UNIVERSITY OF MODENA AND REGGIO EMILIA

PhD Program of Clinical and Experimental Medicine (CEM)

XXXV Doctorate Cycle

Director of the PhD Program: Prof. Marco Vinceti

COMPARISON BETWEEN THE TNM-AJCC 8th EDITION AND THE JAPANESE (JSCCR) GRADING SYSTEMS IN COLON CANCER

Candidata: Dott.ssa CAROLINA CASTRO RUIZ

Relatore (Tutor): Dott.ssa LUISA SAVOLDI

Co-Tutor: Prof. GIACOMO BORGONOVO

Abstract **4**

(English version) **4**

(Italian version) **5**

CHAPTER ONE **8**

1.1 Background **8**

1.2 Epidemiology **9**

1.3 Staging of Colon cancer **10**

1.4 Treatment **11**

1.4.1 Surgical Treatment *11*

1.4.2 Oncological Treatment *13*

CHAPTER TWO **15**

2.1 Prognostic factors in Colon Cancer **15**

2.1.1 Prognostic Impact of pT stage *15*

2.1.2 Negative Surgical Resection Margins *16*

2.1.3 Positive Lymph Nodes *16*

2.1.4 Tumor Deposits *18*

2.1.5 Extranodal extension (ENE) *19*

2.1.6 Isolated Tumor Cells and Micrometatases *19*

2.1.7 Lymphovascular Invasion *20*

2.1.8 Tumor Budding (TB) *20*

2.1.9 Poorly-Differentiated Cluster (PDC) *21*

2.1.10 Tumor-stroma ratio (TSR) *21*

2.1.11 Colon Cancer with perforation or obstruction *21*

2.2 Evolution of Colon Cancer staging **22**

2.2.1 Evolution of N stage *24*

2.2.2. Tumor Deposits *24*

2.3 Staging Systems **25**

2.3.1 TNM-AJCC 8th Ed89 *25*

2.3.2 Japanese (JSCCR) grading system90 *32*

2.3.3 Similarities and Differences between the TNM-AJCC and JSCCR grading systems *37*

CHAPTER THREE **39**

3.1 Rationale of the PhD project **39**

CHAPTER FOUR **40**

4.1 Materials and Methods **40**

4.1.2 Study Design *40*

4.1.2 Population *40*

4.1.3 Study flow-chart *41*

4.1.4 Surgical Phase *41*

4.1.5 Processing of the specimen by the Pathologist *42*

4.1.6 Follow-up Phase *43*

CHAPTER FIVE **44**

5.1 Results **44**

5.2 Statistical Analysis **46**

5.3 Discussion **47**

5.4 Conclusions **48**

Aknowledgements **57**

APPENDIX A **58**

APPENDIX B **59**

APPENDIX C **60**

APPENDIX D **61**

Abstract

(English version)

Background: Surgery remains the most efficient therapeutic approach to colon cancer. Its main targets are the treatment of the primary tumor, determining the lymph node status, and the treatment of metastatic disease. Lymph nodes (LNs) are a significant prognostic factor in predicting disease-free survival (DFS) and overall survival (OS) in patients without metastatic disease.

LN metastases are a risk factor for disease recurrence and the development of metastatic disease. Furthermore, they determine whether or not the patient should undergo adjuvant therapy. Recent studies have stated that the prognosis is not only determined by the number of positive LNs but that their topographic distribution may carry an important role.

Currently, we apply the AJCC-TNM classification, in which a correct nodal sampling is based on the retrieval of at least 12 LNs regardless of their location. On the other hand, the JSCCR (Japanese Society for Cancer of the Colon and Rectum) classification takes into consideration the topographic distribution of the positive LNs. At this moment there are no studies that determine the superiority of one system over the other in terms of predicting 3-year disease recurrence and OS. Due to the important prognostic value that the LN status has, its correct staging is a largely debated argument.

Objectives: Primary aims, 1) Applicability of the JSCCR classification to our population. 2) Agreement between disease stages applying both staging systems. Secondary aims, 1) Evaluate if the JSCCR system can highlight recurrence risk subcategories based on the topographic distribution of positive LN's with a 3-year follow-up. 2) Assess if the JSCCR system can detect a different mortality rate in subcategories based on the topographic distribution of positive LN's.

Methods and Results: This is a monocentric prospective study that aimed to confront these two grading systems. We have determined the main differences and similarities between both staging systems. We enrolled 91 patients with a diagnosis of colon cancer in a 12-month period, from which 6 patients were withdrawn from the study. Inclusion criteria: patients >18 years old with a diagnosis of colon cancer who agree to continue the follow-up period in our institution. Exclusion criteria: patients with a diagnosis of rectal cancer, synchronous solid tumors, oncohematologic diseases, patients who underwent neoadjuvant therapies, and patients with recurrent/metastatic disease. The analysis concerns only the primary aims (feasibility and agreement) because the secondary aims assess the risk of recurrence and related mortality which require a 3-year follow-up. Continuous variables were characterized by median and range. Categorical data were summarized as absolute

and relative frequencies. The JSCCR classification was defined as applicable whenever it was able to define the disease stage. The applicability of the JSCCR classification was calculated as a percentage with a confidence interval of 95% according to Wilson. It was calculated the percentage of cases in which both systems appointed the same stage for each patient with a CI of 95% according to Wilson. The degree of agreement was deterred by Cohen's $\varkappa$ coefficient with a CI of 95%. Statistical analysis was performed with R 4.0.4 software.

Results: The JSCCR classification proved to be applicable in 85/85 cases included and evaluated in the study. Both classifications, TNM-AJCC and JSCCR, showed a strong level of agreement (100%, 95% CI: 94.6-100).

Conclusions: The JSCCR classification can be applied to our population without any variability concerning the vastly used TNM-AJCC classification. The fact that both systems presented such a strong level of agreement (100%) while determining the disease stage could be due to the small sample size. To better assess if the differences between both staging systems could carry an upgrade or downgrade in stage, a bigger sample size is needed. This study is now entering the follow-up phase, which will be completed in December 2024. At the end of the follow-up phase, we will be able to respond to our secondary aims.

(Italian version)

Background: La Chirurgia rimane l'approccio terapeutico più efficace nel trattamento del cancro del colon. I suoi obbiettivi principali sono il trattamento del tumore primario, determinare lo status linfonodale e la terapia della malattia metastatic. La presenza di linfonodi (LNs) positivi sono un fattore prognostico significativo nella predizione del periodo libero di malattia (DFS) e della sopravvivenza (OS) in pazienti senza malattia mestastatica.

Le metastasi linfonodi sono un fattore di rischio per la ricorrenza di malattia e lo sviluppo di malattia metastatica. Inoltre, determinano se il paziente dovrà eseguire chemioterapia adiuvante. Studi recenti hanno dimostrato che la prognosi non è determinata solo dal numero di LNs positivi bensì anche della loro distribuzione topografica.

Al momento attuale, in occidente utilizziamo la classificazione del TNM-AJCC, che definisce una corretta linfadenectomia come l'asportazione di almeno 12 linfonodi, non tenendo conto della loro distribuzione topografica. D'altro canto, la classificazione JSCCR (Japanese Society for Cancer of the Colon and Rectum) tiene in considerazione non solo il numero di linfonodi positivi ma anche la loro distribuzione topografica. Al momento attuale non vi sono studi che determinino la superiorità di un sistema di stadiazione sull'altro in termini di predizione della OS e del tempo di ricorrenza di

malattia a 3 anni. Data l'importanza prognostica dello status linfonodale, la sua corretta stadiazione continua ad essere argomento di dibattito.

