 (0.88-1.34) | 0.431 |
| Tumour histology | Well | 1 | | 1 | |
| | Moderately | 1.24 (0.71, 2.14) | 0.453 | 1.00 (0.57, 1.75) | 0.990 |
| | Poorly | 2.33 (1.31, 4.15) | 0.004 | 1.48 (0.82, 2.67) | 0.195 |
| | Mucinous | 2.56 (0.97, 6.73) | 0.058 | 1.31 (0.49, 3.47) | 0.588 |
| UICC stage | 1 | 1 | | 1 | |
| | 2 | 1.48 (1.11, 1.97) | 0.007 | 1.28 (0.95, 1.71) | 0.107 |
| | 3 | 2.24 (1.71, 2.94) | <0.001 | 1.96 (1.48, 2.60) | <0.001 |
| | 4 | 7.07 (5.28, 9.46) | <0.001 | 5.21 (3.81, 7.13) | <0.001 |
| Resection margin | R0 | 1 | | 1 | |
| | R1/R2 | 3.84 (2.25, 6.53) | <0.001 | 2.06 (1.20, 3.55) | 0.009 |
| Mode of admission | Elective | 1 | | 1 | |
| | Emergency | 2.75 (2.28, 3.31) | <0.001 | 1.91 (1.57, 2.32) | <0.001 |
| Neoadjuvant Chemo | No | 1 | | - | |
| | Yes | 0.96 (0.71, 1.31) | 0.807 | | |
| Adjuvant Chemo | No | 1 | | - | |
| | Yes | 0.89 (0.74, 1.08) | 0.230 | | |

### 4.5.5 Disease-free survival

Overall, 15.8% developed a local or distant recurrence. The 18-40-year-old group had significantly worse disease-free survival (DFS) compared to the older two groups (61%, 80%, 79% *P*=0.022). Similar findings were observed in 5-year cumulative DFS (44%, 78%, 77% *P*=0.022) (Figure 4.2). The univariate Cox proportional regression analysis demonstrated that age, tumour histology, UICC stage, mode of admission, neo-adjuvant chemotherapy and adjuvant chemotherapy were all significantly associated with disease recurrence. Whilst age was noted to be significant in the univariate analysis, this was not the case in the multivariate analysis. Whereas disease stage and neoadjuvant therapy remained significant ($p<0.05$) (Table 4.5)**.** The multivariate results demonstrated that patients with UICC stage 3 disease had over four times more risk of recurrence at any time compared to stage 1 (HR 4.42; 95% confidence interval (CI): 2.81-6.94) p=0.001). Individuals receiving neoadjuvant chemotherapy had a 65% higher risk of recurrence (HR 1.65; 95% confidence interval (CI): 1.06-2.58) p=0.026) (Table 4.5).




| Year | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| **18-40** | 23 | 13.5 | 11 | 10 | 8 | 3 |
| **41-60** | 238 | 175 | 149 | 135 | 110 | 47 |
| **>60** | 791 | 594 | 143 | 143 | 348 | 143 |

**Numbers at risk**

**Figure 4.2 Disease free survival (DFS) stratified by age (Kaplan-Meier)**

**Table 4.5 Univariate and multivariate analyses (Cox proportional hazards model) of patient and factors influencing disease free survival (DFS)**

| Variable | Category | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| | | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value |
| Age | 18 – 40 | 1 | | 1 | |
| | 41 – 60 | 0.38 (0.18, 0.77) | 0.008 | 0.55 (0.26,1.19) | 0.131 |
| | > 60 | 0.43 (0.22, 0.84) | 0.014 | 0.65 (0.31,1.35) | 0.248 |
| Gender | Male | 1 | | - | |
| | Female | 1.01 (0.77, 1.33) | 0.956 | | |
| Tumour Location | Right | 1 | | - | |
| | Left | 0.88 (0.62, 1.25) | 0.465 | | |
| | Rectum | 1.01 (0.73, 1.38) | 0.966 | | |
| Tumour histology | Well | 1 | | 1 | |
| | Moderately | 2.64 (0.65, 10.6) | 0.173 | 1.50 (0.37-6.15) | 0.572 |
| | Poorly | 5.08 (1.22, 21.1) | 0.025 | 2.38 (0.56-10.13) | 0.239 |
| | Mucinous | 1.78 (0.16, 19.7) | 0.637 | 0.98 (0.09-10.77) | 0.976 |
| UICC stage | 1 | 1 | | 1 | |
| | 2 | 1.43 (0.86, 2.38) | 0.164 | 1.34 (0.81, 2.23) | 0.257 |
| | 3 | 4.78 (3.05, 7.50) | <0.001 | 4.42 (2.81, 6.94) | <0.001 |
| Resection margin | R0 | 1 | | 1 | |
| | R1/R2 | 2.91 (0.93, 9.11) | 0.066 | 0.93 (0.22-3.84) | 0.916 |
| Mode of admission | Elective | 1 | | 1 | |
| | Emergency | 1.60 (1.09, 2.35) | 0.017 | 1.37 (0.91-2.07) | 0.13 |
| Neoadjuvant Chemo | No | 1 | | 1 | |
| | Yes | 1.75 (1.15, 2.66) | 0.009 | 1.65 (1.06, 2.58) | 0.026 |
| Adjuvant Chemo | No | 1 | | 1 | |
| | Yes | 2.32 (1.76, 3.05) | <0.001 | 1.29 (0.94-1.76) | 0.115 |

Data are expressed as hazard ratio (HR) with confidence interval (CI) in parentheses

## 4.6 Discussion

The incidence of EOCRC was slightly higher (2.1%) in this study compared to the national study (chapter 3). Similar to the findings in the previous chapter, patients under the age of 40 years are more likely to present with rectal cancer. This results also demonstrated that young age is not an independent prognostic factor for overall and disease-free survival. Age-related differences in clinicopathological characteristics and prognosis in patients with CRC have been described in several institutional studies from the United States and Asia. However, disparities in the patient cohort makes accurate comparison difficult. For instance, some studies included patients with pre-existing CRC predisposing factor such as hereditary gastrointestinal cancer syndrome (e.g. FAP, MAP or Lynch syndrome) and inflammatory bowel disease in their cohort. O'Connell et al [61] performed a systematic review to assess the clinicopathological features and management of CRC in the young population. In their study of 6,425 patients identified from 55 articles, 16% of the patients had CRC predisposing factors. These factors are likely to have over-estimated the incidence of CRC in the young population. In chapter 4, I initially identified 56 patients diagnosed with CRC at the age of 40 or younger. This accounted for 3.8% of the overall cases of CRC in the study period. However, 28 patients in this group were found to have CRC predisposing conditions and were excluded as they have an inherently different type of disease and CRC risk compared with the general CRC population.

The link between hereditary gastrointestinal cancer syndromes and EOCRC has been well-established and described in several retrospective studies [48,74,255]. In a review of 193

individuals with CRC under the of 35 years, Mork et al [48] demonstrated that 35% had identifiable hereditary cancer syndromes of which 23 had LS, 22 had mutation negative MMR deficient tumours, 16 had FAP, two had constitutional MMR deficiency, two had biallelic MUTYH mutations, and one with Li-Fraumeni syndrome. Similarly, in a larger tertiary centre study of EOCRC [255], 26% of patients were found to have a family history of CRC. In the same study, germline sequencing was performed in 315 cases, 79 (25%) individuals had a germline mutation associated with hereditary cancer. The authors concluded that 1 in 5 individuals with EOCRC carries a germline mutation associated with cancer. In my study, 28% of cases had predisposing genetic GI syndrome (Table 4.1)**.** The higher prevalence of individuals with FAP (12%) in my cohort might reflect the fact that our institution is closely affiliated with an established polyposis registry. Clearly identification and management of individuals at risk of these genetic syndromes via detailed family history, appropriate molecular testing and surveillance of CRC is integral to the management of EOCRC. It is unsurprising that various institutions including NICE have recently adopted universal testing of CRC for MMR deficiency.

Similar to chapter 3, the rectum was the most common site of CRC in all three groups with group 1 having the highest rate (57.1%). Compared to LS related CRC which are usually right sided, 70% of all the CRC cases in the patients under the age of 60 years (group 1 and 2) were left sided tumours. These findings are consistent with previously published studies that report a predilection of CRC for the left colon and rectum in younger patients [61,64,250]. This pattern of distribution implies that the majority of cancers are likely to be identified on

screening flexible sigmoidoscopy. This is clinically relevant in terms of endoscopic screening in the United Kingdom: a one-off screening flexible-sigmoidoscopy is usually offered to individuals, albeit at the age of 55 years.

With regards to histopathological features, although not statistically significant, the results demonstrate that patients under the age of 40 years old were more likely to present with poor histological features such as poorly and undifferentiated tumours and advanced disease compared to the other two groups (stage III and IV). The lack of significance is likely due to the smaller sample size compared to the population study in chapter 3 where the difference was statistically significant. In both studies (Chapter 3 and 4), over 60% of cases in the 18-40-year-old group presented with advanced disease (stage 3 and 4) and had a higher rate of poorly differentiated adenocarcinoma compared with the other two groups. These findings are similar to the review by O'Connell study [61] which demonstrated that 66% of patient under the age of 40 years presented with advanced disease (Dukes C & D) and were more likely to have poorly differentiated adenocarcinoma and mucinous carcinoma. Although similar findings have also been demonstrated in other studies [60–62,69], some studies have not identified any age-related differences in histology or disease stage [237,258]. Some authors have suggested that the higher rate of advanced disease seen in the younger patient groups could be attributed to delayed diagnosis because: (1) younger patients are more likely to dismiss red flag bowel symptoms and (2) physicians less likely to refer young patients for endoscopic investigations, attributing their symptoms as benign [60,65,241] and (3) lack of screening. According to one study, the majority of patients with EOCRC are symptomatic at the time of

presentation, therefore it is necessary to maintain a high index of suspicion when assessing young patients with abdominal symptoms [259]. In addition, some researchers have argued the higher rate of advanced disease observed in young patients could also be explained by overestimation or up staging of disease. Young patients with CRC are more likely to undergo aggressive and extensive resection leading to identification of more involved lymph nodes compared to the older group. Although this was not evaluated in chapter 3 and 4 or this thesis, Goldvaser et al [260] demonstrated that that young adults who underwent surgery for CRC had more lymph nodes dissected compared to older adults (median 17 vs. 12, $p < 0.0001$). They also proposed that this could have been necessitated by the intraoperative findings of macroscopic advanced disease. Furthermore, although not statistically significant, Goldvaser et al [260] also demonstrated that young patients were more likely to present acutely or as an emergency with obstructive symptoms (30.6 vs. 21.3 %, $p = 0.06$). This was not the case in the result from our local study; Group 1 were least likely to present as an emergency (10.3% vs 11.8 vs 17.2% p 0.070) **(**Table 4.2**).**

Accurate comparisons of age-related difference in overall survival and disease-free survival using published literature is somewhat difficult due to the different age definitions and stratifications used in individual studies. Some studies stratified their cohort into two groups; young versus old whereas others have used more than two groups. Steele et al [62] stratified their cohorts into four groups: <40, 40 to 49, 50 to 79, and ≥80 year. Their results demonstrated that overall CRC recurrence was highest in the under 40s and 3-year and 5-year survival were highest in the middle-aged group. Similar findings were observed in earlier

studies by Taylor et al [261] and Marble et al [262] and more recent study by Zhao et al [67]. Compared to the findings in chapter 3, the results from the local study demonstrated that group 2 (41-60-year) had best 5-year overall survival compared to the other groups (69%, 77%, 60% *P*<0.001). Similar findings have also been reported in other population and institutional studies [239,249,261]. Furthermore, individuals in group 1 were found to have the worst disease-free survival. The multivariate analysis showed that age itself was not an independent prognostic factor rather UICC stage 3 (HR 4.42; 95% confidence interval (CI): 2.81-6.94 P<0.001) and neoadjuvant chemotherapy (HR 1.65; 95% confidence interval (CI): 1.06-2.58 P=0.026) were associated with an increased risk of recurrence. Published studies have suggested that the worse DFS observed in EOCRC could be attributed to delayed diagnosis and late presentation, whereas others believe it is due to young adults presenting with more histologically aggressive disease [261]. Studies also suggest that although young patients were more likely to have advanced disease and unfavourable histopathological features, their prognosis was similar or better than the older group [61,69,239,241,263,264]. This is because younger patients have better performance status and are therefore more likely to tolerate aggressive treatment (radical curative surgery and adjuvant chemotherapy). Clinicians and oncologists are more likely to recommend chemotherapy in young individuals with good performance status because they are more likely to tolerate its associated toxicities [265,266]. Quah et al [52] demonstrated that younger patients undergoing complete resection of stage I-III colon cancer were more likely to receive adjuvant chemotherapy even in node-negative disease. Our results suggest that despite the increased administration of chemotherapy and neoadjuvant treatment in group 1 (Table 4.3), they still had worse DFS.

These findings should be interpreted with caution due to the small number of young patients with advanced disease in our cohort. Larger studies are required to further evaluate age related difference in chemotherapy administration and CRC recurrence. Unfortunately, we could not evaluate CRC related mortality or disease-free survival in the population study due to lack of data in NCIN and HES database.

## 4.7 Study limitations

There are several limitations to these studies. Firstly, it was based on retrospective data from a single institution which is prone to biases. For instance, factors such as patient's comorbidities, performance status and American Society of Anaesthesiologists (ASA) grade, weight and body mass index, family history of CRC and smoking status were not assessed in this study. These are clinically relevant variables that could affect risk of CRC and survival outcome. A sampling bias is also possible due to the tertiary nature of our institution and therefore the patients described in this study might not be an accurate representation of UK population with CRC. Another limitation is the small sample size especially in the 18-40-year age group. This limited our ability to perform stage dependent stratification of overall survival and disease-free survival between in the 3 groups.

## 4.8 Conclusion

In conclusion, our data suggest that over 28% of cases of EOCRC were found to have hereditary gastrointestinal syndrome. Although young patients aged 18-40 years with CRC present with poorer pathological features and more advanced disease, they do not have

worse prognosis. Furthermore, despite the young group having higher rates of adjuvant and neo-adjuvant treatment, they were found to have worse disease-free survival. Optimising diagnosis and management of hereditary gastrointestinal syndromes is important in the management of EOCRC.

# Section II: OPTIMIZING IDENTIFICATION AND MANAGEMENT OF FAMILIAL ADENOMAOUS POLYPOSIS

# 5 Chapter 5- Attenuated familial adenomatous polyposis (AFAP) - a phenotypic diagnosis but obsolete term?

## 5.1 Study abstract

**Introduction**: Attenuated FAP (AFAP) is characterised by low number (≤ 100) and delayed development of colorectal adenomas. Various definitions have been used, and genotype-phenotype correlations suggested. We aimed to evaluate phenotypic and genotypic correlation in patients with presumed AFAP and assess familial variability.

**Method**: Individuals with AFAP were identified from our registry. Phenotypic AFAP was defined as ≤ 100 adenomas at age 25 years and genotypic AFAP was defined as constitutional pathogenic variant in *APC* region associated with AFAP. Only patients with a germline *APC* pathogenic variant were included in this study. Pathology polyp count (PPC) was used for patients who had undergone surgery and endoscopic polyp count (EPC) for those with intact colon.

**Results**: A total of 69 patients were identified with phenotypic AFAP of which 54 (78%) had pathogenic variant in the AFAP regions of the *APC* gene. Forty-eight (70%) had intact colon (median age at last colonoscopy 43 [25-73] years; median EPC 20 [0-100]) and 21 (30%) had undergone colectomy (median age at surgery 45 [25-54] years; median PPC was 43 [3-100]).

Eighty-three patients were identified with genotypic AFAP of which 54 (65%) had phenotypic AFAP and were described in the previous group. Twenty-nine (35%) had a non-attenuated phenotype and had all undergone colectomy at a median age of 18 [15-26] years; median PPC was 540 [101-2345]. There was evidence of inter- and intra- familial variability.

**Conclusion**: Phenotype in FAP lies on a spectrum - being determined by *APC* genotype and age at adenoma count. Diagnosis of attenuated FAP should be based on phenotype; genotype is not a reliable indicator of an attenuated phenotype. For those with a truly attenuated; management should be personalised according to the phenotype of each individual with the genotype providing supportive information.

## 5.3 Introduction

Attenuated FAP (AFAP) is thought to be a variant of FAP characterised by milder course of disease. Features of AFAP include ≤ 100 colorectal adenomas and later development of colorectal adenomas. It is linked to mutation in the in 5' of codon 233, 3' of 1595 and the alternative spliced region of exon 9 of the *APC* gene [149]. Several authors have explained why mutation in these three regions may be associated with AFAP. Adenomatous polyposis pathogenic variants 5' of codon 233 are the most commonly described mutations associated with AFAP [149]. It is thought that far 5' mutation results in the production of truncated protein which is unable to dimerise with wild-type *APC* protein, so cannot interfere with its function, thereby producing an attenuated phenotype [267]. In patients with variants in the alternative spliced region of exon 9, Su et al [268] suggested that the allele with the pathogenic variant produces an *APC* protein with normal tumour suppressor activity. The attenuated phenotype observed in patients with 3' variants is thought to be due to the production of an almost intact and functional protein [269]. In addition, variants in this region are mutations towards the 3' end are also associated with an increased risk of developing desmoid disease [150,157].

Some authors have defined AFAP by genotype, using the sites associated with AFAP irrespective of the patient's phenotype. However, phenotype variability in patients with variants in these regions has been observed; those variants towards the 5' end result in significant variability in the number of colorectal adenomas and are often phenotypically similar to classical FAP[144,270]. Similarly, intra-familial phenotypic variability has been reported in patients with mutation in the alternatively spliced region of exon 9 [146,152]. Whilst several

diagnostic criteria for AFAP have been proposed [149,154,271], the lack of consensus on the age at which an individual is defined as having AFAP poses diagnostic and surveillance challenge for clinicians, patients and their first-degree relatives. In addition, the true incidence of an attenuated colorectal phenotype in patients with a pathogenic *APC* variant in the attenuated region (attenuated genotype) is not well described in the literature. Indeed, the incidence of AFAP in those with germline pathogenic variant associated with classical FAP is not well established. Attenuated FAP remains a poorly understood entity due to its wide genotypic and phenotypic variability. In this study, we aimed to evaluate: (1) phenotypic and genotypic correlation in patients with presumed AFAP and (2) intra-familial and inter-familial variability in patients and first-degree relatives with genotypic AFAP.

## 5.4 Methods

### 5.4.1 Ethical approval

Ethical approval for this study was obtained from Health Research Authority (HRA) and committee (Research ethics committee reference number 253340; 18/NW/0664) and Research and Development department of London North West University Healthcare NHS trust.

### 5.4.2 Patient selection

The prospectively maintained St Mark's Hospital Polyposis Registry was used to identify individuals with AFAP based on colorectal phenotype and genotype; only patients with a proven germline *APC* pathogenic variant were included. For the purpose of this study,

patients were categorised into two groups: phenotypic and genotypic AFAP. Colorectal phenotype was assessed using endoscopic polyp count (EPC) as documented in the colonoscopy report in patients who had not undergone surgery. In patients who had undergone surgery, pathology polyp count (PPC) was obtained from the post-operative histopathology report.

### 5.4.3 Definitions

We defined phenotypic AFAP as individuals with fewer than or equal to 100 colorectal adenomas at the age of 25 years or older. This age cut-off has been used in a published international collaborative study by Knudsen et al [271]. Genotypic AFAP included three groups as defined by previously published data [144–147,149,156,272]. Group 1 were patients with germline *APC* pathogenic variant towards the 5' end of the gene (codon 1- 233); group 2 had a variant in the alternative spliced region of exon 9 (codon 311- 412); group 3 had a variant 3' of codon 1595. All other germline variants were defined as non-AFAP. Patients under the age of 25 years were excluded from the analysis unless they had an adenoma count of greater than 100 before their 25$^{th}$ birthday, in which case, they were defined as having a non-attenuated FAP. Patients were also excluded if they were over 25 years of age at their first recorded polyp count and had over 100 adenomas.

Patient demographic data including age, location of *APC* germline variant, age at surgery, type of surgery, indication for surgery, adenoma count (pathology or endoscopic) and presence of colorectal cancer were collected.

### 5.4.4 Intra-familial and inter-familial phenotype variability

Intra-familial and inter-familial variability were evaluated for patients with genotypic attenuated AFAP who fulfilled our inclusion criteria. Individuals in each family were deemed to have attenuated phenotype if they had ≤ 100 colorectal adenomas at the age of 25 years or older.

### 5.4.5 Statistical analysis

Descriptive statistics (mean ± standard deviation or median and range) were used for continuous variables and numbers and frequencies for categorical variables were calculated to summarize the patient population. The SPSS statistical software, version 24 was used for analysis.

## 5.5 Results

### 5.5.1 Phenotypic AFAP

A total of 69 patients fulfilled our inclusion criteria (≤ 100 adenomas at the age of 25 years or older) of whom 48 (70%) had intact large bowel and 21 (30%) had undergone colectomy or proctocolectomy (Table 5.1). In the group with intact large bowel, the median age at time of last colonoscopy data was 43 [25-73] years and the median EPC was 20 [0-100]. Of the 48 patients in this group, 41 (86%) had a variant in one of the AFAP regions of the *APC* gene (Table 5.1).

