Serrated Polyps of the Colon: The Winnipeg Experience

By

Diane Bay

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ABSTRACT

BACKGROUND: The pathological distinction between hyperplastic polyps and sessile serrated adenoma/polyps of the right colon is often difficult and may result in misdiagnosed polyps.

OBJECTIVE: To review the proportion and accuracy of serrated polyp diagnosis within a one year retrospective review of colorectal polyp samples, focusing on hyperplastic polyps of the right colon, using criteria set forth by previous studies.

MATERIALS & METHODS: 4096 Winnipeg patient cases from January 2009 to December 2009 were reviewed. The proportion of sessile serrated adenoma/polyps, traditional serrated adenoma and serrated adenoma were determined in the patient population. Additionally, pathological morphological variables were reassessed by two study pathologists to determine the frequency of sessile serrated adenoma/polyp initially diagnosed as hyperplastic polyps within the right colon.

RESULTS: Approximately 5% of all polyps in the patient population where diagnosed as non-hyperplastic serrated polyps (SSA/P, TSA and SA) and 12.5% as hyperpalstic polyps. Of the non-hyperplastic serrated polyps, a majority were diagnosed as SA. Upon reassessment of right sided HP (n=121), 34% were re-classified as SSA/P.

CONCLUSIONS: Winnipeg pathologists diagnose non-hyperplastic serrated polyps with a frequency similar to literature, but are not fully utilizing modern terminology, as majority of non-hyperplastic serrated

polyps are reported as SA without further categorisation. Furthermore, a significant proportion of right sided hyperplastic polyps could be re-classified as sessile serrated adenomas on review. Given the difficulty in distinguishing sessile serrated adenomas from hyperplastic polyps, closer endoscopic surveillance should be considered for all individuals with all serrated polyps (including hyperplastic polyps) in the right colon or alternatively all such polyps should be routinely reviewed by two pathologists.

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III. LIST OF ABBREVIATIONS

ACS	American Cancer Society
AJCC	American Joint Committee on Cancer
APC	adenomatous polyposis coli gene
BMI	body mass index
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CA	conventional adenoma
CAP	College of American Physicians
CAT	computerized axial tomography
CEA	carcinoembryonic antigen
CI	confidence interval
CIMP-	CpG island methylation phenotype negative
CIMP+	CpG island methylation phenotype positive
CI	chromosomal instability
CRC	colorectal carcinoma
CT	computed tomography
CTC	computed tomography colonography
FAP	familial adenomatous polyposis
FIT	fecal immunochemical test
FSIG	flexible sigmoidoscopy
GCHP	goblet cell hyperplastic polyp
gFOBT	guaiac-based fecal occult blood test
GI	gastrointestinal

hMLH1	human MutL homolog 1
hMSH2	human MutS homolog 2
HNPCC	hereditary non polyposis colon cancer
HP	hyperplastic polyp
KRAS	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
MMR	mismatch repair gene
MPHP	Mucin Poor Hyperplastic Polyp
MSI	microsatellite Instability
MSS	microsatellite stable
MVHP	microvesicular hyperplastic polyp
MYH/MUTYH	MutY homolog of *Escherichia coli* human gene
Pap	Papanicolaou
PSA	prostate-specific antigen
PMS2	postmeiotic segregation gene
SA	serrated adenoma
sDNA	stool DNA
SSA/P	serrated sessile adenoma / polyp
TA	tubular adenoma
TNM	tumour, node, metastasis
TSA	traditional serrated adenoma
TVA	tubulovillous adenoma
VA	villous adenoma

IV. Chapter 1 INTRODUCTION

The focus of this introduction is to provide background information on colorectal carcinoma and the precursor lesions that are linked to colorectal carcinoma. This background information is necessary to understand the importance of correctly distinguishing between hyperplastic polyps and sessile serrated adenomas / polyps.

1.1. Colorectal carcinoma (CRC)

Before defining what colorectal carcinoma is, it is vital to understand cancer. Cancer is often defined as any change within a cell that results in the abnormal growth of a cell within a multicellular organism (Compton *et al.* 2008). It is the result of abnormal cell growth within a tissue that produces an abnormal mass of tissue referred to as a neoplasm. Neoplastic formations within a tissue are either benign, where abnormal cells are contained within the tissue of origin, or they are malignant, when the abnormal cells have spread from the tissue of origin into surrounding tissues and organs, becoming life threatening to the afflicted individuals (Odze *et al.* 2010). Cancerous cell growth generally occurs within any living tissue or organ and it can be caused by a variety of diverse factors from the environment or genetic predisposition. In general, cellular changes that result in cancer formation are caused by direct genetic changes to the host cell or indirect changes to systems that maintain the cellular genome such as cell damage (Kumar *et al*. 2010).

A carcinoma of the colon and rectum occurs as a malignant epithelial tumour that forms within the epithelium of the colon or rectum and has penetrated through the muscularis mucosae into the submucosae (Hamilton *et al*. 2010). Epithelial tumours of the colon and rectum are the most common type of colorectal malignancies by far, and of

these adenocarcinomas comprise approximately 95% of colonic carcinomas (Hamilton *et al.* 2010; Snover *et al*. 2010). The remaining 5% of colorectal malignancies include lymphomas, sarcomas and melanomas (Hamilton *et al*. 2010). Other histological types of carcinoma may also occur within the large bowel and are as follows: adenosquamous carcinoma, spindle cell carcinoma, squamous cell carcinoma, and undifferentiated carcinoma (Hamilton *et al.* 2010). Since these types of carcinoma are not relevant to the aims of this thesis, only colorectal adenocarcinomas will be focused on for the remainder of this introduction.

An adenocarcinoma is a specific type of carcinoma that arises within the glands of the epithelial layer (Kumar *et al*. 2010). Adenocarcinomas may form in any tissue that is lined by a glandular epithelium (Hamilton *et al.* 2010). Adenocarcinomas are a major cause of mortality and morbidity worldwide and account for 90% of diagnosed CRC deaths (Kumar *et al*. 2010; Odze *et al*. 2010). Most adenocarcinomas of the colorectum have been thought to arise from a precursor lesion of the colonic epithelium, referred to as an adenoma. Adenomas can develop anywhere within the proximal and distal aspects of the colorectum (Hamilton 2010; Odze *et al*. 2010; Kumar *et al*. 2010).

In general, a polyp is a generic term used to describe any excrescence or growth protruding above a mucosal membrane. In Latin and Greek, a polyp is defined as the sea animal medusa, which has a distinct bulbous or cylindrical shape, with many feet or tentacles; intestinal polyps are often synonymous to this description. Adenomas are most commonly found in the gastrointestinal (GI) tract and are grossly identified as either the pedunculated type, a mushroom shaped protrusion with a fibrovascular stalk, or as a sessile type, with a flat or slightly elevated growth with no fibrovascular stalk (Kumar *et*

al. 2010). The shape, size, and location of an adenomatous polyp predict the potential of development of the polyp into CRC. The larger a polyp becomes the more potential it gains to become cancerous (Rex *et al*. 2006). Polyps larger than two centimeters (about the diameter of a nickel) have a 30-50 % chance of becoming cancerous (Rex *et al*. 2006 and Li *et al*. 2007). A polyp can occur anywhere within the GI tract and the large intestine but the majority are found within the left side of the colon and rectum, specifically in the sigmoid and rectum regions.

1.2 Etiology of CRC according to diet and lifestyle

In most cases, by the time a CRC is detected by a physician, it has likely been present for several years either as an adenoma or as a CRC. CRC have the second highest death rate (the highest being cancer of the lung) within the United States among males in particular (Chang *et al*. 2010). Globally, CRC is the third most commonly diagnosed cancer in the world (Odze *et al*. 2010; Chang *et al*. 2010). It is estimated that 150,000 new cases of CRC will be diagnosed this year and over 50,000 people will die of this disease in the United States alone (Odze *et al*. 2010; Chang *et al*. 2010; Rex *et al*. 2006). CRC were projected to be the third most commonly diagnosed cancer among Canadians in 2010 (Canadian Cancer Society 2010). It is estimated that 22,500 new cases will be diagnosed in Canada this year, and of those cases an estimated 12,400 will occur in men while 10,100 will be women (Canadian Cancer Society 2010). The estimated mortality for CRC is 9,100 Canadians and based on this estimate approximately 5,000 are expected to be males and 4,100 females (Canadian Cancer Society 2010).

CRC is most prominent in westernized countries such as the United States, Canada, Australia, New Zealand, Europe, and other developed nations (Odze *et al*. 2010;

Chang *et al*. 2010) A higher incidence of developing CRC is associated with westernized diets. A western diet is typically rich in animal fat, red meat, and reduced fibre intake (Odze *et al.* 2010). These foods are associated with an increased risk of CRC onset in patients greater than 50 years of age or patients with a family history of CRC ranging from a relative risk of 1.18 (95% confidence interval (CI) of 1.02–1.35) to 11.7 (95% CI of 5.8–23.9) (Randi *et al*. 2010). One study that examined CRC risk in patients adopting a western diet indicated that CRC detection was higher within the distal (left-sided) colon in men in contrast to high CRC rates within proximal (right-sided) colon in women (Randi *et al*. 2010). Diets rich in dietary fibre, which include fruits and vegetables, abundant sources of calcium, folic acid, vitamins A, C, and E have been shown to reduce the occurrence of colorectal cancers (Gonzalez *et al*. 2010; Moore *et al*. 2010). However, the protective effect of a fibre enriched diet was only identified for proximal CRC among both men and women. These non-western fibre enriched diets appeared to reduce overall colorectal cancer risk by 50% or more (Randi *et al*. 2010).

Over the past 40 years, a significant increase in CRC incidence has been gradually observed in developing nations, which adopt more "western" lifestyles and diets (Hamilton *et al*. 2010, Randi *et al*. 2010). The introduction of westernized diets to Africa, Asia, India, and for newly landed immigrants to North America starting in the early 1970's correlated with an increase in CRC diagnosis of approximately 18% from 1973 to 1987 (Edge *et al*. 2010; Hamilton 2010).

Alcohol consumption, cigarette smoking, and sedentary physical activity are also possible factors for the development of CRC (Wallace *et al*. 2009, Abdulkareem *et al*. 2008). The relationship between CRC and cigarette smoking has been extensively studied

but many findings are ambiguous and contradictory. A recently published European large case study (465,879 participants; 2,741 developed CRC) by the European Prospective Investigation into Cancer and Nutrition (EPIC) showed that chronic cigarette smokers had a greater risk of developing CRC (hazard ratio (HR) 1.18; 95% CI, 1.06-1.32) than non smokers (Leufkens *et al*. 2010). The findings also indicated that cigarette smokers who quit for a minimum of 20 years had the same risk of developing colon cancer as non-smokers (Leufkens *et al*. 2010). In contrast, a smaller but more focused study of 151 individuals with multiple serrated polyps (37% were diagnosed with CRC) indicated that current female smokers had decreased odds of developing CRC when compared to non-smoking females (Buchanan *et al*. 2010). Males in this study showed no relationship between those currently smoking and CRC and no statistical evidence of an association between former smoking and CRC for either sex could be determined (Buchanan *et al*. 2010). Hence, a clear relationship between CRC and cigarette smoking appears to depend on the specific variables examined within a study.