Objectives: Obiettivi primari, 1) Applicabilità della classificazione JSCCR alla nostra popolazione. 2) Agreement tra i diversi stadi di malattia applicando entrambi i sistemi di stadiazione. Obiettivi secondari, 1) Valutare se il sistema JSCCR può individuare sottocategorie di rischio in base alla distribuzione topografica dei LN's positivi con un follow-up di 3 anni. 2) Determinare se il sistema JSCCR può individuare sottocategorie con un tasso di mortalità diverso in base alla distribuzione topografica dei LN's positivi.

Methods: Questo è uno studio prospettico monocentrico che ha come obiettivo mettere a confronto questi due sistemi di stadiazione. Abbiamo individuato le principali difference e similitudini di entrambi i sistemi di stadiazione. Abbiamo arruolato 91 pazienti con diagnosi di cancro del colon in un periodo di 12 mesi, dai quali 6 pazienti sono stati esclusi dallo studio. Criteri di inclusione: pazienti >18 anni con diagnosi di cancro del colon che hanno garantito di continuare il periodo di follow-up presso il nostro Instituto. Criteri di esclusione: pazienti con diagnosi di cancro del retto, tumori solidi sincroni, malattie oncoematologiche, pazienti che siano stati sottoposti a chemioterapia neoadiuvante e pazienti con ripresa di malattia/malattia metastatica. L'analisi riguarda solo gli obiettivi primary (fattibilità e grado di agreement) dato che gli obiettivi secondary stabiliscono il rischio di ricorrenza e la mortalità, che richiedono un periodo di follow-up (3 anni).

Le variabili continue sono caratterizzate da media e range. Le variabili categoriche sono state rapportate come assoluti e le loro relative frequenze. La classificazione JSCCR è stata definita come applicabile nei casi nei quali sia in grado di definire uno stadio di malattia. L'applicabilità per la classificazione JSCCR è stata calcolata come una percentuale con un intervallo di confidenza del 95% d'accordo con Wilson score intervals. É stato calcolata la percentuale di casi nei quali entrambi i sistemi determinavano il medesimo stadio di malattia per lo stesso paziente con un CI di 95%. Il grado di agreement è stato determinato con il coefficiente $\varkappa$ di Cohen con un CI di 95%. L'analisi statistica è stata eseguita con il software 4.0.4.

Results: La Classificazione JSCCR ha provato di essere applicabile in 85/85 dei casi inclusi in questo studio. Entrambe le classificazioni, la TNM-JSCCR e la JSCCR, hanno dimostrato un forte grado di agreement (100%, 95% CI: 94.6-100).

Conclusions: La classificazione JSCCR può essere applicabile alla nostra popolazione senza nessuna variability nei confronti della classificazione TNM-AJCC. Il fatto che entrambi sistemi abbiano presentato un forte agreement (100%) nel determinare lo stadio di malattia potrebbe essere dovuto alle ridotte dimensioni del campione.

Per una miglior valutazione delle differenze tra entrambi sistemi e determinare se vi possa essere la possibilità di uno slittamento nello stadio di malattia si richiede un campione più ampio. Questo studio è entrato nella fase di follow-up, che sarà completata a Dicembre 2024. Al termine della fase di follow-up saremo in grado di rispondere a i nostri obiettivi secondari.

CHAPTER ONE

1.1 Background

Colon cancer is one of the main tumors worldwide alongside lung, prostate, and breast cancer. It is the second cause of cancer death (9,4%).[1,2] The World Cancer Research Fund reported the following as risk factors for colon cancer: obesity, consumption of processed meat, cigarette smoking, and alcoholic drinks, whereas calcium supplements and adequate consumption of whole grains, fiber, and dairy products appear to decrease risk. [2,3]

The carcinogenesis of colon cancer is a well-known multistep process that leads to specific types of neoplastic polyps in colonic mucosa. The two common histologic types are hyperplastic and adenomatous. Most colon cancers arise from adenomas (adenoma-to-carcinoma sequence). In some cases, colon cancer generates within a syndromic colon cancer or as sporadic cancer (in 80% to 85% a APC mutation is present).[1,4] The major histotype in colon cancer is adenocarcinoma, which accounts for 90-95% of all large bowel tumors, colloid or mucinous adenocarcinomas represent about 17% of large bowel tumors.[1]

Rectal cancer differentiates from colon cancer due to several biological and clinical hallmarks. The rectum and colon have different embryological origins, anatomy, and function.[5]

Tumor cells can disseminate toward distant organs through two pathways: the vascular and the lymphatic pathway. The vascular hypothesis suggests that blood vessels transport tumor cells directly to distant organs. In the lymphatic pathway, tumor cells may disseminate from regional lymph nodes to distant lymph nodes, reach the systematic circulation and subsequently form organ metastases.[6]

Knijn *et al*, conducted a large study comparing metastatic patterns according to lymph node status of CRC. They found that the most common site of distant metastasis was the liver followed by the lung, peritoneum, and distant lymph nodes, with percentages comparable to the literature. Peritoneal and distant lymph node metastases occurred more often in regional lymph node-positive CRC; while liver and lung metastasis occurred in a similar percentage. They found that alongside with established risk factors for peritoneal carcinomatosis, like T-stage, proximal location, and mucinous carcinoma, regional lymph node metastases are an important risk factor. The omentum is a preferential site of peritoneal metastases and the lymphoid milky spots in the omentum are a homing site for metastatic cancer cells. Tumor cells in the omentum can reach the peritoneal cavity by direct growth.[7]

1.2 Epidemiology

Colorectal cancer (CRC) is by incidence the 3rd most diagnosed tumor, but second in terms of mortality. In Western Europe, colorectal cancer has an incidence rate of 13,3 per 100,000 males and 6,8 per 100,000 females.[2]

Despite being considered for many years age-related neoplasia, in recent times there appears to be a decline in CRC incidence in the population over 50 years old, balanced by an increase in new diagnoses in individuals younger than 50 years.[8]

Declines in colorectal cancer incidence in some high-incidence countries have been attributed to the acquisition of healthier lifestyles by the populations and the uptake of screening. On the other hand, a gradual shift toward right-sided or proximal colon cancers has been observed; this change in the anatomic distribution may be related to the fact that colonoscopy is more effective in preventing left-sided than right-sided colorectal tumors.[2,9]

In Italy, a prevalence of 267,000 cases was estimated for the year 2008. The proportion in Northern Italian regions proved to be 2-fold that in the southern regions (580 vs. 295 for men and 447 vs. 225 per 100,000 for women).[1]

Risk factors for colorectal cancer include animal-source foods, a sedentary lifestyle with decreased activity and increased prevalence of excess body weight, heavy alcohol consumption, cigarette smoking, and consumption of red or processed meat increase the risk.[10]

The USA, presented a 5-year survival rate of 65.5%, from 2000 to 2002, while in Europe the rate was 56.2%. Colon cancer is characterized by a much better response when treated at an early stage, and the large survival differences may therefore reflect the fact that more healthy Americans than Europeans undergo early diagnostic procedures.[11]

There are considerable variations amongst the 5-year survival rate depending on the TNM stage of the disease at the moment of diagnosis: as a matter of fact, it amounts to 91% in the localized disease (stage I-II), 72% in the regional disease (stage III) and it dramatically drops to 14% in the advanced disease (stage IV).[10]

Approximately 9 of 10 patients with colon cancer are diagnosed at 50 years of age or older. While the incidence and mortality rate of colorectal cancer is declining for individuals older than 50 years of age, both are on the rise for those younger than 50. The treatment of patients with colon cancer is guided by the stage at presentation, emphasizing the importance of a comprehensive strategy for the diagnosis, evaluation, and treatment.[12]

1.3 Staging of Colon cancer

Before surgery, the patient undergoes an accurate clinical staging of the disease (cTNM). During this phase, the patients undergo a series of studies to better assess the tumor burden, locally or distantly. A colonoscopy with biopsy is the main study that assesses the presence of colon cancer. Afterward, radiological exams such as an abdomen and chest CT-scan, magnetic resonance (MR), or positron emission tomography (PET) will be performed.

