THE CAUSE OF THE INCREASED COLON CANCER MORTALITY RATE IN NEWFOUNDLAND & LABRADOR: A STUDY COMPARING SURVIVAL IN NEWFOUNDLAND & LABRADOR AND ONTARIO POPULATION-BASED COHORTS

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ABSTRACT

Background: Colorectal cancer (CRC) is the second leading cause of cancer-related death among Canadian men and women. Newfoundland and Labrador (NL) has the highest CRC mortality rate in the country. To determine whether this resulted from increased CRC incidence, later stage or more adverse prognostic factors at diagnosis, or diminished survival by stage, colon cancer data was compared from NL (n=510) and Ontario (ON) (n=906) cohorts.

Methods: Predicted and actual CRC incidence and death rates were obtained. Survival analysis was conducted for stages 1-3 colon cancer patients in the 2 cohorts. Multivariate models included sex, stage and age at diagnosis, microsatellite instability, body mass index, smoking status, and adjuvant treatment.

Results: Estimated age-standardized incidence rates in NL were 34% higher in men and 21% higher in women than in ON, comparatively. Actual NL incidence rates were 55% and 53% higher in men and women than estimated. NL cases had improved survival compared to ON at stage 2 (p=.041), otherwise survival by stage was similar. Other adverse predictors of survival were similar between provinces.

Conclusion: NL's high CRC mortality rate can be attributed to increased incidence and not to adverse prognostic indicators or worse survival. This data supports the need for improved CRC screening strategies.

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ABBREVIATIONS

AC – Amsterdam Criteria

ACII – Amsterdam Criteria II

AJCC – American Joint Committee on Cancer

ASIR – Age-Standardized Incidence Rate

ASMR – Age-Standardized Mortality Rate

BG – Bethesda Guidelines

BMI – Body Mass Index

CAG – Canadian Association of Gastroenterology

CCS – Canadian Cancer Statistics

CDHF – Canadian Digestive Health Foundation

CoxPH – Cox Proportional Hazard

CRC – Colorectal Cancer

CPG – Clinical Practice Guidelines

CTFPHC – Canadian Task Force on Preventive Health Care

FAP – Familial Adenomatous Polyposis

FCCTX – Familial Colorectal Cancer Type X

FOBT – Fecal Occult Blood Test

FT – Fecal Test

FTi – Immunochemical Fecal Test

gFOBT – Guaiac Fecal Occult Blood Test

HNPCC – Hereditary Non-Polyposis Colorectal Cancer

HR – Hazard Ratio

KM – Kaplan Meier

LML – Log Minus Log

LS - Lynch Syndrome

MMR – Mismatch Repair

MSI – Microsatellite Instability

MSI-H – Microsatellite Instability-High

MSI-L – Microsatellite Instability-Low

MSS – Microsatellite stable

NCCSN – National Colorectal Cancer Screening Network

NFCCR – Newfoundland and Labrador Familial Colorectal Cancer Registry

NIH – National Institutes of Health

NL – Newfoundland and Labrador

OFCCR – Ontario Familial Colorectal Cancer Registry

ON – Ontario

OR – Odds Ratio

PHAC – Public Health Agency of Canada

RBG – Revised Bethesda Guidelines

RR – Relative Risk

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Chapter 1 INTRODUCTION

Colorectal cancer (CRC) is cancer of the large intestine including cancers of both the colon and rectum. The colon extends from the cecum, or end of the small intestine, to the rectum. The colon is further comprised of the right, transverse, and left colon. The right side includes the cecum, ascending colon, and hepatic flexure. The left side includes the splenic flexure, descending colon, and sigmoid colon. The rectum then extends from the sigmoid colon to the anus.

Colorectal cancer develops from a polyp, a precancerous bulging tissue mass that protrudes from the epithelial lining of the colon or rectum. Polyps develop on the intestinal wall and continue to grow until they develop into a malignant, cancerous tumour. Adenomas are a group of polyps that account for most CRC tumours.[1] Adenocarcinoma is then cancer that has developed from an adenomatous polyp.

Polyps may develop sporadically and without underlying heritable contribution, leading to sporadic colorectal cancer. They may also develop due to familial risk where cases present in some families at a higher rate than expected by chance. Familial risk is defined as having two or more first- or second- degree relatives with CRC. Causes of familial cancer development are unknown, however possible contributors are similar risk factors in lifestyle, diet or environment.

Polyps may also develop due to a hereditary condition such as Hereditary Non-Polyposis Colorectal Cancer (HNPCC), including cases with Lynch Syndrome (LS) or Familial Colorectal Cancer Type X (FCCTX). LS is an autosomal dominant hereditary predisposition for malignancy explained by a germline mutation in a DNA mismatch repair (MMR) gene.[2] There tends to be multiple generations affected with CRC at an early age (mean of approximately 45 years) with tumour predominance in the proximal colon and an excess of extracolonic cancers.

FCCTX is a condition of autosomal dominant inheritance of CRC that does not have a germline MMR mutation but rather has unknown genetic basis.[3] Compared to LS, FCCTX families have a lower predisposition to CRC (2.3 vs 6.1), are not associated with developing extracolonic cancers, tend to have older mean age at diagnosis (50-60 years vs 45 years), and tumours are more likely to be located in the distal colon or rectum.

Whether sporadic, familial, or heritable, distinct characteristics are evident in the pathway leading to development of CRC. There are different implications in treatment, testing for tumour markers, follow-up and involvement of family members in sporadic vs hereditary CRC patients so it is therefore important to differentiate the underlying nature of CRC.

1.1 Staging

CRC is staged at diagnosis to describe the degree to which cancer is present in the body. Cancer stage is one of the most important factors in determining prognosis and treatment recommendations. Cases included in this thesis were staged using the guidelines of the American Joint Committee on Cancer (AJCC), also known as the TNM system. The TNM system incorporates 3 elements to determine cancer extent:

- "T" examines the extent of the tumour's spread through the various layers that comprise the wall of the colon and rectum;
- "N" indicates if or to what extent the cancer has spread to the lymph nodes;
- "M" describes whether the cancer has metastasized to distant organs.

CRC cases in the Newfoundland Familial Colorectal Cancer Registry (NFCCR) are staged at the time of initial diagnosis as stage1-4 using the TNM system as follows:

Stage I: (T1, N0, M0 or T2, N0, M0) – Cancer has grown through the muscularis mucosa into the submucosa (T1) *or* may have also grown into the muscularis propria (T2), with no spread to lymph nodes or distant sites.

Stage II: (T3, N0, M0 or T4, N0, M0) – Cancer has grown into outermost layers of the colon or rectum but has not reached nearby organs *or* has grown through wall of the colon or rectum into other nearby tissues or organs, with no spread to lymph nodes or distant sites.

Stage III: (T1, N1, M0 or T2, N1, M0) – Cancer has grown through mucosa into submucosa (T1) *or* may have also grown into muscularis propria (T2). It has spread to 1-3 nearby lymph nodes but not distant sites; *or* (T3, N1, M0 or T4, N1, M0) – Cancer has grown into outermost layers of colon or rectum but has not reached nearby organs *or* has grown through wall of colon or rectum and into other nearby tissues or organs. It has spread to 1-3 nearby lymph nodes but not distant sites; *or* (Any T, N2, M0) – Cancer may or may not have grown through wall of colon or rectum, has spread to 4 or more nearby lymph nodes, but not spread to distant sites.

Stage IV: (Any T, Any N, M1) – Cancer may or may not have grown through wall of colon or rectum and may or may not have spread to nearby lymph nodes. It has spread to distant sites such as the liver, lung, peritoneum, or ovary.

1.2 Colon Cancer versus Rectal Cancer

Cancer of the colon is the most common of CRCs comprising about 70%, with rectal cancer comprising 30%.[4] Relatively few studies in the literature differentiate between colon and rectal cancer; analyses and results are generally pooled together as CRC. While CRC is often considered to be one disease, colon and rectal cancers are likely to have etiological differences.[4-6]

Modifiable risk factors do not appear to contribute equally to colon and rectal cancers.[4,7-10] Even right and left subsections of colon cancers may each have distinct etiologies.[4] Further, treatment recommendations differ for colon and rectal cancers.[11] Hereditary CRCs also affect cancers of the colon and rectum uniquely.[4,12] Another important difference is anatomic in that the distal rectum does not have a serosal covering. Because cancer of the colon or rectum each involve unique anatomy and etiology, colon and rectal cancers should be considered different diseases. Analyses for this Master's thesis will focus on cancer of the colon.

1.3 CRC Mortality in Canada

According to Canadian Cancer Statistics, almost half of all Canadians will develop cancer in their lifetime and 1 in 4 Canadians will die from it. Colorectal cancer is the second leading cause of cancer-related death in both men and women in Canada.[13] In 2015 there were 9300 expected CRC-related deaths in Canada, including 5100 men and 4200 women. Estimated age-standardized mortality rates (ASMRs per 100,000 people) of CRC in Canada for 2015 were 22 in males and 14 in females.

1.4 CRC Incidence and Mortality in Newfoundland & Labrador

The lifetime risk of developing CRC is 7%.14 Newfoundland and Labrador (NL) has the highest incidence of CRC of all the Canadian provinces and 27% higher than the Canadian average.[15] Green et al (2007) attribute such high incidence of CRC in NL to hereditary factors, both familial and genetic.[15]

NL has the highest CRC mortality rate in Canada.[13] The predicted ASMR for males was 38 and 21 for females in NL, compared in Ontario (ON) to 20 in males and 13 in females. ON

ASMRs were comparable to the national ASMRs. In 2015, it was estimated that there would be 240 expected deaths from CRC in NL. CRC is the second leading cause of cancer-related death for men and third leading cause of cancer-related death for women in NL.

Higher mortality rates in NL may be due to either increased CRC incidence due to hereditary conditions or exposure to modifiable risk factors, worse prognosis at time of diagnosis, or level of care and treatment, resulting in lower survival rates. Known prognostic indicators of CRC include stage of disease at the time of diagnosis, age, and tumour microsatellite instability (MSI) status.[16,17] MSI exists in tumours that exhibit MMR mutations. MMR proteins repair sections of DNA by removing sequence errors that can lead to cancer development.

1.5 Factors in CRC Incidence and Survival

1.5.1 Modifiable risk factors

Evidence in the literature suggests a relationship exists between lifestyle factors, CRC incidence, and survival. Primary prevention of CRC involves controlling modifiable risk factors such as diet, obesity, smoking, and alcohol consumption. Reducing such risk factors could substantially decrease the risk of CRC development in the general population and complement screening to reduce CRC incidence and improve survival.[9]

Diet

Diet is one of the most important modifiable risk factors linked with CRC. There is inconsistency in the literature in attributing specific dietary factors (red meat vs. poultry, fish or

plant-sourced proteins, fiber, and fruits and vegetables, among others) to CRC incidence and mortality.[9,18-20]

Red meat consumption and its relationship to CRC development has been widely studied with varying results. Chan and Giovannucci (2010) report an increase in colon cancer risk and adenoma development in people with high red meat intake.[9] A meta-analysis of meat consumption and CRC similarly concludes that red meat and processed meat increase risk of CRC.[21] Tumour recurrence, metastasis, and death has been reported significantly higher among CRC patients that followed a high processed meat dietary pattern, characterized by high intakes of red meat, fish, and processed meat and fish.[22] Processed meat may present a stronger risk factor than fresh red meat.[23,24] There may also be an association between how red meat is cooked and CRC risk, as risk may be higher with consumption of heavily browned meat or that cooked at high temperatures for a long period of time.[9] In contrast, it has also been reported that there is little evidence of association between red meat and processed meat consumption and CRC risk.[18] An NL population study on red meat consumption could not conclude that increased red meat consumption increased CRC risk.[25] Similar studies in ON populations found that consuming a greater amount of red meat increased CRC risk in their population.[25,26]

A significant relationship has been reported between pickled red meat consumption and increased CRC risk in NL, with a possible dose-response effect.[25] Pickled red meat (meat preserved in brine solution) is a unique food in NL and is not as commonly consumed in other parts of Canada. Squires et al (2010) compared diets of an NL and ON population. An ON diet may be more representative of the Canadian diet overall as compared to the traditional NL diet. Differences noted between NL and ON diets may be indication that red meat intake is more

likely to work in conjunction with other factors such as genetics or environment, rather than independently, to predict CRC risk.

A diet high in caloric intake has been reported to increase CRC risk,[19,27-31] while there appears to be an inverse relationship between CRC incidence and diets high in protein, fiber, and carbohydrates.[19,28,32-35] While there has been little research on the effects of diet on CRC survival, it has also been reported that a high carbohydrate diet was related to increased CRC survival.[36] Total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, and cholesterol have not been associated with increased CRC risk.[19]

While increasing consumption of fruits, vegetables, and dietary fiber are unlikely to prevent most CRCs, health benefits of a fiber-rich diet may be effective in managing chronic inflammation associated with gastrointestinal disorders which may play a role in increasing CRC incidence risk.[9]

Alcohol

There is inconclusive evidence in the literature regarding the relationship between alcohol consumption and CRC risk. A review by Chan and Giovannucci (2010) cites evidence that high alcohol consumption increases CRC risk,[9] yet other studies have found no impact.[19,37]

A study of NL cases showed that the effect of alcohol consumption on developing CRC may be worsened by obesity.[37] Among obese subjects, alcohol intake was positively associated with CRC risk (OR=2.2), increasing in a linear trend with reported increasing number of drinking years.

Physical activity

An inverse relationship is consistently reported between physical activity and incidence of both colon adenoma and carcinoma in both prospective cohort and case-control studies.[9] This relationship remains true when controlling for other healthy lifestyle factors and is observed in both leisure and occupational physical activity.[9] Physically active people have been found to be at 20-30% decreased risk of colon cancer incidence compared to those less active.[9] Even without weight loss, physical activity itself appears to lower risk, and the association appears to be stronger for colon cancer than rectal cancer.[4,9] Obese individuals with sedentary lifestyles may have higher colon cancer risk than obese individuals leading physically active lifestyles, particularly in those with high abdominal obesity and waist circumference.[38]

Obesity & waist circumference

An association between obesity as measured by Body Mass Index (BMI) and increased risk of CRC has been reported.[9,39,40] The association appears to be stronger in men than women and for colon cancer compared to rectal cancer.[5,9,39,41] The mechanism between the obesity and CRC incidence relationship is not well-established in the literature,[9,38,41] however it may be linked to the obesity-induced effects of insulin, leptin, chronic inflammation, and steroid hormones.[41] Results for women are less consistent than men, possibly due to the effect of estrogen on cancer development.[4] One study reported that individuals categorized as having an overweight BMI had a 30-40% increased colon cancer risk, and those with obese BMI had a 60-80% increased risk compared to individuals with normal BMI.[38,41] Overweight and obese men have been found to have similar CRC risk, irrespective of familial risk of cancer.[41]

A relationship also appears between CRC incidence in both men and women and abdominal obesity as measured by waist circumference and waist-to-hip ratio.[5,9,39] Waist circumference may be a stronger predictor of colon cancer risk than BMI.[5,38,39] A linear effect has been reported between male and female waist size and colon cancer incidence in particular.[38]

Smoking

The relationship between cigarette smoking, adenoma formation, CRC incidence and CRC survival is well documented.[7,9,42,43] A systematic review and meta-analysis of 36 prospective studies investigated the impact of cigarette smoking on CRC risk.[7] Compared to patients who never smoked, current smokers had significantly increased risk of CRC incidence (RR=1.15) and mortality (RR=1.40). Current smokers were also more likely to be diagnosed at a later stage of disease. This may be due to more rapid cancer progression or because current smokers are less likely to have health-conscious behaviours and practices than never or former smokers. Compared to never smokers, former smokers were at significantly increased risk of CRC incidence overall (RR=1.25), and specifically with increased risk for colon cancer incidence (RR=1.10) and mortality (RR=1.27). Further, a significant increased risk of CRC incidence and mortality was reported with increasing daily cigarette consumption, as well as a dose-response gradient between daily cigarette consumption and CRC mortality.

Pre-diagnosis cigarette smoking has been associated with diminished survival for patients with CRC.[42,43] It has been reported that smoking is associated with worse survival particularly in patients diagnosed at early stage disease, yet may have a negligible impact on survival for patients diagnosed with advanced stage disease.[42]

Cigarette smoking has been found to increase CRC risk in a NL study population.[8] Former and current smokers were found to have significantly higher risk of CRC incidence than non-smokers (OR=1.36 & 1.96).[8] Risk increased significantly with the number of smoking years, number of cigarettes smoked daily, and pack-years, and decreased significantly with years absent from smoking. There is conflicting evidence that earlier age of smoking initiation increases CRC risk.[7,8] In comparison with non-smokers, former and current smokers were at significantly elevated risk of CRC.[8] The association was stronger among cases who consumed alcohol and in men.[8]

1.5.2 Familial CRC

Family history is a strong predictor of CRC development. Familial risk is defined as having two or more first- or second- degree relatives with CRC.[44] Approximately 20% of all patients with CRC have a familial predisposition for developing CRC.[44]

Green et al (2007) conclude that genetic, or at least familial, factors are responsible for the excess cancer incidence in NL compared to the Canadian average. This is supported by the significantly higher proportion of high and intermediate risk families when compared to ON and other non-Canadian centres.[15] Indicators of familial risk were significantly higher in NL compared to ON and 13 other population-based studies. Of NL cases, 31% had at least 1 first-degree relative affected with CRC, compared to 20.4% in ON. The NL rate was 85% greater than the mean from 5 other non-Canadian centers included in their analysis.