In those who had undergone colectomy or proctocolectomy, the median age at the time of surgery was 45 [IQR 28-54] years and the median PPC was 42 [IQR 18-80]. Thirteen (62%) of these patients had a variant in one of the AFAP regions of *APC* gene. Of the 21 patients who had undergone surgery, 18 (86%) underwent TC-IRA and three (14%) had proctocolectomy with IPAA or RPC. Two patients underwent RPC due to the presence of rectal cancer and high rectal polyp burden respectively; the indication for RPC in the third is not known. Colorectal cancer was diagnosed in three (14%) patients in the surgery group, at the ages of 38, 41 and 51 years with PPC of 93, 42 and 12 respectively. None of these patients were under surveillance at our registry; all three patients were diagnosed with FAP and CRC simultaneously.

| Table 5.1 Phenotypic Attenuated FAP | | | |
|---|---|---|---|
| | | **Colectomy n=21 (30)** | **Intact colon n=48 (70)** |
| **Median age (years)** | | 45 [25-54] | 43 [25-73] |
| **Median polyp count** | | PPC 42 [3-100] | EPC 20 [0-100] |
| **Genotype** | *Group 1* | 5 (24) | 26 (54) |
| | *Group 2* | 6 (29) | 9 (19) |
| | *Group 3* | 2 (9) | 6 (13) |
| | *Non-AFAP* | 8 (38) | 7 (14) |
| **Type of surgery** | *TC-IRA* | 18 (86) | NA |
| | *RPC* | 3 (14) | NA |

### 5.5.2 Genotypic AFAP

A total of 83 patients (from 48 families) fulfilled our inclusion criteria of which 54 (65%) had phenotypic AFAP and have been described in the previous group (Figure 5.1). Twenty-nine (35%) had a non-attenuated phenotype and all had undergone colectomy. The median age at surgery was 18 [15-26] years (Table 5.2)**.** All patients in this group underwent TC-IRA and the median PPC was 540 [101-2345] (Table 5.2)**.**

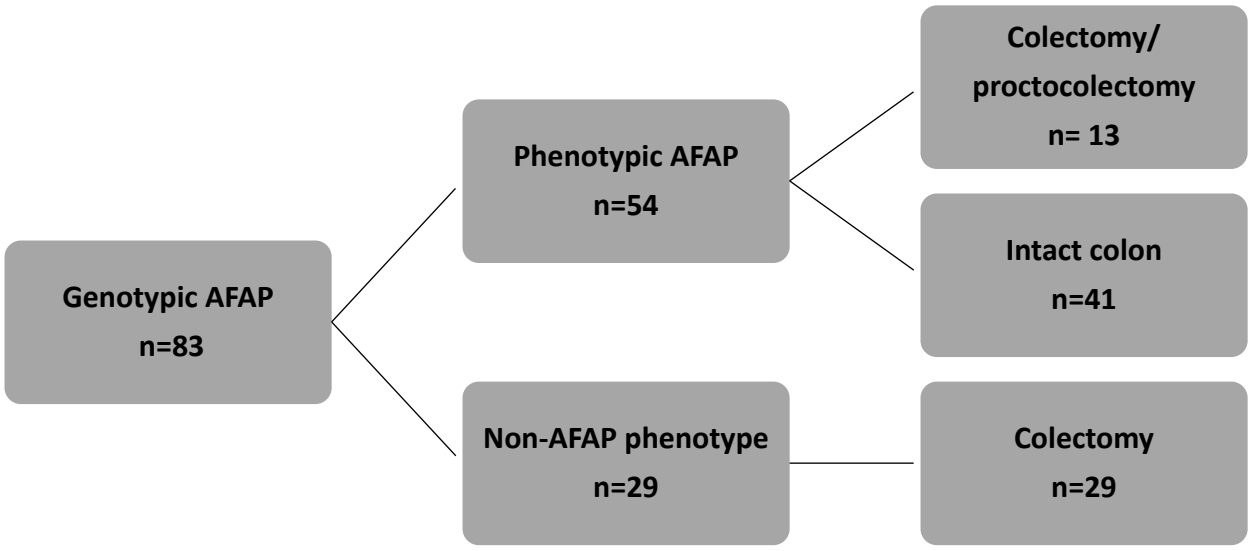



**Figure 5.1 Genotypic Attenuated Familial adenomatous polyposis**

AFAP, attenuated familial adenomatous polyposis: phenotypic AFAP ≤ 100 adenomas at 25 years and non- AFAP phenotype ≥ 100 adenomas at 25 years.

| Table 5.2 Genotypic AFAP with non-AFAP phenotype | | |
|---|---|---|
| | | **Colectomy n=29** |
| **Median age at surgery (years)** | | 18 [15-26] |
| **Genotype** | Group 1 | 28 (97) |
| | Group 2 | 1 (3) |
| | Group 3 | 0 |
| **Polyp count** | 101-500 | 12 (41) |
| | 501-1000 | 11 (38) |
| | >1000 | 6 (21) |
| **Type of surgery** | TC-IRA | 29 (100) |

### 5.5.3 Intra-familial and inter-familial phenotypic variability

We identified 48 families of which 31 had a variant in group 1 (Table 5.3-Table 5.5**),** 11 in group 2 (Table 5.6) and six in group 3 (Table 5.7). In group 3, no variability was observed; all individuals had phenotypic AFAP (table 5.7). Intra-familial variability was observed in two families in group 1: family 18 (variation codon 163) and 23 (variant in codon 170). In family 18, individuals 18I and 18II had polyp counts of 6 and 0 at the age of 27 and 25 years respectively whereas individual 18III had a polyp count of 660 at the age of 25 (Table 5.4)**.**

Inter-familial variability was observed in groups 1 and 2. In group 1, of the four families with a variant in codon 163 of *APC*, three individuals had a non-attenuated phenotype (17I, 17II and 18III) (Table 5.4)**.** Furthermore, families with variants in codons 170, 187, 213, 216 did not appear to have an attenuated phenotype (Table 5.4)**.** Similarly, in group 2, although families 4 and 5 had an identical variant site (codon 358) there was evidence of marked difference in adenoma burden between them; patient 5I had an attenuated phenotype with polyp count of 71 at the age of 72 years old, whereas individuals 4I and II showed a non-attenuated phenotype with polyp counts greater than 100 before the age of 25 years (Table 5.6)**.**

**Table 5.3 Intra and inter-familial variability in patients with variants in 5' region**

| Family | Codon | Generation | Age at polyp count | Number of adenomas |
|---|---|---|---|---|
| 1 | 49 | *I* | 54 | 49 |
| | | *II* | 40 | 69 |
| | | *III* | 40 | 4 |
| | | *IV* | 28 | 9 |
| | | *V* | 41 | 4 |
| | | *VI* | 38 | 3 |
| 2 | 49 | *I* | 53 | 2 |
| 3 | 73 | *I* | 65 | 50 |
| | | *II* | 67 | 37 |
| | | *III* | 38 | 8 |
| 4 | 73 | *I* | 83 | 31 |
| 5 | 96 | *I* | 54 | 72 |
| | | *II* | 25 | 1 |
| 6 | 96 | *I* | 73 | 50 |
| 7 | 96 | *I* | 38 | 3 |
| | | *II* | 38 | 10 |
| | | *III* | 47 | 30 |
| 8 | 104 | *I* | 46 | 11 |
| 9 | 122 | *I* | 22 | 425 |
| 10 | 126 | *I* | 38 | 15 |
| 11 | 141 | *I* | 26 | 60 |
| 12 | 141 | *I* | 77 | 37 |
| 13 | 141 | *I* | 39 | 99 |
| | | *II* | 26 | 60 |
| 14 | 150 | *I* | 64 | 50 |

**Table 5.4 Intra and inter-familial variability in patients with variants in 5' region**

| Family | Codon | Generation | Age at polyp count | Number of adenomas |
|---|---|---|---|---|
| 15 | 159 | *I* | 24 | 258 |
| | | *II* | 22 | 1073 |
| 16 | 161 | *I* | 30 | 30 |
| | | *II* | 25 | 70 |
| 17 | 163 | *I* | 20 | 101 |
| | | *II* | 21 | 117 |
| 18 | 163 | *I* | 27 | 6 |
| | | *II* | 25 | 0 |
| | | *III* | 25 | 660 |
| 19 | 163 | *I* | 42 | 10 |
| 20 | 163 | *I* | 23 | 82 |
| 21 | 163 | *I* | 31 | 50 |
| 22 | 163 | *I* | 37 | 79 |
| 23 | 170 | *I* | 24 | 1258 |
| | | *II* | 17 | 500 |
| | | *III* | 16 | 132 |
| | | *IV* | 16 | 1274 |
| 24 | 177 | *I* | 25 | 18 |
| 25 | 177 | *I* | 25 | 32 |
| 26 | 187 | *I* | 25 | 2345 |
| | | *I* | 18 | 300 |
| 27 | 213 | *II* | 21 | 860 |
| 28 | 213 | *I* | 18 | >200 |
| | | *II* | 17 | 940 |
| | | *III* | 16 | 890 |
| | | *IV* | 18 | 504 |
| | | *V* | 16 | 504 |

**Table 5.5 Intra and inter-familial variability in patients with variants in 5' region**

| Family | Codon | Generation | Age at polyp count | Number of adenomas |
|---|---|---|---|---|
| 29 | 213 | *I* | 21 | 892 |
| | | *II* | 17 | 976 |
| | | *III* | 17 | 501 |
| 30 | 216 | *I* | 23 | 300 |
| 31 | 233 | *I* | 36 | 95 |

**Table 5.6 Intra and inter-familial variability in patients with variants in alternative spliced region of exon 9**

| Family | Codon | Generation | Age at polyp count | Number of adenomas |
|---|---|---|---|---|
| **1** | **332** | ***I*** | **48** | **100** |
| | | *II* | 64 | 50 |
| | | *III* | 50 | 75 |
| | | *IV* | 31 | 2 |
| | | *V* | 26 | 4 |
| 2 | 332 | *I* | 47 | 50 |
| | | *II* | 41 | 12 |
| | | *III* | 49 | 77 |
| 3 | 332 | *I* | 27 | 15 |
| 4 | 358 | *I* | 23 | 400 |
| | | *II* | 15 | 200 |
| 5 | 358 | *I* | 72 | 71 |
| 6 | 399 | *I* | 24 | 0 |
| 7 | 405 | *I* | 45 | 3 |
| 8 | 405 | *I* | 45 | 4 |
| 9 | 405 | *I* | 36 | 3 |
| 10 | 405 | *I* | 34 | 16 |
| 11 | 405 | *I* | 47 | 30 |
| | | *II* | 33 | 19 |

**Table 5.7 Intra and inter-familial variability in patients with variants in 3' region**

| Family | Codon | Generation | Age at polyp count | Number of adenomas |
|---|---|---|---|---|
| 1 | 1619 | *I* | 52 | 42 |
| 2 | 1636 | *I* | 28 | 50 |
| | | *II* | 25 | 0 |
| 3 | 1925 | *I* | 29 | 27 |
| 4 | 1998 | *I* | 62 | 0 |
| 5 | 2079 | *I* | 66 | 3 |
| 6 | 2079 | *I* | 51 | 5 |

## 5.6 Discussion

Previous published studies have described AFAP in small groups of patients or families based on their genotype without assessing the phenotype component. Others have described AFAP in individuals with or without confirmed *APC* germline variant. To our knowledge, only one study to date has evaluated the phenotype and genotype in patients with AFAP. In that collaborative study [271], members of the Leeds Castle Polyposis Group (LCPG) were encouraged to submit clinical and pathological information on patients with presumed phenotypic AFAP based on the definition being ≤ 100 colorectal adenomas by the age of 25.

Only 40% of that cohort had a proven germline *APC* pathogenic variant, of which 52% had variants in the regions associated with AFAP. In our study, we sought to evaluate AFAP using both phenotypic (≤ 100 colorectal adenomas by the age of 25) and genotypic (based on published literature) definitions in patients with confirmed *APC* variants, using data from a prospectively maintained polyposis registry. Of the 69 patients with phenotypic AFAP, 78% had a variant in a region of *APC* gene thought to be associated with AFAP. Similar to published studies [149], the majority had variants in the 5' region. Our data also demonstrate that genotype does not reliably predict colorectal phenotype in AFAP; only 65% of patients with variants in the regions of *APC* gene implicated in AFAP (far 5', alternative spliced region of exon 9 and far 3') appeared to have an attenuated colorectal phenotype.

The emergence of adenomas in AFAP is thought to be delayed by up to 10-20 years compared to classical FAP. However, there is currently no consensus on whether this phenotypic difference should influence the timing of prophylactic surgery in individuals with FAP. Although studies have suggested regular colonoscopy surveillance and polypectomy might be sufficient in managing patients with phenotypic AFAP [151,273], some have also reported CRC in AFAP, even in the presence of few adenomas [144,151,153]. Burt et al [151] reported an average age of CRC diagnosis of 58 (range 29-81) years in two American families with identical constitutional pathogenic variant in AFAP region. In that study, the median number of adenomas was 25 (range 1-470) at a median age of 41 (range 16-79) years at the time of colonoscopy. Twenty five percent of the patients in their cohort had more than 100 adenomas, but their age at the time of endoscopic assessment was not stated. Therefore, it

is impossible to ascertain if all the patients with genotypic AFAP did in fact have phenotypic AFAP.

We recommend caution when interpreting these results because assessing the role of endoscopic surveillance and delayed colectomy in AFAP requires clear distinction between genotypic and phenotypic diagnosis of AFAP. In our study, the majority (69%) of patients with phenotypic AFAP had an intact colon. The median colonoscopy polyp count at last colonoscopy in this group was 20 [range 0-100] at a median age of 43 years [25-73] (Table 5.1). None of these patients had colorectal cancer during surveillance. Furthermore, it is well established that appropriately timed colectomy remains the cornerstone of FAP management. Total colectomy and ileorectal anastomosis (TC-IRA) has been recommended as surgery of choice in patients with AFAP [144,151,274]. In our study, 82% of patients with phenotypic AFAP underwent TC-IRA at median age of 45 [range 25-54] which is clearly older than is usual for patients with FAP. Despite this, the median pathology polyp count in this group of patients was 42. None of these patients had CRC; the three patients with CRC in our phenotypic AFAP group who had undergone colectomy were not under surveillance. They presented with lower gastrointestinal symptoms and were found to have CRC and PPC of <100.

If endoscopic surveillance and management rather than early colectomy is chosen as the preferred management option in individuals with phenotypic AFAP, its aim should be to abolish the risk of CRC. The numbers in this cohort are probably too small to say definitively

that primary endoscopic management has been safe but certainly seem to suggest so. It is important to highlight that the decision making should be individualised based on phenotypic and genotypic manifestation of the disease.

Genotypic and phenotypic variability in families with AFAP have been described in literature [134,147,268]. Sieber et al [134] found that individuals with *APC* variants in the regions thought to be associated with AFAP (far 5', far 3' and alternative spiced region of exon 9) displayed variable colorectal phenotype [134]. They found that individuals with variants in the 5' regions were more likely to display a severe colonic phenotype. Similarly, Soravia et al [144] reported that this group of patients were also more likely to display intra-familial variability in colorectal phenotype. In our results, we also observed more inter- and intra-familial variability in patients with 5' variants (especially between codons of 150 and 180 (Table 5.3). For example, in Family 18 with a variant in codon 163, individuals I and II had attenuated colorectal phenotype whereas, individual III had 660 polyps at 25 (Table 5.4). There was no evidence of intra-familial variability in group 2 and 3. However, inter-familial variability was observed in families with variants in codon 358 (Table 5.6). The reason for these variabilities is uncertain. Several authors suggest they occur due to inconsistency in clinical practice, influence of environmental factors (diet, smoking status and lifestyle) or the role of modifier genes [134,144,275]. Further genetic and registry studies are needed to investigate the role of these factors. Furthermore, although this was not assessed in our study, it is unclear if the phenotypic intra-familial variability observed increases with decreasing genetic relationship between family members.

Evidently, the clinical definition of AFAP is somewhat arbitrary as it relies entirely on the colorectal adenoma count of ≤ 100 at colonoscopy. Studies have shown that the number of colorectal adenomas in FAP is dependent on the genotype, age at colonoscopy and endoscopic technique [232,275]. Estimation of adenoma number is usually based on counting number of adenomas within a sample area or a fixed dimension and correcting for the length of the colorectum. This estimate can be confounded by factors such as the experience of the endoscopist, sensitivity of the naked eye to varying adenoma sizes, use of dye spray and the length of the colorectum [275]. These variabilities in endoscopic techniques pose a genuine risk of underestimation of adenoma count and misclassification of a classical FAP patient as having AFAP. A study carried out by Wallace et al [276] from St Mark's Hospital, highlighted that adenoma count could be underestimated and patients incorrectly labelled as having AFAP if dye spray was not used at colonoscopy. In their study, all patients thought to have AFAP at simple colonoscopy were found to have classical phenotype at dye spray colonoscopy and pathology count following colectomy.

## 5.7 Study Limitations

There are several limitations to this study. Firstly, we did not collect data on size of adenomas nor presence of HGD. Although these parameters need to be considered too regarding timing of surgery, this study is not focused on timing of surgery, rather addressing the overall genotype and phenotype of what has historically been called AFAP. Although these parameters are important, they are not the sole indicator of when colectomy is required,

which indeed is more of a global assessment of polyp burden, genotype, other aspects of FAP history (e.g. personal or family history of desmoid disease) and social convenience. Another illimitation is the discrepancy between endoscopic and histological estimation of adenoma enumeration. Finally, this is an historical series. Endoscopic technology has advanced significantly during this time and therefore there may be variation in adenoma count, based purely on the improved quality of procedures using high definition equipment. Again, because of the retrospective nature of this work, the surgical decision-making process is not clear, especially for those with a very attenuated colorectal phenotype. With growing confidence in endoscopic techniques, no doubt a more conservative approach would be adopted and there is likely to have been changes in our clinical practise during the study period.

## 5.8 Conclusion

Phenotype in FAP lies on a spectrum - being determined by genotype, likely genetic modifiers and environment, and age. Some authors [273] have suggested that clinical decision-making should not depend entirely on the presence of ≤ 100 adenomas, but on the understanding that AFAP is a variant of FAP. And although adenoma count is less than in classical FAP, there is still risk of developing CRC. Therefore, we feel that AFAP is potentially a misleading term and should be abandoned. Surveillance and prophylactic surgery should be tailored to the phenotype of each individual and the genotype may be useful in supporting a more conservative strategy.

# 6 Chapter 6- Polyp progression in paediatric patients with familial adenomatous polyposis - a single centre experience

## 6.1 Study abstract

**Background**: Prophylactic colectomy at a premalignant stage is the cornerstone of management of familial adenomatous polyposis (FAP). Prior to surgery, colonoscopy surveillance is recommended in children with FAP. This study aimed to examine the natural history of FAP in children by evaluating adenoma progression and factors influencing timing of colectomy.

**Method** Patients with FAP under the age of 18 years at first surveillance colonoscopy and who had undergone more than one colonoscopy were identified. Demographic, endoscopic, genetic and surgical data were retrieved. Cumulative adenoma (polyp) counts were obtained whilst accounting for any polypectomies during the study period. The rate of polyp progression and factors influencing the timing of colectomy were evaluated.

**Results**: Eighty-four patients (50% male; mean age at first colonoscopy 13 years [SD 1.97]) were identified, of which 83 had a family history of FAP. At first colonoscopy, 67 (79%) had <100 adenomas and 29 (35%) had colonic polyps identified despite rectal sparing. The median rate of polyp progression per patient was 12.5 polyps/year (range 0-145). Of the 45 (54%) patients who had undergone surgery, 41 (91%) underwent colectomy with ileorectal or

ileodistal sigmoid anastomosis. Polyp progression did not alter the choice of surgical intervention in any patient.

**Conclusion**: Our results suggest that adenoma number remains relatively stable in the majority of children under surveillance. Tailored surveillance intervals according to phenotype are a more appropriate strategy as recommended by recently published guidelines.

## 6.2 Introduction

The management of children with FAP, or at risk of FAP is based around appropriate timing of predictive genetic testing, endoscopic surveillance and prophylactic colectomy (colectomy and IRA or ileodistal sigmoid anastomosis or proctocolectomy (usually restorative, with formation of ileoanal pouch [RPC]) at a premalignant stage. Adenomas generally begin to be detected in adolescence, with some studies reporting a mean age of 16 years at first identification of polyps of [277–279]. The number and size of the adenomas are thought to be dependent on factors including genotype (location of pathogenic variant in the *APC* gene) and age at which the large bowel is examined [280,281].

Published guidelines recommend regular colonoscopy surveillance to assess adenoma number, size and distribution [167,208]. Although endoscopic surveillance has been shown to reduce the risk of CRC in patients with FAP [282], optimal frequency remains contentious. Some authors recommend yearly colonoscopy in all children under surveillance once adenomas have been identified [281,283], whereas recent guidelines recommend adopting an individualised approach based on the patient's genotype and phenotype [168,284]. Similar controversies exist with regard to timing of prophylactic surgery. Generally, indications for surgery include: onset of colorectal symptoms, marked increase in polyp size or number, presence of high-grade dysplasia and patient's choice [208,285]. However, there is no consensus on the exact age, size or polyp number at which surgery should be offered or which surgical procedure should be performed. Some centres recommend colectomy once adenomas have been identified whilst others recommend colectomy at a time that when it will cause minimal disruption to the

child's psychological, social and educational development. It is generally agreed that in those with a milder genotype (pathogenic variant outside the mutation cluster region) and phenotype (fewer than 500 colonic polyps and fewer than 20 rectal polyps), it is reasonable to delay surgery and perform IRA [170,286].

Evidently, there are controversies in the management of paediatric patients with FAP in terms of optimum intervals for colonoscopy surveillance and timing of prophylactic colectomy. Our aim was to better understand the natural history of FAP in this group of patients by evaluating adenoma progression and factors influencing the timing of colectomy.

## 6.3 Methods

### 6.3.1 Patient selection

This study was approved by our institutional review board as a service evaluation project. At our institution, predictive genetic testing for children at risk of FAP (where there is a known constitutional pathogenic variant in an affected family member) is recommended at the age of 12-14 years. Colonoscopy surveillance is commenced if they are proven to have constitutional pathogenic variants in *APC* gene. In those with a family history of FAP but no identified pathogenic variant, screening colonoscopy at the age 14-15 years is recommended. Colonoscopies in children are usually performed under general anaesthetic by a paediatric gastroenterologist. Colorectal adenoma burden is calculated by counting number of adenomas on withdrawal, or if adenomas are too numerous to count individually, it is

estimated as previously described in the literature [232]. Polypectomy is not routinely performed in children at our institution.

The prospectively maintained St Mark's Hospital Polyposis Registry was searched to identify all patients with FAP under the age of 18 years at their first surveillance colonoscopy. FAP diagnosis was defined as confirmed *APC* pathogenic variant on genetic testing or the presence of histologically confirmed adenomas on colonoscopy in an individual with known family history of clinically diagnosed FAP. Data from the Polyposis Registry were supplemented with data from patients' medical, endoscopic, pathology and operative records. Data extracted include: demographic information, family history, genetic results (location of pathogenic variant in *APC*), dates and frequency of endoscopic surveillance, endoscopic findings (number of polyps at each colonoscopy), and type of surgery (e.g. IRA or RPC), age at surgery and indication for surgery.

To adequately assess adenoma progression, we excluded: (1) patients who had undergone only one colonoscopy (2) patients followed up at other institutions and (3) colonoscopy reports without a numerical adenoma count.

