MESORECTAL EXCISION: A SURGICAL AND PATHOLOGICAL AUDIT IN WINNIPEG

By

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Abstract

Introduction: Colorectal cancer (CRC) is the malignant neoplasm of the colon or rectum. Total Mesorectal Excision (TME) refers to the complete sharp surgical resection of the perirectal soft tissue, resulting in a smooth, circumferential radial margin. The completeness of a mesorectal excision can be evaluated based on the integrity of the radial margin, which is crucial for pathological examination, as it is important in predicting local and systemic recurrence. Our objectives of this study are to assess the quality factors of the mesorectal excisions and to discuss the importance of the proper macroscopic and microscopic pathological examinations of rectal and rectosigmoid cancer in Winnipeg.

Material and Methods: This retrospective cohort study of mesorectal excision specimens with rectal carcinomas in Winnipeg from 2012 – 2015 includes 453 cases. Statistical analyses including descriptive statistics, the analysis of variances, and pair-wise comparison are performed.

Results and Conclusions: The local incomplete mesorectal excision percentage is 10.9 % (SD ± 4.1%), which is compatible with the previously published data worldwide.[1] Surgical quality is highly related to radial margin involvement, the surgeon's training background, and volume. Surgical performance and accuracy of pathologic assessment have been slightly increased from 2012 to 2015.

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List of abbreviations

AJCC	American Joint Committee on Cancer
ANOVA	Analysis of variance
AP	Anatomic Pathology
APC	Adenomatous Polyposis Coli
APR	Abdominoperineal Resection
BMI	Body Mass Index
CAP	College of American Pathologists
CCND1	Cyclin-D1
ChXRT	Long Course Chemoradiotherapy
COX-2	Prostaglandin-endoperoxide Synthase 2
CRC	Colorectal Cancer
CRM	Circumferential Radial Margin
DSM	Diagnostic Service of Manitoba
FAP	Familial Adenomatous Polyposis
HNPCC	Hereditary Nonpolyposis Colorectal Cancer
KRAS	Kirsten-Ras
LAR	Low Anterior Resection
LIS	Laboratory Information System
LOH	Loss of Heterozygosity
NASID	Nonsteroidal Anti-Inflammatory Drug
NCP	Non-Canadian trained Pathologist working as a PA
OT	Onsite Trained
PA	Pathologists' Assistant

PHIA	Personal Health Information Act
PME	Partial Mesorectal Excision
PT	Program Trained
SPSS	Statistical Package for The Social Sciences
Sxrt	Short Course Radiation Therapy
TCF family	Transcription Factor Family
TGF-β pathway	Transforming Growth Factor Beta Pathway
TME	Total Mesorectal Excision
TNM	Tumor Nodes Metastases
TSTME	Tumor Specific Total Mesorectal Excision

Chapter I: Introduction

Colorectal cancer (CRC) is the 3rd most commonly diagnosed cancer and is the 2nd most common cause of cancer-related mortality in Canada.[2] In Manitoba, the incidence is 84.4/10,0000, which is even higher than the Canadian average of 76.2/10,0000.[2] Approximately one-third of the CRC occurs in the rectal and rectosigmoid region, which makes it one of the hot spots for developing malignant neoplasms.[3] The current treatment for rectal and rectosigmoid cancer includes the combination of medication, radio/chemotherapy, and surgical excision. The mesorectal excision quality is highly related to the local/systematic cancer recurrence.[4] Therefore the mesorectal excision quality, as well as the surgical specimen macroscopic and microscopic examination, is crucial for improving patient's outcomes and management. In this project, we will discuss the mesorectal excision quality and pathological examination of the surgical specimens from 2012 to 2015. Hopefully, this research will contribute to an improved patient care for future patients.

I.1: Colorectal cancer

I.1.1: Colorectal cancer basics

CRC is the development of a malignant neoplasm in the portion of colon or rectum of the bowel.[5] Adenocarcinoma is the most common histologic type of malignant neoplasm in the large bowel, making up approximately 98% of CRC.[6] The other rare types include carcinoid, lymphoma, squamous cell carcinoma, and sarcoma, which is distinctive from adenocarcinoma with different etiology, pathogenesis, morphology, epidemiology, and prognosis. Since the vast

majority of CRC is adenocarcinoma, which is corresponding to this retrospective cohort study, only adenocarcinomas will be discussed here.

CRC is believed to be highly associated with a low intake of fibre and a high intake of carbohydrates and animal fat in the diet.[7] This also explains the fact that the CRC incidence is higher in the Western developed countries.[8] The idea behind this is that a low fibre intake in the diet is known to cause constipation, leading to an alteration of the intestinal normal flora. A "disrupted" microenvironment of the intestinal normal composition can lead to the accumulation of metabolic bystander products of bacteria which can prove to be potentially harmful and can lead to CRC long term.[9] Although not completely understood, a high fat or carbohydrates diet is believed to result in an increased production of bile and cholesterol in the liver. This excessive secretion of bile and cholesterol can be stressful to the colonic mucosa, which may cause hyperplastic, metaplastic or neoplastic changes.[9]

In addition to dietary factors, changes in lifestyles are associated with CRC as well. Physical activities increase the metabolic levels, which is beneficial to the person's overall health and minimizes the risk of developing CRC by increasing colon motility, reducing blood pressure, and insulin resistance.[8] Carcinogens in cigarette and alcohol are stressful and cause mucosal injury, which is highly associated with CRC carcinogenesis.[8]

NSAIDs, such as aspirin have been proposed to prevent sporadic CRC carcinogenesis, by the inhibition of COX-2 enzyme, which is over-expressed in approximately 90% of CRC.[9] Similarly, some epidemiology studies suggest that these COX-2 inhibitors, such as Rofecoxib, Etoricoxib or Celebrex not only reduce the risk of developing precancerous lesions but also benefit in reducing the cancer recurrence after surgical removal.[8]

Typically adenocarcinomas start as a precancerous polyp in the epithelial lining of the mucosa and gradually become malignant as the result of multiple proto-oncogene mutations. These mutations can be classified into three major categories: (1) genes causing abnormal cellular proliferation; (2) tumour suppressor genes with function lost resulting in the inhibition of apoptosis; (3) defects in the DNA mismatch repair machinery components genes, which decrease DNA replication fidelity. In sporadic CRC, the most frequently altered genes are related to the Wnt and TGF-β signaling pathways, in which *APC* or *β-catenin* mutations can trigger the genomic instability and further downstream genes mutations, for example, *TP53* and *K-RAS*.[10] Approximate 10-20% of CRC are inherited diseases, which are associated with Lynch syndrome (HNPCC), Gardner syndrome, or Familial Adenomatous Polyposis (FAP).[10]

I.1.2: Pathogenesis of adenocarcinoma of colorectal cancer

Similar to the basic concepts behind the onset of carcinogenesis in other organs, adenocarcinoma can develop as a multiple steps process, due to the combination of the multiple gene alterations, such as proto-oncogenes, tumour suppressor genes, genes that regulate apoptosis, and genes involved in DNA repair.[9] The two commonly altered pathways are *APC/ β-catenin* and microsatellite instability pathway.[9] In addition to the genetic factors, other epigenetic abnormalities, such as gene silencing of tumour suppressor genes after hypermethylation also contribute to carcinogenesis in colon and rectum.[9]

In the colon, the majority of CRC develop through an ordered sequence of events, starting from transformation of the normal colonic epithelium to an adenomatous pre-cancerous

lesion and finally to an adenocarcinoma. The most commonly affected gene in this sequence, the *APC*, negatively regulates *β-catenin* in the Wnt signalling pathway.[9] The most exaggerated form of this is in familial cases. When only one copy of mutated *APC* is inherited to these patients, they usually develop FAP, typically an autosomal dominant inherited disease with more than 100 polyps in the colon, which can be precursors to developing tubular/villous colorectal adenocarcinoma.[9] If left untreated, almost all FAP patients will develop adenocarcinoma by the age of 50; some even before age of 30.[9] According to the "Knudson Hypothesis", adenocarcinomas can develop after the "second hit", such as the functional loss of the other copy of *APC*. The function loss is not only due to the genetic mutation but also as a combination of the hypermethylation of the gene promoter. Functional inactivation of both copies of *APC* is essential for the accumulation of β-catenin. Together with TCF family members and other enhancer elements, the β-catenin forms a regulatory complex in the nuclei to promote transcription of genes controlling proliferation, such as *MYC* proto-oncogene, as well as genes regulate cell cycles, like *CCND1*. [9]

In addition, the TGF-β signalling pathway is known to inhibit cell cycles.[10] Genetic alterations in TGF-β signalling pathway genes, such as mutations or gene silencing in tumour-suppressor genes like *SMAD2* and *SMAD4*, are observed commonly in adenocarcinomas, but not in adenomas.[9] Functional loss of the other tumour suppressor gene, *TP53* is observed in 70-80% of adenocarcinomas.[9] The tumour cells with the *TP53* mutation are able to escape from apoptosis, leading to carcinogenesis.[11] *KRAS* mutations are observed more frequently in an adenocarcinoma than adenoma, suggesting that it is associated with the accumulatively genetic alteration during carcinogenesis (Figure 1).[11]

Furthermore, in addition to the classic adenoma to adenocarcinoma pathway caused by APC/β-catenin, some patients also develop CRC due to the DNA mismatch repair pathway deficiency, like HNPCC and are highly associated with microsatellite instability.[9] Microsatellites are short tandem repeats located across the human genome. The stability of the microsatellites located in the promoter or gene-coding region of genes are responsible for normal cellular function, such as cell cycle regulation, cell differentiation, apoptosis and proliferation.[9] HNPCC is one of the most common inherited types of CRC, responsible for approximately 3% of all cases. Similar to the patients with FAP derived adenocarcinoma, HNPCC patients develop CRC at a younger age than sporadic CRC patients.[9]

The "Knudson Hypothesis" can also be applicable to the carcinogenesis of HNPCC.[9] The functional loss of one copy of DNA mismatch repair genes, such as *MLH1*, *MSH2*, *MSH6*, *PMS1*, or *PMS2* can be inherited or sporadically acquired.[9] Carcinogenesis is triggered by the alternation of the other allele, such as sporadic mutations, CpG island hypermethylation, or LOH, which in sequence results in the abnormal expansion of microsatellite and microsatellite instability of many proto-oncogenes or tumour suppressor genes.[9] For example, the gene function loss of *BAX*, encoding a pro-apoptotic protein BAX that inhibit colonic epithelial proliferation, is observed in HNPCC patients with microsatellite instability.[11] Many other genes regulating proliferation, differentiation, and apoptosis are accumulatively mutated in the process of carcinogeneses, such as *BRAF*, *TP53*, *TCF4*, *IGF2R* and etc.[9] The pathogenesis of colorectal carcinogenesis is summarized in Figure 1.

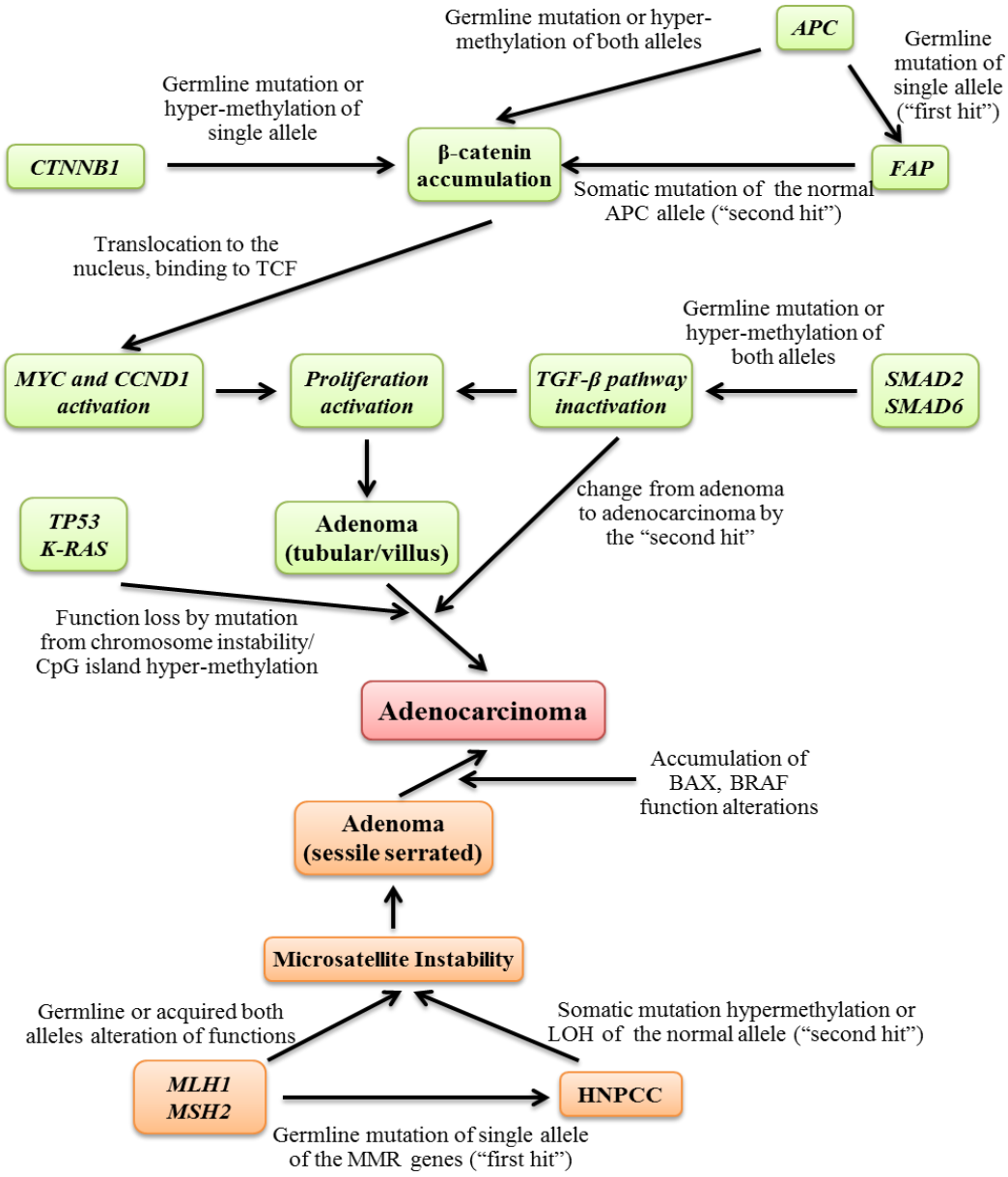


Figure 1: Genetic alterations pathways in the pathogenesis of colonic adenocarcinoma. The "first hit" usually results from a germline mutation, which puts individuals at high risk of developing adenocarcinoma. Loss of function of the second copy of the same gene or other genes termed the "second hit" lead to carcinogenesis. Multiple genes are involved in the sequence of developing tubular/villous adenoma and sessile serrated adenoma to adenocarcinoma and labelled with green and orange, respectively. These genetic changes seem sequential; however, the importance is the accumulation of these changes rather than the specific order.

I.2: Colorectal cancer in Manitoba and Winnipeg

According to the Canadian Cancer Statistics data published by the Canadian Cancer Society, CRC is the 3rd most commonly diagnosed cancer in both genders in Canada.[2] The incidence rate for combined male and female is estimated to be 56 per 100, 000 populations, ranking it the 3rd (13%) most commonly diagnosed cancer.[2] The peak incidence age is between 65-79 for both genders.[2] Approximately 1 in every 14 males and 1 in every 16 females are expected to develop CRC in their lifespans.[2]

In Manitoba, the estimated new cases of CRC are 91.4 and 57.6 per 100,000 population for male and female, respectively. This makes Manitoba the 2nd highest province in Canada of male incidence rate in 2016.[2] CRC is the 2nd most common cause of cancer-related death for males and the 3rd for females, accounting for 12% of all deaths related to cancer.[2] Thanks to the improved diagnosis, better treatment, and the efficient surveillance program advancements that has occurred all over Canada, the age-standardized mortality rate in Canada for CRC gradually dropped from 45 to 28 per 100,000 populations from 1987 to 2016.[2] The overall 5-year survival rate in Canada increased from 55% to 65% during the last 15 years. However, the 5-year survival rate in Manitoba (60%) is slightly lower than that of overall Canadian average (64%) (Table 1).[2] This might be caused by the lower surveillance rate in Manitoba.

Table 1. Colorectal Cancer in Manitoba and Canada

Incidence in 2016 (cases per 100,000 population)

Male	**All cancer**	**Prostate**	**Lung and Bronchus**	**Colorectal**	**Bladder**
Canada	584.4	146.1	85.8	76.2	37.9
Manitoba	554.4	112.5	71.9	84.4	34.9
Female	**All cancer**	**Breast**	**Lung and Bronchus**	**Colorectal**	**Uterus**
Canada	475.9	130.2	62.9	53.9	30.2
Manitoba	485.4	130.1	68.7	48.3	35.4

Source: Canadian Cancer Registry database at Statistics Canada 2016

The "Canadian Cancer Statistics" does not mention the exact percentage of rectal, or distal sigmoid cancers in Manitoba; however, based on the "American Cancer Society" statistics, in the United States, the estimated rectal cancer account for approximately 39,220 of 134,490 (29%) of total colorectal cancer in 2017.[3] This result is supported by the statistics obtained from the "Cancer Research UK", in which 7,327 of 23,282 (32%) and 4,240 of 18,317 (23%) of CRC are rectal cancer identified for male and female respectively from 2010 to 2012 (Figure 2).[12] Therefore, the rectal cancer incidence in Winnipeg is estimated to be approximately 27 and 17 per 100,000 population for male and female respectively. Considering the population of approximately 700,000 people in Winnipeg published by the "Statistics Canada Census Profile",[13] each year we expect to have approximately 190 and 120 rectal cancer in Winnipeg.

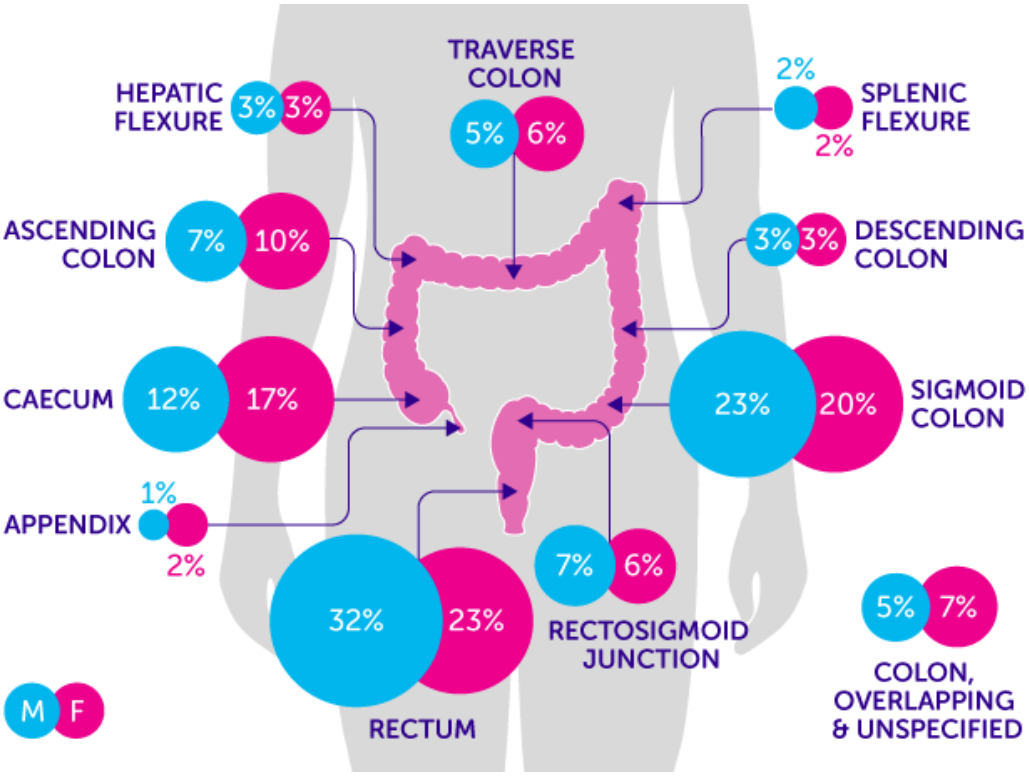


Figure 2. Percentage of colorectal cancer incidence based on anatomical site. (Used with permission of the Cancer Research UK[12])

I.3: Surgical procedure for rectal and rectosigmoid cancer

The surgical treatment for rectal and rectosigmoid cancer depends on the preoperative biopsy result and clinical stages of cancer, including size and possible lymph nodes involvement.[14] Histologically proven malignant polyps, which by definition are invasive adenomatous polyps with the tumour cells breaching the muscularis mucosa, and spreading into the submucosa, are usually removed by polypectomy during endoscopy. No further surgical

treatment is necessary if the resection margin is free of tumour cells and no metastatic lymph nodes are identified on imaging.[15] Similarly, local excision of a small superficial cancer with an adjacent colonic wall does not require a colectomy.[14] Colectomy including total, partial, or segmental is reserved for a larger and more aggressive cancer in the rectal and rectosigmoid colon. The current most commonly used surgical technique for rectal cancer is radical resection, including low anterior resection (LAR) and abdominoperineal resection (APR).[15]

I.3.1 Surgical and anatomical definitions of rectum and function of anal sphincters

The rectum is the most distal portion of the large intestine with a proximate length of 12 cm. It starts from the rectosigmoid junction, at the level of approximately the 3rd sacral vertebra, and ends at the anorectal ring, known as the pectinate or dentate line. It runs against the concavity shape of the sacrum and coccyx, named the sacral flexure. The distal rectum curves posteriorly, forming the peritoneal flexure, which is the site of insertion of the puborectalis muscle, which is part of the levator ani muscle. The rectum has three major transverse mucosal folds (Houston's valves), two on the left and one on the right.

The transition from the sigmoid colon to the rectum is defined clinically at the level of sacral promontory, which is approximately 12 cm from the pectinate line.[15] Unlike the sigmoid colon, the rectum lacks the taeniae coli, appendices epiploicae, sacculations, and semilunar folds. Instead, the rectal wall has circumferential longitudinal muscles.[15]

The definitions of where the rectum starts are slightly different between surgeons and anatomists. Clinically, the tumour is considered a rectal tumour if the location is no more than 16 cm from the anal verge.[15] The rectosigmoid junction is supposed to be at the level of the sacral

promontory, corresponding to the superior rectal artery (superior hemorrhoidal artery), which is always ligated during a surgical resection.[15] This definition might not be applied to every patient as the length of colon, rectum and anus could vary significantly among individuals.

During surgical specimen macroscopic examination, pathologists and pathologists' assistants (PA) use the posterior peritoneal reflection, which almost always corresponds to the level of the superior rectal artery ligature, as the landmark of the rectosigmoid junction. A tumour is considered truly at the rectosigmoid if it straddles the posterior peritoneal reflection (Figure 3). However, because of the concave and convex nature of the rectosigmoid colon, sometimes it is extremely difficult to accurately identify the exact location of a tumour with respect of the level of the rectosigmoid junction, especially when a tumour is in the anterior, antimesenteric aspect. As previously discussed, sometimes, the differences of the distal sigmoid and rectum can be distinguished microscopically, as only the rectum has circumferential longitudinal muscle with the absence of taeniae coli.[15]

The rectum is split into three parts, upper, middle, and lower rectum, with the length of around 4 cm in each segment. The upper rectum extends from the rectosigmoid junction, at the level of the posterior peritoneal reflection, to approximately halfway between the anterior and posterior peritoneal reflections, which is also where the middle rectum starts. The anterior peritoneal reflection is where the middle rectum ends and the lower rectum starts. The lower rectum ends at the dentate line (Figure 3).

There are multiple pelvic floor muscles attached to the distal rectum, such as levator ani muscle, internal and external anal sphincters. Constant muscle tonus of these muscles is important for faecal continence.[15] The internal anal sphincter is made up of smooth muscles and provides permanently high muscle tonus, innervated by the autonomic nervous system.[15] The

external anal sphincter is made up of skeletal muscles with the ability to close the anal canal voluntarily, which is innervated by pudendal nerve.[15] The function of defecation might be maintained after the less aggressive operation, such as LAR, if the structures of the sphincters, as well as the nerves, are preserved.