The relationship between alcohol consumption and CRC has been extensively studied within the past decade and the results also tend to be inconsistent. A recently published large population based case study of the multiethnic population of Hawaii was performed to evaluate the correlation of westernized culture and diets in relation to CRC rates. The major finding from this study was a positive correlation between previous and current alcohol consumption in males and females and the development of CRC (Marchand *et al*. 2010). Another population based case study (Cho *et al*. 2004) set in North American and Europe pooled data from 8 cohort studies in 5 countries for a total 489,979 individuals who were questioned about alcohol consumption and monitored for 6

to 16 years. The conclusion of this study was that individuals who consumed an average sized drink of approximately 30 grams of alcohol per day or more, had a higher risk of developing CRC than non-drinkers (relative risk of 1.16 in females, and 1.41 for males). Overall, alcohol consumption at increased levels in later life appears to be modestly correlated to an increased risk of developing CRC in both males and females based on both studies.

Obesity is a risk factor for many diseases and has recently become of interest for CRC risk over the past decade, since 30% of North Americans are now obese (American Cancer Society 2010; Gunter *et al.* 2006). Recent studies within North America have established obesity associated with type II diabetes as an important contributor in the pathogenesis of CRC, mainly due to insulin-resistance (Frezza *et al*. 2005). Insulin levels increase when an individual's body mass index (BMI; weight in kilograms per height in meters squared) for a healthy male and female (age 20 years or older) is greater than 30 kilograms per meter squared (Odze *et al*. 2010). A BMI greater than 30 increases the risk of developing CRC by 20% and for every 5 kilograms gained, increases the risk by 7% (Thomas *et al*. 1995). The exact genetic mechanism of increased CRC risk as a result of insulin-resistance due to obesity is still unknown.

1.3 Epidemiology of CRC

Studies conducted by the World Health Organization revealed that an estimated 1.23 million new cases of CRC occurred worldwide in 2008 and representing about 9.7% of all new cancers worldwide (Hamilton *et al*. 2010). CRC is listed as the fourth most frequent cancer in men (after lung, prostate and stomach cancer) and the third most frequent in women (after breast and uterine cancer) worldwide (Hamilton *et al*. 2010).

The age standardized incidence of CRC varies widely across the globe. The highest rates of CRC occur in industrialized countries, predominantly in North America and Europe (about 40 -60 per 100 000) with lower rates observed in Asia, India, and Africa (Hamilton *et al*. 2010).

As mentioned previously, the linkage between CRC and sex is in favour of males, and a male: female ratio of 3:1 appears to be consistent in all ethnic backgrounds studied (Abdulkareem *et al*. 2008). The incidence of CRC increases with age. Carcinomas of the colon and rectum are less frequent before the age of 30 to 40 years; except for individuals with a genetic predisposition or factors such as inflammatory bowel disease or a family history of CRC (American Cancer Society 2010; Kumar *et al*. 2010). Age standardized incidence rates for rectal cancer in males are 50% higher than in females whereas colonic rates are 20% higher in males than in females (Hamilton *et al*. 2010). This indicates that males are more vulnerable to the development of CRC than females within an equivalent age group.

1.4 CRC disease staging and grading

The stage and to a lesser extent the grade of a tumour directly determine the type of therapy used to treat a CRC patient and predict their prognosis. The stage of a CRC tumour is determined by its anatomical extent at the time it is removed from a patient (Compton *et al*. 2008; Odze *et al*. 2010; Thomas *et al*. 1995). The stage is determined based on three components, T, N, and M (TNM system), where the extent of the primary tumour (T), the presence or absence of regional lymph nodes metastasis and its extent (N), and the presence or absence of distant metastasis (M) are measured (Sobin *et al*. 2009). Details of each stage T, N, and M are provided on Tables 1.1- 1.3 respectively.

The TNM system of tumour classification involves numerical designations for each of the three components (AJCC 2010). Once a lesion is assigned a TNM stage it directs what type of therapy is selected. Tumour staging also determines the prognosis for the patient; the higher the stage the lower patient survival.

Once the staging process is completed, there are 24 possible stage group outcomes according to the TNM classification manual (4T categories x 3N categories x 2M categories) (Sobin *et al*. 2008). Each stage grouping is assembled based on established survival rates (AJCC 2010, Canadian Cancer Society 2010, Sobin *et al*. 2008). Stage groupings range from 0 to IV, where 0 is cancer in its earliest stage, also known as *carcinoma in situ* and stage IV, representing a metastatic tumour (Sobin *et al*. 2008). Specifically, stage 0 includes all Tis, N0, M0 tumour types and stage I includes T1-T2, N0, M0 tumours. Stage II represents T2 tumours that are node negative and subdivided into three different subgroups stage IIA - IIC based on T-stage. Stage IIA includes T3, N0, M0 tumours (Sobin *et al.* 2008), stage IIB includes T4a, N0, M0 tumours, and stage IIC includes T4b, N0, M0 tumours. Stage III tumours are defined as any T stage tumour with node (N) positivity but no metastasis (M0). Stage III is divided into IIIA to IIIC. Stage IV is divided into stages IVA and IVB where Stage IVA includes any T, and N category tumours and M1a tumours, while stage IVB also includes any T, and N category tumours, as well as M1b tumours (Sobin *et al*. 2008).

The classification of the tumour N- stage relates the absence or presence and extent of regional lymph node metastasis (Sobin *et al*. 2008). The N-stage is divided into NX to N2 designations for the colon and rectum TNM staging as summarized on Table 1.2. The NX stage is used to describe tumours where regional lymph nodes cannot be

assessed (Sobin *et al*. 2008). In N0 stage tumours, no regional lymph node metastasis are observed (Sobin *et al*. 2008). N1 stage tumours have the presence of metastasis in 1-3 regional lymph nodes and are further subdivided into N1a to N1c designations (Sobin *et al*. 2008). In subtype N1a, metastasis is present in 1 regional node, where as in subtype N1b, metastasis is identified in 2-3 regional lymph nodes (Sobin *et al*. 2008). The final N1c subtype is classified as tumour nodules located within the subserosa or in non-peritonealized pericoloic or perirectal soft tissue without regional lymph node metastasis (Sobin *et al*. 2008). The final stage N2describes tumours with metastasis in 4 or more regional lymph nodes and is subdivided into N2a and N2b. For N2a, tumour metastasis is identified in 4-6 regional lymph nodes, where as for N2b, metastasis is present in 7 or more regional lymph nodes (Sobin *et al*. 2008).

Histopathological grading relates to the microscopic examination of the primary tumour within the colon and rectum (summarized in Table 1.4). The grade is based on the amount of glandular differentiation observed within the tumour. G1 to G4 describes a range of well differentiated tumour cell morphology to undifferentiated tumour cell morphology (Sobin *et al*. 2008). A tumour with a G1 designation exhibits glandular formation in greater than 95% of tumour cells examined and it is classified as a well differentiated adenocarcinoma (Hamilton *et al.* 2010; Compton *et al*. 2008). A G2 adenocarinoma has 50-95% glandular formation within its tumour cells (Hamilton *et al.* 2010; Compton *et al*. 2008). A G3 adenocarinoma has 5-50% glandular formation of tumour cells where at least 50% of them have a de-differentiated component (Hamilton *et al.* 2010; Compton *et al.* 2010). A G4 adenocarinoma has less than 5% glandular

formation and is almost entirely composed of undifferentiated cells (Hamilton*et al*. 2010; Compton *et al*. 2008).

The grade can be further classified into high-grade and low-grade categories (Table 1.4) (Hamilton *et al*. 2010; AJCC 2010). High-grade CRC describes G3 to G4, (Hamilton *et al*. 2010; AJCC 2010) where as low-grade CRC describes stages G1 to G2 (AJCC 2010). Mucinous and signet-ring cell carcinomas of the colon and rectum are considered to be poorly differentiated and typically designated as G3 high-grade tumours according to this grading scheme (Hamilton *et al*. 2010).

TNM is a two-tiered system with clinical and pathological tumour classifications. The clinical classification, or 'c', is a pre-treatment classification used to describe a tumour (cTNM) and is based on evidence acquired after physical examination, imaging and endoscopy (Sobin *et al*. 2009). The pathological classification, or 'p', is used to describe a tumour after postsurgical histopathological categorization, and it is based on the microscopic examination of a surgically removed specimen (Sobin *et al*. 2009). The pTNM provides the most accurate T and N stages, if no neoadjuvant therapy is provided. The M stage can be determined using imaging techniques alone. A biopsy can only be used to determine T stage if the material provided included the entire tumour. For example in the colon, this scenario would apply to polypectomies with a completely excised carcinoma within the polyp (Compton *et al*. 2008).

Tumour staging is important for both the prognosis and therapy of the patient. In general, patients with node negative stage II CRC have a better prognosis and can be treated by surgery alone. Patients with node positive CRC are treated by surgery combined with multi-agent chemotherapy post-operatively. Patients with metastatic CRC

are treated with a specific chemotherapeutic protocol, distinct from the therapy offered to node positive CRC patients. The metastatic chemotherapeutic treatment is more aggressive and can prolong life but is not considered curative. Tumour grading is also important for stage II CRC, since patients with poorly differentiated adenocarcinoma and node negativity may also require chemotherapeutic treatments (AJCC 2010).

When staging CRC, regional lymph node involvement is a very most important prognostic indicator. The American Joint Committee on Cancer (AJCC) and the College of American Pathologists (CAP) recommend that an examination of at least 12 lymph nodes is required to confidently assign a stage II designation for CRC (CAP 2010 *et al*. 2011).

1.5 Clinical Presentation of CRC

Many people with colorectal cancer experience no symptoms in its early stages but in some cases patients may present with symptoms such as haematochezia and anemia (Compton *et al*. 2008). Other nonspecific symptoms of early to mid-stage CRC may include fever, malaise, weight loss, and abdominal pain (Hamilton *et al*. 2010). Symptoms vary depending on the size of the neoplasm and its location within the large intestine. The average duration of patient symptoms prior to CRC diagnosis is 14 weeks based on patient case surveys (American Cancer Society 2010). Many patients experience changes in bowel habits, especially constipation, because solidified fecal matter is impeded by the enlarging neoplasm (American Cancer Society 2010). The obstruction of feces may also result in bowel obstruction or perforation and cause a variety of secondary symptoms such as bacterial infections (Hamilton *et al*. 2010). A rectosigmoid lesion can produce tenesmus and rectal bleeding (Jass 2007).