### 6.3.2 Definitions

To facilitate analysis of genotype, the pathogenic *APC* variant was stratified based on the location relative to the mutation cluster region (MCR; codon 1250-1464). The groups were: group 1, pre-MCR (5' of codon 1250); group 2, MCR (codon 1250-1464); group 3, post-MCR (3' of 1464) and group 4, gross deletion [170,280]. The increase in absolute colorectal adenoma

(polyp) counts count per year for each individual was calculated relative to the polyp count at previous colonoscopy, using the formula “rate of polyp progression per year “= (number of polyps documented at a given colonoscopy – polyp count at previous colonoscopy)/ (time between colonoscopies (years)). There is currently no evidence to support polyp regression in patients with FAP, therefore, the rate of polyp progression was assumed to be zero if the polyp count at a given colonoscopy was less than the count at previous colonoscopy.

### 6.3.3 Statistical analysis

Continuous variables were expressed as mean and standard deviation or median and range depending on distribution. Categorical variables were reported as frequency (percentages). In addition to a summary of the changes in category in different time periods, a statistical comparison between time periods was performed. A feature of the data was that the same patients were assessed over time. Due to the binary nature of the outcome (increase or no increase), the analyses were performed using multilevel binary logistic regression. In patients who showed polyp progression, the rate of polyp progression was calculated as described above. The Mann-Whitney U test was used to compare 2 categories and the Kruskal-Wallis test for more than 2 categories.

## 6.4 Results

### 6.4.1 Patient Characteristics

Over the 20-year study period, 142 patients under the age of 18 years with a confirmed diagnosis of FAP were seen at our institution. Of these, 58 were excluded because: they were not followed up at our institution (n=24), only had one surveillance colonoscopy (n=33) or did not have a numerical documented polyp count (n= 1). A total of 84 patients met our inclusion criteria of which 42 (50%) were male. Eighty-three patients (99%) had known family history of FAP and one (1%) had new constitutional pathogenic variant. All patients had undergone genetic testing of which sixty-two (74%) had an *APC* pathogenic variant in the pre-MCR, 13 (15%) in MCR, five (6%) in post-MCR and four (5%) patients had a gross deletion (Table 6.1). Forty-five (54%) patients had undergone prophylactic surgery.

**Table 6.1 Patient demographics**

| Variables | Number (%) |
|---|---|
| Confirmed pathogenic variant | 84 (100%) |
| Median age at first colonoscopy (years) | 13 (range 9-17) |
| Sex | |
| Male | 42 (50%) |
| *APC* mutation | |
| Pre-MCR (5' of 1250) | 62 (74) |
| MCR (1250-1464) | 13* (15) |
| Post MCR (3' of 1464) | 5 (6) |
| Gross deletion | 4 (5) |
| Prophylactic surgery performed | 45 (54) |

*3 patients in the MCR group had constitutional pathogenic variant in codon 1309, which is associated with severe polyposis.

### 6.4.2 Endoscopic surveillance and polyp progression

A total of 293 colonoscopies were carried out over the study period, of which 210 (72%) were performed by a single paediatric gastroenterologist. The median age at first colonoscopy was 13 (range 9-18). Three patients with constitutional pathogenic variant in codon 1309 had

their first colonoscopy before the age of 11 years due to onset of colorectal symptoms. Adenomas were identified in 77 (92%) patients at initial colonoscopy. In 29 (35%) patients, polyps were identified in colon despite rectal sparing. At first colonoscopy 67 (79%) individuals had fewer than 100 polyps, 14 (17%) had between 101-500 polyps, and 3 (4%) had over 500 polyps (Table 6.2). The median adenoma count in the different genotypic groups were: pre-MCR 40 (0-400), MCR 75 (15-1000) Post-MCR 2 (0-15) and gross deletion 50 (10-60) ($p=0.0547$). The median size of the largest adenoma colonoscopy was 3mm (range 1-15mm). After a median follow-up of 3.5 (range 2-8) years and median of 3 (range 2-8) colonoscopies per patient, there was a 26% increase in polyp count per year (95% CI: 20% to 32%; $p<0.001$). The median rate of polyp progression per patient was 12.5 polyps/year (range 0-124). The rate of polyp progression was highest in the MCR group (16 polyps/year (range 5-145)).

| Table 6.2 Endoscopic surveillance | |
|---|---|
| **Variables** | **Number (%)** |
| Total number of colonoscopies | 293 |
| Mean colonoscopy per year per patient | 1 (SD±0.4) |
| Median colonoscopies per patient | 3 (range 2-8) |
| Number of colonoscopies/patient | |
| 2-4 | 64 (76%) |
| ≥ 5 | 20 (24%) |
| Interval of colonoscopy | |
| ≤ 1 year | 79 (94%) |
| Polyp burden at first colonoscopy | |
| *0-100* | 67 (79%) |
| *101-500* | 14(17%) |
| *>500* | 3 (4%) |
| Median size of largest polyp | 2mm (range 1-15mm) |
| Number of patients who progressed to a higher polyp category | 26 (30%) |

### 6.4.3 Surgery

During the study period, 45 patients had undergone prophylactic surgery of which 27 (60%) were male. The mean age at surgery was 17 (range 11-22) years and the rate of polyp progression in this group was 13 polyps/year (0-124). The median polyp count in the resected surgical specimen was 178 (3-3150) and no patient was diagnosed with colorectal cancer.

Forty-one (91%) patients underwent IRA, three (7%) underwent RPC and one (2%) underwent panproctocolectomy and end ileostomy. Restorative proctocolectomy was performed in three patients with 1309 constitutional pathogenic variant due to presence of severe rectal polyposis and each had total pathology polyp count greater than 1000. All three underwent colectomy before the age of 12 years. One patient was scheduled to undergo RPC; however, this was converted to panproctocolectomy and end ileostomy due to the presence mesenteric desmoid. Surgery was mostly (51%) performed as a planned procedure at a time that was least disruptive on the child's social and educational development. The genotype and indications for surgery are summarised in Table 6.3.

| Table 6.3 Prophylactic surgery | |
|---|---|
| **Variables** | **Number (%)** |
| Total | 45 |
| Median age at surgery (years) | 17 (11-22) |
| Median pre-operative colonoscopies per patient | 3 (2-7) |
| Sex | |
| Male | 27 (60%) |
| Type of surgery | |
| IRA | 41 (91%) |
| RPC | 3 (7%) |
| TPC* | 1 (2%) |
| Genotype | |
| Pre-MCR | 34 (55) |
| MCR | 8 (62) |
| Gross exon deletion | 3 (75) |
| Median pathology polyp count | 178 (3-3150) |
| Indications for surgery | |
| Increase in polyp burden or size | 7 (16) |
| Social convenience | 23 (51) |
| Patient/family preference for surgery over surveillance | 15 (33) |

## 6.5 Discussion

Our results support the recently published guidelines from the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) ESPHGAN which recommend that annual colonoscopy is not required for all children with FAP under surveillance, rather surveillance should be individualised based on colorectal phenotype [168]. In addition, polyp progression did not appear to alter the choice of surgical intervention in any patient; the choice of surgery was guided by genotype, colonic phenotype and rectal

polyp burden, which in none of our cases changed sufficiently to alter which operation was recommended. Similar findings were reported in a recent study by Sarvepalli et al [287]. They examined the rate of polyposis progression in 168 patients under the age of 30 years and found that the rate of polyp progression was independently associated with genotype and polyp number at initial colonoscopy. To our knowledge, our study is the first paper to evaluate polyp progression in children with FAP. Other published studies on paediatric patients with FAP have mainly reported on smaller groups of patients or were published prior to the genetic testing era and therefore may have included patients with other adenomatous polyposis syndromes, where tumour biology may differ.

Current evidence suggests that flexible sigmoidoscopy is inadequate in the assessment of adenoma number and distribution in children with FAP. Previous historical data by Bussey [169] demonstrated rectal involvement in all 170 adult cases with colonic polyposis, however, studies have shown that over 80% of children have colonic adenomas at first colonoscopy [288,289]. Furthermore, Munck et al [284] demonstrated that at initial colonoscopy, 11% of children were found to have colonic polyposis despite not having any polyp in the rectosigmoid. Similar findings were observed in our study; at a median age of 13 (9-17) years at first colonoscopy, colonic adenomas were identified in 92% of patients in our cohort and 29 (35%) patients had identifiable colonic polyps despite the absence of polyps in the rectum. Consequently, it is unsurprising that some authors [288] and institutions [168] now recommend colonoscopy rather than flexible sigmoidoscopy as an initial screening tool when predictive genetic testing is not available. Our findings further support the evidence that endoscopic surveillance should be

by colonoscopy in order to adequately assess the entire large bowel and define the patient's colorectal phenotype.

Genotypic and phenotypic correlation in FAP have been reported in published studies [144,290]. Pathogenic variants in the hot-spot regions such as 1309 or between 1250-1450 regions are associated with a more severe phenotype and earlier presentation of disease. These phenomena were observed in our study. The median rate of polyp progression/year was lowest in individuals with less severe phenotype: pre-MCR & post-MCR 15.4 (range 0-124), MCR 16 (range 5-145) and gross deletion 10.4 (range 9-31) although this was not statistically significant (p=0.647). Studies have also described phenotypic variability amongst individuals or families with similar pathogenic variant [140,152,289]. For example, although the median age at initial colonoscopy was 13, three patients with pathogenic variant in codon 1309 (associated with severe polyposis) had first colonoscopy before the age of 11 years due to onset of colorectal symptoms; two of these patients had over 1000 colorectal polyps and one had 150 at first colonoscopy. Furthermore, of the 66 patients with less severe genotype (Pre-MCR and post-MCR), seven patients had no identifiable colorectal polyp at initial colonoscopy and the rate of polyp progression in these seven patients was 1.2 polyps/year. Similar findings were reported in the Cleveland Clinic study [287]. They demonstrated a correlation between the rate of polyposis progression, genotype and polyp count at first colonoscopy. In their study, patients with a pathogenic variant in MCR had the highest rate of progression whereas the lowest rate were observed in patients with a pathogenic variant 5' of codon 151 and in those with fewer than 20 polyps at first colonoscopy [287]. They concluded that low polyp count at

first colonoscopy predicts polyposis progression independent of genotype. These genotypic-phenotypic correlation and inter and intrafamilial variability in polyp count has led to some authors recommending individualisation of surveillance protocol based on the patient's colorectal phenotype with the genotype providing supportive information [151,284,288]. Asymptomatic patients with low polyp burden at initial colonoscopy could be offered a less frequent surveillance compared to those with severe phenotype.

Colectomy remains the definitive management of patients with FAP, however the optimal age at which surgery should be performed, the magnitude of increase in polyp count at which surgery should be recommended and the choice of surgery remains contentious. Recent guidelines suggests that patients should be referred for colectomy if there is concern about polyp size, density and presence of advanced changes [168]. In our study, only 7% of patient underwent surgery due to polyposis progression. The majority (53%) underwent colectomy as a planned procedure at time that was least disruptive to the child's social educational development. This suggests that the decision for colectomy is not solely dependent on the endoscopic evidence of polyposis progression. This is supported by the fact the rate of polyp progression in the patients who had undergone surgery was only slightly higher than those with intact colon continuing surveillance (13.1 vs 10.8 polyps/year). The other factor contributing to timing of colectomy is patient's or family decision to opt for early colectomy over continuing surveillance despite the individual having no concerning endoscopic features. In the 15 patients who opted for earlier surgery in our cohort, the median preoperative endoscopic polyp count was 43 (range 0-110) and all patients underwent IRA. It is likely that

this decision might have been influenced by the surgical outcome in older first-degree relatives who had undergone surgery at similar age. Consequently, we recommend the decision for timing and type of surgery is made by a multidisciplinary team and factors such as genotype, phenotype (colorectal polyp burden and size of polyp), patient's preference, social and educational needs should be considered.

## 6.6 Study limitations

We acknowledge several limitations to our study. This was a single center study from a large polyposis registry which is prone to inherent institutional, referral and data entry biases. For example, our data did not include other factors which may influence polyposis progression and surgical decision making, including but not limited to diet, smoking history, body mass index (BMI), family history of desmoid and size of adenomas. Furthermore, although 72% of the colonoscopies were performed by a single experienced pediatric gastroenterologist, estimation of polyp size and counts especially in cases where actual numerically count could not be done may have been prone to error. Also, we have assumed a linear relationship in description of the polyp progression over time which might not be entirely accurate.

## 6.7 Conclusion

Our results suggest that polyposis progression is slow in the majority of children under surveillance. The need for annual colonoscopy is not supported and tailoring surveillance interval to phenotype is a more appropriate strategy. Our results support the recently published ESPHGAN guidelines.

# 7 Chapter 7- Safety and efficacy of laparoscopic near-total colectomy and ileo-distal sigmoid anastomosis (NT-IDSA) as a modification of total colectomy and ileorectal anastomosis (TC-IRA) for prophylactic surgery in patients with adenomatous polyposis syndromes – a comparative study

## 7.1 Study abstract

**Introduction**: Colectomy in patients with adenomatous polyposis syndromes (AP) demands good oncological and surgical outcome. Total colectomy with ileorectal anastomosis (TC-IRA) is one of the surgical options for these patients. Anastomotic leak rates of 11% have been reported following TC-IRA. Ileo-distal sigmoid anastomosis (IDSA) is a recent modification of our practice. We compare post-operative outcome in patients with AP following Near Total colectomy with IDSA (NT-IDSA) and TC-IRA at a single institution.

**Method**: A prospectively maintained database was reviewed to identify patients with AP who underwent laparoscopic NT-IDSA and TC-IRA. Patient demographics, early morbidity and mortality and outcome of endoscopic surveillance were evaluated.

**Results**: A total of 191 patients with AP underwent laparoscopic colectomy between 2006 and 2017 of which 139 (72.8%) underwent TC-IRA and 52 (27.2%) underwent NT-IDSA. The median age at surgery in the TC- IRA and NT-IDSA groups was 20 years (IQR 17-45) and 27 years (IQR 19-50) years respectively. Grade II complications were comparable between the two groups. There were no anastomotic leaks in the NT-IDSA group compared to 15 (10.8%) in the TC-IRA group (p=0.0125) and no reoperation in the NT-IDSA group compared to 17

(12.2%) in the TC-IRA group (P=0.008). Frequency of polypectomies per flexible sigmoidoscopy was comparable between the two groups.

**Conclusion**: This study demonstrates that laparoscopic NT- IDSA for polyposis is associated with significant improvement in anastomotic leak rates and surgical outcome. It is too soon to tell whether NT-IDSA alters the need for further intervention, either endoscopic polypectomy or further surgery.

## 7.2 Introduction

Colectomy remains the cornerstone of treatment and prevention of colorectal cancer (CRC) in patients with adenomatous polyposis syndromes (familial adenomatous polyposis (FAP) and MutYH associated polyposis (MAP)) and those with a clinical diagnosis but no identified germline pathogenic variant.[138,208] Without surgery, development of CRC is almost inevitable in most of these patients. Compared to sporadic CRC, the majority of patients undergoing colectomy for adenomatous polyposis syndromes (AP) are young, healthy and asymptomatic. For this reason, not only must the procedure be oncologically sound, but also have a low level of surgical risk.[291]

A selective approach to colectomy has been recommended in patients with FAP [173,292]. Patients with a less severe genotype and colorectal phenotype are offered TC-IRA with RPC reserved for those with more aggressive disease [274,286,293,294]. Total colectomy with ileorectal anastomosis TC-IRA has also been shown to be an appropriate surgical procedure for patients with MAP, patients with adenomatous polyposis syndromes who develop CRC whilst on surveillance or those diagnosed with CRC and adenomatous syndromes simultaneously [295]. The risk of rectal cancer and subsequent proctectomy in this group of patients is minimised by regular endoscopic surveillance of the remaining large bowel [296].

Rectal sparing surgery such as TC-IRA or subtotal colectomy and ileosigmoid anastomosis have been shown to confer better bowel functional outcome than RPC [173,297,298]. However, studies have reported anastomotic leak rates of 2%-11% [298–302] in patients with FAP following

TC-IRA [301]. This often requires re-operation and ileostomy formation. Some authors have hypothesized that the high leak rate observed following ileorectal anastomosis using circular stapler could be due to disparity in lumen size and thickness between the ileum and rectum[303]. In order to improve anastomotic leak rates in these groups of patients, a modification of the conventional IRA anastomotic configuration has been carried out at our institution. In near total colectomy with ileo-distal sigmoid anastomosis (NT-IDSA) the inferior mesenteric artery pedicle is preserved with intracorporeal dissection being undertaken to the distal sigmoid and an extracorporeal ileum to distal sigmoid anastomosis (IDSA) is performed.

The aim of this study is to describe and compare early morbidity in patients with adenomatous polyposis syndromes undergoing conventional TC-IRA and NT-IDSA anastomotic configuration. We also compared outcome of endoscopic surveillance of remaining large bowel between the two groups.

## 7.3 Method

### 7.3.1 Patient selection

This study was approved by our local institutional research and development board. A retrospective review of the prospectively maintained St Mark's Hospital Polyposis Registry was performed. Patients with FAP requiring colectomy were eligible for rectum sparing surgery if they had either an *APC* constitutional pathogenic variant outside the MCR (codon 1250-1595) or low-density polyposis phenotype (<500 colonic polyps or <20 rectal polyps with

no polyp larger than 1cm). Patients with MAP were eligible if they fulfilled the same phenotypic criteria. From early 2014, patients eligible for TC-IRA were offered NT-IDSA.

Patients with adenomatous polyposis, FAP and MAP who underwent either laparoscopic TC-IRA or NT-IDSA between January 2006 and June 2017 were identified and their medical records were reviewed. Patients were divided into two groups TC-IRA and NT-IDSA. Patient demographics and operative data such as age, gender, type of polyposis, American society of Anaesthesiologist (ASA) grade, date of operation, anastomotic configuration and concurrent diagnosis of colorectal cancer were retrieved.

Endoscopic follow up data including frequency of flexible sigmoidoscopy examination, adenoma count and polypectomy were also retrieved. Early morbidity and mortality including reoperations and re-admissions were retrieved. All complications occurring during hospital admission or the immediate 30 days after discharge were graded using the Clavien-Dindo classification [235]. Prolonged small bowel ileus was defined as inability to pass flatus or faeces within five days of the operation or nasogastric tube insertion. The level of anastomosis was defined as distance from anastomosis to anal verge and was measured either via on-table flexible sigmoidoscopy or retrieved from postoperative endoscopy surveillance report.

### 7.3.2 Surgical technique

#### 7.3.2.1 *Laparoscopic Total colectomy and ileorectal anastomosis*

The majority of laparoscopic total colectomy and ileorectal anastomosis were performed by two surgeons at our institution. Systematic dissection and ligation of the ileocolic, right colic and middle colic and inferior mesenteric pedicles was performed using clips and energy device (Thunderbeat©). The entire colon is mobilised and the top of total mesorectum excision (TME) plane is entered. Following division of the mesorectum, the specimen is divided at upper rectum using Ethicon ETS 45 ©. The terminal ileum mesentery is dissected and specimen retrieved through pfannestiel incision. The terminal ileum is prepared for anastomosis using the anvil of a Ethicon © CDH 29 circular stapler device. The same circular CDH stapler device gun is introduced per anum and an intracorporeal double stapled ileo-rectal anastomosis is performed. The staple line is over sewn on both sides.

#### 7.3.2.2 *Laparoscopic near total colectomy with ileo-distal sigmoid anastomosis*

Laparoscopic near total colectomy with ileo-distal sigmoid anastomosis (NT-IDSA) was offered to patients with AP providing the rectosigmoid junction and its vascular supply can be oncologically preserved. The procedure was performed by a single surgeon at our institution from January 2014 - May 2017. This technique has been described in previously published article[304]. In contrast to TC-IRA, the inferior mesenteric artery pedicle was preserved. Intracorporeal dissection of the sigmoid mesocolon was undertaken up to the distal sigmoid. To avoid leaving behind a redundant loop of sigmoid, the sigmoid was carefully straightened and the site of anastomosis marked at the distal sigmoid. A small Pfannenstiel incision was

made for extraction of specimen and construction of the anastomosis. An extracorporeal 'side of ileum to a side of distal sigmoid' anastomotic configuration was performed using a linear TLC 75mm (Ethicon) device and a transverse TA 90mm (Ethicon) stapler, between. The staple lines were buried using 3.0 vicryl interrupted sutures. On-table, flexible sigmoidoscopy was performed in the majority of the patients to visualise the anastomosis and measure the length of the residual recto-sigmoid segment (Figure 7.1).

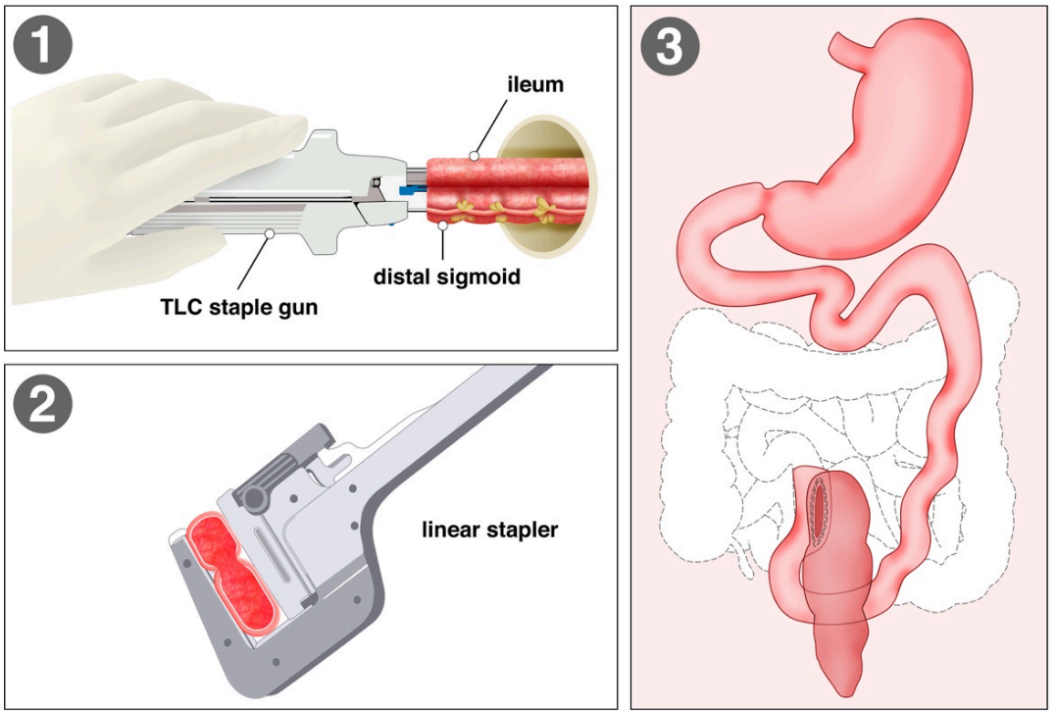



**Figure 7.1 Near total colectomy and ileo-distal sigmoid anastomosis (NT-IDSA)**

### 7.3.3 Statistical analysis

Continuous variables are reported as mean ± standard deviation or median and interquartile range (IQR) and categorical variables as frequencies and percentages. Chi-square or Fisher's exact test was used to compare categorical variables and Wilcoxon's rank-sum test was performed to compare non-parametric continuous variables. Spearman's correlation coefficient was computed to assess the relationship between length of recto-sigmoid segment and number of macroscopic adenomas per patient per year identified in the NT-IDSA group during surveillance. A *P*-value of 0.05 was considered statistically significant. All analysis was conducted using SPSS version 24.0.

