An Assessment of Colonoscopy Recommendations and the Provision of Genetic Counseling for Families of Colorectal Cancer Patients in Newfoundland & Labrador

By

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ABSTRACT

Introduction

Family members of patients who develop colorectal cancer (CRC) are at higher than population risk of developing CRC. Screening can prevent CRC by finding and excising precancerous polyps. Family members at risk of CRC require screening, starting at different ages and varying frequency depending on family history. The purpose of this thesis is to investigate clinical, pathological, demographical and family history variables in patients with CRC in an attempt to predict clinical decisions important in their management: referral to genetic counselors and frequency of colonoscopy in family members.

Methodology

The cohort used was from a familial CRC clinic started in 2010 in urban and rural regions of Newfoundland and Labrador (NL). Patients in the clinic were pathologically confirmed incident CRC patients from the Newfoundland Cancer Registry presenting between 2008-2010. Of the 1091 cases presented to the two health regions, 525 were considered for analysis. Pedigrees were reviewed by genetic counselors and family risk was determined using a variety of criteria. Multivariate models were created to predict the need for genetic counseling and the frequency of screening colonoscopy in family members as recommended by the genetic counselor.

Results

The multivariate model created to predict the need for provision of genetic counseling (GC) included eight predictive variables, had a sensitivity of 81.3% and specificity of 87.4%. Scores in 25 of 134 (19%) patients recommended for GC differed from the decision of the genetic counselor. Review of clinical information demonstrated that the decision of our model was more clinically apt than that of the genetic counselor. The model to predict screening at 5-10-year intervals included nine predictive variables, had a sensitivity of 90.3% and specificity of 76.3%, and identified 30 (9%) patients that differed from the decision of the genetic counselor. The model to predict screening at 2-3-year intervals included 7 predictive variables, had a sensitivity of 69.1% and specificity of 87.4%, and identified 21 (31%) patients that differed from the decision made by the genetic counselor. The model to predict 3-5-year screening intervals compared to 2-3-year intervals included five predictive variables, had a sensitivity of 75.0% and specificity of 55.6%. This prediction of this decision was poor and was not considered to be clinically important.

Conclusion

Reasonable prediction models of patients who had families that needed to see a genetic counselor and of those whose family members needed colonoscopy at 5-10-year, or 2-3-year intervals were obtained. Clinical information on patients where the decision made by the multivariate score differed from the decision made by the genetic counselor suggested the score's decision was more clinically apt. Application of these multivariate scores in a population-based program of incident CRC patients has the potential to reduce mortality rates from CRC in NL.

GENERAL SUMMARY

Family members of patients with colorectal cancer (CRC) have a higher risk than the general population of developing CRC themselves. However, early identification and screening of these family members can identify and remove cancerous precursors before they manifest. This screening is done by colonoscopy, beginning at different ages and repeated at varying frequencies, dependent on family history, among other variables. In NL, these decisions are made by primary care providers, geneticists and genetic counselors.

In a previous study, we determined that the efficiency of these genetic counselor services in NL was poor, and the rate of referral to genetic counselors was high, which was the result of subjective interpretation of CRC patients' family histories by geneticists. Thus, the purpose of this project was to develop and assess the utility of multivariate prediction models that could (1) predict whether referral to a genetic counselor was necessary, and (2) make colonoscopy recommendations for families of patients with CRC. The electronic predictions of these models were then compared to the clinical decisions previously made by primary care providers, geneticists and genetic counselors.

Our models were able to accurately predict which patients required referral to a genetic counselor; as well as whether patients required annual screening, high-frequency colonoscopy screening (every 2-3 years), or low frequency screening (every 5-10 years). Prediction of intermediate-frequency screening (every 3-5 years) was more challenging.

In summary, our models yielded different recommendations than those from the geneticists and genetic counselors. On review of the clinical data, it appears they are more accurate recommendations. Application of these models to a population-based program of incident CRC patients has the potential to reduce colorectal cancer death rates in NL.

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LIST OF ABBREVIATIONS AND SYMBOLS

AC1	Amsterdam Criteria I
AC2	Amsterdam Criteria II
ACMAC	Age and Cancer Modified Amsterdam Criteria
AFAP	Attenuated Familial Adenomatous Polyposis
APC	Adenomatous Polyposis Coli
CI	Confidence Interval
CIN	Chromosomal Instability
CRC	Colorectal Cancer
FAP	Familial Adenomatous Polyposis
FCCTX	Familial Colorectal Cancer Type X
FDR	First Degree Relative
FHS	Family History Score
FIT	Fecal Immunochemical Test
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
HPP	Hyperplastic Polyposis
IBD	Inflammatory Bowel Disease
LS	Lynch Syndrome
MAP	MUTYH-Associated Polyposis
miRNA	MicroRNA
MMR	Mismatch Repair
MSI	Microsatellite Instability
MSS	Microsatellite Stable
NL	Newfoundland and Labrador
OR	Odds Ratio
PJS	Peutz-Jehgers Syndrome
PMG	Provincial Medical Genetics
PPAP	Polymerase Proofreading-Associated Polyposis
ROC	Receiver Operator Characteristic

LIST OF APPENDICES

Chapter 1 – INTRODUCTION

1.1 Colorectal Cancer in Newfoundland and Labrador

Colorectal cancer (CRC) is the third most common form of cancer in Canada, accounting for 13% of all subtypes. It carries a lifetime risk of 6.3%, among which 12% of patients will die – the second highest mortality rate of all cancers [1]. Newfoundland and Labrador (NL) has both the highest incidence and mortality rates of CRC among males and females in Canada, in addition to the highest rate of familial CRC worldwide [2]. In 2019, age-standardized CRC incidence rates per 100,000 people in NL were 114 among males and 75 among females; compared to 72 in males and 51 in females across the rest of Canada. Similarly, age-standardized mortality rates per 100,000 people in NL were 46 among males and 32 among females; compared to 27 in males and 18 in females across the rest of Canada [1].

In a previous study [2], we concluded that NL's high rate of CRC mortality is neither a result of decreased survival after diagnosis, nor more adverse prognostic factors, but rather a direct result of a higher incidence rate. One of our proposed strategies to reduce CRC incidence was through a population-based familial CRC clinic that would provide colonoscopic screening recommendations to families of patients with CRC based on several variables, including a calculated risk score. The process has proven to be inefficient in the management of probands (particularly those at 'Intermediate' risk), in addition to yielding low response rates and inefficient usage of genetic counselors' time. The purpose of the current study was to evaluate the utility of multivariate prediction models for referral to a genetic counselor and for colonoscopy screening recommendations in families with CRC and using the recommendations made by genetic counselors as the comparator. The decisions assessed are outlined in Figures 1 and 2.

Figure 1: Decision Tree for Incident Colorectal Cancer Patients in Newfoundland and Labrador

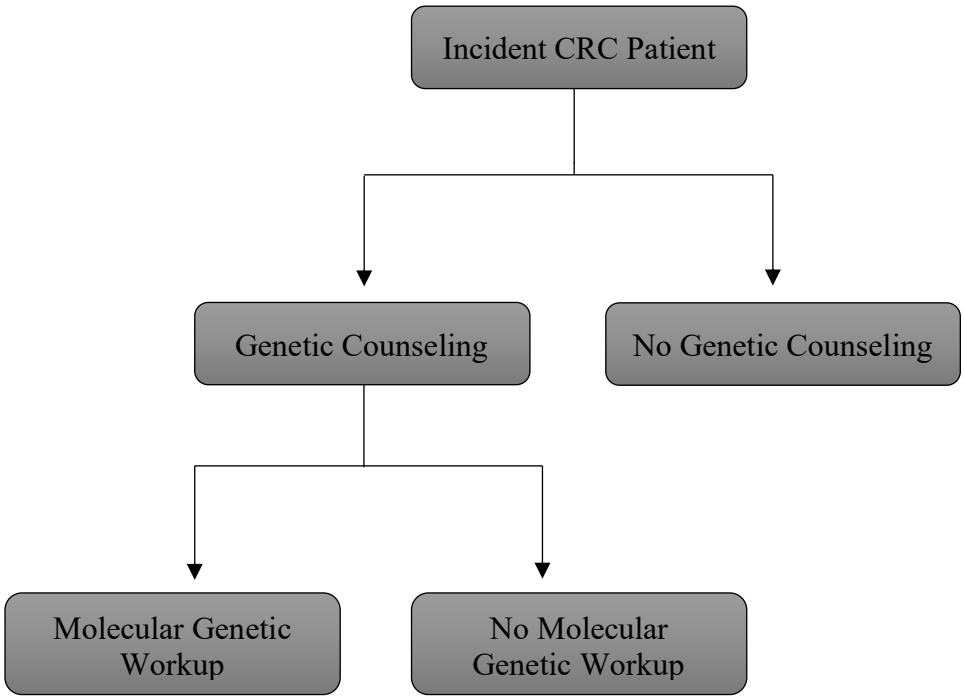
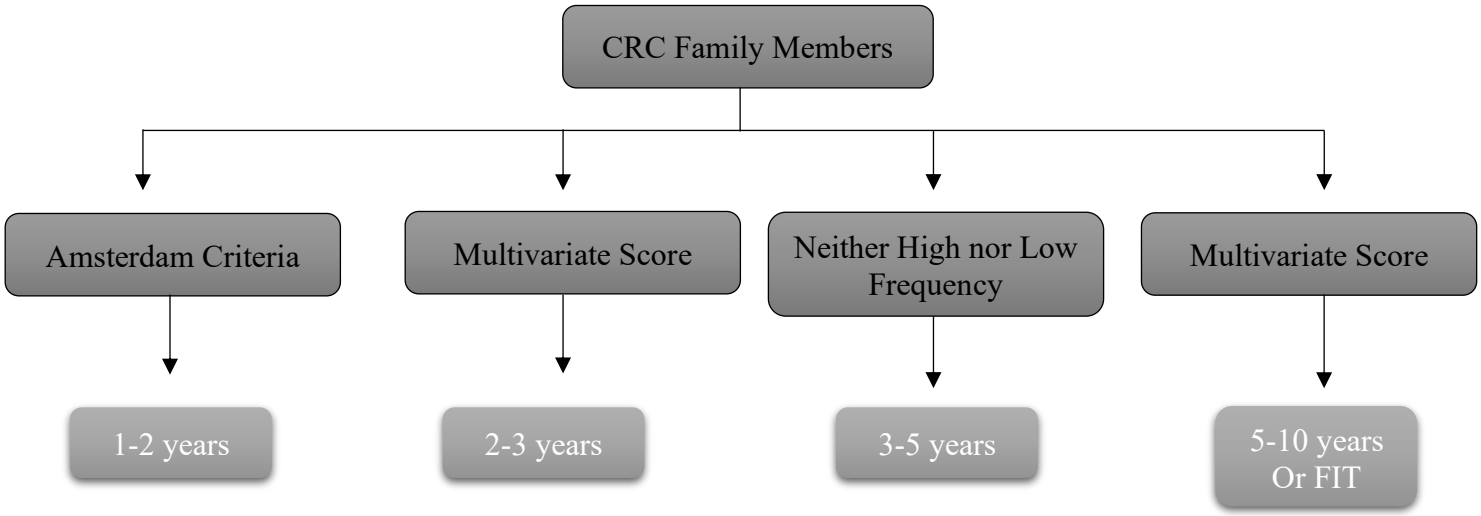


Figure 2: Decision Tree for Family Members of Colorectal Cancer Patients in Newfoundland and Labrador



Chapter 2 – LITERATURE REVIEW

2.1 Literature Search

A comprehensive literature search was conducted using medical databases PubMed, MEDLINE, EMBASE, and The Cochrane Library. The World Wide Web was also searched without using a specific time frame, accessing the websites for key groups. Combinations of the following key words were used to perform the search: “colorectal cancer”, “hereditary colorectal cancer”, “familial colorectal cancer”, “lynch syndrome”, “hereditary non-polyposis colorectal cancer”, “screening”, “genetic counselor”, “genetic counseling”, “polyps”, “tumorigenesis”, “familial colorectal cancer type x”, “genetics”, “polyposis syndromes”, “hamartomatous polyps”, “molecular pathways”, “prevention” and “newfoundland”. When relevant, reference lists of papers were used to identify additional literature.

2.2 Epidemiology

Colorectal cancer is among the most common forms of cancer worldwide, carrying a 6.3% lifetime risk [1]. The prognosis in patients with CRC has steadily improved over the past number of decades in developed countries. In Canada, the 5-year relative survival has reached nearly 65% [3]. Two principal factors in determining survival rate are age at diagnosis and stage. Relative survival decreases with age, while stage at diagnosis is the single most important prognostic factor. A study in the United States reported the 5-year relative survival of patients diagnosed with CRC to be 90.1% for patients with localized stage, 69.2% for patients with regional spread, and 11.7% for patients with distant tumor spread [4].

No single risk factor accounts for most cases of CRC. Apart from age and male sex, several other independent risk factors have been identified in epidemiological studies, including family history of CRC, inflammatory bowel disease, smoking, excessive alcohol consumption, excessive consumption of red and processed meats, obesity and diabetes. Risk increase has been strongest in patients with one or multiple first-degree relatives with CRC, among which lower age of

diagnosis further increased risk. As a result, the focus on inherited etiology of CRC remains an important topic [5, 6].

2.3 Hereditary Colorectal Cancer

Colorectal cancer has a substantial heritable component, with upwards of 25-35% of incident cases being attributable to heritable factors. The two most common forms, Lynch Syndrome and Familial Adenomatous Polyposis occur via autosomal dominant inheritance, and together account for 5 percent of all CRC [7].

Lynch Syndrome (LS), often used synonymously with the term Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is the most common hereditary syndrome associated with CRC, representing 3 percent of incident CRC cases [8]. HNPCC tends to comprise of single adenomas that are difficult to distinguish from sporadic tumors. As such, despite being the most common hereditary syndrome, it is often underdiagnosed [9]. HNPCC refers to a broad spectrum of familial CRC that encompasses disorders that may mimic clinical features of LS, despite not having the mismatch repair gene mutations characteristic to Lynch Syndrome. As such, HNPCC is dichotomized into LS and Familial CRC Type X (FCCTX). LS is characterized by heritable germline mutations to one of four MMR genes: MLH1, MSH2, MSH6 and PMS2. These mutations result in tumor microsatellite instability (MSI) that causes CRC as well as extra-colonic cancer susceptibility (HNPCC also presents itself at extra-colonic sites, namely the endometrium, stomach, ovaries, pancreas, ureter, renal pelvis, biliary tract, brain, sebaceous gland, and small bowel) [10, 11]. FCCTX describes cases of CRC meeting the criteria for LS, but whose tumors are proficient in DNA MMR proteins and limited to the colorectum [12].

Familial Adenomatous Polyposis (FAP) is an autosomal dominant disorder characterized by the development of multiple (>100) adenomas throughout the colorectum [13]. Classical FAP is caused my inherited germline mutations in the adenomatous polyposis coli (APC) gene on chromosome 5q21 [14, 15]. Half of FAP patients develop adenomas before the age of 15, and the lifetime risk of CRC approaches 100% in FAP patients who are not treated by prophylactic colectomy [16].

Lastly, Peutz-Jehgers syndrome (PJS) is another rare autosomal dominant, inherited condition caused by a mutated STK11 gene and characterized by unique gastrointestinal polyps and mucocutaneous pigmentation. PJS polyps can develop anywhere in the gastrointestinal tract, although commonly in the jejunum, ileum and duodenum. These polyps can display a phenomenon known as pseudo-invasion, which tends to mimic the features of an invasive carcinoma [17, 18].

2.4 Nonpolyposis Syndromes: Hereditary Non-Polyposis Colorectal Cancer, Lynch Syndrome and Familial Colorectal Cancer Type X

2.4.1 Hereditary Non-Polyposis Colorectal Cancer

In 1913, Dr. Aldred Warthin published a large pedigree with a number of colorectal cancer cases in the absence of polyposis, in addition to cases with uterine and gastric cancer labelled ‘Family G’ [19]. In 1966, Lynch et al. reported two large Midwestern families labeled families ‘N’ and ‘M’ whose tumors were analogous to Warthin’s Family G [20]. The study of these families, along with several others, were central in defining the original features of LS. In 1993, a genome analysis was performed in several large families with autosomal dominant inheritance of CRC which led to the discovery of 2 loci on chromosomes 2p and 3p [21, 22]. Tumors in these cases were distinguishable from non-familial cases due to instability of DNA microsatellites that implicated errors in MMR – a novel pathway in CRC development at the time [23, 24]. These discoveries provided the basis for cloning the 4 MMR genes most associated with HNPCC, MLH1, MSH2, MSH6 and PMS2 [25-27].

2.4.2 Lynch Syndrome

The term HNPCC was often criticized for its narrow focus on colorectal cancer and has since been replaced with the eponymous Lynch Syndrome to characterize families affected with germline mutations in DNA MMR genes.

MLH1, MSH2, MSH6 and PMS2 code for proteins that are integral to the function in MMR, which correct nucleotide mismatches that have evaded the normal editing function of DNA polymerase. Single-base mismatch and insertion-deletion loop errors that form during DNA replication can result in mutations in genes that regulate cell growth and promote neoplasia. In an intact MMR system, proteins bind to nucleotide base mismatches of double-stranded DNA or loops of microsatellites to target them for repair. A heterodimer complex between MSH2-MSH6 (MutS) recognizes single nucleotide mis-pairs and binds to the mismatched sequence. A second heterodimer complex between MLH1-PMS2 (MutL) binds to MutS to remove bases from the new DNA strand, which allows for re-synthesis of DNA with the correct base pairing [28, 29].

Most individuals with LS have germline mutations in one of the 4 DNA MMR genes. A second 'hit' or somatic event in the wild-type (WT) allele is required to render both copies of the MMR gene inactive. Mutations in the MLH1 and MSH2 genes were originally thought to represent 90% of all LS cases [7]. However, a population-based study evaluating universal molecular tumor testing on consecutive CRC cases found the prevalence of MSH6 and PMS2 gene mutations to be as high as 13% and 9%, respectively [30].

Individuals with LS are at increased risk for developing CRC, as well as cancers of the endometrium, ovaries, stomach, small intestine, urinary tract, brain, pancreas and sebaceous glands. Colorectal cancer and polyps in LS tend to arise at an early age, although age of onset can vary. CRC tumors in LS are predominately right-sided, and the risk of developing metachronous CRC is close to 30% at 10 years after the first diagnosis. Histologically, LS-associated tumors are mucinous, poorly differentiated, have a large number of lymphocytes and more than 90% display a high-level of MSI [7].

The lifetime risk of developing CRC with LS is between 50-80% [7]. Other LS-associated cancer risks may vary according to which MMR gene is mutated. Studies assessing genotype-phenotype correlations have found higher risks for CRC in families with MLH1 gene mutations, in addition to younger age of onset when compared to cases with MSH2 and MSH6 mutations. [31, 32]. The risk of extra-colonic tumors appears higher among MSH2 carriers. MSH6 carriers appear to have a lower risk of CRC and at later ages than MLH1 and MSH2 carriers; however, the risk of

endometrial cancer appears to be higher in some families [33, 34]. In a large study that evaluated gene-specific risk estimates among 537 French families with identified MMR gene mutations, overall cancer risks were lower for MSH6 carriers, compared with MLH1 or MSH2 gene mutation carriers. Although risks of CRC were similar among MLH1 and MSH2 carriers, endometrial cancer risk was highest in MLH1 carriers, and the cumulative risks of other Lynch syndrome cancers were highest among MSH2 gene mutation carriers [35].

Data regarding cancer risk for PMS2 mutation carriers remains limited, but as aforementioned, these individuals appear to have lower overall risk of cancer compared to other MMR mutation carriers. Lifetime risk of CRC ranges from 15-20%, 15% for endometrial cancer, and 25-32% for any other LS-associated cancer up to 70 years of age [36].

The high variability in cancer risks among LS carriers is related in part to modifier genes and environmental factors [37]. Several studies on heterogeneity of age of CRC onset among carriers attribute this to modifier genes that include IGF1, RNASEL and HFE genes [38]. More recent associations have been made between genes regulating the cell cycle, xenobiotic-metabolizing enzymes, telomerase and young age CRC onset in MMR gene mutation carriers [39].

2.4.3 Familial Colorectal Cancer Type X

Familial Colorectal Cancer Type X (FCCTX) describes a subset of colorectal cancer cases that meet the Amsterdam I criteria (AC1) of Lynch Syndrome, but whose tumors are DNA MMR-proficient when tested for MSI or tumor immunohistochemistry [40]. Between 2-5% of CRC cases fulfil the AC1. The heterogenous HNPCC subset of CRC is made up of the 4% linked to LS, the <1% with a Lynch-like syndrome, and the 2-4% classified as FCCTX. Approximately one-third of LS families fulfil the AC1, while several groups have reported that nearly half of CRC cases that fulfil AC1 are now classified as FCCTX [41, 42]. Despite FCCTX representing a major cause of hereditary CRC, it remains both poorly defined and investigated.

FCCTX pedigrees typically display autosomal-dominant inheritance, although the genetic etiology remaining largely unknown. Relative to LS, FCCTX has been associated with less

morbid outcomes. Lindor et al. have reported FCCTX to have lower predisposition to CRC (standard incidence ratio 2.3 vs 6.1), older mean age of diagnosis (50-60 years old vs 40 years old), left-sided in 70 percent of cases, less likely to be associated with synchronous or metachronous adenomas, and lastly, not associated with any extra-colonic cancers [40].

When compared to LS, FCCTX has also been associated with a more tubular growth pattern and less frequent mucinous histology [43]. FCCTX tumors typically show a more sporadic-like phenotype with medium to high differentiation, glandular and infiltrative growth patterns, as well as 'dirty' necrosis [44, 45]. The lack of explicit histopathological features makes the identification of FCCTX-associated cancers challenging. FCCTX surveillance programs target CRC, and feature screening colonoscopies, typically at 3-5-year intervals beginning 5-10 years prior to the earliest age of onset in the family [40, 46].

A number of genome association studies have investigated susceptibility loci in hereditary CRC. While they are not specifically linked to FCCTX, several candidate genetic variants have been identified, namely *CENPE, CDH18, GALNT12, ZNF367, HABP4, GABBR2, BMP4, GREM1, KIF24 AND BCR* [47]. Furthermore, two miRNA, *hsa-mir-491* and *hsa-mir-646* have shown preliminary association, with further data required [48].

The genomic profiles of FCCTX tumors mimic those of sporadic MMR-proficient CRC, making them a challenge to differentiate from one another [49]. Comparative genomic hybridization studies have suggested differences in both genomic and gene expression, most notably the gain of chromosome 20q, which has been specifically linked to FCCTX tumors [50]. Gene expression data from FCCTX tumors has also suggested upregulation of genes involved in peptidyl-amino acid modification, enzyme-linked receptor protein signaling, growth regulation, DNA repair pathways, vascular smooth muscle contraction, and G protein-coupled receptor signaling [51, 52].

In summary, the current understanding of the literature postulates that FCCTX tumor development is linked to inhibition of apoptosis, insensitive growth inhibitory signals and

increased migration that may result in infiltrative growth patterns and ‘dirty’ necrosis. The basic mechanisms of the FCCTX tumor genome continue to be defined.

2.5 Polyposis Syndromes

2.5.1 Familial Adenomatous Polyposis and Attenuated Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is the second most common inherited colorectal cancer syndrome, with a prevalence of 1:10,000. FAP is an autosomal dominant disorder, as a result of an inherited mutation in the adenomatous polyposis coli (APC) gene on 5q21. The majority of mutations that affect APC occur on exon 15, resulting in ‘truncated’ proteins. The APC gene normally encodes a tumor suppressor protein responsible for binding and degrading cytoplasmic β-catenin. However, when β-catenin fails to bind to APC, the gene transcription in the nucleus activates, which enables the proliferation of MYC and cyclin D1. The result is adenomatous polyp formation and eventual carcinoma [53].

Approximately 10-30% of FAP patients develop the disorder spontaneously, without family history [54]. Phenotypic expression is often varied as a result of genetic variation from multiple mutation sites, in addition to environmental and dietary factors [55].

While classical FAP is the second most common hereditary CRC, it accounts for less than one percent of all CRC, yet germline mutations of APC are 100% penetrant [56]. The classical phenotype in FAP patients is the presence of hundreds to thousands of adenomatous polyps of the bowel by adolescence, and typically, CRC by the age of 40 [57]. As a result, patient often undergo prophylactic surgery, removing the bowel. FAP is also associated with other extracolonic disorders, namely upper GI carcinomas, desmoid tumors, epidermoid cysts, osteomas, congenital hypertrophy of the retinal pigmented epithelium and papillary thyroid cancers [58].