The island of NL has a geographically and genetically isolated population comprised almost entirely of people from a limited founder population.[15,45,46] Approximately 95% of Newfoundlanders are from specific regions of England and Ireland.[15] In contrast, there is

considerable ethnic and racial diversity in the ON population and Canadian population overall. NL has been the focus of numerous studies on hereditary diseases involving the province's relatively young population of fewer than 20 generations.[46] Due to its limited founder population, it has been proposed that the high rate of familial CRC in NL is genetic in origin and attributable to founder effects. A founder effect has been observed in several other inherited diseases, which are observed at unusually high or low rates in the NL population.[15]

1.5.3 Hereditary CRC

Approximately 5-10% of CRC cases can be attributed to dominantly inherited, highly penetrant autosomal syndromes. Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is an umbrella term for several hereditary conditions, including Lynch Syndrome (LS) and Familial Colorectal Cancer Type X (FCCTX).

HNPCC and LS are often erroneously referred to synonymously in the literature. It is therefore challenging to distinguish between the two in literature review. While they are overlapping conditions, they are not synonymous.[47,48] The term HNPCC was developed to identify families who presented with certain criteria as outlined in Amsterdam Criteria I and II (Appendix A). However, with the discovery of the genetic mechanism underlying HNPCC in 1993, the term HNPCC should have been abolished.

Lynch Syndrome

LS is a hereditary predisposition to malignancy due to a germline mutation in DNA mismatch repair (MMR) genes.[48] LS is autosomal dominant where first-degree relatives of mutation carriers have a 50% chance of inheritance.[49] LS is the most common of hereditary

CRCs both in NL[15] and globally.[44] Approximately 3% of all CRCs present in families with LS.[16,44] NL has been found to have the highest incidence of LS-related CRC in the world.[15]

LS is associated with inherited genetic mutations in at least one of several MMR genes that lead to microsatellite instability (MSI).[16,44] The human genome contains hundreds of thousands of short, repeated sequences of DNA known as microsatellites.[44] MMR proteins exist naturally in DNA and repair sections of DNA that have not properly replicated by removing sequence errors. Mutations that occur in MMR genes allow for erroneously replicated DNA to exist unchecked and thus microsatellites become unstable, leading to cancer development. Tumours that exhibit MMR mutations are referred to as exhibiting microsatellite instability (MSI). A tumour that does not exhibit MSI is microsatellite stable (MSS). MSI is present in all LS tumours.

MSI tumours have low- (MSI-L) or high-frequency (MSI-H) instability. MSI-L tumours show instability in 1 of 5 tumour markers, while MSI-H tumours show instability in at least 2 of 5 microsatellites. MSI-L and MSS tumours appear to be phenotypically similar in nature.[50] MSI-H tumours have unique clinical and pathological features and are seen in about 15% of all CRC tumours, including hereditary (~3%) and sporadic (~12%) CRC[15,16,50] MSI-H tumours are found more frequently in the proximal colon,[16,44] have unique histopathological features such as poor differentiation,[16,44] and, when matched for stage, are less aggressive than MSI-L and MSS tumours.[50] Whether hereditary or sporadic, MSI-H tumours have slightly better prognosis than MSS tumours, however respond poorly to the standard 5 FU-based chemotherapy.[16,47]

Because MSI is identified in about 12% of sporadic CRC cases, MSI alone is not a diagnostic feature of hereditary CRC. In addition to MMR mutations, environmental and

modifiable risk factors such as smoking and diet may also play a role in the development of MSI tumours, either acting alone or with MMR mutations.[50,51]

While there are no identifiable phenotypic characteristics to distinguish between LS and sporadic CRC,[3,15,52] there are some identifiable features prevalent in LS families. LS-related CRC tends to present at a younger age of onset, at approximately age 45 years, compared to sporadic CRC cases with approximate onset age of 63 years.[16,44] LS cases are also at substantially elevated risk of developing extracolonic cancers, including gynecological cancers such as endometrial and ovarian, as well as cancers of the stomach, small bowel, pancreas, brain, and of the upper uroepithelial tract including renal pelvis and ureter.[2,44,49,52,53]

Familial Colorectal Cancer Type X

The term Familial Colorectal Cancer Type X (FCCTX) was proposed by Lindor et al (2005) to describe families that meet AC but who do not have a germline DNA MMR mutation, named "Type X" due to unknown etiology. Known MMR gene defects account for over 95% of mutations that cause LS,[54-58] however only 50-60% of families that meet the AC have a germline MMR defect.[3,59] A large proportion of apparent hereditary CRC families studied in NL presented with CRC cases in the absence of an MMR defect, thus not qualifying as LS.[45,60]

There are marked differences between LS and FCCTX families. FCCTX families have shown increased incidence of CRC only, yet still less than in germline mutation families.[61] FCCTX families are further characterized as having MSS tumours, lower relative risk of CRC, absence of excess extra-colonic tumours, and a later age of onset of CRC compared to LS families, at approximately age 60.7 years.[16,45,59-62] Compared to LS CRC tumours, FCCTX tumours present more frequently in the distal colon.[60,62] Stage of disease at diagnosis has not

been found to differ between LS and FCCTX cases.[62] Studies have more recently shown the novel genetic pathways of FCCTX that lead to cancer development.[61]

1.6 Hereditary Risk Screening

The Amsterdam Criteria (AC) is a series of criteria originally developed to help practitioners systematically identify LS families.[15,50] Published in 1991, the AC was eventually found to be too strict for clinical use as it excluded patients diagnosed with extracolonic cancers, a feature of LS. To increase sensitivity, AC was modified in 1998 to become Amsterdam Criteria II (ACII) and include pathology other than CRC. Families who meet ACII that have an MMR germline mutation are considered LS, while those without a germline mutation are FCCTX.

There is conflicting evidence on the efficacy of the AC. Some reports have found adherence to ACII to be the most effective system to detect HNPCC families,[59] while others find only 50-60% of families that meet AC have an MMR defect.[3] Because FCCTX patients tend to develop CRC after age 50, they do not meet the AC and may be missed by this screening criteria altogether.

Green et al (2007) compared NL to ON and 13 non-Canadian population-based cohorts to determine the proportion of NL CRC cases that met the AC. They reported that 3.7% of NL cases with high-familial risk met ACII, significantly higher than the aggregate from the high-familial risk cases from the other cohorts.[15] It was higher, although not significantly, compared to the ON cohort.[15]

The Bethesda Guidelines (BG) were developed by the National Cancer Institute in 1997 to identify colorectal tumours that should be tested for MSI, with the goal to identify LS

patients[63] (Appendix B). To improve function, the guidelines were revised in 2004 to become the Revised Bethesda Guidelines (RBG).

There are reports that challenge the BG's accuracy and usability in identifying MSI-potential tumours and LS families. Evidence suggests strict use of the original BG to identify families for gene analysis results in reduced probability of mutation detection.[59] In comparing RBG and universal MMR screening to identify LS families, one study reported that 1.7% of patients met ACII and that, compared to the universal strategy, RBG failed to detect 14.3% of cases with LS and 57.1% of cases with probable non-sporadic MSI tumours.[51] This means that 14.3% of LS patients did not meet RBG. Because the RBG exclude patients over age 60 years, adhering to the criteria could miss a substantial number of LS cases.[64] Morrison et al (2011) implemented a universal approach to LS screening and found it significantly increased the detection rate of potential LS patients to 20.7% from 8.5% through adherence to RBG.[64] Adherence to RBG missed 68.4% of MSI-H tumour cases because the patients were over age 60 years. While a universal screening paradigm found significantly more MSI-H tumours and therefore had increased likelihood of detecting LS families, testing all CRC tumours for MSI may not be clinically or financially feasible.

Differences are evident between LS and FCCTX families in terms of genetic syndrome screening, CRC screening, and cancer risk. Although known hereditary forms of CRC account for only 3-5% of all CRCs, it is important to screen for these conditions due to the very high risk of disease in these families. MMR germline mutation carriers are at approximately 80% lifetime risk of developing CRC[52] as well as at increased risk of developing extra-colonic cancers. Since LS-related CRCs have also been found to respond differently to standard CRC chemotherapies, identifying MMR germline mutation carriers will help determine the best course of treatment.[49]

Failure to detect genetic mutations can pose a serious impediment in implementing a comprehensive genetic and clinical screening program in high-risk families. Genetic screening is important in LS and FCCTX cases because of the lack of phenotype presentation in these cases.[52] At least a three-generation family history has been deemed necessary to adequately determine hereditary-associated cancer.[44] Genetic testing should take place for LS when ACII or RBG have been met.[52] Genetic counseling should occur prior to DNA collection and also when test results are disclosed.[44]

There are benefits of genetic testing beyond identification of hereditary cancer risks. A positive mutation test result has been found to drastically increase patient compliance with colonoscopic screening programs.[65] There are patient risks associated with genetic testing such as psychological distress, anxiety, health insurance issues, and changes in the family dynamic.[52] A risk estimation cut-off that is too high will overlook MMR mutations and lead to possible subsequent morbidity and mortality.[49] One that is too low will mean unnecessary patient investigation that can lead to increased health care costs, patient anxiety, and unnecessary invasive clinical screening.[49]

Patients with CRC can also present with sporadic MSI tumours unrelated to hereditary CRC etiology. MMR mutations can occur naturally due to methylation of DNA, leading to MSI tumours. This happens more frequently in elderly patients. Patients who present with sporadic MSI CRC tumours tend to be older than patients with MSS tumours due to methylation (non-hereditary MSI due to natural MMR defects) which increases with advancing age.[16]

1.7 CRC Screening

CRC screening efficacy is well documented in the literature.[9,44,52,66-69] Screening reduces CRC incidence through early detection of pre-cancerous polyps. It also diagnoses CRC in early stage of disease therefore improving likelihood of survival.[52,66,68] Fecal occult blood testing (FOBT) including immunochemical fecal tests (FTi), sigmoidoscopy, and colonoscopy have all been shown to decrease incidence and mortality of CRC.[52,66,70,71]

The Canadian Task Force on Preventive Health Care (CTFPHC) was established by the Public Health Agency of Canada (PHAC) to develop clinical practice guidelines that support primary care providers in delivering preventive health care. In response to lack of national guidance and variability in provincial screening programs, the CTFPHC released CRC screening recommendations in 2001 for people at normal and above-average risk of developing CRC.[72] In 2016, the CTFPHC released an updated protocol and guidelines to include recommendations on appropriate populations to be screened, most appropriate screening tests, and interval and age to commence and cease screening.[73,74] They recommend screening adults aged 50-74 years at population risk for CRC with FOBT, either guaiac fecal occult blood test (gFOBT) or FTi, every two years or flexible sigmoidoscopy every 10 years. They do not recommend screening adults aged 75 years and older or using colonoscopy to screen those at population risk unless as further follow-up screening post positive FOBT.

In 2004, the Canadian Association of Gastroenterology (CAG) and the Canadian Digestive Health Foundation (CDHF) collaborated to develop guidelines on colon cancer screening for people at average risk.[75] Given that cancer risk is greatly influenced by age, past medical history, and family history, they recommend asymptomatic individuals over age 50 years with negative family history should undergo average risk screening by method determined

by physician, patient preference, evidence and available resources. They recommend these individuals follow one of the following screening strategies: FOBT every 2 years, flexible sigmoidoscopy every 5 years, flexible sigmoidoscopy combined with FOBT every 5 years, double contract barium enema every 5 years, or colonoscopy every 10 years. These guidelines are in keeping with those recommended by both the American Gastroenterology Association and the British Society of Gastroenterology. The CAG further proposes screening guidelines for individuals at increased CRC risk including those with first-degree relatives with CRC, a family history suggesting definable genetic abnormality such as LS, Familial Adenomatous Polyposis (FAP), or long-standing inflammatory bowel disease.[75] In light of technology development and advances in clinical knowledge, the CAG released updated guidelines in 2010.[76]

The CAG recommends colon cancer screening in Canada should be delivered through a programmatic regional or provincial program rather than through opportunistic screening. Opportunistic screening is performed outside of an organized screening program and often delivered through fee-for-service reimbursement of physicians. In comparison, programmatic population-based screening is an organized screening program that contains an explicit policy with specified age categories, a method and interval for screening, a defined target population, a management team responsible for implementation, a health care team responsible for decisions and care, a quality assurance structure, and a method for identifying cancer occurrence in the population.[76] Programmatic screening revolves around the quality of the screening process, including follow-up with program participants, thus providing greater protection against harms of screening including over-screening, poor quality and complications of screening, and poor follow-up of those who test positive.[76]

Population-based CRC screening programs are currently in various stages of development across Canada.[70] In 2007, the Canadian Partnership Against Cancer created the National Colorectal Cancer Screening Network (NCCSN) in an effort to bring focus and attention to providing organized CRC screening to Canadians.[70] The NCCSN collaborates with numerous groups at both the national and provincial levels to plan and implement CRC screening in Canada. In an evaluation of CRC screening program adherence by province, the NCCSN compiled and analyzed quality indicators of fecal test (FT) screening.[70] FTs measure microscopic fecal blood amounts as markers that indicate if further colorectal investigation is required. In particular, FTi is gaining popularity in screening programs as it is more sensitive than other FTs. Individuals who test positive for markers in FTs are then referred for colonoscopy.

Of provinces involved, 70.9% of cancer detected by FT screening was diagnosed at early stage (1 or 2). The number of CRC cases diagnosed at later stage of disease should decrease as screening programs develop and participant recruitment increases in these programs. As of late 2012, CRC screening program adherence had not yet reached the national target of 60%,[70] and there is evidence to indicate fewer than half of eligible adults have participated in timely CRC screening (2012 Canadian Community Health Survey). The NCCSN further reports that screening program adherence in Canada is higher among women than men, and overall participation is highest in those ages 70-74 years.[70] Positivity rates for FOBT testing is higher in males and increases with age.[70]

In 2012, NL released the Newfoundland and Labrador Colon Cancer Screening Program.[70] Program implementation was phased in, first servicing select areas of the province and then expanding to include all regions. FTi screening kits are available to all residents

through mailout delivery or through their general practitioner. The program targets people aged 50 to 74 years that are at population-risk of developing CRC. Without a wide-spread provincial screening program, individuals residing in rural areas have limited access to screening. The provincial screening strategy campaign in NL is expected to increase screening rates and allow for early detection of CRC, resulting in improved CRC-related survival.

In a 2007 study on CRC screening adherence in population-risk individuals, ON had the most up-to-date screening adherence with 20%. NL had lowest screening adherence at 12.6%.[67]

CRC screening rates in Canada have not reached proposed targets.[67,70] Guideline adherence is impacted by test availability, patient characteristics such as age and gender, and public health promotion. As provincial screening programs become more established, screening rates are expected to increase. Understanding screening rates and adherence allows researchers to examine how screening impacts cancer incidence, mortality, and morbidity.

Colonoscopy surveillance in hereditary CRC families has been found to reduce CRC incidence,[44,77] and reduce mortality and morbidity by up to 70%.[44,77,78] LS families, or those who have strong clinical evidence relating to LS, are recommended to get annual colonoscopies starting between ages 20 and 25.[44] Because of early age of onset and increased risk of associated extra-colonic cancers, early and more frequent screening is encouraged for LS families.[3,44] Reports of lower incidence due to screening have been found particularly in the distal colon and rectum.[66] Screening the proximal colon, where LS cases present more frequently with adenomas, can be challenging.[66] In cases of FCCTX, it has been recommended that screening with colonoscopy start 5-10 years before the age of onset of the earliest family member diagnosed with CRC, with a frequency of no less than every 5 years.[3] Screening in FCCTX

families, as in those with LS, is important as high incidence of adenoma formation can lead to carcinoma.

Hereditary CRC families exhibit a different survival pattern than those with familial or sporadic CRC.[44] Hereditary colorectal adenomas tend to develop into carcinomas more rapidly compared to sporadic polyps. Annual colonoscopy is recommended for patients with hereditary CRC as these polyps experience accelerated carcinogenesis compared to sporadic CRC polyps. A sporadic CRC polyp may take 8-10 years to develop into a carcinoma whereas an LS polyp can progress to carcinoma in 2-3 years.[44] This is evidence in support of more frequent cancer screening in hereditary CRC families.

1.8 Treatment

Surgical procedures most commonly performed in colon cancer treatment include local excision or resection to remove polyps or early stage superficial tumours from the colon lining, or bowel resection to remove part of the colon and associated lymph nodes.

Chemotherapy is also a common treatment for colon cancer, depending on the stage of disease at diagnosis and if the patient is at high risk of recurrence. Adjuvant therapy, or chemotherapy administered after surgery, is given to reduce risk of recurrence and increase likelihood of survival. In some cases, neoadjuvant therapy, or chemotherapy administered before surgery, is given to shrink tumours in an effort to allow surgery and improve outcomes. Radiation therapy is not routinely recommended for colon cancer patients.

The Canadian Cancer Society published Clinical Practice Guidelines (CPGs) for management of colon and rectal cancers in the Canadian Cancer Statistics 2011 manual.[13] These guidelines were developed through Cancer Care Ontario's Program in Evidence-Based Care to

guide patients and practitioners in implementing treatment recommendations. For stage 1 colon cancer, surgical resection alone is the standard treatment recommendation. For stage 2 cases, adjuvant therapy is not routinely recommended unless the patient is at high risk for recurrence in which case they may be candidate for adjuvant therapy similar to that recommended for stage 3 cases. Stage 3 recommendations include adjuvant therapy. Recommendations for stage 4 cases include combination chemotherapy and possibly targeted therapy. Several factors must be considered when determining best course of treatment including stage of disease at diagnosis, location of the tumour, and patient characteristics such as age and risk of recurrence.

In 1991, the National Institutes of Health (NIH) published adjuvant therapy recommendations for patients with colon and rectal cancer.[11] For colon cancer, they recommend no adjuvant therapy for stage 1 cases. Adjuvant therapy is also not recommended for stage 2 cases unless patients present with high-risk features. They recommend adjuvant therapy for stage 3 cases. Regarding stage 4 cases, the NIH indicate that adjuvant therapy, by definition, is not applicable to cases that have metastatic disease, therefore these cases are excluded from their recommendations.