## 7.4 Results

### 7.4.1 Patient Characteristics

During the period of study, 191 patients underwent laparoscopic colectomy for adenomatous polyposis. A hundred and thirty-nine patients (72.8%) underwent TC-IRA and 52 (27.2%) underwent NT-IDSA. Of these, 166 (86.9%) patients had FAP, 16 (8.4%) had MAP and nine (4.7%) had clinical adenomatous polyposis with no identifiable pathogenic variant (Table 7.1). There were 100 (52.4%) males and the median age at the time of surgery in the TC- IRA and NT-IDSA groups were 20 (IQR 17-45) and 27 (IQR 19-50) *P*= 0.139. Eleven patients had a pre-operative diagnosis of CRC or high-grade dysplasia and two had an incidental finding of CRC on histology. The cancers were located in ascending colon (n=6), transverse (n=4) and descending (n=3). All cancers in the NT-IDSA group were right sided. Other patient characteristics are summarised in Table 7.1.

**Table 7.1 Patient characteristics**

| Characteristics | TC-IRA (n=139) | NT- IDSA (n=52) | P value |
|---|---|---|---|
| Median age (yr.) | | | 0.139 |
| | 20 (17-45) | 27 (19-50) | |
| Gender | | | 0.627 |
| Male | 71 (51.3) | 29 (55.8) | |
| Female | 68 (48.9) | 23 (44.2) | |
| Underlying disease | | | 0.081 |
| FAP | 123 (88.5) | 43 (82.7) | |
| MAP | 10 (7.2) | 6 (11.5) | |
| Unclassified | 6 (4.3) | 3 (5.8) | |
| ASA Score | | | 0.722 |
| I | 101 (72.7) | 40 (76.9) | |
| II | 37 (26.6) | 12 (23.1) | |
| III | 1(0.7) | 0 (0) | |
| Operative access | | | 0.676 |
| Completely Laparoscopic | 133 (95.7) | 51 (98.1) | |
| Open (conversion) | 6 (4.3) | 1 (1.9) | |
| Defunctioning ileostomy | | | 0.548 |
| Yes | 1 (0.7) | 0 (0) | |
| No | 105 (99.3) | 51 (100) | |
| Malignancy | | | 0.7528 |
| Yes | 9 (6.5) | 4 (7.7) | |
| No | 130 (93.5) | 48 (92.3) | |

### 7.4.2 Perioperative outcomes

There were no 30-day postoperative deaths (Table 7.2). There was no reoperation in the NT-IDSA group whereas 17 (12.2%) patients in the TC-IRA group underwent reoperation for anastomotic leak (n=15) and small bowel obstruction secondary to internal herniation (n=2) (*P*=0.008). Fifteen (10.8%) patients in the TC-IRA group had anastomotic leakage; there were no anastomotic leaks in the NT-IDSA group (*P*=0.0125). One (2%) patient in the NT-IDSA group had a low-volume enterocutaneous fistula. A fistulogram demonstrated the fistula originated away from the anastomosis and was thought to be iatrogenic in origin. Radiological drainage was performed for infected haematoma in one (2%) patient in the NT-IDSA group (Table 7.2). The most common complication across the two groups was small bowel ileus 22 (11.6%). Of these, seven (3.7%) patients required total parenteral nutrition. There was no significant difference between the two groups with regards to Clavien-Dindo Grade II complications (Table 7.2).

**Table 7.2 Early postoperative outcome using Clavien-Dindo classification**

| Outcome | TC-IRA (n=139) | NT- IDSA (n=52) | P -value |
|---|---|---|---|
| Overall complication-free rate | 101 (72.6) | 38 (73.1) | 0.954 |
| 30-day reoperation | 17 (12.2) | 0 (0) | 0.008 |
| Mortality | Nil | Nil | |
| Grade IV & V | Nil | Nil | |
| Grade IIIb complication | | | |
| Anastomotic leak requiring reoperation | 15 (10.8) | Nil | 0.0125 |
| Small bowel obstruction | 2 (1.4) | Nil | |
| Grade IIIa | | | |
| Pelvic collection | 1 (0.7) | 1 (1.9) | 0.471 |
| Grade II | | | |
| Pneumonia | 2 (1.4) | 2 (3.8) | 0.299 |
| Enterocutaneous fistula | 0 | 1 (1.9) | 0.272 |
| Venous thromboembolism | 1 (0.7) | 1 (1.9) | 0.471 |
| Wound infection | 1 (0.7) | 1 (1.9) | 0.471 |
| Ileus | 12 (8.6) | 10 (19.2) | 0.071 |

Data are numbers with percentages in parenthesis or medians with interquartile ranges in parenthesis. Ileus is defined as inability to pass flatus or faeces within five days of the operation or nasogastric tube.

### 7.4.3 Endoscopic surveillance of the rectum and recto-sigmoid following TC-IRA and NT-IDSA

Cumulatively, 182 (95.2%) patients were followed up at our institution for a total of 909 patient years after undergoing TC-IRA or NT-IDSA. The median follow-up was 4.6 years (IQR, 2.4-8.3 years). A total of 1312 flexible sigmoidoscopies were performed with a median of 6 (IQR 3-11) flexible sigmoidoscopies per patient. The length of rectum of recto-sigmoid remnant was significantly longer in the NT-IDSA group (median 25cm; IQR, 22-30) group than in the TC-IRA group (median 18cm; IQR, 18-20) $P<0.001$ (Table 7.3). A total of 10355 (median per patient 8; IQR 4-12) cumulative macroscopic adenomas were identified in the rectum or recto-sigmoid during post-operative surveillance. The median cumulative adenoma count per patient per year was significantly higher in the NT-IDSA group (11; IQR 3-23) compared to TC-IRA (median 6; IQR 1-17) group $P<0.001$. A total of 6212 (median per patient 10; IQR 2-75) polyps were removed endoscopically. Patients in the NT-IDSA group underwent more polypectomies per patient per flexible sigmoidoscopy (median 3; IQR 1-9) compared to the TC-IRA (median 2; IQR 0-7) although this was not statically significant P=0.391. At the time of data collection, no patient had developed rectal or recto-sigmoid cancer.

**Table 7.3 Endoscopic surveillance post TC-IRA and NT-IDSA**

| Variable | TC- IRA (n=139) | NT-IDSA (n=43) | *P* value |
|---|---|---|---|
| Pre-op rectal polyp count | 10 (2-18) | 10 (4-16) | 0.079 |
| Length of anastomosis /cm | 18 (18-20) | 25 (22-30) | P<0.001 |
| Cumulative adenomas/patient/year | 6 (IQR 1-17) | 11 (3-23) | P<0.001 |
| Polypectomies/patient/sigmoidoscopy | 2 (IQR 0-7) | 3 (1-9) | 0.391 |

Data are numbers with percentages in parenthesis or medians with interquartile ranges in parenthesis.

## 7.5 Discussion

This study aimed to demonstrate the utility of NT-IDSA in surgical treatment of patients with adenomatous polyposis syndromes and also compare it to conventional TC-IRA. These data suggest that although the rates of Grade I and II complications were comparable between the two groups, NT-IDSA anastomotic configuration was associated with significant reduction in anastomotic leak rates (p=0.0125). These findings suggest that NT-IDSA extracorporeal anastomotic configuration may provide a safer alternative to conventional TC-IRA in young and otherwise healthy patients requiring surgery for polyposis syndromes. Laparoscopic NT-IDSA leaves behind more mucosal for polyp formation than conventional laparoscopic TC-IRA, however, our data did not demonstrate a significant correlation between length of recto-sigmoid segment and frequency of polypectomies.

Published studies have reported an anastomotic leak rate of 2%-11% in FAP patients following TC-IRA [299–302]. This level of risk is considered high in otherwise healthy individuals undergoing surgery in their late teens or early twenties. In our small cohort of patients who underwent NT-IDSA, no patient suffered an anastomotic leakage. We believe that this significant difference in leak rate could be explained by the extra-corporeal anastomotic technique and configuration. Hyman et al [303] suggested that the use of circular stapler in ileorectal anastomosis may contribute to the high leak rate due to the disparity in lumen side and wall thickness between the rectum and ileum. This is supported by the fact that a higher anastomotic leak rate was reported in FAP patients whose ileorectal anastomosis were performed laparoscopically using circular stapler [301] compared to those in the open era [297,299,300,305]. Furthermore, preservation of the inferior mesenteric artery when oncologically safe, ensures improved vascularity to the anastomosis.

Although the rates of Grade II complications were comparable between the two groups, the overall complication rate was favourable in the NT-IDSA group. The complication rate of 26.9% was similar to those described in published studies of FAP patients undergoing TC-IRA (21%-25%) [299,302,305]. Furthermore, we observed a higher rate of post-operative ileus in the NT-IDSA but this was not statistically significant when compared to the TC-IRA group. The rate of postoperative ileus following TC-IRA specifically in patients with polyposis syndromes is poorly reported in the literature, however rates of 10%-38% have been reported in patients undergoing extended colectomy with IRA or ileosigmoid anastomosis for various indications

including: polyposis syndromes, inflammatory bowel disease and colonic inertia [203,306]. Similar rates were observed in our study in both groups.

The evolution of surgery in FAP is such that the choice of surgery is now guided by the patient's genotype and phenotype. Patients with attenuated genotype, mild colonic phenotype and a low rectal polyp burden are offered TC-IRA. Over 80% of patients in our series underwent either TC-IRA or NT-IDSA for FAP (the remainder underwent surgery for MAP). Several studies have reported on the advantages of adopting this selective strategy in the management of patients with FAP [150,286,293,307,308]. Sinha *et al* [293] showed that this selective approach reduces the risk of rectal cancer and secondary proctectomy compared to use of TC-IRA in all patients. Similar results were observed by Moreira *et al* [286]. In our study, NT-IDSA was offered to patients eligible for TC-IRA using the selective criteria. None of the patients who underwent NT-IDSA had severe colonic polyposis and the median preoperative rectal polyp count in this group was 10 (IQR 4-16). Similarly, rectal sparing surgery is recommended in patients with MAP (8.4% of our cohort) and mild rectal disease [295,309].

As expected, the NT-IDSA group had a longer recto-sigmoid segment and therefore more cumulative adenoma count per patient per year. Historical studies on patients with FAP before the laparoscopic era and the advent of pouch surgery favoured subtotal colectomy and ileorectosigmoid anastomosis for rectal sparing surgery in patient with polyposis reluctant to have an end ileostomy [310–312]. The incidence of rectal cancer following ileorectosigmoid or ileorectal anastomosis ranged from 4 to 37%, [310–313] depending on the

duration of follow-up. Although recent studies have reported subtotal colectomy and ileorectosigmoid anastomosis in patients with FAP [297,298] the majority of these studies predate the identification of the *APC* gene and the routine use of preoperative colonoscopy therefore, patients were offered ileorectosigmoid or ileorectal anastomosis irrespective of the severity of their genotype or colonic phenotype. Furthermore, postoperative endoscopic surveillance was performed using proctoscopy or rigid sigmoidoscopy which may not have adequately visualised the recto-sigmoid segment up to the level of anastomosis. We suspect that the risk of cancer following ileorectosigmoid anastomosis in that era was higher due to the lack of appropriate selection of patients and robust surveillance strategy (flexible sigmoidoscopy). Gleeson *et al* [296] showed that lower gastrointestinal endoscopic surveillance, ablation therapy and polypectomy reduces the risk of progression to advance neoplasia in FAP patients following surgery. All patients in our cohort were suitable for rectum preserving surgery and currently undergo flexible sigmoidoscopic surveillance with or without polypectomy by an experienced endoscopist. Our results show that, the number of polypectomies per patient per scope were comparable between the two groups. Nonetheless, long term follow-up studies are needed to ascertain the risk of cancer in the NT-IDA recto-sigmoid segment. Chapter 8 of this thesis evaluates the role of modern endoscopic techniques such as polypectomy in management of rectal remnant following rectal sparing surgery in FAP.

Although not assessed in this study, one might anticipate bowel function would be better in the NT-IDSA group. Studies have suggested that preserving vascularity during colectomy could prevent bowel and sexual dysfunction [203,297,314]. Similarly, a longer rectal or

rectosigmoid stump have been shown to correlate with better bowel function [203,315]. Lin et al [315] suggested that the rectosigmoid junction performs a breaking mechanism termed 'rectosigmoid break' which helps to limit rectal filling. Removal of this region during surgery is likely to lead to post-operative motility disorder such as increase stool frequency. We acknowledge the inadequate follow-up period and the lack of data to adequately report on bowel function and quality of life between the two groups at this stage is a limitation of the study. Some authors [306,316] suggest that it takes approximately 18 months for bowel function parameters (bowel frequency, faecal incontinence and day and nocturnal bowel movement) to stabilize, therefore, prospective studies with longer follow-up are needed to robustly evaluate this.

## 7.6 Study limitations

In addition to the lack of functional data, we acknowledge several limitations to this study. Firstly, our data did not include patient dependent factors such as body mass index and smoking status which may influence surgical outcome. Secondly, the short-term follow-up in the NT-IDSA group meant we could not report on the risk of cancer and completion proctectomy between the two groups. Larger studies including longer postoperative surveillance are need to further evaluate this. Despite these limitations, our results have demonstrated significant improvement in surgical outcome particularly anastomotic leak rates with NT-IDSA compared to TC-IRA.

## 7.7 Conclusion

Near total colectomy and IDSA anastomotic configuration is safe and associated with good surgical outcome in patients requiring colectomy for adenomatous polyposis syndromes. This is particularly important in young patients undergoing prophylactic colectomy for polyposis syndromes. The long-term oncological and functional impact of this procedure is uncertain.

# 8 Chapter 8- Regular endoscopic surveillance and polypectomy is effective in managing rectal adenoma progression following colectomy and ileorectal anastomosis in patients with familial adenomatous polyposis

## 8.1 Study abstract

**Aim**: Total colectomy with ileorectal anastomosis (TC-IRA) is a surgical option for patients with familial adenomatous polyposis (FAP). Regular endoscopic surveillance of the rectum is recommended to prevent rectal cancer. We aim to evaluate polyp progression in the rectum following TC-IRA and evaluate the role of polypectomy during surveillance.

**Method**: Patients with FAP who underwent TC-IRA between 1990-2017 were identified. Demographic, endoscopic and genetic data were retrieved. Demographic, endoscopic, genetic and surgical data were retrieved. Cumulative rectal adenoma (polyp) counts were obtained whilst accounting for any polypectomies during the study period. The rate of polyp progression and factors influencing secondary proctectomy were evaluated.

**Results:** One hundred and ninety-nine patients fulfilled our inclusion criteria, of which 44% were male. The median age at colectomy was 19 (range 11-70) years and median pre-operative rectal polyp count was 7 (range 0-50). All patients had an *APC* pathogenic variant, of which 151 (79%) were 5' of the mutation cluster region (MCR), 19 (10%) in the MCR, six (3%) were 3' of the MCR and 15 (8%) had a gross deletion. After a median of follow-up of 8.6

(range1-27) years and a median of 11 (range 2-37) flexible sigmoidoscopies per patient, the median rate of polyp progression was 5.5 polyps/year (range 0-70.2). There was no evidence of polyp regression. Eight (4%) patients underwent secondary proctectomy for neoplasia, of which one (0.5%) had rectal adenocarcinoma. A total of 13,527 polyps were removed, a median of 35 polyps/patient (range 0-829). The rate of polyp progression was not significantly associated with genotypic or phenotypic factors.

**Conclusion**: Progression of rectal polyp number following TC-IRA appears to be slow and dependent on the length of follow-up. In the modern era of stringent endoscopic surveillance and therapeutic procedures such as cold snare polypectomy, the rate of secondary proctectomy and the risk of rectal cancer after TC-IRA are very low. These findings are important when counselling patients with regard to the choice of surgery for FAP and implementing endoscopic surveillance.

## 8.3 Introduction

The risk of rectal cancer and secondary proctectomy following rectal sparing surgery in FAP depends on genotype and colorectal phenotype [317,318]. Following TC-IRA, the cumulative risk of developing cancer in the residual rectum is thought to be in the region of 10-25% after 15-25 years and increasing to 29-30% by the age of 60 years [319–323]. The high risk of rectal cancer observed in the majority of these studies reflects the era in which the surgery was performed. Before introduction of restorative proctocolectomy with ileal pouch anal anastomosis (RPC) (the pre-pouch era), rectum sparing surgery was the only option to avoid permanent ileostomy; genotype was unknown and phenotype could not be assessed before the advent of colonoscopy. Studies comparing the risk in the pre-pouch and pouch era have demonstrated a significant difference [171,322]. A selective approach to choice of prophylactic surgery ensures that proctocolectomy with or without ileoanal pouch formation is now reserved for patients with high risk of rectal failure (severe genotype, phenotype and rectal polyposis) [139,170,286].

Regular postoperative endoscopic surveillance is crucial in management of the residual rectum following TC-IRA. Simultaneous removal of large polyps (polypectomy) is currently recommended during surveillance [324,325]. Previously, endoscopic surveillance was performed using rigid sigmoidoscopy or low definition flexible sigmoidoscopy with occasional fulguration or Argon plasma coagulation of adenoma. Neither of these techniques are likely to definitely treat the adenoma and caused scaring and distortion. In the modern endoscopic era, dramatic improvements in endoscopy cameras (high definition) and techniques now allow for

improved optical diagnosis and assessment of adenomas. Furthermore, the Introduction of cold snare polypectomy has revolutionized polyp resection with multiple polypectomies being performed in a safe and efficient manner. Although poorly reported in the literature, these modern endoscopic techniques are likely to further improve management of the rectum in FAP.

There is currently no agreed consensus on the frequency of surveillance or polypectomy what size of polyp should be removed following TC-IRA in FAP. Some institutions recommend biannual whereas others recommend annual flexible sigmoidoscopy in all patients [208,218,325]. Recent guidelines on pre-operative surveillance of colorectum in FAP suggests a role for individualising the frequency of surveillance based on the patient's genotype and phenotype [168]. Furthermore, historical studies have suggested spontaneous regression in adenoma enumeration occurs after TC-IRA [326,327] and in patients on chemoprevention [224,328]. Understanding the rate of adenoma progression or regression and the role of therapeutic procedures such as cold snare polypectomy in management of rectal polyposis will help inform surveillance protocol and surgical decision making. The aim of this study is to: (1) evaluate the rate of adenoma (polyp) progression or regression in the rectum following TC-IRA and (2) report our experience of postoperative endoscopic surveillance and role of polypectomy in management of the rectal polyposis following TC-IRA.

## 8.4 Methods

This study was approved by our institutional review board as a service evaluation project. All patients who underwent TC-IRA between August 1990 and May 2017 were identified from the prospectively maintained St Mark's Hospital Polyposis Registry. The date was chosen to cover the era of routine optical endoscopy surveillance rather than rigid proctosigmoidoscopy. A diagnosis of FAP was defined as confirmed *APC* pathogenic variant on genetic testing or the presence of histologically confirmed adenomas on colonoscopy in an individual with known family history of clinically diagnosed FAP. Data extracted include: demographic information, genetic results (location of pathogenic variant in *APC*), dates and frequency of flexible sigmoidoscopy, endoscopic findings (polyp enumeration at each flexible sigmoidoscope), preoperative rectal polyp count, age at surgery, pathology colonic polyp count, chemoprevention, history of colon cancer, number of polypectomies, indication for proctectomy (rectal cancer, severe polyposis, functional problems and other causes) and length of rectum (measured endoscopically).

Since 2010, the practice at our institution has been that patients are offered TC-IRA if they have: (1) pathogenic variant in *APC* outside of the MCR, (2) colonic adenoma burden <500 and (3) fewer than 20 rectal adenomas, all of which are endoscopically manageable. Post-operatively, during the study period, they underwent biannual surveillance flexible sigmoidoscopy and polypectomy. Endoscopic surveillance of individuals with FAP was usually performed by an experienced gastrointestinal endoscopist with a specialist interest in FAP.

There were no formal criteria for polypectomy, which was done at the discretion of the endoscopist.

To adequately assess adenoma progression in the rectum, we excluded (1) individuals who had undergone TC-IRA but were lost to follow (1) patient who had undergone less than 2 flexible sigmoidoscopies and (3) endoscopic reports without a numerical adenoma count. Endoscopic surveillance of individuals with FAP is usually performed by experienced gastrointestinal endoscopic with specialist interest in FAP. Adenoma burden is usually calculated by counting number of adenomas in the rectum and adenoma size was estimated relative to the open biopsy forceps [233,234]. Polypectomies (predominantly cold snare polypectomies) are routinely performed during surveillance at our institutions.

## 8.5 Definitions

To facilitate analysis of FAP genotypes, the pathogenic *APC* variant was stratified based on the location relative to the MCR (codon 1250-1464) [170]. The groups used include: group 1, pre-MCR (5' of codon 1250); group 2, MCR (codon 1250-1464); group 3, post-MCR (3' of 1464) and group 4, gross deletion. The rate of rectal adenoma (polyp) progression was calculated using a modified version of previously published formula [329]: rate of rectal polyp progression (number of polyps at last flexible sigmoidoscope + total number of polyps removed during surveillance - number of rectal polyps documented at surgery)/(years between last flexible sigmoidoscope and date of surgery).

## 8.6 Statistical analysis

Continuous variables were expressed as mean and standard deviation or median and range depending on the distribution whilst categorical variables were reported as frequency with percentages. A feature of the data was that the same patients were assessed over time. The rate of polyp progression or regression was calculated using the formula described above. Individuals with negative rate of polyp progression were described as showing polyp regression and then excluded from subsequent analysis. The difference between rate of polyp progression between groups were compared using Mann-Whitney U test when only 2 categories were present and the Kruskal-Wallis test for more than 2 categories. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) IBM version 24.0.