Attenuated familial adenomatous polyposis (aFAP) is another product of APC germline mutation. In comparison to FAP, aFAP is a much less severe disorder, characterized by fewer

polyps (<100), a tendency for proximal colonic adenomas, and a later age of onset of CRC at an average age of 59 years [59, 60].

2.5.2 MUTYH-Associated Polyposis and Polymerase Proofreading-Associated Polyposis

MUTYH-Associated Polyposis (MAP) is an autosomal recessive form of FAP that carries an incidence of 1 in 10,000 with near 100% penetrance due to mutations in the MutY homolog (hMUTYH) gene [61]. Similar to classical FAP, MAP is also associated with extracolonic disease that include desmoid tumors, duodenal adenomas, fundic gland polyps, as well as ovarian, bladder and endometrial cancers [62]. MAP results from mutations to the MUTYH gene, a base excision gene that monitors coupling in DNA replication. The mutation causes transversion of G:C to T:A coupling in the APC or KRAS gene [63]. The two most common mutations are Y179C and G396D, which lead to adenomatous polyposis with APC mutations and serrated polyposis with *KRAS* mutations. Increased risk of CRC has also been reported in MutY mutation carriers [64].

Polymerase Proofreading-Associated Polyposis (PPAP) is a highly penetrant autosomal-dominant disorder. This particular syndrome is characterized by less than 100 adenomatous polyps and is linked to germline mutations in DNA polymerase ε and δ [65]. Germline mutations to DNA polymerase δ have been associated with increased risk of endometrial cancer. Both mutations interfere with the proofreading endonuclease function of DNA polymerase, which can result in mutations in the APC and KRAS genes, leading to adenomatous polyposis. The majority of resultant CRC cases are diagnosed between the ages of 30 and 50 [66].

2.6 Hamartomatous Polyps: Peutz-Jehgers Syndrome, Juvenile Polyposis Syndrome, PTEN-hamartoma Tumor Syndrome

Peutz-Jehgers Syndrome (PJS) is an autosomal-dominant disorder caused by germline mutations of STK11 on chromosome 19. Mutations on STK11, a tumor suppressor gene, result in dysfunctional or absent Serine-Threonine kinase enzyme activity, which in turn leads to unregulated cell proliferation and gastrointestinal hamartomatous polyposis – the fundamental

feature of PJS. PJS is also characterized by mucocutaneous pigmentation and extra-colonic cancers. The polyps are predominantly found in the small intestine, although they present in the colon in nearly half of cases as well [67]. PJS carries an incidence of 1:200,000 and 95% penetrance, in addition to a 40% lifetime risk of CRC [68]. Genetic testing for STK11 mutations is recommended for suspected patients. They include individuals with early-onset pigmented lesions, PJS-related hamartomatous polyps, and first-degree relatives of patients with PJS [69].

Juvenile Polyposis Syndrome (JPS) is a sporadic autosomal dominant disorder characterized by five or more juvenile polyps in the colon. It carries an incidence of 1:100,000 and 90% penetrance, in addition to a lifetime risk of up to 50% of developing CRC, and 20% risk of upper GI cancer [65, 70]. Mutations in SMAD4 on chromosome 18q21 and BMPR1A on 10q22 have both been linked to JPS. Both are involved in the TGF-β signaling pathway that regulates cell proliferation. As such, mutations to either have led to polyposis, dysplasia, and adenocarcinoma. Moreover, up to 60% and 15% of JPS patients in the United States have germline mutations in SMAD4 and BMPR1A, respectively [58]. JPS predominantly presents as rectal bleeding, anemia, and polyp prolapse by the age of 10. Colonoscopies are recommended every two years from the time of diagnosis, or at age 15 years for at-risk patients [69].

Lastly, PTEN-hamartoma tumor syndrome, commonly known as Cowden syndrome (CS), is an autosomal dominant syndrome characterized by colonic polyps, macrocephaly, and extra-colonic neoplasms [68]. It carries a lifetime risk of CRC between nine and 18 percent [69]. CS has been linked to germline mutations of the PTEN gene, a tumor suppressing gene on chromosome 10q23 that inhibits the mTOR/AKT signaling pathway used in cell proliferation, cell cycle progression and apoptosis [71]. The resultant mutation leads to increased pathway signaling, and eventually, large bowel polyposis [72]. Individuals with colonic polyps, extra-colonic disorders characteristic of CS, and first-degree relatives with CS are considered at-risk, and candidates for genetic testing and subsequent screening.

2.7 Hyperplastic Polyps

Hyperplastic polyposis (HPP) is a rare condition characterized by multiple large hyperplastic polyps on the colon. Little is known about the etiology, history or incidence of HPP. Diagnostic criteria include 20-30 cumulative hyperplastic polyps of any size, throughout the colon; 5 or more polyps proximal to the sigmoid colon (of which 2 are greater than 10mm in diameter); or 1 or more hyperplastic colonic polyp in first degree relatives of HPP patients [73]. Sessile serrated polyps have recently been added to the polyp histological type as well. HPP is often asymptomatic, and only identified during screening colonoscopies. HPP does present an increased risk of CRC, in the range of 30-35%. Synchronous and metachronous cancers are frequently observed, often in a patient's 50s or 60s [74]. There are currently no precise surveillance strategies against HPP. However, regular colonoscopies every 1-2 years has been widely accepted [75].

2.8 Familial Non-Autosomal-Dominant Colorectal Cancer

Despite approximately 30% of patients diagnosed with colorectal cancer having a family history of the disease, as few as five percent of them carry germline mutations inherited in an autosomal-dominant fashion [41]. As aforementioned in sections 2.4.1 and 2.5.1, the two major forms of autosomal-dominant inherited CRC are FAP and HNPCC, of which the latter is considerably more common. Familial non-autosomal-dominant CRC thus refers to cases with familial traits, but without identifiable genetic causes. This is of particular importance in NL, as it has the highest rate of familial CRC in the world.

A 2007 study by Green et al. [11] investigated the contribution of genetic and environmental factors to the incidence of CRC in NL and compared it with data from Ontario where the same recruitment and risk-assessment criteria are used. These data were then compared to results published from 13 other population-based studies across the world.

Out of 702 cases in NL, n=32 (4.6%) of patients were classified as high risk, compared to 2.7% in Ontario. Among this cohort, n=6 were a result of a diagnosis of family history of FAP. Twenty-four (3.4%) of the 702 cases met Amsterdam I criteria (see 2.11.1), which is 2.8-fold

higher than the aggregate from the 13 other populations ($p<0.0001$). Twenty-six patients met the criteria for Amsterdam Criteria II at a rate of 3.7%, and 1.7-fold higher than the aggregate from the other centers ($p=0.02$). Moreover, of the n=26 meeting Amsterdam criteria, n=15 of them (58%) had microsatellite-stable (MSS) tumors, and thus cannot be attributed to autosomal-dominant genetic factors, despite rampant family history.

Intermediate-risk cases were those that do not meet FAP or Amsterdam criteria, but one or more of the Bethesda criteria (see 2.11.2). This classification included 43% of the NL cases, compared to 31% in Ontario. Thus, the proportion of NL patients meeting Bethesda criteria is 1.4-fold higher than in Ontario ($p<0.001$). Furthermore, 31% of NL patients had at least one first-degree relative affected with CRC, compared to 20.4% in Ontario.

The higher number of intermediate and high-risk cases in NL can be attributed to a number of possibilities. A significant family history can indicate a familial trait, but not necessarily an inherited trait. This is to suggest that perhaps environmental and lifestyle factors specific to NL are responsible for the increased incidence of CRC, among it, familial non-autosomal-dominant cases. The high rate of familial CRC could also be due to the limited number of immigrants who established the NL population, and therefore attributable to founder mutations [11]. The incidence of CRC is 27% higher than the national average, and it is believed to be genetic, or at least familial.

2.9 Molecular Pathways

The pathogenesis of colorectal cancer is complex and varies according to a number of factors. It can be influenced by genetic predispositions, as well as dietary and lifestyle factors. The consensus understanding of these different pathways is characterized by models of genetic instability, clinical manifestations, and pathological characteristics. The majority of CRC follows the microsatellite instability (MSI) pathway or the chromosomal instability (CIN) pathway. Both the MSI and CIN pathways result in genomic instability, a major mechanism of colorectal tumorigenesis. The third novel pathway involved in pathogenesis is the serrated pathway, also

known as the CpG island methylator phenotype (CIMP). Recently, a fourth pathway has been investigated, in which microRNA (miRNA) actively contribute to colorectal carcinogenesis.

2.9.1 Microsatellite Instability

Microsatellites are short, highly repetitive areas of repeat nucleotide sequences spread across the genome. The DNA mismatch repair (MMR) system recognizes and repairs mismatches that occur during DNA replication [76]. Microsatellite instability is caused by inactivity of the MMR system and can be identified by a change in the number of DNA microsatellites. There are five validated microsatellite markers used for reference in the detection of MSI, collectively known as the Bethesda panel. They are *BAT25, BAT26, D2S123, D5S346* and *D17S250*. MSI-high is defined by instability of at least two markers, MSI-low is defined by instability of one marker, while MSS (microsatellite-stable) tumors have no apparent instability [77].

The discovery of MSI, coupled with its link to HNPCC have led to recognition of MSI as a pathway in colorectal carcinogenesis. Genomic instability by way of germline mutation in MMR genes accounts for 95% of HNPCC cases, while somatic mutation or hypermethylation silencing of MMR genes account for approximately 15% of sporadic CRC [78].

The MMR system itself is composed of multiple interacting proteins, including MutS homologue 2 (MSH2) and MutL homologue 1 (MLH1). Other identified members of the MMR system include MSH6, PMS2, MLH3, MSH3, PMS1 and Exo1 [76].

Sporadic, MSI-high CRC is often caused by hypermethylation silencing of MLH1. Moreover, in 95% of HNPCC cases, mutations are present in *h*MLH1 and *h*MSH2 [79]. Clinical manifestations vary, depending on the mutated gene. Mutated MSH2 is associated with a 40-60% increased risk of endometrial cancer, while mutated MLH1 with a 50-80% increased risk of CRC. Moreover, MSH6 mutations are associated with an 11-19% increased risk of gastric cancer, while PMS2 with a 9-12% increased risk of ovarian cancer [80].

2.9.2 Chromosomal Instability Pathway

The chromosomal instability (CIN) pathway is the most common and well-defined colorectal pathway, accounting for 65-70% of sporadic colorectal cancer. Tumorigenesis involves mitotic spindle checkpoint regulators and proteins that influence chromosome stability [81]. The primary initial mutation is that of the APC tumor suppressor gene, involved in both sporadic CIN and FAP [82]. As aforementioned in section 2.5.1, there is a germline mutation of the APC gene that has been identified in 60-80 percent of families with FAP [83]. Following the initial mutations in the CIN pathway, new mutations are promoted, and benign tumors progress to malignant stages.

The proto-oncogene, *K-ras,* determines the transition of an adenoma to a carcinoma. Mutations to *K-ras* happen at codon 12 and 13, which keep the gene in an active state, thus evading apoptosis and continue growing [84]. The later stages of colorectal tumorigenesis often feature loss of function of the *p53* system. Mutations to the *p53* gene results in the loss of cell cycle control and apoptosis, subsequently stimulating high proliferative activity. As reported by Lanza *et al.* [85], loss of function of the *p53* system is associated with loss of heterozygosity of chromosome 18q in 65.4% of cases. Loss of heterozygosity of 18q has been strongly associated with negative prognosis in CRC as a result of high metastatic potential [86].

2.9.3 Serrated Pathway

Serrated adenocarcinoma is a subset of colorectal cancer that accounts for approximately 10 percent of incident cases. It follows another pathway in which serrated polyps serve as the CRC precursor lesion [87]. Serrated polyps form a heterogeneous group of colorectal lesions, namely hyperplastic polyps, sessile serrated adenoma, traditional serrated adenoma and mixed polyps. Molecularly, classical adenoma-carcinoma pathways are governed by CIN and *KRAS* mutations [88]. The serrated pathway, however, included *BRAF* mutations and hypermethylation of gene promoter (CpG island methylator phenotype) [89]. The MSI pathway has also been detected in the serrated pathway, in addition to classical adenoma-carcinoma sequences. Morphologically, serrated carcinomas that develop from serrated adenomas are generally microsatellite stable

(MSS) or MSI-low (MSI-L). Those originating from sessile serrated adenomas are MSI-high (MSI-H). Common histological features include presence of epithelial serrations, clear and abundant cytoplasm, vesicular nuclei, absence of necrosis, mucin production, and presence of cell balls or rods. The presence of serrated lesion in the periphery of the infiltrative carcinoma is also indicative of a serrated carcinoma [90].

Hyperplastic polyps are the most common serrated polyp in the colon – accounting for approximately 90 percent of all serrated polyps, and 15 percent of all polyps of the colon. HPs are generally small (<5mm) and are most frequently located in the distal colon [91].

The most frequent genetic alterations in the serrated pathway involve *BRAF* and *KRAS* mutations. Both *BRAF* and *KRAS* encode for kinases of the mitogen-activated protein kinase (MAPK) cascade that mediates cellular signaling involving cell proliferation, apoptosis and differentiation. Mutations to *BRAF* and *KRAS* result in activation of the MAPK pathway and subsequent uncontrolled cell proliferation [92]. Sefanius et al. reported high frequency of *KRAS* mutations (45.2%) in serrated adenocarcinoma, suggesting a significant amount of *KRAS* mutated CRC originated from serrated polyps [93]. O'Brien et al. reported high frequencies of *BRAF* mutations (V600E) among serrated carcinomas (82%), also suggesting that this mutation is a marker of the serrated pathway. *KRAS* mutations occur predominantly at codon 12, the most common of which are G12D, G12V and G13D which are mutated in 0-73% of serrated polyps, 6-73% of HPs, 7-25% of SSAs, and 0-28% of TSAs. The most frequent mutation in *BRAF* is V600E, occurring in 0-88% of HPs, 32-83% of SSA and 60-76% of TSA [94].

Another molecular alteration in the serrated pathway is MSI. Tumors with two or more unstable markers are considered MSI high (MSI-H), those with only one unstable marker are considered MSI low (MSI-L), while tumors without unstable markers are considered stable (MSS) [95]. Sefanius et al. reported that 20.6% of serrated cancer showed MSI-H [93].

The CIMP phenotype is another feature of the serrated pathway. The methylation of the CpG island is considered to cause transcriptional silencing as well as inhibition of gene expression by binding methyl groups to cytosine-guanine dinucleotide sequences in the promoter regions [96].

This is commonly observed in precursor serrated lesions and colorectal polyps. The CIMP is frequent in serrated polyps in the proximal colon [87]. Among serrated adenomas, CIMP-H is overserved in 44-77% of SSA and 43-80% of TSA [94]. Hypermethylation of CpG island is more frequently associated with *BRAF* mutation than with *KRAS* mutations in serrated CRC. The status of CIMP is also often correlated with MSI status and mutations in both *KRAS* and *BRAF* oncogenes [97].

2.9.4 microRNA

Recently, microRNAs (miRNA) have been proposed to be involved in colorectal cancer pathogenesis. MicroRNAs are short, non-coding single-stranded RNAs ranging from 18-25 nucleotides long [98]. Through the inhibition of mRNA translation, they in turn modulate protein expression. A strong correlation between miRNA expression and tumorigenesis has led to a variety of research to investigate their potential as cancer biomarkers.

Typical disease progression in CRC involves both upregulation of oncogenes, and downregulation of tumor suppressor genes. Similarly, miRNA activity in CRC is also both upregulated and downregulated [99]. The first association between microRNA and CRC was identified in 2003 by Michael *et al.* [100], who observed decreased levels of miR-143 and miR-145 in CRC tissue compared to healthy tissue. Oncogenic miRNAs target and downregulate tumor-suppressor genes in their regulation of pathways, often leading to cancer formation. Conversely, tumor-suppressive miRNAs downregulate growth and metastasis genes.

A major pathway involved in uncontrolled proliferation in CRC is the MAP kinase pathway, which involves the protein RAS. A 2006 study by Bandres *et al.* investigated miRNA expression in both tumoral and non-tumoral CRC tissues [101]. They observed divergent expression of miRNAs in CRC cases with *KRAS* or *BRAF* mutations, suggesting a link between altered miRNA expression and the RAS pathway. miR-143 and miR-145 downregulate RAS and insulin-like growth factor 1 receptor, and act as tumor-suppressive miRNAs in CRC [102]. Loss of apoptotic control is another necessary characteristic for uncontrolled cell growth. miR-195 and miR-491 have been shown to promote apoptosis by targeting certain B-cells [103]. miR-96

is upregulated in CRC, exerting downregulatory effects on p53 activity [104], while miR-34a has been found to increase p53 activity [105].

The diagnostic potential of miRNAs in CRC is important. They have shown promise in improving screening and diagnostic methods for primary and metastatic CRC tumors. The use of miRNAs as a screening tool not only eliminates common discomforts associated with colonoscopies, but also display stable, tissue specific expression – a useful trait for a potential biomarker [106]. Differential miRNA expression can differentiate colon cancers from rectal cancers, and MSI CRC from MSS CRC [107]. Current practice for CRC metastasis testing uses carcinoembryonic antigen as a biomarker. Chang and colleagues [108] have shown that miR-141 can be used in collaboration with CEA to increase predictive capabilities. The discovery of the relationship between miRNA and CRC pathogenesis is relatively novel, however, further understanding of miRNA will see its role in the diagnosis and treatment of CRC increase.

2.10 Screening

2.10.1 Screening Techniques

Despite ranking third among the most commonly diagnosed cancers worldwide, colorectal cancer is also one of the most preventable forms of cancer [109]. Recent treatment modalities for CRC have largely improved disease outcome and patient survival, but at a markedly increased cost. Screening and interventional strategies, however, have been shown to cost-effective strategies in preventing CRC incidence and mortality [110]. Survival is better at earlier stages of CRC, thus screening for early-stage CRC is worthwhile.

As a result of the invasive nature of colonoscopy, there are several widely used non-invasive screening methods for patients with a negative family history, namely fecal occult blood testing (FOBT), computed tomography colonography, flexible sigmoidoscopy and double-contrast barium enema. There are two types of FOBT, the first of which is guaiac-based (gFOBT) which detects peroxidase-like activity of heme. A 2008 study [111] provided level 1 evidence that 2-3 rounds of annual or biennial gFOBT would reduce CRC mortality by 16% (95% CI, 10-22%). However, the gFOBT has been criticized for its limited cancer sensitivity and poor detection of

adenomas. In an effort to improve sensitivity, the fecal immunochemical test (FIT) was developed. The FIT uses antibodies to human globin and can be varied to adjust sensitivity and specificity. Unlike the gFOBT, the FIT has no dietary restrictions, and RCTs have reported a 13-15% higher participation rate than the gFOBT [112, 113]. Cross-sectional studies using colonoscopy for reference observed FIT detecting cancer and advanced adenomas three times more frequently than the gFOBT [112]. Furthermore, in a 2012 study of 1256 participants who underwent both FIT and a colonoscopy, FIT detected 7 of 8 cancers and 38 of 111 adenomas [114]. The results of these studies as well as popular consensus suggests FIT as a viable replacement for gFOBT as the FOBT test of choice.

Computed tomography (CT) colonography is a relatively new, and attractive screening test for a number of reasons, namely its non-invasive nature and high (>90%) sensitivity for cancer and polyps (>1cm) [115]. CT colonography involves the administration of oral contrast agents, followed by bowel distention through anal insufflation of carbon dioxide. A colonoscopy is then ordered if there are any positive findings. Despite some of the attractive features of CT colonography, it's limited by its high cost, its inability to detect small or flat polyps like sessile serrated adenomas, and the fact that if a polyp is indeed found, it requires a follow-up scope to remove it anyway. Moreover, incidental lesions outside the GI tract are often detected, and necessitate further imaging or investigations. A randomized population-based trial also reported participants to find CT colonography more burdensome than colonoscopy due to the bowel dissention without sedation and subsequent disturbed bowel habit [112]. However, CT colonography offers an attractive alternative to double-contrast barium enema for patients unable to undergo colonoscopy.

Flexible sigmoidoscopy (FS) is an endoscopic examination of the rectum and sigmoid colon and is a common alternative to FOBT. It is performed using a flexible instrument inserted into the sigmoid colon through the anus. Both malignant tumors and benign lesions can be detected, the latter of which can be removed in the same session without anesthesia or patient discomfort. Follow-up colonoscopies are often offered. A 2014 randomized controlled trial [116] investigated the effectiveness of FS on CRC incidence and mortality. Compared to the control group, participants in the screening group had reduced CRC incidence at ages 50-54 (Hazard

Ratio 0.68, 95% CI: 0.49-0.94) and ages 55-64 (Hazard Ratio 0.83, 95% CI: 0.71-0.96). Flexible sigmoidoscopy has also proven effective in reducing CRC mortality. Four population-based, prospective, RCTs have shown a 22-31% decrease in CRC mortality using one-off FS [117]. Flexible sigmoidoscopy is limited in that its benefits are confined to distal cancers within reach of the sigmoidoscope.

Lastly, double-contrast barium enema is commonly employed to study the colon through radiologic imaging, involving a barium enema injection. Its strengths include its cost-effectiveness, and ability to detect CRC throughout the entire colon, beyond the range of incomplete colonoscopies [118]. A study comparing double-contrast barium enema to FOBT in detecting neoplastic lesions of the colon associated the procedure with increases in detection rates of cancer or adenoma of 2.3/1000 and 3.8/1000, respectively [119]. The procedure can be limited, however, by its low sensitivity to polyps [119], and a required follow-up colonoscopy to confirm findings.

The majority of CRC develop from a preclinical precursor, the adenoma, which can be identified and removed by colonoscopic polypectomy [120]. As such, worldwide guidelines often endorse screening after the age of 50 [121]. Despite being invasive in nature, the colonoscopy is widely viewed as a comfortable procedure. Coupled with its ability to examine the entire colon, the colonoscopy is the predominant screening strategy worldwide [122]. Despite the invasive nature of colonoscopy, a study of patients within the same practice receiving un-sedated flexible sigmoidoscopy were more than twice as likely not to be screened again versus those screened with sedated colonoscopy [123]. The major advantage of colonoscopy lies in its unmatched ability to detect precancerous lesions and cancer. While CT colonography can also detect cancer, it still requires bowel preparation, has radiation-associated risks and is costly [122]. Colonoscopy appears to be the standard in detection of serrated lesions as well. Neither CT colonography, gFOBT or FIT could detect serrated lesions when tested, while fecal DNA was the only non-invasive test with sensitivity to serrated lesions [124]. Lastly, an important advantage of colonoscopy is its long-lasting protection from CRC. Colonoscopy is the only available test with recommendations at 10-year intervals [121]. Moreover, a recent German case-control study on colonoscopy reported substantial protection against CRC up to 20 years [125].

Disadvantages of colonoscopy lie in its negative perception, high complication rate, high cost and operator dependence. Patients who haven't undergone colonoscopy often cite fear of procedure and need for bowel preparation as major deterrents [126]. Despite the benefit largely outweighing the harm, the occurrence of perforation, aspiration and splenic injuries can be both expensive and fatal. While colonoscopy is merely a screening test, it has a relatively high complication rate. Colonoscopies have been criticized for their high cost, despite being relatively cost-effective [127]. Lastly, subjectivity associated with operator dependence of colonoscopies remains a disadvantage, namely with respect to variance among colonoscopists on adenoma and CRC detection rate [128]. In many cases this has led to more frequent screening intervals (often to 5-year intervals), which threatens the cost-effectiveness of the procedure [129].

While there is a lack of RCT on the impact of colonoscopy on CRC incidence and mortality, there is sufficient evidence to suggest a positive impact on both. Despite improvements in non-invasive screening techniques, colonoscopy remains the most effective screening test due to its unmatched detection abilities, coupled with its ability to examine the entire colon. Furthermore, improvements in technique such as low-volume bowel preparation, and high-definition, wide-angle colonoscopy have produced significant advantages and reduced discomfort.