1.6 Treatments for CRC patients

Surgery is recommended for almost all stages of CRC and it is the main treatment for early stages of the disease (Boland *et al*. 2001; Chang *et al*. 2010). Standard open colectomy surgical resection procedures include right hemicolectomy, left hemicolectomy and subtotal colectomy (American Cancer Society 2010; Compton *et al.* 2008). All open colectomy procedures involve the partial or segmental removal of a section of colon including adjacent lymph nodes.

Another surgical resection technique used in CRC is laparoscopic colectomy. In comparison to an open colectomy, laparoscopic colectomy has a shorter recovery time for the patient and reduces the length of hospital stays (Compton *et al*. 2008). Both methods are equally effective in the removal of the colon and result in similar long term survival of patients with CRC. The major difference between these techniques in the cost; laparoscopic assisted colectomy is less expensive due to shorter hospital stays (American Cancer Society 2010; Compton *et al.* 2008).

Polypectomy and local resection procedures are generally used to remove early stage forms of CRC, primarily stages 0 to I tumours. A polypectomy is the term used to describe the excision of a polyp whereas a local resection describes a transanal approach or endoscopic micro-surgery procedure to remove early stage cancers, primarily of the rectum (Compton *et al.* 2008).

The removal of tumours from the rectum involve one or more of the following procedures: local transanal resections, transanal endoscopic microsurgery, lower anterior resection, abdominoperineal (AP) resection, and pelvic exenteration (Kumar *et al*. 2010;

American Cancer Society 2010). Younger patients diagnosed with synchronous CRC in both the left and right colon often require a subtotal colectomy (Compton *et al*. 2008).

Surgery is only one of the treatments used to manage colorectal cancer. Typically, the stage of the tumour will determine if it is followed up with adjuvant chemotherapy. Recent analysis of CRC patients shows tumour recurrence is most prevalent within the first 2 to 3 years after surgical resection of the neoplasm (Compton *et al*. 2008; National Institute of Health 2010). When chemotherapy is used following cancer resection the long-term disease free survival rate increased by 5% to 10% for stage II tumours (Benson *et al*. 2004). Stage III tumours treated with chemotherapy demonstrate an increase in five year disease free survival rate of approximately 33% (Boland *et al*. 2000). Most chemotherapy regimens use 5-flourouracil, an inhibitor of DNA replication (American Cancer Society 2010). Other medications that are used in conjunction with 5-flourouracil are leucovorin, oxaliplatin and irinotecan in multi-drug regiments (Goodwin *et al*. 2009).

Radiation therapy is generally used to treat advanced stages of rectal cancer in combination with surgery and/or chemotherapy to improve disease control at the primary cancer site (Compton *et al*. 2008). Studies have shown that patients with locally advanced rectal carcinoma who receive neo-adjuvant (pre-operative) chemoradiation may have better clinical outcomes (Odze et al. 2010).

1.7 Screening and Surveillance for CRC

Screening asymptomatic patients for CRC is an important process to identify polyps or tumours at their earliest stages. The goal of CRC screening is to detect and remove colorectal polyps before they develop into the invasive forms of CRC (Levin *et al*. 2008; Gordon 2010). For a screening test to be effective, several criteria must be met.

Firstly, the test should be capable of detecting earlier stage cancers or precancerous lesions. Secondly, treatment and/ or removal of the identified early stage tumours or premalignant lesions should improve patient survival. Finally, the test should be cost-effective with minimal risks to the patient during the process and acceptable to the patients.

The premalignant adenomatous phase of colorectal cancer is a prolonged process, often taking a decade or more to progress into later stages of CRC. Additionally, adenomas can be reliably identified through a variety of cost-effective techniques. Therefore, CRC is an ideal candidate for a screening and surveillance program.

Common methods used for CRC screening and detection are divided into two distinct categories described below that involve six types of tests: 1) guaiac-based fecal occult blood testing (gFOBT), 2) immunochemical-based fecal occult blood testing (FOBT), (FIT), 3) testing stool for exfoliated DNA (sDNA), 4) flexible sigmoidoscopy (FSIG) and colonoscopy, 5) double contrast barium enema (DCBE), and 6) virtual computed tomographic colonography (CTC). The most recent colorectal cancer screening guidelines jointly produced by the American Cancer Society and the U.S. Multi-Society Task force on Colorectal Cancer released a comprehensive description of all six tests and placed them into two major types – i) cancer detection tests and ii) cancer prevention tests. Cancer detection tests encompass tests that predominantly detect cancer as opposed to precursor lesions. These are stool based tests and include gFOBT, FOBT (FIT) and sDNA. Cancer prevention tests can assess anatomic structure. The cancer prevention tests derive most of their additional value from adenoma detection although they can also detect asymptomatic cancers at an earlier more curable stage. Cancer prevention tests

include both endoscopic and radiologic examinations (i.e. FSIG, colonoscopy, and computerized tomographic colonography(CTC)) (Levin *et al*., 2008).

One of the most common symptoms of CRC is chronic blood loss in patient stool. Hence, a stool sample may be collected to test for occult (hidden) blood. The gFOBT is a test that uses the chemical guaiac to detect heme levels in stool (Levin *et al.* 2008; Winawer *et al*. 2006; Rex *et al*. 2006; Brooks *et al*.2008). Heme is the iron-containing component of red blood cell protein hemoglobin. The gFOBT cannot determine the location of blood loss within the colon or from other portions of the digestive tract (such as the stomach) nor its specific cause (Levin *et al*. 2008). Therefore, if the test is positive, a colonoscopy is required to determine if blood loss is due to cancer, polyp development, or other cause of bleeding such as ulcers, hemorrhoids, or inflammatory bowel disease (colitis) (Levin *et al*. 2008; American Cancer Society 2010). Another disadvantage is that this test can become positive due to dietary components, such as excessive intake of red meat. The advantage of this test is its simplicity, its cost-effectiveness and its ability to be self- administered for the screening detection of CRC.

Another type of early detection test similar to gFOBT is the FIT test. Instead of detecting the heme molecule, FIT uses antibodies to detect the human hemoglobin protein itself within the stool sample (Levin *et al*. 2008). Like gFOBT, FIT detects the presence of blood in the stool but the main difference is that FIT uses antibodies to detect haemoglobin at far greater sensitivity, since guaiac based chemical require high concentrations of heme molecules that may not be present leading to false negative tests (Levin *et al*. 2008; Rex *et al*. 2006; Brooks *et al*.2008). Hence, FIT may be a more accurate way to screen for blood in the stool than gFOBT. Similar to gFOBT, if blood is

detected by FIT patients will require a follow-up colonoscopy to determine the cause of blood in the stool (Levin *et al*. 2008). As with gFOBT, FIT will not detect a tumour that is not bleeding.

CRCs contain abnormally replicating DNA which is contained in abnormal cells that are often shed or 'exfoliated' into the stool. A stool sample can be checked for the presence of abnormal DNA by using sDNA testing. This method involves a polymerase chain reaction (PCR) based technique which detects and amplifies only the abnormal DNA sequences using small DNA probes with sequences identical to known regions of abnormal sDNA. If sDNA tests are positive for abnormal DNA in the stool, a colonoscopy is performed (Sima *et al*. 2010; Levin *et al*. 2008). The drawbacks of sDNA testing include high cost, slow turn around time and false positive/negative results (Levin et al. 2008). Many genetic DNA regions used as markers for probes may not always be present in all forms of tumour cells, which can lead to false negatives or abnormal cell DNA may be below detectable limits of the test. The significance of a positive sDNA in the setting of a normal colonoscopy is not yet known. Similar to gFOBT or FIT, this test is not invasive and does not require any special preparation by the patient.

CTC is a non invasive technique which can assess the anatomy of the colon, allowing detection of adenomas and small cancers. However, it is most effective for detecting larger adenomas (greater than 1 centimetre) and is relatively insensitive at detecting smaller lesions (< 5 mm). Additionally, CTC requires a significant commitment in radiological equipment and technical/professional time. Additionally, although it is less invasive than colonoscopy, it is not complication free. Perforation due to colonic inflation can occur and allergies to contrast media are some of the potential limitations.

Additionally, as in non-structural tests, any detected abnormality requires further investigation by colonoscopy.

The gold standard in CRC screening is colonoscopy (Winawer *et al*. 2006; Rex *et al*. 2006; Levin *et al*. 2008; Brooks *et al*.2008). Colonoscopies allow a physician to identify and remove any polyps within the colon and rectum present at the time of the examination (Brooks *et al*. 2008). Colonoscopy has greater than 95% cancer detection accuracy when used effectively (Levin *et al*. 2008; Winawer *et al*. 2006). Colonoscopies are highly demanding on patients requiring proper intensive bowel preparation procedures, and expose patients to complications from the procedure itself (Winawer *et al*. 2006; Brooks *et al*. 2008; Rex *et al*. 2006). However, the benefits of colonoscopy may far out weight the adverse affects it may present to the patient in the long term based on its effectiveness in eliminating precursor forms of CRC and CRC itself. Currently, there is no uniform consensus on the best test for colorectal cancer screening. Many experts suggest "the best test is the one that gets done" ; this statement emphases the need to take into account the local availability of the different tests and the acceptance by the patients.

The ACS and the US Multi-Task Force on CRC recommend surveillance standards to prevent the misuse of colonoscopy screening procedures (Brooks *et al*. 2008). Post-polypectomy colonoscopy is recommended at a three year interval for patients diagnosed with three or more adenomas, high grade dysplasia, villous features or size greater than 1 cm (Winawer *et al*. 2006). Patients with polyps with no high grade dysplasia, two or fewer polyps less than 1 cm and no villous component are recommended to undergo follow up at a five to ten year interval after complete polyp excision. Patients diagnosed with early stage CRC are recommended first repeat

colonoscopy at one year and then at three years and then followed as per the guidelines for surveillance of adenomas (Winawer *et al*. 2006; Brooks *et al*. 2008)

1.8 Colorectal precursor lesions: Polyps

CRC polyps were traditionally divided into two basic groups, hyperplastic polyps (HP) and conventional adenomas (CA). HP are currently subdivided into three different morphological variants referred to as microvesicular hyperplastic polyps (MVHP), goblet cell hyperplastic polyps (GCHP), and mucin poor hyperplastic polyps (MPHP) (Odze *et al*. 2010; Hamilton *et al*. 2010, Snover 2011). CA are subclassified into tubular adenoma (TA), villous adenoma (VA) and tubular villous adenoma (TVA) (Odze *et al*. 2010; Hamilton *et al*. 2010). Serrated polyps (SP) are a more recently described subgroup of polyps that have a distinct serrated morphology and include both sessile serrated adenomas (SSA/P) and traditional serrated adenomas (TSA) (Hamilton *et al.* 2010). The term serrated adenoma (SA) refers to a type of serrated polyp diagnosed prior to the recognition of SSA/P. Upon review, most SA can be re-classified as either SSA/P or TSA (Hamilton et al. 2001).