## 8.7 Results

### 8.7.1 Patient characteristics

Over the 27-year study period, 235 patients were identified. Of these, 44 were excluded because: they were not followed up at our institution (n=26) and only had one recorded surveillance flexible sigmoidoscope (n=18). A total of 191 patients fulfilled our inclusion criteria of which of 84 (44%) were male. The median age at surgery was 19 (range 11-70). All patients had an *APC* pathogenic variant, of which 151 (79%) were in the pre MCR, 19 (10%) in the MCR, six (3%) in the post MCR and 15 (8%) had a gross deletion. The median pre-operative rectal count was 7 (range 0-50) and median pathology colonic adenoma count was 400 (3-

3760). Eight patients (4%) were on chemoprevention of which five were on Indomethacin and three on Sulidinac (Table 8.1).

| **Table 8.1 Baseline characteristics of patients undergoing colectomy and ileorectal anastomosis for FAP** | |
|---|---|
| **Variable** | **N=191** |
| Sex | |
| Male | 84 (44) |
| Median age at surgery (years) | 19 (11- 70) |
| *APC* constitutional pathogenic variant | |
| Pre MCR (5' of 1250) | 151 (79) |
| MCR (1250-1464) | 19 (10) |
| Post MCR (3' of 1464) | 6 (3) |
| Gross deletion | 15 (8) |
| Chemoprevention | 8 (4) |
| Median pre-op rectal polyp count | 7 (0-50) |
| Median pathology colonic polyp count | 400 (3-3760) |
| Colon cancer | |
| Yes | 4 (2) |
| No | 187 (98) |
| Mean length of rectum | 19 (SD ± 3.3) |
| Proctectomy | 10 (5) |
| Rectal Adenocarcinoma | 1 (0.5) * |

*1 patient had squamous cell carcinoma.

### 8.7.2 Endoscopic surveillance and polyp progression

During the study period, the median number of flexible sigmoidoscopies per patient was 11 (range 2-37). A total of 2,440 surveillance flexible sigmoidoscopies were performed of which 97% had concurrent therapeutic procedure. Overall, 13,527 polyps were removed a median, of 35 polyps/patient (range 0- 829). The median size of the largest polyp idenitified was 2mm (range 1-15). After a median follow up of 8.7 years (range 1-27) and accounting for all polypectomies, 185 (97%) had polyp progression. The median rate of polyp progression was 5.5 polyps/year (range 0-70.2). Ninety-seven (51%) patients had polyp progression of less than 5 polyps/year, all of whom had constitutional pathogenic variant in the pre-MCR and post-MCR areas of *APC* gene (Table 8.2). Six patients (3%) showed polyp regression; the median rate of regression was 1.2 polyps/year (range 1-5.2) (Table 8.2).

| Table 8.2 Endoscopic surveillance following colectomy and IRA | |
|---|---|
| **Variables** | **Total** |
| Number with follow up data | 191 |
| Total number of flexible sigmoidoscopies | 2,440 |
| Median follow-up (years) | 8.6 (1-27) |
| Median flexible sigmoidoscopy per patient | 11 (2-37) |
| Total number of polypectomies | 13,527 |
| Median number of polypectomies/patient | 35 (0-829) |
| Polyp progression | 185 (97) |
| Median rate of polyp progression/year | 5.5 (range 0-97.8) |
| ≤ 5 | 97 (51) |
| 6-10 | 36 (19) |
| > 10 | 58 (30) |
| Median rate of polyp progression (polyps/year) | 5.5 (0-70.2) |
| Median size of largest polyp identified | 2 (range 1-15) mm |

### 8.7.3 Rate of rectal polyp progression and rectal failure

The median rate of polyp progression in this group of patients was 5.5 polyps/year (0-46). During the surveillance period, 10 patients (5.2%) underwent secondary proctectomy after a median follow-up of 7.3 (range 2–25) years. Of these, seven had constitutional pathogenic variant in the pre-MCR and three in the MCR. Eight (4.2%) patients underwent proctectomy for neoplasia. Seven had progression in polyp enumeration deemed endoscopically unmanageable and the median rate of polyp progression in these patients as 26.9 polyps/year. One individual had 2cm adenoma initially thought to be high grade dysplasia on biopsy. One patient developed rectal adenocarcinoma. In this patient, rectal adenocarcinoma was found in a 12mm polyp which was initially excised endoscopically before proceeding to completion restorative proctectomy. Histology confirmed stage I rectal adenocarcinoma. The rate of polyp progression in this patient was 2.1 polyps/year. Two patients had proctectomy for non-neoplastic reasons: one for functional difficulties two years after colectomy and one patient chose to have completion proctectomy due to postoperative neurological comorbidity which made surveillance unfeasible (Table 8.3).

**Table 8.3 Indications for secondary proctectomy**

| Indications for proctectomies | Number |
|---|---|
| Rectal adenocarcinoma | 1 |
| Polyposis progression deemed endoscopically Unmanageable | 7 |
| Patient choice | 1 |
| Functional difficulties | 1 |

### 8.7.4 Differences in rate of polyp progression based on genotypic and phenotypic characteristics

The highest rate of polyp progression (97.8 polyps/year) was observed in a patient with constitutional pathogenic variant in codon 1062. Although individuals with pathogenic variant in the MCR region seemed to have the highest rate of polyp progression, this was not statistically significant. Our results also showed an overall trend toward an increase in the rate of polyp progression in individuals with >500 colonic polyps in the resected colon ($P$=0.053) and those on chemoprevention ($P$=0.076). The presence of colon cancer at the time of TC-IRA ($P$=0.981) or genotype ($P$=0.102) were not associated with the rate of polyp progression. Table 8.4 summaries the differences in rate of polyp progression based on patients genotypic and phenotypic characteristics.

**Table 8.4 Differences in rate of rectal polyp progression based on patients clinical, genotypic and phenotypic characteristics**

| Variable | Number (%) | Rate of polyp progression (polyps/year (range)) | *P* value |
|---|---|---|---|
| Chemoprevention | | | |
| Yes | 7 | 11.3 (2.8-42.5) | 0.076 |
| No | 178 | 5.5 (0-70.2) | |
| Genotype | | | |
| Pre MCR (5' of 1250) | 147 (80) | 5.9 (0 -70.2) | 0.102 |
| MCR (1250-1464) | 17 (9) | 8.7 (0.3 - 46.2) | |
| Post MCR (3' of 1464) | 6 (3) | 3.3 (0 - 10.5) | |
| Gross deletion | 15 (8) | 6.1 (2.3 - 34.1) | |
| `Pathology colonic polyp count | | | |
| <500 | 99 (53) | 4.9 (0 - 70.2) | 0.053 |
| >500 | 78 (47) | 6.3 (0.3 - 46.2) | |
| Colon cancer | | | |
| No | 181 (98) | 5.9 (0 - 70.2) | 0.981 |
| Yes | 4 (2) | 7.4 (0 – 16.2) | |

## 8.8 Discussion

This large single-centre study has shown that over a median follow-up of 8.6 (1-27) years, and accounting for all polypectomies, 97% of patients had an increase in adenoma burden at a rate of 5.5 polyps/year (range 0-70.2) and rectal cancer occurred in only one (0.5%) patient. Published historical series in the pre-pouch, pre-flexible sigmoidoscopy era demonstrated higher rates of rectal cancer despite regular surveillance [312,330]. In the paper by Nugent et al[319] from the same institution as the current study, rectal cancer occurred in 10% of the cohort evaluated. Of the 22 patients who developed rectal cancer in that study, 12 (55%) occurred within 10 years of TC-IRA [319]. The better outcomes observed in our study are likely to be due to the use of selection criteria to inform choice of surgery over the last 12 years, improved surveillance with flexible sigmoidoscopy and control of polyp numbers using cold snare polypectomy.

Historically, TC-IRA was the surgical procedure of choice for most individuals with FAP because it is a simple one-stage procedure with fewer post-operative complications than panproctocolectomy, avoids a permanent ileostomy and has good functional outcome. This became contentious after several institutional studies demonstrated significant risk of rectal cancer after TC-IRA despite regular endoscopic surveillance [274,319,320,331]. Consequently, some institutions currently offer RPC as the procedure of choice as it almost eradicates the risk of rectal cancer although cuff or pouch can occur [172,332]. Restorative proctocolectomy has been shown to be associated with a higher risk of post-operative morbidity and poor functional outcome when compared to TC-IRA [173,300,302]. Also, more recent studies have shown the that

the risk of rectal cancer and proctectomy is indeed lower than previously thought when a selective approach is adopted [313,322]. Church et al [313] demonstrated a reduction in the risk of secondary proctectomy from 32% in the pre-pouch to 2% in pouch era and the risk of rectal cancer also reduced from 13% to 0%. Similarly, in the multicentre study by Bulow et al, the rate of rectal cancer reduced from 10% in the pre-pouch era to 2% in the pouch period. In this study, only one out of 191 patients under surveillance at our institution developed stage 1 adenocarcinoma of the rectum. These findings provide further evidence that selective approach to choice of prophylactic operation combined with stringent endoscopic surveillance and polypectomy reduces the risk of rectal cancer and secondary proctectomy following TC-IRA.

To our knowledge, this is the largest study to describe adenoma progression or regression in the rectum following rectal sparing surgery. The natural history of FAP is such that without surgical or endoscopic intervention, increase in adenoma size and enumeration is expected over time. In our study, 97% of individuals showed progression in rectal polyp numbers. Spontaneous regression or resolution of rectal polyposis after TC-IRA has been reported in the literature. In the Feinberg study [326], spontaneous resolution of rectal polyposis occurred in 64% (complete 38% and partial 26%) of FAP patients following TC-IRA. This appeared to occur at a median time of six months after surgery. Similarly, Watne et al [333] demonstrated a reduction in polyp count in 15 out of 17 patients three months after rectal sparing surgery. The consistent patterns in these studies is that adenoma regression occurred within a year of surgery and in both studies, the observed regression was temporary. In the Feinberg study,

55% of individuals with complete resolution redeveloped polyps at a median follow up of 6.8 years. Several theories have been developed to explain the observed regression after colectomy. They include: (1) removal of the colon is thought to induce changes in the faecal flora and bile acid which affects rectal mucosal proliferation and polyp development and (2) increased alkalinity in the ileal content destroys polyps in the rectum [327]. Although in the Shepherd study, rectal regression occurred in patients who underwent a 2-stage procedure in which an end ileostomy was initially formed for a few months prior to IRA [327]. Another suggestion was that there is a degree of reduction and rectal blood supply, however this has also been disputed because good vascularity is required for safe anastomosis. Our results did not demonstrate convincing evidence of polyp regression. In our study, only 6 individuals demonstrated a degree of adenoma regression at a rate of 1.2 polyps/year. Five of these had rate of regression ≤ 2 polyps/year and we propose this is most likely due to observer bias during counting of adenomas rather than actual regression.

Eight individuals received chemoprevention for a portion of the study period. Chemoprevention is thought to reduce adenoma size thus making them flatter and smaller; this means that the adenomas can be more difficult to detect during flexible sigmoidoscopy and as such a “reduction” in polyp count may be artefactual rather than a real effect. It is not possible to interpret the findings of those on “chemoprevention” in a meaningful manner; their phenotype is likely to have been more severe initially, leading to the use of chemoprevention and the drug was stopped in most. Tonelli observed a reduction polyp

count and size in individuals on chemoprevention six months after TC-IRA, however, in all patients adenoma number and size increased again after a mean follow-up of 4 years [334].

Increase in adenoma enumeration and size is the commonest indication for secondary proctectomy. Of the 229 patients who underwent proctectomy in the Bulow study [274], 163 (71%) was due to severe rectal polyposis and 7 (3%) for functional problems. Compared to gastric and duodenal surveillance, the role of therapeutic endoscopic procedures in management of rectal adenoma burden in FAP is poorly reported. Gleeson et al [296] demonstrated positive outcomes in controlling rectal polyp burden and reducing the risk of histological adenoma progression when surveillance is combined with therapeutic procedures such as snare polypectomy [296]. Overall, a total of 13,527 polyps were successfully removed with a median of 35 polyps/patient (range 0- 829). Historically, some of these patients would have been triaged for completion proctectomy. Cold snare polypectomy and in some cases endoscopic mucosal resection (EMR) for large polyps provide a safe and less aggressive method of successfully managing the rectum until surgery is absolutely necessary [324,335,336].

Genotype and phenotype have been documented as independent predictors of advanced adenomas or need for proctectomy following TC-IRA in FAP [170,294]. In a study 427 patients who underwent IRA for FAP, Sinha et al reported that the independent predictors of rectal failure include: colonic adenoma count>500 prior to colectomy, *APC* pathogenic variants in MCR and age <25 at the time of surgery. Similarly, the rate of polyposis progression in the colorectum

prior to surgery has been shown to be independently associated with the location of pathogenic variant on *APC* gene and the number of polyps at the first colonoscopy [287]. In our study, although not statistically significant, there was a trend towards higher rate of adenoma progression in individuals with similar genotypic and phenotypic features (Table 8.4). The lack of significance is likely due to type II error due to small sample size. Further studies with larger cohort are required. Interestingly, individuals on chemoprevention were found to have a higher rate of progression compared to those without (11.3 vs 5.6; *P* 0.076). This should also be interpreted with caution due to the small numbers of patients on chemoprevention.

This study also demonstrated an important finding with regards to the fate of the rectum in individuals with constitutional pathogenic variant in the MCR. In the Bulow paper [274], the majority of secondary proctectomies were performed in patients in who underwent IRA in the prepouch era (irrespective of genotype) . In our study, 19 patients with pathogenic variants in the MCR of *APC* gene underwent TC-IRA with the majority performed in the prepouch era prior to commencement of selective approach at our institution. Of these, only four (21%) had undergone secondary proctectomy for polyp progression after a median follow-up 10 years after primary surgery. Understandably, these cohorts had more polyps removed (median 78 polypectomies/patient vs 35 polypectomies/patient) during the surveillance period. One patient had 829 polyps removed over a 20-year period (41 polypectomies/year and 22 polypectomies/flexible sigmoidoscope). These findings from a single institutional study are not sufficient to suggest TC-IRA is safe in patients with pathogenic variant in the MCR but maybe is useful when counselling patients who chose to

avoid pouch surgery at a young age due to the known complications (functional difficulties, sexual dysfunction and decreased fecundity) associated with pelvic dissection in IPAA [173] for a later stage in their lives.

## 8.9 Study Limitations

Several limitations should be considered when interpreting our findings. This is a single centre study from the oldest and one of the largest polyposis registries in the world. Although all flexible sigmoidoscopies were performed by experienced polyposis endoscopists, there is bound to be variabilities in the inaccuracies of polyp counts an estimation of polyp sizes. Furthermore, despite this being the largest study to address this topic, we acknowledge the small sample size particularly when comparing groups such as individuals on chemoprevention. Our methodology and formula also assumed linearity in the rate of polyp progression. We also did not take into consideration environmental factors such as diet and smoking that could increase or decrease the rate of adenoma development and progression. Also, this study did not take into consideration the evolution of endoscopic techniques and improvement in technology. These factors would have improved the ability to detect and manage rectal polyp progression. Further research is needed to evaluate the differences in polypectomy rates and risk of secondary proctectomy between historic and modern endoscopic eras.

## 8.10 Conclusion

This single centre study has demonstrated no evidence to support polyp regression after TC-IRA for FAP, rather adenoma progression occurs in the majority (97%) of individuals and it is relatively slow. We have also provided further evidence that selective approach coupled with stringent endoscopic surveillance and cold snare polypectomy reduces the rate of secondary proctectomy and rectal cancer after TC-IRA. In individuals who IPAA is indicated based on selective approach, IRA and surveillance plus polypectomy in the modern endoscopic era appears to delay the need for secondary proctectomy. These findings are important when counselling patients with regards to the choice of surgery for FAP and implementing the frequency of endoscopic surveillance.

# SECTION III: OPTIMIZING IDENTIFICATION AND MANAGEMENT OF OPTIMIZING LYNCH SYNDROME

## 9 Chapter 9- Utilization of mismatch repair immunohistochemistry in clinical practice – correlation between resected specimens and non-resected specimen

### 9.1 Study abstract

**Aim:** Mismatch repair Immunohistochemistry (MMR IHC) is predominantly performed on resected CRC specimens as molecular screening for LS. There are scant data to evaluate the reliability of performing MMR IHC on endoscopic biopsies (EB) and metastatic tissues. Also, the reliability of MMR IHC on resected rectal cancer cases that have undergone neo-adjuvant chemoradiotherapy is uncertain. We aimed to evaluate concordance of MMR IHC between pre-operative tissue and their corresponding surgical resection (SR) specimen.

**Methods:** Paired CRC EB, metastatic regional lymph nodes and their matched SR specimens were analysed for MMR IHC (*MLH1*, *MSH2*, *PMS2* and *MSH6*). Abnormal expression was defined as complete loss of MMR protein. Concordance between preoperative tissues and lymph nodes and corresponding SR was assessed.

**Results:** A total of 112 matched cases were analysed in two groups. In group 1, 99 CRC (48 colonic and 51 rectal) paired endoscopic and surgical cases were compared. In the 48 colon cases, 20 had abnormal MMR (dMMR) on SR (13 loss of *MLH1* and *PMS2*, 3 loss of *MSH6* and

*PMS2*, one loss of *MSH6*, one loss of *PMS2* and 2 loss of all 4 MMR proteins). Concordant staining patterns were observed in 47/48 (98%), sensitivity was 100% (95% CI 83.2-100) and specificity was 97.4% (95%CI 86.5-99.9). In the discordant case, the EB showed an isolated loss of *PMS2* and the SR demonstrated normal expression. Of the 51 rectal cancer cases, 40 (78%) had undergone neo-adjuvant chemoradiotherapy of which nine had complete pathological tumour response and were excluded. All cases were normal or proficient MMR (pMMR) and there was 100% concordance in MMR IHC status between EP and SR in all cases. In group 2, thirteen paired metastatic regional lymph nodes and SR were compared from individuals with known abnormal MMR IHC. All metastatic lymph nodes showed 100% concordance in MMR IHC staining with primary resected tumour.

**Conclusion:** MMR IHC on CRC EB and metastatic tissues appear to be as reliable as that on SR specimens. Neoadjuvant chemoradiotherapy does not appear to induce MMR protein loss, but it may result in complete tumour regression making MMR IHC impossible. Therefore, MMR IHC on EB is recommended especially in rectal cancer.

## 9.3 Introduction

Microsatellite instability (MSI) is one of the pathways of development of CRC in both sporadic and hereditary conditions via different molecular alterations [92]. Loss of function of one of the four mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6* or *PMS2* and EPCAM gene upstream of *MSH2*) leads to MSI-H phenotype. Although MSI-H phenotype is the molecular hallmark associated with LS, it is also observed in 15% of sporadic cancers [337]. The majority of the sporadic cases occur via *MLH1* promoter hypermethylation, however, recent studies have also demonstrated loss of MMR expression secondary to biallelic somatic variants [338,339]. Constitutional or germline pathogenic variant in one of the MMR genes leads to the inability to identify and repair DNA replication errors that arise in the genetic proof-reading process [94]. The resulting effect is increased mutation rates, reduced susceptibility to apoptosis and a predisposition to early onset of various cancers, particularly CRC [180,183].

Microsatellite instability testing via polymerase chain reaction is used as a screening test to identify individuals with LS. Currently, this technique is expensive and can only be performed in genetic laboratories. Mismatch repair immunohistochemistry (IHC) is fast, cost-effective with excellent sensitivity and specificity for screening CRC for dMMR [340–344]. Lindor et al [345] reported sensitivity of 92.3% and specificity of 100% which is comparable to MSI. Consequently, universal testing of all CRC cases for dMMR using IHC has been recommended by various societies and institutions to identify patients who require constitutional genetic testing for LS [346]. Identification of individuals with a dMMR tumour is not only important for screening but may also influence oncological therapy. Mismatch repair deficient cancers tend

to be more immunogenic and are thought to respond to targeted immunotherapy therapy. Therefore, availability of tumour MMR status at the time of pathology review could help decide on appropriate oncological treatment.

Currently, MMR IHC is predominantly performed on resected CRC tissue. However, this may be suboptimal or indeed impossible in some cases. For instance, some rectal cancer cases may undergo complete pathological response following neoadjuvant treatment thereby rendering the resected tissue totally void of any cancer cells for MMR IHC testing. Furthermore, the reliability of performing MMR IHC on residual CRC cancer tissue after neoadjuvant treatment is uncertain. Studies suggest chemoradiotherapy induced changes to tumour morphology [347] might alter the outcome of MMR IHC staining [347,348]. Finally, in patients presenting with advanced CRC and deemed unsuitable for surgical resection, non-resected specimen such as EB and metastatic tissues (lymph nodes, liver, lung) may be the only tissue available for MMR IHC testing.

Few studies have shown good concordance of MMR IHC status between EB and resected specimen [349–351]. To our knowledge, there has only been one published study comparing concordance between resected specimen and metastatic tissues [352]. For this reason, we aim to evaluate the concordance in MMR IHC staining between SR specimen and corresponding EB and corresponding metastatic tissues. Furthermore, we aim to evaluate concordance between pre-treatment EB and post neoadjuvant chemoradiotherapy SR rectal cancer specimen.

## 9.4 Methods

### 9.4.1 Ethical approval

Ethical approval for this study was obtained from Health Research Authority (HRA) and committee (Research Ethics Committee (REC) reference number 16/LO/1857) and Research and Development department of London North West University Healthcare NHS trust (RD16/066).

### 9.4.2 Patient selection

Colorectal cancer diagnosed between 2009 and 2019 were identified from our institution database. Patients who had both preoperative biopsy and cancer resection performed at our institution were included in the study and their EB and matched SR specimens were retrieved. A mixture of patients with dMMR and pMMR tumours were required for this study. Twenty cases of previously known dMMR on SR specimen as part of routine diagnostic assessment were randomly selected. For consistency, the MMR IHC staining on these resected tissues were re-evaluated for the purpose of this study. We also retrieved metastatic regional lymph nodes tissues from cases with advanced disease and known dMMR. Patient demographic and clinical data, histopathological characteristics (tumour location, histological characteristics), neoadjuvant therapy and presence and location of metastasis were recorded.