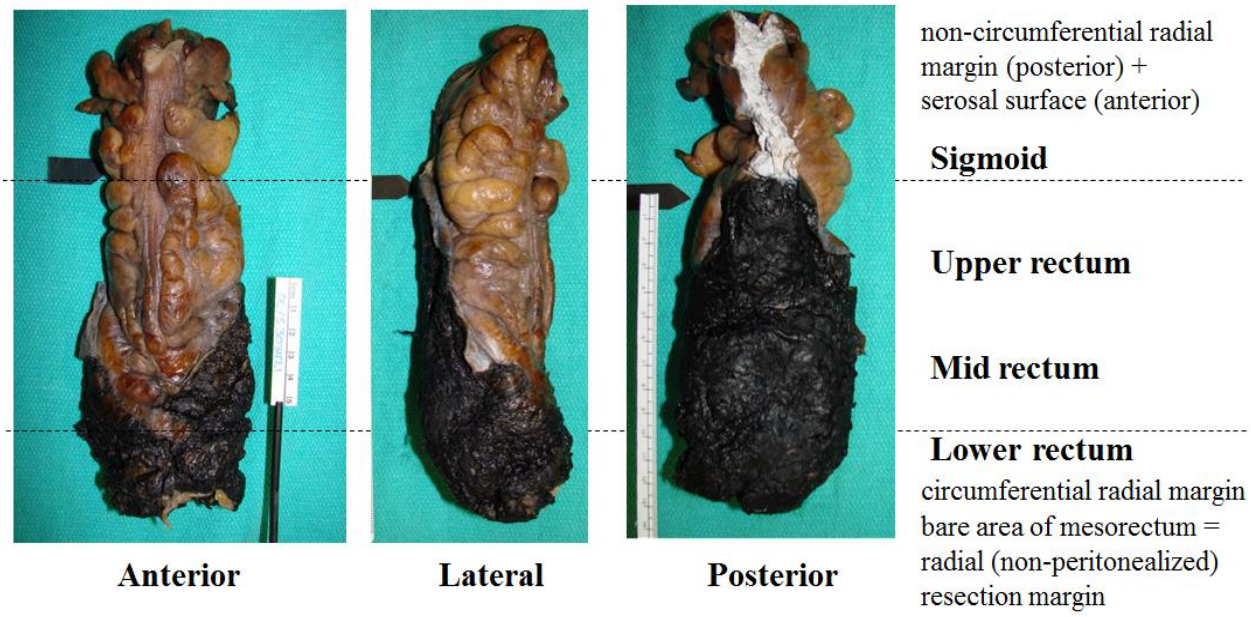


Figure 3. Gross specimen showing anatomical landmarks of the upper, middle and lower rectum. White ink marks the sigmoid mesenteric margin, and Indian ink marks the mesorectal margin. (Used with permission of Mr. L. Fuczek and Dr. R. Wightman, Diagnostic Service of Manitoba (DSM))

I.3.2 Total mesorectal excision and partial mesorectal excision

The mesorectum is the pericolonic fatty tissue, with the associated blood vessels, nerves and lymphatics surrounding the rectum, approximately 2-3 cm in thickness, invested by a fascia.[16] Total mesorectal excision (TME) refers to the sharp dissection surgical technique of the complete mesorectum along the fascial plane, between the visceral and parietal layers of pelvic fascia.[16] It is the standard surgical procedure for a tumour situated in the middle and lower rectum and can be used during LAR or ultra-low anterior resection.[16] In contrast, the partial mesorectal excision (PME) is essentially a similar dissection procedure as TME where the rectum and mesorectum are transected at a right angle to the rectal wall, 4-5 cm below the tumour site.[16] PME is generally performed for the sigmoid or upper third rectal tumour.[16]

Lymph node with cancer metastasis tends to be along the lymphatic drainage from the site of a tumour extending along the lymphatic chain along with the inferior mesentery vessels.[17] Therefore, complete local excision of mesorectum in the vicinity of a tumour is critical for both assessing the regional lymph node involvement and complete cure of a tumour and potential metastatic lymph nodes. Thanks to the introduction of the technique of TME by Dr. Bill Heald (UK) in 1982,[18] and better neoadjuvant chemoradiotherapy, the local recurrence for rectal cancer has been progressively decreasing dramatically during the last 3 decades.[19]

I.3.3 Low anterior resection

For a patient with a localized distal sigmoid cancer or proximal two thirds rectal cancers, LAR can be performed laparoscopically or openly in conjunction with a PME or TME. Occasionally, severe perforated diverticulitis, inflammatory bowel disease, or other medical

conditions can be treated with LAR. Compared to abdominoperineal resection, sphincters and levator ani muscles are be generally preserved in LAR, which permits a better life quality after the procedure.

Pre-operative chemoradiotherapy, also called neoadjuvant therapy is routinely applied to T3/4 or N1 clinical staged tumours on preoperative imaging, like CT, MRI or endorectal ultrasound.[20] The courses of treatment can vary from 1 week to 5-6 weeks. Many of these high-grade tumours significantly shrink after neoadjuvant therapy, with a reduction in the chance of local and systemic recurrence and improvement of survival; some completely response to the treatment, and are cured by subsequent surgical excision.[20] The complete treatment options based on preoperative assessment are listed in Table 2. Generally, a sphincter-sparing option is preferred if a tumour is not in an advanced stage, and the location is high enough for a clear distal margin and anastomosis.[21] However, the possibility to use a sphincter-sparing operation technique like LAR also depends on the BMI, gender, and neoadjuvant chemoradiotherapy treatment. For example, an overweight male with a narrow pelvic cavity, adhesions from previous surgeries, and a long course of radiation can make a LAR with complete TME and proper anastomosis extremely challenging. The possibility of an anastomotic leak is expected to be higher than the conventional cases.[21]

Table 2. Treatment schedule for rectal cancer patients depending on preoperative staging and tumour location.

Cancer Location	cT stage	cN stage	Treatment
Upper rectum	T1 or T2	and N0	LAR
	T3	or N1	sXRT/ChXRT → LAR or LAR → ChXRT
Middle rectum	T1 or T2	and N0	LAR
	T3	or N1	sXRT/ChXRT → LAR or LAR → ChXRT (if imaging not clear)
Lower rectum	T1	and N0	APR
	T2	and N0	APR → ChXRT
	T3	or N1	sXRT/ChXRT → APR or APR → ChXRT (if imaging not clear)
Any	T4	any N	sXRT/ChXRT → APR or APR → ChXRT

Note: ChXRT: long course chemoradiotherapy; sXRT: short course radiation therapy; (adapted from Surgical Procedure of Rectal Cancer Ronald Bleday and Julio Garcia-Aguilar[21])

The LAR or ultra LAR procedure starts with a patient's general anaesthesia, after a complete bowel preparation.[22] A laparoscopic-assisted LAR can be performed for rectal cancer, with the benefit of reduced chances of infection, recovery time, and urogenital function lost. It gives a better visualization of the sympathetic hypogastric nerve plexus and parasympathetic pelvic nerve plexus for preservation.[23] However, other reports demonstrated increased complications of an anastomotic leak.[24] Here, the discussion in this article is limited to only the traditional open surgery, which is more commonly used in Winnipeg.

Generally, an incision is made from the area of right lateral to the umbilicus to the pubic symphysis along the midline.[21] A portion of the sigmoid colon and small bowel is mobilized and

reflected laterally, exposing the inferior mesenteric vessels and branches to the left colic vessels and the superior rectal vessels.[21] During this process, the presence of metastatic nodule is inspected in the abdomen. The superior rectal and middle arteries are ligated.[21] The proximal margin is stapled and transected. A segment of sigmoid colon is usually resected together with the upper and middle rectum, because both have a common blood supply from the superior rectal artery, which is ligated, and the branches of the sigmoid artery are inadequate to supply the anastomosis after transection of the superior rectal artery.[21]

The left ureter and autonomic nerve plexuses are identified and reflected sideways to ensure the dissection is in the anterior and medial aspect, from posteriorly to laterally and then anteriorly, following in the perimesorectal plane.[21] For a male, the dissection is supposed to include at least one layer of the Denovillier's fascia, to ensure the integrity of the seminal vesicles.[21] For a female, the incision should include the base of the rectovaginal pouch or the pouch of Douglas.[21] Once the mesorectum is completely divided, the rectum is transacted at least 2 cm distal to a tumour.[21] The anastomosis between the proximal segment of colon with the anus or rectal remains is possible if a tumour is located at least 7 cm from the anal orifice.[21] Otherwise, a side-to-end anastomosis for LAR or J pouch anastomosis for ultra LAR is recommended.[21] A leaking test is performed to ensure the quality of anastomosis. Finally, fascia and skin are closed by looped suture and staples respectively. Ideally, this sphincter-sparing operation can cure cancer, with the preservation of urogenital functions.

I.3.4 Abdominoperineal resection

In addition to LAR, another mainstream surgical procedure for rectal cancer is the abdominoperineal resection (APR).[21] A perineal resection can be performed simultaneously by a second perineal surgeon from distal to proximal as the abdominal resection proceeds from proximal to distal.[21] As previously discussed, APR is usually performed for a lower rectal cancer. Compared with LAR, APR removes the entire anus and lower rectum. Many patients received neoadjuvant or adjuvant chemoradiotherapy. Therefore, APR is considered as a more radical procedure that affects patient's quality of life more than LAR.

An APR can also be performed laparoscopically or open. A recent multicentric, retrospective study of 667 laparoscopic APR and 2,443 open APR demonstrated that the group of patients with laparoscopic APR has a lower morbidity rate, reduced recovery time and cost, with similar mortality and recurrence rates.[24]

The perineal resection procedure starts from the incision of the perineal body anteriorly, going laterally to the area of the ischial tuberosity and finally posteriorly to the coccygeal tip. As the incision cut through the skin, subcutaneous fatty tissue and perineal membrane, the levator ani muscles are exposed and divided.[21] The abdominal surgeon and perineal surgeon join at the precoccygeal space posteriorly by separation of ligaments posterior to the rectum.[21] The dissection is completed by the perineal surgeon who separates tissue laterally then anteriorly and cautery.[21] Once the specimen including sigmoid colon, rectum, anus with attached soft tissue is removed, the fascia, ischiorectal fatty tissue, subcutaneous fatty tissue, and skin are closed sequentially.[21] The final step is creating an ostomy site in the anterior abdominal wall.[21]

A major predictor of APR outcome is the number of operations performed by a surgeon. A large cohort study of 2815 LAR or APR performed for rectal cancer patients concluded that

both hospital and surgeon procedure volumes have a significant positive correlation with the patient's outcome.[25] Similar results are observed by multiple groups.[26]

I.4: Quirke's method for macroscopic examination of rectal cancer specimen

As previously discussed, the concept of TME and its importance to local recurrence was first recognized by Dr. Heald in 1982.[18] Furthermore, the importance of a complete TME and a clear radial (non-peritonealized) margin was emphasized in 1986 by Dr. Philip Quirke (University of Leeds, UK).[27] He concluded that the most important risk factor for high local recurrence is the involvement of the radial margin, partially due to an incomplete surgical resection.[27] Other factors like high tumour stage, poorly differentiated tumour, and infiltrating border also have somewhat associated with the local recurrence.[27] Obviously, if the tumour tissue is not removed completely during the procedure, it can proliferate and recurrent locally. Therefore, complete pathological assessment, including evaluation of the completeness of a tumour excision in a surgical specimen is extremely helpful in determining if further adjuvant chemoradiotherapy is necessary.

I.4.1: Quirke's method in TME specimen macroscopic examination

To recognize his great contribution to the pathological assessment of TME specimens, the current grossing technique of a TME specimen is commonly named as the "Quirke method", which was initially established in 1998.[28] This technique emphasizes the great importance of TME quality assessment, as patients with complete TME has a local recurrence <5%, compared to a local recurrence around 30-40% with an incomplete TME.[29] The proper assessment of a

TME specimen requires the cooperation between the surgeon, oncologist, and the pathological team including the pathologist and PA. The result of a TME specimen assessment can provide important prognostic estimation to the patient, and help the surgeon and the oncologist to decide if further surgical or chemoradiotherapy is necessary. Additionally, the critical surgical performance quality assessment provides surgeons feedback, encouraging them to improve their surgical techniques.

The pathological assessment of a TME specimen requires the collaboration of macroscopic examination usually done by a PA, and microscopic examination done by a pathologist. Generally, there are three steps in the macroscopic examination using the Quirke methodology.[30]

Step 1 is the **"identifying of landmarks in a TME specimen and initial grading of the TME quality at fresh state."** Upon receiving a TME, either a LAR or APR, the opening PA, resident or pathologist should identify the anterior and posterior peritoneal reflections, as well as the posterior rectal artery.[30] The sigmoid colon is considered as the portion proximal to the posterior peritoneal reflection (at the level of the ligature of superior rectal artery), which has only the posterior mesenteric root margin. The lower rectum is distal to the anterior peritoneal reflection, which has a circumferential radial margin. The upper and middle rectum is in between the sigmoid and lower rectum with a flaring posterior radial margin gradually becomes circumferential below the anterior peritoneal reflection (Figure 3). The integrity of the radial margin, also known as the TME quality is better assessed in a fresh state. The criteria of TME grading are listed in Table 3.[30] Basically, the key points of grading TME are the presence and the depth of surgical defects in mesorectum. A high quality, "complete" TME should follow the mesorectal fascia plane with a bulky, smooth, and intact mesorectum. No visible surgical defects

deeper than 0.5 cm should be identified.[30] Whereas "incomplete" TME has little bulk to the mesorectum with defects down to the muscularis propria level.[30] Coning effect refers to the shape of the distal portion. Pronounced coning effect towards the distal end is associated with high local recurrence and is considered an "incomplete" TME.[30] The gross appearances of "complete", "nearly complete" and "incomplete" TME specimens are demonstrated in Figure 4.

Table 3. Criteria for macroscopic grading of TME specimen completeness

		TME descriptors			
Resection plan	**Grade**	**Mesorectum**	**Defects**	**Coning**	**CRM**
Mesorectal plane	**Complete** (Grade 3)	Bulky, smooth, intact, minor irregularities	No deeper than 0.5 cm	None	Smooth, intact
Intramesorectal plane	**Nearly complete** (Grade 2)	Moderate bulk, some irregularities	Deeper than 0.5 cm, but no muscularis propria is visible except the insertion of levator ani muscle	Moderate	Moderate irregular
Muscularis propria plane	**Incomplete** (Grade 1)	Little bulk, irregular	Deep to the muscularis propria, may have infraperitoneal perforations	Pronounced	Irregular

Note: The mesorectum, defects, and coning effect of TME can be assessed at the fresh state. The circumferential radial margin (CRM) is assessed at the fixed state after serial cross sections at around 0.5 cm intervals.

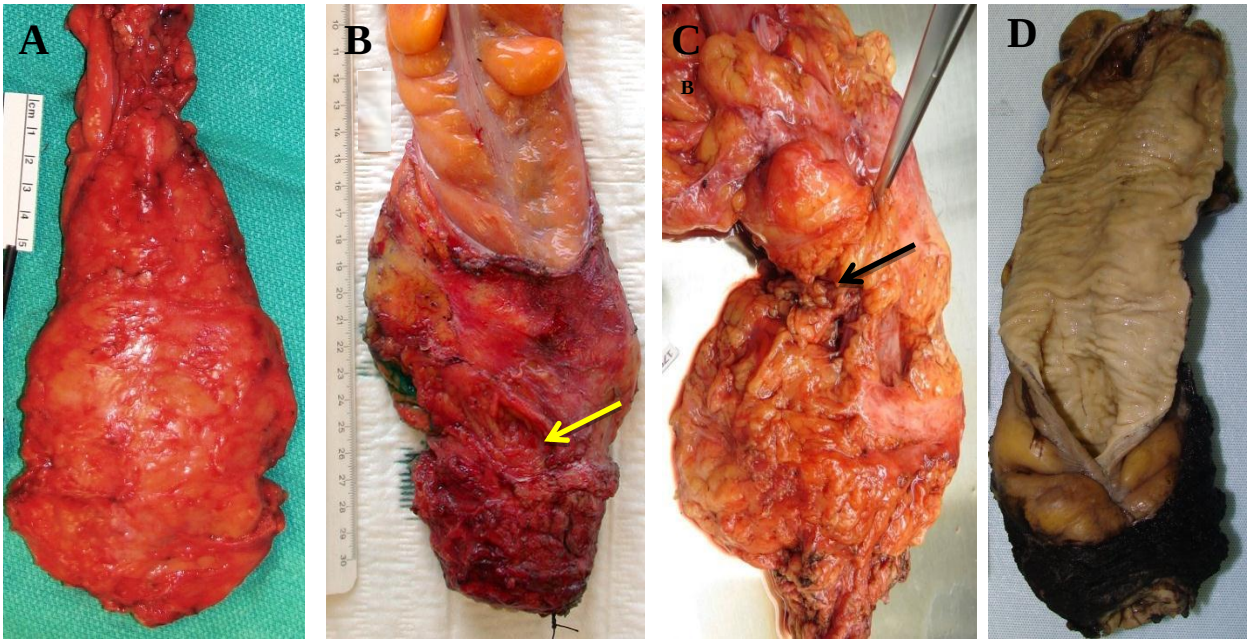


Figure 4. Macroscopic assessment of TME completeness for rectal cancer. **(A)** Complete TME, the resection follows in the mesorectal plane, resulting in smooth and bulky mesorectum with minimal irregularity. **(B)** Nearly complete TME, the specimen shows moderate irregularity with surgical defects greater than 0.5 cm, but not exposing the muscularis propria, marked by a yellow arrow. **(C)** Incomplete TME, the specimen has minimal bulky to the mesorectum with surgical defects down to the muscularis propria, marked by a black arrow. **(D)** A TME specimen partially opened anteriorly with the tumour area intact and inked black. (Figure 4.A: used with permission of Mr. L. Fuczek and Dr. R. Wightman, DSM; Figure 4.B: used with permission of Dr. N. West, Leeds Institute of Molecular Medicine, U.K.)

In current local practice in Winnipeg, as per DSM SOP # 170-81-07, if the TME is graded as "incomplete" by a PA at the initial macroscopic examination, the proper descriptors are used in the gross description and the assessment is confirmed by the pathologist. Photographs showing muscularis propria defects are taken at multiple angles, and the pathology report will be provided to the surgeon afterwards.

Once the initial TME grading is completed, photographs are taken when necessary and the radial margin is painted with the Indian ink. Again, the sigmoid colon only has the posterior mesenteric root margin. The upper rectum generally has the posterior radial margin, which is continuous with some additional lateral radial margin in the middle rectum. The lower rectum and anus, if resected, has the circumferential radial margin. Any surgical defects deeper than 0.5 cm, are supposed to be inked with a second colour, for microscopic examination of any extramural discontinued tumour nodules or a tumour replaced lymph nodes close to defect (<0.1 cm), resulting in a reported positive radial margin.[30]

After inking the radial margin and deep surgical defect, the specimen should be opened anteriorly from both proximal and distal ends, leaving the segment of bowel intact at the level just approximately 1.5 cm above and below the tumour or residual tumour bed. In those cases after neoadjuvant chemoradiotherapy, the residual tumour bed can be subtle or even invisible. Although sometimes difficult, identification of the residual tumour bed is usually possible by palpation of ulcerated or indurated areas. In addition, the tumour bed is usually marked by "tattoo" ink at the time of endoscopy.

After opening the specimen, the intact segment of tumour region needs to be packed with formalin-soaked gauze in the lumen for at least 48 hours prior to grossing, to ensure sufficient formalin exposure of the tumour area or residual tumour bed (Figure 4. D).

Step 2 is the "**fixed specimen gross sectioning and examination**". After proper fixation, the mesorectal excision completeness graded by the opening PA should be confirmed with the grossing PA. In case of any discrepancies with the initial opening assessment, the pathologist on duty needs to be consulted to ensure the accuracy of TME quality grading. Sometimes, additional photographs are helpful.

Serial cross-sections of the intact tumour area at approximately 0.5 cm intervals will be placed down on a work surface. A slice mapping photograph is taken with proper scale, case number and labels, designating anterior/posterior, left/right and proximal/distal orientations, which can be compared with pre-operative MRI images if necessary (Figure 5).

As per required by DSM Grossing manual, gross description of a TME specimen should include the following elements: (1) the type of procedure; (2) the presence of any other adjacent structure, like uterus, adnexa, vaginal wall, seminal vesicles and etc.; (3) the length, diameter of the specimen; (4) proximal and distal margins, with respect to the location of landmarks, like anterior and posterior peritoneal reflection; (5) the depth, weight and appearance of mesorectum, including the TME grading; (6) appearance of the outer surface of serosa, including any adhesion, puckered area or tumour perforation; (7) location, size, gross appearance of tumour/ residual tumour bed; (8) maximal depth of tumour invasion, the closest distances to proximal, distal, radial margins and serosal surface; (9) the number, appearance of possible lymph nodes dissected from mesorectum and sigmoid mesocolon (mesenteric fat); (10) distant between the radial margin and the gross positive lymph nodes or extramural discontinuous tumour nodes; (11) any other mucosal or intramural abnormalities.

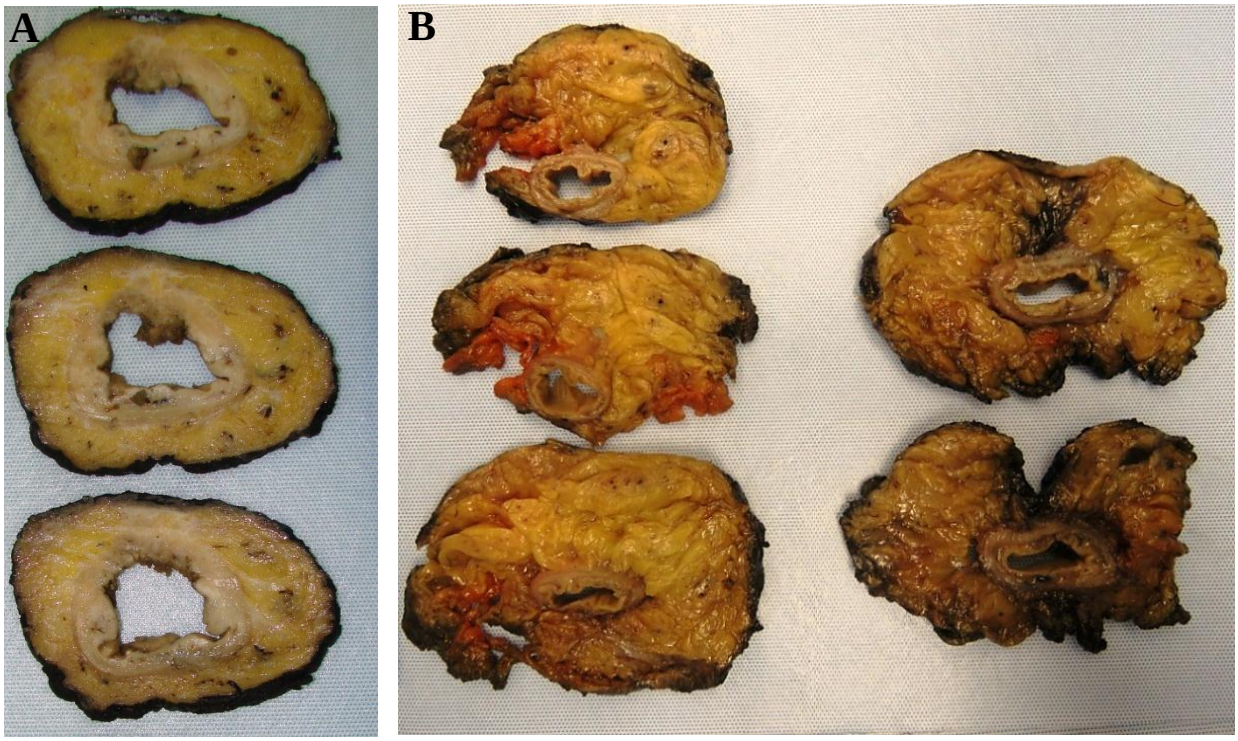


Figure 5. Cross sections of TME specimens. **(A)** A complete TME specimen with a smooth and bulky appearance of mesorectum. **(B)** An incomplete TME specimen showing surgical defect down to the muscularis propria inked orange.

Pericolonic fat including mesorectum and sigmoid mesocolon is stripped after the examination of a tumour and closest margins. However, a rim of fat in the vicinity of a tumour is ideally left attached to the tumour; in case of further sections of the tumour with pericolonic fat involvement is required.

S**tep 3** is "**sectioning and sampling for histology**". Sections for a TME specimen should include the followings:

(1) Margins: Closest radial (non-peritonealized) margin and longitudinal sections of proximal and distal margins. In an APR, longitudinal sections of distal margin should include the cutaneous margin and anal. Additional sections at the dentate line with a tumour if close by can be used to demonstrate the involvement of anal canal.