Wirtzfeld et al (2009) investigated concordance with CPGs in NL and ON colon cancer patients.[79] Patients were from the same data set as this thesis. Patients in both provinces diagnosed at stages 1 and 3 were treated according to CPGs. The proportion of low- to high-risk patients at stage 2 who had adjuvant treatment did not differ significantly between NL and ON. While patients diagnosed as high-risk stage 2 were more likely to receive adjuvant treatment than low-risk stage 2 patients, other information was used more frequently in addition to CPGs to determine best course of treatment for the high-risk cases.[79] For example, patient age (<50 years) influenced their course of treatment more so than risk status. This is of interest since

MSI-H tumours are linked to younger age of onset and also may not respond to widely-used 5-FU-based standard chemotherapeutics,[16] and yet a strong trend of chemotherapy use was reported in younger groups, independent of high-risk status. Local factors such as resource allocation and access to treatment may also affect CPG adherence.[79]

Treatment management requires the consideration of many patient and tumour characteristics.[16,80] Boland and Goel (2010) note the potential that no two CRCs are alike, which presents a challenging task in treatment planning.[16] Understanding variations in clinical practices and outcomes will help improve quality of care for patients with CRC.

1.9 Survival

Stage of cancer at the time of diagnosis is well supported as being the strongest predictor of CRC survival.[6,12,17,69,80-85] Cases diagnosed at later stage of disease have worse prognosis than those diagnosed in earlier stages.

There is conflicting evidence in the literature regarding gender as a predictive prognostic factor in CRC survival. Some studies have reported that gender had no significant effect on survival[6,80] while others reported women had improved survival compared to men.[1,12,81,86,87]

A meta-analysis of 13 retrospective cohort studies and 1 randomized controlled trial reported that females had significantly better overall survival than males.[86] After adjusting for all known baseline patient characteristics, sex remained an independent prognostic factor in CRC survival. Evidence suggests that CRC survival differences between genders can be attributable to hormonal, genetic, immunological, or environmental differences.[86,87]

A study on metastatic CRC reported that young women aged 18-44 years survived longer than young men, however older women aged 55+ had significantly worse survival than older

men.[87] Hormones may play a role in CRC prognosis as pre-menopausal women with metastatic CRC have shown improved survival, however as they age through menopause, their prognosis worsens.

Patient age at diagnosis is another predictor of CRC survival noted in the literature.[1,6,80-82,87,88] Younger cases tend to have improved survival compared to older cases as prognosis diminishes with increasing patient age. A study on patients under and over age 65 reported that cases under age 65 showed improved survival compared to those over age 65.[80] CRC was diagnosed in older patients at a later stage, which can subsequently influence treatment regimens and prognosis.

It has been reported that patients diagnosed before age 50 show more advanced stage disease and have more aggressive tumours compared to patients ages 80+, yet younger patients have better survival.[1] Worse prognosis in older patients may not be due to delayed diagnosis, but rather that delayed diagnosis may be more prevalent in younger patients. Consequently, improved survival in younger patients may be due to their receiving more aggressive treatment.[1] Adjuvant therapy is more frequently administered in younger patients compared to their elderly counterparts, and elderly cases also tend to have more comorbidities.[88] Treating physicians may believe the gain in giving aggressive treatment is reduced by presence of other illnesses and the physical toll treatment takes on older patients.

Adjuvant treatment has a positive effect on prognosis and survival.[17,82,84,85] Improvements in survival due to adjuvant therapy have been reported for advanced CRC, including in patients over age 70 years.[82]

Adjuvant chemotherapy is associated with improved survival in younger patients, and survival trends have differed significantly by age where stage has been associated with

significant improvement in survival for patients over age 75.[17] Improvement over time in younger patients diagnosed at stage 3 in particular has been reported, potentially due to improvements in adjuvant treatment for these cases. Cancer survival is likely to improve with advancements in diagnostic and treatment procedures.

Obesity has been associated with reduced survival after CRC diagnosis.[40] A study on obesity as measured by BMI and waist-hip circumference in an Asian population reported that relative to normal BMI (18.5-24.9), cases categorized as having low (<18.5) and obese (≥30) BMI showed worse survival.[89] Overweight BMI (25.0-29.9) and waist-to-hip ratio were not associated with risk of death in CRC. BMI as a predictor of CRC mortality is represented in a U-shaped pattern, where cases in higher and lower BMI categories have worse prognosis.

BMI may affect colon cancer survival in men and women differently.[90] The relationship has been reported to be stronger in men in linear fashion across all BMI categories greater than 25.0, and strongest for BMI ≥32.5. In women, BMI ≥30 has shown significantly increased risk of colon cancer mortality. Men have a greater tendency for central adiposity which may be a contributing factor to the relationship between CRC and poor prognosis. Prolonged elevated insulin levels in high BMI patients may also impact colon cancer survival by acting as a tumour growth promoter. In women, reduced risk may be related to possible protective effects of estrogen.

There is inconclusive evidence on the effects of cigarette smoking on CRC survival. A study conducted on cases of the same cohorts included in this thesis reported pre-diagnosis cigarette smoking to be independently predictive of worse prognosis in CRC patients.[10] Current smoking was significantly associated with increased risk of mortality, and a step-wise gradient was reported of decreasing mortality risk with increasing years of smoking abstinence for former

smokers. Smoking was associated with worse prognosis for colon cancer cases specifically, and decreased survival was reported in males only. Smoking was significantly associated with worse survival in patients diagnosed in early stage disease, suggesting newly diagnosed patients with early stage disease could see survival benefit by immediate smoking cessation. They also report smoking had little impact on survival in MSI-H tumour patients but was associated with high mortality risk in cases diagnosed with MSS/MSI-L tumours.

In contrast, another study reported that smoking is associated with increased CRC mortality in both men and women, with a significantly higher mortality risk from rectal cancer in males and colon cancer in females.[91] Other reports indicate a relationship between smoking and increased mortality after CRC diagnosis with especially pronounced negative effects for MSI-H cases.[92]

Patients that have MSI-H tumours are shown to have better survival rates than those with sporadic CRC.[12,93-95] Patients with MSI tumours have longer survival times (even when diagnosed at stage 4), earlier stage of disease at diagnosis, and are less likely to have metastatic cancer compared to MSS patients.[16] Evidence shows that the detection of MSI in CRC patients is a positive prognostic factor, particularly in young patients. There is limited evidence in the literature to differentiate between how LS MSI and sporadic MSI patients respond to traditional chemotherapy treatment.[16] Since sporadic MSI CRC cases are more common than LS cases in MSI treatment response studies, it is possible that sporadic cases are those represented in these treatment studies.[16]

In a meta-analysis looking at relationship between MSI and CRC prognosis, they found an association between MSI and improved survival of CRC patients.[95] They also found a

significant beneficial effect of 5-FU therapy for MSS tumour but could not make a clear conclusion for MSI tumours. MSI can be used as a prognostic and predictive marker of survival.

Investigating colon cancer specifically, one study found MSI-H status is an independent prognostic factor that improves survival in colon cancer.[12] They found that significantly more females than males had MSI-H tumours. MSI-H was a favorable factor for overall survival. The average age of cases with MSI-H and MSI-L/MSS tumours was the same so improved survival was not due to earlier age at diagnosis. MSI-H had a favorable prognosis over MSI-L and MSS tumours.

Another study compared survival of HNPCC patients to sporadic CRC patients diagnosed under age 65 years and found that relative survival rates for HNPCC cases were higher than in sporadic cases.[94] Stage at diagnosis was similar in both groups so this difference was not due to stage. They suggest the malignant potential of HNPCC tumours does not fully develop as it does with sporadic cases, and that the same genetic defect that is responsible for the tumour development may also reduce the viability of the cancer cells.

Having family history of CRC is related to increased incidence of the disease however it has also been investigated as being a predictor of survival. Evidence suggests that family history itself does not appear to be related to better survival.[1,96] Family history showed no significant effect on survival.[1] Patients with family history of CRC are more likely to have MSI-H tumours, and MSI-H cases tend to have improved survival over MSS or MSI-L cases. Family history has been found not to be a determinant of tumour characteristics.[1]

1.10 Thesis Objectives

The primary objectives of this thesis are:

1. To assess whether there is a difference in overall survival between NL and ON colon cancer patients
2. To determine why cases from NL appear to have a worse prognosis when diagnosed with colon cancer compared to their ON counterparts
3. To evaluate whether there are differences in mortality, predictors at presentation, and treatment associated with colon cancer survival between NL and ON

The secondary objectives of this thesis are:

1. To develop a strong knowledge of the steps and processes involved in conducting expert ethical research that will allow for a future career in clinical epidemiology
2. To contribute to the knowledge already present in colorectal cancer by providing relevant new information for the care and treatment of individuals with colon cancer

Chapter 2 METHODS

2.1 Ethical Considerations

Ethical approval was sought and granted from the Human Investigation Committee of the Faculty of Medicine at Memorial University of Newfoundland. Data were collected and analyzed directly from existing patient files and questionnaires obtained through previous research projects in accordance with the Newfoundland Familial Colorectal Cancer Registry (NFCCR) and Ontario Familial Colorectal Cancer Registry (OFCCR). For this thesis project, only de-identified patient information using unique personal code was entered into an SPSS database. Electronic files were accessible to the investigator through use of a computer password. All patient files were kept in locked cabinets where only the investigator and designated researchers had access.

2.2 Cancer Statistics

Estimated numbers of new incident cases and age-standardized incidence rates (ASIRs) for Canada, ON, and NL were obtained from the Canadian Cancer Statistics (CCS) manual (2011). Actual numbers of new incident cases and ASIRs for Canada were also obtained from the CCS manual (2011). Actual ASIRs in ON and NL were confirmed through reports from provincial agencies from 1996-2006, prepared for the Colorectal Cancer Network (CRCNet), an initiative of the Canadian Partnership Against Cancer. Similarly, estimated numbers of new incident cases and age-standardized mortality rates (ASMRs) for Canada, ON, and NL were obtained from the CCS manual (2011). The actual number of deaths and ASMRs for Canada, ON, and NL were obtained through CCS (2011-2016) and further for ON through provincial

agencies as prepared for CRCNet report. Actual ASMRs for NL were available for 2006, 2009, 2010 and 2012 only.

2.3 Case Ascertainment

The NFCCR was established in 1999 through Canadian Institutes of Health Research funding and modelled based on the OFCCR. The NFCCR is a population-based cohort that has reported incident cases of colorectal carcinomas identified through the NL provincial cancer registry and diagnosed between January 1st, 1999 and December 31st, 2003. Patient follow-up data was collected up to April 30th, 2010. Patients between the ages of 20 and 74 years and diagnosed within the specified inclusion timeframe were invited to participate. Cases completed a family history questionnaire and risk factor questionnaires, and were also asked to provide a blood sample and allow access to their tumour tissue and medical records. For this thesis project, 510 incident cases of colon cancer were recruited from the NFCCR. For deceased subjects, their proxies were invited to participate.

The OFCCR was established in 1997 and is one of several international sites involved in the National Cancer Institute-funded Cooperative Familial Registry for Colorectal Studies. The OFCCR utilized the population-based Ontario Cancer Registry to recruit incident colorectal cancer diagnoses spanning January 1st, 1997 to December 31st, 2000. Follow-up data collection was completed in 2010 for OFCCR cases. Registrants with incident cases of colon cancer were asked to participate and 906 cases were recruited. The OFCCR included 25% of low familial risk cases. No proxies were recruited as the OFCCR did not obtain proxy consent, which systematically excluded patients who died 1-2 years after diagnosis.

An amendment to a previously submitted application was reviewed by the NIH-funded Colon Cancer Family Registry Steering and Advisory Committee, the central hub of guardianship for several familial colorectal cancer registries including the OFCCR. Permission for the data originally was sought by a previous investigator on a separate CRC research project on the adherence to treatment guidelines involving OFCCR participants diagnosed between January 1999 and December 2000 only.[79] The amended application was submitted and subsequently approved which provided data on colon and rectal cancer patients diagnosed over the 4-year span between January 1999 and December 2003.

2.4 Data Collection

Prognostic indicators on each member of the NFCCR and OFCCR were obtained through a series of standardized chart reviews including pathology reports, operative records, oncology progress notes, and general medical records. Pathology reports were used to confirm patient diagnosis information. Once consent was obtained, individuals provided information on family history, epidemiological data, personal demographics and personal history, and also provided a DNA sample and consented to allow researchers access to tissue blocks.

2.5 Survival Analysis Models

Survival analysis is used for outcome variables that are time-to-event in nature. Time-to-event in this case is survival time. Survival time is defined by the period beginning at time of diagnosis and ending at death. Follow-up was censored in all participants who were still alive at the end of the study or lost to follow-up.

Kaplan-Meier Survival Analysis

Kaplan-Meier (KM) survival analysis is a univariate model used to compute the survival curves and compare between 2 groups. The Log-rank test is a chi-squared test that compares observed and expected frequencies over categories. If the Log-rank test is used to compare two groups, it tells us if a statistically significant difference exists between the groups. If Log-rank is used to compare more than two groups, it tells us if a significant difference exists overall but not between which groups the difference lies. With KM, survival of different groups can be plotted and compared using Log-rank tests. However, the KM survival analysis does not allow assessment of multiple explanatory variables.

Cox Proportional Hazards Multivariate Model

The Cox Proportional Hazards (CoxPH) Model allows for multivariate modelling survival analysis and allows for multiple explanatory (independent) variables. CoxPH computes an estimate of the magnitude of effect for independent variables, which is the Hazard Ratio (HR). The hazard function is the rate (not a probability) at which an event might occur given that the subject has survived up until that time. The precision of this HR estimate is indicated using a Confidence Interval (CI) of 95%. Interaction terms between independent variables can also be examined in a CoxPH model. To meet one of the main model assumptions for CoxPH, the HR must be constant over time and the hazard for one group must be proportional to the hazard for another group. To evaluate the Proportional Hazards Model assumption, the log minus log (LML) graph function is used. If the relationship between the 2 groups is proportional, the 2 lines will be parallel. The Omnibus Tests of Model Coefficients (-2 Log Likelihood or -2LL test) will verify if the chosen model fits the data better than no model. For

this data, the -2LL value in the Omnibus Tests of Model Coefficients is statistically significant with p-value less than 0.05 (p=.000) indicating the CoxPH model fits the data.

2.6 Statistical Analysis

All data were analyzed using SPSS v.18 (IBM SPSS Inc. (2010)). Prognostic variables included in statistical models were gender, age at time of diagnosis, MSI status, adjuvant treatment administered, BMI, smoking status, and stage of disease at time of diagnosis. Age was categorized as ≤49 years, 50-59 years, 60-69 years, or ≥70 years. MSI status was defined as MSI-H (≥30% of markers tested unstable), MSI-low (MSI-L, >0% and <30% of markers unstable) or microsatellite stable (MSS). Treatment included chemotherapy administered pre-surgery (neoadjuvant) and post-surgery (adjuvant) and excluded palliative chemotherapy. BMI was categorized as Low (BMI <18.5), Normal (BMI 18.5-24.9), Overweight (BMI 25.0-29.9) or Obese (BMI ≥30). Smoking status was defined as never, former, or current smokers. Never smokers were qualified as never having smoked 1 cigarette a day for 3 months or more. Former smokers were those who were still smoking 1 or more cigarettes a day 2 years ago. Current smokers were those who currently smoked 1 or more cigarettes a day. Stage of disease was defined according to the TNM system whereby "T" examines the extent of the tumor's spread through the various layers that comprise the wall of the colon and rectum, "N" indicates if or to what extent the cancer has spread to the lymph nodes, and "M" describes whether the cancer has metastasized to distant organs.

Counts and percentages were calculated to describe all categorical variables. The distribution differences between groups were examined by chi-squared (X^2) tests. The Fisher

Exact test was used for any expected cells <5. A statistically significant relationship existed at a p-value of less than 0.05.

KM univariate survival analysis was performed to examine the survival curves between the provinces, stratified by stage. The clinical endpoint of time to death or last follow-up, reported in months, was used. Cases were censored at time of last follow-up, or at any other event, if the outcome was not reached. Because of ascertainment bias in ON, cases from both NL and ON diagnosed at stage 4 were excluded from the analysis.

A Cox PH model for multivariate survival analysis was used to examine the association between the independent variables and survival after controlling for prognostic factors.

NL cases included in this study were compared to 289 NL patients that were in the NFCCR but declined study entry to determine if a difference existed between these 2 groups. Variables available for inclusion for non-enrolled cases were gender, age at diagnosis, and vital status.

Chapter 3 RESULTS

3.1 Predicted Age-Standardized Incidence Rates

Figures 1A and 1B show the CRC predicted ASIRs for males and females in Canada, ON, and NL from 1996-2011. In 2006, Canada predicted ASIRs for CRC were 62 per 100,000 in men and 41 per 100,000 in women. In the same year, ON predicted ASIRs were 61 in men and 42 in women, compared to 82 in men and 51 in women in NL. In men, predicted ASIRs were 34% higher in NL than in ON, and 21% higher in NL women compared to ON.

3.2 Actual Age-Standardized Incidence Rates

Figures 2A and 2B show the CRC actual ASIRs for males and females in Canada, ON, and NL from 1996-2006. In 2006, actual CRC ASIRs in Canada were 60 per 100,000 in men and 40 per 100,000 in women. In 2006 in ON, ASIRs were 60 in men and 40 in women, compared to 93 in men and 61 in women in NL. In men, actual ASIRs were 55% higher in NL than in ON. In women, actual ASIRs were 53% higher in NL than in ON.

3.3 Predicted Number of Deaths

Tables 1A and 1B show the CRC predicted number of deaths for males and females in Canada, ON, and NL for 2006 and 2009, respectively. In 2006, the total population of Canada was estimated at 32,570,505, including 12,661,566 in ON and 510,584 in NL. In 2009, the total population of Canada was estimated at 33,628,571, including 12,997,687 in ON, and 516,729 in NL.