2.10.2 Hereditary CRC Screening

Thirty percent of incident CRC cases have a heritable component, among which 5% arise from established inherited disorders such as the aforementioned LS, FAP and MAP [64]. Early identification and screening of these high-risk individuals is important, likewise with individuals with a family history of CRC without a confirmed genetic mutation. The lifetime risk of CRC in patients with LS varies upon the mutated MMR gene, but the average age of CRC diagnosis ranges between 44 to 61 years, as compared to 69 years in sporadic CRC patients. Screening for LS, however, saves lives, as LS patients have improved stage-to-stage survival of CRC than sporadic CRC patients [130]. Individuals suspected of LS with confirmed germline mutations of one of their MMR genes are recommended to undergo colonoscopy screening every 1-2 years beginning between the ages of 20-25, or ten years earlier than the youngest CRC onset in the

family [131]. Primary treatment for LS-affected patients with CRC is colectomy with ileorectal anastomosis [130].

As aforementioned in 2.5.1, FAP is an autosomal dominant inherited syndrome caused by germline mutation in the APC gene. Depending on the mutation location, FAP can manifest in either classical or attenuated form. Fifty percent of classical FAP patients develop adenomas by age 15, while 95 percent develop them by age 35. If left untreated, FAP patients develop CRC at an average age of 39. Patients with aFAP carry a 70% lifetime risk of CRC, about 12 years later than in classical FAP [132]. Patients at risk of FAP are recommended to be screened between the ages of 10-12. APC gene testing is the test of choice, but if it cannot be obtained, annual sigmoidoscopy or colonoscopy is recommended between the ages of 10-15. Each subsequent decade, screening frequency can be reduced by a year until age 50, when screening should take place every 3 years [132]. Individuals suspected of aFAP are recommended for gene testing if more than 20 cumulative colorectal adenomas are found. Patients at risk for aFAP should begin screening with colonoscopy at age 12, 15, 18 and 21, then every 2 years [132]. Prophylactic colectomy should be performed upon diagnosis of FAP to prevent CRC development [64].

MUTYH-associated polyposis (MAP) is caused by germline mutation of both alleles of the MUTYH gene, and its colonic phenotype mimics that of aFAP. The risk of CRC in patients with MAP is 19% by age 50 and 43% by age 60, with an average age of onset of 48 years. Relatives of MAP patients with a heterozygous MUTYH mutation have a similar risk of CRC to that of sporadic CRC FDRs [133]. Patients with MAP are recommended for colonoscopy every 1-2 years. If endoscopy fails or CRC develops, subtotal colectomy is recommended [130].

2.11 Diagnostic Criteria to Measure Family Risk

2.11.1 Amsterdam Criteria

The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (IGN-HNPCC) met in 1991 to develop the original Amsterdam Criteria (AC1) in an attempt to standardize diagnostic criteria for multicenter studies on HNPCC, and ultimately improve uniformity in the literature. The AC1 were subsequently developed as a high-specificity

guideline for identifying HNPCC families for the purposes of genetic research and analysis [134]. Fulfilment of AC1 requires that: i) at least 3 relatives should have CRC; ii) one of the relatives is a first-degree relative of the other two; iii) two successive generations are affected; iv) at least one individual was diagnosed before 50 years of age; v) Familial Adenomatous Polyposis is excluded; and vi) the tumors are verified by a pathological exam.

While widely accepted, the AC1 were also criticized as some investigators felt that the criteria excluded extracolonic cancers that are part of the syndrome. The criteria were revised in 1998 with the aim of increasing sensitivity by improving physician's identification of families and extracolonic cancers associated with HNPCC, now termed the AC2 [135]. The extracolonic cancers include cancers of the endometrium, ureter, renal pelvis and small bowel.

The Amsterdam criteria were ultimately developed for the selection of families for research and were therefore more focused on specificity than sensitivity. Thus, many HNPCC families may be missed if the criteria were applied in a clinical setting. As a result, investigators developed additional criteria with increased sensitivity to identify other potential HNPCC families, chief among them the Bethesda Guidelines.

2.11.2 Bethesda Guidelines

Criticism of the Amsterdam criteria's clinical sensitivity coupled with improved understanding of clinical and histologic manifestations of HNPCC led to the development of the Bethesda Guidelines at a 1996 National Cancer Institute workshop [136]. The goal of the Bethesda Guidelines was to identify colorectal tumors that should undergo MSI testing. If they are found to have MSI, then MMR testing is recommended for them. A follow-up workshop was conducted to further aid in the identification of HNPCC kindreds for genetic testing, leading to the Revised Bethesda Guidelines release in 2004 [137]. The criteria are as follows: 1) a CRC patient who is less than 50 years of age; 2) presence of synchronous or metachronous CRC or other HNPCC-related tumor, any age; 3) CRC with MSI-high histology diagnosis in a patient who is less than 60 years of age; 4) individual and one first-degree relative with an HNPCC-related tumor with one of the cancers being diagnosed under the age of 50 years old; 5)

individual and 2 first- or second-degree relatives with HNPCC-related tumors, at any age. HNPCC-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, renal, pelvic, biliary tract, brain (glioblastoma), small intestine, sebaceous gland adenomas and keratoacanthomas.

A 2007 assessment of both the revised Bethesda and Amsterdam criteria determined the sensitivity for detection of mutation carriers was 90% and 40%, respectively [46]. The positive predictive value of the revised Bethesda and Amsterdam criteria was 50% and 10-20%, respectively. Ultimately the assessment determined that the revised Bethesda criteria were appropriate for patients whose tumors require MSI testing.

2.11.3 Provincial Medical Genetics Criteria

The Provincial Medical Genetics (PMG) program developed criteria for the cascade testing protocol for MMR mutation identification. These criteria were developed by local expert opinion following review of the literature [2]. Families meeting these criteria had tumors and DNA sent to Toronto for cascade testing to diagnose HNPCC or LS. The criteria are collectively referred to as the age and cancer modified Amsterdam criteria (ACMAC), and are as follows:

PMG1: ACMAC

(a) Three or more relatives with colorectal cancer or an HNPCC-associated cancer (colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain, sebaceous gland and small bowel carcinoma) *AND*
(b) Colorectal or HNPCC-associated cancer in *at least* two generations *AND*
(c) One or more colorectal or HNPCC-associated cancers diagnosed before age 60

Or one of the following PMG criteria:

PMG2*:* Colorectal carcinoma before age 40

PMG3*:* Endometrial carcinoma before age 45

PMG4*:* Sebaceous tumor (adenoma, carcinoma, epithelioma) before age 50 or multiple tumors at any age

PMG5*:* Presence of multiple HNPCC-related cancers in an individual, with one cancer before age 60

2.11.4 Family History Score

The Family History Score (FHS) was developed on the basis that a consistent predictor of cancer is family history of the disease. The score is calculated by describing the observed deviations from expected risk for each family during an observation period, while also taking into account family structure and risk covariates of family members like age, sex, race and birth cohort.

The expected risk of CRC for each family member is calculated using data from the Surveillance, Epidemiology and End Results (SEER) program from the National Cancer Institute [8]. It multiplies age-, race- and time-specific US cancer incidence rates by age-, race- and birth cohort-specific person-years at risk. A negative FHS indicates that a family contains fewer members with cancer than expected, while a positive FHS indicates more. The magnitude of negative values is primarily determined by family size and average age of family members. Larger families with older average age among members without a family history of CRC have larger negative FHS values because the expected values were high. Similarly, small families with younger average age and a family history of CRC have larger positive FHS scores [138].

2.11.5 MMRpredict Score

The diagnosis of Lynch Syndrome or HNPCC is a complex process. One of the more efficient strategies at doing so has been through the use of MMR mutation prediction algorithms that use patient and family history to estimate the likelihood of being a MMR mutation carrier. Several models have been developed to predict the occurrence of these mutations in high-risk patients and families, namely the Lieden, PREMM, MMRpro and MMRpredict models. A 2009 study by Green et al. [8] compared several of these models with one another and assessed their performance on CRC patients from the general population. Risk scores were calculated from each of the models for all 725 patients participating in the study. The risk score represented the

probability that the patient with CRC was carrying a pathogenic mutation in an MMR gene. While all four models could discriminate between carriers and noncarriers of MMR mutations, the best performing model was MMRpredict which achieved a sensitivity of 94% and specificity of 91% when using a cutoff criterion of 1.66 and adjusting for family size. MMRpredict also identified a smaller proportion (11%) of patients than the revised Bethesda criteria (50%) as those who require molecular testing.

Variables in the MMRpredict model include age at diagnosis of CRC, sex, location of tumor, multiple CRCs, occurrence of, and age at, diagnosis of CRC in first-degree relatives, and occurrence of endometrial cancer in any first-degree relative [139].

2.12 Colorectal Cancer Risk Factors

Unlike most other cancers, there is no single risk factor accounting for the majority of colorectal cancer cases. Epidemiological studies have identified family history of CRC, smoking, alcohol consumption, obesity, diabetes, inflammatory bowel disease and consumption of red and processed meats as prominent risk factors [140].

2.12.1 Family History

Among the most prominent risk factors for developing colorectal cancer is family history. Specifically, the number of first-degree relatives (FDRs) with CRC, and their age at diagnosis. A 2013 meta-analysis based on 8091 cases of CRC in 16 studies reported a RR of 1.80 (95% CI: 1.61-2.02) for CRC development when an FDR was also affected [141]. Another meta-analysis of 27 studies reported a RR of 2.25 (95% CI: 2.00-2.53) under the same conditions [142]. That same meta-analysis reported increased RR (4.2; 95% CI: 3.01-6.08) if more than one FDR was affected, as well as if an FDR was 45 years or younger when affected (RR: 3.87; 95% CI: 2.40-6.22). Furthermore, RR was reduced to 1.82 if the FDR was 59 years or older when diagnosed (95% CI: 1.47-2.72).

2.12.2 Inflammatory Bowel Disease

Patients with inflammatory bowel disease (IBD) are also at a substantially increased risk of colorectal cancer development, although incidence of IBD-associated CRC has steadily decreased in the western world [143]. Both duration and extent are the most important risk factors when considering IBD-associated CRC. A meta-analysis [141] using data from 13 cohort studies observed the RR of IBD-associated CRC to be 2.93 (95% CI: 1.79-4.81).

2.12.3 Dietary Effects

Dietary factors have long been a conflicting topic in reference to their implication as a colorectal cancer risk factor; namely alcohol consumption, red and processed meat consumption, fruit- and vegetable-rich diets, and high-fiber diets. Alcohol consumption has been linked to slight increases in risk of CRC. An analysis of 8 cohort studies reported that alcohol consumption increased risk of CRC significantly (RR:1.23; 95% CI: 1.07-1.42) [144]. Consumption of both red and processed meats have also been linked to CRC, likely due to carcinogens present in processed meat, and produced in charred red meat [145]. A meta-analysis reported the linear trend between processed meat and CRC risk to be statistically insignificant [141]. Conversely, the same meta-analysis observed both a significant and positive correlation between red meat consumption in excess of four servings per week, and CRC (RR:1.13, 95% CI: 1.09-1.16). A fruit and vegetable-rich diet has been suggested to reduce CRC risk, however a meta-analysis of 9 studies found insignificant and non-linear results [141]. Lastly, despite a large European cohort study [146] reporting reduced risk of CRC in association with a high-fiber diet, many other cohort studies [147, 148] have reported the contrary.

2.12.4 Lifestyle, Smoking

Metabolic syndrome is a cluster of emerging risk factors associated with a primary clinical outcome of cardiovascular disease [149]. These risk factors include abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance, proinflammatory state and prothrombotic state. The American National Heart, Lung, and Blood Institute considers the "obesity epidemic" as being primarily responsible for the rising prevalence of the syndrome, as it has been shown to contribute to many of these risk factors (hypertension, high serum LDL, low HDL, and hyperglycemia). Patients meeting the criteria for metabolic syndrome have consistently been associated with a 50% increased risk of colorectal cancer, as well [150]. A 2010 review [151] of 1378 cases of CRC observed odds ratios (OR) in men of 1.27 (95% CI, 0.95 – 1.69) for diabetes, 1.24 (95% CI, 1.03 – 1.48) for hypertension, 1.14 (95% CI, 0.82– 1.75) for hypercholesterolemia and 1.26 (95% CI, 1.08 – 1.48) for men with BMI greater than 25. Moreover, in both men and women, the OR of CRC increased in correspondence with the number of metabolic syndrome components. The corresponding ORs were 0.95 (95% CI, 0.84 – 1.06), 1.15 (95% CI, 0.98 – 1.37), and 1.69 (95% CI, 1.23 – 2.33) for 1, 2 and 3 components of metabolic syndrome, respectively.

Despite the mechanism by which it happens remaining unclear, there is widespread epidemiological evidence that strongly indicates an association between cigarette smoking and risk of CRC. While several large cohort studies and meta-analyses have linked smoking to CRC [152-154], others have refuted the association [155, 156]. A 2008 systematic review and meta-analysis [157] of 106 observational studies, however, reported a statistically significant pooled relative risk of 1.18 (95% CI, 1.11 – 1.25). The same systematic review observed CRC mortality of smokers versus non-smokers in 17 cohort studies, reporting a pooled relative risk of 1.25 (95% CI, 1.14 – 1.37). Similarly, a 2013 meta-analysis [141] investigated the effects of smoking measured in pack-years on relative risk of CRC using data from 9399 CRC cases. Compared to non-smokers, the relative risks of CRC were 1.06 (95% CI, 1.03 – 1.08), 1.11 (95% CI, 1.07 – 1.16), 1.21 (95% CI, 1.13 – 1.29) and 1.26 (95% CI, 1.17 – 1.36) for 5, 10, 20 and 30 pack-years, respectively. The consistency of evidence suggests that cigarette smoking increases risk of CRC. Both the extent and the mechanisms by which it does, however, remain unclear.

2.13 Colorectal Cancer Prevention

The chemoprevention of colorectal cancer refers to the use of pharmaceutical compounds intended to prevent adenomatous polyp formation, and if CRC is already present, inhibit its progression. A number of therapies have emerged as potential chemopreventative agents against CRC. Among them are non-steroidal anti-inflammatory drugs (NSAIDs), statins, hormone replacement therapy (HRT) and COX-2 inhibitors.

The potential of NSAIDs – namely Aspirin – as CRC chemopreventative agents has received the most attention and provided the most promise in terms of results. Briefly, Aspirin works mechanistically through inhibition of prostaglandin E2 synthesis by inhibiting cyclooxygenase (COX-1&2) activity that is characteristic of CRC. Aspirin has also been shown to exhibit chemopreventative potential against CRC through the modulation of the wnt/ß – catenin signaling pathway [158].

A significant body of evidence has suggested that 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, commonly referred to as Statins, may have a role in colorectal chemoprevention, specifically as an anti-neoplastic agent in the colon. Statins have exhibited growth-inhibitory and pro-apoptotic effects in a number of human colorectal cancer cell lines in vitro [159]. While the mechanisms of which statins modulate colorectal cancer cell growth remain poorly understood, the mechanisms by which statins are believed to act are via increased oxidative stress, endoplasmic reticulum stress, and altered expression of apoptotic and proliferative signalling molecules of CRC cells [160].

While the use of hormone replacement therapy (HRT) in the treatment of CRC has received considerable interest, previous research has generated inconsistent results. A 2017 study, however, observed the use of HRT in women diagnosed with CRC to reduce the risk of CRC mortality by 26% (HR =0.67, 95% CI: 0.56-0.79) [161].

No therapy is currently recommended as a chemopreventative agent. Despite the current lack of evidence, the fact that both adenomatous polyp development and existing CRC progression appear to be influenced by chemopreventative agents is promising.

2.14 Study Rationale

The rationale of this study is rooted in the results of a previous study [2] by many of the same authors. In 2010, a familial colorectal cancer clinic was started in two regions of NL to provide screening recommendations to families of incident CRC patients based on family risk of CRC because guidelines recognize family members of CRC patients as high-risk individuals who require screening colonoscopies, and because incidence of familial CRC in NL is high. The aforementioned study provided two conclusions concerning the clinic: (1) the experience with the clinic in NL was disappointing considering the response rate was only 52% of the probands, and the efficiency of genetic counselor services was poor; and (2) the rate of referral to a genetic counselor for probands with CRC was high (28%) and the decision seemed to reflect a subjective assessment of the pedigree. This study also surmised that the decisions for genetic counselor referral for patients in the intermediate risk groups was likely based on subjective interpretation of family history by geneticists, as there was little difference in other criteria that contribute to screening recommendation decisions i.e. Family History Score, MMRpredict score, Bethesda criteria. Furthermore, the efficiency in the provision of counseling was poor as only 30% of high and intermediate-high risk families were seen by genetic counselors during the duration of the study. The poor efficiency was the result of delays in ensuring family history accuracy, and obtaining information from hospitals, which in turn created a large waitlist for referral to genetic counselors for high and intermediate-high risk patients. This produced a workload that was too large for genetic counselors.

The NL population is a valuable resource for the hereditary study of colorectal cancer for a number of reasons. For one, approximately 90% of its 510,000 inhabitants are descendants from its original 20,000 - 30,000 founders [162]. Secondly, the population has been genetically isolated, homogenous, had low in-or-out-migration rates, and families were consistently large over several generations [163]. Finally, in a study of thirteen founder populations, the

Newfoundland population had the greatest genetic generalizability to Caucasian populations when compared to the other twelve [164].

The abovementioned value of the NL population for studying heritable colorectal cancer, coupled with advances in our understanding of genetics and recent emphasis on preventative medicine [165] were important factors in rationalizing this study. Furthermore, the large workload required of genetic counselors, the inefficient use of their time, as well as the unavoidable subjectivity in interpreting family history and proving screening recommendations highlight the need to determine whether an algorithmic approach to risk definition and screening recommendations was possible. This subsequently makes the identification and management of families with high susceptibility of CRC an important topic of research and ultimately provides the rationale for this study.

2.15 Study Objectives

The first objective of this study was to assess the effectiveness with which the previously established population-based familial colorectal cancer clinic provided colonoscopic screening recommendations to families at different degrees of risk of CRC. The second objective of this study was to develop and assess the utility of multivariate prediction models that could facilitate screening recommendations in families with CRC and compare the electronic predictions to the clinical decisions previously made by genetic counselors.

Chapter 3 – METHODOLOGY

3.1 The Cohort

The cohort used to develop the model was from a Familial colorectal cancer clinic that was started in 2010 in urban (Eastern Health) [Figure 3] and rural (Central Health) [Figure 4] regions of NL to provide risk-based screening recommendations to families of patients with incident CRC [2]. The rationale behind the clinic was that family members of CRC patients are recognized as a high-risk group who require screening colonoscopies [156], and that NL has a high incidence of familial CRC [7]. Patients invited to the clinic were pathologically confirmed incident CRC patients from the Newfoundland Cancer Registry who presented between 2008-2010. Patients were asked to provide a family history, including cancer occurrence in first and second-degree relatives. When necessary, medical records and release of information consent was requested of family members. Patients and family members were afforded the right of refusal, and their reasons for doing so were recorded. Family pedigrees were reviewed by a medical geneticist and genetic counselor. Family risk for HNPCC and CRC was determined using the Amsterdam criteria, age and cancer modified Amsterdam criteria (ACMAC), Bethesda criteria, presence of multiple adenomatous polyps in a first-degree relative, and local expert opinion.

From 2008-2010, n=784 incident CRC cases were presented to Eastern Health, while n=307 presented to Central Health. Following the abovementioned process, of the n=1,091 CRC cases presented at the two health regions, n=529 were enrolled into the clinic. Genetic counselors stopped working at the end of 2013 when funding for this project ceased. All patients received recommendations for colonoscopy screening in family members pending work-up for Lynch Syndrome. Contact information was available for 99.6% of patients. Incident endometrial and ovarian cancer patients were also contacted but are not included in this report. Of the n=529 patients enrolled in the clinic, n=525 were considered for analysis, as n=4 patients had no information available, and were listed as ‘Waiting on Records’.

Patients' demographic information including age at diagnosis, sex, and region was collected from the NFCCR. Clinical information including histologic type, name of tumor site and tumor grade were collected from pathology reports.

Patients with families at high or intermediate-high risk of CRC were referred to attend the genetic counselor to provide colonoscopy screening recommendations to families and obtain consent if necessary, for tumor/DNA cascade testing to diagnose possible LS. Patients with family at low or intermediate-low risk of CRC received a letter or phone call summarizing the family history and were provided with screening recommendations thereafter.

At Eastern Health, clinical staffing included a genetic counselor, a clerk, an information technology and data management research assistant and a subject matter expert research assistant. At Central Health, clinical staffing included a nurse and a clerk.

Ethics approval was granted by the Health Research and Ethics Board of Memorial University of Newfoundland (#2016.147); Researcher Portal File (#20170239) [Appendix C].

Figure 3: The Geographic Boundaries of Eastern Health, NL [166]

Image of the Eastern Health Region with site locations depicted. Retrieved from: http://www.easternhealth.ca/AboutEH.aspx?d=1&id=1995&p=73

Figure 4: The Geographic Boundaries of Central Health, NL [167]

Image of the Central Health Region with site locations depicted. Retrieved from: https://www.centralhealth.nl.ca/about-us

3.2 Variables

3.2.1 Risk Classification

The definitions of family risk for developing colorectal cancer used by genetic counselors are as follows. ***Low***: No criteria of increased family risk of CRC. ***Intermediate-Low***: Increased family risk of CRC but not necessary to see a genetic counselor. ***Intermediate-High***: Increased family risk of CRC such as to make it necessary to see a genetic counselor. ***High***: Fulfilled Amsterdam or ACMAC criteria, necessary to see a genetic counselor.

3.2.2 Family History of Colorectal Cancer

Familial colorectal cancer was defined as having at least one first-degree relative (FDR) with CRC. Family History was classified according to the Amsterdam criteria definition.

3.2.3 Family History Score

The Family History Score (FHS) compares each family member to age and sex-matched population controls with respect to probability of cancer. The score compares the observed number of cases in a family over a specific period of time to the expected number of cases and is calculated based on family member covariates such as age, sex and race, as well as overall family structure [8].

3.2.4 MMRpredict Score

The MMRpredict score is a model for identifying colorectal cancer patients at high risk of carrying a DNA MMR gene mutation and thus require LS screening. It has been shown to be the best-performing model as compared to other similar models in showing presence of DNA MMR gene mutations, where a cutoff criterion of 1.66 showed optimal specificity (91%) and sensitivity (94%) when corrected for family size. Variables in the model include age at diagnosis of CRC, sex, location of tumor, multiple CRCs, occurrence of, and age at, diagnosis of CRC in first-degree relatives, and occurrence of endometrial cancer in any first-degree relative.

3.2.5 The Amsterdam Criteria

***Amsterdam I Criteria*:** at least 3 family members with colorectal cancer plus:

- One is a first degree relative of the other two
- Two successive generations represented
- At least 1 individual younger than 50 years at diagnosis
- FAP excluded
- Tumors verified by pathological examination

***Amsterdam II Criteria*:** 3 family members with HNPCC-related cancer (CRC, endometrial, small bowel, ureter, renal pelvis) plus:

- One is a first degree relative of the other two
- Two successive generations represented

- At least 1 individual younger than 50 years at diagnosis
- FAP excluded
- Tumors verified by pathological examination

3.2.6 The Bethesda Criteria

The Revised Bethesda Criteria:

1) CRC Patient who is less than 50 years of age.
2) Presence of synchronous or metachronous CRC or other HNPCC-related tumor, any age.
3) CRC with MSI-H histology diagnosis in a patient who is less than 60 years of age.
4) Individual and one first-degree relative with an HNPCC-related tumor with one of the cancers being diagnosed under the age of 50 years old.
5) Individual and two first- or second-degree relatives with HNPCC-related tumors*, any age.

* HNPCC-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, renal, pelvic, biliary tract, brain (glioblastoma), small intestine, sebaceous gland adenomas and keratoacanthomas.

[Appendix A]

3.2.7 ACMAC

***PMG1*:** ACMAC

(d) Three or more relatives with colorectal cancer or an HNPCC-associated cancer (colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain, sebaceous gland and small bowel carcinoma) *AND*
(e) Colorectal or HNPCC-associated cancer in *at least* two generations *AND*
(f) One or more colorectal or HNPCC-associated cancers diagnosed before age 60

Or one of the following PMG criteria:

***PMG2*:** Colorectal carcinoma before age 40

***PMG3*:** Endometrial carcinoma before age 45

***PMG4*:** Sebaceous tumor (adenoma, carcinoma, epithelioma) before age 50 or multiple tumors at any age

***PMG5*:** Presence of multiple HNPCC-related cancers in an individual, with one cancer before age 60

[Appendix B]

3.2.8 Screening Recommendations and Colonoscopy Frequencies

Screening recommendations to family members are presented as age recommended for first colonoscopy, frequency of subsequent colonoscopies, and referral for tumor/DNA cascade testing for Lynch syndrome.