(2) Tumour sections: Three or more sections demonstrating tumour maximal depth of invasion, and at least additional two sections are required to include the closest radial margin (Figure 6). Generally, if the depth of invasion cannot be identified grossly, additional sections of suspicious areas are submitted more generously. If a tumour is no more than 2.0 cm in the greatest dimension, the entire tumour with adjacent tissue is sectioned and submitted for histology. One of the tumour sections should include adjacent normal appearing bowel.

(3) Lymph nodes: Possible lymph nodes identified after pericolonic fat dissection are submitted in their entireties, apart from an obvious tumour replaced ones, which only require one representative section each. Possible lymph nodes close to the radial margin, especially those with a similar gross appearance of a tumour, needs to be submitted together with the adjacent margin for microscopic assessment of margin involvement (Figure 6).

(4) Other organs: It is necessary to make at least one representative section of other attached organs, such as the uterus, adnexa, seminal vesicles, prostate and etc. In case of possible tumour involvement of these organs, additional sections might be required (Figure 6).

(5) Additional pathological findings: Representative sections of any other abnormalities described in the gross report are required for further microscopic examination, such as polyps, diverticulum, ulceration, submucosal nodules, stricture, tattoo, and etc.

(6) Anastomosis ring: Sometimes, the true proximal and distal margins are placed in an Anvil after anastomosis, which is rarely involved by a tumour. However, representative sections of the doughnut-shaped bowel are examined microscopically to rule out the potential of a positive proximal/distal margin.

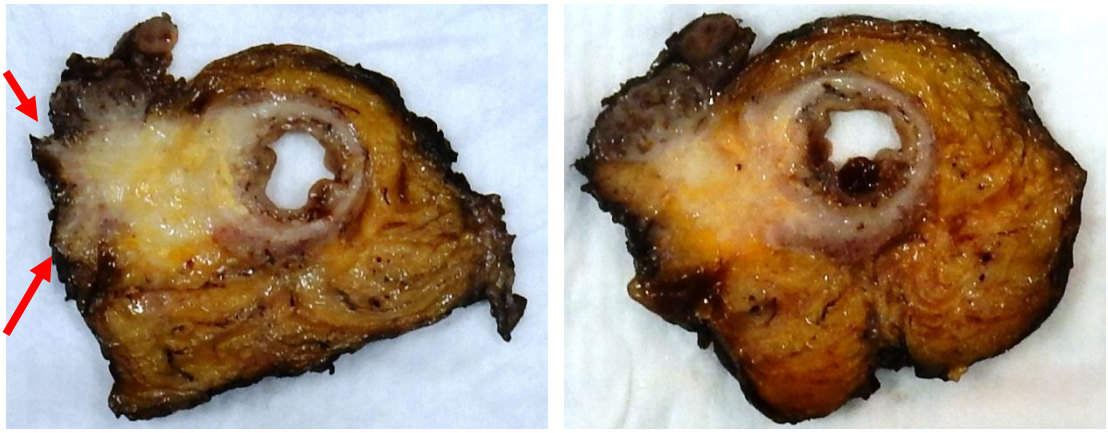


Figure 6. Cross sections of a TME specimen. A tumour extends through the full-thickness of the bowel wall, involving the mesorectum and the seminal vesicles. Grossly, the tumour is at the inked margin, indicated by red arrows.

I.4.2: Rationale and potential drawback of using the Quirke's method

APR and LAR are the two types of specimens required to be grossed according to the Quirke methodology. The rationale for this examination technique is its established superiority to routine examination of tumour extent and radial margins. For example, the tumour invasion of the adjacent organ is obvious in the cross sections, and the distance between a tumour and radial margin can be measured accurately macroscopically and validated microscopically (Figure 6). Furthermore, this technique provides a standardized gross examination of mesorectum excision quality with the assignment of a TME "grade", which is useful for surgical quality assurance. It has been demonstrated by multiple centres that the local recurrence is highly related to TME grade and positivity of radial margin. Feedback from pathological report might be beneficial to improve the surgical quality and to decrease the radial margin positivity rate.

Despite the benefit of pathological assessment and a possible better patient care, there are potential drawbacks of using the Quirke's method in grossing TME specimen. Ischemic time is relatively long with this method than a traditional cut-open fixation. A tumour and pericolonic fat are left intact at initial opening without sufficient exposure to formalin. The autolysis rate in colon and rectum is remarkable higher than other organs, due to the residence of multiple bacterial flora strains. This autolysis process might make the accurate interpretation of special staining almost impossible at the molecular level. The era of designing personalized treatment strategy is developing in many tumour areas like the breast.[31] Six molecular subtypes of colorectal cancer are discovered recently with possible future group specific clinical treatment plans.[32] Therefore, the prolonged ischemic intervals of a TME grossed by the Quirke method might reduce the sensitivity and specificity of ancillary molecular studies.

I.5 Microscopic examination of rectal cancer

In Canada, pathological report of colorectal cancer mainly follows the cancer protocol template published by College of American Pathologists (CAP). "Protocol for the Examination of Specimens From Patients With Primary Carcinoma of the Colon and Rectum" is based on the information from the American Joint Committee on Cancer (AJCC), cancer staging manual 8th edition.[15] The key elements in this template protocol related to this research project are to be discussed in this section.

I.5.1 synoptic report elements

1. Tumour site: As previously discussed in the section: I.3.1, the site of a rectal and rectosigmoid tumour is largely dependent on its anatomical location with respect to the anterior and posterior peritoneal reflections. Although the clinical definition is slightly different, tumours proximal to the posterior peritoneal reflection are defined as true sigmoid tumour anatomically; whereas, tumours distal to the anterior peritoneal reflection and proximal to the dentate line are lower rectal tumours.[15] The segment of bowel between posterior and anterior peritoneal reflections is upper and/or mid rectum. A tumour is considered as truly rectosigmoid if it straddles the posterior peritoneal reflection.[15]

2. Histologic Type: The vast majority (98%) of colorectal cancers are adenocarcinomas, deriving from the glandular epithelial cells in the colon.[15] Adenocarcinoma show tumour cells that invade into the submucosa, muscularis propria and adjacent structures.[15] Microscopically, the cells have the morphology of tubular glands, with some central necrosis. The mucinous subtype of adenocarcinoma has abundant mucin production associated with the carcinoma. Some high grade, poorly differentiation carcinoma have intracellular mucin production with a nucleus

in the periphery, giving the cell a "signet-ring" appearance.[15] Other rare types are medullary carcinoma, neuroendocrine carcinoma, squamous cell carcinoma and etc.[15] Some histologic types of carcinoma are highly related to the prognosis, and therefore are extremely important elements in the pathological examination of tumour.[15]

3. Histologic Grade: A widely used grading scheme for colorectal cancer is based on the degree of differentiation. There are a total of 4 grades. Grade 1 (well differentiated, more than 95% glandular formation); Grade 2 (moderately differentiated, between 50% to 95% glandular formation) are generally considered less aggressive, low-grade carcinomas; whereas, the Grade 3 (poorly differentiated, no more than 50% glandular formation) and Grade 4 (undifferentiated, no glandular formation) are essentially more aggressive, high-grade carcinomas. A two-tiered system is also commonly used that combines the Grade 1/Grade 2 to be low-grade carcinomas, and Grade 3/Grade 4 to be high-grade carcinomas.

Although the grading of a tumour can be highly subjective and vary significantly between observers, the histologic grade can be a strong and powerful prognostic predictor.[33] Therefore, an accurate microscopic assessment of histologic grade is extremely helpful for the clinician to design a proper post-operative treatment strategy.

4. Extent of invasion: In TNM staging system, each individual category has both clinical and pathological staging, with a letter of "c" and "p" in front of the staging element, respectively. The "T" of the pathologic TNM staging is the most important prognostic factor reported by a pathologist in colorectal cancer. According to the AJCC, this element is based on the extent of the primary tumour invasion of anatomical structures.[34] The T stages with the corresponding maximum depth of extension are listed in Table 4. Stage T4b refers to the direct extension of a tumour into adjacent structures locally. Distant metastasis of a tumour into liver,

lung, bone, kidney, brain and etc. are staged as "M" in TNM,[34] and will be discussed in the distant metastasis section.

Table 4. T stage of CRC with the corresponding anatomic extent

pT stage	Maximum depth of extension
pTx	Information is incomplete, depth of invasion cannot be assessed
pT0	No evidence of a primary tumour
pTis	Carcinoma in situ with a tumour involving the mucosa, including epithelium, connective tissue, and a thin layer of muscularis mucosa
pT1	Beyond the muscularis mucosa into the submucosa, but not the thick layer of muscularis propria
pT2	Muscularis propria involvement
pT3	Beyond muscularis propria, with involvement of subserosa, a thin layer of connective tissue underneath the serosa, or non-peritonealized pericolorectal fat (adventitia)
pT4a	Involving the visceral peritoneum layer of serosa; however, still covered by a layer of normal serosa
pT4b	Breach the full-thickness of the bowel, involving the adjacent structure, usually another segment of small/large bowel, bladder, gonads or coccyx.

Note: The size of a tumour is not a staging parameter for colorectal cancer and is an optional element in TNM staging.

5. Lymph nodes: Regional lymph nodes metastasis, "N" stage is the second key staging parameter in TNM staging system. It describes the degree of regional lymph nodes involvement. The definition of the regional lymph node is largely based on the anatomical site for lymphatic drainage. In the rectal and rectosigmoid colon, the regional nodes include the sigmoid,

pericolonic, superior rectal/hemorrhoidal, mesorectal, and inferior rectal/hemorrhoidal lymph nodes.[15] Eventually, they drain into the afferent lymph nodes, either through the inferior mesenteric or internal iliac nodes, which are considered as a distant lymph node. Although it is difficult to distinguish the exact type of lymph nodes in a single surgical specimen, the lymph nodes recovered from the pericolonic fat are generally considered as regional lymph nodes conventionally; whereas, the distant lymph nodes, are usually collected as separate specimens for pathological assessment. The definition of different "N" categories of lymph nodes involvement is listed in Table 5.

The possibility of identifying lymph node metastasis microscopically is largely related to the number of lymph nodes recovered from a surgical specimen. Therefore, it is extremely important for the PAs to find as many lymph nodes as possible for the pathologists to exam.[15] Quality control studies carried by the American College of Surgeons suggest a minimal number of "12" lymph nodes retrieved is essential to accurately stage a colorectal tumour to reliable predict prognosis[35]. Variations in lymph node yield can be related to multiple factors, like the physical condition of a patient (age, amount of pericolonic fat), surgical technique, location and size of the lymph nodes, neoadjuvant treatment, as well as the grossing technique of PA, which will be discussed furthermore in the result section.[15]

Table 5. N stage of CRC with the corresponding number of regional lymph nodes metastasis

pN stage	Regional lymph nodes metastasis
pNx	Information is incomplete, lymph nodes metastasis cannot be assessed
pN0	No evidence of regional lymph node metastasis
pN1a	1 regional lymph node metastasis
pN1b	2 – 3 regional lymph nodes metastasis
pN1c	Extramural discontinuous tumour deposits, or no evidence of residual lymphatic tissue
pN2a	4 – 6 regional lymph nodes metastasis
pN2b	7 or more regional lymph nodes metastasis

Note: The total number of lymph nodes assessed is to be included in a pathological report. Gross re-examination to find more lymph nodes is usually required in the case where less than 12 lymph nodes are identified in the initial submission. An insufficient number of lymph nodes (below 12) should be included in the report.

6. Treatment Effect: Neoadjuvant chemoradiotherapy has been proven to be significantly beneficial to CRC patients, especially those with rectal cancer [36]. Many tumours have a complete response to the treatment leaving a fibrous scar; while others may shrink in size and depth of invasion. However, a portion of patients has only minimal or no response to the treatment. The degree of tumour regression after neoadjuvant treatment is graded by the "Ryan scheme".[37] Although there can be interobserver variation in grading the treatment response, the Ryan scheme is a widely accepted system to determine treatment response, which is currently in practice in Winnipeg to predict prognosis of a colorectal cancer patient after neoadjuvant

treatment. In general, the degree of the primary tumour regression is defined from "complete response" to "poor response", with a score of "0" to "3" (Table 6).[15]

Table 6. Criteria for primary tumour regression assessment based on the "Ryan Scheme"

Tumour regression score	Response grade	Description
0	Complete response	Complete tumour ablation; no residual tumour cells are identified
1	Nearly complete response	Single cells or small cluster of viable tumour cells
2	Partial response	Large cluster of viable tumour cells with evidence of tumour regression
3	No or poor response	Tumour has no significant response to treatment, extensive residual tumour

7. Lymphovascular involvement: Several studies emphasized the prognostic significance of tumour involvement of small vessels, including capillaries, arterioles, venules, and lymphatics, which are thin-walled vessels, lack of smooth muscles. In contrast, large vessels have the smooth muscle layer. The invasions of small or large vessels are to be reported separately as they have different prognostic significances.[15]

The extramural venous invasion has an increased possibility of liver metastasis and is considered as a poor prognostic marker than intramural venous invasion.[15] Extramural perineural invasion is another poor prognostic indicator and should be reported separately when identified.[15]

8. Margins: Margins include the proximal, distal and radial (non-peritonealized) margins, which are to be inked at the gross examination. Associated serosa, like a puckered area, is not a margin, but inking with a second colour is helpful for the pathologists to assess the possibility of microscopic serosal involvement. It is rare to have an anastomosis recurrence if there is a clearance of more than 5 cm from a tumour to margin.[15] Usually, 2 cm of a tumour free margin resection is believed to be sufficient to prevent anastomotic recurrence. In some early stage carcinomas, like T1 or T2 tumours, 1 cm may be acceptable.[15]

Radial margin status is one of the most important prognostic predictors. It is well demonstrated that the positive radial margin will increase the possibility of local recurrence by 3.5 folds and also increase the mortality rate by 2 folds.[38] Aforementioned, to be assessed as a positive radial margin, the carcinoma cells or carcinoma component in a metastatic lymph node has to be within 0.1 cm of the inked margin. Radial margin status reflects the surgical quality and predicts the prognostic outcome. The importance of radial margin status and its relationship with mesorectal excision quality will be discussed in the result section, Chapter V.2.

9. pTNM: The final pathological grading of a tumour is based on the "T" - tumour; "N"- node; and "M"- metastasis classifications. It is a different grading scheme than the AJCC staging "I" to "IV" system. However, it provides a more detailed classification for the surgeon and oncologist and should be in use together with the AJCC staging.

In TNM, a prefix "y" should be used as an identifier of a tumour with neoadjuvant chemoradiotherapy, which is a part of the audit discussed in result section, Chapter V.4. The suffix "m" indicates multiple primary foci of a tumour. The highest "T" stage should be reported

for cases with multiple foci of carcinoma, while “N” and “M” stages are graded together. For example, the TNM staging of a tumour with multiple foci is pT4a(m)N2bM1.

“M” stage for distant metastasis is required only if biopsies from distant organs are collected for microscopic examination. If the distant metastasis cannot be assessed due to the lack of sampling, which is the situation of most cases, the “M” stage should not be included in the synoptic report. The term “Mx” used to be stated for these cases, but is not in used currently as per most recent CAP cancer protocol, and should not be included in the synoptic report.[15]

Chapter II: Literature review:

II.1 Quirke method is becoming mainstream for grossing TME specimen

II.1.1 Importance of TME and radial margin

The importance of total mesorectal excision, particular the radial margin status was initially highlighted by Dr. Bill Heald in 1982.[18] This retrospective study compared the local recurrence rates between traditional surgical removals of a rectal tumour and over than 100 total mesorectal excisions, suggesting that the intact mesorectum excision for middle and lower rectal cancer can significantly prevent cancer from the extrarectal spread.[18] Furthermore, the same group Heald *et al*. (1986) followed up these patients for 3.5 to 7.5 years and concluded that the distal margin clearance is not as important as the integrity of mesorectum excision.[39] The surgeries in this study were performed by a single experienced surgeon, who routinely performed TME. Therefore, the 5 years local recurrence rate of these patients averaged at 3.7%, which is remarkably lower than the numbers (30-40% recurrence rate) reported by others in the 1980s.[40] This is not based on a perfectly balanced statistical analysis; however, the importance of TME was successfully brought to public attention. This series study also suggested that the anal sphincter-saving LAR or ultra-LAR should be performed if the stapled anastomosis is 3-5 cm above the anal verge, and it is possible to excise the entire mesorectum intact.[39]

The quality of TME is also important to predict rectal tumour patients' prognosis.[27] Quirke *et al.* (1986) concluded that poor surgical quality, resulting in incomplete mesorectal excision and positive radial margin, dramatically increase the local recurrence and mortality rates.[27] Obviously, incomplete excision of all tumour cells or tumour cells implanted in the pelvis due to surgical instrumentation is the most important factor. Therefore, in their study, 14% of

patients who underwent "curative surgery" with incomplete mesorectal excision, ended up with subsequent pelvic or liver metastasis, which was not detected prior to the initial surgery. Using the Quirke method to gross mesorectal excision specimens, the possibility to detect a positive margin involvement and predict local/systemic recurrence is about 92%.[27]

In a more recent study, Beaufrère et al (2017) performed a statistical analysis between the local recurrence rate and the tumour free distance of radial margin in a cohort of 321 middle or lower rectal cancers.[40] Interesting, the group of patients with less than 0.4 mm radial margin clearance have a significantly worse prognosis. Patients with margin clearance between 0.4 mm to 1.0 mm show no statistically prognosis differences than those with negative radial margin, suggesting that the radial margin clearance should be measured very carefully and included in the pathological assessment.[40]

II.1.2 Widely used Quirke method for mesorectal excision specimen

The original idea of the proper assessment of radial margin was brought by Quirke *et al* (1986).[27] As previously mentioned, he further established the standard macroscopic examination protocol for mesorectal excision specimen in 1998.[28] Since then, many publications emphasized the importance of mesorectal excision quality as well as the radial margin clearance distance in reducing the local recurrence rate.[41,42,43,44,45]

The Quirke method used in the macroscopic examination of a rectal tumour specimen has been described by multiple groups and summarized in literature.[30,45,46,47] The modified Quirke method is currently in use as a standardized grossing technique in DSM as per protocol DSM # 170-81-07.

II.2 Factors associated with TME quality

As an important predictor of local recurrence, the completeness of the mesorectal excision is highly related to many factors, including the low volume of surgery performed, female patients with deep pelvic, deviated body mass index (BMI) patients, demonstrated by Campa-Thompson M, *et al* (2015).[47]

Surgical technique is the most important factor of the mesorectal excision quality. More experienced surgeons from a high volume hospital generally performed better mesorectal excision with low incomplete rate due to more experiences gained during practice.[25,48] Ideally, the excision should follow the mesorectal plane. The chance of recurrence increases significantly when the excision is at the muscularis propria plane.[49] In addition, pathological assessment and documentation of the surgical quality encourage and promote the surgeons to improve the mesorectal excision quality and reduce the incomplete percentage.[49]

Other factors like the BMI is also shown to be related to incomplete excision. Obese patients have limited operation space in the pelvic cavity, making it difficult for the surgical tools to access the mesorectal envelope. On the other hand, low BMI patients have minimal mesorectum overlapping the tumour, which can increase the chance of positive radial margin with even shallow surgical defects.[47]

Leonard D *et al* (2010) indicated other independent factors predicting the completeness of mesorectal excision including the position of a tumour, clinical tumour staging, neoadjuvant chemoradiotherapy, and type of procedure.[48]

II.3 Mesorectal excision quality reported by other medical centres

An incomplete mesorectal excision percentage depends on multiple factors, and varies dramatically from 5% to 40%, reported by different groups.[1] A recent retrospective chart review of 56 patients performed by Mendez MA, et al (2016), shows overall 89% complete, 5% nearly complete, and 5% incomplete mesorectal excision. The incomplete mesorectal excision is related to the tumour location being in the lower rectum.[1] Another retrospective cohort study of 359 patients carried out by Garc á-Granero E *et al*, (2009) demonstrated that the incomplete mesorectal excision percentage decreased from 41.7% (2000-2001) to 7.7% (2006-2007), averaged at 17.7%.[50] The significant difference is mainly caused by the delivery of pathological grading of mesorectal excision quality to the surgical team, which started in the year of 2001. This result is a strong evidence of the positive effect of pathological assessment on improving surgical quality.

A meta-analysis of 4034 patients carried out by Mart ínez-P érez A et al. (2017) reported the noncomplete percentage of 13.2% for laparoscopic surgery and 10.4% for open surgery of mesorectal excision.[51] Ferko A *et al* (2014) reported the overall incomplete percentage in a 125 patients retrospective study to be as high as 24.5% and 43.1% for laparoscopic and conventional surgeries, respectively.[52] In addition to the real surgical quality variations between different medical centres, the great variation of incomplete percentage is highly associated with differences in understanding and interpreting the grading criteria among individual PA. Therefore, more detailed explanation of grading criteria with illustration is strongly suggested.

Chapter III: Objectives and Hypothesis:

In this retrospective study, there are three main factors being examined (1) mesorectal excision quality in Winnipeg between January 2012 and December 2015, (2) macroscopic assessment of the mesorectal excision specimens and (3) microscopic assessment and TNM staging of these specimens. The main focus of this study is to discover the factors associated with "incomplete" mesorectal excision and recommendations to improve the quality of pathological assessment of the mesorectal excision specimens, resulting in a better patient care.

III.1 Mesorectal excision surgical quality audit in Winnipeg (2012-2015)

Our learning objective in the surgical audit sections is to evaluate the differences of mesorectal excision completeness by surgeons and the factors associated with surgical quality. We hypothesized that high volume surgeons, who performed more surgeries, tend to result in a better quality mesorectal excisions. On the other hand, low volume surgeons who might not be as experienced may result in a possibly lower quality surgery.

Another major factor associated with surgical quality might be the surgeons' training background. We are going to summarize the differences in incomplete percentage among the three groups of surgeons: general surgeons, GI specialized surgeons, and surgical oncologist to evaluate if the fellowship trained surgeons having a better mesorectal excision quality.

Other factors like gender, age, tumour locations, and the number of surgeries performed in a month, will be summarized and compared, in order to predict the completeness of a mesorectal excision.

III.2 Macroscopic assessment of the mesorectal excision specimens

The fundamental of statistical significance is the normal distribution of the variables. The variances between individual observers are supposed to be minimized, in order to get standardized grading of the mesorectal excision quality. As per DSM standard grossing protocol, the completeness of mesorectal excision was included in the gross description since 2011. Our learning objective in this section is to assess the percentage of specimens which were grossed according to the "Quirke Method" from 2012 to 2015 and to note any improvements of grossing technique during this period of time. It is hypothesized that thanks to the better education; more specimens are expected to be grossed based on the "Quirke Method".

The macroscopic examination of mesorectal excision specimens is performed by four groups of individuals, (1) on-site trained PA, (2) non-Canadian-trained pathologist working as a PA, (3) program-trained students/PA, and (4) resident. Another objective is to compare if there are any grossing skill differences between these groups. For example, the percentage of "Quirke Method" application, the number and accuracy of lymph nodes retrieval, and the clarity/accuracy of gross description are to be compared. Due to the differences in training background, individuals from different groups might have significant differences in grossing skills.

III.3 Microscopic assessment and TNM staging the mesorectal excision specimens

Pathologists use the standardized synoptic reports to report rectal cancer stages. It is well organized and consistent in the layout of tumour characteristics and is the most important information provided to the surgeon or medical oncologist to decide if any further treatment is necessary. Our objective in this section is to evaluate the consistency and integrity of synoptic

reports of mesorectal excision specimens. CAP routinely updates the colorectal cancer protocol. There might be a few synoptic reports having misapplied grading elements or missing elements. The hypothesis is the percentage of synoptic reports with misapplied grading or inconsistency between the microscopic description and TNM stage would decrease from 2012 to 2015, suggesting the pathologists are more aware of the recent changes in the CAP cancer protocol.

Chapter IV: Materials and Methods:

IV.1 Selection of mesorectal excision cases and establishing the database

DSM is a multicentre organization in charge of laboratory tests for diagnostic purposes. As a retrospective chart review study, this research involves many patients' personal information, such as name, age, gender and clinical history. Therefore, as per requested by the Personal Health Information Act (PHIA), the methods in this study has been evaluated and approved of by the University of Manitoba Research Ethics Board (REB) (ETHICS #: HS21215), prior to the data selection, to ensure the confidentiality of patients during and after the research.