### 9.4.3 Immunohistochemical staining and analysis

Mismatch repair immunohistochemistry is a well-established molecular screening tool for LS CRC. Consequently, the IHC staining and interpretation were performed by an NHS approved

laboratory and gastrointestinal histopathologist respectively. This was deemed appropriate by the thesis supervisors due to the clinical and genetic implication of the results. Formalin-fixed, paraffin embedded tissue blocks containing representative tumour were retrieved from archive tissue store. Sections were cut at 3-4µm onto positively charged IHC slides (Leica BioSystems PLUS). Slides were left standing and air dried at room temperature for 30 minutes, followed by baking at 60C for one hour. Slides were carefully evaluated to ensure that invasive adenocarcinoma was present on all serial sections. Individual slides were processed for IHC (or stored at 4C until ready to use). MMR IHC staining for all four proteins was performed on the Leica Bond III platform using the Leica Refine DAB kit for antibody detection, according to manufacturer's instructions. The antibody panel used is described in Table 9.1.

**Table 9.1 Antibody panel used for MMR IHC**

| Antibody | Host species | Working dilution |
|---|---|---|
| *MLH1* | Mouse monoclonal ES05 | 1/200 |
| *MSH2* | Mouse monoclonal FE11 | 1/50 |
| *MSH6* | Mouse monoclonal EP49 | 1/50 |
| *PMS2* | Mouse monoclonal A16-4 | 1/300 |

Loss of expression of an MMR protein was defined as complete absence of nuclear staining within tumour cells in the presence of normal staining of nuclei of internal non-neoplastic

cells. Tumours showing nuclei staining for an MMR protein were classified as having no loss of MMR protein. The stained slides from EB, SR and metastatic slides were reviewed by a single blinded (i.e. without knowledge of the staining results of the samples with known dMMR) specialist gastrointestinal pathologist.

### 9.4.4 Statistical analysis

Continuous data were expressed as mean ± standard deviation or median and range whilst categorical data were expressed as frequency and percentages. Sensitivity and specificity with 95% confidence intervals were also calculated. All statistics were performed using IBM® SPSS®, Version 24.0.

## 9.5 Results

### 9.5.1 Patient demographics and clinicopathological data

A total of 112 matched cases (median age at diagnosis was 65 (range 35-95)) were included of which six (5%) cases were known to have LS. The majority of the CRC tumours (89%) were moderately differentiated adenocarcinoma (Table 9.2). Forty-six (41%) were right-sided tumours, 15 (13%) left-sided and 51 (46%) rectal. Forty (36%) of the rectal cancer cases had undergone preoperative chemoradiotherapy (Table 9.2).

The cohort were divided into two groups. In group 1, 99 SR CRC cases (48 colon and 51 rectal) with matched EB were compared. In group 2, 13 colon cancer cases with known dMMR IHC

on resected tumour and their matched metastatic regional lymph nodes tissues were compared.

| Table 9.2 Patient demographic and histopathological features | |
|---|---|
| **Variable** | **N (%)** |
| Total | 112 |
| Age at diagnosis/yrs. | 65 (35-93) |
| Known Lynch syndrome | 6 (5) |
| Tumour location | |
| Right sided | 46 (41) |
| Left sided (descending and sigmoid) | 15 (13) |
| Rectum | 51 (46) |
| Neoadjuvant treatment | 40 |
| Differentiation | |
| Well | 1 (1) |
| Moderately | 100 (89) |
| Poor | 11 (10) |
| Mucinous | 18 |
| TILS present | 6 (5) |

TILS= Tumour infiltrating lymphocytes

### 9.5.2 Group 1 Endoscopic biopsy versus surgical resected specimen

#### 9.5.2.1 *No neoadjuvant chemoradiotherapy*

Of the 99 cases in group 1, 59 (48 colon and 11 rectal) cases which had not undergone neo-adjuvant treatment were evaluated for concordance between EB and corresponding SR specimens. Of these, 20/59 (34%) showed loss of MMR protein expression on IHC on the SR specimen (Figure 9.1-Figure 9.3). Concordant staining patterns between EB and SR specimens were observed in 58/59 (98%). The sensitivity for EB was 100% (95% CI 83.2-100) and specificity was 97.4% (95%CI 86.5-99.9). In the discordant case, the EB showed a loss of *PMS2* whilst the SR had normal expression (Table 9.3). In all of the cases with known a germline or constitutional MMR pathogenic variant, both EB and SR specimens demonstrated abnormal IHC staining for the corresponding MMR protein.

**Table 9.3 Correlation of mismatch repair protein expression between EB and resected specimen in group without neoadjuvant treatment**

| MMR protein loss on resected specimen | Number of cases | MMR protein loss on biopsy | Number of cases | Concordance |
|---|---|---|---|---|
| *MLH1+PMS2* | 13 | *MLH1+PMS2* | 13 | Yes |
| *MSH6+PMS2* | 3 | *MSH6+PMS2* | 3 | Yes |
| *MSH6* | 1 | *MSH6* | 1 | Yes |
| *PMS2* | 1 | *PMS2* | 1 | Yes |
| All 4 MMR proteins | 2 | All 4 MMR proteins | 2 | Yes |
| Normal | 1 | *PMS2* | 1 | No |
| None | 39 | None | None | yes |

#### 9.5.2.2 *Neoadjuvant chemoradiotherapy*

Forty cases had undergone neoadjuvant treatment prior to tumour resection of which nine had complete pathological response and were therefore excluded from the analysis. All of the remaining 31 cases had normal MMR IHC and there was 100% concordance in MMR IHC status between EB and post-neoadjuvant therapy SR indicating a specificity of 100%.

### 9.5.3 Group 2- Resected specimen versus metastatic specimen

A total of 13 cases with known abnormal MMR IHC on SR specimen had metastatic regional lymph nodes tissues available for analysis. The MMR protein losses are described in Table 9.4.

All cases showed 100% concordance between primary resected tumour and corresponding metastatic lymph nodes.

**Table 9.4 Concordance of mismatch repair protein expression between resected specimen and corresponding metastatic lymph nodes**

| MMR protein loss on resected specimen | Number of cases | Concordance with metastatic lymph node |
|---|---|---|
| *MLH1* | 3 | Yes |
| *MSH2* | 1 | Yes |
| *MSH6* | 1 | Yes |
| *MLH1* & *PMS2* | 4 | Yes |
| *MSH2* & *MSH6* | 4 | Yes |

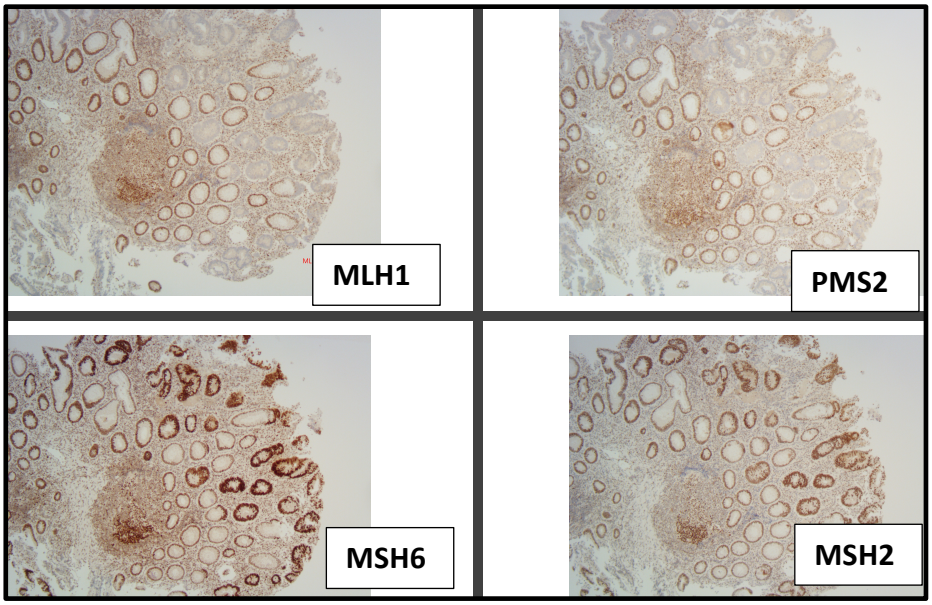



**Figure 9.1 H&E micrograph of colorectal adenocarcinoma resected specimen exhibiting normal mismatch repair immunohistochemistry staining**

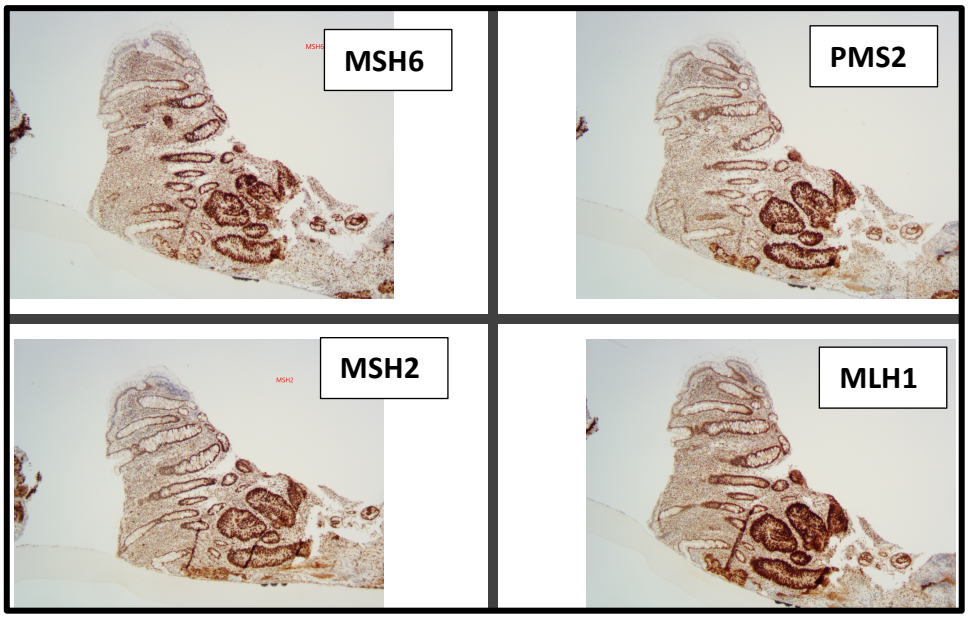



**Figure 9.2 H&E micrograph of colorectal adenocarcinoma endoscopic biopsy exhibiting normal mismatch repair immunohistochemistry staining**

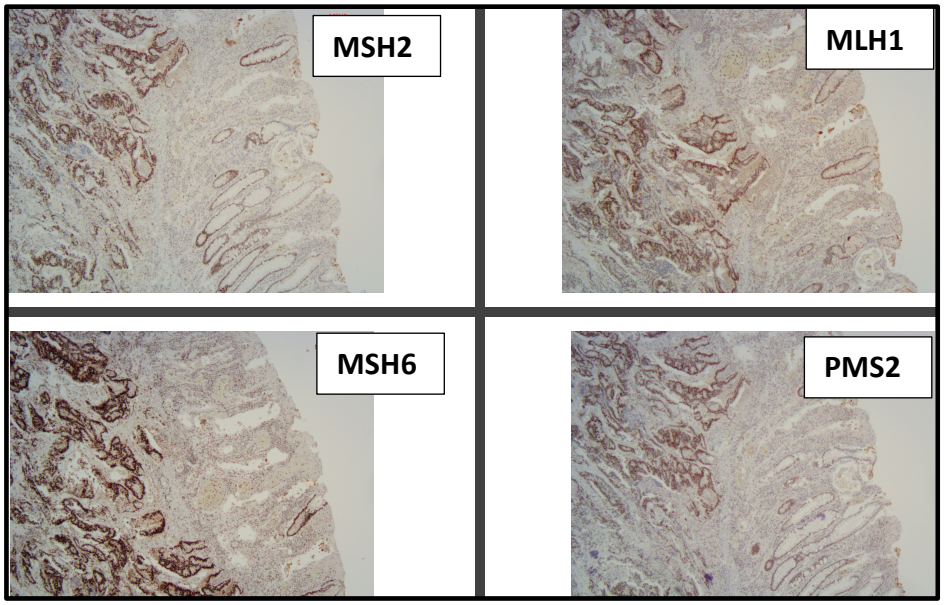
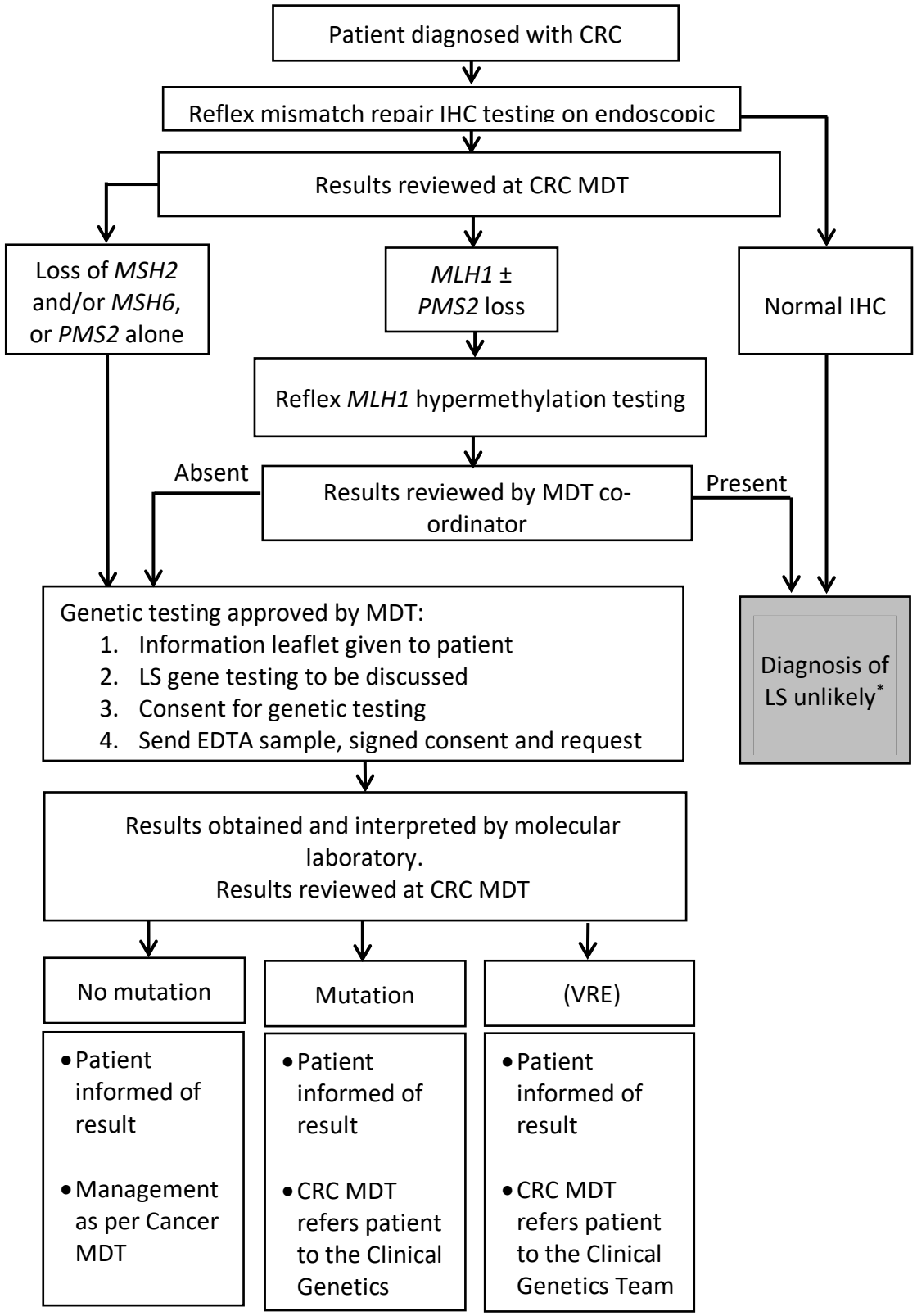



**Figure 9.3 H&E micrograph of colorectal adenocarcinoma exhibiting abnormal mismatch repair immunohistochemistry in all 4 MMR proteins**




***** Consider referral to Genetics if early-onset (<50yrs), significant family history, or multiple cancer diagnoses
VRE -Variant requiring evaluation

**Figure 9.4 Recommended algorithm for mismatch repair immunohistochemistry testing for Lynch syndrome using endoscopic biopsies**

## 9.6 Discussion

This study sought to evaluate concordance between SR CRC specimen and pre-operative EB or metastatic samples. Our findings confirm previous findings of excellent concordance between EB and corresponding SR tissues [349–351,353]. In addition, we did not any find any evidence to suggest that neoadjuvant chemoradiotherapy induces MMR protein loss in rectal cancer tissues. We have also demonstrated that regional lymph node samples are a reliable alternative for MMR IHC in individuals with advanced CRC not suitable for surgical management. Although loss of MMR protein on IHC does not guarantee a diagnosis of LS (especially when loss of *MLH1+/-PMS2* is demonstrated), performing MMR IHC on EB has the advantage of being available a few weeks in advance, thereby initiating earlier genetic testing if required. In cases where MMR IHC demonstrates a loss of *MLH1 +/- PMS2*, the first line would be to perform $BRAF^{V600E}$ mutational analysis or hypermethylation studies to determine whether promoter hypermethylation of the gene is present before proceeding to genetic sequencing [354]. In a population-based screening program for LS in Australia, 75% of individuals with loss of expression for *MLH1* or its partner (*PMS2*) expression on MMR IHC demonstrated $BRAF^{V600E}$ pathogenic variant [355].

It is well-established that LS is associated with an increased risk of developing metachronous CRC (mCRC) especially in individuals with *MLH1* and *MSH2* pathogenic variant. Consequently, some authors and institutions have suggested pre-operative testing in patients with CRC to facilitate decision making with regards to extended rather than segmental colectomy especially in young adults [218]. This is currently not applicable in clinical practice because of

the time it takes to obtain genetic testing. Although this might be possible in patients where surgery is delayed for administration of neoadjuvant therapy. Abnormal MMR IHC is not a diagnosis of LS and should therefore not be used in isolation to decide the extent of surgical resection. In the setting of a very strong family history of LS, abnormal IHC in a young patient may prompt MDT discussion and consideration of extended resection especially in young patients with *MLH1* and *MSH2* pathogenic variant. There is currently insufficient evidence to demonstrate any benefit of extended colectomy in *MSH6* and *PMS2* carriers.

Although several studies have demonstrated good concordance between EB and SR, the majority have also reported few cases of discordance between known germline pathogenic variant status and IHC. In the study by Warrier et al [351], of the 66 cases compared, two patients with known germline pathogenic variant demonstrated discordant results between known germline pathogenic variant status and MMR IHC status on preoperative EB. In both cases (*PMS2* and *MSH6*), the biopsy tissue demonstrated the presence of all four MMR proteins. This discrepancy could be explained by the presence of missense pathogenic variant in which the protein although present and can identified by the antibody used in the assay, but it is non-functional [351]. Salahshor et al [356] also described intact *MLH1* staining in 2 out of 15 cases with known *MLH1* germline pathogenic variant. Our study demonstrated 100% concordance in all of cases of known germline pathogenic variant and the corresponding loss of MMR protein on IHC. Similarly, discrepancies in IHC between biopsies and resected specimens have been reported. In the Shia study, discordance was observed in 7.2% of cases. The authors attributed this to tissue artefact secondary to neoadjuvant treatment. We

identified one patient had abnormal isolated staining for *PMS2* in EB despite normal staining in SR specimen. On the basis of the clinical implication of this finding, we informed the responsible the clinician for further investigation of this patient.

The discordance or discrepancies observed in interpretation of MMR IHC could be attributed to poor staining quality which is usually due to the suboptimal tissue fixation in formalin. Surgical resections tend to have poorer tissue fixation due to the large size and delay in fixation after surgery. In contrast, EB are smaller in size with a larger surface area which results in better formalin fixation making IHC staining and interpretation easier. As a result, EB is certainly as good as a SR and indeed might be preferable for MMR IHC testing. Kumarasinghe et al [349] observed better uniformity of staining in EB compared to SR. They concluded that the accuracy and ease of interpretation of IHC staining was better in EB compared to SR. Similarly, Viking et al [353] demonstrated significantly higher qualitative scoring of all MMR protein IHC staying in endoscopic material compared to SR ($P<0.001$).

Another advantage of performing MMR IHC on endoscopic tissue is the availability of tissue containing cancer cells in patients who have undergone neoadjuvant treatment for rectal cancer. In individuals who have had complete pathological response to neoadjuvant treatment, there might be no tissue available for analysis. In our cohort, nine cases were excluded following neoadjuvant treatment due lack to tumour cells for staining in the SR. Furthermore, chemoradiotherapy is thought to induces changes in CRC which is likely to make interpretation of staining more difficult and in some cases alter the MMR repair protein

expression [347,357]. In a study comparing MMR IHC staining in pre-neoadjuvant and corresponding post-neoadjuvant tissue in 32 rectal cancers with and a control group of 39 patients without neoadjuvant treatment, Vilkin et al found significantly higher discordance in the neoadjuvant group (18.5%) compared to the control group (7.7%) (P= 0.009)[348]. Despite these advantages, availability of endoscopic tissues could pose some difficulties. The volume of tissue biopsied during colonoscopy or flexible sigmoidoscope depends on the size, site, accessibility and location of the cancer in colorectum. Encouraging larger or multiple tumour biopsies could be beneficial for MMR IHC testing.

MMR IHC on biopsies is not only important for LS screening but may also influence oncological therapy in a multidisciplinary (MDT) setting. Approximately 20% of patients diagnosed with CRC have inoperable advanced disease at the time of presentation. In some of these cases, the preoperative tissue might be the only tissue available for analysis or screening. Patients with Dukes B CRC and poor histological features would usually be eligible for single agent 5-fluorouracil (5-FU) treatment. Studies have demonstrated that individuals with MMR deficient tumours are less likely to benefit from 5-FU based adjuvant or neoadjuvant chemotherapy [125,257,358]. Also, MMR deficient tumours have recently been shown to have better response to PD-1 inhibitor therapy [359,360]. Therefore, availability of results of IHC at the time of multi-disciplinary team discussion would facilitate prompt commencement of appropriate oncological treatment. In some cases of advanced disease, metastatic tissues such as lymph node, liver or lung biopsies might be the initial or only tissue available for analysis. In our results, we have demonstrated that metastatic regional lymph nodes can be

reliably used to detect MMR deficiency. Unfortunately, we did not have solid organ (liver or lungs) biopsies to compare but we believe this will be equally as reliable as demonstrated by Haraldsdottir study [352]. In their study, the authors reported 100% concordance between 50 metastatic tissues (26 regional lymph nodes and 24 metastatic tissue) and their corresponding primary resection.