The recommended screening frequencies are as follows: (i) 1-2 years, (ii) 2-3 years, (iii) 3-5 years, and (iv) 5-10 years. For 1-2 years, those recommended for yearly and 1-2 years were included; for 2-3 years, those recommended for 2 yearly or 2-3 yearly were included; for 3-5 years, those recommended for 3 yearly, 4 yearly or 3-5 yearly were included; for 5-10 years, those recommended for 5 yearly, 10 yearly or 5-10 yearly were included.

Screening options for patients over the age of 65 years with a negative family history of CRC could also include fecal immunochemical testing (FIT) every 2 years in lieu of colonoscopy.

3.3 Statistical Methods

Statistical analysis was performed using IBM's SPSS version 25.0, in which a database was constructed with patient demographics and tumor characteristics, as well as various categorical, nominal and continuous variables pertaining to colorectal cancer risk, family history and screening information. Specifically, patient demographic information refers to a unique and confidential study ID, the region of the clinic attended, gender, age at diagnosis, and whether the patient had been recommended for a meeting with a genetic counselor, received a phone call, or

a letter in the mail. Among variables pertaining to risk of CRC were risk as defined by a genetic counselor, family history of CRC, Family History Score, MMRpredict Score and whether or not they fulfil Amsterdam, Bethesda or Provincial Medical Genetics criteria. Variables associated with recommended CRC screening interval were colonoscopy frequency and age at first colonoscopy.

Pearson chi-square tests were performed to test for baseline differences and possible associations among gender, risk and frequency of colonoscopy. The test is applied to sets of categorical data, often within a contingency table, and Pearson's X^2 statistic is used to test the independence of the jointly multinomial variables in the rows and columns [168].

Binary logistic regression models were used to assess the relationship between outcome variables and the independent clinical and demographic variables associated with subsets of patients. This relationship was estimated using Odds Ratio. Separate univariate logistic regressions were performed for each variable. Variables with a $p < 0.20$ were tested in subsequent multivariate regression models. Variables with $p<0.050$ were included in the final multivariate model, with their respective Adjusted Odds Ratio (AOR). The multivariate logistic regression models, along with sensitivity and specificity, were used to identify and assess predictive variables among the three brackets of screening frequencies, as well as to compare patients recommended for genetic counseling to those who were not. For high and low risk patients especially, sensitivity was important as a matter of accurately ruling in patients.

The multivariate regression models produced the following equation:
$Y = ß_0 + ß_1x_1 + ß_2x_2 + \cdots + ß_nx_n$; where ß represents the regression coefficient of the multivariate model; and x represents variables in the model. The multivariate model scores were then converted to a probability score (P) between 0-1; where:

$$Y = \log\left(\frac{P}{1-P}\right) \qquad e^Y = \left(\frac{P}{1-P}\right) \qquad P = \frac{e^Y}{1+e^Y}$$

A receiver operator characteristic (ROC) curve is produced using sensitivities and specificities for different values of a continuous test measure. The result is a list of various test values and corresponding sensitivity and specificity of the test at that value. The graphical ROC curve is produced by plotting sensitivity on the y-axis and 1-specificity on the x-axis for various values tabulated [169]. Positive and negative predictive values are used as estimates of the accuracy of the test. Specifically, the positive predictive value (PPV) estimates the fraction of patients who are diagnosed/classified correctly as positive, while the negative predictive value (NPV) estimates the fraction of patients who are diagnosed/classified correctly as negative [170].

The area under the ROC curve is a measure of the ability of a test to discriminate whether or not a specific condition is present. An area of 0.5 represents a test with no discriminating ability, while an area of 1.0 represents a test with perfect discrimination. Selecting an optimal threshold requires careful consideration of the significance of false positives and false negatives. Giving equal importance to sensitivity and specificity is the most commonly used approach. This is achieved by choosing the point nearest the top-left corner of the curve, known as the Youden Index [171]. However, selecting a threshold at the Youden Index assumes that the balance between false-positive and false-negative rates is not clinically important. Thus, the selection of a threshold depends on the purpose of the test and not simply giving equal weight to sensitivity and specificity in order to achieve higher accuracy [172].

As identification of the Amsterdam criteria in a family indicates screening colonoscopy at 1-2-year intervals in family members, these 10 families were omitted from the multivariate modelling. Multivariate models were created for prediction of:

(1) Referral to a genetic counselor
(2) Screening colonoscopy recommended every 5-10 years in family members
(3) Screening colonoscopy recommended every 2-3 years in family members
(4) Screening colonoscopy recommended every 3-5 years in family members

Chapter 4 – RESULTS

4.1 Results from the Newfoundland Colorectal Cancer Clinic

The task flows in the Familial colorectal cancer clinic from 2010-2013 are outlined in Figures 7 and 8. Of n=1091 CRC patients eligible to attend clinic contact was made with n=1085 (99.6%), n=691 (63.7%) agreed to participate and n=558 of 1085 (51.4%) provided a completed Family History Questionnaire. Of those n=558 patients, n=29 were members of existing families, while n=4 were waiting on records, leaving n=525 patients eligible for analysis.

In comparing patients who provided a family history to those who did not, n=233 (43%) versus n=147 (55%) were older than 75 years, n=316 (58%) versus n=157 (57%) were male, and n=355 (60%) versus n=202 (76%) were from Eastern Health Region. Thus, on average family history was more likely to be available from younger probands and those from Central Health.

Reasons for refusal to attend the clinic were as follows: of n=267 who refused, 52.4% (n=140) had no interest, 19.4% (n=52) did so because they had no family history of CRC, 17.6% (n=47) were too old or too sick, n=15 (6%) said family were already in screening program, and the remaining 4.9% (n=13) had miscellaneous reasons. A further n=134 (12.3%) agreed to provide a family history but never did so.

Of the n=256 patients who were asked to complete a release of information form to confirm tumor pathology, n=146 (57%) completed this task, comprising 292 tumors.

Twenty percent of probands had familial CRC (n=106). Only n=10 (1.9%) families fulfilled Amsterdam I or II criteria, while 18.7% (n=98) fulfilled ACMAC criteria, and 37.0% (n=194) Bethesda criteria. Fifty-seven percent (n=300) of families were considered to be at low risk for CRC by the geneticist, 15% (n=81) intermediate low, 23.4% (n=123) intermediate high and 4.0% (n=21) high risk for CRC. The latter 2 groups were asked to attend the genetic counselor.

The distribution of FHS is shown in Figure 5. Twenty-three percent fulfilled the Provincial Medical Genetics Program criteria for Lynch Syndrome testing. Twenty-four percent had an MMRpredict score > 1.66. Distribution of MMRpredict is provided in Figure 6.

Figure 5: Distribution of Family History Scores

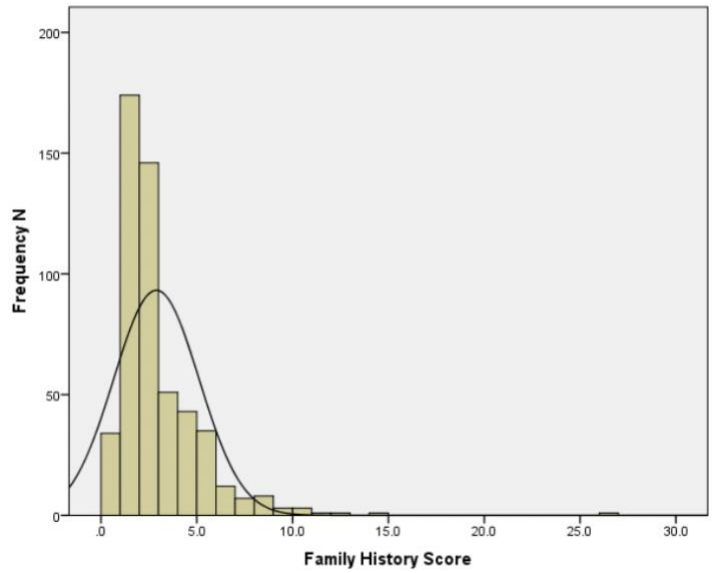
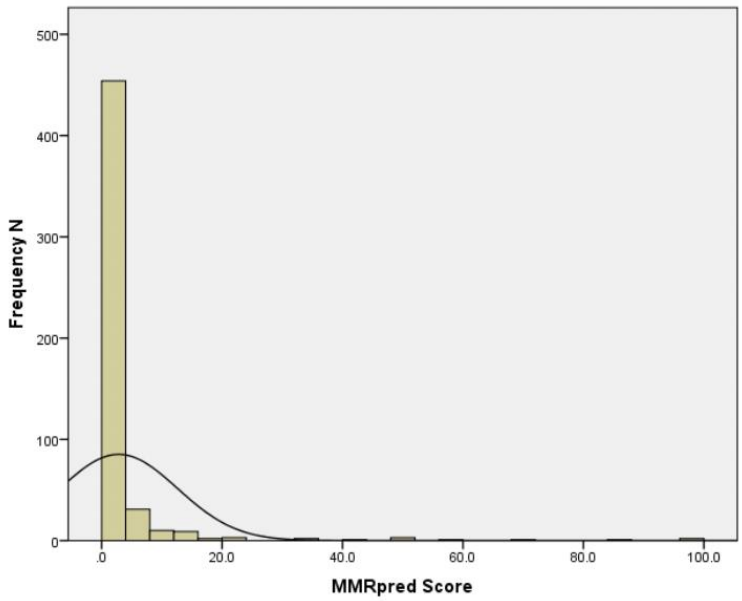


Figure 6: Distribution of MMRpredict Scores



Population Descriptives

Table 1: CRC Patients Classified by Risk

Table 2: CRC Patients Classified by Frequency of Colonoscopy for Family Members

Table 3: CRC Patients Classified by Age Brackets

These tables include number of cases (n), percentages for given variables, and p-values for Pearson chi-square tests when applicable. The Pearson chi-square test was performed to test for baseline differences and possible association among variables. The test is applied to sets of categorical data, often within a contingency table, and Pearson's statistic is used to test the independence of the jointly multinomial variables in the rows and columns [163].

The clinical and family characteristics of the patients by family risk of CRC as defined by the geneticist are outlined in Table 1. Mean ages were significantly lower when comparing high risk patients to the rest. Among the rest, there was little difference in patient age. Similarly, Family History Scores and MMRpredict scores were significantly higher among the high-risk patients. There was little difference in both scores among intermediate high and intermediate low patients. Patients at intermediate high risk were recommended for significantly more frequent screening than those in intermediate low, however. Rates of ACMAC fulfilment, polyps and family history of CRC were also significantly different among intermediate high and intermediate low patients.

Clinical and family characteristics of patients by frequency of colonoscopy recommended to family members are outlined in Table 2. Fifteen percent of the families were recommended for screening colonoscopies in the 1-3-year brackets, 21% in the 3-5-year bracket, and the remaining 64% in the 5-10-year bracket. More frequent screening recommendations were associated with higher FHS and MMRpredict scores, proportions fulfilling Amsterdam criteria and ACMAC, history of polyps, and a lower mean age at diagnosis.

Figure 7: Risk-based Classification of Patients from the CRC Clinic

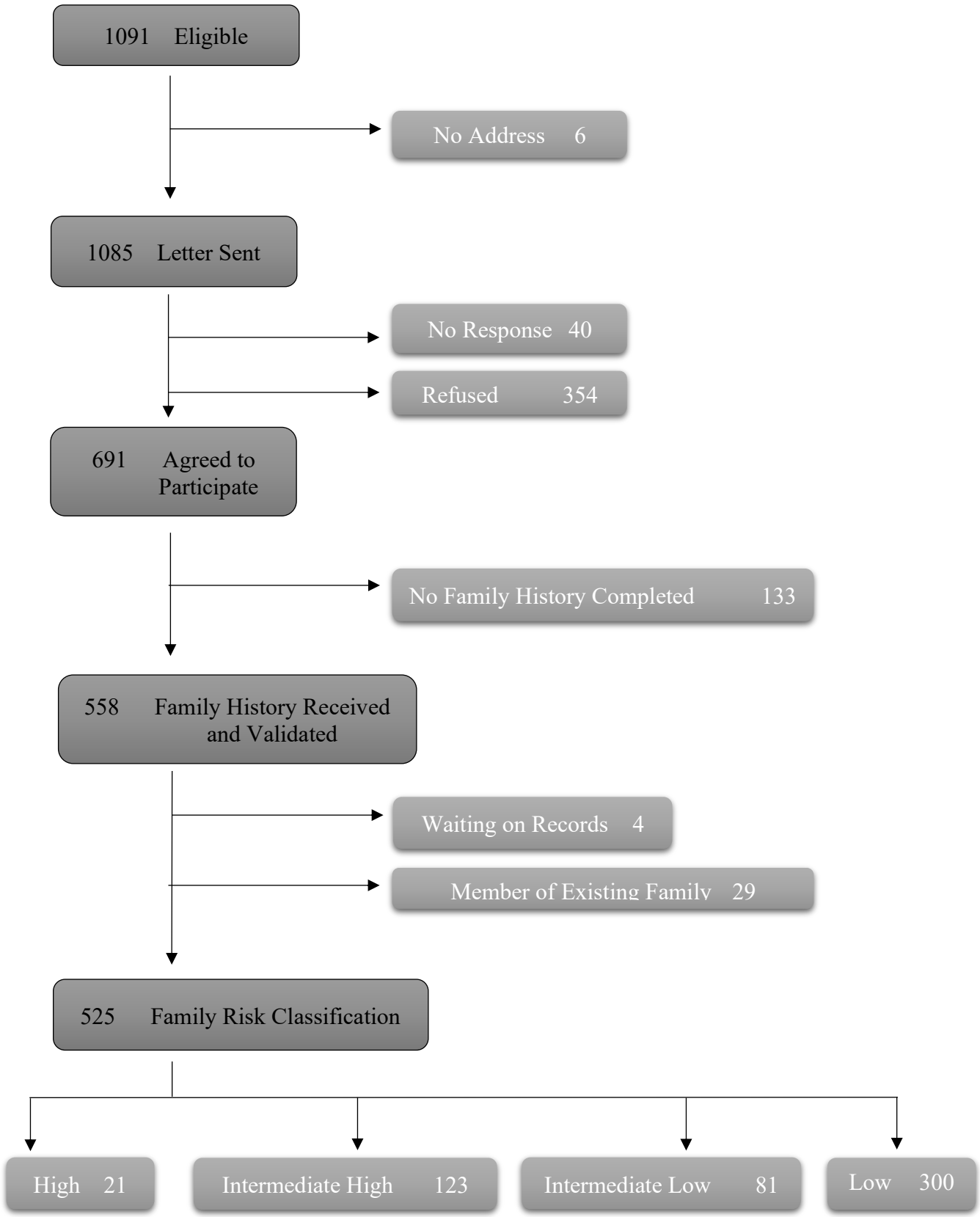


Figure 8: Screening Frequency-based Ascertainment of Patients from the CRC Clinic

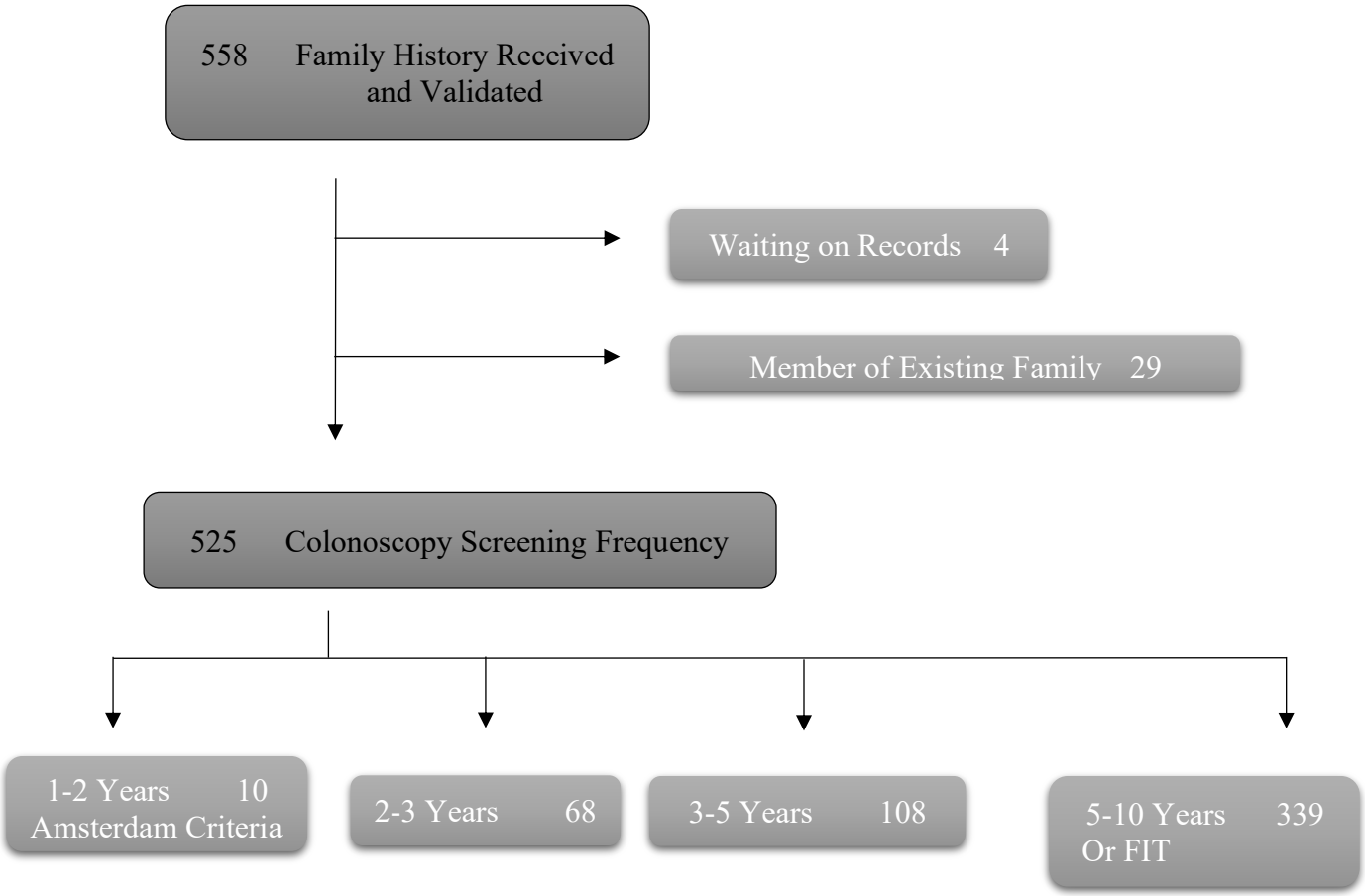


Table 1: CRC Patients Classified by Risk

Risk (N)	High (21)	Int High (123)	Int Low (81)	Low (300)	p-value
Region					
GFW, N (%)	6 (3.4)	26 (14.9)	29 (16.7)	110 (63.2)	
SJ, N (%)	15 (4.2)	97 (27.3)	52 (14.6)	190 (53.5)	0.010
Sex					
Male, N (%)	9 (3.0)	66 (21.6)	47 (15.4)	179 (58.7)	
Female, N (%)	12 (5.4)	57 (25.4)	34 (15.2)	121 (54.0)	0.190
Screening Frequency					
1-3, N (%)	19 (24.4)	54 (69.2)	4 (5.1)	1 (1.3)	
3-5, N (%)	2 (1.9)	53 (49.1)	34 (31.5)	19 (17.6)	
5-10, N (%)	0 (0.0)	16 (4.7)	43 (12.7)	280 (82.6)	0.000
Age Bracket					
≤50	7 (26.9)	9 (34.6)	7 (26.9)	3 (11.5)	
51-64	9 (5.0)	53 (29.3)	16 (8.8)	103 (56.9)	
≥65	5 (1.6)	61 (19.2)	58 (18.2)	194 (61.0)	0.000
Mean Age ± SD	56.2 ± 15.0	65.0 ± 10.9	68.1 ± 11.5	68.9 ± 9.9	
FHS Median	7.8	3.1	2.5	1.9	
Q1 – Q3	5.1-9.8	1.9-4.9	1.8-4.1	1.4-2.5	
MMRpred Median	7.8	1.0	0.8	0.2	
Q1 – Q3	2.5-34.2	0.3-3.2	0.2-2.8	0.1-0.6	
%>1.66	12.2	34.1	24.4	27.6	0.000
Amsterdam N (%)	9 (90.0)	1 (10.0)	0 (0.0)	0 (0.0)	0.000
AC I	8 (88.9)	1 (11.1)	0 (0.0)	0 (0.0)	0.000
AC II	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.000
Bethesda N (%)	17 (8.4)	108 (53.5)	74 (36.6)	3 (1.5)	0.000
B1	7 (26.9)	9 (34.6)	7 (26.9)	3 (11.5)	0.000
B2	2 (4.8)	22 (52.4)	18 (42.9)	0 (0.0)	0.000
B3	2 (13.3)	10 (66.7)	3 (20.0)	0 (0.0)	0.000
B4	5 (20.0)	13 (52.0)	7 (28.0)	0 (0.0)	0.000
B5	6 (4.7)	73 (57.5)	48 (37.8)	0 (0.0)	0.000
Polyps, N (%)	0 (0.0)	11 (64.7)	5 (29.4)	1 (5.9)	0.000
FamCRC, N (%)	18 (17.0)	56 (52.8)	32 (30.2)	0 (0.0)	0.000
ACMAC (PMG1)	11 (11.2)	60 (61.2)	27 (27.6)	0 (0.0)	0.000
PMG2	1 (11.1)	6 (66.7)	2 (22.2)	0 (0.0)	0.007
PMG3	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	0.060
PMG4	2 (9.1)	10 (45.5)	10 (45.5)	0 (0.0)	0.000
PMG5	0 (0.0)	8 (80.0)	2 (20.0)	0 (0.0)	0.000
Ready For, N (%)					
Genetic Counseling	21 (13.7)	122 (79.7)	0 (0.0)	9 (5.9)	
Low Risk Letter	0 (0.0)	0 (0.0)	8 (2.8)	280 (97.2)	
Phone Call/Letter	0 (0.0)	0 (0.0)	3 (42.9)	1 (14.3)	0.000

Table 2: CRC Patients by Frequency of Colonoscopy Recommended to Family Members

Screening Freq (N)	1-3 Years (78)	3-5 Years (108)	5-10 Years (339)	p-value
Region				
GFW, N (%)	23 (13.5)	28 (16.4)	120 (70.2)	
SJ, N (%)	55 (15.5)	80 (22.6)	219 (61.9)	0.154
Sex				
Male, N (%)	43 (14.3)	59 (19.6)	199 (66.1)	
Female, N (%)	35 (15.6)	49 (21.9)	140 (62.5)	0.692
Family Risk of CRC				
High	19 (90.5)	2 (9.5)	0 (0.0)	
Int High	54 (43.9)	53 (43.1)	16 (13.0)	
Int Low	4 (4.9)	34 (42.0)	43 (53.1)	
Low	1 (0.3)	19 (6.3)	280 (93.3)	0.000
Age Bracket				
≤50	13 (50.0)	11 (42.3)	2 (7.7)	
51-64	35 (19.3)	45 (24.9)	101 (55.8)	
≥65	30 (9.4)	52 (16.4)	236 (74.2)	0.000
Mean Age ± SD	60.8 ± 13.3	64.7 ± 11.4	69.7 ± 9.3	
FHS Median	4.4	2.7	2.0	
Q1 – Q3	2.4-6.4	1.9-4.4	1.4-2.6	
MMRpred Median	2.5	0.6	0.2	
Q1 – Q3	0.5-10.4	0.2-2.3	0.1-0.7	
%>1.66	33.9	26.4	39.7	0.000
Amsterdam N (%)	10 (100.0)	0 (0.0)	0 (0.0)	0.000
AC I	9 (100.0)	0 (0.0)	0 (0.0)	0.000
AC II	1 (100.0)	0 (0.0)	0 (0.0)	0.057
Bethesda N (%)	67 (33.2)	84 (41.6)	51 (25.2)	0.000
B1	13 (50.0)	11 (42.3)	2 (7.7)	0.000
B2	11 (26.2)	13 (31.0)	18 (42.9)	0.008
B3	8 (53.3)	6 (40.0)	1 (6.7)	0.000
B4	13 (52.0)	8 (32.0)	4 (16.0)	0.000
B5	35 (27.6)	59 (46.5)	33 (26.0)	0.000
Polyps, N (%)	7 (41.2)	4 (23.5)	6 (35.3)	0.005
FamCRC, N (%)	47 (44.3)	42 (39.6)	17 (16.0)	0.000
ACMAC (PMG1)	37 (37.8)	40 (40.8)	21 (21.4)	0.000
PMG2	4 (44.4)	2 (22.2)	3 (33.3)	0.034
PMG3	1 (33.3)	1 (33.3)	1 (33.3)	0.500
PMG4	5 (22.7)	6 (27.3)	11 (50.0)	0.334
PMG5	6 (60.0)	3 (30.0)	1 (10.0)	0.000
Ready For, N (%)				
Genetic Counseling	73 (48.0)	61 (40.1)	18 (11.8)	
Low Risk Letter	0 (0.0)	9 (3.1)	279 (96.9)	
Phone Call/Letter	4 (4.9)	37 (45.7)	40 (49.4)	0.000