In 2006, Canada predicted CRC-related deaths in 4600 males and 3900 females. In the same year in ON, 1650 and 1450 CRC-related deaths were predicted for males and females,

respectively. In comparison for 2006, 120 deaths in males and 95 deaths in females were predicted in NL.

In 2009, Canada predicted CRC-related deaths in 4900 males and 4200 females. In the same year in ON, 1800 and 1500 CRC-related deaths were predicted for males and females, respectively. In comparison for 2009, 130 deaths in males and 100 deaths in females were predicted in NL.

3.4 Predicted Age-Standardized Mortality Rates

Figures 3A and 3B show the predicted CRC ASMRs for males and females in Canada, ON, and NL for 1996 to 2011.

In 2006, Canada predicted ASMRs for CRC were 27 per 100,000 in men and 17 per 100,000 in women. In the same year in ON, ASMRs of 26 in men and 16 in women were predicted. In comparison, ASMRs of 40 in men and 26 in women were predicted in NL. In 2006, predicted ASMRs were 54% higher in NL men than in ON men, and 63% higher in NL women than ON women.

In 2009, Canada predicted ASMRs for CRC were 26 per 100,000 in men and 16 per 100,000 in women. In the same year in ON, ASMRs of 24 in men and 15 in women were predicted. In comparison, ASMRs of 41 in men and 26 in women were predicted in NL. Predicted ASMRs were 71% higher in NL men compared to those in ON, and 73% higher in NL women compared to ON.

3.5 Actual Number of Deaths

Tables 2A and 2B show the CRC actual number of deaths for males and females in Canada, ON, and NL for 2006 and 2009, respectively. Actual death counts for NL were available for these years only.

In 2006, there were 4400 CRC-related deaths in males and 3800 in females in Canada. In the same year in ON there were 1650 and 1400 CRC-related deaths for males and females. In comparison for 2006, there were 130 deaths in males and 75 deaths in females in NL.

In 2009, there were 4600 and 4000 actual CRC-related deaths in Canada in males and females, respectively. In the same year in ON, there were 1650 and 1450 actual CRC-related deaths in males and females, respectively. In comparison, there were 130 deaths in males and 90 deaths in females in NL.

3.6 Actual Age-Standardized Mortality Rates

Figures 4A and 4B show the CRC actual ASMRs for Canada, ON, and NL for 2006 and 2009. ASMRs for NL were available for these years only.

In 2006, actual ASMRs for CRC in Canada were 24.8 per 100,000 in men and 15.7 per 100,000 in women. In the same year in ON, ASMRs were 24.3 in men and 15.1 in women. In comparison, ASMRs were 42 in NL men and 20 in women. That year, actual ASMRs were 73% higher in NL men than in ON men, and 32% higher in NL women than those in ON.

In 2009, actual ASMRs in Canada were 23.3 per 100,000 in men and 15.1 per 100,000 in women. In the same year in ON, ASMRs were 22 in men and 14 in women. In comparison, ASMRs were 42 in men and 23 in women in NL. In 2009, actual ASMRs were 91% higher in NL men than ON, and 64% higher in NL women than ON women.

3.7 Recruitment

From Newfoundland and Labrador, 799 patients diagnosed with colon cancer met inclusion criteria and were eligible for study enrollment. Of 799 cases, 510 (63.8%) patients consented and enrolled, consisting of 294 (57.6%) males and 216 (42.4%) females. NL patients were eligible for enrollment through proxy consent. Of 510 cases, 166 (32.5%) enrolled through proxy consent and 344 (67.5%) enrolled through direct patient consent (Figure 5).

From Ontario, 906 patients diagnosed with colon cancer enrolled in the study, comprised of 466 (51.4%) males and 440 (48.6%) females (Figure 6). ON patients were not eligible for inclusion through proxy consent therefore all cases from ON were enrolled through direct patient consent.

3.8 Patient Demographics and Clinical Characteristics

Patient demographics and clinical characteristics are seen in Tables 3 and 4. This included cases at stages 1-4, unknown stage, and cases recruited through proxy consent.

Gender

In the NL dataset, there were 294 (57.6%) males and 216 (42.4%) females compared to 466 (51.4%) males and 440 (48.6%) females in ON (p=.024).

Age

Age of patients at time of diagnosis in NL was 60 (11.8%) at age <=49, 128 (25.1%) age 50-59, 212 (41.6%) age 60-69, and 110 (21.6%) age >=70 years. In ON, 111 (12.3%) were

diagnosed at age <=49, 231 (25.5%) at age 50-59, 419 (46.2%) at age 60-69, and 145 (16.0%) at age >=70 years (p=.063).

Adjuvant Treatment

Adjuvant treatment was administered in 198 (39.1%) of cases in NL and 395 (44.6%) of cases in ON (p=.045). Stratified by stage of disease, a statistically significant relationship was evident in stage 3 cases who received adjuvant treatment (p=.017) where significantly fewer NL cases diagnosed at stage 3 received adjuvant treatment compared with ON. When cases recruited through proxy consent were excluded from the analysis there was no significant difference between the groups (Table 5).

MSI Status

MSI tumour counts in NL included 71 (14.4%) MSI-H cases and 423 (85.6%) MSI-L/MSS cases. In ON, 162 (19.8%) cases where diagnosed with MSI-H tumours and 655 (80.2%) cases with MSI-L/MSS. The difference was statistically significant (p=.012).

BMI

Excluding cases with unknown BMI, NL BMI category counts included 6 (1.3%) Low, 140 (31.0%) Normal, 175 (38.7%) Overweight, and 131 (29.0%) Obese. In ON, 15 (1.8%) cases were categorized BMI Low, 288 (34.6%) Normal, 366 (43.9%) Overweight, and 164 (19.7%) Obese (Table 6). The difference was statistically significant (p=.002).

Smoking Status

As seen in Table 3, 143 (30.2%) NL cases never smoked, 233 (49.2%) cases were former smokers, and 98 (20.7%) were current smokers at time of diagnosis. In ON, 344 (39.9%) cases never smoked, 441 (51.2%) formerly smoked, and 77 (8.9%) were current smokers (p=.000). When stratified by sex, statistical significance remained in both males (p=.000) and females (p=.000) between NL and ON (Table 7).

Stage at Diagnosis

When cases between the provinces were compared stratified by stage a statistically significant relationship existed (p=.000). Including all cases diagnosed at stages 1-4 in NL, 73 (14.3%) were diagnosed at stage 1, 171 (33.5%) at stage 2, 142 (27.8%) at stage 3, and 124 (24.3%) at stage 4. In ON, 167 (19.4%) of cases were diagnosed at stage 1 disease, 363 (42.1%) at stage 2, 256 (29.7%) at stage 3, and 77 (8.9%) at stage 4. This difference is likely related to the non-recruitment of patients who had died before the study had started, as proxies were not allowed from ON.

Excluding Stage 4 Cases

Excluding stage 4 cases, in NL the proportion of cases diagnosed at stage 1 was 18.9%, 44.3% at stage 2 and 36.8% at stage 3, and respective proportions in ON were 21.2%, 46.2% and 32.6% (p=.325).

Excluding Proxy Consented Cases

When the 166 NL cases recruited through proxy consent were excluded and provinces were again compared by stages 1-4 (NL=344; ON=863; total=1207) the proportions were comparable between provinces (p=.911). NL cases diagnosed at stage 1 were 66 (19.2%), 140 (40.7%) at stage 2, 103 (29.9%) at stage 3, and 35 (10.2%) at stage 4.

Non-Enrolled Cases

Patient demographic characteristics for NL colon cancer cases eligible for study enrollment but who declined entry are seen in Table 8. Groups were compared based on gender (p=.140), age at diagnosis (p=.206), and vital status (p=.000).

3.9 Univariate Survival Analysis

Analysis of all cases including unknown stage cases in ON resulted in a statistically significant survival distribution between NL and ON cases (p=.010) with median survival of 94.2 months in NL and 85.2 months in ON (Table 9).

Excluding unknown stage cases, a statistically significant survival distribution remained for stages 1-4 overall between NL and ON cases (p=.019) with median survival of 94.2 months in NL and 84.7 months in ON (Table 10).

Stage 1

KM analysis of stage 1 cases included 73 from NL with median survival 133.2 months and 167 from ON with median survival 101.8 months (p=.760) (Figure 7A, Table 11A).

Stage 2

In NL, there were 171 cases diagnosed as stage 2 with median survival 139.8 months and 363 cases from ON with median survival 91.0 months (p=.006) (Figure 7B, Table 11B).

Stage 3

In NL, 142 cases were diagnosed at stage 3 with median survival 105.0 months and 256 cases in ON with median survival of 77.6 months (p=.259) (Figure 7C, Table 11C).

Stage 4

Stage 4 case counts for NL and ON were 124 and 77, respectively, with improved survival in ON (p=.021). Median survival was 15.0 months in NL and 30.6 months in ON (Figure 7D, Table 11D). This is likely influenced by selection bias when proxies were not enrolled in ON.

Excluding Stage 4

Due to disproportionately low counts of stage 4 cases in ON, these cases for both provinces were excluded and stages 1-3 cases only were included in subsequent survival analyses and multivariate modeling. KM survival analysis of cases diagnosed at stages 1 to 3 from both provinces resulted in a statistically significant difference in favour of improved survival for NL cases (p=.028) (Table 12).

Median survival in NL cases increased from 94.2 months including stage 4 cases to 133.2 months excluding stage 4 cases. In ON, median survival was 84.7 months including stage 4 cases compared to 89.7 months excluding stage 4 cases.

3.10 Multivariate Survival Analysis

Cox Proportional Hazards Model multivariate survival analysis compared NL and ON cases diagnosed at stages 1-3 including variables for gender, stage at diagnosis, age at diagnosis, adjuvant treatment, MSI status, BMI, and smoking status (Table 13). There were 328 NL and 659 ON cases included in the analysis.

There was a statistically significant difference in survival between NL and ON cases, with improved survival for NL (p=.023). Additional statistically significant prognostic variables included stage of disease at diagnosis (p=.000 for stage 1 compared to 3, and p=.000 for stage 2 compared to 3), male gender (p=.034), receiving adjuvant treatment (p=.000), and being a never smoker compared to a current smoker (p=.040).

Stage 1

Analysis of stage 1 cases only revealed no significant differences between provinces (Table 14A).

Stage 2

At stage 2, the survival of colon cancer patients in NL was significantly better compared to ON (p=.041) (Table 14B).

Stage 3

Stage 3 analysis comparing provinces indicated no significant difference (Table 14C).

Independent of adverse prognostic factors at diagnosis, colon cancer patients in NL showed significantly improved survival compared to those in ON (p=.023).

Chapter 4 DISCUSSION

There is a marked difference in both predicted and actual age-standardized CRC incidence rates between the Ontario and Newfoundland and Labrador populations according to Canadian Cancer Statistics. In both men and women, NL's ASIRs are higher than those reported in ON, while ON rates are comparable to the Canadian national rate overall. In men, predicted ASIRs were 34% higher in NL than in ON, and 21% higher in NL women compared to ON. In men, actual ASIRs were 55% higher in NL than in ON. In women, actual ASIRs were 53% higher in NL than in ON.

There is also a clear difference in the age-standardized mortality rates between the provinces, where both predicted and actual rates are higher in NL men and women compared to ON and Canada rates overall. In 2009, predicted ASMRs were 71% higher in NL men compared to those in ON, and 73% higher in NL women compared to ON. Actual ASMRs were 91% higher in NL men than ON, and 64% higher in NL women than ON women.

Significant differences are evident in baseline prognostic variables between the NL and ON registries. Compared to ON, NL had a higher proportion of males diagnosed with CRC, a lower proportion of cases treated with adjuvant treatment when including cases recruited through proxy consent, a lower proportion of cases categorized as MSI-H, a greater proportion of cases categorized as obese (BMI $\geq$30), a greater proportion of cases characterized as current smokers at time of diagnosis, and a greater proportion of cases diagnosed at stage 4 disease when including cases recruited through proxy consent.

In univariate analysis of stages 1-4 overall, NL cases showed improved survival compared to ON. When each stage was analysed separately, NL cases had significantly

improved survival at stage 2 disease, while ON had significantly improved survival in cases diagnosed as stage 4.

When stage 4 cases were excluded due to the effect of cases recruited through proxy consent, univariate survival indicated significantly improved survival for NL cases overall and, when stratified by stage, for those diagnosed at stage 2 disease compared to ON.

In multivariate survival analyses, NL's improved survival rate remained at stage 2. There was no significant difference in survival between the provinces in cases diagnosed at stages 1 or 3 of disease. Prognostic variables significantly related to survival were stage at diagnosis, gender, adjuvant treatment, and smoking status.

Overall, NL cases exhibit a higher rate of CRC incidence compared to their ON counterparts, however do not exhibit increased mortality due to CRC.

4.1 Canadian Cancer Statistics

The Canadian Cancer Statistics (CCS) manual is published annually and reports cancer statistics related to predicted and actual incidence and mortality rates in men and women in Canada overall and by province. Provincial cancer registries provide CCS with actual data values and consult with CCS affiliates to determine estimated values. Estimated data are determined through statistical models. Cancer registries reveal patterns that can be observed and compared across populations, providing valuable data for cancer research and screening programs.

Actual ASIRs for NL and ON were available up to 2006, thus incidence rates were compared and interpreted based on values from that year. Predicted and actual ASIRs for ON were comparable, with ON rates similar to Canadian rates overall. Predicted ASIRs for NL in

2006 were highest by province in Canada, and actual ASIRs for NL were even higher than predicted. NL's estimated CRC ASIR in 2006 was 6.1% higher than that of ON. ASIRs were higher in males compared to females in Canada, ON, and NL.

Predicted and actual NL incidence rates are likely higher than reported. In NL, actual mortality rates were not available prior to 2006 as the provincial cancer registry did not receive information on cancer-related deaths diagnosed post-mortem through death certificates until 2005. As a result, CRC actual incidence in NL may be underestimated. Case ascertainment for incidence and ASIRs of cancers in NL has also been less complete in previous years in comparison to those more recent due to lack of reported cases from some regions of the province. Actual incidence values reported prior to 2005 should be interpreted with caution.

Further, because CCS predicted values are generated through statistical models using actual values from previous years, this may provide an explanation for the substantial discrepancy between NL predicted and actual values we see in CCS today. Because actual values may be erroneously low, predicted values also appear low. Further investigation is required to determine why NL incidence rates are so high, and why they are so much higher than other provinces.

According to CCS, for the past decade or more NL has had the highest predicted CRC mortality rate in Canada. In 2006, the ASMR for CRC-related death in ON was in keeping with the overall Canadian predicted value for men and women. For NL in the same year, predicted ASMRs for men and women were substantially higher than the Canadian average. In fact, NL had the highest predicted ASMR of all provinces in Canada.

Actual ASMRs for Canada and ON were available up to and including 2006 and for 2009. ASMRs for Canada overall and ON were comparable and these values reflected predicted

rates. Actual ASMRs for NL were available for 2006 and 2009 only. Actual ASMRs for NL were higher than all other provinces, but were similar to those values predicted. ASMRs were higher in males compared to females in Canada, ON, and NL. This investigation was undertaken to explore possible causes for such a high mortality rate in NL due to CRC.

4.2 Differences in Prognostic Variables

A comparison of the NL and ON data sets revealed several statistically significant differences in the proportion of prognostic factors that can affect CRC survival.

A significant relationship existed between province and sex. There were more males and fewer females diagnosed with colon cancer in the NL data compared with ON cases.

When including cases diagnosed through proxy consent, NL had significant fewer cases treated with adjuvant therapy compared to ON. Stratified by stage, statistically significant differences existed between stage 3 cases only. However, when proxy cases were excluded from the analysis, similar frequencies of use of adjuvant chemotherapy were observed, as expected from concordance with clinical practice guidelines reported previously.[79] Including cases recruited through proxy consent, 89.4% of NFCCR stage 3 cases and 95.6% of OFCCR stage 3 cases received adjuvant treatment. Excluding proxy cases, 98.1% of NFCCR cases diagnosed at stage 3 received adjuvant treatment.

Compared to ON, NL had a lower proportion of cases categorized as MSI-H. In the literature, one would expect approximately 15% of tumours to present as MSI-H, including 3% hereditary and 12% sporadic. In NL, 14.4% of cases had MSI-H tumours versus 19.8% in ON, indicating NL counts were more in keeping with expected values. Overall in both NL and ON there were significantly more MSI-L/MSS tumours than MSI-H.

There was an overall statistically significant relationship between province and BMI. Compared to ON, NL had a greater proportion of cases categorized as obese (BMI ≥30). When stratified by sex, NL males had significantly higher reported BMI than ON males. Stratified by gender, ON males had a higher reported proportion of Overweight BMI (BMI 25.0-29.9) than NL males (55% in ON vs 41.3% in NL), while NL males had a higher reported proportion of Obese BMI (BMI ≥30.0) than ON males (33.7% in NL vs 22.4% in ON).

Increased BMI and waist circumference have been associated with increased risk of CRC incidence in the literature. The association between BMI and CRC appears to be stronger in men than in women and for colon rather than rectal cancer development. There also appears to be a dose-response, where higher BMI means higher CRC risk, particularly in men. Findings in this analysis support existing evidence in the literature of the relationship between obesity and colon cancer incidence in males. Compared to the rest of Canada, obesity is more prevalent in the Atlantic provinces, including NL.

Smoking status was significantly different between NL and ON cases. Compared to ON, NL had a greater proportion of cases characterized as current smokers at time of diagnosis overall, while ON had a greater proportion of never smokers compared to NL. By gender, females in NL had a significantly higher proportion of current smokers than ON. Females from both provinces had a greater proportion of cases in the never smoker group (45.2% in NL vs 50.2% in ON) compared to males (19.3% in NL vs 29.9% in ON).