The initial data mining was performed Ms. D. Kiesman, DSM, using the keywords "Rectum (REC)" for "spectype" to find out all the surgical specimens from rectum; and "syncolon7" for "insertedcodes" (which is an insert code used to generate the colonic synoptic report template for colon cancers in the laboratory information system (LIS)) to exclude the non-malignant or pre-malignant tumour conditions, such as low/high-grade dysplasia, adenoma, inflammatory disease, diverticulitis, ischemic diseases and etc. The search of the DSM LIS results in a table of 462 patients who underwent rectal surgery to remove a malignant tumour from 01/01/2012 to 12/31/2015. This table contains the following elements: (1) the year of surgery; (2) Delphic Anatomic Pathology (AP) number; (3) LIS number; (4) data registration date and time; (5) name of signing pathologist.

Using the Delphic AP number, the synoptic reports were retrieved from the DSM LIS database. Patients' personal information, including name, birthday, address, and Delphic AP number was de-identified and replaced by a unique study ID, which is stored in the DSM secured computer, as per requested by REB.

Each individual pathological report was reviewed and clinical data were captured, forming a master table with the following elements: (1) unique study ID; (2) surgery year; (3) surgery month; (3) patient age; (4) patient gender; (5) surgeon name; (6) pathologist name; (7) grossing person; (8) mesorectal excision completeness; (9) number of possible lymph nodes retrieved grossly; (10) procedure type; (11) tumour location; (12) histology type; (13) histology grade; (14) margins status including radial margin; (15) number of lymph nodes examined microscopically; (16) treatment effect; (17) TNM staging.

9 out of the 462 cases were resected for rectal metastasis from non-colorectal primaries and therefore excluded in the study. None of the patients is pediatric at the time of surgery, resulting in a master table of 453 cases included in this study. Information about (18) the surgeons' training background is provided by Dr. D. Hochman, and (19) category of PA training background is provided by Mr. L. Fuczek. These factors are included in the master list as additional variables.

IV.2 Interpretation of the mesorectal excision grading in the gross description

Criteria for macroscopic grading of mesorectal excision specimen are listed in Table 3. In reality, many PAs failed to mention the specific grade of the excision quality and instead, most of them used descriptors to describe the quality. However, due to the previous absence of detailed mandatory elements in the grossing protocol, it was not required for the PAs to include all the descriptors in the gross description report. Part of this audit is to study the completeness of mesorectal excision quality description in the gross report.

The followings descriptions are considered as “inadequate gross description”, although the quality of mesorectal excision can be graded. Some only used the term “smooth and bulky” to describe the quality of mesorectal excision; conventionally it is considered as “complete” excision in this study. In many cases, the terms of “moderate bulky with some irregularity”, or “mesorectum is ragged” are used, and interpreted as “nearly complete”. Occasionally, some grossing reports included the presence of defects, without describing the depth of defects, or whether they expose muscularis propria or not. Those cases are graded as “nearly complete” because PAs usually mention the surgery is at the muscular propria plane if the excision quality is “incomplete”. Due to the limited space in the pelvic cavity and special anatomical structure of some patients, it is difficult for the surgeon to pull out the specimen out from the body, causing tearing effects; therefore graded as “nearly complete” in this study, as long as the mesorectum can be put back together, forming an intact surface.

There are approximately 5% of gross reports which gave the grade of mesorectal excision without including any detailed explanation or descriptors. Those are considered as an “incomplete gross description” as well. Some reports failed to mention the mesorectal excision quality at all, which are excluded from statistical analysis.

IV.3 Statistic analysis

The IBM Statistical Package for the Social Sciences (SPSS) software version 19.0 was used to perform the statistical analysis. Simple descriptive statistics were used to study the frequencies, percentages and plots of each variable. All the statistical significances were based on a p-value of < 0.05.

The mean value differences between a categorical variable were studied by "Compare mean" under the function of "Analyze".

Chi-square (χ^2) test measures the existence of a relationship between two categorical variables. For example, in this study, to study the relationship between the "type of operation" with "year", a chi-square test was performed under the function of "Crosstabs" in the "Descriptive statistics". If the chi-square test shows a significant relationship, we can make the conclusion that the type of operation changed over the 4 years, with a *p*-value of < 0.05, representing the confidence of the conclusion.

In contrast, the Pearson correlation test measures the relationship between two linear quantitative variables, such as "height" and "weight". In our study, the only linear independent variable is the "age of patient". Therefore, the correlation studies were not performed by the Pearson correlation test.

Analysis of variance (ANOVA) is a powerful tool to investigate the relationship between independent categorical variables, such as "surgeon category", "site of tumour" and dependent ordinal variables, such as "mesorectal excision quality". One-way ANOVA can measure one independent variable; while, the two-way ANOVA can study the relationship of 2 independent variables with respect to the dependent variable at the same. For example, two-way ANOVA can be used to study if surgeon's training background and the background of the PA have an impact on the mesorectal excision quality; to study if a certain group of surgeon performs better than others; or to study if the significant difference is only caused by the variation of PA training background.

One-way ANOVA was performed under the "Compare mean" function of the "Analyze". Two-way ANOVA was carried out under the "Univariate" function of the "General linear

model". The post-hoc test is typically conducted after a significant ANOVA, looking for differences between levels of the dependent variables, testing each possible pair of groups. In this study, Tukey's tests were performed, because it has the ability to compare the differences of means between groups and to keep the overall 95% confidence level. For two-way ANOVA, the interactions between the two dependent variables are measured by the *F* test. To quantify the "quality of the mesorectal excision", the independent variable is coded as "complete" = 1; "nearly complete" = 2; and "incomplete" = 3. A smaller score indicates better surgical quality. Similarly, the response of neoadjuvant therapy is quantified as "Grade 0" = 1; "Grade 1" = 2; "Grade 2" = 3; "Grade 3" = 4, with a smaller number demonstrating better response.

Chapter V: Results

V.1 Tumour characteristics of the 453 cases from 2012 to 2015

The characteristics of the 453 rectal cancer cases from 2012 to 2015 are discussed in this section. Statistical analyses were performed to examine the changes in tumour characteristics over the years, the risk factors for a high-grade rectal tumour, and the factors impacting neoadjuvant therapy.

V.1.1 Descriptive statistics of the tumour characteristics of the 453 cases

The frequencies and percentages of each variable clustered by year are summarized in Table 7.

Table 7. Tumour characteristics for the 453 cases, clustered by year of operation

	2012	2013	2014	2015	Total
Total	96	109	121	127	453
Type of operation					
LAR	81 (84.4%)	77 (70.6%)	92 (76.0%)	92 (72.4%)	342 (75.4%)
APR	12 (12.5%)	25 (22.9%)	23 (19.0%)	28 (22.0%)	88 (19.4%)
Others*	3 (3.1%)	7 (6.4%)	6 (5.0%)	7 (5.5%)	23 (5.1%)
Patient gender					
Female	36 (37.5%)	37 (33.9%)	44 (36.4%)	48 (37.8%)	165 (36.4%)
Male	60 (62.5%)	72 (66.1%)	77 (63.6%)	79 (62.2%)	288 (63.6%)
Patient age group					
Young (25 – 49)	9 (9.4%)	8 (7.3%)	14 (11.6%)	14 (11.0%)	45 (9.9%)
Mid (50 – 79)	76 (79.2%)	87 (79.8%)	87 (71.9%)	93 (73.2%)	343 (75.7%)
Old (80 -93)	11 (11.5%)	14 (12.8%)	20 (16.5%)	20 (15.7%)	65 (14.3%)
Site of tumour					
Rectosigmoid junction	5 (5.2%)	10 (9.2%)	10 (8.3%)	11 (8.7%)	36 (7.9%)
Upper rectum	11 (11.5%)	6 (5.5%)	9 (7.4%)	13 (10.2%)	39 (8.6%)
Upper/mid rectum	6 (6.3%)	11(10.1%)	22 (18.2%)	20 (15.7%)	59 (13.0%)

Table 7 (Continued)

	2012	2013	2014	2015	Total
Total	96	109	121	127	453
Site of tumour (Continued)					
Mid/ lower rectum	11 (11.5%)	12 (11.0%)	15 (12.4%)	16 (12.6%)	54 (11.9%)
Lower rectum	32 (33.3%)	41 (37.6%)	30 (24.8%)	45 (35.4%)	148 (32.7%)
Ano-rectal junction	0 (0.0%)	1 (0.9%)	4 (3.3%)	1 (0.8%)	6 (1.3%)
No residual tumour	1 (1.0%)	3 (2.8%)	6 (5.0%)	4 (3.1%)	14 (3.1%)
No description	7 (7.3%)	6 (5.5%)	12 (9.9%)	3 (2.4%)	28 (6.2%)
Histology type					
No residual tumour	12 (12.5%)	7 (6.4%)	20 (16.5%)	11 (8.7%)	50 (11.0%)
Adenocarcinoma	79 (82.3%)	97 (89.0%)	87 (71.9%)	107 (84.3%)	370 (81.7%)
Mucinous (mixed)	4 (4.2%)	4 (3.7%)	10 (8.3%)	6 (4.7%)	24 (5.3%)
Signet-ring cell	0 (0.0%)	0 (0.0%)	2 (1.7%)	1 (0.8%)	3 (0.7%)
Neuroendocrine	1 (1.0%)	0 (0.0%)	1 (0.8%)	1 (0.8%)	3 (0.7%)
Squamous cell	0 (0.0%)	0 (0.0%)	1 (0.8%)	0 (0.0%)	1 (0.2%)
GIST	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.8%)	2 (0.4%)
Histology grade					
Not determined after treatment	16 (16.7%)	15 (13.8%)	22 (18.2%)	16 (12.6%)	69 (15.2%)
Not available	1 (1.0%)	1 (0.9%)	0 (0.0%)	0 (0.0%)	2 (0.4%)
Low-grade**	76 (79.1%)	86 (78.9%)	93 (76.9%)	102 (80.3%)	357 (78.8%)
High-grade**	3 (3.1%)	7 (6.4%)	6 (4.9%)	9 (7.1%)	25 (5.5%)
Neoadjuvant therapy					
yes	36 (37.5%)	51 (46.8%)	53 (43.8%)	64 (50.4%)	204 (45.0%)
no	60 (62.5%)	58 (53.2%)	68 (56.2%)	63 (49.6%)	249 (55.0%)
pT stage					
ypT0	13 (13.5%)	7 (6.4%)	20 (16.5%)	11 (8.7%)	51 (11.3%)
pT1/ypT1	12 (12.5%)	8 (7.3%)	11 (9.1%)	12 (9.4%)	43 (9.5%)
pT2/ypT2	13 (13.5%)	23 (21.1%)	27 (22.3%)	35 (27.6%)	98 (21.6%)
pT3/ypT3	46 (47.9%)	53 (48.6%)	51 (42.1%)	54 (42.5%)	204 (45.0%)
pT4a/ypT4a	11 (11.5%)	12 (11.0%)	9 (7.4%)	13 (10.2%)	45 (9.9%)
pT4b/ypT4b	1 (1.0%)	6 (5.5%)	3 (2.5%)	2 (1.6%)	12 (2.6%)
pN stage					
N0/yN0	53 (55.2%)	60 (55.0%)	76 (62.8%)	79 (62.2%)	268 (59.2%)
N1a/yN1a	12 (12.5%)	17 (15.6%)	17 (14.0%)	14 (11.0%)	60 (13.2%)
N1b/yN1b	8 (8.3%)	6 (5.5%)	10 (8.3%)	14 (11.0%)	38 (8.4%)
N1c/yN1c	11 (11.5%)	10 (9.2%)	6 (5.0%)	5 (3.9%)	32 (7.1%)
N2a/yN2a	4 (4.2%)	8 (7.3%)	7 (5.8%)	8 (6.3%)	27 (6.0%)
N2b/yN2b	8 (8.3%)	8 (7.3%)	5 (4.1%)	7 (5.5%)	28 (6.2%)

Note: * The other types of operations include pelvic exenteration, removal of the rectum and sigmoid colon with vaginal wall, reproductive organs, bladder and other segments of small or large bowel.

** The histology grade of "low-grade" includes well differentiated and moderately differentiated carcinoma; whereas "high-grade" includes poorly differentiated, moderately to poorly differentiated carcinoma.

V.1.2 Changes in tumour characteristics from 2012 to 2015

Chi-square analyses were performed to study the relationship between the year and other categorical variables (Table 8), to investigate if there are changes over the years, regarding the tumour type, location, grade, response to treatment and etc. In fact, none of the all the variables is statistically unrelated to the variable of the years, indicating basic characteristics of rectal cancer didn't change over the years.

Table 8. Chi-square tests for multiple categorical variables with respect to year

Categorical variables	χ^2 value	df	N	*p* value	Significance
Type of operation	6.467	6	453	0.373	no
Patient gender	0.441	3	453	0.932	no
Patient age group	3.281	6	453	0.773	no
Site of tumour	39.086	27	453	0.062	no
Histology type	17.094	18	453	0.517	no
Histology grade	8.571	9	453	0.478	no
Neoadjuvant therapy presence	3.885	3	453	0.274	no
pT stage	19.801	15	453	0.180	no
pN stage	12.794	15	453	0.618	no

Note: df = degree of freedom is calculated by the sum of each variable levels – 1.

V.1.3 T stage, N stage, tumour site with the choice of neoadjuvant therapy

Chi-square tests indicated that the pT stage and pN stage are not statistically related to the years (Table 8); therefore the frequencies of T and N stages were analyzed together from 2012 to 2015. The most frequently observed combination is the pT3N0 and pT2N0, with 103/453 (22.7%) and 69/453 (15.2%) cases, respectively (Figure 7 A).

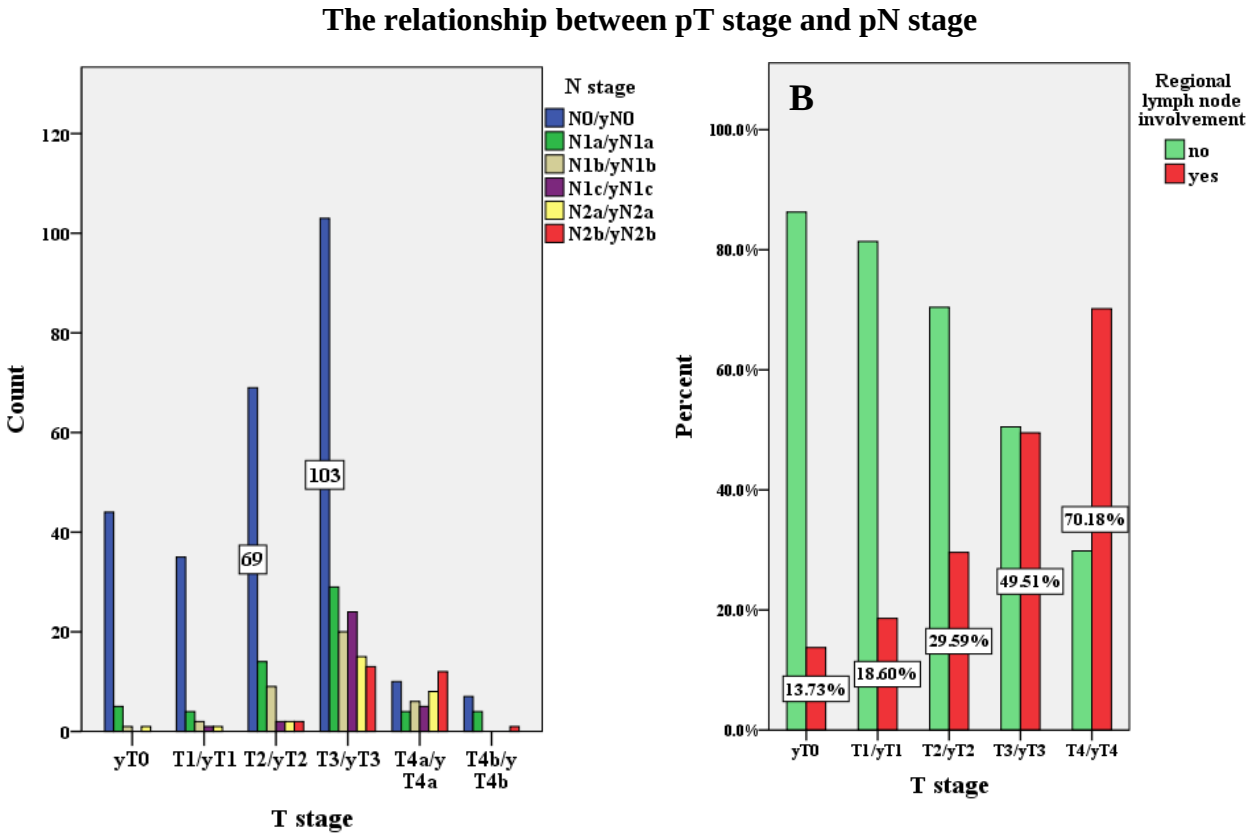


Figure 7. The relationship between pT stage and pN stage. **(A)** The number of cases in pT stage groups, clustered by N stage, 2012 to 2015. **(B)** The percentage of positive regional lymph nodes in different T stages.

The correlation study shows a significant correlation between the T stage and N stage, χ^2 (25, 453) = 109.288, $p < 0.001$. The percentage of patients have regional lymph node involvement is low in the early T stage tumour: ypT0 – 7/51 (13.7%), pT1/ypT1 – 8/43 (18.6%). As the T stage increases, more percentage of patients shows positive lymph nodes: pT2/ypT2 – 29/98 (29.6%) and pT3/ypT3 - 101/204 (49.5%). Once a tumour breaches the serosa in T4a/T4b, 40/57 (70.2%) of patients have regional lymph nodes involvement (Figure 7 B).

There is a significant correlation between the tumour site and the choice of operation type, χ^2 (14, 411) = 139.964, $p < 0.001$. Most 67/81 (82.7%) cases of APR were performed for lower rectal tumour (Figure 8 A). In fact, almost half (46.6%) of the lower rectal tumour was resected by LAR/ultra LAR, mainly to preserve anal continence if a good distal margin clearance is a possible or significant response to neoadjuvant therapy. When a tumour is at the anorectal junction, an APR or pelvic exenteration has to be carried out (Figure 8 A).

The choice of neoadjuvant therapy is also highly related to the tumour site, χ^2 (7, 411) = 99.297, $p < 0.001$. As a tumour is approaching the anal verge from proximal to distal, the percentage of patients with neoadjuvant therapy dramatically increased (Figure 8 B). Patients without neoadjuvant therapy have a relatively equal distribution in tumour site (Figure 8 B).

Surgical type and neoadjuvant therapy based on tumour location

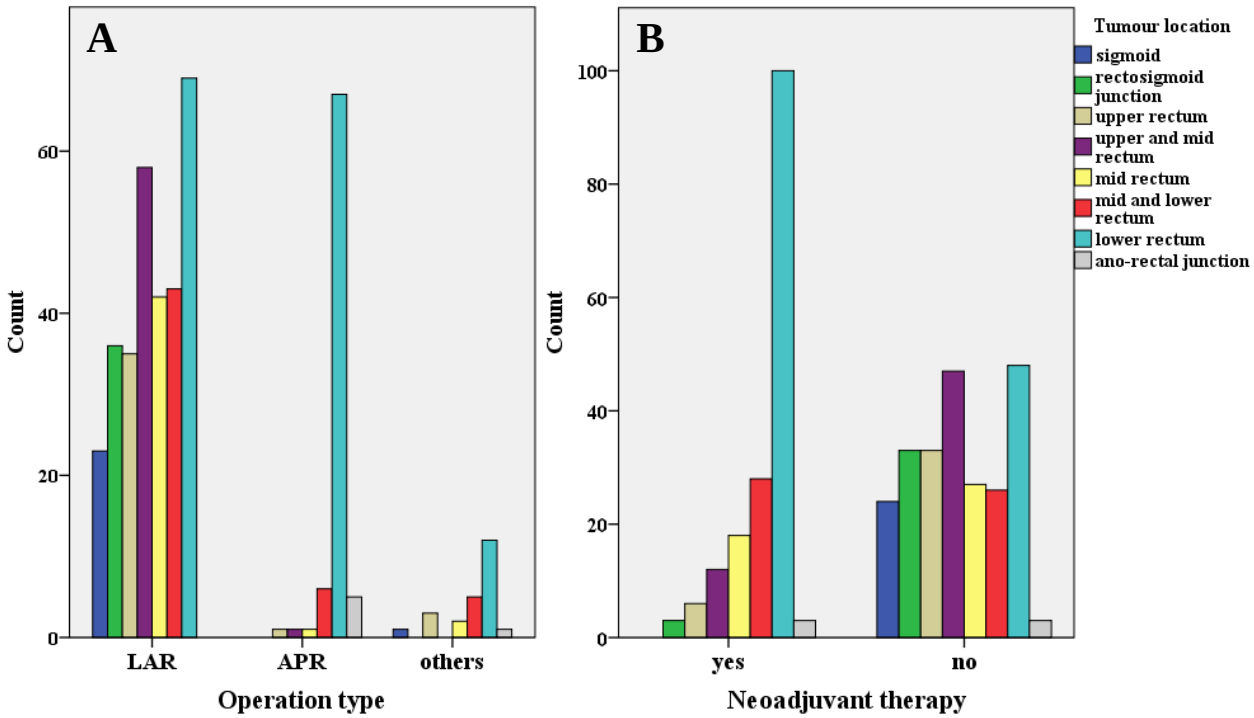


Figure 8. Surgical type and neoadjuvant therapy based on tumour location, 2012 to 2015. **(A)** The number of different surgical procedures, clustered by tumour location. **(B)** The number of patients who received neoadjuvant therapy, clustered by tumour location.

V.1.4 Factors related to neoadjuvant therapy response

Neoadjuvant radio/chemotherapies are widely used to shrink or even eliminate a tumour, especially for lower rectal tumours. It is important to find out the factors related to a good response to the preoperative treatment, in order to predict the outcome of future rectal cancer patients and to aid in the decision if neoadjuvant therapy is necessary.

Series of One-way ANOVAs were performed to study the factors associated with neoadjuvant response (Table 9).

Table 9. One-way ANOVA to study the factors related to complete pathological response

Independent variables	*F* value	dfn*	dfd**	*p* value	Significance
Year of operation	1.891	3	200	0.132	no
Patient gender	0.874	1	202	0.351	no
Surgeon category	1.473	3	200	0.223	no
Site of tumour	1.546	6	163	0.166	no
Histology type	0.801	3	155	0.495	no
Histology grade	59.406	2	201	**< 0.001**	**yes**
Patient age group	2.976	2	201	0.053	no
ypT stage	67.567	5	198	**< 0.001**	**yes**
ypN stage	5.743	5	198	**< 0.001**	**yes**

Note: * - dfn (degrees of freedom numerator). **- dfd (degrees of freedom denominator)

As expected, the histology grade had a significant impact on the treatment outcome, F (2, 201) = 59.406, $p < 0.001$. None of the high-grade carcinoma (moderately/poorly or poorly differentiated) has a complete response (grade 0) to the neoadjuvant therapy. Patients with high-grade carcinoma, such as moderately or poorly differentiated carcinoma tend to respond worse to the neoadjuvant treatment than the low-grade carcinoma, such as well differentiated (Figure 9 A, 9 B).

The ypT stage and ypN stage are also significantly associated with the neoadjuvant therapy response, with the p values less than 0.001, indicating the depth of invasion and lymph node involvement had a great impact on the treatment outcome (Figure 9 C, 9 D).

Although the "patient age group" did not have a significant impact on the neoadjuvant therapy outcome, the p-value is 0.053, which is slightly greater than the 0.05 threshold. Furthermore, homogeneous subsets statistical studies successfully separated the treatment effect into two groups: a good response group, with more young patients; and a bad response group with more old patients, suggesting the age might have some impact on predicting neoadjuvant therapy response, although not statistically significant in this particular study.