Table 3: CRC Patients Classified by Age Bracket

Age Bracket (N)	≤50 (26)	51-64 (181)	≥65 (318)	p-value
Region				
GFW, N (%)	7 (4.1)	48 (28.1)	116 (67.8)	
SJ, N (%)	19 (5.4)	133 (37.6)	202 (57.1)	0.061
Sex				
Male, N (%)	12 (4.0)	107 (35.5)	182 (60.5)	
Female, N (%)	14 (6.3)	74 (33.0)	136 (60.7)	0.457
Screening Frequency				
1-3, N (%)	13 (16.7)	35 (44.9)	30 (38.5)	
3-5, N (%)	11 (10.2)	45 (41.7)	52 (48.1)	
5-10, N (%)	2 (0.6)	101 (29.8)	236 (69.6)	0.000
Risk				
High	7 (33.3)	9 (42.9)	5 (23.8)	
Int High	9 (7.3)	53 (43.1)	61 (49.6)	
Int Low	7 (8.6)	16 (19.8)	58 (71.6)	
Low	3 (1.0)	103 (34.3)	194 (64.7)	0.000
FHS Median	4.1	2.5	1.9	
Q1 – Q3	2.7-6.9	1.9-4.3	1.3-3.0	
MMRpred Median	2.8	0.6	0.2	
Q1 – Q3	1.4-9.3	0.3-2.6	0.1-0.8	
%>1.66	13.0	48.0	39.0	0.000
Amsterdam N (%)	5 (50.0)	2 (20.0)	3 (30.0)	0.000
AC I	4 (44.4)	2 (22.2)	3 (33.3)	0.000
AC II	1 (100.0)	0 (0.0)	0 (0.0)	0.000
Bethesda N (%)	26 (12.8)	66 (32.7)	110 (54.5)	0.002
B1	26 (100.0)	0 (0.0)	0 (0.0)	0.000
B2	1 (2.4)	11 (26.2)	30 (71.4)	0.300
B3	3 (20.0)	9 (60.0)	3 (20.0)	0.001
B4	0 (0.0)	8 (32.0)	17 (68.0)	0.453
B5	8 (6.3)	45 (35.4)	74 (58.3)	0.669
Polyps, N (%)	0 (0.0)	8 (47.1)	9 (52.9)	0.397
FamCRC, N (%)	7 (6.6)	32 (30.2)	67 (63.2)	0.451
ACMAC (PMG1)	7 (7.1)	44 (44.9)	47 (48.0)	0.017
PMG2	1 (11.1)	0 (0.0)	8 (88.9)	0.079
PMG3	0 (0.0)	1 (33.3)	2 (66.7)	0.919
PMG4	1 (4.5)	7 (31.8)	14 (63.6)	0.954
PMG5	0 (0.0)	5 (50.0)	5 (50.0)	0.495
Ready For, N (%)				
Genetic Counseling	16 (10.5)	68 (44.7)	68 (44.7)	
Low Risk Letter	3 (1.0)	92 (31.9)	193 (67.0)	
Phone Call/Letter	6 (7.4)	21 (25.9)	54 (66.7)	0.000

The clinical and family characteristics of the patients by age are outlined in Table 3. Fifty percent (n=13) of patients under 50 years old were in the most frequent screening bracket of 1-3 years. Conversely, 74% of patients 65 years and older were in the least frequent screening bracket of 5-10 years. The median of MMRpredict scores was 2.8 in patients under the age of 50, compared to 0.2 in those greater than 65 years old. Sixty-one percent of patients were ≥ 65 years of age, of whom 20.8% had families at high or intermediate-high risk of CRC, compared to 37.7% of patients under 65 years of age. Nevertheless, 34.6% of patients older than 65 years of age fulfilled at least one Bethesda criterion, compared to 40.6% of patients under 65 years old. Moreover, 14.8% of patients 65 years and older fulfilled ACMAC, while 21.1% had a family history of CRC.

Twenty-nine percent (n=152/525) were referred to the genetic counselor, n=21 (14%) of whom had high family risk and n=122 (80%) intermediate high risk. No patients with intermediate low risk were referred to a genetic counselor, however, n=9 (6%) of low-risk patients were.

4.2 Electronic Prediction versus Clinical Recommendations

For the purposes of this study specifically, screening frequency and age brackets were both amended from their originals referenced in section 4.1. Recommended colonoscopy screening frequencies were reduced from four categories to three, now reading: 2-3 years, 3-5 years and 5-10 years. Age brackets were adjusted to classify patients as ≤50, 51-64, or ≥65 years old. Patients fulfilling Amsterdam criteria (n=10) were excluded from the univariate and multivariate regression models, on the basis of being too high a predictive variable and skewing the model. Thus, any patient fulfilling Amsterdam criteria was automatically recommended for a screening frequency of 1-2 years. The Bethesda 1 criteria (CRC diagnosis in a patient who is less than 50 years of age) was excluded from the univariate and multivariate regression models in favor of the 'Age at Diagnosis' brackets, to avoid possible confounding factors.

FHS and MMRpredict data was excluded from multivariate regression models on account of potential collinear data stemming from their multivariate nature.

4.2.1 Prediction of Referral to a Genetic Counselor

Every patient fulfilling Amsterdam criteria were referred to see a genetic counselor, so for the purposes of this particular analysis, they were omitted. Moreover, the test variable of 'Family history of CRC' was amended to include only patients with a family history of CRC but *without* fulfilling ACMAC, termed: 'FamCRC without ACMAC'.

We compared patients recommended for genetic counseling to those who were not [Table 5]. In univariate analysis, patients referred to a genetic counselor were more likely to fulfil ACMAC ($p<0.0001$), have a family history of CRC without fulfilling ACMAC ($p<0.0001$), have a FHS in the fifth quintile ($p<0.0001$), a MMRpredict score >1.66 ($p<0.0001$), fulfil Bethesda 2 ($p<0.0001$), Bethesda 3 ($p<0.0001$), Bethesda 4 ($p<0.0001$) and Bethesda 5 ($p<0.0001$) criteria, and have polyps ($p=0.001$). Patients referred to a genetic counselor were also more likely to fulfil ACMAC criteria ($p=0.008$), PMG3 criteria ($p=0.003$), PMG4 criteria ($p=0.002$), and be 50 years old or younger ($p<0.0001$).

In multivariate analysis, independent factors predicting referral to a genetic counselor were ACMAC (OR= 12.5; $p<0.0001$), have family history of CRC independent of ACMAC (OR= 10.3; $p<0.0001$), fulfil Bethesda 2 criteria (OR= 8.4; $p<0.0001$), Bethesda 3 criteria (OR= 5.6; $p=0.042$), Bethesda 4 criteria (OR= 3.9; $p=0.050$) and have polyps (OR= 16.5; $p<0.0001$). Patients referred to a genetic counselor were also more likely to be 50 years old and younger (OR= 8.7; $p=0.001$), or between the ages of 51-64 (OR= 2.7; $p=0.001$), when compared to patients aged 65 years and older.

There is a strong statistically significant association between the predictive variables and the referral to a genetic counselor [$X^2= 244.4$; $df=9$; $p<0.0001$]. Using a cut-off of 0.4, the model had a sensitivity of 81.3% and a specificity of 87.4%. The model also had a PPV of 69.4% and an NPV of 93.0%. The c-statistic of this model, which represents Area Under the Curve (AUC) of the Receiver Operator Characteristic curve was 0.912 [Table 4].

Table 4: Descriptive Data from the Multivariate Model Predictive of GC Referral

Multivariate Model Descriptive Statistics	
Number of patients	514
Patients referred to GC	134
Patients not referred to GC	380
Chi-square Statistic, X^2	244.4
Degrees of Freedom	9
P-value	<0.0001
Model's Predicted Probability of GC Referral	
Cut-off	0.400
Mean	0.261
Standard Deviation	0.300
Minimum	0.032
Maximum	0.996
Sensitivity	81.3%
Specificity	87.4%
Positive Predictive Value	69.4%
Negative Predictive Value	93.0%
Area Under the Curve	0.912

The multivariate model yielded the following equation, where 'Y' represents patients referred for genetic counseling:

$$Y = ß0 + ß1x1 + ß2x2 + ß3x3 + ß4x4 + ß5x5 + ß6x6 + ß7x7 + ß8x8$$

$$Y = -3.399 + 2.331\ [\text{FamCRC w/o ACMAC}] + 2.125\ [\text{Bethesda 2}] + 1.723\ [\text{Bethesda 3}] + 1.357\ [\text{Bethesda4}] + 2.803\ [\text{Polyps}] + 2.527\ [\text{ACMAC}] + 2.164\ [\text{Age}\geq 50] + 0.991\ [\text{Age51-64}]$$

Table 5: Univariate and Multivariate Odds Ratios to Predict Referral to a Genetic Counselor: Yes (n=134) versus No (n=381)

Predictor Variable	**N, % H+IH**	**N, % IL+L**	**OR[a], Univariate (95% CI)**	**p-value**	**AOR[b], Multivariate (95%CI)**	**p-value**
Amsterdam	10 (6.9)	0 (0.0)	∞	0.000	ni[c]	ni
FamCRC w/o ACMAC	28 (20.9)	17 (4.5)	5.7 (3.0 – 10.7)	0.000	10.3 (3.3 – 32.5)	0.000
FHS in 5th Quintile	52 (39.1)	42 (11.0)	5.1 (3.2 – 8.2)	0.000	ni	ni
MMRpredict Score >1.66	51 (38.3)	64 (17.0)	3.0 (2.0 – 4.7)	0.000	ni	ni
Bethesda 1	11 (8.2)	10 (2.6)	3.3 (1.4 – 8.0)	0.008	ni	ni
Bethesda 2	24 (17.9)	18 (4.7)	4.4 (2.3 – 8.4)	0.000	8.4 (3.4 – 20.7)	0.000
Bethesda 3	12 (9.0)	3 (0.8)	12.4 (3.4 – 44.6)	0.000	5.6 (1.1 – 29.5)	0.042
Bethesda 4	17 (12.7)	7 (1.8)	7.8 (3.1 – 19.2)	0.000	3.9 (1.0 – 15.2)	0.050
Bethesda 5	79 (59.0)	48 (12.6)	10.0 (6.3 – 15.8)	0.000	ns[d]	ns
Polyps	11 (8.2)	6 (1.6)	5.6 (2.0 – 15.4)	0.001	16.5 (4.8 – 57.3)	0.000
ACMAC (PMG1)	70 (52.2)	27 (7.1)	14.3 (8.5 – 24.0)	0.000	12.5 (4.0 – 39.5)	0.000
PMG2	6 (4.5)	2 (0.5)	8.9 (1.8 – 44.6)	0.008	ns	ns
PMG3	2 (1.5)	1 (0.3)	5.8 (0.5 – 64.0)	0.154	ns	ns
PMG4	12 (9.0)	10 (2.6)	3.6 (1.5 – 8.6)	0.003	ns	ns
PMG5	8 (6.0)	2 (0.5)	12.0 (2.5 – 57.4)	0.002	ns	ns
Age ≤50	11 (8.2)	10 (2.6)	3.3 (1.4 – 8.0)	0.008	8.7 (2.4 – 31.6)	0.001
Age 51-64	67 (50.0)	136 (35.7)	1.8 (1.2 – 2.7)	0.004	2.7 (1.5 – 4.8)	0.001
Age ≥65	56 (41.8)	235 (61.7)	0.4 (0.3 – 0.7)	0.000	1.0 (Reference)	0.000

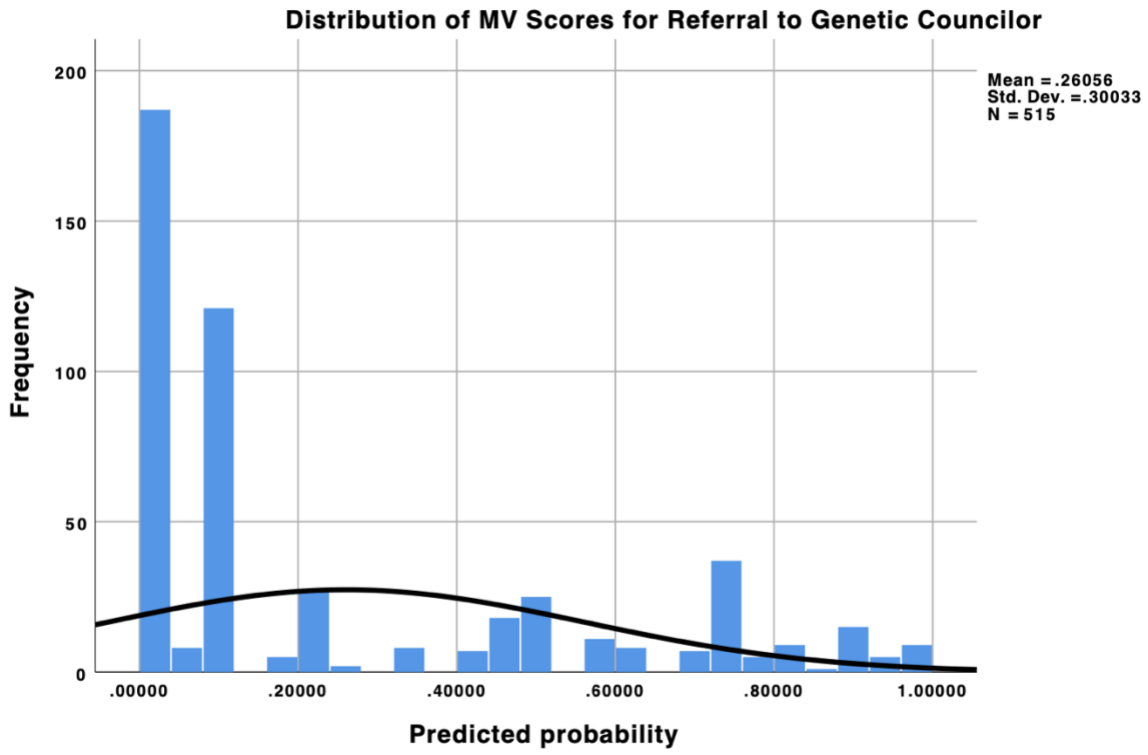
a: Odds Ratio estimated by binary logistic regression
b: Adjusted Odds Ratio estimated by multivariate logistic regression
c: Not included for potential confounding reasons
d: Not significant

Table 6: Odds Ratios for variables predictive of referral to a Genetic Counselor

Predictive Variable	**Odds Ratio**	**95% CI**
Adenomatous polyps in a first-degree relative	16.5	4.8 – 57.3
Age and Cancer Modified Amsterdam Criteria (ACMAC)	12.5	4.0 – 39.5
At least one first-degree relative with Colorectal cancer, but without ACMAC	10.3	3.3 – 32.5
Age at diagnosis less than 50 years	8.7	2.4 – 31.6
Presence of synchronous or metachronous colorectal, or other HNPCC-associated tumors, regardless of age	8.4	3.4 – 20.7
Colorectal cancer with MSI-high histology diagnosed in a patient less than 60 years of age	5.6	1.1 – 29.5
Colorectal cancer diagnosed in 1 or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years	3.9	1.0 – 15.2
Age at diagnosis between 51 – 64 years	2.7	1.5 – 4.8

The multivariate model scores were then converted to a probability score between 0-1 for the clinical decision made by genetics. The distribution of these scores in in Figure 9.

Figure 9: Distribution of Multivariate Model Equation Scores for Patients Recommended for Referral to a Genetic Counselor



Probability (P) predicted by the model (Y); where:

$$Y = \log\left(\frac{P}{1-P}\right) \qquad e^{Y} = \left(\frac{P}{1-P}\right) \qquad P = \frac{e^{Y}}{1+e^{Y}}$$

The optimal sensitivity and specificity for the probability score was obtained at a cut-off of 0.4. Table 9 reveals the utility of the score >0.4 compared to the clinical decision made by genetics to refer the patient to a genetic counselor. The sensitivity was 81% and the specificity was 87%.

The biggest concern in using the multivariate score for clinical decision-making is the harm associated with false negative scores: 25 of the 134 patients referred to genetics would not be referred if the decision was based on the score. However, review of the clinical data for each of the 25 patients [Table 7, Appendix D] revealed that 10 were over 50 years of age without any predictive variable for screening. In addition, 3 patients were younger than 50 years of age with no indicators, and 12 patients fulfilled Bethesda 2 or 3 criteria, dependent on pathology results, and no family history of CRC. It thus appears that 10 of the patients referred by genetics should not have been referred based on their clinical data.

Table 7: Frequencies of Predictive Variable Combinations for False Negatives of Multivariate Model Predictive of Genetic Counselor Referral (n=25)

Predictive Variables Fulfilled	Age Bracket	n
None	≤ 50 51-64 ≥ 65 *Total*	3 5 5 *13*
Bethesda 2	51-64 ≥ 65 *Total*	3 7 *10*
Bethesda 3	51-64 *Total*	2 *2*

Another concern is false positive results: patients not referred to a genetic counselor who would have been referred based on the multivariate score. Of 380 patients not referred by genetics, 48 had scores >0.4. Review of predictor variables in the 48 patients [Table 8, Appendix E] revealed that 26 should have been referred because their family fulfilled ACMAC criteria and a further 3 patients fulfilled Bethesda criteria. Of the remaining 19 patients, 15 had a family history of CRC without fulfilling ACMAC criteria and 4 had a family history of polyps only. Thus, it appears that 29 of the 48 patients with a score that indicated referral to genetics should have been referred although they had not been.

Table 8: Frequencies of Predictive Variable Combinations for False Positives of Multivariate Model Predictive of Genetic Counselor Referral (n=48)

Predictive Variables Fulfilled	Age Bracket	n
Age and cancer modified Amsterdam criteria (ACMAC)	51-64 ≥ 65 *Total*	5 10 *15*
ACMAC; Bethesda 2	51-64 ≥ 65 *Total*	1 3 *4*
ACMAC; Bethesda 2; Bethesda 3	≥ 65 *Total*	1 *1*
ACMAC; Bethesda 4	≥ 65 *Total*	5 *5*
ACMAC; Adenomatous polyps in a first-degree relative; Bethesda 3	51-64 *Total*	1 *1*
Family History of CRC without ACMAC	≤ 50 51-64 ≥ 65 *Total*	1 1 13 *15*
Family History of CRC without ACMAC; Bethesda 4	≥ 65 *Total*	2 *2*
Adenomatous polyps in a first-degree relative	≥ 65 *Total*	4 *4*
Adenomatous polyps in a first-degree relative; Family History of CRC without ACMAC; Bethesda 2	≥ 65 *Total*	1 *1*

Assuming that the research team had collected all the data used by genetics to make decisions, then it appeared that 10 of the 25 false negative and 29 of the 48 false positive groups should have had a different clinical decision. This would alter the sensitivity of the multivariate score to 90% and the specificity to 95% [Table 10].

Table 9: Predicted versus Observed Classification Table for Referral to a Genetic Counselor

	Genetics Decision	
MV Score	**GC Yes**	**GC No**
GC Yes	109	48
GC No	25	332

Table 10: The Utility of the Multivariate Score if the Decision Made by Genetics had been Correctly Assigned based on the Clinical Data

	Genetics Decision	
MV Score	**GC Yes**	**GC No**
GC Yes	138	19
GC No	15	342

4.2.2 Prediction of 5-10-year Screening Colonoscopy Frequency

We compared the patients in the 5-10-year recommended screening frequency bracket (n=339) to the rest (n=186) [Table 12]. In univariate analysis, patients in the 5-10-year bracket were more likely to have no family history of CRC ($p<0.0001$), less likely to fulfil ACMAC ($p<0.0001$), Bethesda 2 ($p=0.003$), Bethesda 3 ($p=0.001$), Bethesda 4 ($p<0.0001$) or Bethesda 5 ($p<0.0001$) criteria, have presence of multiple adenomatous polyps in a first-degree relative ($p=0.015$), be between the ages of 51 and 64 ($p=0.004$) or 65 years and older ($p<0.0001$). The cohort were also less likely to have an FHS in the fifth quintile ($p<0.0001$) or an MMRpredict score >1.66 ($p<0.0001$).

In multivariate analysis, every variable remained significantly associated with patients in the 5-10-year bracket with the exception of ACMAC, PMG2, PMG3 and PMG5 criteria. Specifically, having no Family History of CRC [OR=8.1; $p<0.0001$]; fulfilling Bethesda 2 criteria [OR=0.1; $p<0.0001$]; fulfilling Bethesda 3 criteria [OR=0.1; $p=0.018$]; fulfilling Bethesda 4 criteria [OR=0.1; $p=0.001$]; fulfilling Bethesda 5 criteria [OR=0.1; $p<0.0001$]; presence of multiple adenomatous polyps in a first-degree relative [OR=0.1; $p=0.002$]; and fulfilling PMG4 criteria [OR=6.6; $p=0.016$]. These patients were also more likely to be 65 years and older [OR=103.2; $p<0.0001$], or between the ages of 51-64 [OR=23.8; $p<0.0001$], when compared to patients in the Age ≤50 bracket.

There is a strong statistically significant association between the predictive variables and the 5-10-year screening bracket [$X^2= 289$; $df=9$; $p<0.0001$]. Using a cut-off of 0.5, the model had a sensitivity of 90.3% and a specificity of 76.3%. In this analysis, a low specificity that rules out patients for 5-10-yearly colonoscopy means that they would get more frequent colonoscopy and not be exposed to failure to diagnose a CRC. The c-statistic of this model, which represents Area Under the Curve (AUC) of the Receiver Operator Characteristic curve was 0.901 [Table 11].