The MMR protein is known to function as two dimers of *MLH1*/*PMS2* and *MSH2*/*MSH6*. The stability of *PMS2* requires and intact *MLH1* and stability of *MSH6* requires an intact *MSH2*, however, *MLH1* and *MSH2* function can be maintained despite the loss of their corresponding dimers [361]. However, MMR IHC can produce unusual results and patterns making interpretation in clinical practice complicated. One example is the concurrent loss of 4 MMR protein sometimes referred to as MMR protein 'null' phenotype. This was observed in 2 cases in our study (Table 9.3). To our knowledge there have been only 2 published cases describing this phenotype. Wang et al reported a case of an 80-year-old lady with colon cancer that exhibited 'null' IHC staining pattern. Subsequent testing revealed that the loss of all four proteins was due to concurrent promoter hypermethylation of *MLH1* and bi-allelic somatic truncating pathogenic variants in *MSH2* [361]. In the other study by Hagen et al, the pattern was due to germline pathogenic variant of *MSH2* and somatic loss of *MLH1*[339]. Also, discordance between MMR IHC findings and MSI status have been reported by several clinical studies. In a larger series by Lindor et al [345], 27/818 tumour were found to be MSI high despite showing intact staining for *MLH1* and *MSH2*. This limitation in IHC assay has led to some authors

recommending increase awareness of unusual staining patterns and supplementing MMR IHC with MSI and where possible next-generation sequencing [351].

## 9.7 Study limitations

Limitations of the study include its retrospective nature, small sample size, single center experience. Also, in the neoadjuvant group, we did not assess whether neoadjuvant chemoradiotherapy reverses MMR protein loss in tumours from individuals with known germline pathogenic variant or loss of MMR on endoscopic biopsy. We could not assess the reliability of MMR IHC on metastatic tissue from solid organs such as omentum, liver or lung due to unavailability of matched specimen. Further studies are required to evaluate this. Finally, although we defined the outcome of MMR IHC as either present or absent, we did not evaluate the quality of the staining. Studies have shown that in cases of focal and or weak, patchy or indeterminate IHC staining can be indicative of d-MMR. Sarode et al [362] demonstrated that 34% of cases with indeterminate IHC expression were found to have LS. The authors concluded that guidelines for interpretation of equivocal MMR IHC staining are necessary to improve the sensitivity and specificity of IHC assay.

## 9.8 Conclusion

Our study suggests that MMR IHC on endoscopic biopsies and metastatic tissue appears to be as reliable, or better as that on surgical resected specimens. This is clinically relevant when screening for LS and planning oncological therapy. In addition, although neoadjuvant chemoradiotherapy does not appear to induce MMR protein loss, several cases had complete

pathological response and were ineligible for MMR IHC on surgical. Therefore, in the setting of rectal cancer, MMR IHC on endoscopic biopsies is recommended. Based on our findings, we propose a diagnostic and management algorithm for screening for LS. Consequently, we recommend a modification of current mainstreaming screening for LS using EB rather than resected as demonstrated in Figure 9.4.

# 10 Chapter 10 -Risk of metachronous colorectal cancer following colectomy in Lynch syndrome: a systematic review and meta-analysis

## 10.1 Abstract

**Aim**: Lynch syndrome (LS) accounts for 2-4% of all colorectal cancer (CRC) cases, and is associated with an increased risk of developing metachronous colorectal cancer (mCRC). The role of extended colectomy (EXTC) in LS CRC is controversial. There is limited evidence comparing the risk of mCRC following segmental (SEGC) and EXTC. The objective of this systematic review is to evaluate the risk of developing mCRC following SEGC and EXTC for LS CRC and endoscopic compliance

**Method**: A systematic review of major databases was performed using predefined terms. All original articles published in English comparing the risk of mCRC in LS patients after SEGC and EXTC from 1950 to January 2016 were included.

**Results**: The search retrieved 324 studies. Six studies involving 871 patients met the inclusion criteria. 705 (80.9%) underwent SEGC and 166 (19.1%) EXTC. Average follow-up was 91.2 months. mCRC rate was 22.8% and 6% in the SEGC and EXTC groups respectively. SEGC group were over four times more likely to develop mCRC (OR 4.02, 95% CI: 2.01-8.04, $p<0.0001$). mCRC occurred in patients after SEGC despite 1-2 yearly postoperative endoscopic surveillance.

**Conclusion**: This result suggests that EXTC reduces the risk of mCRC by over four-fold compared to SEGC. mCRC occurred in the SEGC group despite postoperative endoscopic surveillance. This needs to be considered when deciding the appropriate surgical management of LS patients with CRC. We recommend that EXTC should be considered for patients with confirmed LS CRC.

## 10.2 Introduction

Regular high-quality colonoscopy surveillance has been shown to reduce the lifetime risk of developing CRC in individuals with LS [192]. The current guidelines recommend 1-2 yearly colonoscopies in affected individuals [363]. Consequently, there is insufficient evidence to recommend prophylactic colectomy in these patients. In the event of CRC occurrence, the appropriate extent of surgical resection has been controversial due to the elevated risk of developing mCRC. The primary aim of surgery is to treat the cancer by removing the site containing tumour and its surrounding lymph nodes. In colon cancer, segmental resection such as right hemicolectomy, extended right hemicolectomy, left hemicolectomy or sigmoid colectomy are the standard of care. Similarly, in rectal cancer, anterior resection or abdominoperineal excision are offered depending on the site of the cancer. However, in LS, to reduce the risk of mCRC and the need for future surgical resection, extended resection such as TC-IRA, subtotal colectomy and ileosigmoid anastomosis have been suggested. This option has to be balanced with the increased morbidity associated with extended resections and potentially poorer bowel function [203]. Regular endoscopic surveillance of the remaining colon or rectum is strongly recommended in either case [364].

There have been no RCT comparing SEGC and EXTC in management of LS related CRC. The evidence for EXTC arises largely from retrospective studies and level III expert recommendation. The primary aim of this systematic review and meta-analysis is to evaluate the risk of developing metachronous colorectal cancer following limited (segmental) and

extended colectomy in patients with LS. We also aim to evaluate the compliance with endoscopic surveillance and staging of mCRC at the time of diagnosis.

## 10.3 Methods

A systematic review was performed in adherence with the Preferred Reporting Items for systematic Reviews and Meta-Analysis (PRISMA) statement [365].

### 10.3.1 Search strategy and information sources

The search strategy was designed by two authors according to the PRISMA guidelines. The following databases were searched for articles: Embase (1950 to present), MEDLINE from PubMed (1950 to present), Google Scholar and Cochrane Data-base of Systematic Reviews.

The search terms were devised to cover Lynch syndrome, metachronous colorectal cancer and surgery or colectomy. This was performed by using the following text words (including their synonyms/variants) and Medical Subject Headings (MESH terms): ‘metachronous’, ‘colorectal’, ‘neoplasms’, ‘hereditary non-polyposis colorectal cancer’, 'Lynch syndrome’, ‘colectomy’ and ‘bowel resection’. The search terms were combined using the Boolean AND/OR operators.

Articles were also sought by hand-searching the reference lists of the selected articles and included if they met the inclusion criteria. The last search date was January 15th 2016.

### 10.3.2 Study selection

All articles published in English language between 1950 to January 2016 were included in the review. Other inclusion criteria were: studies which reported CRC in patients with LS, who underwent treatment in the form of surgical resection or colectomy (segmental, subtotal or total) and subsequent development of metachronus colorectal cancer. Studies were included if the patients have proven germline pathogenic vaiants in one of the five genes known to alter mismatch repair function.

Exclusion criteria included: case reports, conference abstracts and review articles. We also excluded studies that did not specify the management of the CRC (i.e. polypectomy or surgery) or extent of surgical resection (segmental and extended) for the index CRC or mCRC. Studies that reported mCRC in patients from families meeting the Amsterdam criteria, without evidence of a germline mutation affecting mismatch repair status, were also excluded.

The two reviewers independently performed the searches. Each author screened titles and abstracts for relevance and excluded studies that did not meet the inclusion criteria. Differences in selected studies were discussed between the two reviewers and a consensus was reached. In the case of dispute, senior reviewers (supervisors) acted as adjudicators.

### 10.3.3 Data collection

The following data were extracted from the selected studies: year of publication, authors' name, country and institution, number of patients, patient demographics, site of index

cancer, type of colectomy, duration of endoscopic follow up, rate of mCRC, endoscopic compliance, stage of mCRC and interval between index cancer and mCRC. For the purpose of this study, SEGC includes: all hemicolectomies, anterior resection of rectum and abdominoperineal excision of rectum. Extended colectomy was defined as either subtotal colectomy with ileosigmoid anastomosis or total colectomy with ileorectal anastomosis. Panproctocolectomy or restorative proctocolectomy were not included as these should completely abolish the risk of mCRC by removing the entire large bowel and rectum.

### 10.3.4 Quality assessment and bias

The quality of the studies was assessed by the two authors using a Modified Newcastle-Ottawa Scale (NOS) for cohort studies. This was evaluated by examining three factors: patient selection, comparability of segmental and extended colectomy and assessment of outcome (in this case mCRC). The maximum available score for each study is nine points.

### 10.3.5 Statistical and Sub-Group Analyses

Data from the included studies were summarised and collated in a Microsoft Excel spreadsheet. Basic descriptive statistics such as percentages and weighted averages were used to summarise the data. For data analysis, Microsoft Excel (Microsoft Redmond, Washington USA) and the software package RevMan 5 version 5.3.5 (The Cochrane Collaboration, http://www.cc-ims.net/RevMan) were used. The Odds Ratio (OR) of developing mCRC in segmental versus extended colectomy was calculated for each study using a random effect model. The Confidence Interval (CI) was set at 95% and a p value of

0.05 or less was deemed statistically significant. In addition, sub-group analyses based on study quality using the validated NOS as well as study size (number of patients in study) were carried out.

## 10.4 Results

Figure 10.1 details the study selection flow chart. The search strategy retrieved a total of 324 studies, of which 312 were identified electronically and 12 were obtained by searching the references of retrieved articles. Of these, 295 were excluded as they did not meet the eligibility criteria. Of the 29 remaining studies, 17 were excluded as they did not specify the type of treatment for the index CRC (n=12), were systematic reviews (n=2), case reports (n=1) or the cohort studied was not specifically LS (n=2), leaving a total of 12 studies. Of these, six were excluded because they reported mCRC in patients from families meeting the Amsterdam criteria, leaving a total of six studies which were examined fully and included in the data synthesis. One of these studies was excluded from the meta-analysis as they only reported rates of mCRC after SEGC for rectal cancer and did not offer a comparative mCRC rate for the total colectomy group.

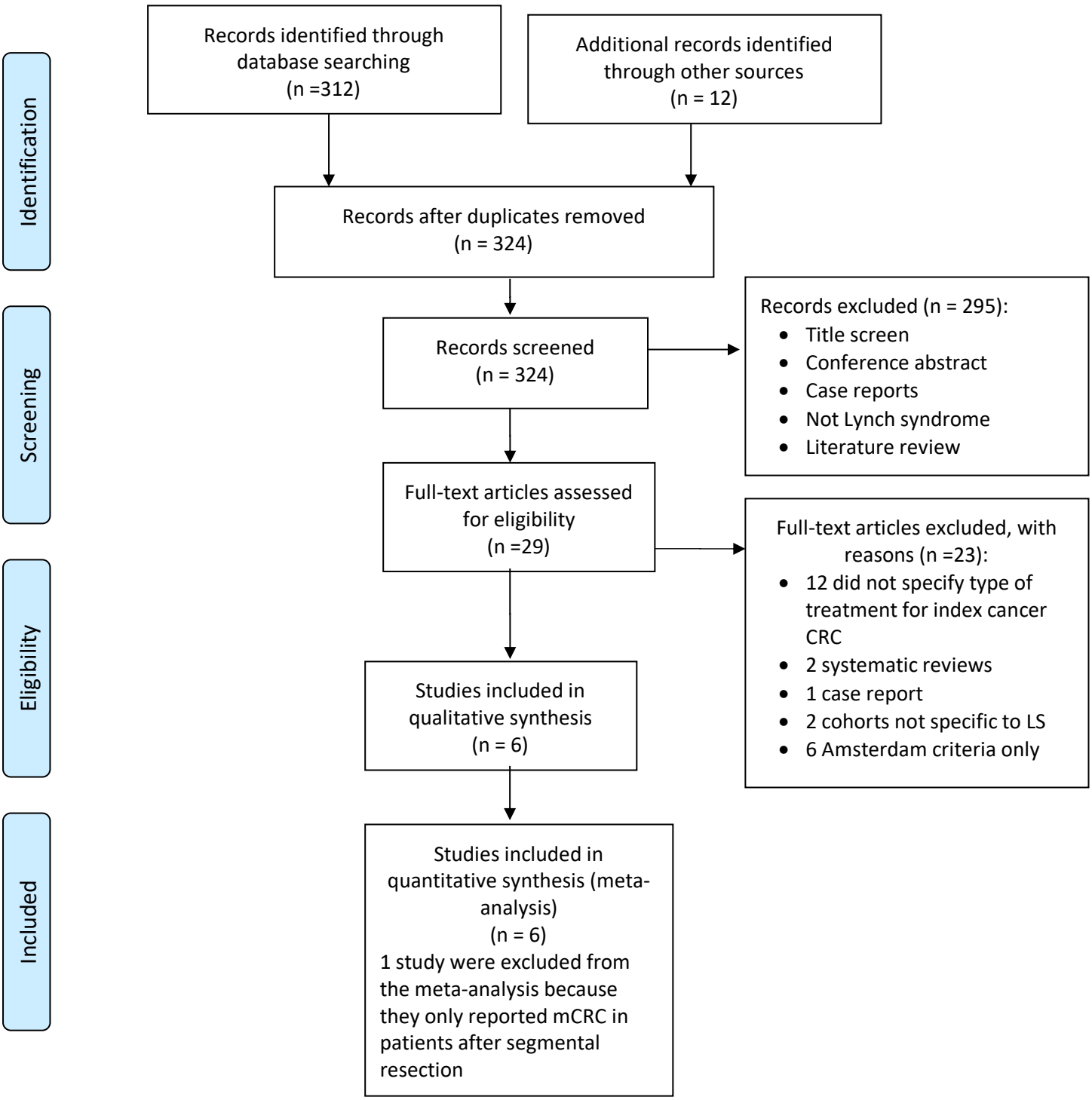



**Figure 10.1 PRISMA flow diagram to demonstrate the selection of studies**

### 10.4.1 Study characteristics

Across the six original studies, a total of 871 patients with LS underwent bowel resection (segmental, subtotal or total colectomy) for CRC (Table 10.1). Across the studies, the average age at the time of index operation was 36.4 years and 35.1% of subjects were male. A total of 705 (80.9%) and 166 (19.1%) patients underwent segmental (SEGC) and extended (EXTC) colectomy respectively. The weighted average duration of follow up was 91.2 months.

All the included studies were retrospective reviews of prospectively collected data from family cancer databases or registries. Three studies [193,366,367] reported mCRC in patients with germline pathogenic variant in any of the four MMR genes. Two studies [200,368] reported on patients with *MLH1* and *MSH2* gene pathogenic variants only. Aronson et al [369] reported mCRC in individuals less than 35 years old with proven mutation in any of the four MMR genes.

**Table 10.1 Study characteristics**

| Author | Year | Country | n | Mean age (years) | Male (%) | Segmental Colectomy (n) | Extended Colectomy (n) | Median Follow-Up (months) |
|---|---|---|---|---|---|---|---|---|
| De Vos tot Nederveen [193] | 2002 | Netherland | 139 | NA# | NA† | 110 | 29 | NAø |
| Natarajan [200] | 2010 | USA | 106 | 45.5 | 39.6 | 69 | 37 | 144 |
| Stupart [368] | 2011 | South Africa | 60 | 42.5 | 56.7 | 39 | 21 | 87.6 |
| Parry [366] | 2011 | New Zealand | 382 | 46 | 51 | 332 | 50 | 106.4 |
| Win [367] | 2013 | New Zealand | 79 | 46.2 | 44.3 | 79 | 0 | 132 |
| Aronson [369] | 2015 | Canada | 105 | 29.7 | NA | 76 | 29 | 74.4 |
| **Summary *** | | | **871** | **36.4** | **36.11** | **705** | **166** | **91.17** |

*Weighted means
NA - Not Available
# mean age reported separately: 46 (range 24-78) and 46 for segmental and extended group respectively
† % male only reported for extended group (51.7%)
ø 6.8 years and 5 years for segmental and extended group respectively

Of the six studies, one scored the maximum nine points (Parry). Another three studies scored eight points (Stupart, Natarajan, Win) whilst two scored seven points (De Vos tot Nederveen and Aronson) (Table 10.2**).**

**Table 10.2 Assessment of study quality using the Newcastle-Ottawa Scale**

| | | Selection | | | | Comparability | Outcome | | | Total Score |
|---|---|---|---|---|---|---|---|---|---|---|
| **Studies** | **year** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | |
| De Vos tot Nederveen | 2002 | * | * | * | * | * | * | | * | 7 |
| Natarajan | 2010 | * | * | * | * | * | * | * | * | 8 |
| Stupart | 2011 | * | * | | * | ** | * | * | * | 8 |
| Parry | 2011 | * | * | * | * | ** | * | * | * | 9 |
| Win | 2013 | * | * | * | * | * | * | * | * | 8 |
| Aronson | 2015 | | * | * | * | * | * | * | * | 7 |

### 10.4.2 Follow- up and rate of metachronous cancer

The average (weighted mean) length of follow up was 91.7 months (range 74.4-144). In this period, mCRC occurred in 19.6% (n=171) of the total population after colectomy (Table 10.3). The rate of mCRC was 22.8% among patients who underwent SEGC and 6% in those who had EXTC. Using the random effect model on five out of six studies (Figure 10.2) that adequately compared the two types of resection, the SEGC group were more than four times more likely to develop mCRC (OR 4.02, 95% CI: 2.01-8.04, p<0.0001).

**Table 10.3 Metachronous CRC (mCRC) after segmental and extended colectomy in Lynch syndrome**

| Author | Year | n= | SEGC (n=) | mCRC after SEGC: n (%) | EXTC (n) | mCRC after EXTC n (%) |
|---|---|---|---|---|---|---|
| De Vos tot Nederveen [193] | 2002 | 139 | 110 | 13 (11.8) | 29 | 1 (3.5) |
| Natarajan [200] | 2010 | 106 | 69 | 23 (33.3) | 37 | 4 (10.8) |
| Stupart [368] | 2011 | 60 | 39 | 8 (20.5) | 21 | 2 (9.5) |
| Parry [366] | 2011 | 382 | 332 | 74 (22.3) | 50 | 0 |
| Win [367] | 2013 | 79 | 79 | 21 (26.6) | 0 | nr |
| Aronson [369] | 2015 | 105 | 76 | 22 (29.0) | 29 | 3 (10.3) |
| **Summary*** | | **871** | **705** | **161 (22.8)** | **166** | **10 (6.0)** |

*Weighted mean

NR - Not Reported

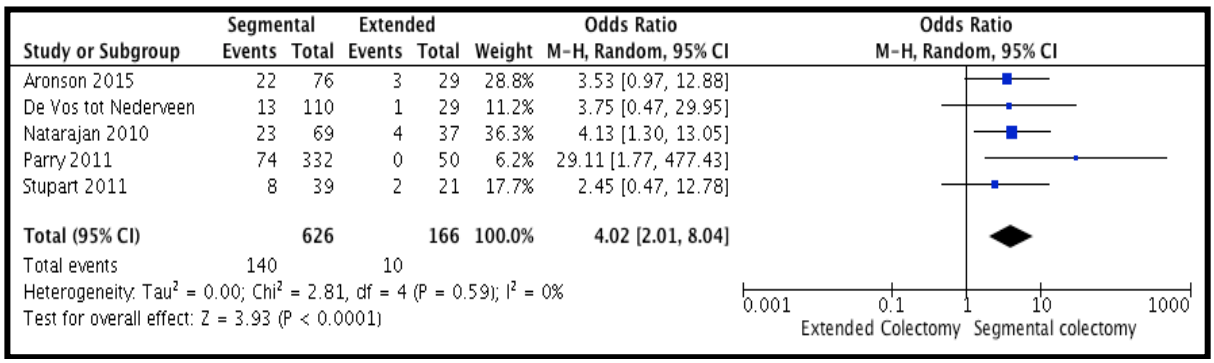



**Figure 10.2 Forest plot showing pooled Odds ratio of developing metachronous colorectal cancer after segmental and extended colectomy**

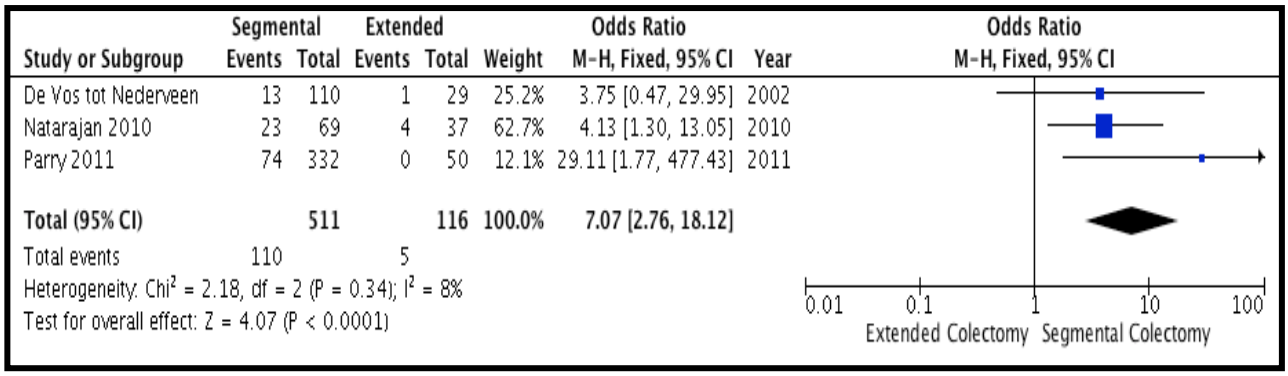



**Figure 10.3 Forest plot showing pooled Odds ratio of developing metachronous colorectal cancer after segmental and extended colectomy based on the three largest studies**

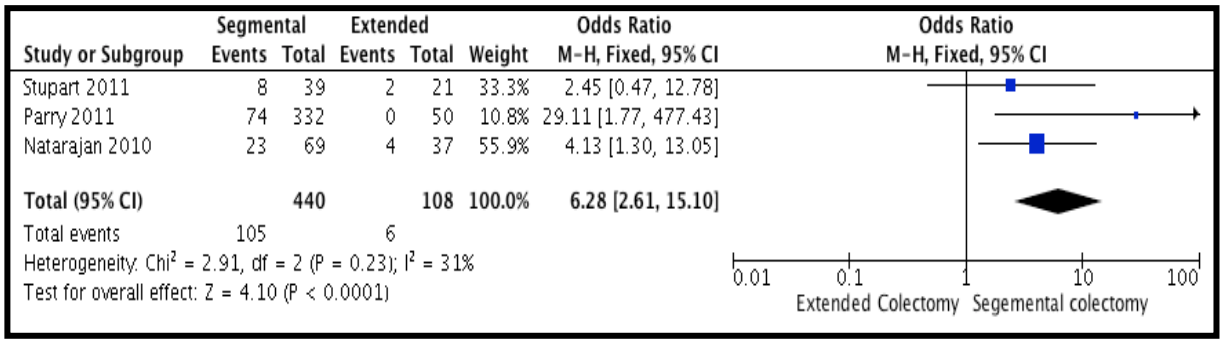



**Figure 10.4 Forest plot comparing risk of mCRC in segmental and extended colectomy based on the three highest-scoring studies on the Newcastle–Ottawa scale**

### 10.4.3 Frequency of endoscopic surveillance and metachronous cancer

Endoscopic follow-up was reported in three of the six studies. Parry et al [366] reported the frequency of colonoscopy or sigmoidoscopy in 57 patients who developed mCRC. Of these, 77.2% (44) were undergoing one to two yearly endoscopic surveillance. Similarly, Win et al [367] reported that 78.9% of patients who developed mCRC underwent one to two yearly surveillance colonoscopy. In the Stupart study [368], two of the eight mCRC in the SEGC group were diagnosed in patients who developed symptoms less than a year after a normal colonoscopy. The remainder developed in patients who had defaulted colonoscopy surveillance for at least two years. In the EXTC group, one mCRC developed one year after a normal surveillance sigmoidoscopy and the other in a patient who had defaulted surveillance sigmoidoscopy for 4 years.