A thorough discussion of HP and other serrated polyps is provided in the Literature Review section and details of HP are summarized on Table 1.5. Traditionally HP are considered to be benign and non-neoplastic polyps that are predominately located in the left sided (distal) colorectum (Snover *et al*. 2011; Noffsinger 2009). A diagnosis of HP has not been considered a risk factor for CRC, but recent studies suggest that some of these lesions, particularly if right sided and greater than 1 centimetre in size may evolve into the SSA/P (Noffsinger 2009; Snover 2011).

Conventional adenomas are classified based their size, villous morphology and degree of dysplasia (Snover 2011; Aust *et al*. 2010) and a summary of these features is provided in Table 1.5. In general, a larger sized adenomas correlate with the degree of villous formation and high grade dysplasia (Noffsinger 2009). Any single finding (*ie*. high grade dysplasia, villous morphology or size greater than 1 cm) defines an adenoma as an "advanced colorectal neoplasm" and results in shorter surveillance interval recommendations for the patient (Rex *et al*. 2006; Sima *et al*. 2010).

Neoplastic serrated polyps are divided into two groups, (SSA/P) and (TSA). The most common neoplastic serrated polyp is the SSA/P (Snover *et al*. 2010; Hamilton *et al*. 2010) and a summary of its distinguishing features is also provided on Table 1.5. Controversy regarding the terminology used to define SSA/P because SSA/P do not demonstrate true dysplasia (Tolakovic & Snover 2006; Snover *et al*. 2010). SSA/P are diagnosed according to their abnormal architecture. SSA/P are predominately located in the right side (proximal) of the colon and account for up to 20% of all serrated polyps (including HP) (Snover 2011). SSA/P demonstrate an association with right sided CRC and these cancers demonstrate molecular and morphologic features distinct from adenocarcinomas of the usual type (Venkatachalam *et al*. 2010). A more detailed discussion of SSA/ P is provided in the Literature Review section. TSA are less common lesions (Torlakovic *et al*.1996) that predominately develop within the left sided (distal) region of the colon and are associated with cytologic dysplasia of the "serrated" type (Snover *et al*. 2010). A summary of the characteristic features specific to TSA is provided in Table 1.5. TSA are characteristically protruberant or pedunculated, and have serrated morphology (Leggett *et al.* 2010). These lesions are typically larger than one

centimetre, with uniform cytological dysplasia, and demonstrate architectural serration with a characteristic eosinphilic cytoplasm (surface serration) (Bauer *et al*. 2008; Aust *et al*. 2010; Li *et al*. 2007). Ectopic crypt formation unrelated to muscularis mucosae is a finding considered diagnostic of TSA (Leggett *et al.*2010).

1.9 Genetics of CRC

The genetics of CRC have been recently summarized in a review by Snover in January of 2011. The majority of CRC evolve through a defined precursor lesion with distinct molecular characteristics into distinct types of CRC. The classic pathway involves evolution from a CA into adenocarcinoma of the usual type. On a molecular basis these cancers move through the suppressor pathway with, mutations in the adenomatous polyposis coli (APC) gene being an important initiating factor, with evolution of chromosomal instability (CI) occurring as the carcinoma evolves. Approximately 60% of carcinomas follow this pathway. Additionally, patients with familial adenomatous polyposis coli (FAP) follow this pathway, due to the presence of a germline mutation in the APC gene. In the last decade investigators have become aware of a second carcinogenic pathway. This pathway is felt to evolve through SSA/P and is the mutator (microsatellite instability (MSI)) pathway. The key molecular event in this pathway is the loss of mismatch repair (MMR) gene function. MMR genes are important in repair during DNA replication and their loss results in a marked increase in mutation rates. This is reflected by changes in microsatellite DNA and result in MSI. It is believed that the initial molecular event in this pathway is a BRAF mutation in a subset of HP. These polyps then evolve into SSA/P at which point MMR genes are inactivated by methylation of CpG island promoter regions, becoming “CIMP +”. Inactivation of MMR

proteins correlates with the development of dysplasia and carcinoma. Approximately half of SSA/P related carcinomas evolve through this pathway with the remainder demonstrating methylation of other poorly defined genes. These CIMP+ carcinomas are microsatellite stable (MSS). As with the better established suppressor pathway, the mutator pathway is also associated with a familial cancer syndrome. Lynch syndrome (hereditary non-polyposis colon cancer syndrome (HNPCC)) is caused by germline mutations in MMR genes. As a result patients develop CRC and other cancers (including breast and endometrial) at an early age. Overall, approximately 2 to 5% of patients with CRC will have a well-defined cancer syndrome such as FAP, other polyposis syndromes or Lynch syndrome. Summaries of both pathways are provided in Figures 1.1 and 1.2.

All cancers evolving along the mutator pathway demonstrate MSI, which can be simply detected through PCR analysis of paraffin embedded tumour tissue. MSI does not distinguish between sporadic MSI cancers and cancers associated with Lynch syndrome. Another way to diagnose adenocarcinomas arising from the mutator pathway is immunohistochemical staining for the most common MMR proteins. The most common proteins are MLH1, MSH2, MSH6, and PMS2 (Shia et al. 2010; de Maat *et al*. 2010). Absence of any one of these proteins strongly correlates with MSI. Generally, negativity for MLH1 is associated with the absence of MSH6 and negativity for MSH2 is associated with the absence of PMS2. An additional benefit of using immunohistochemistry is that while patients negative for MSH6/PMS2 are almost always suffering from Lynch syndrome (Shia et al. 2010), MLH1 negativity can be seen in either sporadic SSA/P related cancers or Lynch syndrome. In addition, poorly defined genetic pathways exist

for a significant minority of CRC. For example, TSA appear to evolve through a distinct CIMP + MSS pathway (Figures 1.1 and 1.2).

Table 1.1 Summary of tumour (T) pathological classifications of colon and rectal tumours based on International Union Against Cancer TNM Classification of Malignant Tumours 7th ed. (Sobin *et al*.2009).

T- Primary Tumour		
Tx	Primary tumour cannot be assessed	
T0	No evidence of primary tumour	
Tis	Carcinoma *in situ* / intraepithelial or invasion of lamina propria	
T1	Tumour invades submucosa	
T2	Tumour invades muscularis propria	
T3	Tumour invades subserosa or into non-peritonealized pericolic or perirectal tissues	
T4	Tumour directly invades other organs or structures and/or perforates visceral peritoneum	
	T4a	Tumour perforates viseral peritoneum
	T4b	Tumour directly invades other organs or structures

Table 1.2 Summary of regional lymph nodes (N) pathologic classification of colon and rectal tumours based on International Union Against Cancer TNM Classification of Malignant Tumours 7th ed. 2009 (Sobin *et al.* 2009).

N - Regional Lymph Nodes		
NX	Regional lymph nodes cannot be assessed	
N0	No regional lymph node metastasis	
N1	Metastasis in 1-3 regional lymph nodes	
	N1a	Metastasis in 1 regional lymph node
	N1b	Metastasis in 2-3 regional lymph nodes
	N1c	Tumour deposit(s), i.e, nodules in the subserosa, or in non-peritonealized pericolic or perirectal soft tissue without regional lymph node metastasis
N2	Metastasis in 4 or more regional lymph nodes	
	N2a	Metastasis in 4-6 regional lymph nodes
	N2b	Metastasis in 7 or more regional lymph nodes

Table 1.3 Summary of distant metastasis (M) pathologic classification of colon and rectal tumours based on International Union Against Cancer TNM Classification of Malignant Tumour, 7th ed. 2009 (Sobin *et al.* 2009).

M - Distant Metastasis		
M0	No distant metastasis	
M1	Distant metastasis	
	M1a	Metastasis confined to one organ (liver, lung, ovary, non-regional lymph node(s))
	M1b	Metastasis in more than one organ or the peritoneum

Table 1.4. Summary of Histolopathological Grading (G) classification of colon and rectal tumours based on International Union Against Caner TNM Classification of Malignant Tumours 7th ed. 2009 (Sobin *et al.* 2009), and the World Health organization, Tumours of the colon and rectum (Hamilton et al. 2010).

G - Histopathological grading (TNM classification manual)	
GX	Grade of differentiation cannot be assessed
G1	Well differentiated
G2	Moderately differentiated
G3	Poorly differentiated
G4	Undifferentiated

Table 1.5 A summary of the morphological and microscopic criteria to distinguish specific polyps and adenomas diagnosed within the colon and rectum.

					Microscopic Features				
Type of polyp	**Frequency of Diagnosis (%)**	**Location**	**Elevation**	**Diameter (cm)**	**Crypts**	**Serration**	**Nuclei**	**Cytological atypia / Architectural dysplasia**	**References**
HP	75%	LC & R	Slight	<0.5	Elongated	Yes	small, uniform, basal	No	Aust *et al.* 2010
TSA	1-6%	LC (60 %); RC (30-40%) & R	Polyploid	0.1	ECF	Yes	vesicular and stratified	Yes; IEN, CE	Aust *et al.* 2010
SSA	15-20%	RC	sessile	> 0.5	T- & L-shaped branching at base; Inverted below the muscularis mucosae; Columnar	Yes	Vesicular nuclei with nucleoli	Yes; Mature goblet cells at the base of the crypts, Shifting of the proliferation zone to the middle 1/3 of the crypts	Aust *et al.* 2010

Continued on next page.

Table 1.5 Continued.