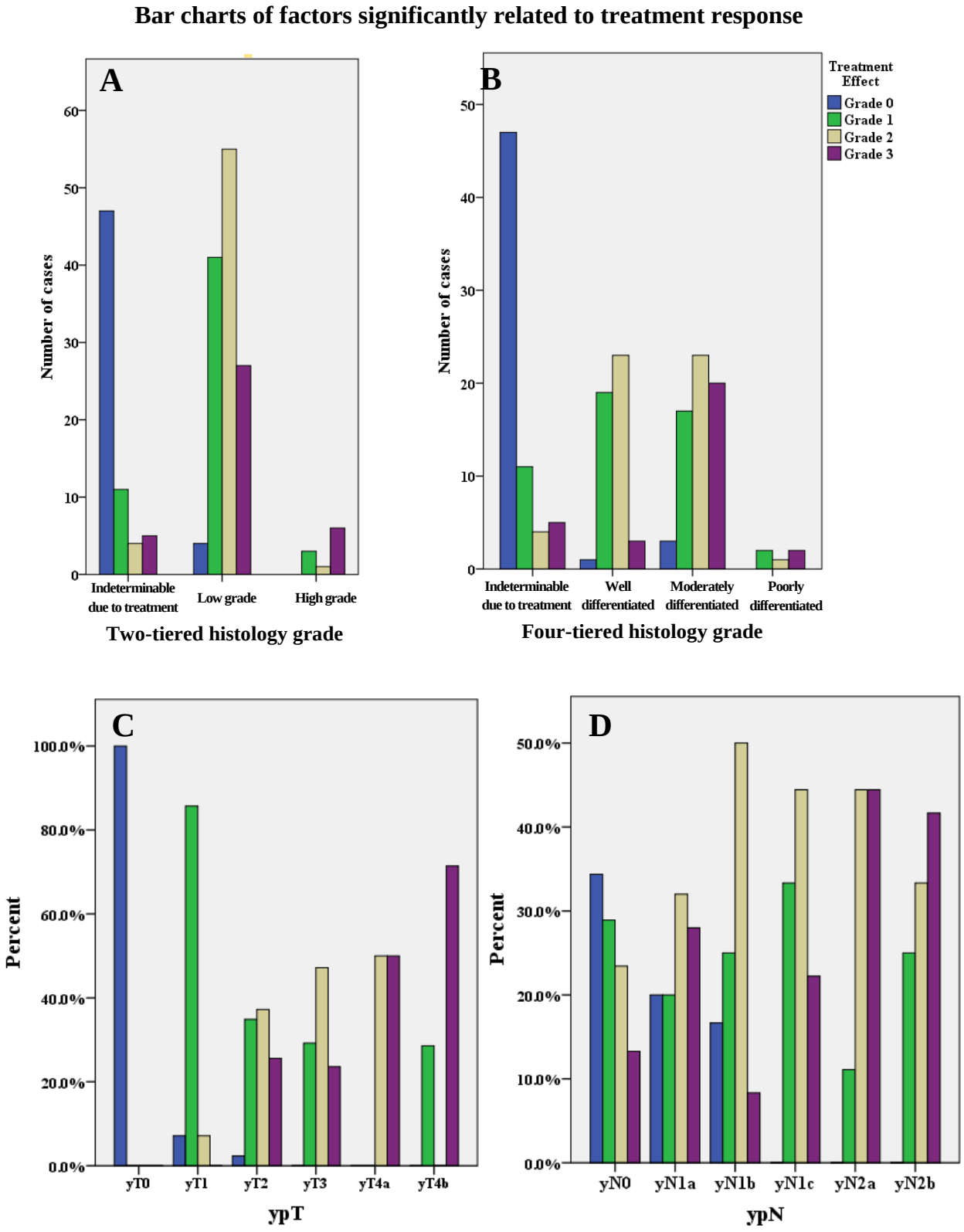


Figure 9. Bar charts of factors significantly related to treatment response. (**A)** Tumour histology grade clustered by the two-tiered histology grade. Generally, better response to the neoadjuvant

therapy is observed in low-grade tumours. **(B)** Tumour histology grade clustered by the four-tiered histology grade. None of the cases was diagnosed as undifferentiated. The cases diagnosed as "moderately to poorly differentiated", as well as the cases diagnosed as "well to moderately differentiated" were excluded in the statistical analysis. (**C)** Tumour stage clustered by neoadjuvant therapy response and (**D)** Lymph node stage clustered by neoadjuvant therapy response. Both show a strong correlation between the staging and response, suggesting high-grade tumours response worse than low-grade tumours.

V.1.5 Impact of age and gender on the pT stage, pN stage and histology grade

Age is one of the most important risk factors for colorectal cancer. In our cohort, 408/453 (90.1%) of patients are older than 50 years. One-way ANOVA shows that none of the pT stage, pN stage and histology grade is significantly related to age, with the *p*-value of 0.15, 0.43 and 0.57, respectively. However, we observed some association between pT stage and pN stage with patient age (Figure 10). The mean age of patient slightly increases from pT0 to pT4a and from pN1 to pN2a. Interestingly, the highest pathological stages, pT4b and pN2b have the youngest patients in average (Figure 10), suggesting a possible complex etiology for the highest pathological stage rectal carcinoma.

The patient gender does not have a significant correlation with the pN stage, χ^2 (5, N=454) = 4.583, p = 0.434 or tumour histology grade, χ^2 (5, N=451) = 3.235, p = 0.664. Only the pT stage shows a significant relationship with the gender, χ^2 (5, N=453) = 11.058, p = 0.050. Although lower in total number, women are more likely to get higher pT stage tumour than men.

For the patients with a complete response of neoadjuvant therapy, ypT0, 70.6% are male and only 29.4% are female. For the early pT stage, for example, pT1/ypT1, 62.8% are male and only 37.2% of female. However, more female patients are observed in the highest pT stage, pT4b/ypT4b. In this group, 58.3% are female and only 41.7% are male, suggesting females patients tend to develop higher pT stage carcinoma, despite the higher overall incidence of males for rectal carcinoma (Figure 11).

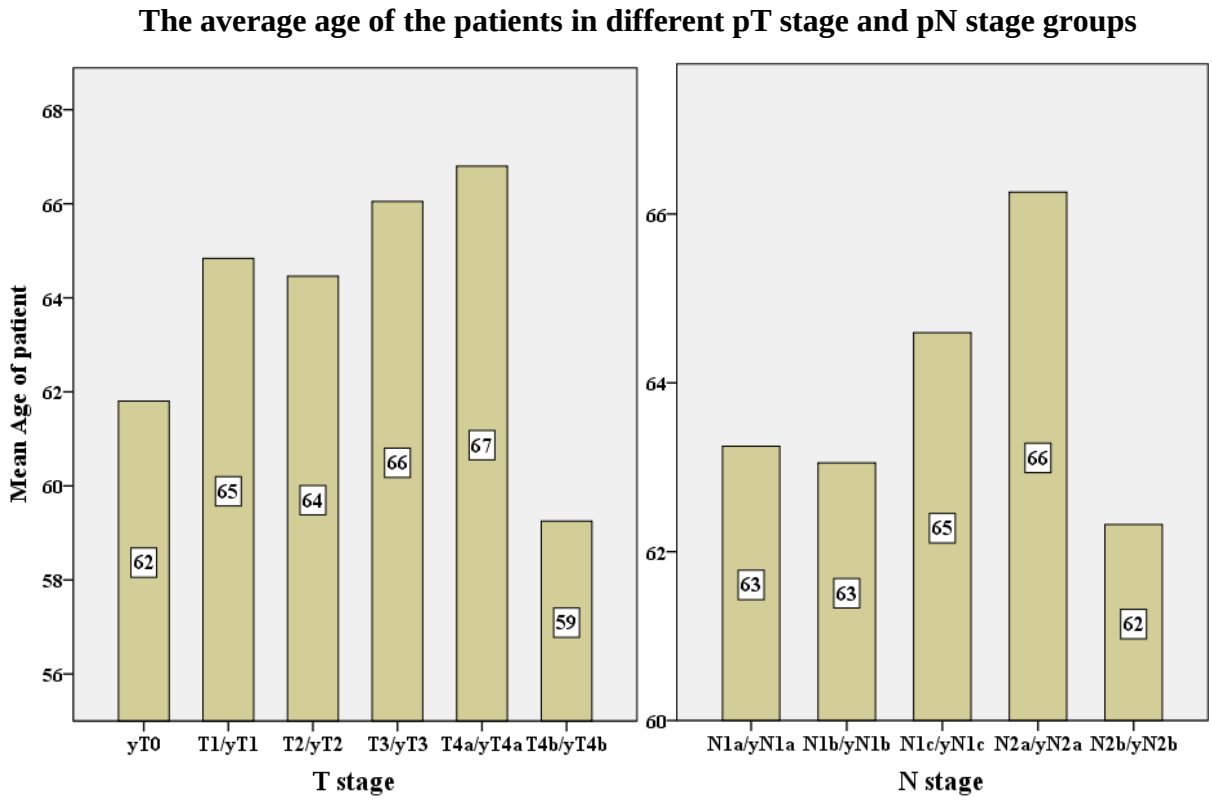


Figure 10. The average age of the patients in different pT stages and pN stage groups. Both graphs show a decrease of average age in the highest stage group.

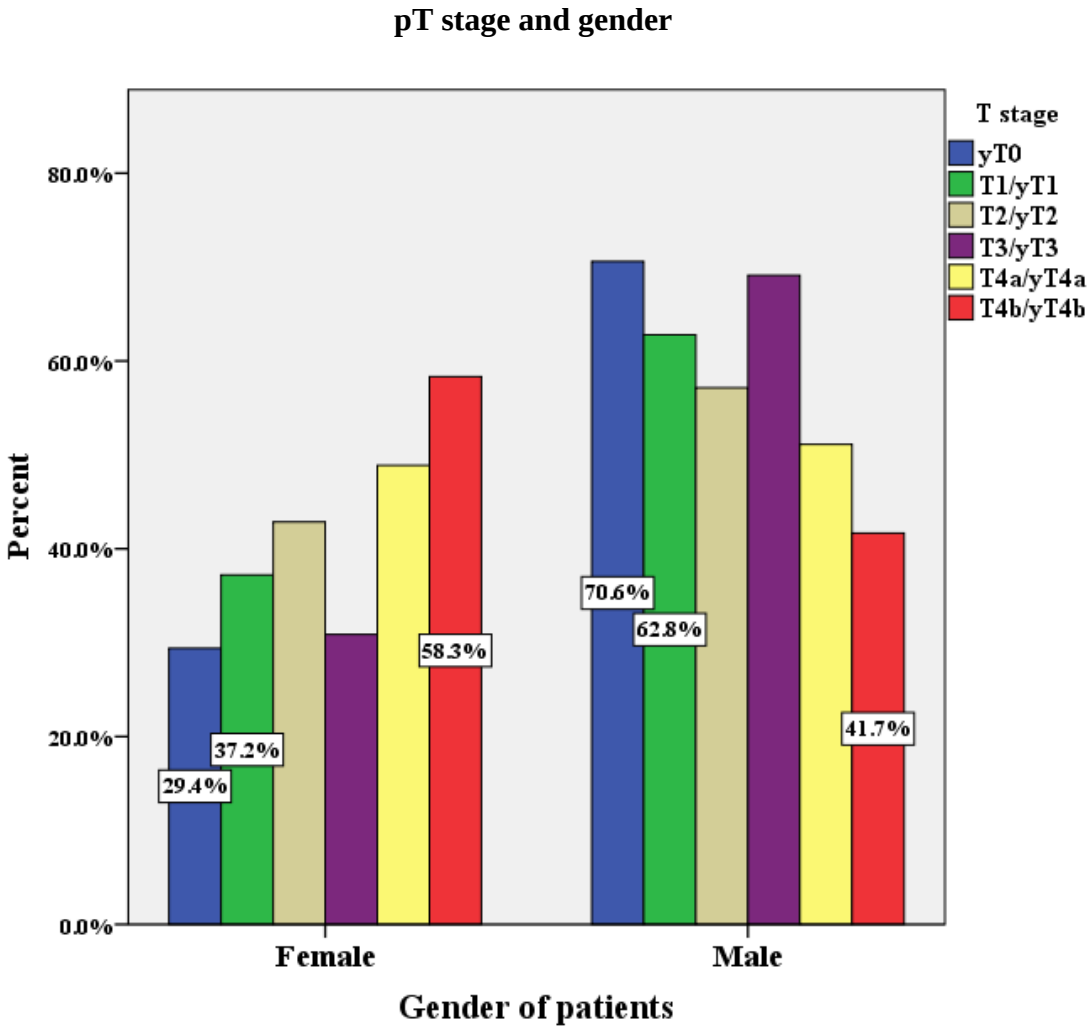


Figure 11. The percentage of each gender in different pT stage groups. Females tend to have more pT4a and pT4b rectal tumour than males.

V.2 Mesorectal excision quality audit

As previously mentioned, the incomplete mesorectal excision is highly correlated to increased local, systemic recurrence.[4] The quality of mesorectal excision of the 453 cases is discussed in this section, to study the factors related to the quality of the surgical procedure.

V.2.1 Mesorectal excision quality changes from 2012 to 2015

The importance of a complete mesorectal excision is well understood by surgeons and pathologists for more than a decade. The application of total mesorectal excision within the fascia plane has been carried out for many years. Therefore, the mesorectal excision quality is not hypothesized to be changed dramatically. One-way ANOVA shows no significant differences in mesorectal excision quality from 2012 to 2015. F (3, 419) = 0.910, p = 0.436. The percentage of each excision quality levels are clustered by year and shown in Figure 12. PAs did not provide the macroscopic examination of the mesorectal excision in 33/453 cases. The percentage of this decreased over the 4 years from 12.5% to 6.3%, indicating an improvement in the completeness of macroscopic mesorectal excision quality description, thanks to the better education. An average of 58.9% and 27.2% of surgeries were graded as complete and nearly complete, respectively. The incomplete percentages stabilized to the level of 10.9% with minimal variations between years, which is compatible with the current published data discussed in the introduction section.

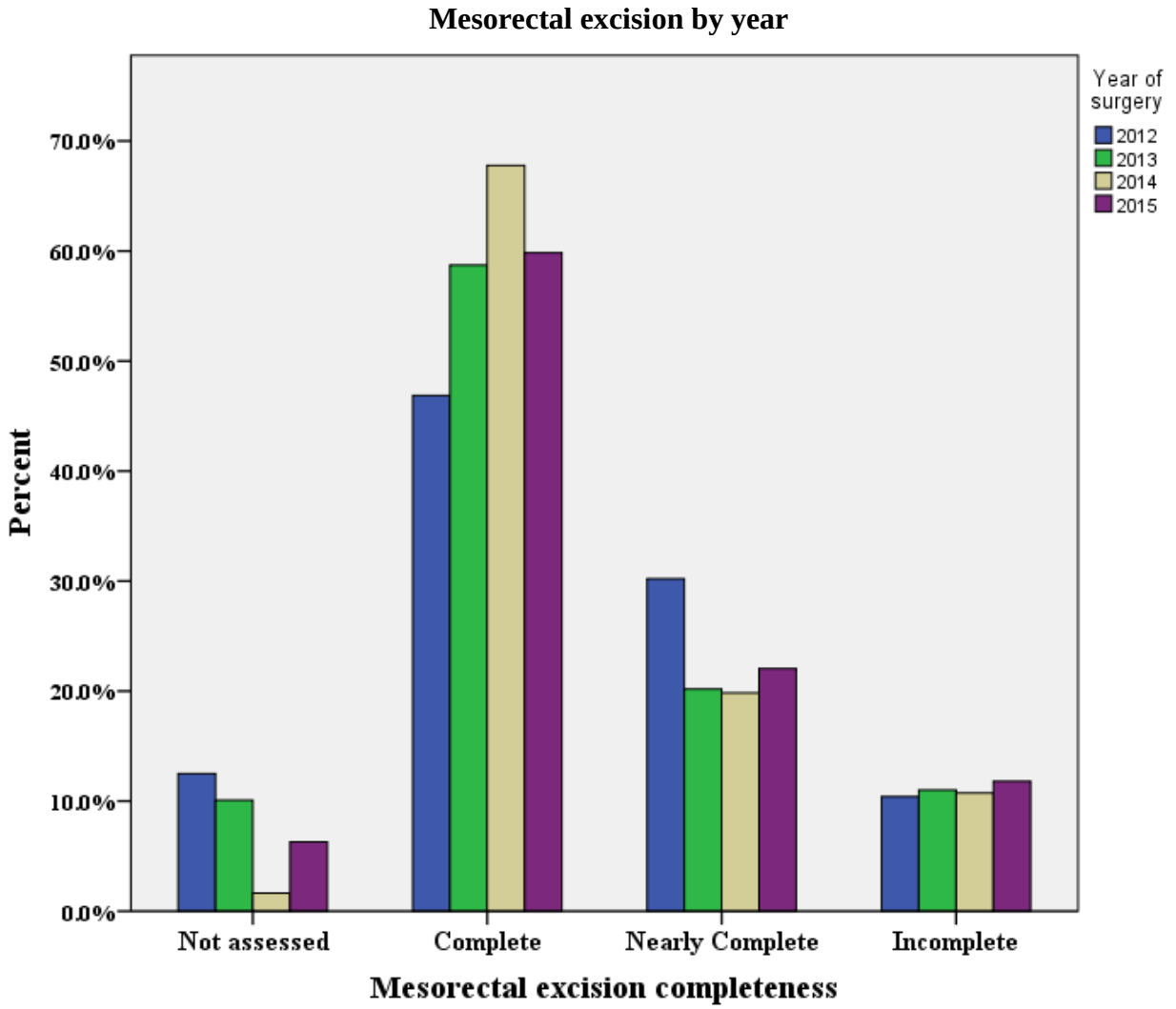


Figure 12. Mesorectal excision quality, clustered by year. Statistical analysis showed no significant changes in the surgical quality over the entire study period.

V.2.2 Potential factors associated with mesorectal excision quality

Since the operation year is not a factor associated with surgical quality, we combined the 4 years data and studied the association between other potential factors listed in Table 10 affecting the mesorectal excision quality, using chi-square analysis.

Table 10. Univariate analysis of relationship between factors and mesorectal excision quality

Factors	Complete	Nearly complete	Incomplete	Total	χ^2	p value/ Significance
	267	103	50	420		
Age					6.621	0.157/no
25-49 years	35 (79.5%)	8 (18.2%)	1 (2.3%)	44 (100.0%)		
50-79 years	194 (61.8%)	79 (25.2%)	41 (13.1%)	314 (100.0%)		
80-93 years	38 (61.3%)	16 (25.8%)	8 (12.9%)	62 (100.0%)		
Gender					4.215	0.122/no
Female	92 (59.0%)	47 (30.1%)	17 (10.9%)	156 (100.0%)		
Male	175(66.3%)	56 (21.2%)	33 (12.5%)	264 (100.0%)		
Margin					14.612	**0.001/yes**
Negative CRM	259 (65.6%)	94 (23.8%)	42 (10.6%)	395 (100.0%)		
Positive CRM	8 (32.0%)	9 (36.0%)	8 (32.0%)	25 (100.0%)		
Tumour grade					6.769	0.343/no
Indeterminable due to treatment	44 (64.7%)	14 (20.5%)	10 (14.7%)	68 (100.0%)		
Low-grade [a]	99 (67.8%)	32 (21.9%)	15 (10.3%)	146 (100.0%)		
High-grade [b]	124(60.5%)	56 (27.3%)	25 (12.1%)	205 (100.0%)		
Tumour stage					4.21	0.838/no
yT0	30 (61.2%)	11 (22.4%)	8 (16.3%)	49 (100.0%)		
T1/yT1	24 (58.5%)	13 (31.7%)	4 (9.8%)	41 (100.0%)		
T2/yT2	57 (61.3%)	22 (23.7%)	14 (15.0%)	93 (100.0%)		
T3/yT3	121 (65.0%)	45 (24.2%)	20 (10.8%)	186 (100.0%)		
T4/yT4	35 (68.6%)	12 (23.5%)	4 (7.8%)	51 (100.0%)		
Regional lymph node involvement					0.695	0.706/no
no	163 (64.9%)	58 (23.1%)	30 (12.0%)	251 (100.0%)		
yes	104 (61.5%)	45 (26.6%)	20 (11.8%)	169 (100.0%)		

Table 10 (continued)

Factors	Complete	Nearly complete	Incomplete	Total	χ^2	p value/ Significance
	267	103	50	420		
Tumour location [c]					20.8	**0.016/yes**
Rectosigmoid junction	25 (78.1%)	5 (15.6%)	2 (6.3%)	32 (100.0%)		
Upper rectum	23 (74.2%)	6 (19.3%)	2 (6.5%)	31 (100.0%)		
Upper-mid rectum	39 (70.9%)	11 (20.0%)	5 (9.1%)	55 (100.0%)		
Mid rectum	27 (65.8%)	10 (24.4%)	4 (9.8%)	41 (100.0%)		
Mid-lower rectum	35 (68.6%)	12 (23.5%)	4 (7.8%)	51 (100.0%)		
Lower rectum	83 (57.2%)	37 (25.5%)	25 (17.3%)	145 (100.0%)		
Ano-rectal junction	1 (16.7%)	3 (50.0%)	2 (33.3%)	6 (100.0%)		
Operation type					14.233	**0.007/yes**
LAR	214 (67.9%)	68 (21.6%)	33 (10.5%)	315 (100.0%)		
APR	42 (50.0%)	26 (31.0%)	16 (19.0%)	84 (100.0%)		
Neoadjuvant therapy					2.357	0.308/no
no	148 (66.4%)	53 (23.8%)	22 (9.9%)	223 (100.0%)		
yes	119 (60.4%)	50 (25.4%)	28 (14.2%)	197 (100.0%)		
Surgeon training					44.951	**<0.001/yes**
Colorectal surgeon	121 (75.2%)	26 (16.1%)	14 (8.7%)	161 (100.0%)		
General surgeon	114 (57.9%)	57 (28.9%)	26 (13.2%)	197 (100.0%)		
Surgical oncologist	32 (55.2%)	20 (34.5%)	6 (10.3%)	58 (100.0%)		
Unspecified [d]	0 (0.0%)	0 (0.0%)	4 (100.0%)	4 (100.0%)		
Surgeon volume [e]					16.997	**0.036/yes**
High (21 - 54)	159 (63.9%)	63 (27.0%)	21 (9.0%)	233 (100.0%)		
Mid (11-20)	53 (63.9%)	20 (24.1%)	10 (12.0%)	83 (100.0%)		
Low (1-10)	65 (62.5%)	20 (19.2%)	19 (18.3%)	104 (100.0%)		
PA category					28.412	**<0.001/yes**
Non-Canadian pathologist [f]	113 (80.1%)	20 (14.2%)	8 (5.7%)	141 (100.0%)		
On-site trained [g]	49 (58.3%)	24 (28.6%)	11 (13.1%)	84 (100.0%)		
Program trained [h]	97 (55.4%)	52 (29.7%)	26 (14.9%)	175 (100.0%)		
Resident	8 (40.0%)	7 (35.0%)	5 (25.0%)	20 (100.0%)		

Note: a - “Low-grade” includes well differentiated and moderately differentiated carcinoma.

b -“High-grade” includes poorly differentiated, moderately to poorly differentiated carcinoma.

c- “Tumour location” is based on the location of a tumour, with respect to the anterior/posterior peritoneal reflections in the gross description.

d- “Unspecified” in surgeon training background indicates the group of surgeons with other specialities, for example, gynecologists, urologists and etc., who might perform the mesorectal excision in an emergency situation or as a part of the pelvic exenteration.

e – “Surgeon volume” is the variable calculated by the total number of surgery performed by each surgeon from 2012-2015. High volume means the surgeons in this group performed more than 20 cases in this time period; while, the mid and low volume surgeons performed 11-20, and less than 10 cases, respectively.

f – “Non-Canadian pathologist” represents a group of PA who received pathology training abroad and currently practices as a PA in Winnipeg.

g – “On-site trained” represents a group of PA received job training in the local pathology lab.

h – “Program trained” represents UofM PA student or graduate. The provincial leading PA, who is in charge of the UofM PA program training, is classified in this group, despite the on-site trained background, as the PA students/graduates are largely educated and influenced by the lead PA.

In summary, the mesorectal excision quality is not statistical significantly related to the age ($p = 0.157$), gender ($p = 0.122$), T stage ($p = 0.838$), N stage ($p = 0.706$), tumour grade ($p = 0.555$), or the application of neoadjuvant therapy ($p = 0.308$). It is significantly related to the CRM positivity ($p < 0.001$), tumour location ($p = 0.016$), operation type ($p = 0.007$), surgeon

category ($p < 0.001$), surgeon volume ($p = 0.036$), and PA category ($p < 0.001$). Each related factors are to be discussed in the later sections.

There are two additional factors, Patients' Body Mass Index (BMI) and follow-up data on recurrence, which are not included in the study due to the limited access to the patient medical chart, and might be investigated in the further studies.

V.2.3 Mesorectal excision quality is highly associated with CRM positivity

Due to the limited information on patients' follow-up data, the impact of the mesorectal excision quality and circumferential radial margin (CRM) positivity cannot be correlated with recurrence. Alternatively, the status of the CRM is another important diagnostic element and largely affects patient's prognosis and treatment. We showed a strong correlation between the mesorectal excision completeness and the CRM positivity, χ^2 (2, N=420) = 14.612, p = 0.001. CRM positivity is observed in 8/50 (16.0%) of the "incomplete"; 9/103 (8.7%) of the "nearly complete"; and only 8/267 (2.9%) of the "complete" mesorectal excision specimens, indicating a strong positive correlation between the mesorectal excision completeness and the CRM positivity (Figure 13).