Colonoscopy with biopsy

Screening is used to detect colorectal cancer (CRC) at an early stage when it is more likely to be curable. Screening can also result in the detection and removal of colorectal polyps before they become cancerous. It has been shown that colonoscopy and polypectomy can reduce the incidence of CRC. Colonoscopy has become the most important method of screening because of its high diagnostic sensitivity and specificity, the ability to procure tissue from identified lesions for histologic analysis, and the ability to completely remove polyps during the procedure.[13-15]

Capsule Colonoscopy

A capsule study of the large bowel is another method for screening. The capsule has evolved from a small bowel capsule to one that is suitable for colonoscopic investigation. The preparation for the capsule involves filling the colon with fluid so the capsule may easily pass through the large bowel, taking pictures both forward and backward as it traverses the large intestine. Because of the long transit time to reach the cecum, the capsule becomes dormant for several hours as it passes through the small bowel before becoming active again to take images of the large bowel. The accuracy is lower than colonoscopy for polyps, although it does seem to be a technique of emerging interest.[16]

Radiological Exams

CT scan: CT scan of the chest, abdomen, and pelvis with oral and intravenous contrast or noncontrast CT scan of the chest and abdominal MRI is recommended for colon cancer staging. CT scan identifies the site of the tumor, size of the tumor, infiltration into surrounding structures, and metastatic spread. A meta-analysis highlighted that the CT scan has good sensitivity for the detection of colon cancers with tumor invasion beyond the bowel wall (T1-T2 vs T3-T4). However, it remains a challenge to detect tumor invasion of 5mm or more (T1-T3ab vs T3cd-T4). The use of thin (<5mm) slices improves the detection of tumor invasion beyond the bowel wall, as well as the detection of malignant lymph nodes, and is, therefore, advocated.[12,17,18]

MRI: MRI is now routinely used for preoperative staging of rectal cancer and provides an accurate assessment of the tumor spread to the mesorectal fascia. This identifies patients at risk of local recurrence and those likely to benefit from neoadjuvant therapy. Compared with CT and ultrasound, MRI is more reliable for the evaluation of the extent of locoregional disease, planning radiation therapy, and assessing postoperative changes and pelvic recurrence. In colon cancer, MRI plays an important role in patients allergic to iodine contrast who can't undergo a CT scan. Multiple studies have shown that MRI is superior to CT for the detection of liver metastasis. Both the introduction of diffusion-weighted imaging and the use of liver-specific hepatobiliary contrast agents have contributed to the superior results of MRI in detecting small liver lesions.[18,19]

PET/CT: it seems to be a useful tool in the evaluation of colorectal cancer by metabolically characterizing undetermined lesions suspected to be recent disease to perform a complete pre-surgical staging and to identify occult metastatic disease. PET and PET/CT have been shown to change the therapy in almost a third of patients with advanced primary rectal cancer. Currently, PET/CT is recommended only for the assessment of suspected recurrences of colorectal cancer and in pre-operative staging prior to metastasectomy. PET/CT colonography is recommended in patients with obstructing colorectal cancers that cannot be traversed colonoscopically to obtain additional information.[20,21]

1.4 Treatment

1.4.1 Surgical Treatment

Surgical treatment is the mainstream in colorectal cancer. The traditional approach to surgical colon cancer resection involves the removal of the primary tumor with adequate proximal and distal reception margins, and a clear circumferential resection margin (which may require en bloc resection of the abdominal wall or other viscera) together with an anatomically defined mesenteric lymph-vascular pedicle. The necessity to include resection of the lymphovascular mesentery is based on the tenet that in addition to the hematogeneous spread, colonic tumors most commonly spread initially via the lymphatic system, which anatomically follows the colonic arterial supply. With respect to the operative harvesting of nodes, it is recommended to perform a "high tie" of the vascular pedicle to maximize the number of lymph nodes within the colonic mesentery.[22]

Laparotomic colectomy has been the *"gold standard"* for the past 100 years. Even though it was proven to be highly effective, it carries grave traumatic stress to patients with a high morbidity rate and a slow recovery. The *"laparoscopic revolution"* started in 1987 with the publication of the first laparoscopic cholecystectomy, and the benefits for the patient were seen immediately. Patients presented a shorter recovery, less postoperative pain, shorter length of stay, and a return to their normal activities in less time, with an important improvement in their quality of life.[23,24]

The first laparoscopic colon resection was published by Jacobs M, *et al* in 1991, they presented a small case series of 20 laparoscopic hemicolectomies.[25]

In the beginning, the oncological safety of this surgical approach was a real concern. It was believed that the tumor cells could spread within the abdominal cavity due to the use of CO_2 and that the gas could enable the implantation of tumor cells in the port sites. The COLOR *Study Group* proved the immediate benefits of laparoscopic surgery in the treatment of colorectal cancer. The COST *Study Group,* on the other hand, aimed to prove the 5-year outcomes after laparoscopic surgery of colorectal cancer. They found a 5-year survival rate and a disease recurrence that overlapped with that of patients operated by laparotomic techniques. The MRC CLASICC *Trial Group* confirmed an overlap in a 3-year overall survival rate and the disease-free survival between patients operated with laparotomic technique and laparoscopic technique. In 2016 Diejen *et al*, have reported in the COLOR I trial a 10-year follow-up that laparoscopic surgery for colorectal surgery for the non-metastatic disease had a disease-free rate, overall survival, and disease recurrences rate similar to those of the patients who underwent laparotomic surgery.[26-29]

Based on the TME experience, the group from Erlangen in Germany has advocated for CME in conjunction with CVL for colon cancer. CME is reported to differ from traditional colon cancer surgery by achieving a far more radical excision of the lymphovascular pedicle and mesocolon. In addition, the CME technique promotes resection of the specimen with an intact visceral peritoneum together with proximal and vital resection margins of at least 10 cm. Arterial supply to the affected segment of the bowel is taken at its origin from the superior mesentery artery (right and transverse colon) and the aorta (left colon), described as CVL. CME has been shown to lead to increased lymph node harvest and more mesocolic tissue.[22]

Several publications have demonstrated that colonic tumors are capable of far wider spread than can be predicted by arterial anatomy. In particular, it appears that right-sided and transverse colon tumors possess highly variable lymphatic spread. This can include spread to lymph nodes associated with neighboring vascular pedicles. Although there is significant variation in lymphatic drainage patterns, there is also evidence of variation in arterial supply. Of note is the variable origin and branching patterns of the right and middle colonic arteries. It could be envisaged that with CME

advocating a dissection of the vessels to the origin of the superior mesenteric artery for the right-sided tumors this may also harvest non anatomically distributed lymph nodes. At present, the specific lymphatic drainage pattern of individual tumors cannot radiologically or otherwise be identified. This implies that those patients who may benefit from such wider lymphadenectomy cannot reliably be selected. Clearly, if this wider resection is performed uniformly on an unselected population, it may lead to increased morbidity. Identification of which patient or tumor subgroups appear to spread more widely is necessary to enable a more targeted approach to selective wider lymphadenectomy, such as CME.[22]

The main surgical procedures in colon cancer, whether they are performed with open- or laparoscopic-technique, are the following: right hemicolectomy, transverse colon resection, left hemicolectomy, segmentary colon resection (for splenic flexure tumors), and sigmoidectomy. [30-32]

1.4.2 Oncological Treatment

The 3 chemotherapy agents utilized to treat patients with early-stage colon cancer are 5-fluorouracil (5FU), capecitabine (Xeloda), and oxaliplatin (Eloxatin).