The literature indicates a relationship exists between smoking and CRC development, while the association appears to be stronger in rectal cancer development than colon cancer. According to Statistics Canada, by province NL has the highest smoking prevalence in Canada. In 2009, NL was above the Canadian average by approximately 3% for smoking prevalence.

Prevalence was reportedly higher in males than females. ON was below the Canadian average by approximately 1.5%.

When including cases recruited through proxy consent, NL had a significantly greater proportion of cases diagnosed at stage 4 disease compared to ON. 24.3% of NL cases were diagnosed stage 4 while 8.9% of cases were diagnosed stage 4 in ON. A literature search was conducted to determine if stage proportions in the NFCCR and OFCCR cohorts were comparable to other population-based studies in Canada. In particular, stage at diagnosis was compared to an incident cohort in ON, diagnosed in 1997-8 in whom ascertainment bias was not limited by age, family history, or early death. NL proportions of advanced disease cases in stages 3 and 4 were similar to the literature. The proportion of cases diagnosed at stage 4 in OFCCR data was uncharacteristically low compared to NFCCR cases and the literature. The proportion of stage 4 disease was 8.9% in ON, yet one would expect up to 20%.[97]

The low proportion of ON stage 4 cases was attributed to lack of proxy consent recruitment in the ON registry. Rather than exclude cases recruited through proxy consent, which would cause both NL and ON stage 4 counts to be disproportionately low, all stage 4 cases were excluded from the survival analysis. With stage 4 cases excluded, there was no significant difference in stage proportion between the provinces.

When comparing NL cases who were enrolled in the database to those who were eligible for enrollment but declined, no significant difference was found in gender or age at diagnosis. There was a statistically significant difference between groups in vital status where a greater proportion of cases enrolled in the study were deceased compared to non-enrolled cases.

4.3 Univariate Survival Analysis

KM univariate survival analysis comparing NL and ON cases diagnosed at stages 1-4 revealed a statistically significant difference in survival between the provinces. Stratified by stage, a statistically significant difference in survival in stage 2 cases was evident, with improved survival for NL cases.

A significant difference also existed for stage 4 cases as expected, with improved survival for ON cases. The NFCCR recruited cases through both direct patient consent and proxy consent. By nature, cases recruited through proxy consent are more likely to be deceased upon entry into the data base. They are also then more likely to have been diagnosed at advanced stage of disease. The OFCCR recruited cases through direct patient consent only, meaning all ON cases had to be alive to be included in the cohort. Lack of ON's proxy consent recruitment created a survivor bias in the data, particularly obvious when interpreting stage proportions between the provinces.

Stage 4 cases would likely have been more numerous in ON with proxy consent recruitment however without proxies the proportion of stage 4 cases appeared low. Due to disproportionately low counts of stage 4 cases in ON, stage 4 cases for both provinces were excluded from subsequent survival analyses and multivariate modeling. In an effort to limit survivor bias present in the data, only cases diagnosed at stages 1-3 were henceforth included.

In univariate survival analyses stratified by stages 1-3 overall, a statistically significant difference remained between the provinces. Excluding stage 4 cases, median survival in NL increased from 94.2 to 133.2 months, a difference of 39.0 months. In ON, median survival increased by only 4.1 months, from 85.2 to 89.2.

Stratified by stage, NL cases continued to show significantly improved survival compared to ON at stage 2 disease. Contributing factors to this difference in survival were unclear and further investigation was required in multivariate modelling to determine why NL cases showed improved survival over ON cases diagnosed at stage 2 disease.

4.4 Multivariate Survival Analysis

Multivariate analysis compared NL and ON cases while controlling for gender, age at diagnosis, stage of disease at diagnosis, MSI status, if adjuvant treatment was administered, BMI, and smoking status.

The CoxPH model assumption was evaluated through a graphical technique involving the log-log (LML) survival curve to ensure a proportional relationship existed between the variables. A proportional relationship was found between the LML curves and therefore amongst the variables, indicating that CoxPH model assumptions were met.

Factors found to independently predict survival between the cohorts included stage at diagnosis, gender, adjuvant treatment, and smoking status.

Independent of adverse prognostic variables at time of diagnosis a statistically significant difference existed in survival between NL and ON cases. Compared to ON, NL showed improved survival overall. Stratified by stage, no difference in survival existed at stages 1 or 3. However when diagnosed at stage 2, improved survival was evident in favour of NL cases. It is not clear why such a difference exists at this particular stage and further investigation is required to understand this relationship.

As expected, later stage of disease at diagnosis was an independent predictor of colon cancer survival. Survival decreased with increasing stage at diagnosis. Compared to stage 1,

cases diagnosed at stage 2 and 3 had significantly worse prognosis. Stage is well known to be the strongest predictor of CRC survival in the literature.

Male gender was found to be a significant predictive factor in multivariate survival analysis. Compared to females, males had worse prognosis. Although there is some inconsistency with evidence in the literature, this finding is consistent with some support in previous research.

Adjuvant treatment was also a significant factor in predicting survival. Compared to those who did not receive adjuvant treatment, cases who did had improved prognosis. Cases diagnosed at later stage of disease are more likely to receive adjuvant therapy in concordance with treatment guidelines. Similar frequencies of use of adjuvant chemotherapy were observed between the provinces. In particular, amongst stage 3 cases, 89.4% of NFCCR and 95.6% of OFCCR cases received adjuvant treatment.

Smoking status was found to be a significant predictor of CRC survival as well. Cases that were categorized as current smokers showed decreased survival compared to never smokers. It does not appear that smoking status alone would be responsible for such a high mortality rate in NL due to CRC compared to ON and the rest of Canada, however it appears it may be a contributing factor.

In multivariate Cox PH survival analysis, independent of adverse prognostic factors at time of diagnosis, the NL cohort showed improved colon cancer survival compared to their ON counterparts. Based on two population-based cancer registries, we can conclude that mortality due to colon cancer has not been found to be worse in NL compared to ON cases.

4.5 Limitations

There were several limitations to note in this analysis. One limitation was survivor bias present in the ON data set. Because proxy consent was not sought from ON cases, those that were deceased in ON could not be included in the study. Cases diagnosed at a later stage of disease had lower likelihood of survival, meaning many ON patients who died within 1-2 years of diagnosis were systematically excluded from the data set. Where NL did obtain proxy consent and therefore could include information on deceased cases, it appeared that NL had worse survival than ON cases. Stage 4 disease cases were excluded from both NL and ON data sets to reduce the effect of evident survivor bias. In OFCCR another bias occurred as proxies were not used to enrol incident cases who died within the first year or so after diagnosis. Consequently, we controlled for this bias by excluding patients with stage 4 disease, patients who were most likely not to have been enrolled in the OFCCR. Exclusion of proxies from the NL sample, which makes the research design more comparable to that for ON, favours improved survival in NL.

Ascertainment bias was also present, as only incident colon cancer patients under 75 years of age were enrolled in both provinces. Even though approximately 15-20% of colon cancer cases appear in this age demographic, the analysis excluded cases over 75 years of age in NL or ON. This was because both NFCCR and OFCCR data bases did not include incident colon cancer cases diagnosed after age 75. Including cases in people over 75 would have furthered the impact of the survivor bias present since ON did not obtain proxy consent. Presumably many cases over age 75 would be deceased, and if NL obtained proxy consent from these cases but ON did not, the results would be skewed if proxies were included.

The OFCCR cohort is limited to inclusion of 25% familial colon cancer cases. The NL population is a founder population which includes a greater proportion of familial cancer than

other provinces. It is therefore likely that the NFCCR cohort is comprised of more than 25% familial cancer. Family history of CRC is a factor associated with increased incidence. Familial colon cancer cases have not been found to have improved survival in the literature, however cases that have family history of CRC may be more likely to have MSI-H tumours which have been found to predict improved survival.

This study was retrospective in design in which patients are enrolled after treatment for CRC. Data on treatment and outcome has been collected retrospectively. The limitations of this design include potential for lower enrollment rate compared to a design that would enrol patients prospectively, inaccuracy associated with chart reviews, and inaccuracy because all relevant data may not be recorded in medical charts.

CONCLUSION

The results of this study suggest that NL's high mortality rate due to CRC cannot be attributed to later stage of disease at diagnosis, higher rate of adverse prognostic factors at baseline, or to diminished survival following diagnosis. Survival of NL patients following colon cancer diagnosis was not worse than that of ON, and may even be better as shown by comparing long term survival rates. This was not the result of differential use of adjuvant therapy, different rates of adverse prognostic factors at baseline, or of ascertainment bias, as failure to enroll patients who died soon after diagnosis would favour ON.

I conclude that NL's high rate of CRC mortality, the highest in Canada, is as a result of also having the highest incidence rate of CRC in Canada. Consequently, strategies to reduce incidence by population-wide primary prevention and screening initiatives are necessary. CRC surveillance in family members of high-risk families may also decrease the incidence of CRC in NL. General practitioners are the first point of patient contact and are the gateway to preventative medicine and implementing appropriate cancer screening for their patients. Contact with a general practitioner has been associated with increased CRC screening rates.[67] A detailed family history is required so that the Amsterdam and Bethesda criteria can be applied where necessary. Due to the high risk of HNPCC families and since the genetic pathway of FCCTX remains relatively unknown, priority should be placed on taking a thorough family history, especially in NL where hereditary CRC rates are high.

Early detection and treatment of CRC are crucial. CRC screening will decrease incidence through polyp removal before stage of malignancy. The mortality rate should also decrease as screening can diagnose carcinoma in early stage therefore increasing likelihood of survival. There is currently provincial-wide FTi screening accessible to all regions of NL. Screening

promotion through public health outlets and general practitioner contact will remain important in encouraging eligible Newfoundlanders and Labradorians to be screened for CRC.

Many factors can impact CRC survival. Some have clear indication such as stage at diagnosis, while others present with mixed evidence. Regardless it is clear that screening is important to decrease incidence or to diagnose CRC cases in early stage. Cancer registries are valuable tools for analysis of true epidemiological trends. Interpreting survival patterns is important for formulating provincial and national cancer management strategies.

The implications of these results are that a focus on prevention of CRC is needed. Modifying lifestyle factors that are related to decreased survival will likely improve prognosis. Promoting a healthy lifestyle and developing health programs to reduce likelihood of CRC incidence would be an asset in decreasing the CRC incidence. Efforts to limit the high rate of obesity through promoting healthy dietary habits and physical activity, as well as promoting smoking cessation and limiting excessive alcohol consumption are likely to decrease NL's high CRC incidence rates and to play a positive role in decreasing the CRC burden.

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Table 1A: CRC Predicted Number of Deaths for Males and Females in Canada, ON, and NL in 2006

	Canada	ON	NL
Males	4600	1650	120
Females	3900	1450	95

Table 1B: CRC Predicted Number of Deaths for Males and Females in Canada, ON, and NL in 2009

	Canada	ON	NL
Males	4900	1800	130
Females	4200	1500	100

Table 2A: CRC Actual Number of Deaths for Males and Females in Canada, ON, and NL in 2006

	Canada	ON	NL
Males	4400	1650	130
Females	3800	1400	75

Table 2B: CRC Actual Number of Deaths for Males and Females in Canada, ON, and NL in 2009

	Canada	ON	NL
Males	4600	1650	130
Females	4000	1450	90

Table 3: Prognostic factors at diagnosis in incident colon cancer patients in NL and ON

		Colon Cancer Cases		
		NL N=510(%)	**NL* N= 344(%)**	**ON N=906(%)**
Mean Age		61.4	61.1	60.9
Sex	**Male**	294 (57.6)	197 (57.3)	466 (51.4)
	Female	216 (42.4)	147 (42.7)	440 (48.6)
Stage	**1**	73 (14.3)	66 (19.2)	167 (18.4)
	2	171 (33.5)	140 (40.7)	363 (40.1)
	3	142 (27.9)	103 (29.9)	256 (28.3)
	4	124 (24.3)	35 (10.2)	77 (8.5)
	Unknown	0	0	43 (4.7)
Adjuvant Treatment Received	**Yes**	198 (38.8)	158 (45.9)	395 (43.6)
	No	309 (60.6)	184 (53.5)	491 (54.2)
	Unknown	3 (0.6)	2 (0.6)	20 (2.2)
MSI	**High**	71 (13.9)	59 (17.2)	162 (17.9)
	Low/Stable	423 (83.0)	273 (79.4)	655 (72.3)
	Unknown	16 (3.1)	12 (3.5)	89 (9.8)
BMI	**≤18.5 (Underweight)**	6 (1.2)	2 (0.6)	15 (1.6)
	18.5-24.9 (Normal)	140 (27.4)	91 (26.5)	288 (31.8)
	25.0-29.9 (Overweight)	175 (34.3)	119 (34.6)	366 (40.4)
	≥30.0 (Obese)	131 (25.7)	91 (26.5)	164 (18.1)
	Unknown	58 (11.4)	41 (11.9)	73 (8.1)
Smoking Status	**Never**	143 (28.0)	92 (26.7)	344 (38.0)
	Former	233 (45.7)	162 (47.1)	441 (48.7)
	Current	98 (19.2)	60 (17.4)	77 (8.5)
	Unknown	36 (7.1)	30 (8.7)	44 (4.8)

*Excluding cases recruited through proxy consent

Table 4: Patient demographics compared by province (chi-squared)

	X^2	p	df
Sex	5.1	.024	1
Age	7.3	.063	3
Stage	62.9	.000	3
Adjuvant treatment	4.0	.045	1
MSI	6.3	.012	1
BMI	14.5	.002	3
Smoking	40.4	.000	2

Table 5: Adjuvant treatment administered in NL and ON by stage

	Adjuvant treatment administered	NL, including proxies N=507 (%)	NL, excluding proxies N=342 (%)	ON N=886 (%)
Stage 1	Yes	2 (2.7)	2 (3.0)	5 (3.0)
	No	71 (97.3)	64 (97.0)	161 (97.0)
	Total	**73 (100)**	**66 (100)**	**166 (100)**
Stage 2	Yes	54 (32.0)	46 (33.3)	132 (36.6)
	No	115 (68.0)	92 (66.7)	229 (63.4)
	Total	**169 (100)**	**138 (100)**	**361 (100)**
Stage 3	Yes	126 (89.4)	101 (98.1)	240 (95.6)
	No	15 (10.6)	2 (1.9)	11 (4.4)
	Total	**141 (100)**	**103 (100)**	**251 (100)**
Stage 4	Yes	16 (12.9)	9 (25.7)	14 (21.5)
	No	108 (87.1)	26 (74.3)	51 (78.5)
	Total	**124 (100)**	**35 (100)**	**65 (100)**

Table 6: BMI in NL and ON by Gender

	BMI	NL N= 452 (%)	ON N= 833 (%)
Male	Low <18.5	2 (0.8)	2 (0.5)
	Normal 18.5-24.9	64 (24.2)	94 (22.2)
	Overweight 25.0-29.9	109 (41.3)	233 (55.0)
	Obese ≥30	89 (33.7)	95 (22.4)
	Total	**264 (100)**	**424 (100)**
Female	Low <18.5	4 (2.1)	13 (3.2)
	Normal 18.5-24.9	76 (40.4)	194 (47.4)
	Overweight 25.0-29.9	66 (35.1)	133 (32.5)
	Obese ≥30	42 (22.3)	69 (16.9)
	Total	**188 (100)**	**409 (100)**

Table 7: Smoking Status in NL and ON by Gender

	Smoking Status	NL N= 474 (%)	ON N= 862 (%)
Male	Never	53 (19.3)	131 (29.9)
	Former	170 (61.8)	266 (60.7)
	Current	52 (18.9)	41 (9.4)
	Total	**275 (100)**	**438 (100)**
Female	Never	90 (45.2)	213 (50.2)
	Former	63 (31.7)	175 (41.3)
	Current	46 (23.1)	36 (8.5)
	Total	**199 (100)**	**424 (100)**

Table 8: Non-Enrolled NL Colon Cancer Cases

Non-Enrolled NL Colon Cancer Cases N=289(%)		
Sex	**Male**	151 (52.2)
	Female	138 (47.7)
Age at diagnosis	**<=49**	25 (8.7)
	50-59	67 (23.2)
	60-69	118 (40.8)
	>=70	79 (27.3)
Vital Status	**Dead**	92 (31.8)
	Alive	197 (68.2)

Table 9: Univariate Analysis Overall Stages 1-4 and Unknown Stage Cases Comparing NL and ON

Province	Total N	N of Events	Censored	
			N	Percent
NL	510	250	260	51.0%
ON	906	322	584	64.5%
Overall	1416	572	844	59.6%

Province	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
NL	81.9	2.6	76.8	87.0	94.2	7.6	79.2	109.1
ON	84.2	1.8	80.6	87.7	85.2	2.7	80.0	90.4
Overall	85.9	1.6	82.9	89.0	93.8	2.3	89.3	98.4

	Chi-Square	df	Sig.
Log-Rank (Mantel-Cox)	6.677	1	.010

Table 10: Univariate Analysis Overall Stages 1-4 Excluding Unknown Stage Cases Comparing NL and ON

Province	Total N	N of Events	Censored	
			N	Percent
NL	510	250	260	51.0%
ON	863	314	549	63.6%
Overall	1373	564	809	58.9%

Province	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
NL	81.9	2.6	76.8	87.0	94.2	7.6	79.2	109.1
ON	83.6	1.8	80.0	87.2	84.7	2.7	79.4	90.0
Overall	85.5	1.6	82.4	88.6	93.8	2.7	88.6	99.0

	Chi-Square	df	Sig.
Log-Rank (Mantel-Cox)	5.484	1	.019

Table 11A: Univariate Survival Analysis for Stage 1 Colon Cancer in NL & ON

Province	Total N	N of Events (%)	Censored	
			N	Percent
NL	73	21	52	71.2%
ON	167	30	137	82.0%
Overall	240	51	189	78.8%