Type of polyp	Frequency of Diagnosis (%)	Location	Elevation	Diameter (cm)	Microscopic Features				References
					Crypts	Serration	Nuclei	Cytological atypia / Architectural dysplasia	
CA	60%	LC & R	Pedunculate or protuberant	< 1	Straight – irregular glands with variable Branching	V	Nuclear enlargement and stratification, mitoses and nuclear apoptosis are frequent	Yes; CE, presence of IEL and enterocytes, Dystrophic goblet cells	Pai *et al*. 2010 Li *et al*. 2007
AP	Rare	V	V	Combination of two or more polyps/ adenomas listed above	V	V	V	V	Aust *et al*. 2010

Abbreviations: left colon (LC); right colon (RC), rectum (R), ectopic crypt formation (ECF), intraepithelial neoplasia (IEN), cytoplasmic eosinophilia (CE), conventional adenoma (CA), mixed/ admixed polyp (AP), intraepithelial lymphocytosis (IEL), and Variable (V)

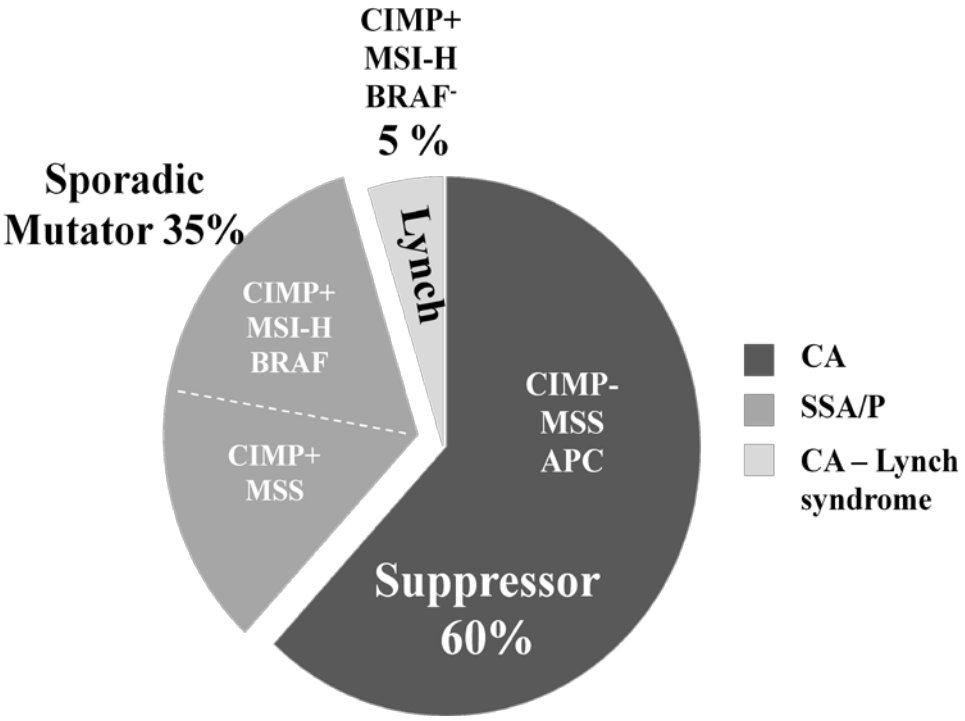

Figure 1.1. A chart summarizing the molecular pathways of carcinogenesis and its associated precursor lesion resulting in CRC development. Each of the three major pathways, suppressor (dark grey), serrated (grey), and mutator (light grey) are shown by percentage of total CRC. Precursor lesions are provided in the legend on the right side of the chart. A summary of genetic characteristics that distinguish each pathway is provided on each facet of the chart.

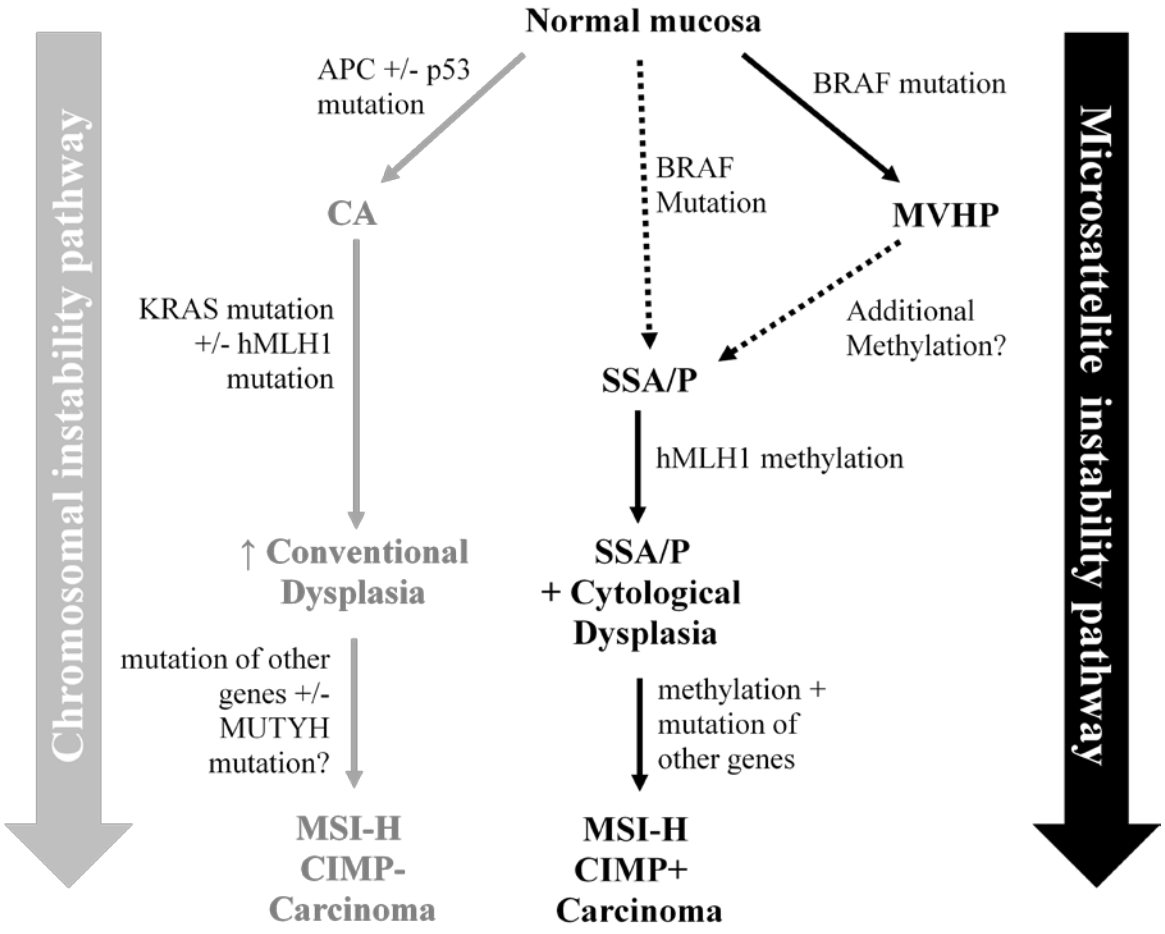

Figure 1.2. A summary of the serrated carcinogenic pathway of serrated adenomas / polyps to carcinomas. Both subdivisions of the serrated pathway, chromosomal instability (in grey arrows and font) and microsatellite instability (in black arrows and font) are shown on each side of the diagram.

V. Literature review

Adenocarcinomas that develop within the colon originate from a variety of different precursor lesions and molecular pathways. The distinction between SSA/P and HP is important because of the difference in clinical significance of these lesions as SSA/P are now recognised precursors to CRC, whereas the relationship of HP to SSA/P and/or CRC is still to be fully elucidated. Histologic examination is not entirely specific in distinguishing these lesions. Accordingly, the focus of this section is to summarize and clarify the differences between HP and SSA by highlighting their differences at the morphological and genetic level. Understanding the differences between HP and SSA is important for correctly diagnosing polyps and will improve treatments for afflicted patients to prevent CRC.

Over the last decade, histologic features of SP have been refined, and correlated with molecular pathways of carcinogenesis. SSA/P and HP are distinguished by their architectural rather than cytological features. HP are the most common serrated lesion, accounting for greater than 75% of SP (Goldstein et al.2003). They are most common within the distal left colon and rectum, where as SSA/P are more common in the right colon (Goldstein 2005). HP are generally small lesions ranging from less than 0.5cm up to 1.5cm. SSA/P are often larger lesions measuring greater than 1 cm in diameter.

HP are classified into three histological subtypes: (i) microvesicular type (MVHP), (ii) goblet cell–rich type (GCHP) and (iii) mucin poor type (MPHP). The classifications are based on distribution, morphology and molecular characteristics and are summarized in Table 1.5 (Li *et al*. 2007). All three subtypes are histologically similar due to the presence of crypt elongation, luminal dilation and their variable degree of

serration. In addition, HP subtypes possess small hyperchromatic nuclei that lack cytological dyplasia in the base of the crypts (Compton *et al*.2008).

GCHP are exclusively observed in the left colon and composed primarily of goblet cells with subtle crypt serration. Some GCHP variants tend to display high susceptibility to KRAS mutations and they are infrequently associated with SSA (Huang *et al*. 2010).

MVHP are histologically defined by straight serrated crypts and typically do not display cytological atypia. MVHP have proliferative changes located within the basal portion and also contain epithelial cells which are composed of microvesicular mucin (Hamilton *et al*. 2010). In addition, MVHP have a varying degree of goblet cells dispersed around the luminal serrations (Li *et al.* 2007;Snover *et al*. 2010). A subset of MVHP typically demonstrate molecular findings found in SSA/P, most specifically BRAF mutations that are associated with increased susceptibility to hypermethylation (Jass *et al*. 2000, Goldstein *et al*. 2003, Huang *et al*. 2010). This finding supports the theory proposed by J.R. Jass in 2000 and Hawkins *et al.* 2001, that HP particularly MVHP, represent the precursor lesion for SSA/P and the initial precursor lesion for right sided MSI carcinoma.

MPHP occur very rarely and little is currently known about this variant form of HP. The morphologic architectural differences that are known distinguishing MPHP from other HP, are listed in Table 1.5.

The term SSA/P was initially coined from a colorectal serrated polyp reappraisal study conducted in 1996 and this study has since served as a standard for describing differences between SSA/P, HP and TSA (Torlakovic *et al*.1996). SSA/P appear to

represent 15 to 25% of all serrated polyp types, and may represent a majority of SP in the right colon (Lu *et al*. 2010). They appear endoscopically as sessile, slightly elevated, lesions, with a variable degree of mucin typically covering the lesion. SSA/P are generally larger than right-sided HP, but can be difficult to histologically differentiate from HP due to their similar morphological appearance to MVHP variants in the right colon (Li *et al*. 2007). Studies examining SSA/P located in the right side of the colon have indicated that these lesions are associated with a high frequency of CRC (Lu *et al*. 2010).

SSA/P are diagnosed through the identification of atypical architectural features not generally found in HP. To be considered an SSA/P, the polyp must include the following architectural features: (i) abnormal-asymmetrical proliferation away from basal crypt, (ii) decreased apoptosis, (iii) excess serration located near the basal crypt,(iv) branching and dilation of basal crypts (as an L or T- shaped growth pattern),(vi) and parallel (horizontal) growth of crypts to adjacent muscularis mucosa (Figures 1.3 and 1.4) (Snover *et al*. 2005). Subtle nuclear alterations will also be present in SSA/P and include: (i) prominent nucleoli, (ii) open chromatin, (iii) irregular nuclear contours and (iv) mitoses within the upper third of the crypt or on the surface of the adenoma (Table 1.5). The archetypical cell type in the crypts of a SSA/P are seen as prominent mature epithelial cells with increased goblet cell or gastric foveolar cell phenotype situated at the base of the crypt which replace the normal proliferative zones demonstrating the L or T-shaped pattern (Li *et al*. 2007, Snover *et al*. 2005). In general, pathologists do not rely on cytologic features in the diagnosis of SSA/P, as architectural features are easier to assess. It is recommended that increasing the frequency of endoscopic follow-up exams for

patients diagnosed with SSA/P is essential to preventing the development MSI CRC (Snover 2011). SSA/P by definition do not demonstrate cytologic dysplasia. When cytologic dysplasia evolves in these lesions, they are classified as SSA/P with dysplasia and are felt to have progressed along the serrated pathways of carcinogenesis. The development of dysplasia is felt to be a mandatory precursor to carcinoma in the serrated pathway. However, recent studies suggest that even SSA/P without dysplasia may evolve into cancer more quickly than routine adenomatous polyps. Recent surveillance recommendations reflect this thinking, and SSA/P are considered equivalent to "advanced colorectal neoplasia" in surveillance recommendations by some authors (Snover 2011; Trediman *et* al.2010). SSA/P are diagnosed in 1- 4 % of the North American population and they represent 1 – 9 % of all colorectal polyps (Snover 2011). The average patient diagnosed with SSA is approximately 61 years of age and female in gender (Carr *et al*. 2009, Goldstein *et al*. 2003, Sandmeier *et al*. 2007).