5FU is a nucleotide analog that can inhibit thymidylate synthase (TS), an enzyme crucial for pyramid nucleotide synthesis. The 5FU metabolite, fluorodeoxyuridine triphosphophate (FdUTP), also disrupts RNA synthesis. 5FU may be administered as an intravenous infusion or bolus schedule, with prolonged infusion inhibiting TS and bolus infusion leading to the incorporation of FDUTP into RNA. Leucovorin is administered with 5FU to enhance clinical activity.[33]

Capecitabine is the oral pro-drug for 5FU, and thus both have shown to have equal efficacy in the adjuvant and metastatic settings.

Oxaliplatin is a platinum drug and is an alkylating agent that inhibits DNA synthesis. It may be administered intravenously in combination with either 5FU or capecitabine. Adjuvant therapy is given over the course of 6 months, either single-agent 5FU or capecitabine, or a doublet combination of 5FU/oxaliplatin or capecitabine/oxaliplatin.[34]

Patients with early-stage colon cancer are carefully evaluated by oncologists to determine whether they should recommend adjuvant chemotherapy largely based on the risk of cancer recurrence and the amount of benefit the patients will receive with treatment. Patients with stage II colon cancer generally have good prognosis and survival (5-year overall survival is estimated to be 80%), and the added benefit in survival with adjuvant chemotherapy may not be more than 5%.[34]

A large retrospective study found that patients with stage II colon cancer who have MSI-high tumors show an improved survival outcome over patients with microsatellite stable (MSS) tumors. In addition, patients with MSI-high colon cancer do not benefit from adjuvant 5FU chemotherapy.

5FU or 5FU/oxaliplatin chemotherapy may be offered to stage II colon cancer with the following high-risk tumor features: T4 stage, bowel perforation, bowel obstruction, poorly differentiated histology (and MSS), lymphovascular invasion, perineural invasion, less than 12 lymph nodes examined, close or positive surgical margins.

On the other hand, stage III cancer has a risk of recurrence after surgery of 50% to 60%, and adjuvant 5FU/oxaliplatin chemotherapy can reduce the risk of death by 20%. (Christina WU)

In metastatic colorectal cancer, the chemotherapy agents used in the firs- and second-line metastatic settings are 5FU, capecitabine (Xeloda), oxaliplatin (Eloxati), and irinotecan. The chemotherapy backbone in the metastatic setting is generally the combination of infusion 5FU/irinotecan (FOLFIRI) or 5FU/oxaliplatin (FOLFOX) with the addition of a biological targeted agent.[35]

CHAPTER TWO

2.1 Prognostic factors in Colon Cancer

2.1.1 Prognostic Impact of pT stage

Primary tumor staging is a predictive factor for local recurrence (LR) of colon cancer. Statistically significant differences between tumors confined to the muscular wall (stages I and II) and those that go beyond it (stages III and IV) have been found.[35] Jung et al, investigated the risk factors for tumor recurrence and the long-term outcomes in stage I CRC. They found an overall incidence of recurrence in stage I CRC after curative radical resection of 4.6%. Having left-sided colon cancer, a pT2 tumor, a tumor size > 5 cm, or LVI were independent risk factors for recurrence.[36]

It has been demonstrated the adverse effects of peritoneum invasion after evaluating the relationship between the tumor and the surface of the peritoneum, establishing it as an independent risk factor for local and regional recurrence.[37-39]

Baguena *et al*, found that pT4a was an independent risk factor for worse oncologic results after curative resection for locally advanced colon cancer.[35]

Increasing tumor size correlates with higher nodal positivity, higher T stage, and decreases 5-year overall survival. Furthermore, it has been proven that tumor size is associated with progression-free and cancer-specific survival. The median tumor size proportionally increases with increasing tumor grade from 3.5 to 5.2 cm. Saha *et al*, found that a higher grade was positively correlated with a tumor size of 6 cm or greater (gamma value 0.26). In each quartile, as tumor size increased, so did the percentage of patients with higher T stage (gamma value 0.54). The median tumor size for T1 patients was 1.9 cm versus 5.5 cm for T4 patients. As tumor size increased, the percentage of patients with nodal metastasis also increased (gamma value 0.25); 79% of patients with tumor size 0 to 2 cm were node negative versus 52% of those with greater than 6 cm tumor size who were node positive. As tumor size increases, 5-year overall survival significantly decreases (66%, 52%, 46%, and 41% for tumor sizes 0 to 2, >2 to 4, >4 to 6, and >6 cm, respectively).[40]

2.1.2 Negative Surgical Resection Margins

Surgical resection remains the mainstay of curative treatment for carcinoma of the colon and rectum. Historically it has been defined that intestinal resection margins in colon cancer should be 5 cm, on both sides of the tumor.[41]

Local or locoregional (LR) recurrence implies the reappearance of carcinoma after an intended complete removal of the tumor. It is usually defined as tumor regrowth at the anastomosis or immediately within or contiguous to the operative area. Solid tumors grow in three dimensions, and good oncological surgery requires distally and circumferentially clear margins to achieve the lowest LR recurrence rates.[42]

It is unclear whether compromised surgical margins may lead merely to anastomotic recurrence or also to all kinds of recurrence. Rocha *et al,* observed that patients with intestinal margins shorter than 5 cm had more early recurrences than patients with intestinal margins of 5 cm or higher (21.8 vs 32.3 months). Furthermore, the overall 5 years survival was slightly higher in patients with margins superior to 5 cm, even though it was statistically significant.[43]

The circumferential resection margin (CRM) refers to the distance in millimeters between the deepest point of tumor invasion in primary cancer and the margin of resection in the retroperitoneum or mesentery. Pathologically, CRM involvement (also called CRM positivity) should be defined as the presence of remnant tumor cells after resection. In ascending and descending colons, CRM refers to the distance to the margin of the section in the retroperitoneum, just like how CRM is measured in rectum cancer. In the transverse colon and sigmoid colon, CRM refers to the distance to the margin of resection in the mesentery. It is has been found a survival difference between CRM-negative and CRM-positive patients.[44]

CRM-positivity has been associated with tumor size, tumors <11 mm had a CRM-positivity rate of 5.6% in comparison with tumors >20 mm that presented a CRM-positivity rate of 12.7%. Signet-ring cell histology presented a CRM-positivity rate of 23.3% vs. 11.3% for non-mucinous adenocarcinomas and 13.6% for mucinous adenocarcinomas, respectively. Pathologic T-stage has been proven by far the most predictive factor for CRM-positivity, pT4 patients presented an overall rate of CRM-positivity of 27%.[45]

2.1.3 Positive Lymph Nodes

Adequate assessment of nodal status depends on the total number of retrieved lymph nodes that are available for histological evaluation. Fielding *et al* stated the ideal minimum was 12 nodes, below

this cut-off value there is a high risk of false-negative reporting of lymph node involvement due to inadequate sampling.[46-47]

There are many factors that can impact LN recovery, including patient age, gender, body habits; immune response; tumor site, size, and length of colon resected; the experience of the surgeon; and the diligence and experience of pathology grossing personnel.