Province	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
NL	107.3	5.5	96.4	118.1	133.2	15.7	102.3	164.0
ON	103.9	4.2	95.6	112.1	101.8	5.89	90.3	113.3
Overall	106.8	3.4	100.2	113.4	107.6	4.4	99.0	116.2

	Chi-Square	df	Sig.
Log-Rank (Mantel-Cox)	.093	1	.760

Table 11B: Univariate Survival Analysis for Stage 2 Colon Cancer in NL & ON

Province	Total N	N of Events	Censored	
			N	Percent
NL	171	51	130	70.2%
ON	363	112	251	69.1%
Overall	534	163	371	69.5%

Province	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
NL	108.2	3.8	100.7	115.7	139.9	---	---	---
ON	88.0	2.6	82.8	93.1	91.0	5.5	80.3	101.8
Overall	97.1	2.4	92.5	101.8	101.9	4.745	92.6	111.2

	Chi-Square	df	Sig.
Log-Rank (Mantel-Cox)	7.704	1	.006

Table 11C: Univariate Survival Analysis for Stage 3 Colon Cancer in NL & ON

Province	Total N	N of Events	Censored	
			N	Percent
NL	142	62	80	56.3%
ON	256	104	152	59.4%
Overall	398	166	232	58.3%

Province	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
NL	87.8	4.7	78.7	97.0	105.0	8.1	89.1	120.9
ON	79.6	3.5	72.8	86.5	77.6	3.9	69.9	85.2
Overall	86.6	3.0	80.8	92.4	88.3	4.5	79.4	97.1

	Chi-Square	df	Sig.
Log-Rank (Mantel-Cox)	1.277	1	.259

Table 11D: Univariate Survival Analysis for Stage 4 Colon Cancer in NL & ON

Province	Total N	N of Events	Censored	
			N	Percent
NL	124	116	8	6.5%
ON	77	68	9	11.7%
Overall	201	184	17	8.5%

Province	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
NL	25.6	2.6	20.6	30.6	15.0	2.1	10.8	19.2
ON	34.8	2.8	29.4	40.2	30.6	2.2	26.3	34.8
Overall	29.4	2.0	25.6	33.2	21.0	1.9	17.3	24.7

	Chi-Square	df	Sig.
Log-Rank (Mantel-Cox)	5.368	1	.021

Table 12: Univariate Analysis Overall Stages 1-3 Comparing NL and ON

Province	Total N	N of Events	Censored	
			N	Percent
NL	386	134	252	65.3%
ON	786	246	540	68.7%
Overall	1172	380	792	67.6%

Province	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
NL	100.0	2.7	94.7	105.3	133.2	21.7	90.5	175.8
ON	88.6	1.9	84.8	92.3	89.7	3.2	83.5	96.0
Overall	95.5	1.7	92.2	98.7	101.2	3.0	95.4	107.1

	Chi-Square	df	Sig.
Log-Rank (Mantel-Cox)	4.841	1	.028

Table 13: Multivariate Survival Analysis Stages 1-3

		N = 1172 (%)
Cases available in analysis	Event	316 (27.0)
	Censored	671 (57.3)
	Total	987 (84.2
Cases dropped	Cases with missing values	185 (15.8)
	Censored before earliest event	0

	Sig.	**Exp(B)**	**95.0% CI for Exp(B)**	
			Lower	**Upper**
NL compared to ON	.023	.753	.589	.961
Males compared to females	.034	1.305	1.021	1.669
Receiving compared to not receiving adjuvant treatment	.000	.486	.337	.699
Never smokers compared to current smokers	.040	.663	.448	.981
Stage 1 compared to Stage 3	.000	.198	.121	.324
Stage 2 compared to Stage 3	.000	.385	.268	.552

Table 14A: Multivariate Survival Analysis Stage 1

		N = 240 (%)
Cases available in analysis	Event	39 (16.3)
	Censored	158 (65.8)
	Total	197 (82.1)
Cases dropped	Cases with missing values	42 (17.5)
	Censored before earliest event	1 (0.4)

	Sig.	**Exp(B)**	**95.0% CI for Exp(B)**	
			Lower	**Upper**
NL compared to ON	.213	1.647	.751	3.609

Table 14B: Multivariate Survival Analysis Stage 2

		N = 534 (%)
Cases available in analysis	Event	133 (24.9)
	Censored	308 (57.7)
	Total	441 (82.6)
Cases dropped	Cases with missing values	92 (17.2)
	Censored before earliest event	1 (0.2)

	Sig.	**Exp(B)**	**95.0% CI for Exp(B)**	
			Lower	**Upper**
NL compared to ON	.041	.669	.455	.983

Table 14C: Multivariate Survival Analysis Stage 3

		N = 398 (%)
Cases available in analysis	Event	144 (36.2)
	Censored	203 (51.0)
	Total	347 (87.2)
Cases dropped	Cases with missing values	51 (12.8)
	Censored before earliest event	0

	Sig.	**Exp(B)**	**95.0% CI for Exp(B)**	
			Lower	**Upper**
NL compared to ON	.079	.711	.486	1.040

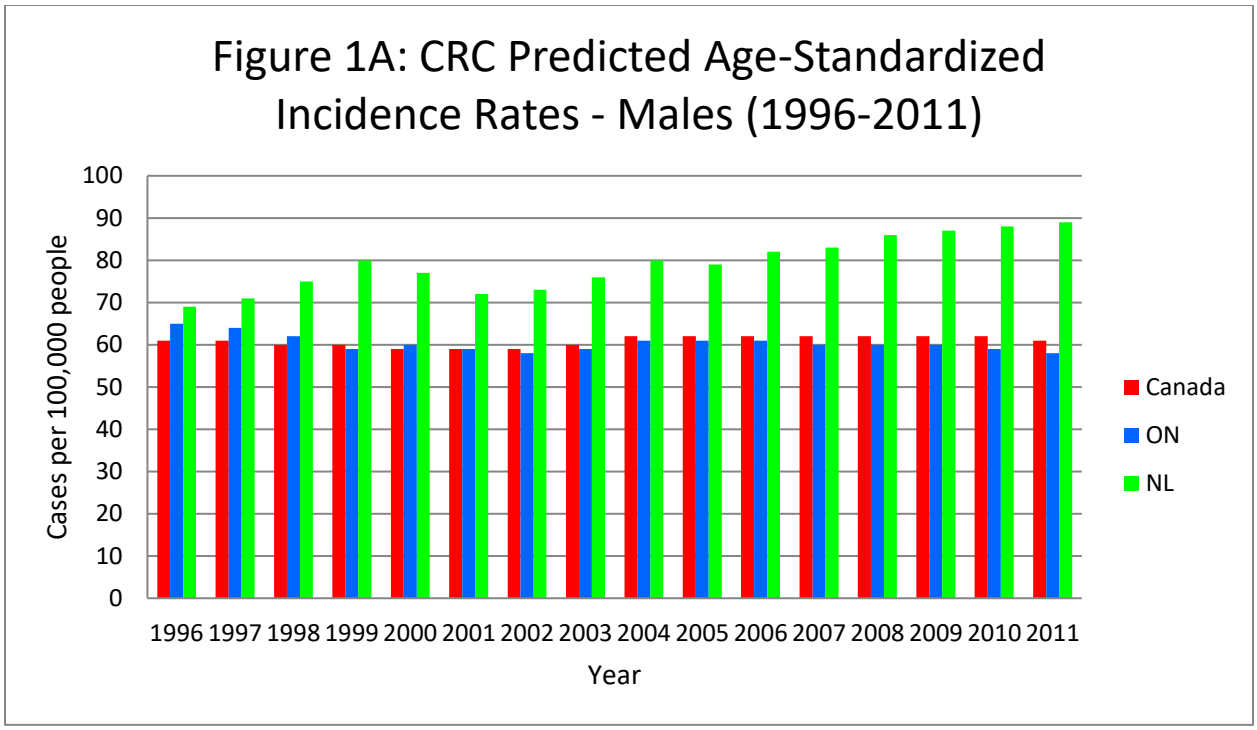


Figure 1A: CRC Predicted Age-Standardized Incidence Rates - Males (1996-2011)
Cases per 100,000 people
100
90
80
70
60
50
40
30
20
10
0
1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011
Year
Canada
ON
NL

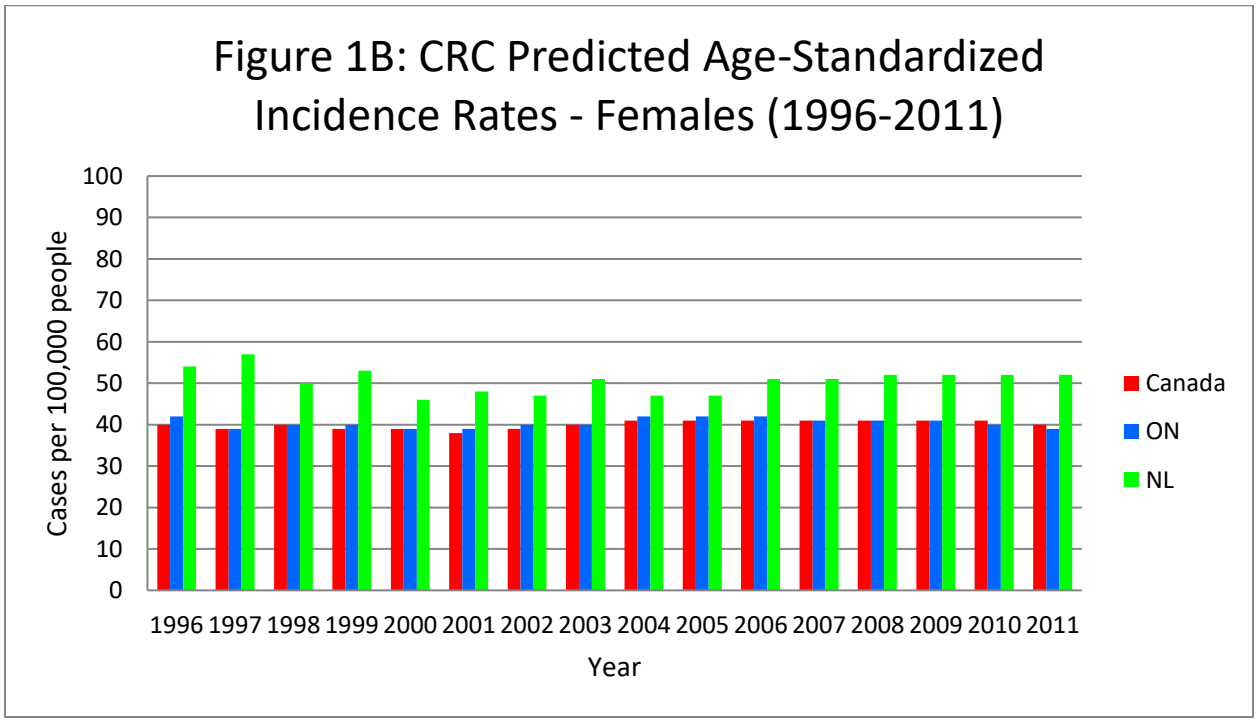


Figure 1B: CRC Predicted Age-Standardized Incidence Rates - Females (1996-2011)
Cases per 100,000 people
100
90
80
70
60
50
40
30
20
10
0
1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011
Year
Canada
ON
NL

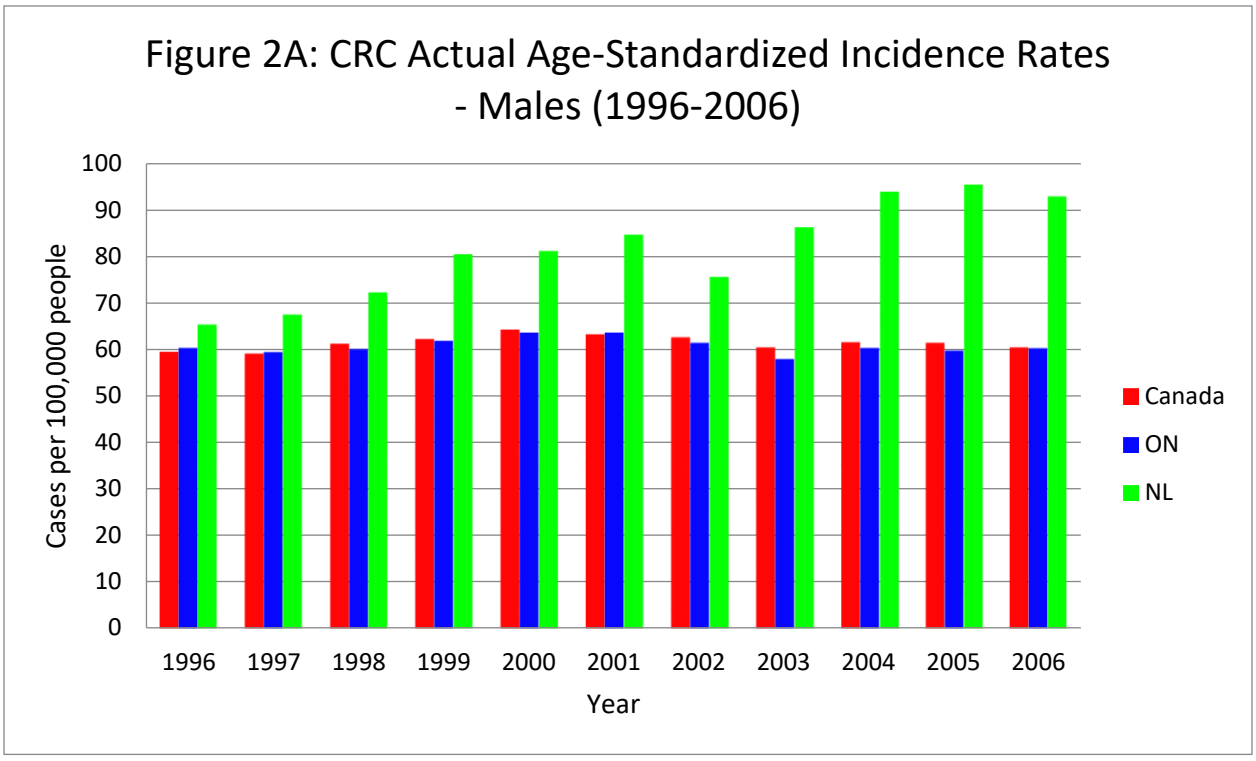


Figure 2A: CRC Actual Age-Standardized Incidence Rates - Males (1996-2006)
Cases per 100,000 people
100
90
80
70
60
50
40
30
20
10
0
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
Year
Canada
ON
NL

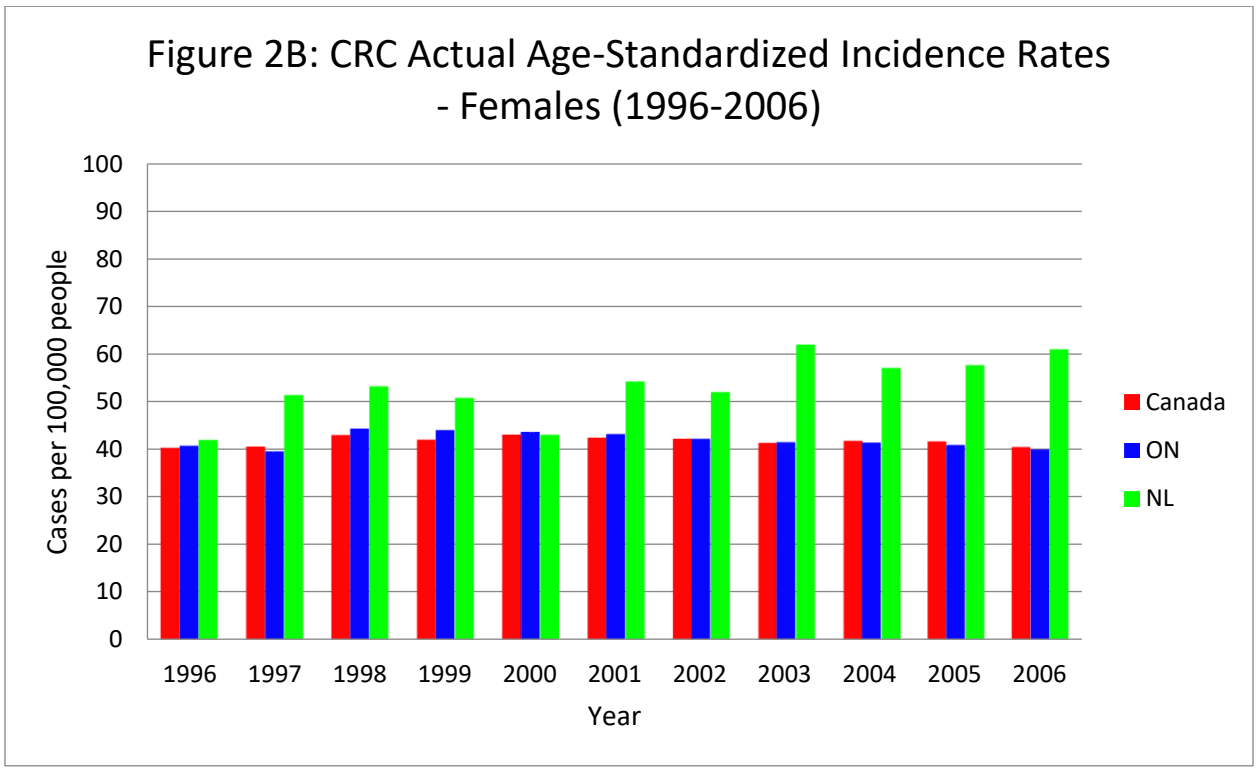


Figure 2B: CRC Actual Age-Standardized Incidence Rates - Females (1996-2006)
Cases per 100,000 people
100
90
80
70
60
50
40
30
20
10
0
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
Year
Canada
ON
NL