Table 11: Descriptive Data from the Multivariate Model Predictive of 5-10 yearly Colonoscopy

Multivariate Model Descriptive Statistics	
Number of patients	525
Patients recommended for 5-10 yearly colonoscopy	339
Patients recommended for more frequent colonoscopy	186
Chi-square Statistic, X^2	289.4
Degrees of Freedom	9
P-value	<0.0001
Model's Predicted Probability of 5-10 yearly Colonoscopy	
Cut-off	0.500
Mean	0.640
Standard Deviation	0.337
Minimum	0.000
Maximum	0.944
Sensitivity	90.3%
Specificity	76.3%
Positive Predictive Value	86.0%
Negative Predictive Value	81.9%
Area Under the Curve	0.901

The multivariate model yielded the following equation, where 'Y' represents patients in the 5-10-year screening bracket:

$$Y = ß0 + ß1x1 - ß2x2 - ß3x3 - ß4x4 - ß5x5 - ß6x6 + ß7x7 + ß8x8 + ß9x9$$

$$Y= -3.800 + 2.053\ [NoFamCRC] – 1.751\ [Bethesda\ 2] – 2.075\ [Bethesda\ 3] – 2.255\ [Bethesda4] – 1.934\ [Bethesda\ 5] – 1.866\ [Polyps] + 1.894\ [PMG4] + 4.563\ [Age65] + 3.194\ [Age5164]$$

Table 12: Univariate and Multivariate Analysis of the Prediction of 5-10 yearly Colonoscopy (n=339) vs More Frequent Colonoscopy (n=186) as Recommended by the Genetic Counselor

Predictor Variable	**N, % 5-10 years**	**N, %, Rest**	**OR[a], Univariate (95% CI)**	**p-value**	**AOR[b], Multivariate (95% CI)**	**p-value**
No FamCRC	322 (95.0)	98 (52.7)	17.0 (9.6 – 30.0)	0.000	8.1 (3.7 – 17.4)	0.000
FHS in 5th Quintile	31 (9.2)	70 (38.9)	0.2 (0.1 – 0.3)	0.000	ni[c]	ni
MMRpredict Score >1.66	48 (14.3)	73 (40.6)	0.2 (0.2 – 0.4)	0.000	ni	ni
No Bethesda 1	2 (0.6)	25 (13.4)	25.0 (5.8 – 111.1)	0.000	ni	ni
No Bethesda 2	18 (5.3)	24 (12.9)	2.6 (1.4 – 5.0)	0.003	14.3 (4.7 – 47.5)	0.000
No Bethesda 3	1 (0.3)	14 (7.5)	27.8 (3.6 – 200.0)	0.001	13.9 (1.6 – 125.0)	0.018
No Bethesda 4	4 (1.2)	21 (11.3)	10.6 (3.6 – 31.3)	0.000	9.9 (2.6 – 37.0)	0.001
No Bethesda 5	33 (9.7)	94 (50.5)	9.4 (5.9 – 14.9)	0.000	7.4 (4.0 – 13.9)	0.000
No Polyps	6 (1.8)	11 (5.9)	3.5 (1.3 – 9.6)	0.015	7.1 (2.1 – 24.4)	0.002
ACMAC (PMG1)	21 (6.2)	77 (41.4)	0.1 (0.06 – 0.2)	0.000	ns[d]	ns
PMG2	3 (0.9)	6 (3.2)	0.3 (0.06 – 1.1)	0.065	ns	ns
PMG3	1 (0.3)	2 (1.1)	0.3 (0.03 – 3.0)	0.289	ns	ns
PMG4	11 (3.3)	11 (5.9)	0.5 (0.2 – 1.3)	0.152	6.6 (1.4 – 31.0)	0.016
PMG5	1 (0.3)	9 (4.8)	0.06 (0.01 – 0.5)	0.007	ns	ns
Age ≤50	2 (0.6)	24 (12.9)	0.04 (0.01 – 0.2)	0.000	1.0 (Reference)	0.000
Age 51-64	117 (34.5)	88 (47.3)	0.6 (0.4 – 0.8)	0.004	23.8 (5.1 – 111.0)	0.000
Age ≥65	220 (64.9)	74 (39.8)	2.8 (1.9 – 4.4)	0.000	103.2 (21.4 – 496.5)	0.000

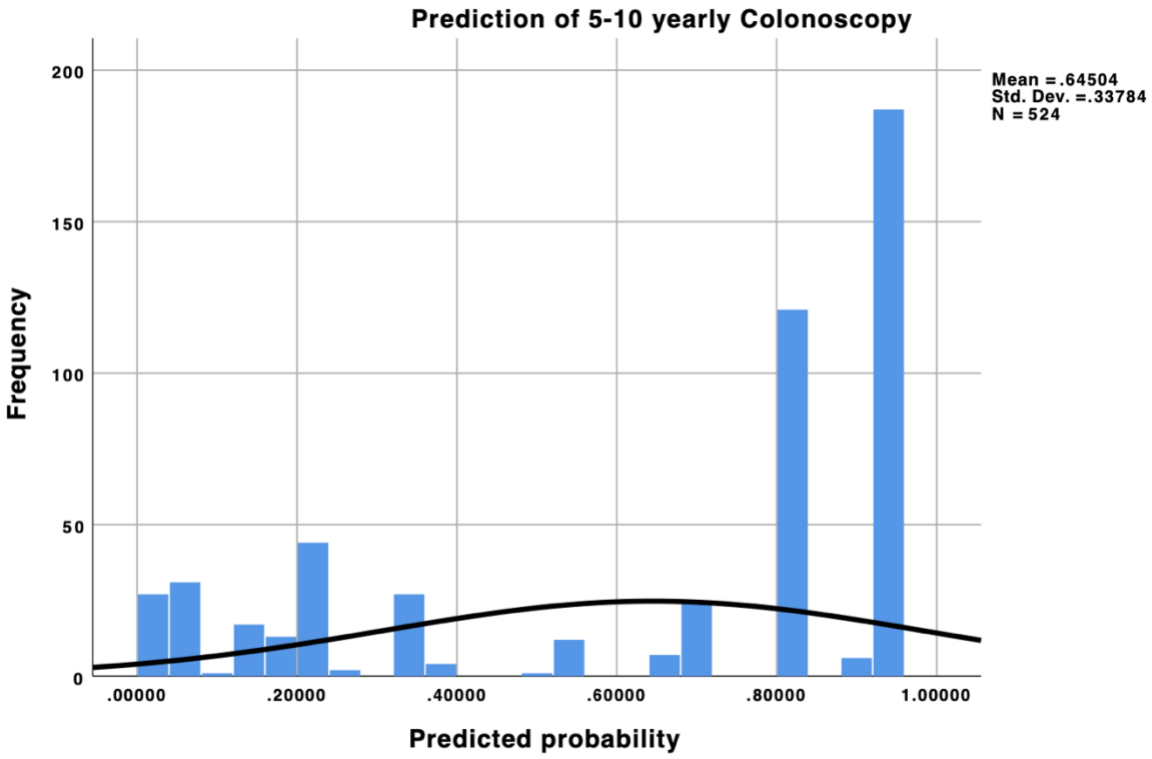
a: Odds Ratio estimated by binary logistic regression
b: Adjusted Odds Ratio estimated by multivariate logistic regression
c: Not included for potentially confounding reasons
d: Not significant

Table 13: Odds Ratios for variables predictive of referral to a 5-10 yearly colonoscopy screening

Predictive Variable	**Odds Ratio**	**95% CI**
Age at diagnosis 65 years and older	103.2	21.4 – 496.5
Age at diagnosis between 51 and 64 years old	23.8	5.1 – 111.0
No Presence of synchronous or metachronous colorectal, or other HNPCC-associated tumors, regardless of age	14.3	4.7 – 43.5
No Colorectal cancer with MSI-high histology diagnosed in a patient less than 60 years of age	13.9	1.6 – 125.0
No Colorectal cancer diagnosed in 1 or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years	9.9	2.6 – 37.0
No first-degree relatives with Colorectal cancer	8.1	3.7 – 17.4
No Colorectal cancer diagnosed in 2 or more first- or second-degree relatives with HNPCC-related tumors, regardless of age	7.4	4.0 – 13.9
No Adenomatous polyps in a first-degree relative	7.1	2.1 – 24.4
Sebaceous tumor (adenoma, carcinoma, epithelioma) before age 50 or multiple tumors at any age	6.6	1.4 – 31.0

The multivariate model scores were then converted to a probability score between 0-1 for the clinical decision made by genetics. The distribution of these scores in in Figure 10.

Figure 10: Distribution of Multivariate Model Equation Scores for Patients Recommended for 5-10-year Screening Intervals versus the Rest



Probability (P) predicted by the model (Y); where:

$$Y = \log\left(\frac{P}{1-P}\right) \qquad e^{Y} = \left(\frac{P}{1-P}\right) \qquad P = \frac{e^{Y}}{1+e^{Y}}$$

For the decision to refer family members for 5-10 yearly colonoscopy, the implication of a false negative result is more frequent colonoscopy. In the false negative group who had scores below the cut-off of 0.5, there were 30 families whose first-degree family members were actually advised to have colonoscopy at 5-10-year intervals. All of them had a Bethesda criterion in

support of the decision except for 3 patients, 2 of whom were aged 50 years or younger [Table 14, Appendix F].

The specificity of the model was 73% with 50 patients having a score above the cut-off, but not receiving advice for 5-10-yearly colonoscopy, whose family members would consequently receive more frequent colonoscopy. Twenty-seven of the 50 patients had no family history of CRC, had no criteria for more frequent colonoscopy, and were older than 50 years of age. These families should have more appropriately been offered 5-10 yearly colonoscopy [Table 15, Appendix G].

Correction of the clinical decision in the one false negative patient and 27 of the false positive patients increases the sensitivity of the model to 92% and the specificity to 86% [Table 17].

Table 14: Frequencies of Predictive Variable Combinations for False Negatives of Multivariate Model Predictive of 5-10 yearly Colonoscopy (n=30)

Predictive Variables Fulfilled	Age Bracket	n
Bethesda 4	≥ 65 *Total*	1 *1*
Bethesda 5	51-64 ≥ 65 *Total*	3 8 *11*
Bethesda 2; Bethesda 5; PMG4	51-64 *Total*	1 *1*
Bethesda 2; Adenomatous polyps in a first-degree relative; PMG4	≥ 65 *Total*	1 *1*
No Family History of CRC	≤ 50 ≥ 65 *Total*	2 1 *3*
No Family History of CRC; Bethesda 2	51-64 ≥ 65 *Total*	1 1 *2*
No Family History of CRC; Bethesda 5	51-64 ≥ 65 *Total*	5 1 *6*
No Family History of CRC; Bethesda 2; Bethesda 5	≥ 65 *Total*	1 *1*
No Family History of CRC; Bethesda 4; Bethesda 5	≥ 65 *Total*	1 *1*
No Family History of CRC; Bethesda 5; Adenomatous polyps in a first-degree relative	≥ 65 *Total*	1 *1*
No Family History of CRC; Bethesda 2; Adenomatous polyps in a first-degree relative; Sebaceous tumor before age 50 or multiple tumors at any age (PMG4)	51-64 *Total*	1 *1*
No Family History of CRC; Bethesda 2; Bethesda 3; Bethesda 5; PMG4	≥ 65 *Total*	1 *1*

Table 15: Frequencies of Predictive Variable Combinations for False Positives of Multivariate Model Predictive of 5-10 yearly Colonoscopy (n=50)

Predictive Variables Fulfilled	Age Bracket	n
None	≥ 65 *Total*	2 *2*
Bethesda 2	≥ 65 *Total*	1 *1*
No Family History of CRC	≤ 50 51-64 ≥ 65 *Total*	2 18 5 *25*
No Family History of CRC; Bethesda 2	51-64 ≥ 65 *Total*	4 3 *7*
No Family History of CRC; Bethesda 5	51-64 ≥ 65 *Total*	1 7 *8*
No Family History of CRC; Adenomatous polyps in a first-degree relative	≥ 65 *Total*	2 *2*
No Family History of CRC; Bethesda 2; PMG4	51-64 ≥ 65 *Total*	3 2 *5*

Table 16: Predicted versus Observed Classification Table for 5-10 yearly Colonoscopy

	Observed Cases	
Predicted Cases	**5-10 Yes**	**5-10 No**
5-10 Yes	308	50
5-10 No	30	136

Table 17: The Utility of the Multivariate Score if the Decision Made by Genetics had been Correctly Assigned based on the Clinical Data

	Observed Cases	
Predicted Cases	**5-10 Yes**	**5-10 No**
5-10 Yes	335	23
5-10 No	29	137

4.2.3 Prediction of 2-3-year Screening Frequency versus the Rest

We compared the patients in the 2-3-year recommended screening frequency bracket (n=68) against the rest (n=447) with the aim of identifying predictive variables for family members recommended for high frequency screening [Table 19]. Patients fulfilling Amsterdam criteria are automatically placed in the 1-2-year bracket, thus for the purpose of this analysis, these families were excluded. In univariate analysis, patients in the 2-3-year bracket were more likely to fulfil ACMAC ($p<0.0001$), have a family history of CRC independent of ACMAC ($p<0.0001$), have a FHS in the fifth quintile ($p<0.0001$), a MMRpredict score >1.66 ($p<0.0001$), fulfil Bethesda 2 ($p=0.012$), Bethesda 3 ($p<0.0001$), Bethesda 4 ($p<0.0001$) and Bethesda 5 ($p<0.0001$) criteria, and have a family member with polyps ($p=0.002$). Patients in the 2-3-year bracket were also more likely to fulfil PMG5 criteria ($p<0.0001$) and were 50 years old or younger ($p=0.001$).

In multivariate analysis, variables that were statistically significantly predictive of the 2-3-year screening bracket were the fulfilment of ACMAC (OR= 11.1; $p<0.0001$), family history of CRC independent of ACMAC (OR= 9.9; $p<0.0001$), fulfilment of Bethesda 4 criteria (OR = 4.0; $p=0.005$), presence of polyps in a family member (OR= 14.6; $p<0.0001$) and fulfil PMG5 criteria (OR= 11.1; $p=0.001$). Patients in the 2-3-year bracket were also more likely to be 50 years old or younger (OR= 11.9; $p<0.0001$), or between the ages of 51 and 64 (OR= 3.1; $p=0.001$), when compared to patients aged 65 years and older.

There is a strong statistically significant association between the predictive variables in the 2-3-year screening bracket [$X^2 = 130$; df=8; $p<0.0001$]. With a cut-off value to 0.3, sensitivity was 69.1%, while specificity was 87.4%. A high sensitivity is important in this bracket, as it rules in high frequency colonoscopy. The c-statistic of this model, which represents Area Under the Curve (AUC) of the Receiver Operator Characteristic curve was 0.897 [Table 18].

Table 18: Descriptive Data from the Multivariate Model Predictive of 2-3 yearly Colonoscopy

Multivariate Model Descriptive Statistics	
Number of patients	515
Patients recommended for 2-3 yearly colonoscopy	68
Patients recommended for less frequent colonoscopy	447
Chi-square Statistic, X^2	130.5
Degrees of Freedom	8
P-value	<0.0001
Model's Predicted Probability of 2-3 yearly Colonoscopy	
Cut-off	0.300
Mean	0.132
Standard Deviation	0.190
Minimum	0.015
Maximum	0.946
Sensitivity	69.1%
Specificity	87.4%
Positive Predictive Value	45.6%
Negative Predictive Value	94.9%
Area Under the Curve	0.897

The multivariate model yielded the following equation, where 'Y' represents patients in the 2-3-year screening bracket:

$$Y = ß0 + ß1x1 + ß2x2 + ß3x3 + ß4x4 + ß5x5 + ß6x6 + ß7x7$$

$$Y = -4.157 + 2.290\ (\text{FamCRC w/o ACMAC}) + 1.376\ (\text{Bethesda 4}) + 2.679\ (\text{Polyps}) + 2.403\ (\text{ACMAC}) + 2.410\ (\text{PMG4}) + 2.473\ (\text{Age50}) + 1.128\ (\text{Age5164})$$

Table 19: Two-three-year Screening Frequency (n=68) versus Less Frequent (n=447)

Predictor Variable	N, % 2-3 years	N, %, Rest	OR[a], Univariate (95% CI)	p-value	AOR[b], Multivariate (95% CI)	p-value
Amsterdam	10 (12.8)	0 (0.0)	∞	0.000	ni[c]	ni
FamCRC w/o ACMAC	15 (22.1)	30 (6.7)	3.9 (2.0 – 7.8)	0.000	9.9 (3.9 – 25.3)	0.000
FHS in 5th Quintile	32 (47.1)	62 (14.1)	5.4 (3.1 – 9.4)	0.000	ni	ni
MMRpredict Score >1.66	35 (51.5)	80 (18.1)	4.8 (2.7 – 8.2)	0.000	ni	ni
Bethesda 1	8 (11.8)	13 (2.9)	4.5 (1.8 – 11.2)	0.001	ni	ni
Bethesda 2	11 (16.2)	31 (6.9)	2.6 (1.2 – 5.4)	0.012	ns[d]	ns
Bethesda 3	8 (11.8)	7 (1.6)	8.4 (2.9 – 24.0)	0.000	ns	ns
Bethesda 4	12 (17.6)	12 (2.7)	7.8 (3.3 – 18.1)	0.000	4.0 (1.5 – 10.4)	0.005
Bethesda 5	35 (51.5)	92 (20.6)	4.1 (2.4 – 7.0)	0.000	ns	ns
Polyps	7 (10.3)	10 (2.2)	5.0 (1.8 – 13.7)	0.002	14.6 (4.2 – 50.0)	0.000
ACMAC (PMG1)	36 (52.9)	61 (13.6)	7.1 (4.1 – 12.3)	0.000	11.1 (5.2 – 23.3)	0.000
PMG2	3 (4.4)	5 (1.1)	4.1 (1.0 – 17.5)	0.058	ns	ns
PMG3	1 (1.5)	2 (0.4)	3.3 (0.3 – 37.1)	0.330	ns	ns
PMG4	5 (7.4)	17 (3.8)	2.0 (0.7 – 5.6)	0.187	ns	ns
PMG5	6 (8.8)	4 (0.9)	10.7 (2.9 – 39.0)	0.000	11.1 (2.5 – 49.0)	0.001
Age ≤50	8 (11.8)	13 (2.9)	4.5 (1.8 – 11.2)	0.001	11.9 (3.5 – 40.5)	0.000
Age 51-64	38 (55.9)	165 (36.9)	2.2 (1.3 – 3.6)	0.003	3.1 (1.6 – 6.1)	0.001
Age ≥65	22 (32.4)	269 (60.2)	0.3 (0.2 – 0.5)	0.000	1.0 (Reference)	0.000

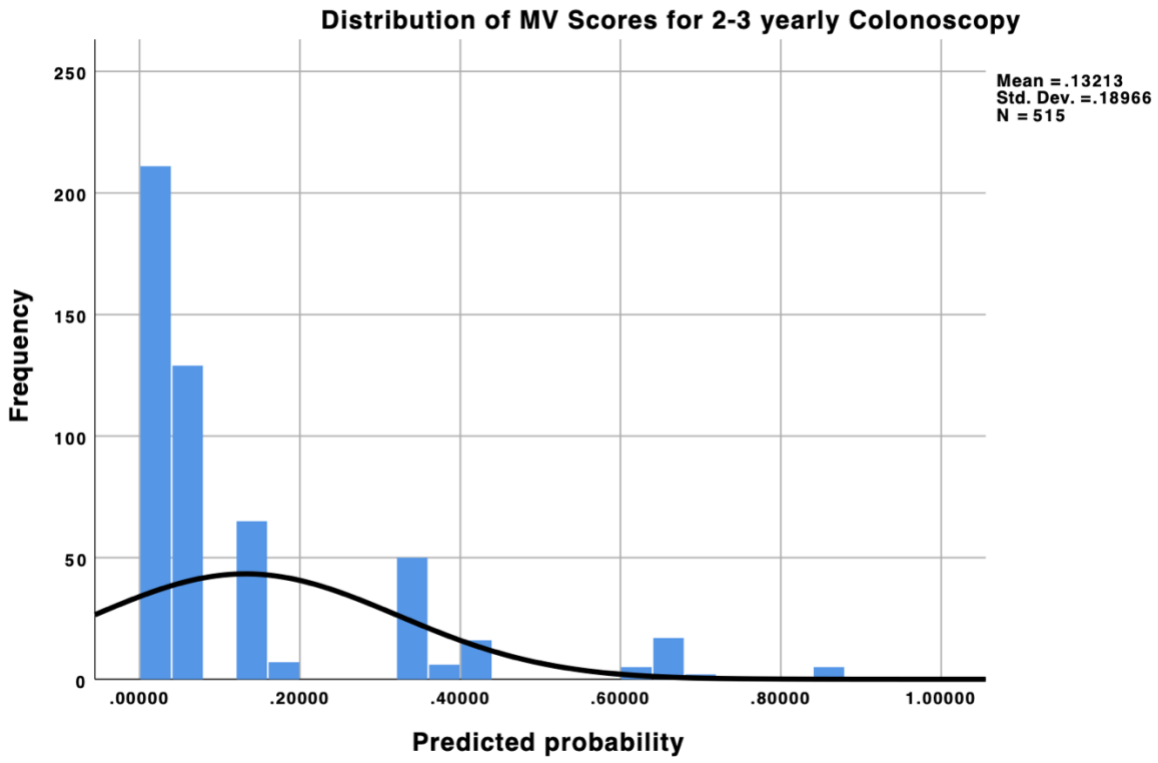
a: Odds Ratio estimated by binary logistic regression
b: Adjusted Odds Ratio estimated by multivariate logistic regression
c: Not included for potential confounding reasons
d: Not significant

Table 20: Odds Ratios for variables predictive of 2-3 yearly colonoscopy screening

Predictive Variable	**Odds Ratio**	**95% CI**
Adenomatous polyps in a first-degree relative	14.6	4.2 – 50.0
Age at diagnosis less than 50 years of age	11.9	3.5 – 40.5
Age and Cancer Modified Amsterdam Criteria (ACMAC)	11.1	5.2 – 23.3
Presence of multiple HNPCC-related cancers in an individual, with one cancer before age 60	11.1	2.5 – 49.0
At least one first-degree relative with Colorectal cancer, but without ACMAC	9.9	3.9 – 25.3
Colorectal cancer diagnosed in 1 or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years	4.0	1.5 – 10.4
Age at diagnosis between ages 51-64	3.1	1.6 – 6.1

The multivariate model scores were then converted to a probability score between 0-1 for the clinical decision made by genetics. The distribution of these scores is in Figure 11.

Figure 11: Distribution of Multivariate Model Equation Scores for Patients Recommended for 2-3-year Screening Intervals versus the Rest



Probability (P) predicted by the model (Y); where:

$$Y = \log\left(\frac{P}{1-P}\right) \qquad e^{Y} = \left(\frac{P}{1-P}\right) \qquad P = \frac{e^{Y}}{1+e^{Y}}$$

For the decision to refer family members for 2-3 yearly colonoscopy, the implication of a false negative result is more frequent colonoscopy. In the false negative group who had scores below the cut-off of 0.3, there were 21 patients whose first-degree family members were actually advised to have colonoscopy at 2-3-year intervals. Four of the 21 families fulfilled none of the predictive variables and were older than 50 years of age. Two of the 21 patients were 65 years of

age or older, and the only predictive variable they fulfilled was adenomatous polyps in a first-degree relative [Table 21, Appendix H].

The specificity of the model was 87% with 56 patients having a score above the cut-off of 0.3, but not receiving advice for 2-3-yearly colonoscopy, whose family members would consequently receive less frequent colonoscopy. Among the 56 false positives patients, 41 of them fulfilled ACMAC. These families should have more appropriately been offered 2-3-yearly colonoscopy [Table 22, Appendix I].

If six of the 21 false negative patients and 41 of the 56 false positive patients were re-classified based on their clinical information, the sensitivity of the model would improve to 85%, and the specificity would improve to 96% [Table 24].

Table 21: Frequencies of Predictive Variable Combinations for False Negatives of Multivariate Model Predictive of 2-3 yearly Colonoscopy (n=21)

Predictive Variables Fulfilled	Age Bracket	n
None	≤ 50 51-64 ≥ 65 *Total*	4 3 1 *8*
Age and cancer modified Amsterdam criteria (ACMAC)	≥ 65 *Total*	9 *9*
Adenomatous polyps in a first-degree relative	≥ 65 *Total*	2 *2*
Family History of CRC without ACMAC	≥ 65 *Total*	2 *2*

Table 22: Frequencies of Predictive Variable Combinations for False Positives of Multivariate Model Predictive of 2-3 yearly Colonoscopy (n=56)

Predictive Variables Fulfilled	Age Bracket	n
None	≤ 50 *Total*	1 *1*
Age and cancer modified Amsterdam criteria (ACMAC)	≤ 50 51-64 ≥ 65 *Total*	4 22 3 *29*
ACMAC; Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years (Bethesda 4)	51-64 ≥ 65 *Total*	1 8 *9*
ACMAC; Adenomatous polyps in a first-degree relative	51-64 *Total*	1 *1*
ACMAC; Presence of multiple HNPCC-related cancers in an individual, with one cancer before age 50 (PMG5)	≥ 65 *Total*	2 *2*
PMG5	51-64 ≥ 65 *Total*	1 1 *2*
Adenomatous polyps in a first-degree relative	51-64 *Total*	2 *2*
Family History of CRC without ACMAC	≤ 50 51-64 ≥ 65 *Total*	1 2 2 *5*
Family History of CRC without ACMAC; Bethesda 4	51-64 ≥ 65 *Total*	1 2 *3*
Family History of CRC without ACMAC, adenomatous polyps in a first-degree relative	≥ 65 *Total*	2 *2*

Table 23: Predicted versus Observed Classification Table for 2-3 yearly Colonoscopy

	Observed Cases	
Predicted Cases	**2-3 Yes**	**2-3 No**
2-3 Yes	47	56
2-3 No	21	390

Table 24: The Utility of the Multivariate Score if the Decision Made by Genetics had been Correctly Assigned based on the Clinical Data

	Observed Cases	
Predicted Cases	**2-3 Yes**	**2-3 No**
2-3 Yes	88	15
2-3 No	15	396

4.2.4 Prediction of 2-3-year versus 3-5-year Screening Frequency

We compared n=68 patients in the 2-3-year recommended screening frequency bracket (excluding Amsterdam) against those in the 3-5-year bracket (n=108) with the aim of identifying predictive variables that can sensitively and specifically distinguish the 2-3-year bracket from the 3-5 yearly group [Table 26].