Both the total number of regional LNs removed and the number of positive LN involvement are prognostically important and thus should be reported. Studies have shown that the total number of LN removed correlates with survival, likely because of optimal mesenteric resection but the surgeon and increased accuracy in staging.[48]

Lymph node ratio (LNR). LNR is defined as the ratio of metastatic lymph nodes (LN) over a total LN examined. A large number of studies showed that the prognostic significance of the lymph node ratio is superior to that of the absolute number of involved lymph nodes.[49-52] LNR has been established as a prognostic indicator in several non-colorectal malignancies, such as breast cancer, esophageal and gastric cancer, medullary and papillary thyroid cancer, non-small cell lung cancer, and oropharyngeal cancer. Recent studies have proved that LNR is an independent prognostic factor and allows for a prognostic separation that is superior to that of the nodal stage alone in terms of OS, DFS, and cancer-specific survival. However, there is no consensus on the cut-off to use when applying LNR.[53] Notably, lymph node ratio remains to be an independent prognostic factor even after neoadjuvant therapy, despite a reduction of the absolute number of retrieved nodes. [54]

Log odds of positive lymph nodes (LODDS). LODDS is defined as the logarithm of the ratio of metastatic lymph nodes to negative lymph nodes. When applied to colon cancer, LODDS was proven effective in discriminating between patients with overlapping LNR values.[53]

Occhionorelli *et al*, proved that LODDS was the only nodal category able to independently predict prognosis in 320 patients with colon cancer receiving emergency surgery.[55]

Lymph node metastases distribution (LND). Anatomical pathways of lymphatic spread in colon cancer are not fully understood. So far, two opposing theories have been advocated. In the *Halsted model*, originally described in breast cancer, lymphatic spread follows a process in which cancer cells migrate in a predictable and stepwise fashion: primary tumor cells spread to paracolic LNs first, then to intermediate and central LNs, and finally to other organs such as the liver. The Halsted model is based on the assumption that invasion to an intermediate LN station will not happen without invasion of the paracolic LN station, and that resection of all invaded nodes may result in

cure. In contrast, the *Fisher model*, which is considered accurate in breast cancer, holds that the spread of invaded LNs occurs randomly and metastases to distant organs occur regardless of the location of invaded LNs.[56]

In left-sided colon cancer, LN metastases tend to spread toward the oral side of the tumor rather than the anal side. In all CRC, LN metastases rarely occur in epicolic/paracolic LNs located more than 5 cm from pT1 tumor and in epicolic/paracolic LNs located more than 5 cm from the distal edge of CRC tumor. In such tumors, omitting the dissection of these LNs might be warranted if it is technically difficult to obtain a resection margin of 10 cm of the bowel from the tumor. As for vertical LNs, the incidence of metastasis in the intermediate or main LNs increased as the pathological T stage advanced.[57]

Huh *et al*, demonstrated that LND is an independent prognostic indicator of survival in patients who have undergone curative resection for sigmoid and rectal cancer. However, a growing amount of data have shown different tumor entities between right- and left-sided colon cancer. For example, a higher percentage of poorly differentiated and locally advanced tumors and peritoneal carcinomatosis are more frequently found in right-sided colon cancer. It has been shown that the location rather than the number of metastatic nodes is the most important prognostic variable associated with CRC. It also has been reported that LND subcategories had a wider distribution range and 5-year survival rate than did the TNM staging system. The TNM staging classification based on the number of positive nodes alone may not provide an accurate assessment after preoperative chemoradiation. It has been argued that the treatment decreases the median number of involved lymph nodes.[58]

2.1.4 Tumor Deposits

Tumor Deposits (TD) are defined as focal aggregates of adenocarcinoma located in the pericolic fat discontinuous with the primary tumor and unassociated with a lymph node. Al Sahaf *et al*, reported a 5-year disease-free survival (15% vs 37%) ($p = 0.005$) and overall survival (11% vs 39%) ($p = 0.003$) significantly lower in patients with soft-tissue TDs. They confirmed TDs to be an independent prognostic factor affecting overall survival.[59,60]

The prognostic implications of TDs alone have been found to be similar to those of LNs alone, whereas tumor deposits concomitant with LNs have exhibited significantly worse prognoses. The presence of TDs and LNs significantly increase the risk of liver metastasis, as compared with those with LNs alone.[59]

A retrospective analysis performed on 19,991 patients with colorectal cancer pooled from the SEER database found that the N1c category is associated with a prognosis similar to that of the N1b category.[61] Mayo *et al*., performed a different analysis on the same database and showed that the presence of TDs is associated with lower 3-year OS in multivariable models.[62] Interestingly, the presence of TDs is associated with a worsening hazard ratio in lower N stages. A phase III trial in colon cancer patients receiving adjuvant chemotherapy (IDEA France) also demonstrated a significantly higher risk of recurrence or death in patients with TDs, regardless of LNM substatus.[63]
A retrospective review classified TDs in invasive-type (TD (iTD) (vascular invasion lymphatic invasion, perineurial invasion, and undefined cancer clusters) or nodal-type TD (nTD) (cancer aggregates without iTD components): DFS was significantly shorter in both node-negative and node-positive, iTD/nTD+ patients compared to TD - patients. Among node-negative patients, disease-specific survival (DSS) differed significantly between the iTD/nTD+ and TD- groups, while in node-positive patients presence of nTD had no impact on DSS.[63]

2.1.5 Extranodal extension (ENE)

Extranodal extension (ENE) is defined as the extension of tumor cells through the nodal capsule into the perinodal fatty tissue. ENE could theoretically identify a more aggressive disease. Early evidence of its role in colorectal cancer was collected in a systematic review that included 4 series of patients with lower gastrointestinal tract malignancies, where the presence of ENE identified patients with significantly worse long-term prognoses.[53,64]
A systematic review with meta-analysis evaluating 1,336 patients with colorectal cancer from 13 different trials, reported that ENE was associated significantly with higher stage and grade of disease, increased risk of all-cause mortality (HR = 1.69, 95% CI 1.32-2.17, $P < 0.0001$) and increased risk of recurring disease (HR = 2.31, 95% CI 1.54-3.44, $p < 0.0001$).[65]

2.1.6 Isolated Tumor Cells and Micrometatases

The prognostic significance of isolated tumor cells (ITCs) or micrometastases in colon cancer remains controversial. ITCs are defined as single cancer cells or small clusters of tumor cells measuring ≤0.2 mm and classified as N0 (i+). Micrometastases are defined as tumor clusters measuring greater than 0.2 mm but ≤2.0 mm in greater dimensions, and classified as N1 (mic). A meta-analysis showed that micrometastases are a significantly poor prognostic factor. Most pathologists have always considered micrometastases as positive lymph nodes. On the other hand, the prognostic value of ITCs is less clear.[48]

Patients with micrometastatic disease represent a subgroup of patients with stage I-II cancer with lower survival and higher recurrence rates, where patients may benefit from adjuvant chemotherapy, as patients with stage III colon cancer do.[66] The development of recurrence in node-negative patients could also be attributed to residual micrometastasis because of inadequate lymphadenectomy.[67]

2.1.7 Lymphovascular Invasion

Lymphovascular invasion (LVI) (characterized by the extension of tumor cells into lymphatic and/or blood vessels) is used as an adjunct to TNM staging in stage II CRC.[68] Based on several studies, lymphovascular invasion (LVI) positivity in colorectal cancer is widely accepted as an independent indicator of unfavorable prognosis. According to the United States National Comprehensive Cancer Network, the presence of LVI is considered a high-risk feature in stage II colon cancer.[69]