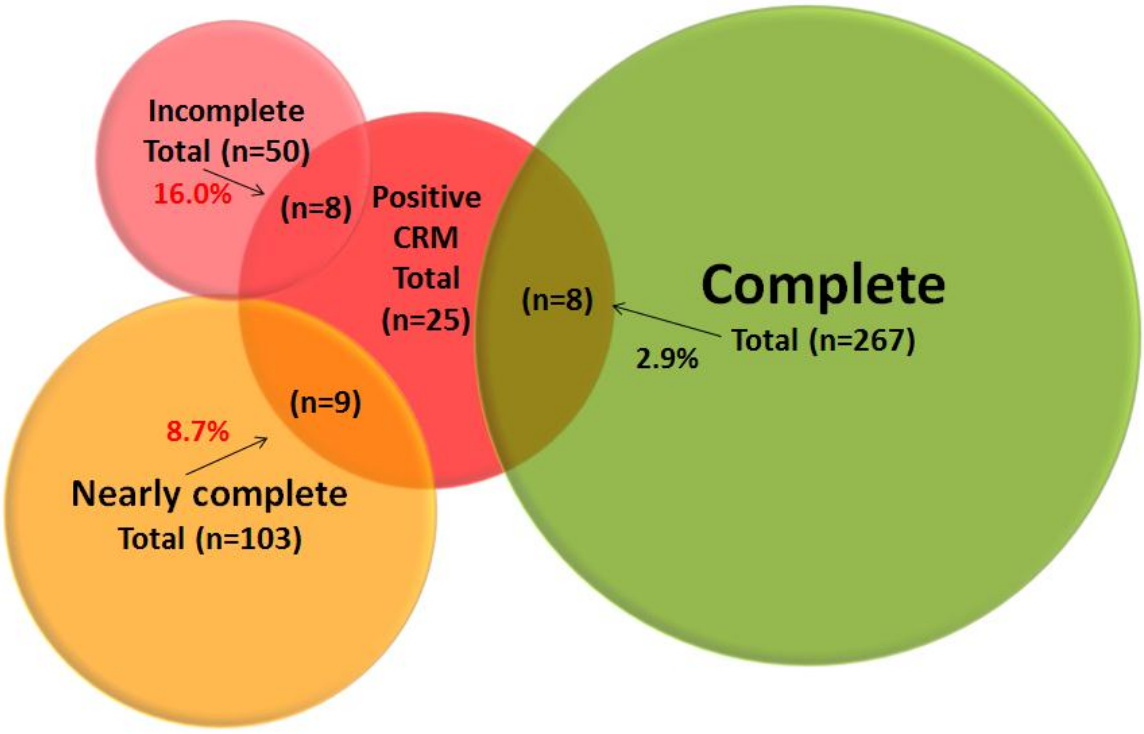


Figure 13. Venn diagram showing the percentage of the mesorectal excision specimen with a positive CRM, clustered by mesorectal excision completeness.

V.2.4 Mesorectal excision quality is highly associated with tumour location

The pelvic cavity is narrow and can be extremely difficult for surgeons to get access to. The difficulty can be magnified with obese patients or patients with adhesions from previous surgery. Therefore, a more distal rectal tumour, close to the anal verge, is expected to be more difficult to excise completely than a proximal rectal tumour, which is partially peritoneal covered. Indeed, a strong relationship between the mesorectal excision quality and tumour location is observed, χ^2 (12, N=420) = 20.816, p = 0.016. The percentage of incomplete excisions elevated from 6.3% to 33.3%, as the tumour location moves from rectosigmoid junction to anorectal junction, indicating the difficulty of surgery increases when a tumour is deeper in the pelvis (Figure 14).

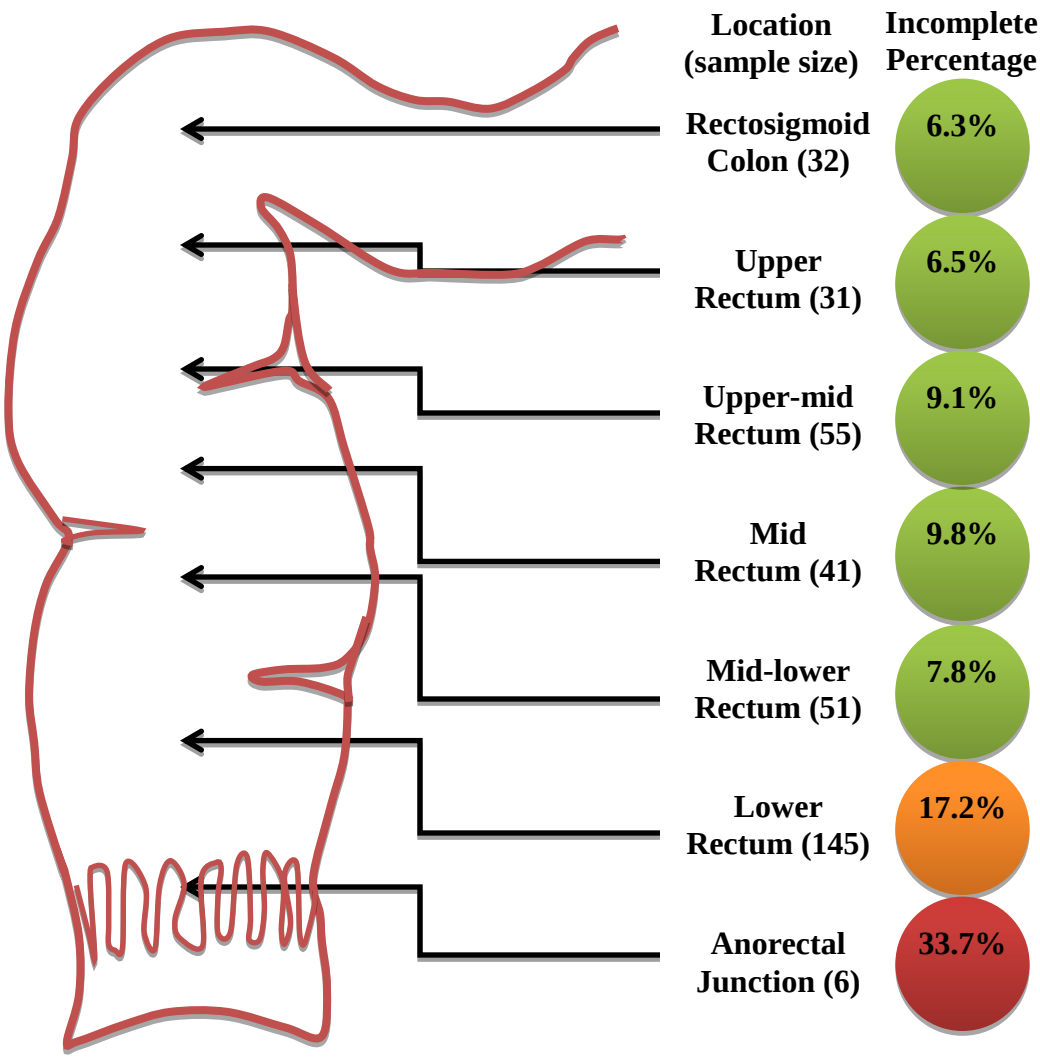


Figure 14. Incomplete mesorectal excision percentage at different tumour location. Tumour location is defined with respect to the anterior and posterior reflections. The ones straddle the anterior peritoneal reflection, are defined as a mid-lower rectal tumour. If the proximal edge is below the anterior peritoneal reflection, the tumour is defined as a lower rectal tumour. The tumour location can be different from the clinical definition, which is mainly based on distal from the anal verge. (Illustration image is used with permission of Netter images, ELSEVIER)

V.2.5 Mesorectal excision quality is highly associated with surgeon training background and the volume of surgery performed

Colorectal surgeons (CRS) are specialized surgeons with minimal 2 years colorectal surgery subspecialty training, defined by the Canadian Society of Colon and Rectal Surgeon.[53] Similarly, surgical oncologists (SO) are a group of subspecialty trained surgeons focusing on surgical treatment of tumours. The additional subspecialty fellowship training can be beneficial in providing additional knowledge and experiences to treat colorectal cancer to improve surgical quality, including the mesorectal excision. The correlation study showed a significant impact of surgeon training background on mesorectal excision, χ^2 (6, N=420) = 44.315, p <0.001. The weighted average incomplete excision percentages are 8.7%, 10.3% and 13.2% for CRS, SO and general surgeon (GS), respectively (Figure 15 A). In addition, pairwise comparison analysis shows significant differences in surgical quality between each pair, except the CRS/SO and GS/SO. The mean differences and a *p*-value of each pair comparison are listed in Table 11.

The volume of mesorectal excision surgeries performed is another significant factor associated with surgical quality, χ^2 (5, N=420) = 16.997, p = 0.036. High volume surgeon appears to have a higher complete resection percentage, from the greater experience with a large volume of surgery. On the other hand, low volume surgeons, although also well trained in surgical technique, may have limited opportunity to perform mesorectal excisions, like 1-2 cases per year in average, with a relatively higher incomplete percentage. The weighted average incomplete excision percentages are 9.01%, 12.05% and 18.27% for the high, mid and low volume surgeons, respectively (Figure 15 B).

Surgical quality with surgeon training background and volume

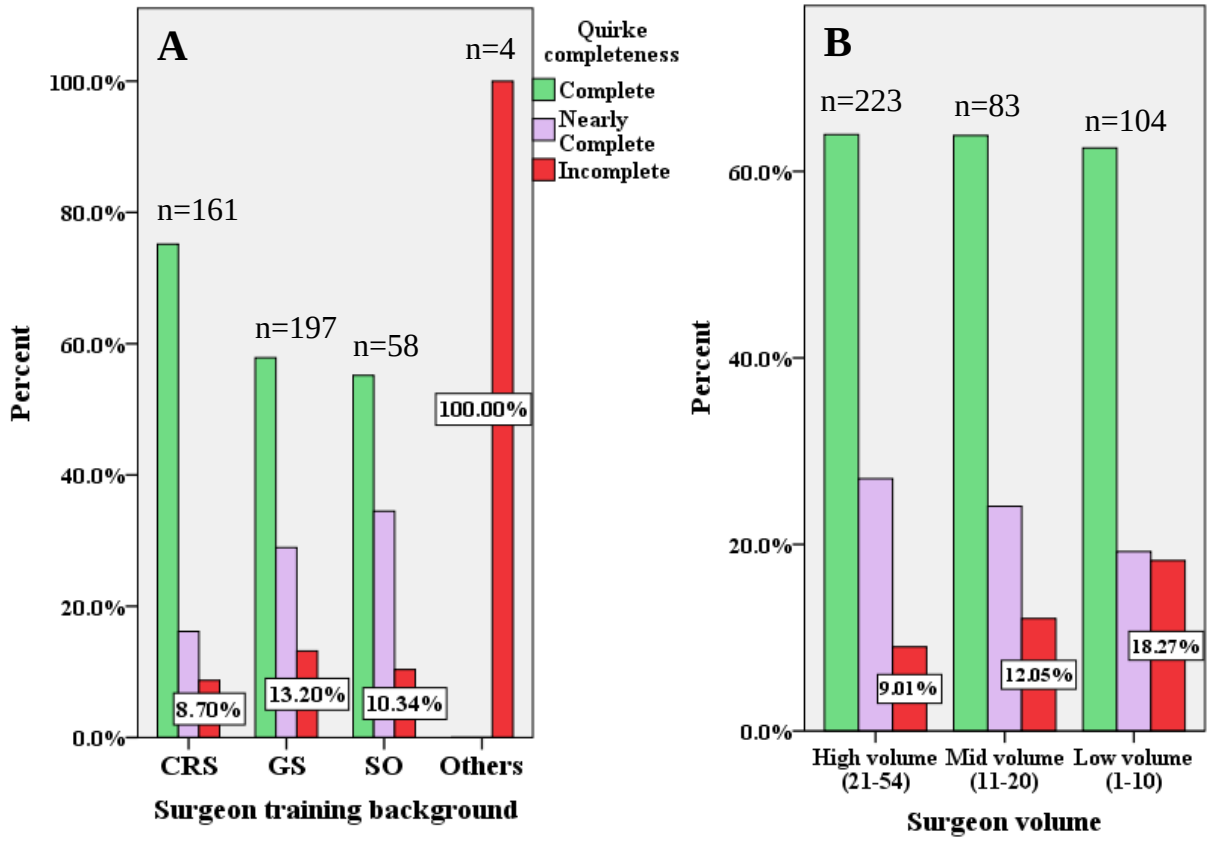


Figure 15: Percentage of incomplete mesorectal excision associated with surgeon's training background and volume. **(A)** surgeon training background. **(B)** The number of surgery performed on the mesorectal excision quality. Significant differences were observed within levels of both variables. CRS – colorectal surgeon; GS – General surgeon; SO – surgical oncologists; Others – unspecified surgeons, like gynecologists, urologists and etc., who don't normally perform colorectal surgery on regular bases.

Table 11: Pairwise comparison of the surgical quality by surgeon groups

Dependent Variable: Quirke completeness

(I) Surgeon group	(J) Surgeon group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
CRS	GS	-.230	.103	.026	-.433	-.027
	SO	-.164	.135	.227	-.429	.102
	Others	-1.593	.358	.000	-2.298	-.889
GS	CRS	.230	.103	.026	.027	.433
	SO	.067	.133	.616	-.195	.328
	Others	-1.363	.358	.000	-2.066	-.660
SO	CRS	.164	.135	.227	-.102	.429
	GS	-.067	.133	.616	-.328	.195
	Others	-1.430	.368	.000	-2.154	-.706
Others	CRS	1.593	.358	.000	.889	2.298
	GS	1.363	.358	.000	.660	2.066
	SO	1.430	.368	.000	.706	2.154

Note: Pairwise comparison of the average surgical quality difference between the groups of surgeons with different training background. A smaller mean indicates better surgical quality. The numbers with green circles are not significantly different, suggesting similar surgical quality. The others are significantly different, suggesting different surgical quality. CRS – colorectal surgeon; GS – General surgeon; SO – surgical oncologists; Others – unspecified surgeons, like gynecologists, urologists and etc., who don't normally perform colorectal surgery on regular bases.

V.2.6 Mesorectal excision quality and month effect

In addition, Chi-square test shows no significant relationship between the month of surgery and incomplete percentage, χ^2 (11, N=420) = 7.535, p = 0.754. And the number of surgeries performed in each month is not significantly related to the incomplete percentage, R^2 = 0.123, p = 0.291, Some correlations between the two variables are observed. For example, the highest number of surgery, 46 was observed in October, and the incomplete percentage is also the highest, 19.57% (Figure 16). However, the high p-value is due to several outliners, like June, where the incomplete percentage is the lowest, 4.55%, and the number of surgeries performed is the second highest, 44 (Figure 16).

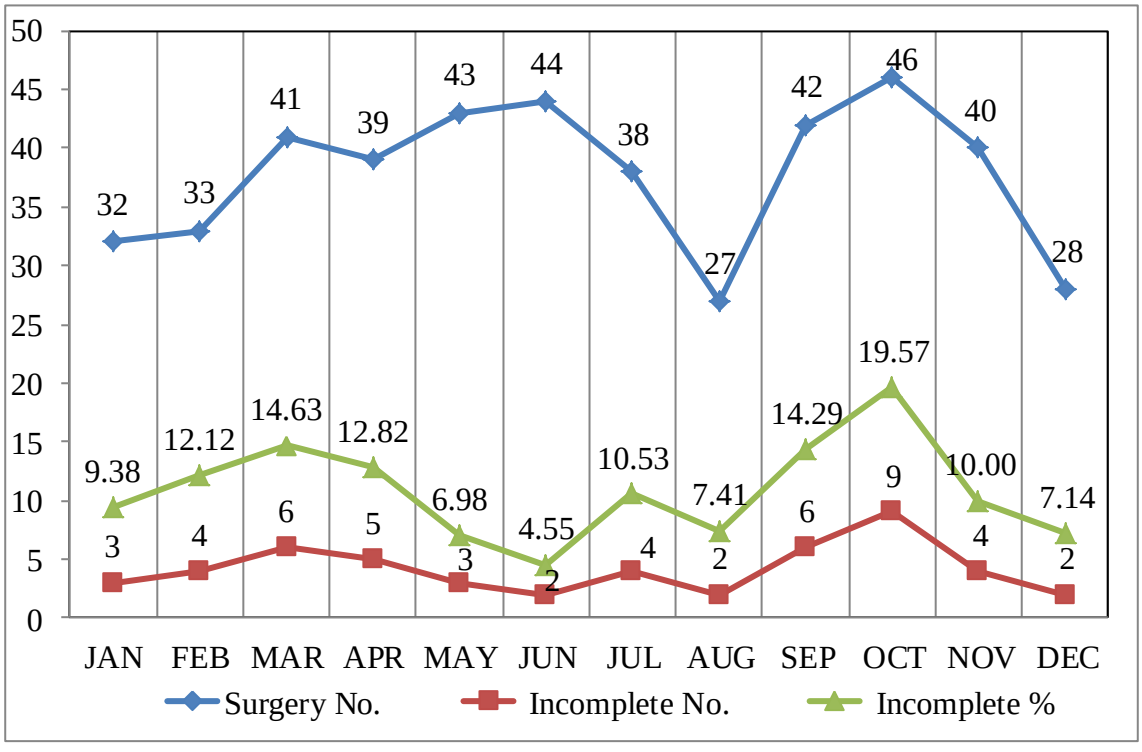


Figure 16. Plot chart of the number and percentage of incomplete mesorectal excision with the number of surgeries performed in each month of the year.

V.2.7 Grading of mesorectal excision quality is highly associated with PA training background

Four categories of PA performed macroscopic examinations of the mesorectal excision, including PAs who were non-Canadian trained pathologists, on-site trained PAs, program trained PAs and residents. The percentage of described incomplete mesorectal excision varies from 5.7% (graded by non-Canadian trained pathologist PAs) to 25.0% (graded by residents). Therefore, the assessment of the surgery quality is statistical significant associated with the PA training background, χ^2 (6, N=420) = 28.412, p <0.001 (Figure 17).

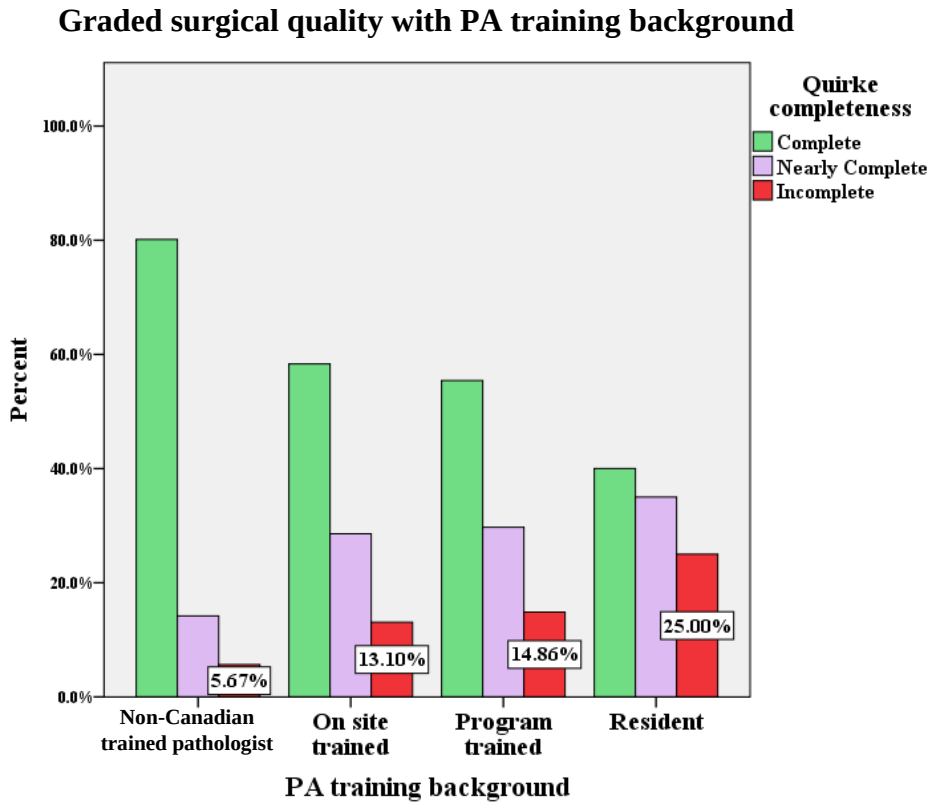


Figure 17. PA training background has a great impact on the grading of mesorectal excision, suggesting a standardized assessment criteria is to be established.

Additionally, a pairwise comparison was performed showing significant differences in the estimated marginal mean values of the non-Canadian trained pathologist with any other group of PA training background (Table 12), indicating a different understanding of the macroscopic examination criteria. Therefore, a better education with a detailed standardized grossing protocol is to be established.

Table 12: Pairwise comparison of the surgical quality by PA groups

Dependent Variable: Quirke completeness

(I) PA category	(J) PA category	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
NCP [a]	OT [b]	-.567	.192	.003	-.945	-.190
	PT [c]	-.602	.142	.000	-.881	-.322
	Resident	-.766	.216	.000	-1.190	-.342
OT	NCP	.567	.192	.003	.190	.945
	PT	-.034	.219	.875	-.464	.396
	Resident	-.199	.272	.466	-.734	.337
PT	NCP	.602	.142	.000	.322	.881
	OT	.034	.219	.875	-.396	.464
	Resident	-.164	.240	.494	-.636	.307
Resident	NCP	.766	.216	.000	.342	1.190
	OT	.199	.272	.466	-.337	.734
	PT	.164	.240	.494	-.307	.636

Note: A smaller mean indicates better surgical quality. Numbers in the red circles indicate significant differences in incomplete percentage assessment, between the two groups of PAs.

a – "NCP" represents Non-Canadian trained pathologist, who is currently working as a PA in Canada.

b – "OT" represents on-site trained PA.

c – "PT" represents program trained students or graduate.

V.2.8 Two-way ANOVA shows the surgeon training background and PA training background affect the graded mesorectal excision quality independently

Since the surgeon and PA training background variables have a significant impact on the graded mesorectal excision quality. We studied the two-way ANOVA between the two variables and found: (1) there was a significant PA training background effect on the graded surgical quality, F (3, 405) = 3.435, p = 0.017, partial $\eta 2$ = 0.025; (2) there was a significant surgeon training background effect on the graded surgical quality, F (3, 405) = 6.789, p <0.001, partial $\eta 2$ = 0.048; (3) however, the "interaction" of two variables of "PA group" and "surgeon group" is not significant F (8, 405) = 0.565, p = 0.807, partial $\eta 2$ = 0.011 (Table 13). Therefore, the PA group and surgeon group variables are entirely unrelated to each other, in term of the impact on surgical quality. In other words, the surgical quality differences between surgeons with different training background are unrelated or independent of the category of PA assessed and graded the specimen. Although there are variations of the surgical quality graded by different groups of PA, they graded the specimens collected by different groups of surgeons in a similar fashion, suggesting the observed differences in the surgical quality were not caused by PA assessment variations (Figure 18).

Table 13: Two-way ANOVA test of the interaction between surgeon group and PA group
Dependent Variable: Quirke completeness

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
PA group	4.558	3	1.519	3.435	.017	.025
Surgeon group	9.009	3	3.003	6.789	.000	.048
PA group * Surgeon group	1.999	8	.250	.565	.807	.011

Note: The interaction is not significant, indicating the two variables affect the mesorectal excision quality independently. Red circles indicate significantly different, and green circles indicate not significantly different.