In univariate analysis, patients in the 2-3-year bracket were more likely to fulfil Bethesda 4 criteria (p=0.04); have a family history of CRC (p=0.045), a FHS in the 5th quintile (p=0.020) an MMRpredict score >1.66 (p=0.006) and fulfil ACMAC (p=0.039).

In multivariate analysis, variables that were statistically significant and predictive of the 2-3-year bracket were the fulfilment of ACMAC (OR= 2.2; p=0.025), family history of CRC (OR=2.5; p=0.011), have a family member with polyps (OR= 5.8; p=0.013), fulfilment of PMG5 (OR=5.9; p=0.020), and have an age at diagnosis of 65 years or older (OR= 0.4; p=0.014).

There is a strong statistically significant association between the predictive variables in the 2-3-year screening bracket [X^2 = 23.814; df= 5; p<0.0001]. Using a cut-off value of 0.5, the sensitivity of the model was poor at only 35.3%, while the specificity was 89.8%. Using a cut-off value of 0.4, the model had a sensitivity of 75.0% and a specificity of 55.6%. The c-statistic of this model, which represents Area Under the Curve (AUC) of the Receiver Operator Characteristic curve was 0.707 [Table 25].

Table 25: Descriptive Data from the Multivariate Model Predictive of 2-3 yearly versus 3-5-yearly Colonoscopy

Multivariate Model Descriptive Statistics	
Number of patients	176
Patients recommended for 2-3 yearly colonoscopy	68
Patients recommended for less frequent colonoscopy	108
Chi-square Statistic, X^2	23.8
Degrees of Freedom	5
P-value	<0.0001
Model's Predicted Probability of 2-3 yearly Colonoscopy versus 3-5 yearly	
Cut-off	0.400
Mean	0.386
Standard Deviation	0.176
Minimum	0.118
Maximum	0.914
Sensitivity	75.0%
Specificity	55.6%
Positive Predictive Value	51.5%
Negative Predictive Value	77.9%
Area Under the Curve	0.707

The multivariate model yielded the following equation, where 'Y' represents patients in the 2-3-year screening bracket:

$$Y = ß0 + ß1x1 + ß2x2 + ß3x3 + ß4x4 – ß5x5$$

$$Y= -1.111 + 0.930\ (FamCRC) + 1.761\ (Polyps) + 0.781\ (ACMAC) + 1.769\ (PMG5) – 0.905\ (Age65)$$

Table 26: Two-three-year Screening Frequency (n=68) vs 3-5 Year Screening Frequency (n=108)

Predictor Variable	**N, % 2-3 years**	**N, % 3-5 years**	**OR[a], Univariate (95% CI)**	**p-value**	**AOR[b], Multivariate (95% CI)**	**p-value**
FamCRC	37 (54.4)	42 (38.9)	1.9 (1.0 – 3.5)	0.045	2.5 (1.2 – 5.2)	0.011
Family History Score in 5th Quintile	32 (47.1)	31 (28.7)	2.2 (1.2 – 4.2)	0.014	ni[c]	ni
MMRpredict Score >1.66	35 (51.5)	32 (30.5)	2.4 (1.3 – 4.6)	0.006	ni	ni
Bethesda 1	8 (11.8)	12 (11.1)	1.1 (0.4 – 2.8)	0.894	ni	ns
Bethesda 2	11 (16.2)	13 (12.0)	1.4 (0.6 – 3.4)	0.437	ns[d]	ns
Bethesda 3	8 (11.8)	6 (5.6)	2.3 (08 – 6.8)	0.147	ns	ns
Bethesda 4	12 (17.6)	8 (7.4)	2.7 (1.0 – 6.9)	0.043	ns	ns
Bethesda 5	35 (51.5)	59 (54.6)	0.9 (0.5 – 1.6)	0.683	ns	ns
Polyps	7 (10.3)	4 (3.7)	3.0 (0.8 – 10.6)	0.091	5.8 (1.4 – 23.4)	0.013
ACMAC (PMG1)	36 (52.9)	40 (37.0)	1.9 (1.0 – 3.5)	0.039	2.2 (1.1 – 4.3)	0.025
PMG2	3 (4.4)	2 (1.9)	2.4 (0.4 – 15.0)	0.334	ns	ns
PMG3	1 (1.5)	1 (0.9)	1.6 (0.1 – 26.0)	0.742	ns	ns
PMG4	5 (7.4)	6 (5.6)	1.3 (0.4 – 4.6)	0.633	ns	ns
PMG5	6 (8.8)	3 (2.8)	3.4 (0.8 – 14.0)	0.092	5.9 (1.3 – 26.1)	0.020
Age ≤50	8 (11.8)	11 (10.2)	1.2 (0.4 – 3.1)	0.743	ns	ns
Age 51-64	38 (55.9)	48 (44.4)	1.6 (0.9 – 2.9)	0.140	ns	ns
Age ≥65	22 (32.4)	49 (45.4)	0.6 (0.3 – 1.1)	0.088	0.4 (0.2 – 0.8)	0.014

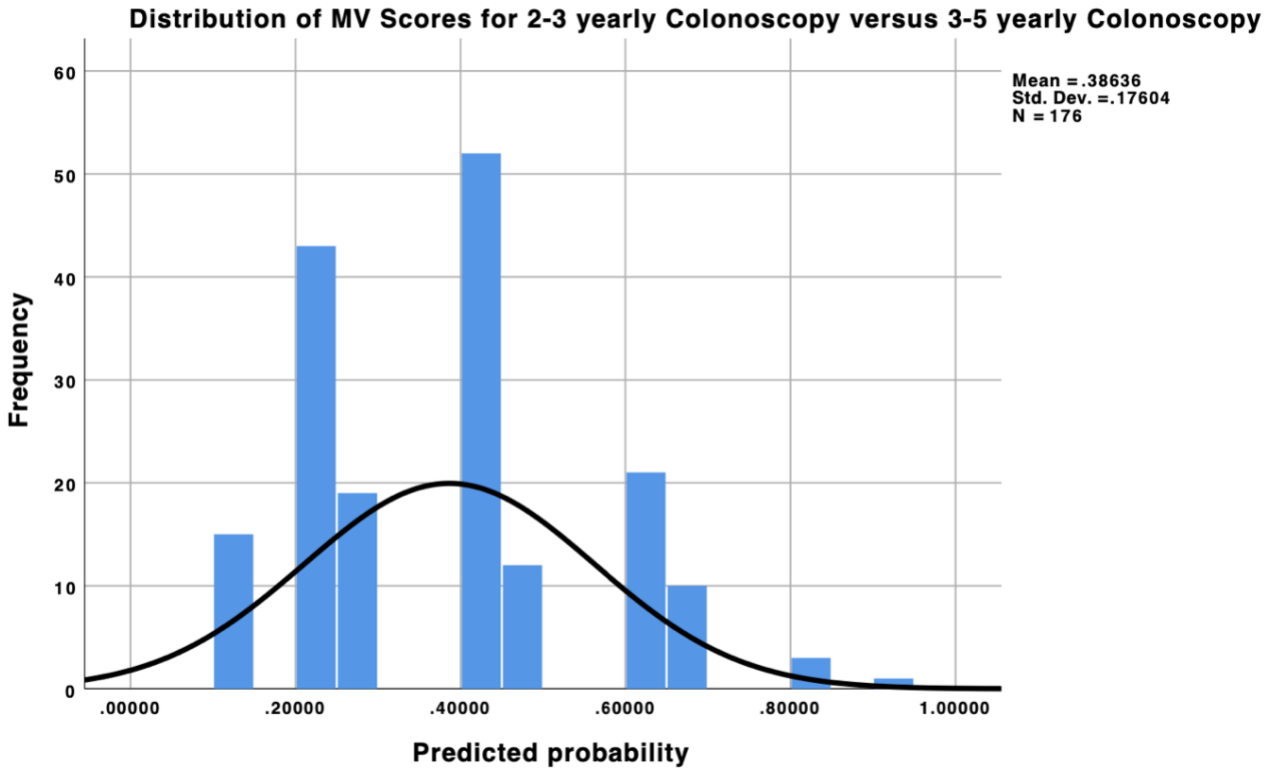
a: Odds Ratio estimated by binary logistic regression
b: Adjusted Odds Ratio estimated by multivariate logistic regression
c: Not included for potential confounding reasons
d: Not significant

Table 27: Odds Ratios for variables predictive of 2-3 yearly colonoscopy screening versus 3-5 yearly

Predictive Variable	**Odds Ratio[b]**	**95% CI**
Presence of multiple HNPCC-related cancers in an individual, with one cancer before age 60	5.9	1.3 – 26.1
Adenomatous polyps in a first-degree relative	5.8	1.4 – 23.4
At least one first-degree relative with Colorectal cancer	2.5	1.2 – 5.2
Age and Cancer Modified Amsterdam Criteria (ACMAC)	2.2	1.1 – 4.3
Age at diagnosis at 65 years and older	0.4	0.2 – 0.8

The multivariate model scores were then converted to a probability score between 0-1 for the clinical decision made by genetics. The distribution of these scores is in Figure 12.

Figure 12: Distribution of Multivariate Model Equation Scores for Patients Recommended for 2-3-year Screening Intervals versus 3-5-years



Probability (P) predicted by the model (Y); where:

$$Y = \log\left(\frac{P}{1-P}\right) \qquad e^{Y} = \left(\frac{P}{1-P}\right) \qquad P = \frac{e^{Y}}{1+e^{Y}}$$

This poor prediction of decisions recommending 2-3-yearly colonoscopy compared to 3-5-yearly frequency was not considered to be clinically important as by default, families would be recommended for 3-5 yearly colonoscopy if not recommended for 2-3 yearly colonoscopy or 5-10 yearly colonoscopy.

Table 28: Predicted versus Observed Classification Table for 2-3 yearly versus 3-5 yearly Colonoscopy

	Observed Cases	
Predicted Cases	**2-3 Yes**	**2-3 No**
2-3 Yes	51	48
2-3 No	17	60

Chapter 5 – DISCUSSION

5.1 Introduction

In this research project, we investigated the effectiveness with which the previously established population-based familial colorectal cancer clinic provided colonoscopic screening recommendations to families at different degrees of risk of CRC. In doing so, we identified important predictive variables with which we could develop and assess the utility of multivariate prediction models to facilitate future screening recommendations in families with CRC. We compared the predictions made using these models to the clinical decisions previously made by genetic counselors.

5.2 The Newfoundland Familial Colorectal Cancer Clinic

The results from the Newfoundland Familial Colorectal Cancer Clinic (NFCCC) supported five conclusions: (i) the clinic had a relatively low response rate (51%) and the efficiency of genetic counseling was poor; (ii) there was a high rate of referral to genetic counselors at 27%; (iii) risk-specific colonoscopic screening recommendations are necessary for all family members, regardless of proband age; (iv) a family history first approach to identify risk of Lynch Syndrome proved inefficient; and (v) the efficiency in the provision of counseling was poor, as only 30% of the high and intermediate-high risk families were seen by genetic counselors during the duration of the study. This was a result of delays in family history checks and obtaining information from hospitals, which in turn resulted in long waitlists to genetic counseling and a workload that was too great.

Incident cases with CRC were invited to attend the clinic but only 51% provided sufficient family history to provide screening recommendations to their family members. The majority of non-responders had no interest in the project, most of whom were 75 years and older. Upon pedigree assessment by the medical geneticist, 27% of incident cases were referred to a genetic counselor. Among intermediate-high and intermediate-low risk patients, a clinical decision was made based on family history interpretation. While guidelines exist for the genetic/familial high-

risk assessment of CRC, their applicability to specific families remains unclear [169]. Newfoundland families are large and family information is extensive, thus clinical interpretation is often necessary when assessing family risk and providing colonoscopic screening recommendations. Thus, decisions for the purpose of this study were made by experienced geneticists, while considering a multitude of parameters.

The role of genetic counselors in family cancer clinics includes review and assessment of family pedigrees, provision of colonoscopic screening recommendations, and obtaining consent for tumor/genomic DNA cascade testing when required. If a mutation is identified, the genetic counselor will discuss the results with the proband and detail them in a letter to share with relatives. If no mutation is identified but significant family history is present, the genetic counselor provides screening recommendations to the patient and asks them to share them with relatives.

The efficiency in the provision of genetic counseling in the NFCCC was poor, as only 30% of high and intermediate-high risk families were seen by genetic counselors as a result of delays in family history checking and release of information from hospitals. This resulted in a long waitlist and subsequent burden on genetic counselors. It is worth noting that the decision not to refer intermediate-low risk patients to a genetic counselor was driven by the limited resources of genetic counseling, and the probability of further increasing the waitlist. It is therefore possible that some families with Lynch Syndrome were missed.

The prevalence of high-risk CRC families was lower in this study than in a previous population-based cohort study from Newfoundland [42]. When comparing the previous study to the current, Amsterdam I and II criteria were fulfilled in 3.7% vs 1.7%, familial CRC was present in 31% vs 19.8%, and low risk families comprised 52.7% vs 57%, respectively. We proposed that the lower prevalence of high-risk families was a direct result of screening, as families with previously identified HNPCC have received screening already.

When examining high risk families classified by age of proband, the rate of higher risk families decreased the older the proband. Nevertheless, the prevalence was still quite high in probands 75

years and older: 19.4% had familial CRC, while 31.3% fulfilled at least one Bethesda criterion. Screening colonoscopy frequency recommended to family members was in the 5-10-year bracket for 23% of families.

The family history-first approach of assessing Lynch Syndrome failed to assess 48% of incident cases of LS risk due to a lack of family history provided. However, 36% of those who did not provide a family history were 75 years and older thus at lower risk of having a LS mutation. Among responders, 23% fulfilled the criteria for tumor/genomic DNA cascade testing for LS mutations. If families at risk of LS as defined by MMRpredict were referred to genetic counselors, the proportion of probands referred would have been 24%. The process in which patients were identified as requiring tumor/genomic DNA cascade testing for LS mutations was inefficient, as 66% of high/ intermediate-high risk families were waiting to see a genetic counselor by the end of the study. Of the 48 patients seen by the genetic counselor, 50% were referred for LS testing. Of the 95 patients not seen by genetic counselors, all received recommendations for colonoscopic screening based on family history, but data on those who needed work-up for LS is not yet available. The efficiency of the process could be improved by narrowing criteria for referral to genetic counselors, hiring more genetic counselors, or exploring the use of electronic predictive models.

An alternative process to the family history first approach is a tumor first approach with immunohistochemistry testing for the four MMR proteins and or microsatellite instability testing in CRCs at the time of surgery. Universal tumor MMR testing among CRC probands had a greater sensitivity for identification of LS compared to multiple alternative strategies, although the increase in diagnostic test yield was modest [169]. The decision to undertake universal testing will be influenced by the utility of defining MMR deficiency for immunotherapies [173].

We concluded that the high CRC mortality rate in NL is likely the result of high CRC incidence, and because the rate of familial CRC in the province is high, we recommended the development of population-based screening strategies to target families at risk of CRC, and algorithmic approaches to defining risk and providing screening recommendations should be investigated. The strategy in this study proved inefficient in the management of high and intermediate risk

families. The workload of genetic counselors was too broad despite being limited to high and intermediate-high risk families.

The age recommended for starting colonoscopies in family members, were determined primarily by family history, generally 10 years earlier than the youngest person in the pedigree with CRC. The provision of screening frequency recommendations requires information on the patient (age, tumor pathology, incidence of HNPCC cancers) and on the family (age of CRC, polyps, and HNPCC in first- and second-degree relatives), which requires expertise. The geographical and financial limitations for genetic counseling led us to investigate the use of predictive models to determine whether electronic algorithms could accurately reflect screening recommendations made by genetic counselors [174].

The clinical decisions tested using multivariate models were (1) referral of the patient to a genetic counselor, (2) colonoscopy at 5-10-year intervals for family members, (3) colonoscopy at 2-3-year intervals for family members. No model was necessary for patients who fulfilled Amsterdam criteria, as these family members automatically require colonoscopy at 1-2-year intervals. Furthermore, we examined the logic that if patients did not have scores suggesting high or low interval colonoscopy, they would automatically require screening at 3-5-year intervals, by modelling 2-3-year colonoscopy frequency versus 3-5-year frequency.

5.3 Electronic Prediction of Referral to a Genetic Counselor and Screening Recommendations

5.3.1 Referral to a Genetic Counselor

Families at high and intermediate-high risk were referred for genetic counseling by the geneticist, including all 10 families fulfilling Amsterdam I or II criteria. In the remaining 515 families that did not fulfil Amsterdam criteria, multivariate analysis identified the following factors as significant and independent predictors of referral: Family history of CRC independent of ACMAC, Bethesda criteria 2, 3 and 4, presence of adenomatous polyps in a family member, ACMAC, age less than or equal to 50, and age between 51-64 [Tables 5, 6]. The multivariate model score cut-off had an area under the curve (AUC) of 0.912. The sensitivity of the

multivariate score cut-off was 81.3% for prediction of referral and specificity 87.4% using a cut-off of 0.4.

We compared the decision made by the multivariate score to that made by the geneticist and identified 25 (19%) patients recommended for genetic counseling, whose multivariate score fell below the cut-off of 0.4. There is concern in using multivariate scores for clinical decision-making on the basis of the harm associated with false negatives. However, review of the clinical data revealed that 10 of the patients referred by genetics should not have been referred. Specifically, they were over 50 years of age without any of the predictive variables for referral. We also identified 48 patients not referred to a genetic counselor, whom on the basis of their multivariate score, should have been. Review of the clinical data of these 48 patients identified 29 patients with a score indicative of referral to a genetic counselor that should have been, although they were not. Specifically, 26 should have been referred on account of fulfilling ACMAC criteria, while a further 3 patients fulfilled Bethesda criteria. Had these 10 false negative patients and 29 false positive patients had the proper clinical decision, the sensitivity of our model would have increased to 90%, and our specificity to 95%. Nevertheless, it appears that the multivariate model was more clinically accurate than the geneticist.

If applied to all incident CRC patients recorded in the Newfoundland Colorectal Cancer Registry, our model would screen patients for referral to genetic counseling, and diminish the labour required by geneticists to assess the patient.

5.3.2 Decision on 5-10-year Screening Colonoscopy

The majority of families (n=339) were recommended to have screening colonoscopy at intervals between five and ten years. When compared to the families who were recommended for more frequent intervals, the decision of the geneticist was influenced by age, absence of family history of CRC, any of the Bethesda criteria, some of the PMG criteria and polyps in family members [Tables 12, 13].

In this instance, the sensitivity is ruling in patients with an appropriate score to have low frequency colonoscopy and ruling out patients not in the range for higher frequency colonoscopy. Thus, they would be unlikely to suffer from failing to get low frequency colonoscopy. We compared the computer-generated cut-off for the multivariate score to decisions made by geneticists. The sensitivity was 90%, the specificity was 76% and the area under the curve was 0.901.

We identified 30 patients recommended for 5-10 yearly colonoscopy whose multivariate scores fell below the cut-off of 0.5. Review of the clinical data determined that 1 of these 30 false negative patients did not fulfil any Bethesda criteria, nor an age of diagnosis that would support the decision, and therefore should have been offered less frequent colonoscopy than they were. We also identified 50 false positive patients, who had multivariate scores above the model's cut-off of 0.5, but who weren't advised for 5-10 yearly colonoscopy. Review of their clinical data identified 27 patients who had no family history of CRC, no other criteria for more frequent colonoscopy, and who were older than 50 years of age. Per our model, these 27 families should have been offered 5-10 yearly colonoscopy instead. Had the one false negative patient and the 27 false positive patients had the proper clinical decision, the sensitivity of our model would improve to 92%, and the specificity to 86%.

This is a significant result both for this cohort and in terms of application to future families who may be screened less frequently than their data indicates.

5.3.3 Decision on 2-3-year Screening Colonoscopy

As it is accepted practice to provide colonoscopy at 1-2 yearly intervals in families who fulfil Amsterdam criteria, that is what was recommended in the 10 families we excluded from the multivariate models.

The decision by the genetic counselor to recommend frequent screening at 2-3-year intervals was influenced primarily by age, fulfilment of Amsterdam, Bethesda and Provincial Medical

Genetics criteria, in addition to family history of CRC and presence of polyps in a family member [Tables 19, 20].

When we compared the computer-generated cut-off for the multivariate score to the decisions made by genetic counselors, the sensitivity was 69.1% while the specificity was 87.4% when using a cut-off value of 0.3. The area under the curve (AUC) of the model was 0.897.

The relatively low sensitivity of 69.1% was a concern, but in reviewing the clinical data we determined that four of the 21 false negative patients did not fulfil any of the predictive variables and were older than 50 years of age. A further two patients aged 65 years and older only fulfilled the predictive variable of adenomatous polyps in a first-degree relative. We also identified 56 false positive patients, who had multivariate scores above the cut-off of 0.3 but weren't advised for 2-3-yearly colonoscopy. Review of their clinical data identified 41 of those patients fulfilled ACMAC. Thus, it appears that genetic counselors are ignoring the fulfilment of ACMAC when deciding on the provision of frequent colonoscopy, despite it being listed first among criteria used in the testing algorithm for suspected HNPCC families developed by the Provincial Medical Genetics program. Had the six false negative patients and the 41 false positive patients had the proper clinical decision, the sensitivity of our model would have increased to 85%, and our specificity to 96%. Nevertheless, it appears that the multivariate model was more clinically accurate than the genetic counselor.

This is a significant result both for this cohort and in terms of application to future families who may be screened less frequently than their data indicates.

5.3.4 Decision on 2-3-year Screening Colonoscopy versus 3-5-year

By a process of elimination, patients without Amsterdam criteria, and who do not have scores consistent with either High (2-3 year) or Low (5-10 year) frequency colonoscopy should have colonoscopies at 3-5-year intervals. Nevertheless, we tried to create a multivariate model to compare the 3-5-year interval group to the 2-3-year group.

The multivariate model identified significant and independent factors as more likely to have a family history of CRC, have presence of multiple adenomatous polyps in a family member, fulfil ACMAC, PMG5 criteria, and be 65 years of age and older [Tables 26, 27]. Using a cut-off value to 0.4, the model's sensitivity was 75.0%, and the specificity 55.6%.

The area under the curve (AUC) of our multivariate model was 0.707 which is borderline. These results further reinforce that distinguishing between these two brackets remains difficult and may still require interpretation from genetic counselors, as well as more data on the effectiveness of the two screening intervals. Nevertheless, our model did outperform Family History Score, and the identification of four new predictive variables will improve decision-making for genetic counselors, particularly for intermediate-risk patients.

To reiterate points made in sections 5.3.1 and 5.3.3, the application of this model for future use will still address several of our previously observed limitations, particularly in reducing the burden on genetic counselors through reduced referral rates, improved efficiency in their time and provision of counseling.

This poor prediction of decisions recommending 2-3-yearly colonoscopy compared to 3-5-yearly frequency was not considered to be clinically important. By default, families would be recommended for 3-5 yearly colonoscopy if not recommended for 2-3 yearly colonoscopy or 5-10 yearly colonoscopy.

5.4 Implications

Failure to accurately screen colorectal cancer patients' family history places family members at increased risk of CRC if a positive family history is missed. Genetic counselling services have a long waitlist and often are referred patients whose families are not at increased risk. In addition, family members undergo colonoscopy at more frequent intervals than indicated by their family history.

This study demonstrates that important clinical decisions can be made for families using electronically generated scores provided that patient and family history confirmation are collected appropriately. Table 29 details the information required to generate multivariate scores to help make important decisions on whether to refer to genetic counselors and how often family members should have colonoscopies. A summary of the predictive variables that contribute to these decisions can be found in Table 30.