TSA are characterized by a pedunculated endoscopic appearance, an overall villiform growth pattern and serrated morphology, and demonstrate a specific type of dysplastic epithelium of "serrated" type. These cells are elongated and pencillate with eosinophilic cytoplasm. More recently TSA have been defined by the presence of so called "ectopic crypt foci" (ECF) an architectural abnormality in which crypt basis are not anchored to the underlying muscularis mucosae (Torlakovic *et al*. 2008). TSA are very rare and less common than other serrated polyps of the colon and rectum. TSAs are diagnosed at a rate of 1% or less (Snover *et al*. 2010). These serrated polyps are usually found in the distal colon and rarely cause clinical symptoms. It is suggested that TSA evolve into carcinoma through the development of "conventional dysplasia" utilizing the

chromosomal instability pathway. However, the malignant potential and molecular pathways of TSA are presently poorly defined, given that older literature did not distinguish between TSA and SSA/P (Snover 2011; Li & Burgart 2007). The architectural presentation of TSA is further defined in Table 1.5.

In summary, it is still uncertain whether SSA/P can arise from pre-existing HP. Morphologic and molecular similarities suggest that this is the case. However, it is likely that this evolution is largely restricted to the right colon. Recent literature has clarified the diagnostic criteria distinguishing SSA/P from HP (Snover 2011). Although inter-observer reliability is poor, partly due to concern over diagnostic accuracy, recent clinical recommendation suggest that all right sided HP and all HP greater than 1 cm in dimension be treated as neoplastic lesions (Tradiman *et al.* 2010). This is seen in our retrospective study along with current literature on this topic

VI. Objectives and Hypothesis

The primary objectives of this study were two-fold. Firstly, the diagnostic frequency of HP and serrated polyps (SSA/P with and without dysplasia, TSA, and SA) in Winnipeg, Manitoba was determined over a recent specified interval, in relation to the total polyps diagnosed. The frequency of occurrence of serrated lesions was compared to previously published national and international studies to determine whether the serrated polyps are being under or over diagnosed.

The second objective of this study was to review a subset of right sided HP diagnosed as HP to determine if serrated polyps were being under diagnosed in this group. The hypothesis of this study was that a significant number of these lesions may have been diagnosed as HP and would meet the criteria of SSA/P since SSA/P are a relatively new diagnostic category. Based on information documented within the literature review, the majority of revised serrated polyp diagnoses were expected to occur in right sided HP of the large intestine.

VII. Materials and Methods

VII.1 Consent/ permission for the colorectal polyp study.

Consent for the polyp slide review for this study was granted by the chair of the Diagnostic Services Manitoba (DSM) Quality Committee. Permission was obtained to revise reports based on the findings from this survey according to DSM discrepancy review guidelines. Re-classification to a diagnosis of SSA/P or TSA was considered to be minimally disruptive to patients as management adjustments would fall within recommended colonoscopy surveillance intervals.

VII.2 Case selection for this study.

All colorectal polyps selected for this study were obtained from the patient report archive of the Diagnostic Services of Manitoba Delphic Anatomical Pathology (DSM AP) system. This laboratory information system (LIS) contains all surgical pathology reports reported in AP laboratories within the city of Winnipeg, Manitoba.

The patient population for this retrospective analytical study consisted of all available cases meeting polyp inclusion criteria and resulted in a total of 4,096 initial cases. Cases were limited to patients who underwent colonic polypectomies and/or biopsies of the right or left colon over one year (January 1, 2009 to December 31, 2009). Reports were searched in the text as well as the diagnosis section for the following features: date (January 1, – December 31, 2009), site (colon or rectum), and key words ‘hyperplastic polyp’, ‘adenoma’, ‘adenomatous polyp’, ‘sessile’ and ‘serrated’. All retrieved reports were manually reviewed by the author.

Regions of the proximal and distal large bowel were designated according to the AJCC cancer staging manual, 5th editon guidelines (AJCC 2000) and measured starting

from the anal verge. Polyps located at sites less than 82 cm from the anal verge were defined as 'distal' colonic lesions and polyps at sites greater than 82 cm from the anal verge were defined as 'proximal'. Locations described as 'rectal', 'rectosigmoid', 'sigmoid' and/or 'descending colon' were considered to be part of the 'distal' colon in this study. In contrast, locations referred to as 'splenic flexure' 'transverse', 'hepatic flexure', 'ascending colon' and 'cecum' was considered to be part of the proximal colon. Proximal and distal serrated polyps were classified into the subtypes of HP and serrated polyps: TSA, SSA/P, SA and mixed SSA/P.

VII.3 Selection criteria used for reassessment of HP within the right sided colon.

As outlined by the second objective of this study, a total of 204 cases of HP were selected for pathologic reassessment. This included 121 right sided HP from patients without associated adenomatous polyps. Eighty eight additional left sided HP were included in the review to eliminate expectation bias and to test the hypothesis that re-classification might be more common in right sided than left sided HP. All cases underwent a preliminary slide review by Dr. H.R.W. Cases that were deemed 'suspicious for possible SSA' were further reviewed by a second GI pathologist Dr. R.G. and a consensus diagnosis was achieved between both pathologists.

All calculations used to analyze serrated polyps in this study were calculated using statistical formula in Microsoft Office Excel 2007. The only exception was the calculations for the two tailed Fisher exact test which was performed using the online website GraphPad Quick Calcs.

Diagnostic criteria used for both the preliminary and final HP reassessment was made based on established criteria. In brief, SSA that demonstrated no evidence of

cytologic dysplasia but demonstrated dysmaturational crypt formation in the form of basal crypt dilation, and serration were selected for the study. Additional architectural features included in the diagnostic criteria were the presence or absence of horizontal crypts, branch crypts, and inverted crypts. Cytological features considered in the diagnostic criteria also included nuclear rounding with prominent nucleoli and upper crypt mitotic activity. It is understood that in diagnostic practice, the distinction between SSA and HP relies predominately on architectural abnormalities that reflect abnormal cell proliferation. Diagnostic criteria used to distinguish TSA from HP were considered to be serrated polyps with cytologic atypia amounting to dysplasia. A summary of these diagnostic criteria is outlined in detail in Table 2. An example of a reassessed SSA/P found during the HP reassessment is provided in figures 1.3 and 1.4.

Table 2. A summary of the histological evaluation criteria used for the reassessment of HP. Reassessment criteria were determined from the histological evaluation guidelines described by Farris *et al*. 2008.

Histological evaluation criteria used during reassessment of selected HP to SSA

1. **Determine if increased mitotic activity within the middle and/or superficial crypt region is present.**
2. **Determination of predominant nuclear shape**
   - **round to oval**
   - **mixed with columnar or flat in superficial crypt region**
3. **Assess the degree of nucleolar prominence**
   - **Detect the presence of eosinophilic (prominent) in middle and/or superficial crypt region.**
4. **Indentify if serrated architecture starting at basilar crypt region is present**
5. **Determine if other architectural features are present:**
   - **horiztonal crypts**
   - **branched crypts**
   - **inverted crypts adjacent to muscularis mucosae**
6. **Determine if dilation of $\geq$ 10% of crypt base is present within the polyp**
7. **Determine if minimal or no epithelial nuclear maturation in $\geq$ 50% of crypts has occurred**
8. **Assess if other characteristic features suggestive of abnormal maturation are present**
   - **Goblet cells in crypt bases**
   - **distended L-shaped or T-shaped crypt bases**
   - **abundant eosinophilic cytoplasm in superficial crypt area**

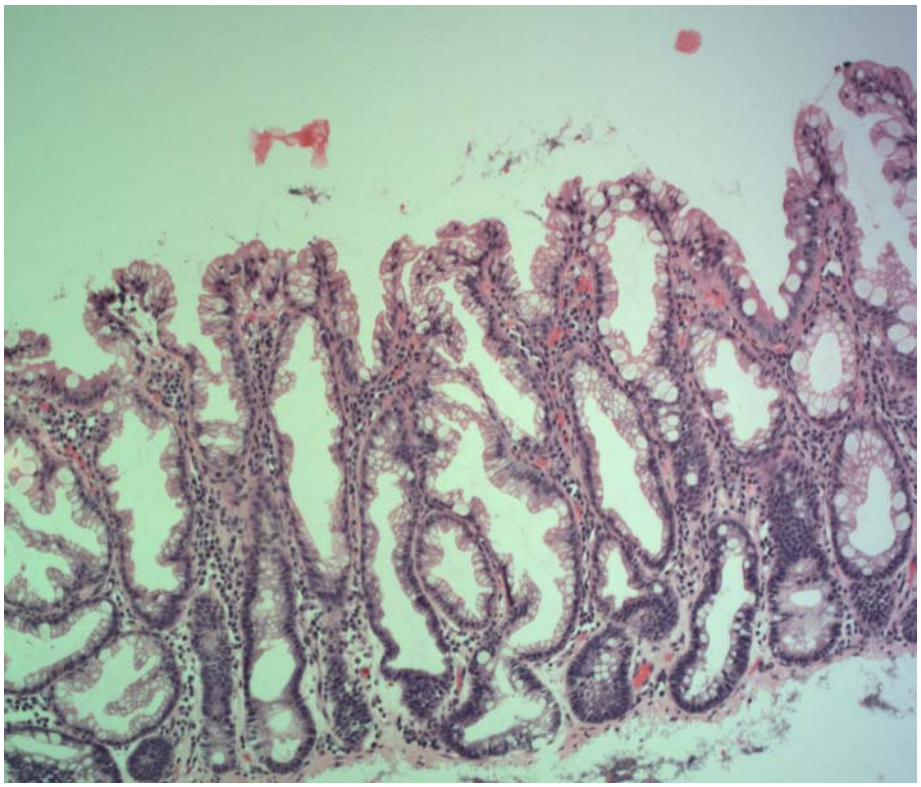

Figure 1.3 A randomly selected image of a typical SSA/P within the right sided colon of a patient from this HP reassessment that displays dilated basal crypts and luminal serration.