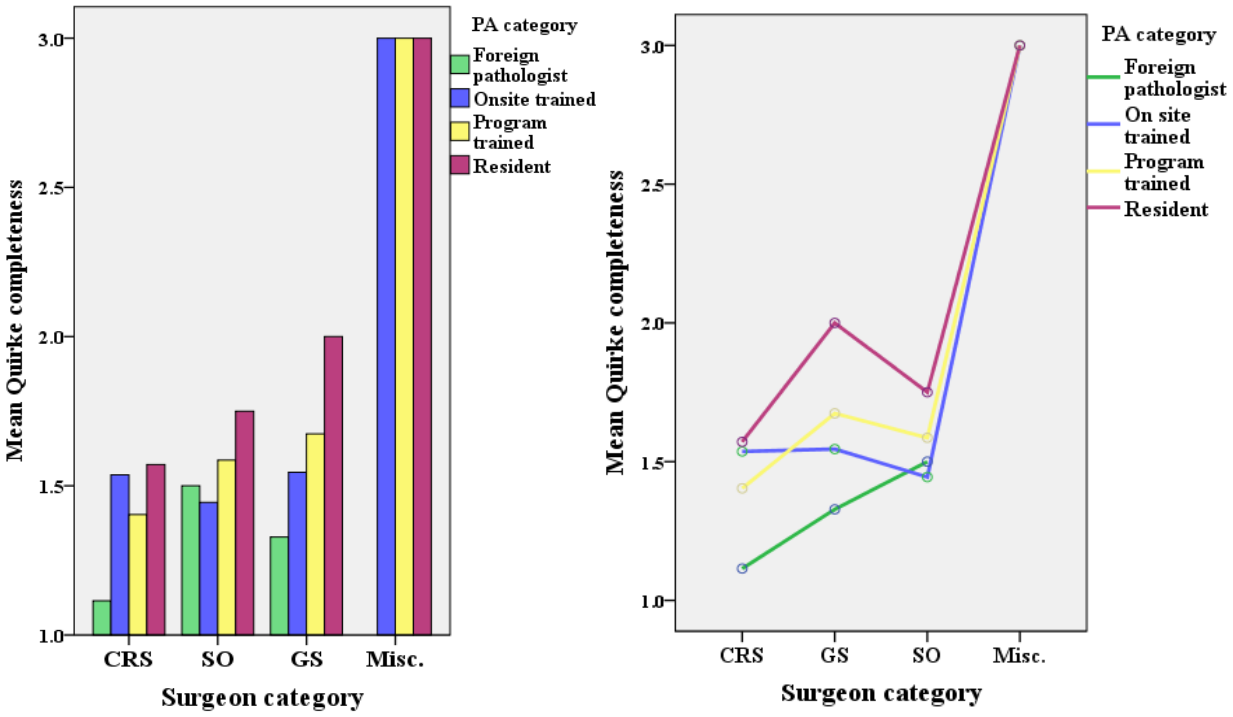


Figure 18. Bar and plot charts of mesorectal excision quality changes among surgeon group, clustered by PA group. Smaller mean Quirke completeness suggests better surgical quality.

V.3 Audit of the macroscopic examination performed by PA

The macroscopic examination is usually performed by PAs, occasionally by residents. An accurate, concise gross description is critical in helping pathologists to understand and assess the specimen. Adequate and effective sampling is important to make the accurate and effective diagnose. Finding a representative number of lymph nodes is crucial for TNM staging; however, factors like pre-surgical neoadjuvant treatment can make this challenging at times. Therefore, an accurate macroscopic examination, effective sampling as well as lymph node yield is important in the pathological assessment of mesorectal excision specimens, and are to be discussed in the following section.

V.3.1 Proper grossing descriptors for mesorectal excision quality examination

The macroscopic examination of the mesorectal excision specimen was carried out by 4 groups of individuals, non-Canadian-trained pathologists, working as a PA (NCP), On-site trained PAs (OT), program-trained PAs (PT), and residents. A significant relationship between the PA group and the year of surgery was observed, χ^2 (9, N=453) = 67.057, p <0.001 (Figure 19). In addition, the percentages of cases grossed by NCP and residents increased from 13.54% to 50.39% and from 2.08% to 11.02%, respectively. In contrast, the percentages dropped from 25.00% to 7.09% and from 59.38% to 31.50% for the OT and PT, respectively.

There is a concern about the number of cases grossed by all residents, who performed only 8 cases in total from January 2012 till December 2014. We should have at least 6 residents grossing at different affiliates for grossing clinical rotations; however, only 8/(6*3) = 0.44 cases of rectal cancer were grossed on average per resident per year, suggesting more grossing

experiences might be needed for residents. The situation was better in 2015, with 14/6 = 2.3 cases were grossed by each resident on average.

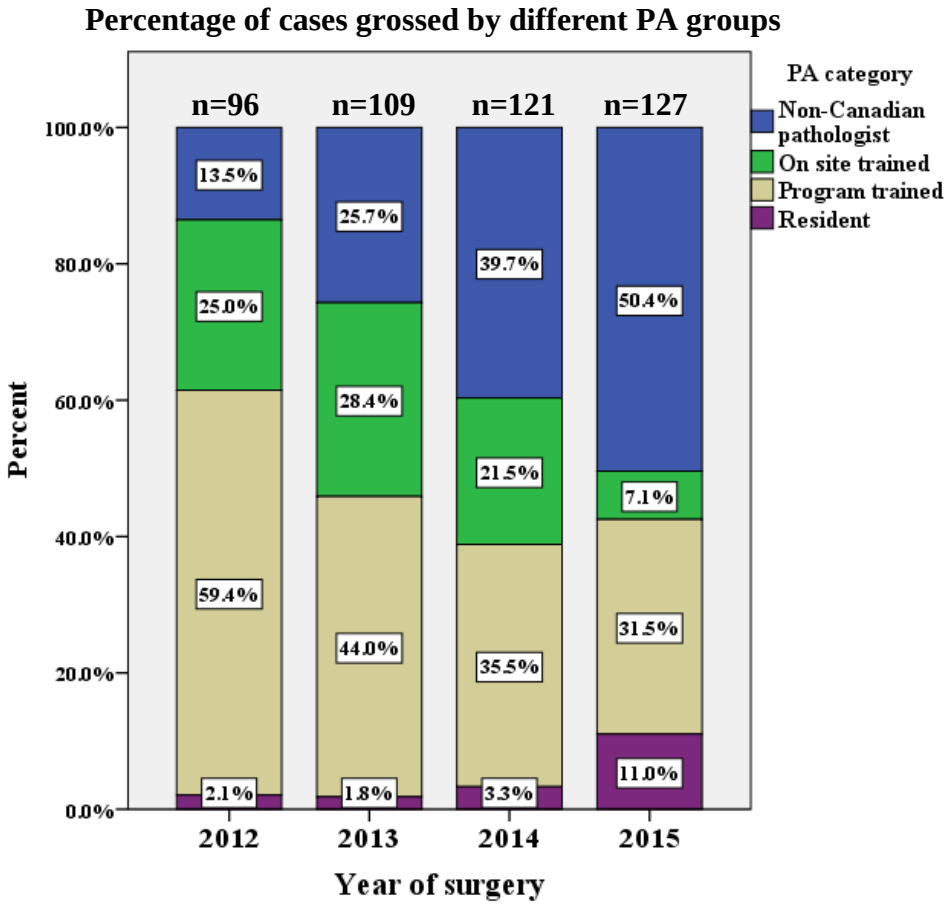
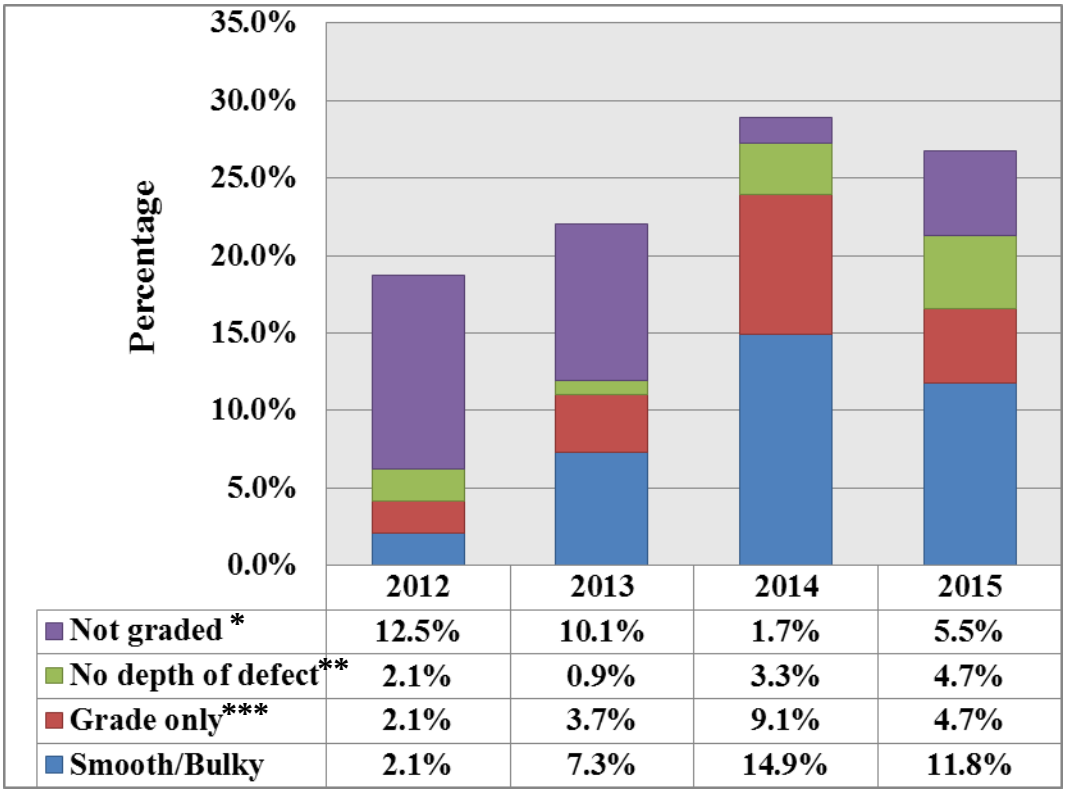


Figure 19. Percentage of cases grossed by different PA groups. An increase was observed for NCP and resident; while a decrease was observed for OT and PT. Each resident grossed less than 0.5 cases per year on average from 2012 to 2014.

The gross description of mesorectal excision should include the amount of mesorectum (bulky, moderate bulk, little bulk or irregular); presence and depth of defect; coning effect; and circumferential margin (smooth, moderate, or irregular) (Table 3). Many gross descriptions only provided the excision grade, without any these descriptors. Others only described the

mesorectum as “smooth and bulky”, presuming “complete excision”. Occasionally there were cases that did not include the depth of defects or have not even to comment on the surgical quality at all. The percentage of each situation, clustered by year is summarized in Figure 20.



	2012	2013	2014	2015
Not graded *	12.5%	10.1%	1.7%	5.5%
No depth of defect**	2.1%	0.9%	3.3%	4.7%
Grade only***	2.1%	3.7%	9.1%	4.7%
Smooth/Bulky	2.1%	7.3%	14.9%	11.8%

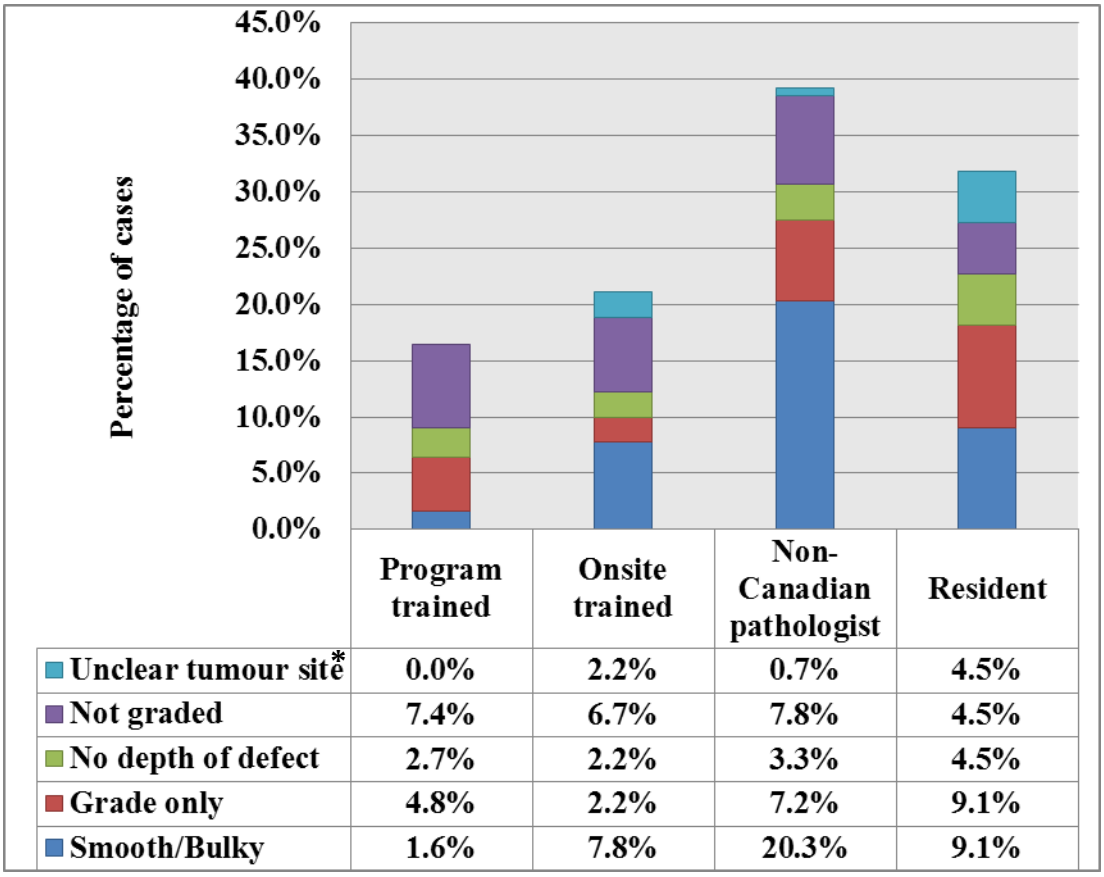
Figure 20. Changes in inadequate PA gross description by year. The term “smooth and bulky”, without other descriptors, was more commonly used in 2014 and 2015. Percentage of cases with ungraded mesorectal excision decreased from 2012 to 2014 and slightly increased in 2015.

* - “Not graded” are cases without complete grading of mesorectal excision.

** - “No depth of defect” are cases mentioned the presence, but not the depth of a defect.

*** - “Grade only” are cases with only a final grade of excision quality, without explanation of the individual factors.

One-way ANOVA shows a significant difference in the inadequate PA gross description percentage between the PAs with different training background, F (3, 450) = 13.688, $p < 0.001$. Post Hoc test, using Tukey HSD for multiple comparisons, successfully separated the PA training background into two groups: A- program trained and Onsite trained PAs, and B – resident and non-Canadian trained pathologist ($p < 0.001$) (Figure 21).



	Program trained	Onsite trained	Non-Canadian pathologist	Resident
Unclear tumour site*	0.0%	2.2%	0.7%	4.5%
Not graded	7.4%	6.7%	7.8%	4.5%
No depth of defect	2.7%	2.2%	3.3%	4.5%
Grade only	4.8%	2.2%	7.2%	9.1%
Smooth/Bulky	1.6%	7.8%	20.3%	9.1%

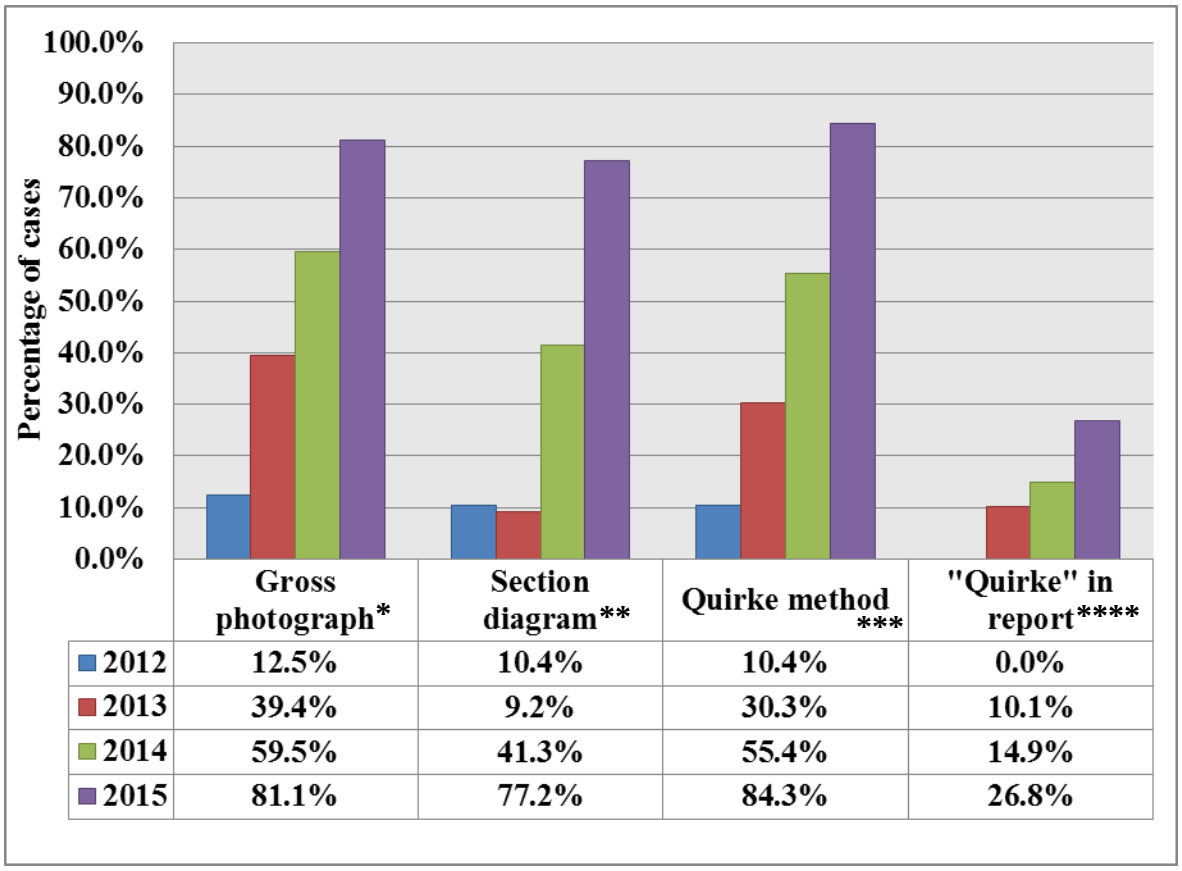
Figure 21. The difference of inadequate gross description between PA groups. The percentage of inadequate gross description is lower in the program trained and onsite trained PA group, but higher in the other two groups, suggesting the differences in grossing experience and skill.

* -"Unclear tumour site" are cases without a description of the tumour location with respect to the anterior/posterior peritoneal reflections or the dentate line.

As per requested by the DSM protocol # 170-81-07, the mesorectal excision specimens are to be grossed according to the modified Quirke method. Photographs with mapping of the sections are extremely helpful for pathologists to understand the orientation and location of the sections collected. We observed a significant increase in the application of the Quirke method, closely followed by the DSM protocol, from 10.4% to 84.3%, 2012 to 2015. In fact, by the end of the year 2015 (October to December 2015), 94.6% (35/37) cases were grossed according to the Quirke method, suggesting a significant improvement of the grossing technique of the PAs in Winnipeg (Figure 22).

The difference in PA training background also has an impact on the percentage of cases grossed by the Quirke method (Figure 23). NCP and PT groups have a higher percentage of cases grossed by the Quirke method. It appears that NCP PAs use photographs and section diagrams more often than other PA groups. Most of the OT PAs gross mesorectal excision specimens without the Quirke method. However, the using of the Quirke method is relatively new policy, and the OT PAs perhaps have not adapted to the new method as quickly, with an average of 30% cases grossed by the Quirke method (Figure 23).

Application of the Quirke method in grossing by year



	Gross photograph*	Section diagram**	Quirke method ***	"Quirke" in report****
2012	12.5%	10.4%	10.4%	0.0%
2013	39.4%	9.2%	30.3%	10.1%
2014	59.5%	41.3%	55.4%	14.9%
2015	81.1%	77.2%	84.3%	26.8%

Figure 22. The changes in the percentage of specimens grossed by the Quirke method from 2012 to 2015.

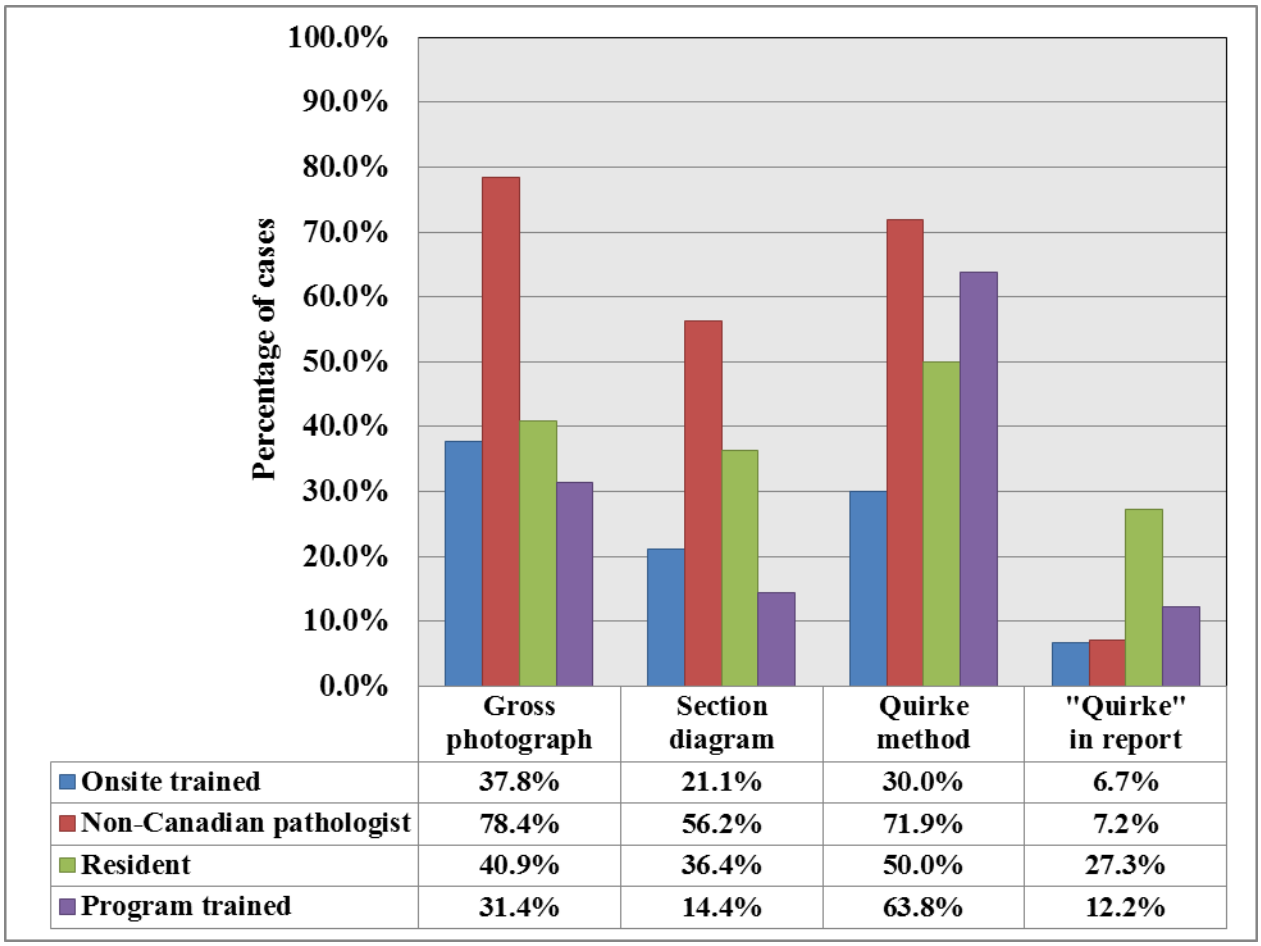
* - "Gross photograph" represents specimens that have been photographed at either fresh or fixed state.

** - "Section diagram" represents a photograph of the sectioned specimen with mappings and demonstration of where the sections were taken is provided.

*** - "Quirke method" represents specimens grossed using the Quirke method, including a macroscopic assessment of the mesorectal excision quality, and cross-sectioning of a tumour region with a photograph.

**** - "Quirke in report" represents the using of Quirke method is detailed in the gross description.

Application of the Quirke method between PA groups



	Gross photograph	Section diagram	Quirke method	"Quirke" in report
Onsite trained	37.8%	21.1%	30.0%	6.7%
Non-Canadian pathologist	78.4%	56.2%	71.9%	7.2%
Resident	40.9%	36.4%	50.0%	27.3%
Program trained	31.4%	14.4%	63.8%	12.2%

Figure 23. Comparing the application of Quirke method between PA groups. The difference of using the Quirke method in grossing between PA groups suggests a better education of the Quirke method and DSM SOP is necessary to ensure the macroscopic examination quality.