It has been widely recognized that LVI is a poor prognostic factor related to lymphatic metastasis in early-stage CRC. LVI has also been suggested as a risk factor for micrometastases or skip metastasis, and this may explain the metastatic potential of LVI, even in patients without LN metastases.[36] LVI has been associated with advanced T and N stage tumors, high grade, tumor budding, PNI, and mucinous histology. Among patients with nodal involvement, LVI+ tumors tend to present a higher number of metastatic lymph nodes. Such a relationship of LVI and N status, at some level, confirms the hypothesis suggesting that LVI may be considered to be a precursor of, and therefore associated with, lymph node metastasis, including occult ones, eventually defining LVI as a potential predictor of patient outcome.[70]

2.1.8 Tumor Budding (TB)

The presence of de-differentiated single cells or small clusters of up to 4 cells at the invasive front of colorectal cancer has been termed tumor budding (TB). TB is an indicator of aggressive tumor biology that may be driven by an epithelial-mesenchymal transition (EMT)-like process.[71]

TB is an effort of the tumor cells to separate from the main tumor mass and create metastasis.[72] The presence of TB in polyps may allow the identification of patients at high risk for nodal metastasis as candidates for surgical resection.[71]

TB can be stratified into peritumoral budding (PTB, tumor buds at the tumor front) and intratumoral budding (ITB, tumor buds in the tumor center). PTB can only be assessed in endoscopic or surgical resection specimens, whereas ITB can be assessed in both colorectal cancer biopsies and resection specimens.[73]

Association between tumor budding and nodal involvement has been found in many studies. TB was observed to be associated with worse survival in stage II CRC, more particularly in pathological T3N0M0 patients and especially for considering the option of administering adjuvant chemotherapy in high-risk node-negative CRC patients.[72]

Patients with tumors with high budding have a worse prognosis compared to patients with low budding.[74]

2.1.9 Poorly-Differentiated Cluster (PDC)

In recent years, histologic patterns at the invasive front have been correlated with tumor behavior and oncologic outcome. The invasive front represents a dynamic interaction of the tumor and host, with cellular dedifferentiation and single and clustered cells advancing and interacting with the host immune system.[75] PDC are defined as cancer clusters in the storm composed of ≥ 5 cancer cells and lack a gland-like structure. They may reflect the biological aggressiveness of colon cancer. It is shown to be an independent prognostic factor in colon cancer patients. Although TB and PDC sound similar, the fundamental difference is that the former assesses small tumor aggregates or single tumor cells (<5), while the latter refers to large clusters of tumor cells (≥ 5).[76]

2.1.10 Tumor-stroma ratio (TSR)

TSR is a biomarker based on the microenvironment of the tumor and has proven to be a strong prognostic parameter. The TSR is based on the relative amount of stroma in the primary tumor. Patients with a tumor containing > 50% stroma (stoma-high) have a worse prognosis, compared to patients with a tumor of ≤ 50% storm (stoma-low).[77] Low TSR (high amount of stroma) is associated with a significantly higher risk of vascular and perineurial invasion together with a higher T- and N-category compared to tumors with a high TSR. The reason for high stroma content being prognostically unfavorable is probably multifaceted, but increasing evidence suggests cellular components of the stroma to be triggering the epithelial-mesenchymal transition in the invasive zone of the tumor and hence increasing the risk of metastatic spread. [78]

2.1.11 Colon Cancer with perforation or obstruction

Colon cancer with perforation comprises 3-10% of the initial presentation of colon cancer, and that with obstruction comprises 8-40%. In the European Society for Medical Oncology and National Comprehensive Cancer Network guidelines, colon cancer with perforation or obstruction is considered a poor prognostic factor. Chen *et al*. compared the outcomes between patients with

perforated vs obstruction colon cancer at presentation, they found that colon cancer with perforation had poorer progression-free survival, a higher local recurrence rate, and a higher distant metastasis rate compared with that of obstruction. The overall survival was identical.[79] Yang *et al*., confirmed a lower disease-free survival rate among patients with colon cancer who initially presented with perforation than among those who presented with obstruction. Perforated patients have a higher overall recurrence rate than obstruction patients. They found that patients with perforation or obstruction had an overall 1-year survival rate of 77.3% and a 3-year survival rate of 53.3%, these curves were very similar to that of all patients with stage IIIc colon cancer.[80]

2.2 Evolution of Colon Cancer staging

The goal of cancer staging systems is to group patients with similar prognoses. The characteristics of a good staging system are: a) the patient survival rate decreases as the stage group increases (Monotonicity), b) the groups have clearly different survival rates (Distinctiveness), and c) within a group, the survival rate is similar (Homogeneity).[81]

One of the first staging systems in CRC was the Duke's classification, this staging system was originally published by C.E. Dukes in 1932 for rectal cancer only, it did not include distant metastases.[82] This system was based on the correlation between worsening patient prognosis and progressive tumor invasion of the bowel wall and regional lymph nodes.[83] Reporting colorectal cancer comprised two phases: the careful collection of pathological data, and the division of patients into groups with different prognoses. Duke's classification was the outcome of this dual approach.[84] In 1949 it was adapted for colon and rectum by Kirkland and later by Astler and Collar in 1953. This system was then revised in 1967 by Turnbull, to include a stage for unresectable tumors and distant metastases. Astler-Coller and Turnbull staging systems are also called Dukes or modified Astler-Coller. This system introduced the "D" stage to indicate the presence of distant spread.[82,84]

Modified Dukes staging

Stage A: Tumor limited to mucosa.

Stage B1: Tumor limited to the submucosa, no lymph node invasion.

Stage B2: Tumors confined to the muscle layer, no lymph node invasion.

Stage C1: The tumor did not exceed the bowel wall, lymph node metastasis.

Stage C2: Tumor exceeded the intestinal wall and lymph node metastasis.

Astler-Coller staging

T: Primary tumor

Tx: Primary tumor of unknown

T0: No primary tumor

Tis: Carcinoma in situ

T1: Tumor invades submucosa

T2: Tumor invades muscularis propria

T3: Tumor invasion to subserosa or to pericolic/perirectal tissue

T4: Tumor invasion to neighboring organs or structures and/or visceral peritoneum is perforated

N: Regional lymph nodes

Nx: Regional lymph nodes can not be assessed

N0: No lymph node metastases

N1: One to three lymph node involvement

N2: Four or more lymph node involvement

M: Distant metastasis

Mx: Distant metastasis can not be assessed

M0: No distant metastasis

M1: Distant Metastasis [85]

The first staging manual base on the tumor, lymph node, and metastasis **(TNM)** was introduced in 1953.[86] This system is a logical form of cancer staging that may be applied to all organs. It should be noted, however, that "T" does not have a uniform meaning. For many sites, it refers to the size of the primary growth, but for colorectal cancer, it has been adapted to cope with the extent of direct spread in continuity. The TNM system is not based on research but is merely a method of encoding pathological and clinical data.[85] The TNM system has been jointly developed by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC), correlating the TNM and Dukes systems, TNM stages 0 and 1 correspond to Duke's B and C, respectively. Metastatic spread, stage IV using the TNM system, correlates with stage D in later modification of the Dukes system.[83]

The TNM system has been updated during the years since its first publication in the *Cancer Staging Manual* in 1977, the latest version being the 8th edition. These revisions were vital in order to address improvements in oncology including advancements in early detection, patient management, treatment, and discovery of new prognostic and predictive factors.[8]

2.2.1 Evolution of N stage

Lymph node staging is of crucial importance for the prognosis and therapy stratification in colon cancer. The occurrence of lymph node metastases is associated with an adverse clinical course with an indication for adjuvant chemotherapy.[87]

Cases with low lymph node harvest might be prone to missing positive lymph nodes and understaging. In contrast, high numbers of evaluated lymph nodes could prevent understaging. Actually, the number of investigated lymph nodes are associated with favorable outcome in colon cancer. A stage migration effect also called Will Rogers phenomenon introduced by Feinstein *et al*[88] would take place resulting in proven survival curves both for stage II and III cancers. The elimination of false node-negative cases within the collective of stage I/II cases and the shift of relatively early nodal positive cases into the correct stage III category is believed to cause such a phenomenon.