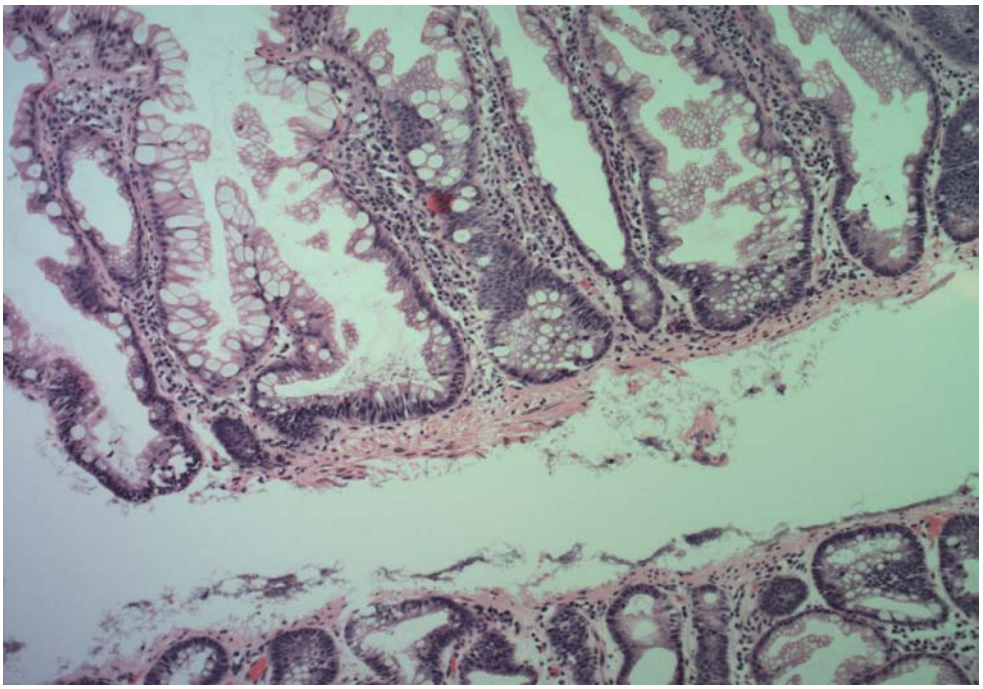

Figure 1.4 A randomly selected image of a typical SSA/P found during the reassessment of the right sided HP in this study. This image of an SSA/P (top left sided quadrant if the image) displays all the characteristic branching and dilation of the crypts as L or T shaped pattern along with exaggerated deep crypt serration.

VIII. Results

The primary aim of this study was to determine the extent of serrated polyp diagnoses and the accuracy of HP diagnosed from a one year survey of colorectal polyps in Winnipeg, Manitoba.

VIII.1 Serrated lesions determined in the original survey generally follow literature norms.

A review of all serrated polyps (SSA/P, TSA, SA, and SSA/P with dysplasia) was conducted from the 4,096 colorectal polyp cases. A summary of all serrated polyps surveyed for this study is provided in Table 3.1. Only polyps diagnosed as HP from either the right or left sided bowel were included in the pathologic reassessment. Serrated polyps were not pathologically reviewed. The average age of serrated lesion occurrence for both male and female patients ranged from 62-65 years in this survey and was higher than the 55 year average reported in previous studies (Edge *et al*. 2010; Kumar *et al*. 2010). After surveying all serrated polyps in the original 2009 samples, the total percentage of HP in the large intestine (on both the right and left side) consisted of 71% of all serrated lesions in the large intestine. Previous studies have reported that HP should account for more than 75% of all serrated lesions in the large intestine (Huang *et al*.2010; Snover *et al*. 2010).

As shown in Figures 2.1 and 2.2 along with Table 3.1, serrated polyps make up approximately 5% of the total polyps in patients and this number is consistent with the literature (Snover 2011). Additionally, the majority of serrated polyps are identified in the proximal colon (62%) and in particular, 70% of SSA/P are identified in the proximal colon. These findings are consistent with the literature (Lu *et al*. 2010; O'Brien 2007).

Within the overall group of non-HP serrated polyps, 54% of the were classified as SA. TSA were rarely diagnosed (2.5% of the non-HP serrated polyps). Mixed SSA/P represent 4.6% and SSA/P's represent 39% of the non-HP serrated polyps. Given the limited number of TSA, further subgroup analysis was not indicated. The SA group will be further addressed in the discussion.

VIII.2 Reassessment of HP

One hundred and twenty one proximal HP in patients without associated adenomas were pathologically reassessed as well as 88 randomly selected left colon HP. Five right sided polyps were excluded as they were felt to demonstrate normal mucosa or mucosal prolapse. The polyps were reviewed by Dr. H.R.W. and subgroups of "possible" SSA/P were further reviewed with Dr. R.G. A consensus was achieved by discussion (Tables 3.2 & 3.3). Based on the final HP reassessment, the conversion of HP to SSA/P was 20% in males and 44% in females within the proximal colon, for an overall conversion rate of 34% in the proximal colon (Table 3.2). The overall conversion rate of HP to SSA/P in the left colon was 3%. The difference in re-classification rates between the right and left colon was highly significant (< 0.0001 *P* value). This finding is consistent with the general consensus that SSA/P are much more common in the proximal (right) colon (Sandmeier *et al.* 2007; Terdiman *et al.* 2010). The difference in conversion rates between males and females was also statistically significant although completely unexplained.

SSA/P were the only serrated polyp to be identified after the final HP reassessment. No conventional dysplasia, TSA or malignancy was identified in the reviewed material (Table 3.2).

Table 3.1 A summary of serrated polyps (SSA, TSA, SA, mixed SSA/P) from the 2009 survey.

	Right-side of colon		Left-side of colon		Total	
	Males	**Females**	**Males**	**Females**	**Total**	**% value***
Number of all patients in survey					4,096	
Number of patients with serrated polyp	60	72	38	38	208	5.0%
Average age of patient	64.7 ±9.8	65.4 ±12.2	63.1 ±10.5	62.0 ±11.3		
Average number of lesions/ patient	1.3	1.3	1.3	1.2	1.3	
Total serrated polyps*						
Total SSA/P	33	33	18	10	94	39%
Total TSA	0	3	3	0	6	2.5%
Total SA	42	32	28	28	130	54%
Total Mixed SSA/P	2	4	4	1	11	4.6%
Total for all non HP serrated polyps	77	72	53	39	241	100%

*Serrated polyps include: SSA/P, TSA, SA, Mixed SSA/P

Table 3.2 Summary of findings from the right and left colon HP reassessment.

	Right sided colon			Left-sided colon		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
Total original HP diagnoses	53	68	121	248	266	514
HP selected for preliminary reassessment	50	66	116	33	55	88
HP selected for double headed review (Final Reassessment)	19	39	58	7	19	26
Total SSA/P after reassessment	10	29	39	0	3	3
Ratio total SSA/P / total original HP	0.20	0.44	0.34	0	0.05	0.03
***P* values for Right vs. Left sided SSA/P, males**						<0.005
***P* value for Right vs. Left sided SSA/P, females**						<0.0001
***P* value for Right vs. Left sided SSA/P, total**						<0.0001
***P* value conversion rate, males vs. females**						<0.01

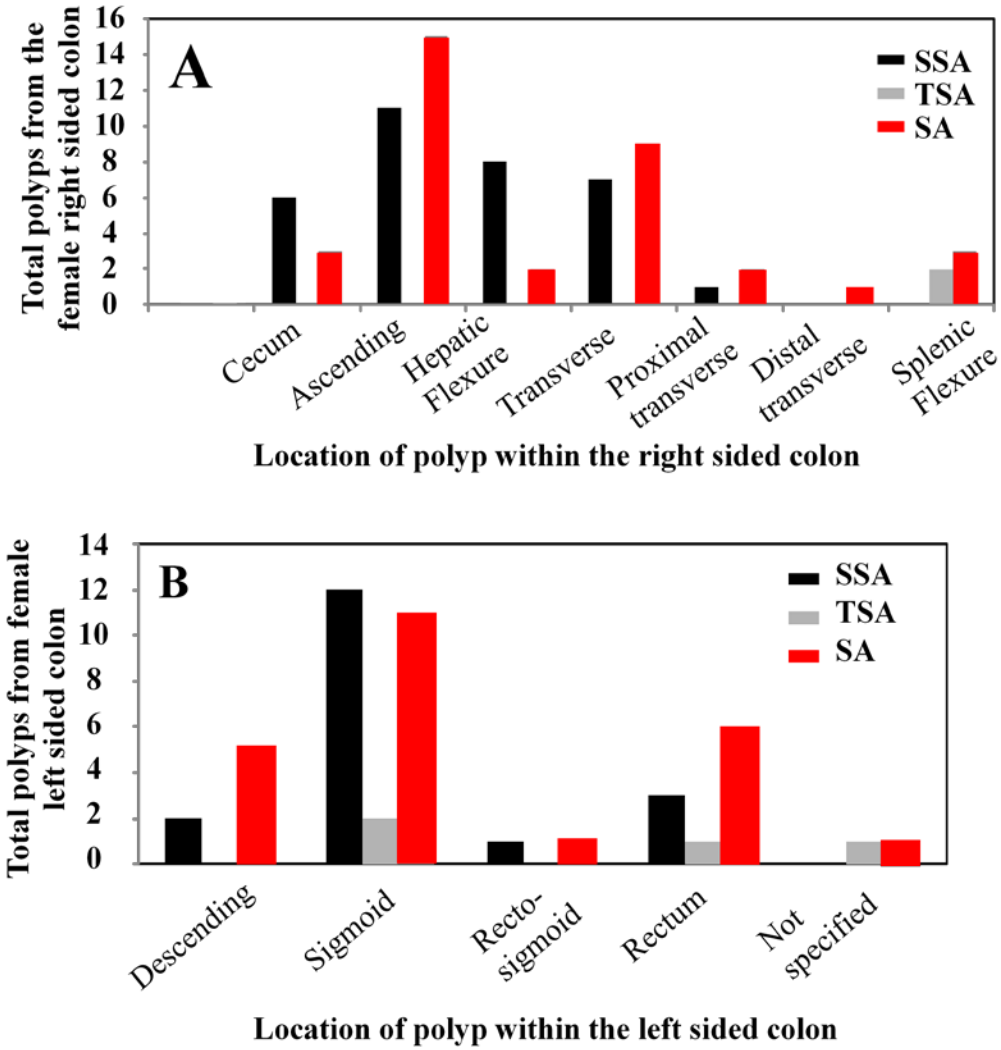

Figure 2.1 A summary of total serrated polyps surveyed from 2009 and their location within the right and left side of large intestine of female patients. Both panels show total female SSA/P, TSA, and SA identified within the right (A) and left (B) side of the colon and specific regions within the colon that have identified HP are listed on the x-axis.