V.3.2 Lymph node dissection quality audit

As previously mentioned, the lymph nodes yield in mesorectum and pericolonic fat is extremely important in pathological assessment and TNM staging. An experienced PA usually can retrieve more lymph nodes for pathologists to review and assess lymph node status accurately. A minimal of 12 lymph nodes is required for accurate reporting of lymph node stage, as per CAP protocol.

Although slightly increased, one-way ANOVA shows no significant differences in the number of possible lymph nodes identified in each specimen from the year 2012 to 2015, F (3, 434) = 1.974, p = 0.117 (Figure 24 A). In addition, the lymph node accuracy is not significantly increased from year 2012 to 2015, F (3, 436) = 0.646, p = 0.586 (Figure 24 B).

The number of lymph nodes identified in each specimen is significantly different between the groups of PAs with different training background, F (3, 437) = 5.593, p = 0.01 (Figure 25 A). The program trained PA retrieved 26.8 possible lymph nodes on average, which is significantly higher than the 21.0 possible lymph nodes identified by the on-site trained PA, $p < 0.001$. However, the lymph nodes accuracy is not significantly different from the four PA training background, F (3, 436) = 1.210, p = 0.306 (Figure 25 B).

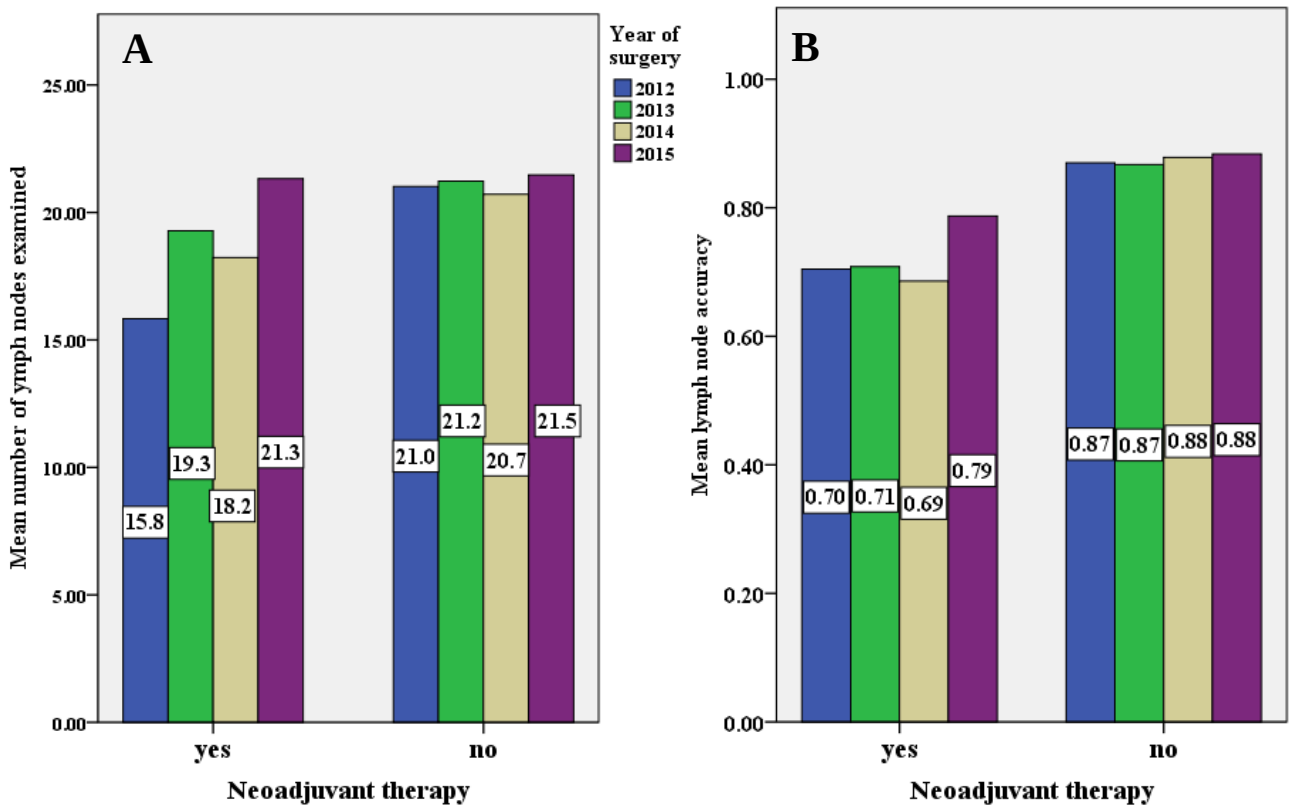


Figure 24. Average number and accuracy of possible lymph nodes identified of each mesorectal excision specimen from 2012 to 2015, clustered by the preoperative therapy. **(A)** The number of lymph nodes identified is slightly increased for the specimens with neoadjuvant therapy. **(B)** The accuracy of lymph node represents the number of lymph nodes confirmed microscopically divided by the number reported in the gross examination. The accuracy is also slightly higher in the specimens with neoadjuvant therapy.

Average number and accuracy of lymph nodes identified by PA training background

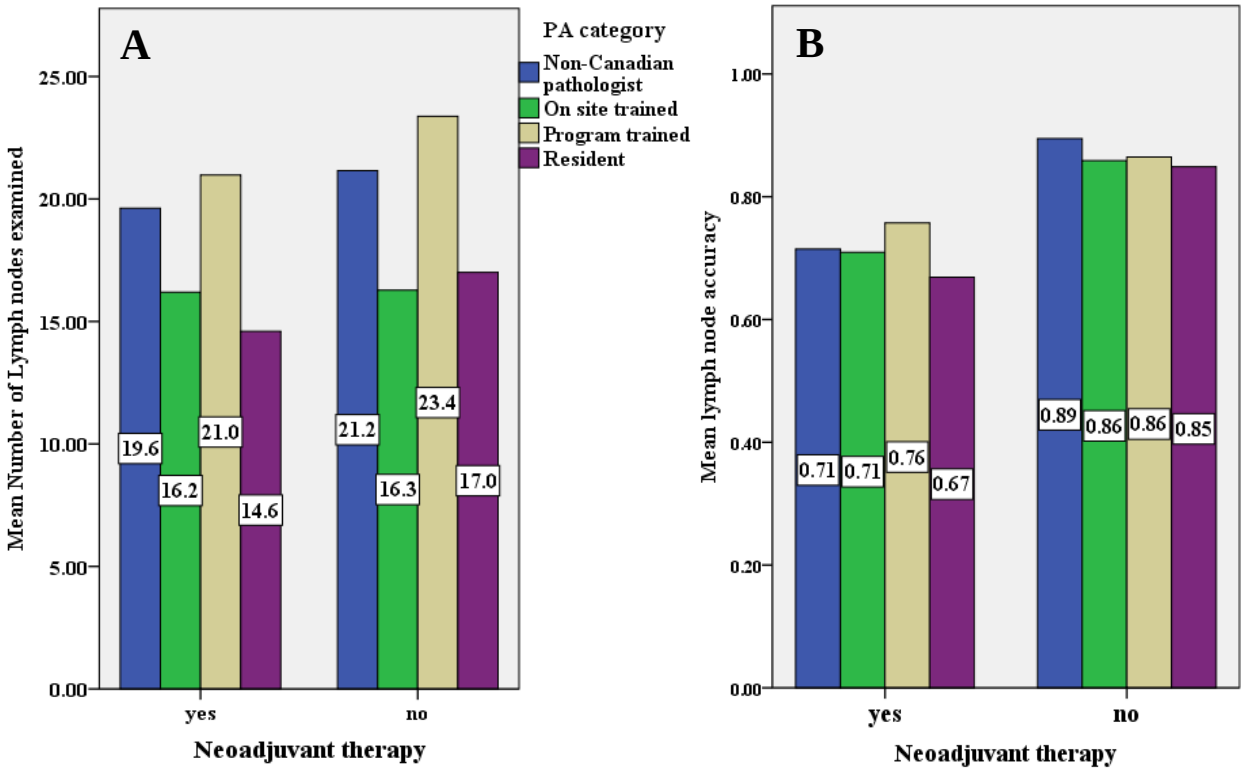


Figure 25. Average number and accuracy of possible lymph nodes identified in each mesorectal excision specimen by different PA training background and clustered by the preoperative treatment. **(A)** The number of possible lymph nodes identified by PT PAs, compared to OT PAs, $p < 0.001$. **(B)** The accuracy of lymph node identified is not significantly related to the PA training background.

V.4 Microscopic examination audit

Currently, all pathologists in Winnipeg report CRC results using a synoptic report, which is an organized and consistent method to report tumour stage and characteristics. The synoptic reports of all the 453 cases were reviewed individually and the minor issues are to be discussed in the following section.

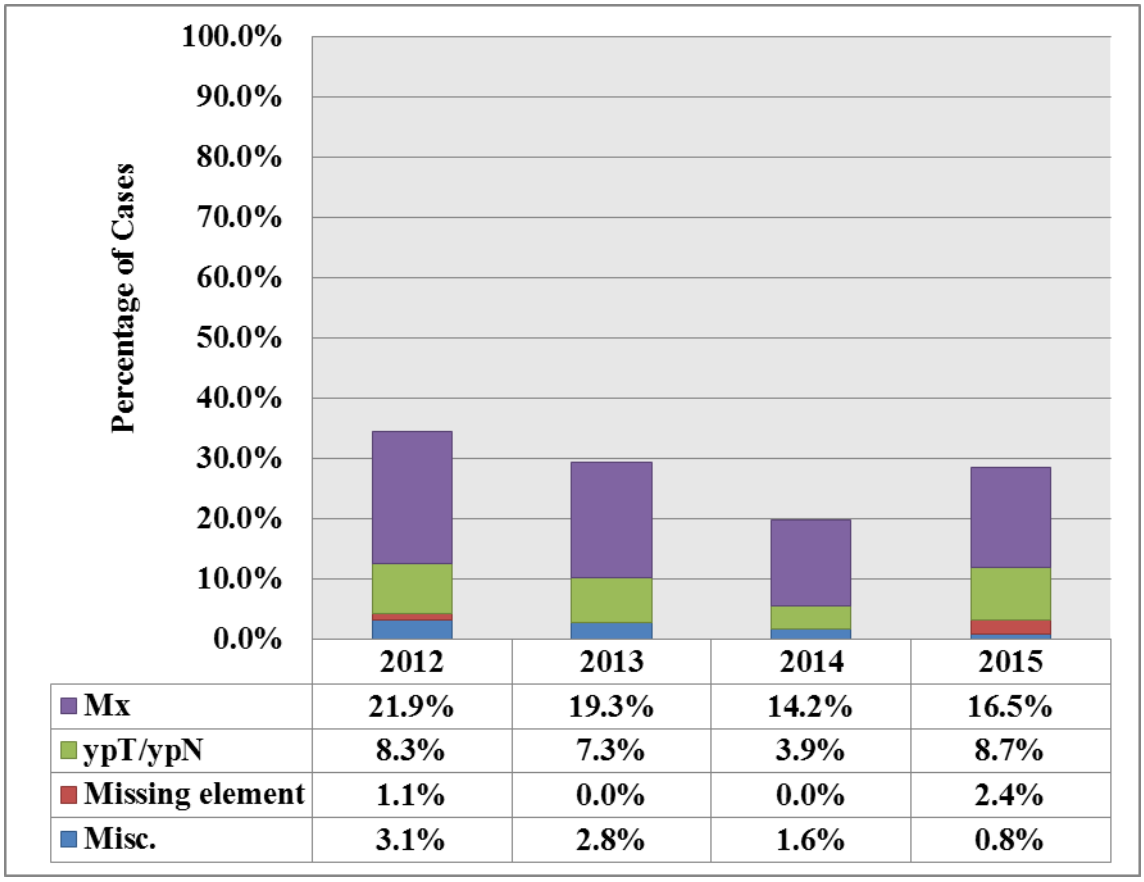
The most common misapplied staging element in the synoptic report is the usage of "Mx", which was dropped by the AJCC 7th edition. CAP protocol indicated that if there's a solid proof of the absence of distant metastasis, "M0" is assigned; or if there's a solid proof of the presence of distant metastasis, depending on the organs involved, "M1a" or "M1b" is assigned. Otherwise, if the metastasis status is undeterminable, the "M" stage should not be mentioned in the synoptic report. In our cohort, 80/453 (17.6%) reports, the "Mx" term was still in use. The percentages of cases using "Mx" slightly dropped from 21.9% (2012) to 16.5% (2015), suggesting more pathologists were aware of the recent changes in CAP protocol (Figure 26).

Another common minor misapplied staging element is the absence of the "y" prefix descriptor in the pathologic stage for the cases with neoadjuvant therapy. For example, the TNM stage of "ypT3N0" is supposed to be included in the report, instead of "pT3N0". In our cohort, 28/453 (6.2%) of reports didn't use the "y" prefix descriptor (Figure 26).

14/453 (3.1%) of reports didn't provide an accurate N stage, reflecting the number of the regional lymph nodes involved specifically in the report. For example, in a synoptic report lymph node section, it was reported as: "Five of thirty-nine lymph nodes contain metastatic adenocarcinoma (5/39)." However, the final TNM stage was diagnosed as "pN2", instead of "pN2a".

In addition, there are 7/453 (1.5%) of reports have missing synoptic report elements, including 2 missing histology grade, 3 missing treatment effect, and 2 missing radial margin involvement (Figure 26).

Other miscellaneous misapplied synoptic reports are listed in Table 13. The microscopic descriptions do not match with the pathological stage diagnosed in 4 cases. No additional amendment reports were filed for the 4 cases, suggesting clinicians might use the misleading information of the pathological stage in the reports.



	2012	2013	2014	2015
Mx	21.9%	19.3%	14.2%	16.5%
ypT/ypN	8.3%	7.3%	3.9%	8.7%
Missing element	1.1%	0.0%	0.0%	2.4%
Misc.	3.1%	2.8%	1.6%	0.8%

Figure 26. Summary of the accumulative percentage of the synoptic reports with minor misapplied synoptic report element.

Table 13. Miscellaneous discrepancies in synoptic reports.

Microscopic description	Diagnose*	Correct stage**
Extramural Discontinuous tumour nodule: present (Down staged)	pN0	pN1c
Regional Lymph Nodes: Positive for metastatic carcinoma: 3/12	pN2	pN1b
Extension of a tumour: confined to the muscularis propria (Up staged)	pT3	pT2
No definite carcinoma beyond the muscularis propria is appreciated	pT3	pT2
16/31 positive lymph nodes	pT3	pT3N2b
Tumor invades less than 1 mm beyond the border of the muscularis	pT3a***	pT3
Procedure: Right hemicolectomy (It is a rectal tumour case)		Left Hemi.****

Note:

* - "Diagnose" represent what written in the synoptic reports.

** - "Correct stage" is the presumably accurate stage based on the microscopic description. No additional amendment reports are in the LIS.

*** - "pT3a" is not an appropriate grade according to the CAP protocol.

**** - "Left Hemi." is short for Left Hemicolectomy.

Chapter VI: Discussion and Conclusion

VI.1 Factors related to the mesorectal excision quality

Mesorectal excision quality is highly related to the local recurrence, which greatly impacts on patients' prognosis and determines if a further surgical or adjuvant chemoradiotherapy is necessary. In our cohort study, using the 453 mesorectal excision surgical specimens for multiple statistical analyses, such as ANOVA and coefficiency test, several factors are identified to be significantly related to the surgical quality, including the tumour location, operation type, surgeon training background, surgical volume performed, and PA training background.

Our data indicate that more distal tumours show a higher incomplete excision rate. This incomplete percentage increases from 6.3% at the rectosigmoid junction to 33.7% at the anorectal junction, suggesting a higher surgical complexity for more distal rectal/anus tumours, which can result in an increased possibility of incomplete mesorectal excision. This also explains the higher incomplete percentage for the APR (19.0%) than the LAR (10.5%). The difference in the incomplete percentage is due to the limited space in the pelvic cavity, which limits access and increases the surgical complexity of more distal tumours.

Surgeon's training background shows a strong correlation with the incomplete percentage. Generally, subspecialized surgeons, such as the colorectal surgeons and surgical oncologists, benefit from the specialized surgical training. They have incomplete percentages of 8.7% (colorectal surgeons) and 10.3% (surgical oncologists), which is significantly lower than the ones without fellowship training, such as a general surgeon, 13.2%. Occasionally, an incidental rectal

tumour can be identified in an emergency bowel conditions, such as colitis, ischemia, diverticulitis, obstruction, perforation and etc. Those patients need medical and surgical attention immediately, and the surgery can be performed by unspecified types of surgeons at the emergency, who might not be very experienced to perform total mesorectal excision. In our study 4 cases were performed by unspecified surgeons, all of which are incomplete mesorectal excision, suggesting the surgeon's training background has a great impact on the surgical quality.

In addition, the volume of surgery performed by a surgeon is highly related to the quality. The weighted average incomplete percentage is 9.01% for the surgeries done by high volume surgeons; whereas, the number increases to 18.27% for the low volume surgeons, who performed no more than 10 mesorectal excisions within 4 years. This suggests that the surgical performance can be improved by repetition and higher surgical volumes. A better surgical outcome may be obtained if mesorectal excision surgeries are centralized and performed by more experienced surgeons.

In addition, PA training background is associated with the graded "incomplete" percentage, due to the different understanding of the grading criteria for mesorectal excision specimens. A major grading inconsistency is how to distinguish a surgical defect from a tearing effect. Many specimens have defects on the mesorectum, with visible muscularis propria; however, the defects can be just tearing effects when the surgeon was pulling out the specimens from the pelvic cavity. Those tearing effects can usually be reassembled easily with a relatively smooth surface, indicating no missing of mesorectum tissue. Some PAs grade these effects as "incomplete" following the grading criteria strictly; while other PAs are familiar of the tearing effect, respect the complexity of the surgery, and grade "complete" with the professional judgments. Therefore, a more detailed SOP with an explanation of common scenario and pitfalls

will be helpful to set up a more reliable and accurate method to perform the macroscopic examination of mesorectal excision quality.

Fortunately, in our cohort study, two-way ANOVA shows no significant interaction between the two variables: surgeon training background and PA training background, suggesting the observed difference of incomplete percentage among surgeon training groups is independent of the difference of incomplete percentage graded by PA groups. Therefore, the difference in mesorectal excision quality among surgeons with different training background is statistically significant.

Although not statistically significant, the number of surgery performed in each month shows some relationship with the surgical quality, with only a few outliners, suggesting the surgical quality might be compromised if the OR time is cut down, and the surgeries are done in a limited operation time.

VI.2 Pathological examination of mesorectal excision specimens

The macroscopic examination is one of the most important steps in the pathological examination. Accurate and complete descriptions of the mesorectal excision quality by well-trained PAs are critical for the grading of the surgical quality, which is recently becoming a mandatory element in the synoptic report for local practice. For educational purpose, the establishment of multiple 3D printed colour models for the typical “complete”. “nearly complete” and “incomplete” specimens might be helpful for the PAs to standardized the grading criteria. Photographs of the specimen, as well as the section diagrams with standardized descriptors in the gross report, can help the pathologists accurately visualize the appearances of

the mesorectal excision quality. In complex specimens where it is difficult to assess the excision quality, surgeons and pathologists should be contacted, for example, to distinguish the defects with possible tearing effects. Furthermore, a deep defect, down to the muscularis propria, which is quite distant from a primary tumour, might have limited clinical significance than a shallow defect of the mesorectum directly overlapping the tumour. Therefore, if an irregular mesorectal margin and deep defects are identified, the distance from the defect to a tumour is another important measurement that might be important to be included in the gross description.

Pathologists report on rectal tumours, using the standardized synoptic reports, which are organized and consistent with the layout of tumour characteristics. There are approximately 20% to 30% of the synoptic reports, which have minor misapplied elements, such as using the "Mx" for M stage, missing "y" prefix to stage of neoadjuvant cases, and other missing synoptic elements. Although the majority are only format issues, these misapplied elements should be corrected from the pathological reports, to ensure the accuracy and consistency.

In four cases, the diagnosed TNM stage does not accurately reflect the given microscopic description. One is a case with 3/12 regional lymph nodes are positive for metastatic carcinoma, but staged as "N2", instead of "N1b". Two are cases have no definite carcinoma involvement beyond the muscularis propria, but staged as "T3", instead of "T2". These three cases reported a higher pathological stage than what described. In another case, that extramural discontinuous tumour nodule present, the N stage was diagnosed as "N0", rather than "N1c", which is the only case, reported a lower pathological stage than the microscopic description. No amended pathological reports for the four cases are available in the LIS, indicating the clinician might not be aware of the discrepancies in the synoptic reports and potential risk of inappropriate treatment cannot be excluded.

VI.3 Limitation of the study

This is a retrospective cohort study of the mesorectal excision specimen in Winnipeg from January 2012 to December 2015, containing 453 primary malignant rectal and rectosigmoid tumours, resected by 42 different surgeons. Although the total number of 453 is good for most statistical analysis, there are a few tests, regarding the relationship of surgeon's training background and surgical quality which needs a larger population number to get more robust results.

Ideally, data on local and systemic recurrence is supposed to be included in the study to study the clinical significance of incomplete mesorectal excision. However, due to the limited access to the Cancer Care Manitoba cancer registry database, the correlation of tumour recurrence and surgical quality is not included in the study but maybe a future direction for further analysis. Instead, the percentage of CRM positivity among the different surgical quality groups was compared. Only 2.9% of complete mesorectal excision has positive CRM; while 16.0% for the incomplete mesorectal excision has positive CRM, suggesting incomplete mesorectal excision might increase the possibility of recurrence. BMI is another potential factor related to the incomplete mesorectal excision, which was not included in the study, due to the limited access to patients chart.

The variation of the surgical quality graded by PAs is high among individuals, which increase the false discovery rate of the study about the conclusions drawn from the statistical analysis. Although the two-way ANOVA shows an independent impact on the incomplete percentage of the two variables of the PA training background and surgeon training background, the possibility of a false positive discovery cannot be completely excluded.

In the synoptic report audit section, the study was limited to the information in the synoptic report. No microscopic review was performed for the cases with the discrepancy between the microscopic description and pathologic stage. A further review of the H&E staining slides on the positive lymph node numbers, depth of invasion and involvement of extramural discontinuous nodes will be helpful to determine if inappropriate diagnoses were made.

VI.4 Future directions

As previously discussed, patients recurrence and BMI information will be collected and compared to the surgical quality to further study the clinical significance of complete mesorectal excision in Winnipeg.

More importantly, the surgical quality provided by the pathology team is to be reported to the surgeons, and surgical performance of each surgeon is to be reviewed routinely, establishing a performance "scorecard", in order to encourage surgeons to improve surgical performance for a better patient care.

Laparoscopic-assisted resection of mesorectum has been demonstrated to reduce infection, recovery time and urogenital function lost. In addition, it might be associated with reduced incomplete mesorectal excision percentage. Therefore, a future mesorectal excision quality audit will include the variable of resection method. If the laparoscopic-assisted resection shows significant reduced incomplete excision percentage, a recommendation can be made to the surgical team of adapting more laparoscopic-assisted surgical technique, rather than conventional open surgery.

The macroscopic examination of mesorectal excision specimens will be continuously monitored to ensure the standardized grading of surgical quality, and the incomplete percentage will be compared to the number included in this study. Recent emphasis on the importance of mesorectal excision quality is hypostasized to have a positive influence of surgeons, promoting an overall better surgical performance. Therefore, the incomplete percentage is hypothesized to slightly decrease in the future.

A further review of the cases with inconsistent microscopic description and reported pathologic stages is necessary. H&E stained slices of the lymph nodes and depth of extensions will be reviewed microscopically. Amendment reports are to be filed if necessary.

VI.5 Conclusion

Our study shows the mesorectal excision quality is highly related to the site of a tumour, surgeon's training background and surgeon's volume of operation. PA training background also has an impact on the final grading of the surgical quality, and it is independent of the tumour location and surgeon's variations. The percentage of incomplete mesorectal excision is not significantly changed from 2012 to 2015.

The application of the Quirke method for macroscopic examination of the mesorectal excision has been increasing from 2012 to 2015, suggesting PAs in Winnipeg are using a more standardized and effective grossing method for APR and LAR.

Synoptic report of a rectal tumour is the most systematic and organized method of delivering tumour information from pathologist to clinician. The percentage of the reports with misapplied or discrepant synoptic report elements decreased from 2012 to 2015, indicating more pathologists in Winnipeg are keeping up to date with the current CAP protocol.

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