This prognostic impact of high lymph node yields prompted the demand for more intensive lymph node evaluations with up to 30 lymph nodes or even more. Because insufficient lymph node harvest has been identified as an adverse prognostic factor adjuvant chemotherapy is recommended for patients with less than 12 identified lymph nodes regardless of the nodal status.[88]

The TNM-AJCC 7th and 8th edition state it is important to obtain and examine at least 12 LNs. The prior 6th edition suggested a range of 7 to 14 LNs that should be obtained. Even if less than the suggested number of LNs was identified, the actual N stage rather than Nx should be provided. Compared with the 6th edition, the 7th edition further subdivided N1 into N1a, N1b, and N1c; and N2 into N2a and N2b. N1c is a newly introduced category in the 7th edition, which is defined by TD in subserosa, mesentery, or nonperitonealized pericolonic and perirectal/mesorectal tissue without any regional nodal metastasis. The 8th edition does not have significant changes in N staging definitions compared with the 7th edition but rather clarifications.[48]

2.2.2. Tumor Deposits

TDs are present in approximately 10% of colorectal cancers, 2.5% of colon cancer, and 3.3% of rectal cancer have TDs without positive LNs. The classification of these tumor nodules as TDs versus LNs has been debated over the years. TDs are thought to either represent discontinuous tumor spread, a totally replaced LN, venous invasion with extravascular extension, and/or less commonly, small-vessel or perineurial invasion. The concept of TD was first introduced in the AJCC/TNM staging system in the 5th edition (1997). The classification was determined by TD size (tumor nodule >3 mm was considered regional LN metastasis; tumor nodule ≤ 3 mm was

considered a discontinuous extension and classified in the T category). In the 6th edition (2002), the classification was based on the form and contour of the TD rather than the size (tumor nodule with the form and smooth contour of an LN is considered LN; tumor nodule with irregular contour was classified in the T category as either discontinuous spread or venous invasion). In the 7th (2010) edition, the size and shape of the contour are not criteria, but TD is simply defined by no evidence of residual LN tissue but within the lymphatic drainage of the primary carcinoma. The new nodal subclassifications category N1c is used if there is TD but no concurrent positive LN. N1c category was created to allow data collection and outcome analysis in the hopes of a better understanding of its prognostic significance. Of note, N1c is not worse by definition than N1a or N1b. The number of TDs should not be added to the number of LNs for assessing the adequacy of LN dissection. The number of LNs and TD is reported separately. In the 8th edition, the fundamental definition of TD is not changed compared with the 7th edition, but rather clarified. If a vessel wall or neural structure is identifiable, the nodule should be classified as lymph-vascular invasion or perineurial invasion correspondingly. To help vessel wall identification, the use of special stains such as the elastin stain may be considered to supplement the routine H&E stains. In addition, 1 to 4 TDs or 5 or more TDs should be recorded.[48]

2.3 Staging Systems

In this chapter, the TNM-AJCC and the JSCCR staging system will be accurately illustrated. Excluding any specifications for rectal cancer, since it is not included in this study.

2.3.1 TNM-AJCC 8th Ed[89]

The staging procedure aims to establish the local and distant extent of the disease to provide a framework for discussing therapy and prognosis.

The Tumor, Node, Metastasis (TNM) staging system of the combined American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) is the preferred staging system for colorectal cancer. This staging system has widely replaced the Astler-Coller modification of the Duke's classification, which its use is nowadays widely discouraged. The most recent version of the TNM staging classification is the 8th edition, 2017 (See Appendix A).

The TNM-AJCC staging system dives the colon anatomically into four parts: the right or ascending colon, the middle or transverse colon, the left or descending colon, and the sigmoid colon. The sigmoid colon is continuous with the rectum, which terminates at the anal canal.

Anatomy

Primary site

The large intestine (colon and rectum) extends from the terminal ileum to the anal canal. Excluding the vermiform appendix and the rectum, the colon is divided into four parts: the right or ascending colon, the middle or transverse colon, the left or descending colon, and the sigmoid colon. The sigmoid colon is continuous with the rectum, which terminates at the anal canal (Fig.1-2).

The ascending colon begins with the cecum, a 6- to 9-cm much that arises as the proximal segment of the right colon at the end of the terminal ileum It is covered with a visceral peritoneum (serosa). The ascending colon continues from the cecum and measures about 15 to 20 cm in length. The ascending colon ends at the hepatic flexure, which transitions the ascending colon into the transverse colon, passing just inferior to the liver and anterior to the duodenum.

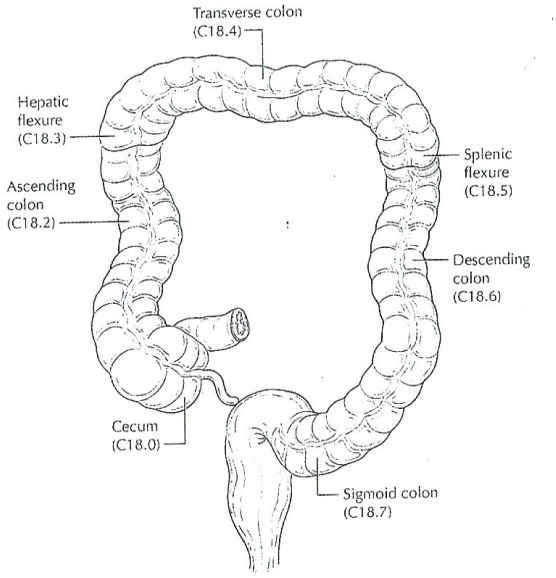


Fig.1 Anatomic subsites of the colon

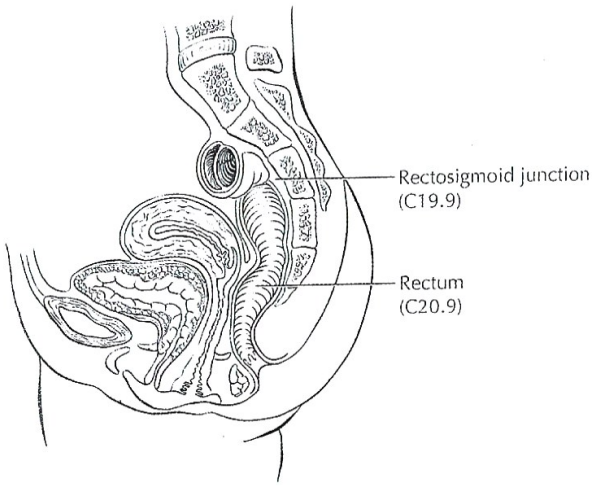


Fig. 2 Anatomic subsites of the rectum

The transverse colon is entirely intraperitoneal, about 18 to 22 cm long, and supported on a mesentery that is attached to the pancreas. Anteriorly, its serosa is continuous with the gastrocolic ligament. The transverse colon ends at the splenic flexure, which transitions into the descending colon.

The descending colon passes inferior to the spleen and anterior to the tail of the pancreas. The posterior aspect of the descending colon lacks serosa and is in direct contact with the retroperitoneum, whereas the lateral and anterior surfaces have serosa and are intraperitoneal. The descending colon measures about 10 to 15 cm in length.