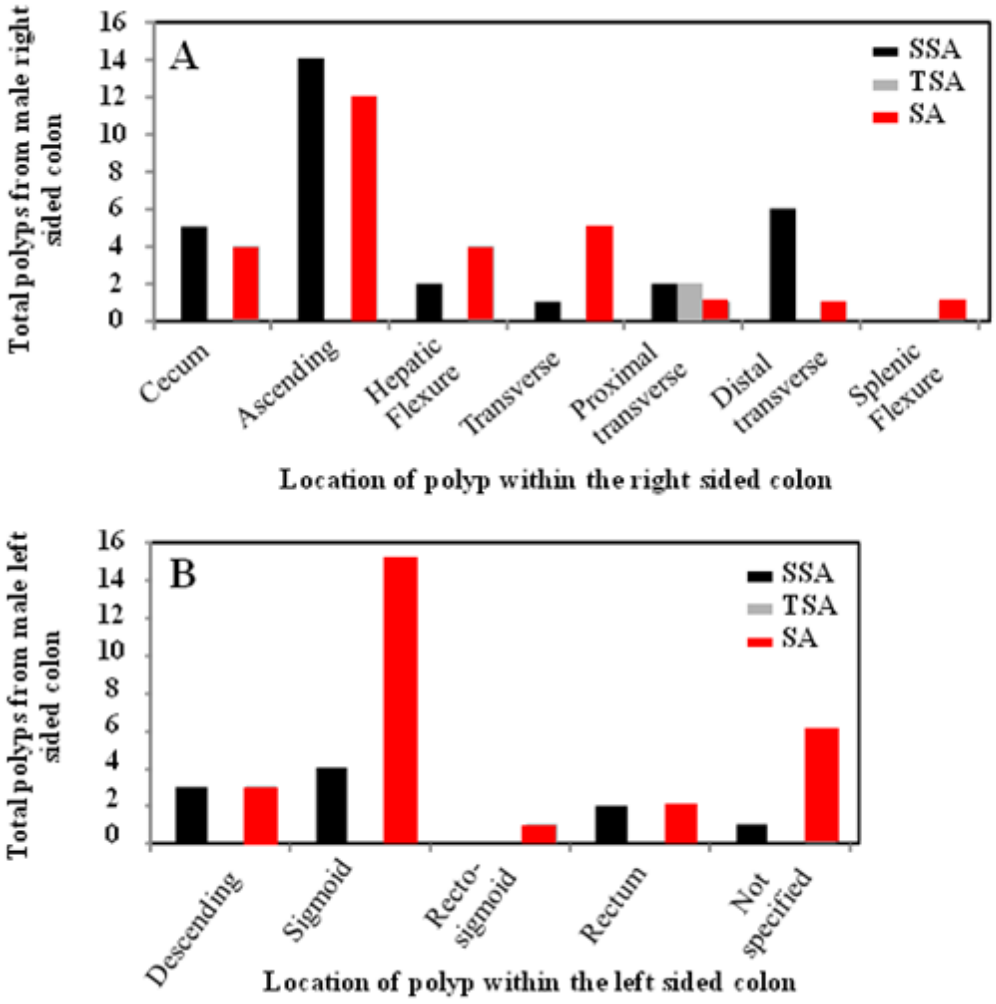

Figure 2.2 A summary of total serrated polyps surveyed over 2009 and their location within the right and left side of large intestine of male patients. Both panels show total male SSA/P, TSA, and SA identified within the right (A) and left (B) side of the colon and specific regions within the colon that have identified HP are listed on the x-axis.

IX. Discussion

CRC is the second most common cause of cancer mortality in Europe and North America, behind lung cancer. Unlike lung cancer, CRC has a well defined non-invasive precursor lesion (neoplastic polyps) which can be detected with reasonable sensitivity by a widely available technology (endoscopy). Prospective studies have suggested that endoscopic surveillance and polypectomy may reduce CRC mortality rates in a screened population by greater than 40% (Brooks *et al.* 2008; Levin *et al.* 2008; Rex *et al.* 2006). These results are excellent, and they can only be matched by PAP screening. The survival benefits of prostate and breast screening programs do not even remotely approach this number (American Cancer Society 2010). Interestingly, there has been an apparent "right shift" with an increase in right sided CRC as opposed to left sided CRC (Baker *et al.* 2004). Additionally, surveillance is less effective in decreasing mortality in proximal CRC as opposed to distal CRC. Explanations for the decreased effectiveness are attributed to the difficulty in fully assessing the proximal colon but also the possibility that the precursor lesions in the proximal colon may progress more rapidly, or be more difficult to appreciate endoscopically or diagnose pathologically.

In 1990, Longacre & Fenglio-Preiser described "serrated adenomas" as a distinct form of colorectal neoplasia. In this study, they initially described the lesion as one demonstrating a serrated morphology without obvious features of dysplasia. The term 'serrated adenoma' gradually evolved into a serrated neoplasm that demonstrated a distinctive variant of cytological dysplasia. This corresponds to the current definition of TSA. This interpretation of the Longacre & Fenglio-Preiser study is supported by the fact that most of the cases in their study were located in the left colon as opposed to the right.

In 2003, Goldstein and colleges defined a subset of HP-like polyps associated with right (proximal) colonic cancers. In the same year, Torlakovic and colleagues critically assessed serrated polyps in the right colon and defined them as subset of HP with "abnormal proliferation" but no true dysplasia. Theses HP-like polyps showed many similarities to those polyps identified within the Goldstein paper. These HP-like polyps are currently termed SSA/P and are believed to be the precursor of some of the right colonic cancers. SSA/P can be difficult to appreciate endoscopically due to their sessile nature and the frequent presence of obscuring mucin. Furthermore, concordance studies have demonstrated that SSA/P are difficult to distinguish from other SP even in a research setting (Aust *et al.* 2010; Huang *et al.* 2010).

Of the 4,096 patients in our study, a total of 208 patients were diagnosed with serrated polyps (SSA/P, TSA, SA and SSA/P with dysplasia) and these lesions amounted to 5% of the total patient population. This number is approximately equivalent to several population based studies in the literature (Carr *et al.* 2009; Lu *et al.* 2010; Goldstein *et al.* 2003). However, recent literature suggests that the vast majority of serrated polyps are SSA/P and the diagnosis of an SA is (as opposed to TSA or SSA/P) considered archaic. Only 39% of the total serrated polyp group was classified as SSA/P, where 54% were diagnosed as SA and only 2.5% as TSA. Overall, less than 0.2% of the total patients were diagnosed with a TSA and this value is lower than recent literature (Snover *et al.* 2005; Bauer *et al*. 2008). However, TSA are considered to be the rarest of all serrated polyps and their biologic potential is uncertain (Snover 2011). Obviously, the SA population contains an admixture of SSA/P, TSA, and CA with serrated architecture. This group was

not further assessed pathologically in this study as there was no significant risk of under diagnosis in this population.

The SSA/P population within this study did demonstrate the expected predominance within the right (proximal) colon. 70% of all SSA/P cases were diagnosed on the right side. With some rare exceptions, previous studies have reported similar or even higher predominance of right sided SSA (Goldstein *et al*. 2006; Lu *et al.* 2010)

The pathologic reappraisal of right sided HP demonstrated several interesting findings. This group was specifically selected as having right sided HP in the absence of associated adenomatous polyps. This was the group considered to be at most risk of under-surveillance. This was a relatively small group making up approximately 2.5% of the total patient population. After review by two pathologists with a special interest in GI pathology, 34% of right sided HP were re-classified as SSA/P, a total of 39 patients. Interestingly, no TSA, CA or malignancies were diagnosed in this group. This finding is consistent with the ability of pathologists to reliably diagnose conventionally dysplastic lesions.

The pathologic reassessment was based on established predominantly architectural criteria. The accuracy of this assessment was supported by a marked difference in the re-classification rate between right and left sided HP. The latter were mixed into the review material in a blinded fashion, eliminating expectation bias. Only 3.4% of the left sided HP were re-classified as SSA/P in comparison to 34% of right sided HP (P value <0.0001). It is noteworthy that the right sided HP of females were re-classified more frequently than right sided HP from males (44% versus 20%, P value <

0.01). The reason for this finding is unknown to us. To date, SSA/P have not been identified to demonstrate a female predominance.

The conversion rate of right sided HP to SSA/P in this study is consistent with other population based studies in the literature (Carr *et al*. 2009; Lu *et al*. 2010). In a recent study by Lu *et al*. 2010 (from Vancouver, British Columbia), a review of HP demonstrated that 50% of right sided HP were re-diagnosed as SSA/P. In an Australian study by Carr *et al*. 2009, 32% of HP were re-classified as serrated polyps and 25% of these were diagnosed as SSA/P. This latter study included cases as recent as 2006 in contrast to the Vancouver study which ended in 2001. The lower HP conversion rates noted in the Carr 2009 study and the conversion rates of this analysis relate to "partial" SSA/P diagnosis of right sided SP and the later year of diagnosis, when the serrated polyps have become a more widely recognised entity.

SSA/P are considered by some to have at least an equivalent risk of malignant progression as CA, despite their lack of dysplasia (Snover 2011). The recent Vancouver study by Lu *et al*. 2010 suggests that there is a significantly higher risk of CRC progression for SSA/P than routine adenomatous polyps. Clinical recommendations for the follow up of SSA/P suggest that they should be considered equivalent to "advanced colorectal neoplasia" in terms of surveillance intervals and treatments (Tradiman *et al.* 2010). Similar to adenomas demonstrating larger size (greater than 1 centimetre), high grade dyplasia or significant villousity, a 3 year surveillance interval is recommended after complete SSA/P polyp removal. Furthermore, some authors recommend that all right sided serrated polyps including HP and all large left sided serrated polyps be treated and followed as SSA/P. This suggestion is understandable, given the difficulty in

diagnosing these lesions and the relatively small number of patients affected. However, the patients with right sided HP would be over-treated by this approach. Alternatively, the sign out pathologist should consider routine consultation in these cases, preferably with a colleague experienced in this area. Given the relatively small number of right sided HP and SP in a given population, any increase in work load would be minimal.

X. Conclusion

In summary, this study suggests that serrated polyp rates in the 2009 patient population are relatively similar to those reported in previous studies. However, the majority of these serrated polyps are diagnosed using archaic terminology. A diagnosis of "serrated adenoma" without a qualifier is no longer acceptable. In cases where a distinction between a TSA or a SSA/P cannot be made a comment reflecting this ambiguity can be made in the pathology report. Furthermore, SSA/P are still being significantly under diagnosed in the population of right sided HP and SP. As patients with right sided HP without adenomas are relatively uncommon, this potential under-diagnosis affects only 1% of our studied population. The complete absence of under-diagnosed conventional dysplasia confirms pathologic consistency in this area. We recommend that right sided HP be routinely reviewed prior to pathologic sign out, particularly in those patients without associated adenomas detected elsewhere in the colon. As SSA/P are potentially aggressive lesions and pathologic inter-observer variability is relatively high, one should consider treating all right sided serrated polyps, including HP as neoplastic precursors with appropriate excision and follow up.

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