Table 29: Information Required to Generate Multivariate Scores for Family Members

Type	**Data Required**
Demographical	• Age of the individual
Tumor	• Is it Synchronous or Metachronous? • Does it have MSI-high Histology? • Age of the individual at onset • Is it an HNPCC-associated tumor? • Is it Sebaceous?
Family History	• Amsterdam Criteria • ACMAC • Family History of CRC (independent of Amsterdam or ACMAC) • Bethesda Criteria • Adenomatous polyps in a first-degree relative

The challenges are (1) identifying CRC patients, (2) collecting accurate family histories, (3) defining criteria for the scores in an electronic decision tool, (4) operationalizing the use of multivariate scores in the health system.

We suggest that (1) patients should be identified through the NL Colorectal Cancer Registry at the NL Cancer program, (2) patients should be provided with a family history form to collect information on age of CRC, HNPCC tumors and diagnosis of polyps in first- and second-degree relatives. Alternatively, a tumor-first approach in which colorectal cancers undergo universal

immunohistochemical testing for the four MMR proteins and/or MSI testing at the time of initial surgery can be explored. (3) The decision form needs to include the criteria for calculation of the scores and the backend of the e-tool needs the equations to calculate the multivariate scores. The e-tool should be accessible via an icon on the electronic health record, 'Health-e-NL'. A healthcare professional should enter the data, whether it be a family physician, nurse practitioner, or an employee within the Cancer Care Program. Completion of the e-tool will require patient-specific data, tumor pathology and family history on hand.

If a patient needs referral to Provincial Medical Genetics, the initial work-up will have been completed and available on Health-e-NL saving the counselor hours of work. A decision to obtain hospital level documents on individual family members can be made by the counselor, along with a decision on whether molecular genetic work-up is necessary.

The scores on recommended frequency of colonoscopy will be available to both the referring doctor and the colonoscopist, thus facilitating accurate appraisal of the available data. This should help high risk family members get frequent colonoscopies and low risk family members get infrequent colonoscopies or FIT. A population-based approach to familial CRC has the potential to prevent CRC in NL and reduce the high mortality rates from CRC observed in NL.

Table 30: Summary of Predictive Variables for Each Decision

Decision	**Predictive Variables**
Referral to a Genetic Counselor	• ACMAC • Family History of CRC independent of ACMAC • Bethesda 2, 3 or 4 criteria • Adenomatous polyps in a first-degree relative • Age 50 years or younger • Age between 51 and 64
5-10-yearly Colonoscopy	• No family history of CRC • No Bethesda 2, 3, 4 or 5 criteria • No adenomatous polyps in a first-degree relative • No PMG4 criteria • Age between 51-64 • Age 65 years or older
3-5-yearly Colonoscopy	• No Amsterdam Criteria • Not recommended for 2-3-yearly colonoscopy • Not recommended for 5-10-yearly colonoscopy
2-3-yearly Colonoscopy	• ACMAC • Family History of CRC independent of ACMAC • Bethesda 4 criteria • Adenomatous polyps in a first-degree relative • PMG5 criteria • Age 50 years or younger • Age between 51 and 64 years
1-2-yearly Colonoscopy	• Amsterdam criteria

5.5 Comparable Studies

Similar practices exist in New Zealand, where the incidence of colorectal cancer is also high by international standards. In 2014, the age-standardized incidence rate was 91 cases per 100,000 people (51 males, 40 females) [175]. This compares to 99 cases per 100,000 people (59 males, 40 females) in Canada, and 139 (86 males, 53 female) in NL over the same time period [176]. Practices in New Zealand also follow a family-first approach similar to that in NL. Risk stratifications depend on number and age of FDRs diagnosed with CRC, and are termed 'Slightly Increased', 'Moderately Increased' and 'Potentially High'. The latter are referred to genetic services or the New Zealand Familial Gastrointestinal Cancer Registry (NZFGCR) for a more accurate risk assessment and surveillance plan. Moreover, NZ patients with a history of polyps receive a risk assessment upon examination and are offered one of three colonoscopy screening frequencies according to the NZFGCR: Low Risk (every 5 years), Intermediate Risk (every 3 years) and High Risk (every year) [177].

An exploratory study by authors in New Zealand and Australia has also investigated the utility of a similar electronic risk assessment and prediction tool used to implement precision screening in primary care [178]. The rationale for the study cites many of the same complications experienced in NL, namely the poor identification of individuals at increased risk of CRC, and over-referral of individuals at average risk.

The paper describes the protocol for a phase II randomized controlled trial exploring the utility of a colorectal cancer risk predictor (CRISP) tool - a web-based risk assessment tool used to compute absolute risk of developing CRC based on analysis of the Colon Cancer Family Registry [179]. The CRISP tool presents the risk information and provides clinical decision support about recommended screening. The CRISP tool used a 2.5% 5-year absolute risk of CRC as the threshold for switching from biennial FOBT testing to colonoscopy in 5-year intervals. The CRISP tool also identifies individuals with family histories indicative of inherited syndromes (Lynch Syndrome, FCCTX, etc.) and these individuals are referred to family cancer clinics rather than the provision of screening advice.

The objective of the CRISP tool was to evaluate its use in general practice compared to the provision of generic cancer prevention information, and to assess whether it increases the proportion of participants who undergo risk-appropriate CRC screening.

5.6 Limitations

One of the primary limitations of this study is the lack of evidence supporting our comparison group: genetic counselors. While recommendations for screening by a genetic counselor are the standard of care, it cannot be considered the gold standard unless it has been shown to be superior to any other means to denote risk and link it to screening recommendations. Thus, in the absence of a trial comparing outcomes both with and without the involvement of a genetic counselor, we don't know what is best.

Many limitations of this study come from the data arising from the familial colorectal cancer clinic itself. First and foremost, we were limited by the low response rate, with only 51% of the 1091 incident CRC cases providing a family history. (2) The disparity in clinical staffing at the two sites may have influenced the quality of data collection. At the Eastern Health site, clinical staff included a genetic counselor, a clerk, an information technology and data management research assistant and a subject matter expert research assistant. Conversely, clinical staffing at Central Health only included a nurse and a clerk. (3) Subjective decision making by genetic counselors may have occurred because the disparity in decisions made by the genetic counselor and by the multivariate model more favored the accuracy of the score. Indeed, the score may be the preferred method, not the genetic counselor. (4) In a few instances there was either missing data or inaccurate recording of data in the database, but the risk of bias is mitigated by our large data set. Inaccurate recordings were corrected when possible, and the few instances of missing data should have minimal impact on the results, if any. (5) Assessment of an accurate pedigree is probably best provided by subject matter experts in a central location. However, in the absence of such a clinic, algorithmic approaches to defining risk and subsequent screening recommendations are dependent on getting accurate family histories on colorectal cancer, polyps and HNPCC-related cancer in first- and second-degree relatives. (6) Family history data is likely

to be more accurate if there are dedicated health professionals encouraging patients to collect information from family members rather than a dependence on a variety of health care professionals. In addition, the need for patient tumor information to complete the score provides a further burden on a health care professional to obtain the data. (7) Although this project was really an evaluation of a health care intervention it benefitted from the rigor associated with research. In the real world, data collection may not be as good as some details are cumbersome to collect (8) Use of the multivariate scores in medical practice may have low acceptance and better penetration of the health system may occur if the interventions were propagated and operationalized by the NL Cancer Care program.

Chapter 6 – SUMMARY AND CONCLUSION

Colorectal cancer mortality rates in Canada have declined significantly among both males (-2.3% per year between 2004-2015) and females (-1.7% per year between 1984-2015). This decline is driven by a decrease in incidence, and improvements in treatment. Half of CRCs in Canada are detected at stages III or IV. Given the well-documented relationship between stage at diagnosis and survival for CRC, the implementation of accurate and effective screening recommendations will reduce CRC mortality rates even further [1]. This is of particular importance in NL, as we have the highest mortality rates for colorectal cancer in the country.

Between 2009-2011, a CRC screening program was organized for average-risk individuals between the ages of 50-74 in five Canadian provinces (B.C., Saskatchewan, Manitoba, Nova Scotia, P.E.I.). The program has since expanded to every province in the country, including NL since 2012. Among similar initiatives worldwide, participation rates in Canada were the lowest of any, at 16.1% [180]. Similarly, the aforementioned Newfoundland familial CRC clinic was limited by the same plight: low participation rates coupled with low response rates. Moreover, the World Health Organization are estimating an increase of 77% in the number of incident CRC cases, and an increase of 80% in deaths due to CRC by 2030 [181]. These data confirm the need to urgently incorporate measures to improve both participation in screening programs and more accurate and efficient provision of screening, including among high-risk families, and particularly in NL, where we have the highest rate of familial CRC in the world, and where 44% of the population are 50 years of age and older [182].

We believe the results from this research project are relevant to future colorectal screening and prevention practices. Specifically, we were able to accurately predict which patients required referral to a genetic counselor, and whether patients require high-frequency colonoscopy screening (2-3-years), low-frequency colonoscopy screening (5-10-years), at high sensitivity and specificity. No score was necessary for 1-2 yearly interval screening because this is the standard of care for Amsterdam criteria families. Furthermore, review of clinical data to assess false positive and false negative results of the scores suggest that the scores were more accurate than the decision of the genetic counselor, or that data not recorded influenced the decision made by

the geneticist. Prediction of colonoscopic screening recommendations for patients at intermediate risk was more challenging from an analytical viewpoint, but from a practical perspective, families that were non-Amsterdam, with low multivariate scores for both high and low frequency screening would automatically require 3-5 yearly screening.

In summary, the models based on validated risk score components yielded different recommendations than those from the geneticists and genetic counselors, and on review it seems they are better recommendations. We feel that our study can improve both the efficiency and accuracy of interventions important to families with colorectal cancer: referral to genetics and the provision of colonoscopy screening in family members. Prospective validation of the screening recommendations arising from the models should be done in the future.

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Chapter 8 - APPENDICES

Appendix A

Amsterdam Criteria II cancers

CRC, **endometrium, small bowel, ureter, or renal pelvis.**

The Revised Bethesda Guidelines are as follows:

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.
2. Presence of synchronous or metachronous colorectal, or other HNPCC-associated tumors,* regardless of age.
3. Colorectal cancer with the MSI-H histology‡ diagnosed in a patient who is less than 60 years of age.§
4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years.
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.

* HNPCC-related tumors include colorectal, endometrial, **stomach, ovary, pancreas**, ureter and renal pelvis, **biliary tract, and brain (usually glioblastoma** as seen in Turcot syndrome) tumors; **sebaceous gland adenomas and keratoacanthomas** in Muir–Torre syndrome, and carcinoma of the small bowel.

‡ Presence of tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.

§ There was no consensus among the Workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep less than 60 years of age in the guidelines.

For Progeny:
Red ones are AC2.
Blue ones are Bethesda.

TCC – Transitional Cell Bladder will be coded green.
RCC – Renal Cell Cancer Kidney will be coded green
TCC – Transitional Cell Ureter and Renal Pelvis will be coded Red
TCC – Transitional Cell Kidney will be coded Red.

Appendix B

Testing algorithm for suspected HNPCC families
Provincial Medical Genetics Program

Families meeting the following criteria:
Age & Cancer Modified Amsterdam Criteria

a) Three or more relatives with colorectal cancer or an HNPCC-associated cancer (colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain, sebaceous gland and small bowel carcinoma) *AND*
b) Colorectal or HNPCC-associated cancer in *at least* two generations *AND*
c) One or more colorectal or HNPCC-associated cancers diagnosed before age 60

Or one of the following additional criteria:

- Colorectal carcinoma before age 40
- Endometrial carcinoma before age 45
- Sebaceous tumour (adenoma, carcinoma, epithelioma) before age 50 or multiple tumours at any age
- Presence of multiple HNPCC-related cancers in an individual, with one cancer before age 60
- Colorectal carcinoma before age 60 with specific pathological features (tumour-infiltrating lymphocytes, Crohn's-like lymphocyte reaction, mucinous/signet ring differentiation, medullary growth pattern or undifferentiated pattern – solid or cribiform)

Are eligible for the following testing protocol through the PMGP:

Mutation unknown in family
Obtain tumour tissue on appropriate individuals
(and bank DNA on affected individual)

↙
MSI / IHC studies –
Blocks transferred to Mount Sinai
(IHC for MLH1, MSH2, MSH6, PMS2)

↓
Abnormal MMR studies

↓
Sequencing to be done
in external labs

↓
Mutation found
Confirm in clinical lab

Mutation known in family

↓
Proceed directly to clinical mutation testing

↘
Normal MMR studies

↓
Consider polyposis syndromes
Counsel as "Hereditary Colorectal Cancer Type X";
Screening recommendations per family history

No mutation found
Consider BRAF testing if MSI-H and MLH1-deficient

Send blocks to: Dr. Aaron Pollett, Staff Pathologist
Pathology and Laboratory Medicine
Suite 6-500-9, Mount Sinai Hospital
600 University Avenue
Toronto, Ontario M5G 1X5
416-586-4457

Modified January 25, 2010

Appendix C

NEWFOUNDLAND AND LABRADOR
HREB
HEALTH RESEARCH ETHICS BOARD

Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

July 12, 2016

12 Winter Place
St. John's, NL
A1B 1J6

Dear Mr. McNicholas:

Researcher Portal File # 20170239
Reference # 2016.147

RE: "Health Policy For Screening in Families of Colorectal Cancer Patients"

Your application received an expedited review by a sub-committee of the Health Research Ethics Board (HREB). ***Full approval*** of this research study is granted for one year effective **July 11, 2016**.

This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approval from the Regional Health Authority (RHA) or other organization as appropriate. You can refer to the HREA website for further guidance on organizational approvals.

This is to confirm that the HREB reviewed and approved or acknowledged the following documents (as indicated):

- Application, approved
- List of variables, approved

MARK THE DATE

This approval will lapse on July 11, 2017. It is your responsibility to ensure that the Ethics Renewal form is submitted prior to the renewal date; you may not receive a reminder. The Ethics Renewal form can be found on the Researcher Portal as an Event form.

If you do not return the completed Ethics Renewal form prior to date of renewal:

- ***You will no longer have ethics approval***
- *You will be required to stop research activity immediately*
- *You may not be permitted to restart the study until you reapply for and receive approval to undertake the study again*
- *Lapse in ethics approval* ***may result in interruption or termination of funding***

You are solely responsible for providing a copy of this letter, along with your approved HREB application form; **to Research Grant and Contract Services** should your research depend on funding administered through that office.

Modifications of the protocol/consent are not permitted without prior approval from the HREB. **Implementing changes without HREB approval may result in your ethics approval being revoked, meaning your research must stop**. Request for modification to the protocol/consent must be outlined on an amendment form (available on the Researcher Portal website as an Event form) and submitted to the HREB for review.

The HREB operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), the Health Research Ethics Authority Act (HREA Act) and applicable laws and regulations.

You are responsible for the ethical conduct of this research, notwithstanding the approval of the HREB.

We wish you every success with your study.

Sincerely,

Ms. Patricia Grainger

Chair, Non-Clinical Trials Health Research Ethics Board

CC: Dr P Parfrey

Appendix D

False Negatives (n=25) of Multivariate Model Predictive of Genetic Counselor Referral

Study ID	Age	Predictive Variables
2862	82	Bethesda 2
60012	76	
60076	55	
60105	79	
60111	59	Bethesda 2
60165	69	Bethesda 2
60181	72	
60183	65	Bethesda 2
60229	81	Bethesda 2
60321	32	
60348	51	Bethesda 3
60606	69	Bethesda 2
60612	54	Bethesda 2
60636	49	
60757	72	
60833	60	
60967	57	
60972	62	
61300	57	Bethesda 3
61485	69	Bethesda 2
61549	58	
61582	68	
61599	52	Bethesda 2
61606	73	Bethesda 2
61609	40	

Appendix E

False Positives (n=48) of Multivariate Model Predictive of Genetic Counselor Referral

Study ID	Age	Predictive Variables
1610	65	ACMAC, Bethesda 2
60005	65	ACMAC
60007	66	Family History of CRC without ACMAC
60085	73	Polyps
60088	57	ACMAC, Polyps, Bethesda 3
60103	73	ACMAC
60138	67	ACMAC, Bethesda 4
60144	72	ACMAC, Bethesda 4
60145	71	Family History of CRC without ACMAC, Bethesda 4
60147	47	Family History of CRC without ACMAC
60238	71	Family History of CRC without ACMAC
60240	84	Family History of CRC without ACMAC
60267	74	ACMAC
60283	67	ACMAC
60295	91	ACMAC
60296	66	Family History of CRC without ACMAC
60300	70	ACMAC, Bethesda 2
60638	68	ACMAC, Bethesda 2
60647	65	ACMAC
60656	57	ACMAC
60683	89	Family History of CRC without ACMAC
60688	82	ACMAC
60722	72	Family History of CRC without ACMAC
60759	75	Family History of CRC without ACMAC
60780	84	ACMAC
60806	60	Family History of CRC without ACMAC
60861	68	Family History of CRC without ACMAC
60896	57	ACMAC
60897	58	ACMAC
60912	73	ACMAC, Bethesda 4
60918	68	ACMAC, Bethesda 4
60941	68	ACMAC
60945	60	ACMAC
60956	68	ACMAC, Bethesda 4
60975	81	Family History of CRC without ACMAC, Bethesda 4
60984	82	Polyps, F. History of CRC without ACMAC, Bethesda 2
61254	71	Family History of CRC without ACMAC
61434	77	ACMAC

61469	69	Family History of CRC without ACMAC
61491	65	Family History of CRC without ACMAC
61538	77	Polyps
61541	70	Polyps
61542	57	ACMAC
61636	76	ACMAC, Bethesda 2, Bethesda 3
61656	70	Family History of CRC without ACMAC
61682	71	Polyps
61737	75	Family History of CRC without ACMAC
61782	64	ACMAC, Bethesda 2

Appendix F

False Negatives (n=30) of Multivariate Model Predictive of 5-10 yearly Colonoscopy

Study ID	Age	Predictive Variables
1610	65	No Family History of CRC, Bethesda 2
60005	65	Bethesda 5
60007	66	Bethesda 5
60084	50	No Family History of CRC
60103	73	Bethesda 5
60138	67	No Family History of CRC, Bethesda 4, Bethesda 5
60145	71	Bethesda 4
60173	58	No Family History of CRC, Bethesda 5
60176	69	Bethesda 5
60206	64	No Family History of CRC, Bethesda 5
60270	61	Bethesda 5
60278	60	No Family History of CRC, Bethesda 5
60286	61	No Family History of CRC, Bethesda 2, Polyps, PMG4
60382	76	No Family History of CRC, Bethesda 2, Bethesda 5
60564	73	Bethesda 5
60638	68	Bethesda 2, Bethesda 5, PMG4
60647	65	No Family History of CRC, Bethesda 5
60683	89	Bethesda 5
60806	60	Bethesda 5
60833	60	No Family History of CRC, Bethesda 5
60861	77	No Family History of CRC
60874	43	No Family History of CRC
60897	58	Bethesda 5
60945	60	No Family History of CRC, Bethesda 5
60984	82	Bethesda 2, Poylps, PMG4
61254	71	Bethesda 5
61434	77	Bethesda 5
61541	70	No Family History of CRC, Bethesda 5, Polyps
61636	76	No Family History of CRC, Bethesda 2, Bethesda 3, Bethesda 5, PMG4
61782	64	No Family History of CRC, Bethesda 2

Appendix G

False Positives (n=50) of Multivariate Model Predictive of 5-10 yearly Colonoscopy

Study ID	Age	Predictive Variables
20705	76	
60040	56	No Family History of CRC
60064	62	No Family History of CRC, Bethesda 2, PMG4
60111	59	No Family History of CRC, Bethesda 2, PMG4
60117	63	No Family History of CRC, Bethesda 2, PMG4
60127	67	No Family History of CRC, Bethesda 2
60165	69	No Family History of CRC, Bethesda 2, PMG4
60181	72	No Family History of CRC
60183	65	No Family History of CRC, Bethesda 2, PMG4
60219	85	No Family History of CRC, Bethesda 5
60247	59	No Family History of CRC
60262	87	Bethesda 2
60280	61	No Family History of CRC
60334	77	No Family History of CRC, Bethesda 5
60358	72	No Family History of CRC, Bethesda 5
60377	73	No Family History of CRC, Bethesda 5
60386	86	
60549	87	No Family History of CRC, Bethesda 2
60591	91	No Family History of CRC
60592	56	No Family History of CRC
60606	69	No Family History of CRC, Bethesda 2
60624	72	No Family History of CRC, Bethesda 5
60627	76	No Family History of CRC, Polyps
60671	55	No Family History of CRC
60698	57	No Family History of CRC
60706	52	No Family History of CRC
60723	61	No Family History of CRC
60757	72	No Family History of CRC
60885	53	No Family History of CRC
60923	66	No Family History of CRC
60937	58	No Family History of CRC
60948	79	No Family History of CRC, Polyps
60952	57	No Family History of CRC, Bethesda 2
60967	57	No Family History of CRC
60972	62	No Family History of CRC
60982	69	No Family History of CRC, Bethesda 5

60989	55	No Family History of CRC
61253	56	No Family History of CRC
61342	54	No Family History of CRC
61383	69	No Family History of CRC
61485	69	No Family History of CRC, Bethesda 2
61493	52	No Family History of CRC
61512	67	No Family History of CRC
61549	58	No Family History of CRC
61597	83	No Family History of CRC
61604	63	No Family History of CRC
61606	73	No Family History of CRC, Bethesda 2
61758	78	No Family History of CRC, Bethesda 5
61763	76	No Family History of CRC, Bethesda 2
61781	68	No Family History of CRC, Bethesda 5

Appendix H

False Negatives (n=21) of Multivariate Model Predictive of 2-3 yearly Colonoscopy

Study ID	Age	Predictive Variables
20419	71	ACMAC
60219	85	ACMAC
60262	87	Family History of CRC without ACMAC
60321	32	
60348	51	
60358	72	ACMAC
60371	81	ACMAC
60373	40	
60624	72	ACMAC
60627	76	Polyps
60660	74	Family History of CRC without ACMAC
60700	71	ACMAC
60706	52	
60736	82	ACMAC
60838	68	ACMAC
60948	79	Polyps
60972	62	
61190	84	ACMAC
61376	28	
61606	73	
61609	40	

Appendix I

False Positives (n=56) of Multivariate Model Predictive of 2-3 yearly Colonoscopy

Study ID	Age	Predictive Variables
1610	65	ACMAC, PMG5
21134	51	ACMAC
60002	61	Family History of CRC without ACMAC
60005	65	ACMAC
60008	53	Family History of CRC without ACMAC, Bethesda 4
60009	60	ACMAC
60016	60	ACMAC
60038	63	ACMAC
60039	51	Polyps
60049	82	Bethesda 4, ACMAC
60071	60	ACMAC
60079	57	Family History of CRC without ACMAC
60088	57	Polyps, ACMAC
60111	59	PMG5
60129	48	ACMAC
60134	56	ACMAC
60138	67	Bethesda 4, ACMAC
60144	72	Bethesda 4, ACMAC
60145	71	Family History of CRC without ACMAC, Bethesda 4
60147	47	Family History of CRC without ACMAC
60173	58	ACMAC
60180	49	ACMAC
60227	61	ACMAC
60270	61	ACMAC
60278	60	ACMAC
60286	61	Polyps
60300	70	ACMAC, PMG5
60303	60	ACMAC
60338	84	Family History of CRC without ACMAC, Polyps
60350	71	Bethesda 4, ACMAC
60359	81	PMG5
60383	46	ACMAC
60647	65	ACMAC
60725	46	ACMAC
60731	56	ACMAC
60745	60	ACMAC
60806	60	ACMAC
60897	58	ACMAC
60909	65	Family History of CRC without ACMAC
60912	73	Bethesda 4, ACMAC
60918	68	Bethesda 4, ACMAC
60945	60	ACMAC

60956	68	Bethesda 4, ACMAC
60975	81	Family History of CRC without ACMAC, Bethesda 4
60984	82	Family History of CRC without ACMAC, Polyps
61327	54	ACMAC
61486	71	Bethesda 4, ACMAC
61491	65	Family History of CRC without ACMAC
61542	57	ACMAC
61558	60	ACMAC
61567	61	Bethesda 4, ACMAC
61581	51	ACMAC
61609	40	
61636	76	ACMAC
61767	60	ACMAC
61782	64	ACMAC