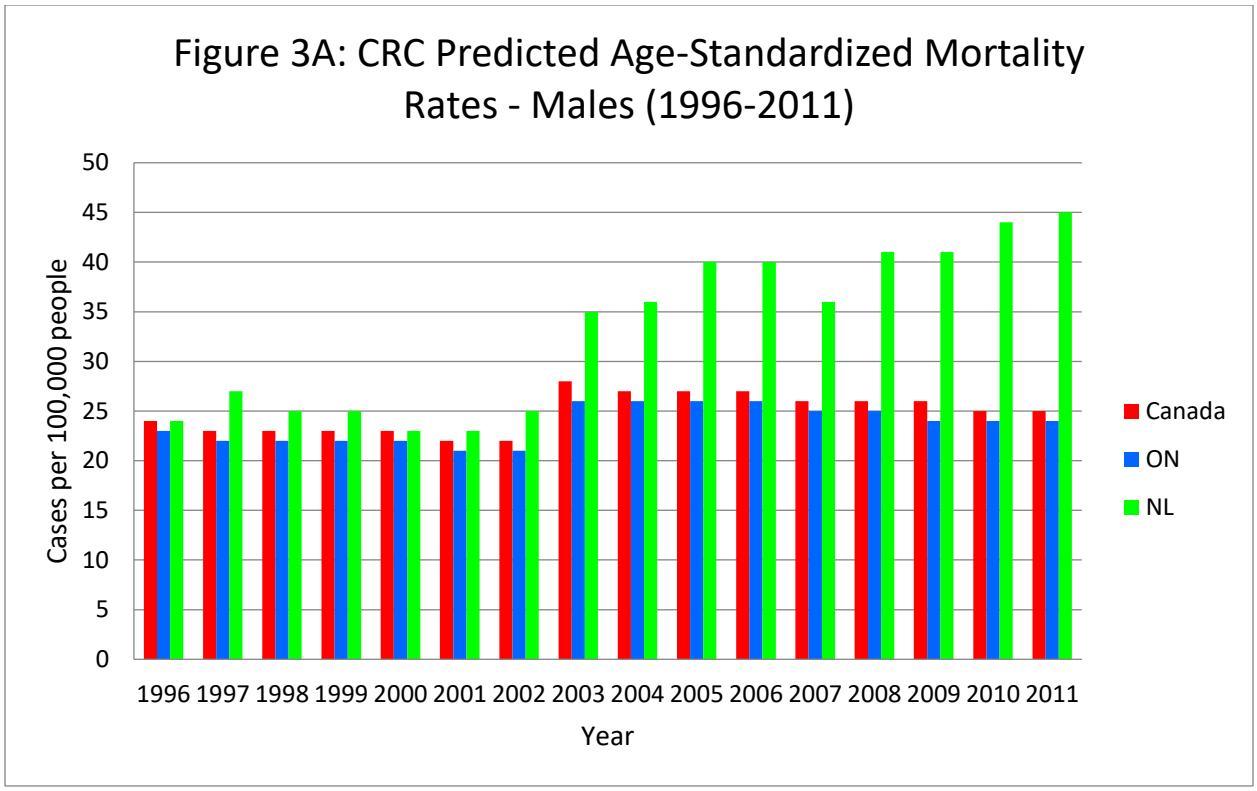


Figure 3A: CRC Predicted Age-Standardized Mortality Rates - Males (1996-2011)
Cases per 100,000 people
50
45
40
35
30
25
20
15
10
5
0
1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011
Year
Canada
ON
NL

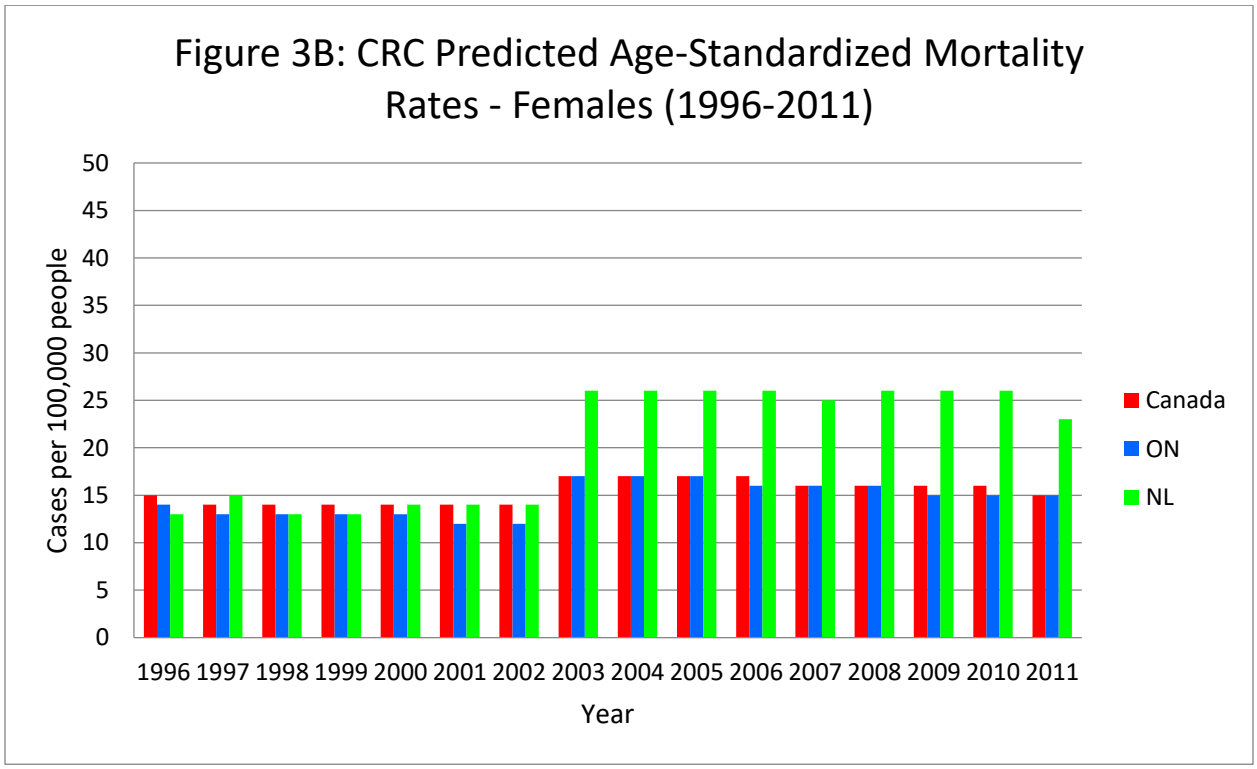


Figure 3B: CRC Predicted Age-Standardized Mortality Rates - Females (1996-2011)
Cases per 100,000 people
50
45
40
35
30
25
20
15
10
5
0
1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011
Year
Canada
ON
NL

Figure 4A: CRC Actual Age-Standardized Mortality Rates - Males (2006, 2009)

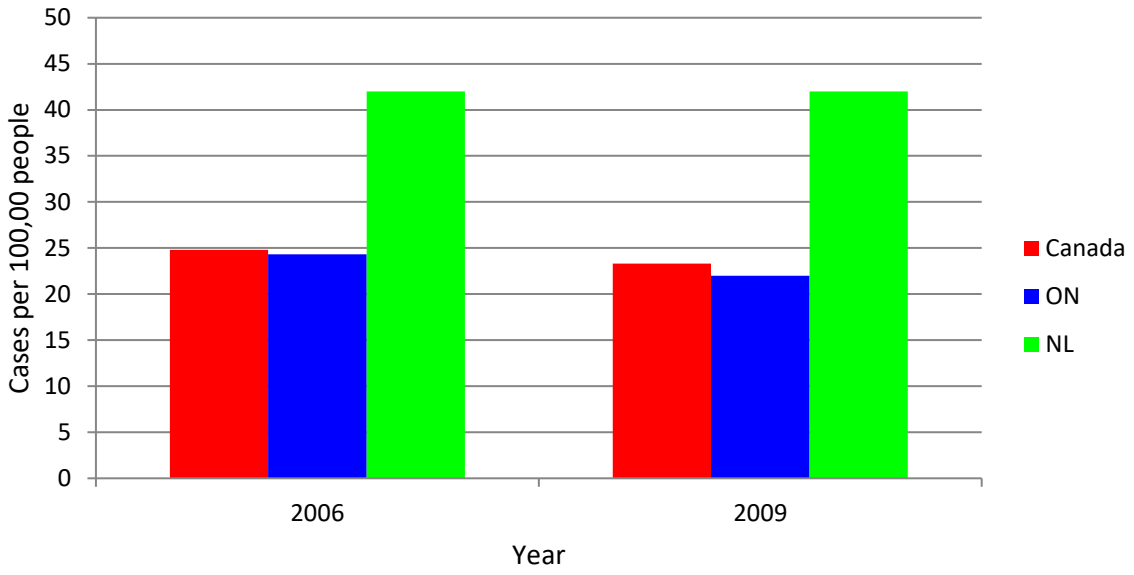


Figure 4B: CRC Actual Age-Standardized Mortality Rates - Females (2006, 2009)

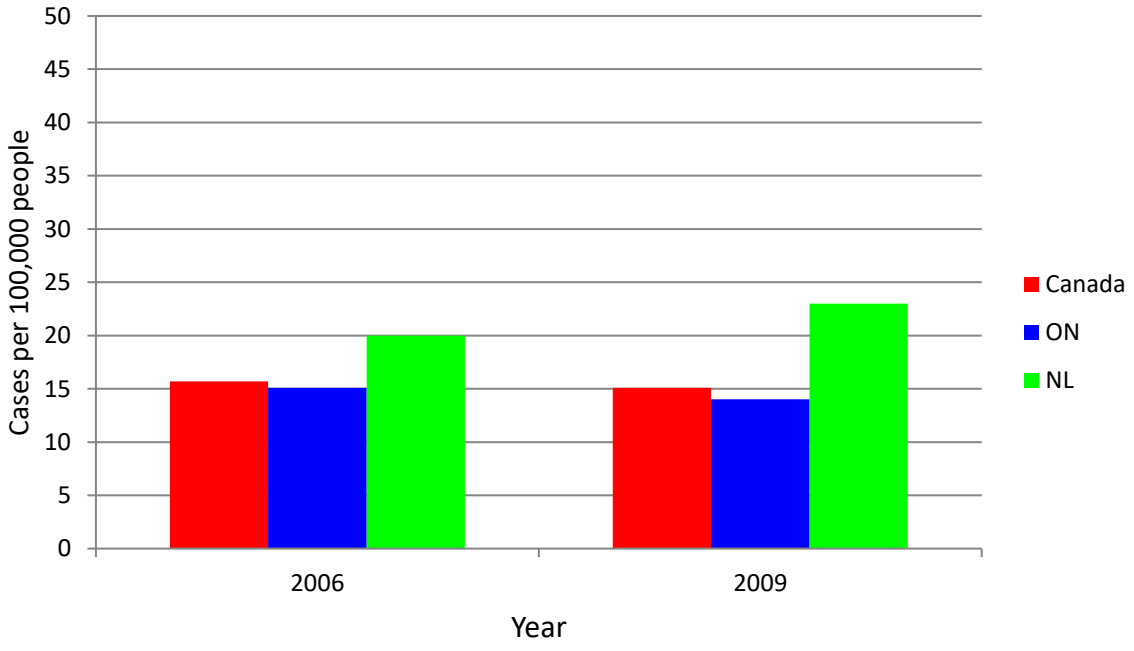
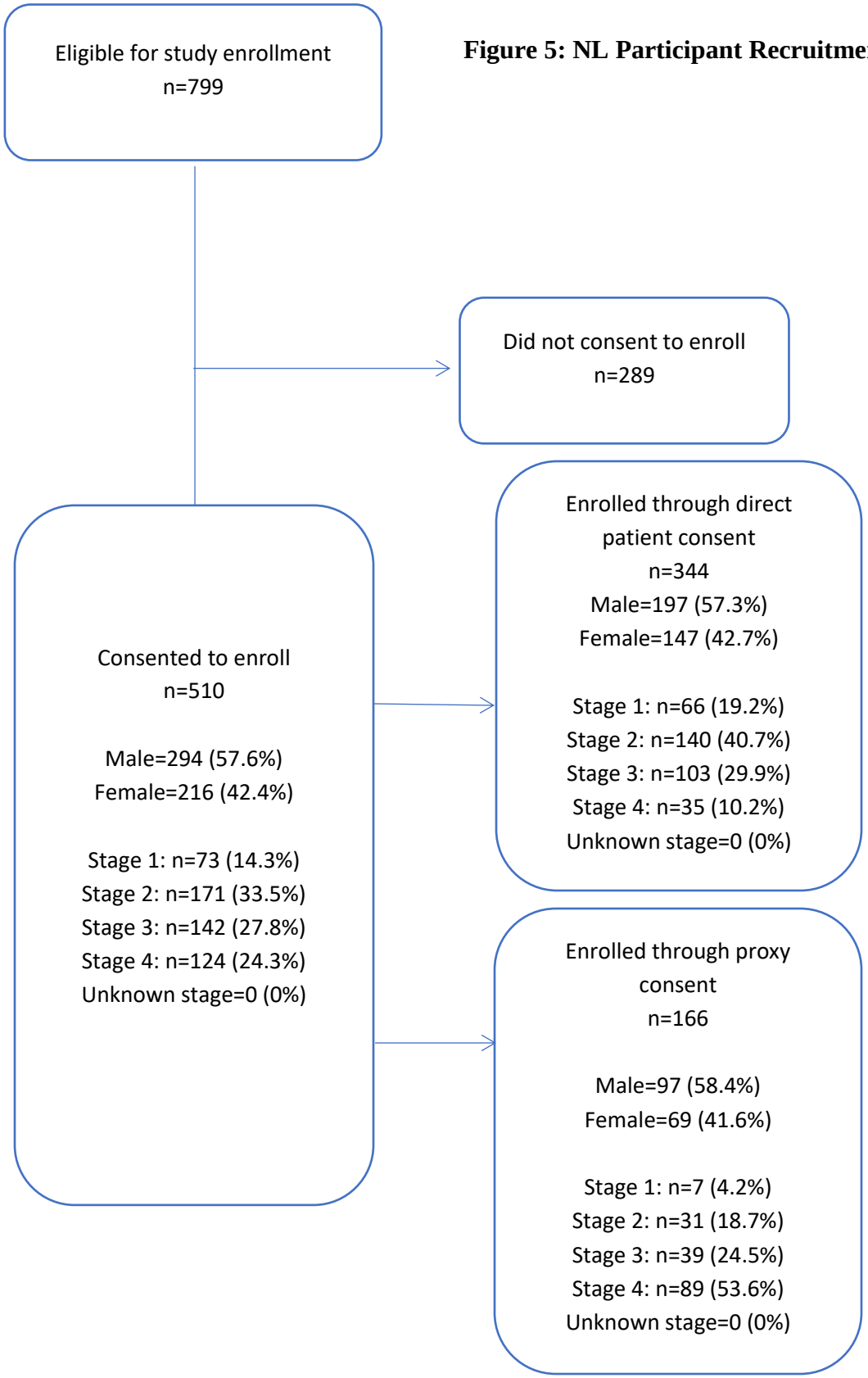