### 10.4.4 Sensitivity analysis and publication bias

Sensitivity analysis for the odds of developing mCRC after segmental and extended colectomy was performed to evaluate the stability of the result. When the largest three studies were analysed (Figure 10.3), the risk of mCRC was greater in the segmental group (OR 7.07, 95% CI: 2.76-18.12, p<0.0001). Similarly, analysis of the top three studies on the basis of the NOS assessment also generated a similar result (OR 6.28, 95% CI: 2.61-15.10, p=0.0001) (Figure 10.4). Publication bias could not be assessed because there were fewer than 10 included studies.

## 10.5 Discussion

The findings of this systematic review and meta-analysis are that CRC patients with LS who have a SEGC are four times more likely to develop mCRC than patients who have EXTC. This is also about four times more than is described in sporadic CRC overall [370]. The sub-group analyses of the largest published studies and the highest quality studies showed a higher odd of 7.3 and 6.8 respectively. Furthermore, mCRC occurred in some patients after SEGC despite adequate postoperative endoscopic surveillance (1-2 yearly). Although segmental resection remains the mainstay of managing sporadic CRC, given the potential for mCRC, extended resection such as subtotal colectomy or total colectomy with ileorectal anastomosis should be considered and discussed in patients with LS related CRC [364]. The majority of the recommendations outlining best practice in terms of surgical resection and endoscopic follow up of LS patients comes from retrospective studies and level III recommendation. As yet, there

have been no RCT comparing segmental resection to extended resection or extended resection versus endoscopic surveillance.

In 2013, Henegan and colleagues [371] published a systematic review and meta-analysis of six studies, comparing the rate of metachronous adenoma and carcinoma after segmental and extended resection in patients with hereditary non-polyposis colorectal cancer (HNPCC). Although the authors demonstrated the mCRC rate to be 23.5% and 6.8% in segmental and extended group respectively, there was no statistical difference between the two groups. Furthermore, three out of the six studies in their review included patients from families which fulfilled the Amsterdam criteria only, without evidence of MMR deficiency. However, meeting the Amsterdam criteria does not confer definite diagnosis of LS. Indeed in one study [372], 60% of the patients with CRC who were from families that fulfil the Amsterdam criteria did not actually have features of MMR deficiency. Using the Amsterdam criteria alone, therefore, gives a very heterogenous cohort, including not only LS but also conditions such as familial colorectal cancer type X, which has a lower rate of mCRC than LS. This is a flaw in the systematic review performed by Henegan and colleagues. Our 6-study review of the risk of mCRC in patients with a confirmed genetic diagnosis of LS, shows that the rate of mCRC after SEGC compared with EXTC was 22.8% vs 6% respectively. Our meta-analysis suggests that EXTC decreases the risk of developing mCRC by just over four-fold compared to SEGC in LS CRC. These findings must be interpreted with caution as it is difficult to quantify the patients' background risk of cancer, which might vary with germline pathogenic variant, compliance

with colonoscopy surveillance, availability of preoperative LS diagnosis and site of index cancer.

The lifetime CRC risk varies depending on the MMR gene affected with *MLH1* and *MSH2* conferring the highest cancer risk [180,183]. *MLH1* and *MSH2* pathogenic variants lead to higher degree of penetrance and therefore increased risk of CRC compared to *MSH6* and *PMS2* [343]. In our systematic review, only two studies [366,367] reported mCRC according to the individual MMR gene (*MLH1*, *MSH2*, *MSH6* or *PMS2*) involved. Parry et al [366] showed that of the 74 patients with mCRC following SEGC, 33 (45%) were *MLH1*, 38 (45%) *MSH2* and three (4%) *MSH6* gene pathogenic variant carriers. No mCRC occurred in the *PMS2* group although the numbers were small. Similarly, Win reported mCRC in five (23.8%) *MLH1* and 16 (77%) *MSH2* pathogenic variant carriers. This supports the observation of a more severe phenotype associated with pathogenic variants in *MLH1* and *MSH2*. Furthermore, Stupart [368] and Natarajan [200] only reported on patients with *MLH1* and *MSH2* gene pathogenic variants. This could be a potential source of bias as it would suggest that surgical decision making should be influenced by germline pathogenic variants, however the data are probably not robust enough to make firm recommendations. Further studies quantifying gene-specific risk are warranted.

Several studies have shown that regular colonoscopy surveillance reduces the incidence of LS CRC and its' related mortality [192,194,196,197,373] and shorter interval (1-2 yearly) between colonoscopies is associated with early tumour stages in patients under surveillance [194,197,364].

However, in our study, Parry and Win reported mCRC in 77.2% and 78.9% of SEGC patients undergoing 1-2 yearly postoperative endoscopic surveillance respectively. These findings might be explained by quality of the endoscopic surveillance and the rapid adenoma-carcinoma sequence associated with LS [194,196]. Identification and removal of these adenomas via improved endoscopic techniques such as pan-colonic chromoendoscopy could decrease the overall risk of developing interval CRC [192,196,374]. In addition to regular endoscopic surveillance, the CAPP2 randomised control trial [234] demonstrated that chemoprevention with aspirin reduces the risk of developing CRC in LS. Their results showed that after a mean follow up of 55.7 months, 600mg of Aspirin daily has a protective effect against colorectal cancer with an incidence rate ratio of (IRR) of 0.56 (95% CI 0.32–0.99, p=0.05). The effect was higher in those taking aspirin for 2 years or more; IRR 0.37 (95% CI 0.18–0.78, p=0.008). Chemoprevention in combination with stringent endoscopic surveillance could potentially reduce the risk of mCRC in both SEGC and EXTC group.

## 10.6 Study limitations

There are some limitations to this study. It is uncertain if the preoperative diagnosis of MMR pathogenic variant was available. Currently, identification of MMR disease status is mainly carried out by performing MMR immunohistochemistry (MMR-IHC) on the resected CRC specimens, followed by further testing if abnormal. Although, Chapter 9 has demonstrated that preoperative diagnosis of MMR deficiency via MMR IHC could be reliably performed on endoscopic biopsy, it is not sufficient to influence surgical planning and or recommend EXTC.

Furthermore, data on morbidity, bowel functional and quality of life between the two groups were not reported. Haanstra and his colleagues [204] reported no difference in QoL of life after partial and subtotal colectomy in LS patients. However, functional outcome such as stool frequency and defecation problems were worse after subtotal colectomy. Conversely, You et al [203] reported that median daily stool frequency and quality of life were better after segmental colectomy compared to subtotal and total colectomy. The extent of resection must also be balanced against this surgical morbidity associated with an extended colectomy, as well as the functional outcome. It is important to highlight that complete elimination of mCRC risk requires a proctocolectomy with end ileostomy or ileoanal pouch. This is associated with significant morbidity and worse functional outcome and is not currently recommended in LS.

This systematic review is also limited by the paucity of reported variables such as the anatomical site of the index and mCRC, endoscopic follow up and or chemoprevention. For instance, reporting the location of the index cancer and mCRC might be a significant factor in evaluating the risk of mCRC. It is well known that CRCs in LS are more likely to develop proximal to the splenic flexure. Therefore, it is uncertain if the risk of mCRC differs in patients after a right sided segmental resection compared to distal segmental resection. Other factors that may influence choice of surgery (e.g. patients' preference, mode of surgery (emergency or elective) and WHO performance status) were not reported. Furthermore, the colonoscopy surveillance data from the studies were sparse.

## 10.7 Conclusion

This review set out to compare the rate of mCRC after segmental and extended colectomy for CRC in Lynch syndrome. We found evidence to suggest that extended resection reduces the risk of mCRC four-fold. Surgeons and patients should be aware of the risk of mCRC after SEGC despite 1-2 yearly postoperative endoscopic surveillance. This risk appears to be higher in *MLH1* and *MSH2* although more studies are needed to evaluate the risk of mCRC in the individuals with specific MMR germline pathogenic variant. Therefore, in CRC patients with confirmed MMR germline pathogenic variant (particularly *MLH1*, *MSH2*), we recommend that the patient should be offered the option of EXTC; however, age, function, co-morbidity and attitudes towards colonoscopy need to be borne in mind. Careful preoperative counselling of the patient is essential. There is currently insufficient evidence to recommend EXTC in individuals with *MSH6* and *PMS2* germline pathogenic variants.

# 11 Chapter 11 – Thesis Discussion, Conclusion and Future work

## 11.1 Thesis discussion

This thesis aimed to evaluate the incidence of EOCRC (CRC in young adults < 40 years old) and optimise identification and management of hereditary GI syndromes. The first section of this thesis evaluated the incidence of EOCRC and assessed whether EOCRC had worse tumour biology and prognosis compared to LOCRC. This was achieved using two data sets: a local cohort of over 1000 patients and an administrative HES database in England comprised of over 300,000 cases. The age at CRC diagnosis was stratified into three age groups: group 1 (18-40 years), Group 2 (41-60), Group 3 (>60 years old). Comparable to values reported in the literature, the incidence of EOCRC in the national and local studies were 1.4% and 2.1% respectively. Both studies demonstrated that rectum was the most common site of cancer in all three groups. The national study also demonstrated with statistical significance that individuals with EOCRC were more likely to present with poor histological features and advanced disease. Although similar trends were observed in the local study, these were not statistically significant. The higher incidence of EOCRC in the local dataset may reflect the tertiary nature of our institution. Individuals with EOCRC (with or without known hereditary GI syndromes) are likely to be referred to a tertiary and specialist institution such as St Mark's Hospital for further investigations and management. Individuals in group 1 (EOCRC) were found to have the worst tumour biology and disease stage. Despite increased administration of systemic adjuvant treatment compared to the older patient groups, EOCRC had worse disease-free survival (44%, 78%, 77% *P*=0.022). The multivariate analysis concluded that

young age itself was not an independent prognostic factor for worse disease outcome. The findings in this study add to current evidence recommending increase awareness of the risk of CRC in young patients presenting with red flag bowel symptoms. The poor outcome (DFS) observed in EOCRC is likely due to a combination of poor histological features and late presentation. Consequently, some authors have even recommended commencing bowel cancer screening at the age 50 years to reduce incidence of young onset CRC [375]. The American cancer society (ACS) recently recommended CRC screening in adults aged 45 years and older with an average risk of CRC either via high-sensitivity stool based test (FOBT or FIT) or a structural (visual) examination [376]. I demonstrated in this section that 28% of individuals with EOCRC had an underlying genetic predisposition to CRC; this is similar to figures reported in the literature [48,74]. However, as a specialist center with a large inherited CRC service, this value might not be an accurate reflection of EOCRC as a whole. This chapter concluded that in addition to highlighting the risk of EOCRC in the general population, optimization of screening and surveillance of these GI syndromes in at risk individuals is important in the overall prevention and management of EOCRC.

Section II of this thesis focused on improving management of individuals with FAP. Using data from St Mark's polyposis registry, I evaluated the genotypic and phenotypic variabilities in patients with presumed "AFAP" and demonstrated that patients with a constitutional pathogenic variant in the region of the *APC* gene thought to be associated with AFAP displayed marked phenotypic variability. Of the 83 patients investigated, 29 (35%) displayed features consistent with classical FAP (colorectal adenoma count >100 adenomas at age 25

years). Similar variabilities were also observed within kindreds and between families with the same constitutional pathogenic variant. These results provide further evidence that relying solely on a genotypic definition of AFAP may incorrectly classify individuals. This is particularly relevant when deciding on initiation of and frequency of endoscopic surveillance, as well as the choice of prophylactic surgery. We proposed that AFAP is part of the FAP spectrum of disease, rather than a distinct disease. In the era of personalised management, the term attenuated should be abandoned and phenotypic description based on serial colonoscopy findings of number of adenomas at first colonoscopy and rate of increase in polyposis enumeration are the important factors upon which important management decisions are based.

There is a clear move towards better personalisation of care in FAP. As a result, recent guidelines recommend adopting individualised surveillance strategy in children with FAP. This recommendation was based on low level of evidence. Historically, it was suggested to perform annual flexible sigmoidoscope on paediatric patients at risk of FAP, for whom predictive genetic testing was not possible. However, in chapter 5, we demonstrate that colonoscopy is the best screening modality, as 35% had colonic adenoma despite rectal sparing. With a median rate of polyp progression of 12.5 polyps/year (0-145) in the entire cohort, we concluded that a tailored surveillance interval based on phenotype, as recommended by ESPGHAN, is a more appropriate surveillance strategy than the previously recommended annual surveillance. Furthermore, the progression to colectomy and choice of surgery is dependent on various factors such as genotype, phenotype (polyp progression)

and patient factors. Overall, 91% of patients in our cohort were suitable for rectal sparing surgery. In 51% of patients, the main determinant of timing of surgery was social and educational convenience, rather than polyp progression. These findings (chapter 6) provide further evidence to support individualisation of endoscopic surveillance and surgical management of FAP.

Total colectomy and IRA an appropriate operation for those with adenomatous polyposis syndrome and an appropriate rectal phenotype [170,286,354]. However, the reported anastomotic leak rate in the laparoscopic era has been problematic [301]. Especially in young adults with FAP, the dual objectives of low surgical morbidity and oncological safety need to be juxtaposed. Poor surgical outcome in this group can have disastrous consequences for them as well as avoidance of surgery amongst first degree relatives. Historically, patients requiring prophylactic surgery for FAP underwent laparotomy and handsewn side-to-end anastomoses with lower anastomotic leak rates reported [297]. In the laparoscopic era, most surgeons undertaking TC & IRA have moved to circular staplers. In chapter 7, we hypothesized that preserving the IMA and exteriorising the ends of the bowel to perform extracorporeal IDSA using linear stapler creates a more robust anastomosis, which may reduce anastomotic leak rates and improving overall surgical outcome. My results demonstrated that NT-IDSA when compared to conventional TC-IRA significantly improved anastomotic leak rates ($P$=0.0125) and has comparable perioperative and postoperative surgical outcome in individuals undergoing prophylactic surgery for adenomatous polyposis syndrome. As with conventional TC-IRA, postoperative endoscopic surveillance beyond the anastomosis is paramount.

Robust regular endoscopic surveillance of the rectum or rectosigmoid remnant following rectal or rectosigmoid sparing surgery reduces the risk of rectal cancer and secondary proctectomy [296]. However, the fate of rectum with regards to rate of polyp progression and endoscopic management of rectal adenoma enumeration is poorly reported in the literature. In chapter 8, we demonstrated that rate of adenoma progression in the rectum is relatively stable at a rate of 5.5 polyps/year and there was no evidence to suggest adenoma regression occurred following rectal sparing surgery for FAP. Furthermore, this chapter is the first study to described the role of cold snare polypectomy in the management of the rectum in the modern endoscopy era. Overall, over 13,000 polypectomies were performed to control adenoma progression in our cohort. Consequently, of the 191 patients studied, rectal failure occurred in 10 (5%) patients and only 1 (0.5) patient developed rectal adenocarcinoma. Interestingly, 15 out of the 19 patients with constitutional pathogenic variants in MCR who underwent rectal sparing surgery in the prepouch era still had their rectum albeit having more polypectomies during the study period. This is an important finding as it demonstrates that regular surveillance and polypectomies in the modern endoscopic era is effective at managing the rectum and delays the need for secondary proctectomy, even in groups whose genotype is currently used as an indication for consideration of proctocolectomy. In the era of patient choice, our findings suggest that IRA with stringent surveillance and polypectomies can be safe in patients who would usually be counselled for pouch surgery but opt to delay it due to personal and social factors. This has to be balanced with the risk of developing small bowel desmoid disease which will then preclude subsequent pouch surgery. Larger multicentre studies with longer follow-up are required to evaluate this further.

The final two chapters, focused on improving identification of and diagnosing LS and LS related CRC. In chapter 9 we showed that colorectal EB and metastatic lymph node tissues are a reliable source of tissue for MMR IHC testing. This has potential diagnostic, clinical and oncological benefits for patients and clinicians in the management of CRC. The availability of the results at the time of the multidisciplinary team discussion ensures tailored approach to systematic chemotherapy and immunotherapy for individuals with metastatic disease or poor tumour biology. It is also important in individuals for whom surgery is not considered where previously IHC would never have been done as no surgical specimen upon which to perform IHC. Similarly, testing on biopsy is also important in individuals undergoing neoadjuvant treatment of rectal in the event of complete tumour response. No evidence was found to suggest that chemoradiotherapy induces MMR protein loss.

The risk of CRC in LS has been well established and recent high-quality data suggest a risk of up to 46% by the age of 75 years, depending on the MMR gene involved [377]. Unlike in FAP, there isn't enough evidence to recommend prophylactic surgery. Once CRC has developed, there remains a risk of mCRC, which has been demonstrated by various retrospective studies [200,201]. In chapter 9, a systematic review and meta-analysis was performed to evaluate the risk of mCRC following segmental and extended colectomy for LS CRC. This demonstrated that extended colectomy was associated with a four-fold decrease in the risk of mCRC compared to segmental colectomy. In addition, mCRC occurred despite post-operative endoscopic surveillance. These findings are important when discussing surgical management of LS-related CRC especially in young individuals with high *MLH1* and *MSH2*. Further long-term

follow-up studies are required to assess the risk in individuals with *MSH6* and *PMS2* pathogenic variants.

## 11.2 Thesis Limitations

The limitations of each study have been described in the individual chapters. The data from the HES administrative database was limited by the availability of important variables and lack of diagnostic coding for hereditary GI predisposition syndromes. Similarly, both local and national database did not account for other CRC predisposing modifiable and non-modifiable risk factors such a risk factors for CRC and survival. Also, the local cohort was flawed by potential ascertainment bias, due to the tertiary nature of the institutions practise. This would have led to an overestimation of the prevalence of genetic predisposing syndrome due to referrals of EOCRC from other secondary institutions. The mainstreaming of diagnostic pathways for hereditary GI syndromes might not be easily replicated is some centres or institutions due to limited personnel and resources. Finally, an agreed international consensus on the definition of EOCRC is required to facilitate accurate comparison of data from institutions.

In section 2 of this thesis, the method of estimating polyp count did not take into consideration the variations in polyp distribution in patients with FAP. Some patients have a left sided predominance of polyps and others, such as those with an attenuated phenotype, have a right sided predominance. There is currently no agreed flawless method of accurately

estimating adenoma numbers other than counting individual polyps, which might be impossible in some patients. All forms of estimation will inadvertently be prone to errors. Furthermore, the studies did not assess the progression in the adenomas size which may reflect overall mucosal instability and be an important indication for surgery. In addition, the post-operative risk of developing desmoid disease was not assessed. These parameters are important dimensions that influence overall mucosa stability and progression to surgery.

The systematic review is limited by the quality of the assessed data from the individual retrospective studies. Given that not all pathogenic variants in the different mismatch repair genes confer the same of level of risk, the bias towards *MLH1* pathogenic variant carriers would have resulted in an overestimation of the risk of mCRC. Furthermore, the study is also limited by the lack of genetic diagnosis in some patients and clarity on the frequency and quality of endoscopic follow-up in the segmental colectomy group. Finally, with CAPP2 trial demonstrating a significant reduction in the risk of CRC, it is likely that the risk of mCRC will also be reduced by taking regular aspirin.

## 11.3 Thesis Conclusion

The studies in this thesis demonstrate that awareness of the risk of EOCRC is needed and that familial genetic predisposition syndromes such as LS and FAP, are important aspects in the management of EOCRC a. In FAP, we demonstrated that tailored pre-operative and post-operative surveillance protocol can be achieved based on colorectal phenotype and post-operative surgical outcome can be improved by NT-IDSA. With regards to LS, the good

concordance demonstrated between primary CRC and corresponding biopsy or metastatic is likely to lead to early identification of patients with a MMR deficient tumour. This is important in improving screening for LS and to aid tailored and targeted oncological therapy. In LS CRC, the risk of mCRC should be given serious consideration when counselling young individuals with high risk MMR pathogenic variants.

## 11.4 Future work

We have demonstrated that NT-IDSA is a safe alternative to TC-IRA in patients undergoing rectal sparing surgery for adenomatous polyposis syndromes. Future studies will be undertaken to assess the long-term outcomes, adenoma progression, ease of surveillance and functional outcome in these individuals. There is undoubted variation in polyp progression after prophylactic surgery and future prospective studies also will be performed to evaluate changes in gut microbiota and metabolomics from the pre-operative and postoperative period to try and establish causes for this variation in polyp progression.

The role of pubertal staging and paediatric age parameters such as weight height as cofounders in colorectal adenoma progression and progression to surgery needs further evaluation. In conjunction with a paediatric auxologist, the role of pubertal staging in management of FAP in children will be evaluated. Finally, future studies should evaluate the role of modifier genes in phenotypic variabilities in FAP.

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