Figure 5: NL Participant Recruitment



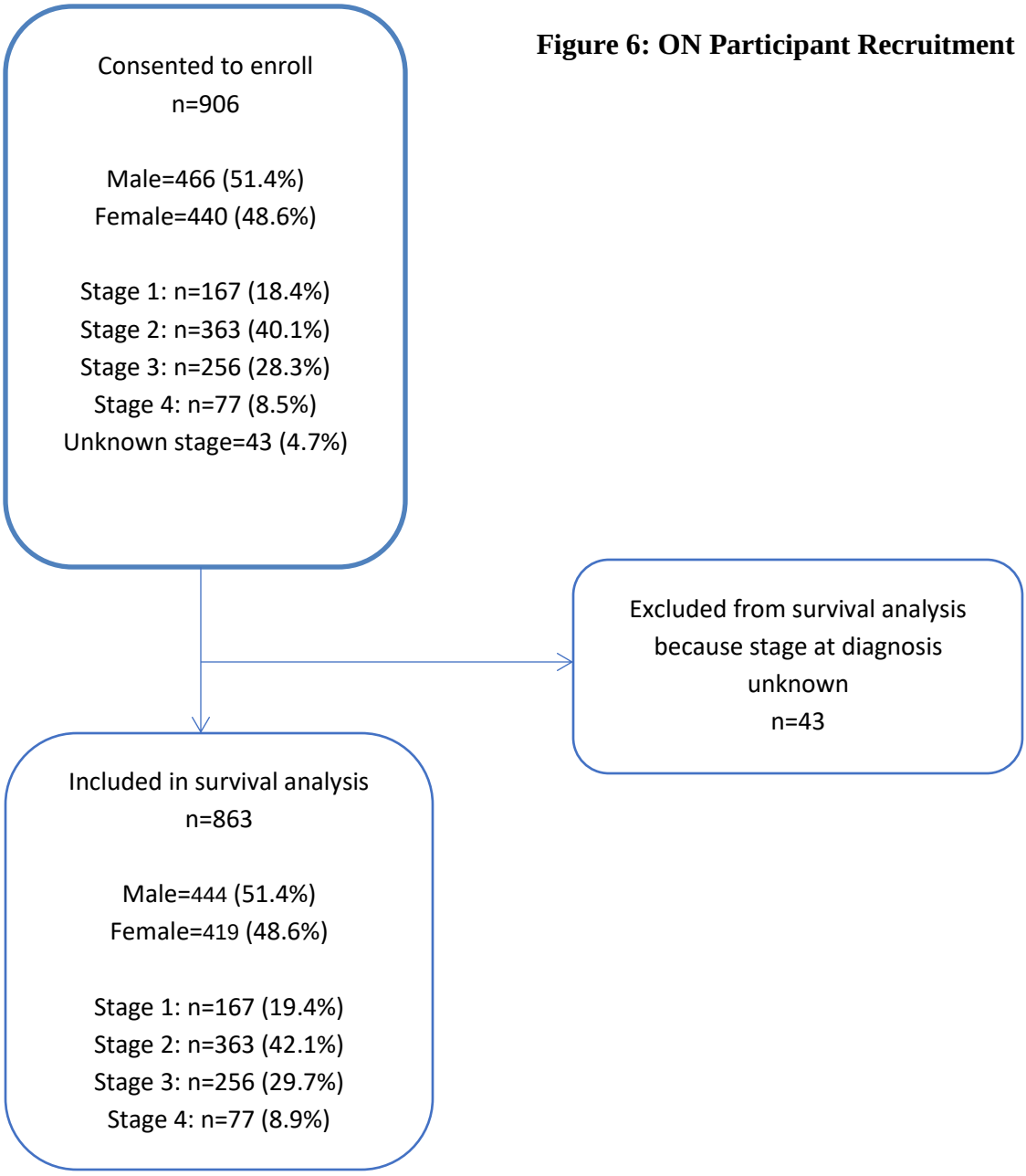


Figure 6: ON Participant Recruitment

Figure 7A: Univariate Survival Analysis for Stage 1 Colon Cancer in NL & ON

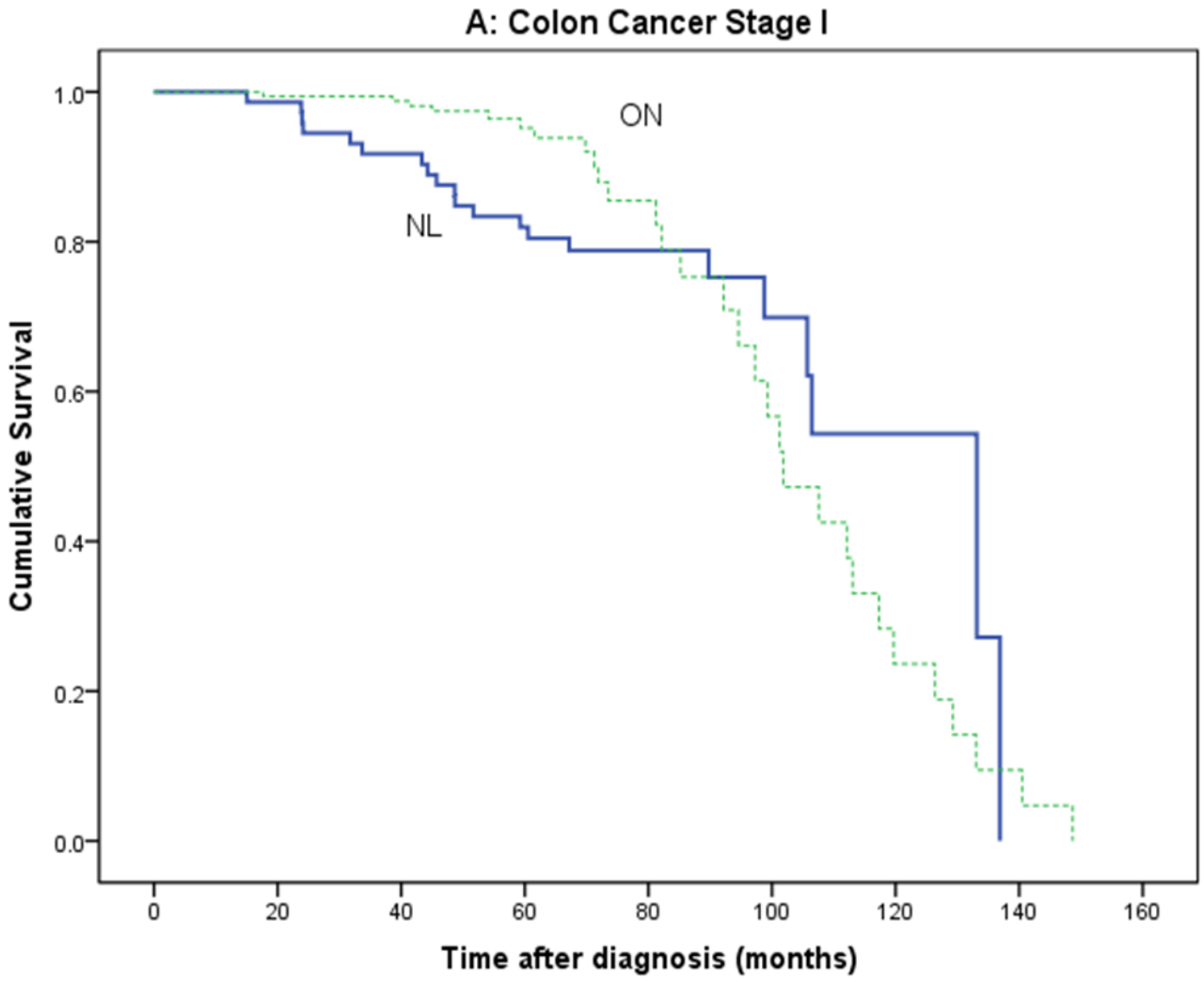


Figure 7B: Univariate Survival Analysis for Stage 2 Colon Cancer in NL & ON

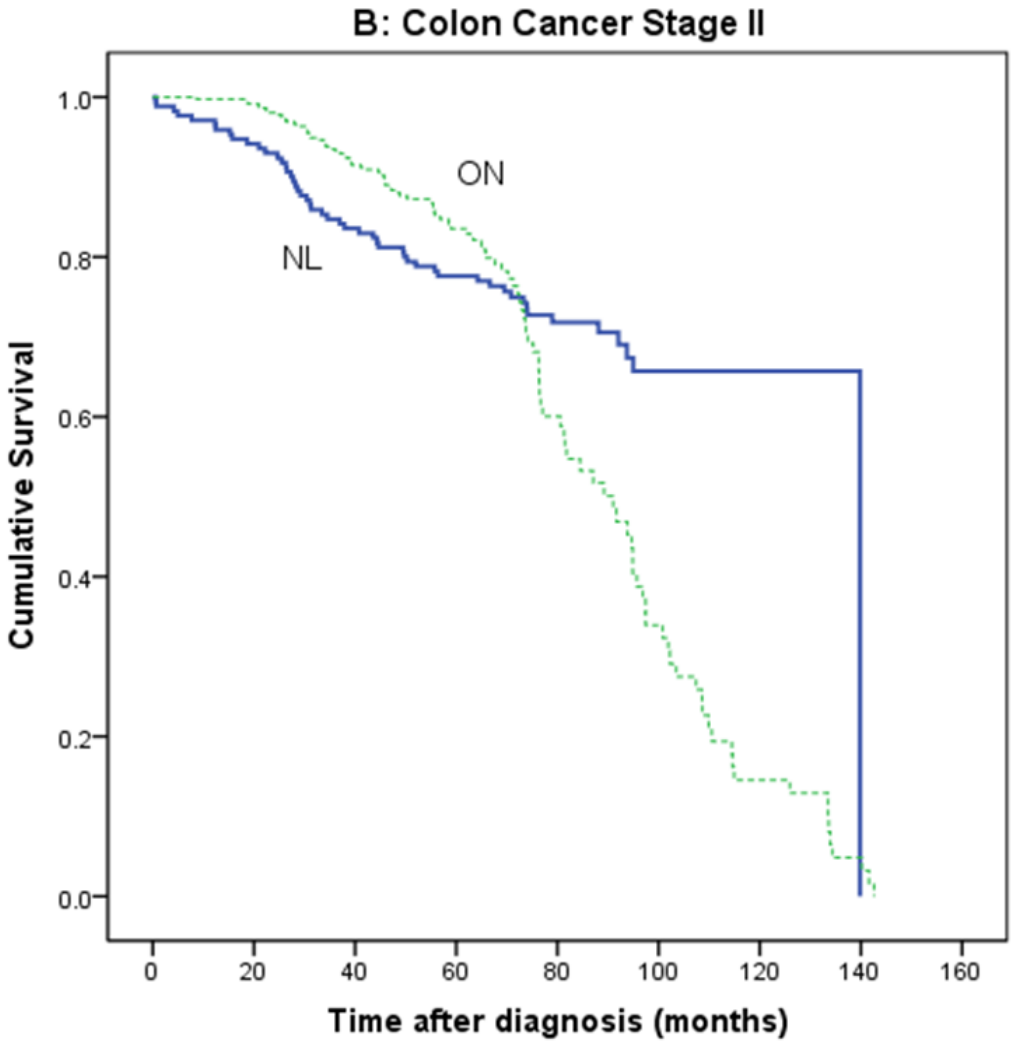


Figure 7C: Univariate Survival Analysis for Stage 3 Colon Cancer in NL & ON

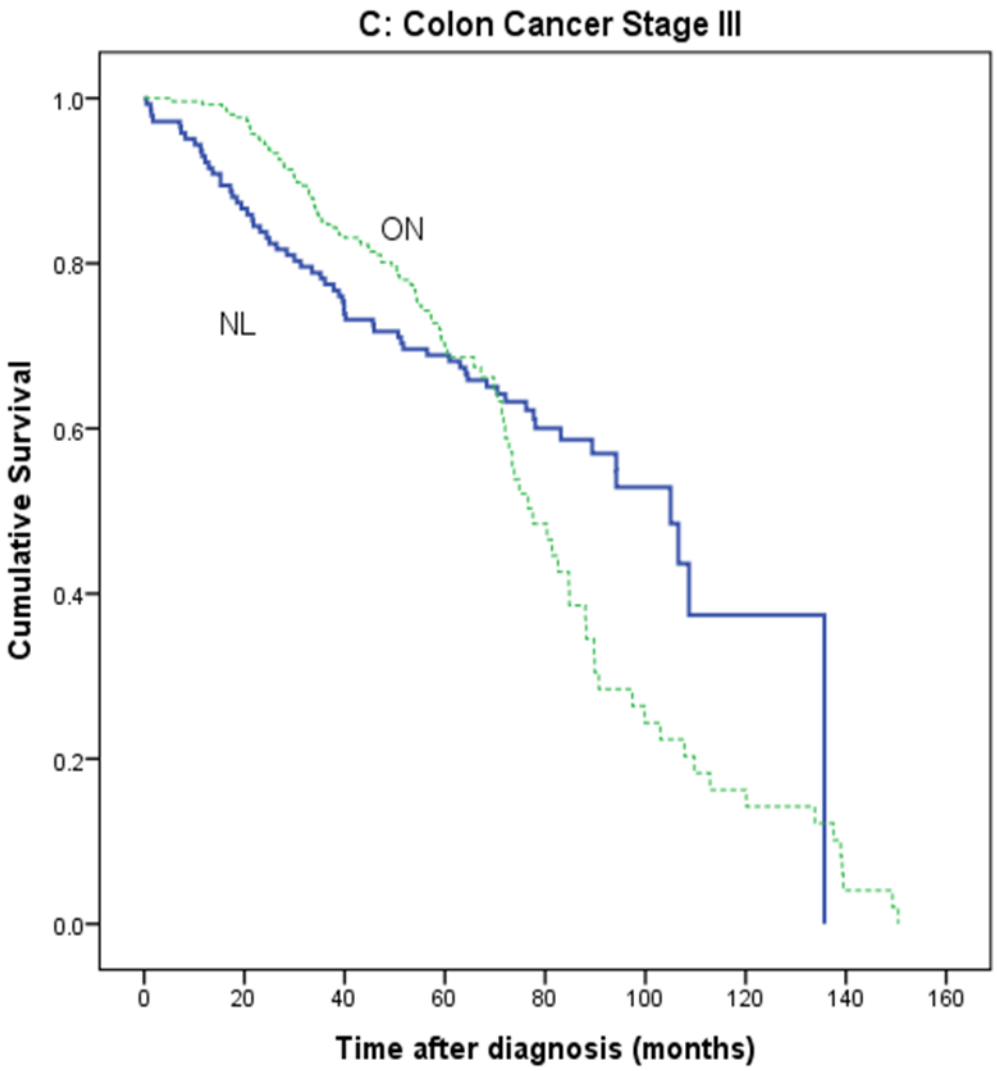
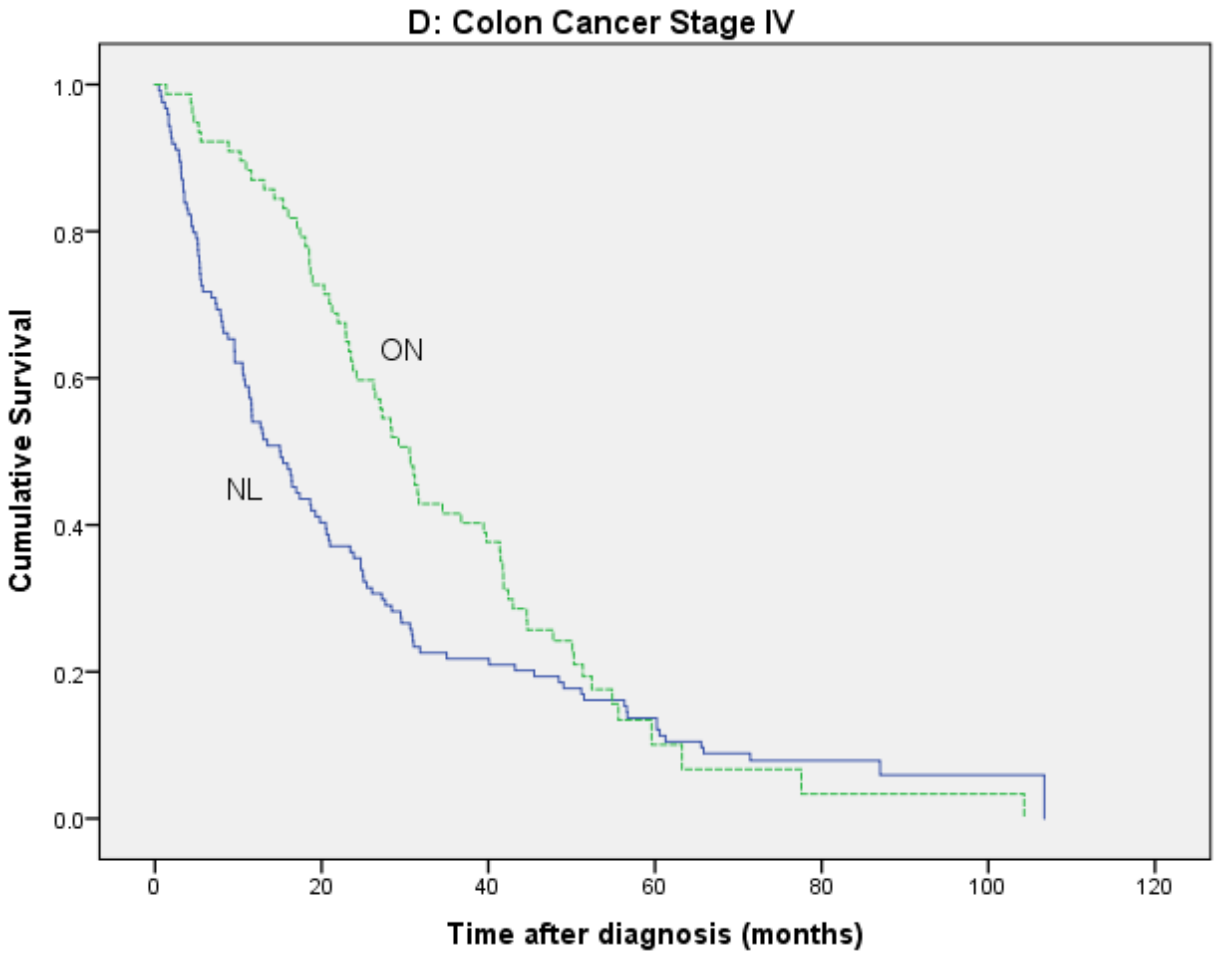


Figure 7D: Univariate Survival Analysis for Stage 4 Colon Cancer in NL & ON



APPENDIX A

Amsterdam Criteria I (1991)

- At least three relatives have histologically verified colorectal cancer (CRC); one of them should be a first-degree relative of the other two. Familial adenomatous polyposis (FAP) should be excluded
- At least two successive generations should be affected
- One of the relatives should be below 50 years of age when the CRC is diagnosed

Amsterdam Criteria II (1998)

- There should be at least three relatives with an HNPCC-associated cancer (CRC, endometrial, small bowel, ureter, or renal pelvis malignancy)
- One affected relatives should be a first-degree relative of the other two
- At least two successive generations should be affected
- At least one malignancy should be diagnosed before age 50 years
- FAP should be excluded in the CRC(s)
- Tumours should be verified by pathological examination

APPENDIX B

Original Bethesda Guidelines (1997)

Tumours should be tested for MSI in the following situations:

- Individuals with cancer in families that meet the Amsterdam Criteria
- Individuals with two HNPCC-related cancers, including synchronous and metachronous CRCs or associated extracolonic cancers*
- Individuals with CRC and a first-degree relative with CRC and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma; one of the cancers diagnosed at age less than 45 years, and the adenoma diagnosed at age less than 40 years
- Individuals with CRC or endometrial cancer diagnosed at age less than 45 years
- Individuals with right-sided CRC with an undifferentiated pattern (solid/cribriform) on histopathology diagnosed at age less than 45 years
- Individuals with signet-ring-cell-type CRC diagnosed at age less than 45 years
- Individuals with adenomas diagnosed at age less than 40 years

Revised Bethesda Guidelines (2004)

Tumours should be tested for MSI in the following situations:

- CRC diagnosed in a patient who is less than 50 years of age
- Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumours*, regardless of age
- CRC with the MSI-H histology diagnosed in a patient who is less than 60 years of age

- CRC diagnosed in one or more first-degree relatives with an HNPCC-related tumour, with one of the cancers being diagnosed under age 50 years
- CRC diagnosed in two or more first- or second-degree relatives with HNPCC-related tumours, regardless of age

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