The sigmoid colon is completely intraperitoneal, once again with a mesentery that develops at the medial border of the left psoas major muscle and extends to the rectum. The transition from sigmoid

colon to rectum is marked by the fusion of the taenia of the sigmoid colon to the circumferential longitudinal muscle of the rectum. The sigmoid colon is approximately 15-20 cm long.

The proximal rectum is defined by the fusion of the taenia, which typically occurs at the level of the sacral promontory. The distal boundary of the rectal reservoir or ampulla is the puborectalis ring, which is palpable as the anorectal ring on digital rectal examination. The rectal mucosa extends below this ring into the functional anal coal to the dentate line. This feature is critical to understanding how rectal cancer may occur within the functional ("surgical") anal canal. The rectum is approximately 12 to 16 cm in length. It is covered by peritoneum in front and on both sides in its upper third and only on the anterior wall in its middle third. The peritoneum is reflected laterally from the rectum to form the perirectal fossa and, anteriorly, the uterine or rectovesical fold. Depending on body habitus and gender, this fossa may be widely variable and may extend to the pelvic floor.

Regional Lymph Nodes

Regional nodes are located 1) along the course of the major vessels supplying the colon and rectum, 2) along the vascular arcades of the marginal artery, and 3) adjacent to the colon that is, along the mesocolic borders of the colon. The regional lymph nodes are termed pericoli and perirectal/ mesorectal and also are found along the ileocolic, right colic, middle colic, left colic, inferior mesenteric, superior rectal (hemorrhoidal), and infernal iliac arteries.

The regional lymph nodes for each segment of the large bowel are designated as follows:

Cecum: pericoli, ileocolic, right colic

Ascending colon: pericolic, ileocolic, right colic, right branch of the middle colic

Hepatic flexure: pericolic, ileocolic, right colic, middle colic

Transverse colon: pericolic, middle colic

Splenic flexure: pericolic, middle colic, left colic

Descending colon: pericolic, left colic, sigmoid, inferior mesenteric

Sigmoid colon: pericolic, sigmoid, superior rectal (hemorrhoidal), inferior mesenteric.

Rectum: mesorectal, superior rectal (hemorrhoidal), inferior mesenteric, internal iliac, inferior rectal (hemorrhoidal).

Metastatic Sites

Although carcinomas of the colon and rectum can metastasize to almost any organ, the liver and lungs are the most commonly affected.

Clinical Classification

Clinical assessment is based on medical history, physical examination, radiology, and endoscopy with biopsy. Radiological examinations are performed to demonstrate the presence of extracolonic metastases, they may include chest radiographs, computed tomography (CT; abdomen, pelvis, chest), magnetic resonance (MR) imaging, positron emission tomography (PET), of sued PET/SCT scans. Clinical stage (cTNM) then may be assigned. Pathological stage (pTNM) is assigned based on the resection specimen. Preoperative determination of the carcinoembryonic antigen (CEA) is recommended, as it may reflect the likelihood that subclinical or clinical liver or lung metastases are present. In the event of recurrence or synchronous metastases, it is recommended that the status of the genes KRAS, NRAS, and BRAF be evaluated and MSI or mismatch repair (MMR) be measured.

Carcinomas that arise in the colon or rectum are spread by direct invasion into the mucosa, submucosa, muscular propria, and subserosal tissue (or adventitia) of the bowel wall and each level of penetration is annotated by a T category. Primary tumors also spread by invading lymphatics and blood vessels to form metastases in lymph nodes or blood vessels to form metastases in lymph nodes or distant sites; this is annotated by the N and M categories, respectively. In addition, carcinomas may spread and grow in the adventitia as discrete nodules of cells called tumor deposits.[89]

Pathological Classification

Cancer of the colon is pathologically staged after microscopic examination of the resected specimen (pTNM) resulting from surgical exploration of the abdomen and cancer-directed surgical resection.

Primary tumor

Tis and T1. Lesions confined to the epithelial layer of crusts and lack invasion through the basement membrane into the lamina propria are defined as high-grade dysplasia. The term intraepithelial carcinoma is synonymous with high-grade dysplasia but is rarely used. Hight-grade dysplasia should not be intended as Tis, because this lesion lacks the potential for tumor spread. However, Tis is assigned to lesions confined to the mucosa in which cancer cells invade into the lamina propria and may involve but not penetrate through the muscular mucosa. These lesions are defined intramucosal carcinoma. The term invasive adenocarcinoma is used for colorectal cancer if the tumor extends through the muscular mucosal into the submucosa or beyond.

Carcinoma in a Polyp. These lesions are classified according to the pT definitions adopted for colorectal carcinomas. For instance, invasive carcinoma limited to the muscular mucosae and/or

lamina propria is classified as pTis, whereas a tumor that has invaded through the muscularis mucosae and has entered the submucosa of the polyp head or stalk is classified as pT1. pTis in a polyp resected with a clear margin during endoscopy is a Stage 0 carcinoma with nodal and metastatic status unknown, but with a sufficiently low probability of nodal involvement that node resection is not justified. The probability of metastasis is similarly low.

T1, T2, and T3. Are defined as tumors that involve the submucosa, penetrating through the submucosa into but not through the muscularis propria, and penetration through the muscularis propria respectively.

T4. Tumors that involve the serial surface (visceral peritoneum) or directly invade adjacent organs or structures are assigned to the T4 category. For both colon and rectum, T4 is divided into 2 categories (T4a and T4b) based on different outcomes. T4a tumors are characterized by involvement of the serial surface (visceral peritoneum) by direct tumor extension. Tumors with perforation in which the tumor cells are continuous with the serial surface through inflammation also are considered T4a. The significance of tumors that are <1mm from the serial surface and accompanied by serial reaction is unclear, with some studies indicating a higher risk for peritoneal relapse. Multiple-level sections and/or additional tissue blocks of the tumor should be examined in these cases to detect serial surface involvement. If the latter is not present after additional evaluation, the tumor should be assigned to the pT3 category. In portions of the colorectal that are not peritonealized (e.g. posterior aspects of the ascending and descending colon, lower portion of the rectum), the T4a category is not applicable.

Lymph Nodes

In the assessment of pN, the number of lymph nodes sampled should be recorded. The number of nodes removed and retrieved from an operative specimen has Benn reported to correlate with improved survival, possibly because of increased accuracy in staging. For nodal sampling to be accurate, it is important to obtain and examine at least 12 lymph nodes in radical colon and rectum resection in patients who undergo surgery for cure. In cases in which tumor is resected for palliation, or in patients who have received preoperative radiation or chemo radiation, fewer lymph nodes may be present. A pN0 determination is assigned if all nodes are histologically negative, even if fewer than the recommended number of nodes have been analyzed.

Regional lymph nodes are classified as N1 or N2 according to the number involved by metastatic tumor. Involvement of one to three nodes by metastasis is pN1: involvement of four or more nodes by tumor metastasis is pN2. The number of nodes involved with metastasis influences outcome in tight the N1 and N2 groups. pN1 is subdivided further into pN1a (metastasis in one regional lymph

node) and pN1b (metastasis in two or three regional lymph nodes), and pN2 is subdivided into pN2a (metastasis in four to six regional lymph nodes) and pN2b (metastasis in seven or more regional lymph nodes). Lymph nodes outside the regional drainage of the primary tumor should be characterized as distant metastases; for example, external iliac or common iliac node involvement in a rectosigmoid carcinoma would be M1a. Micrometastases have been defined as clusters of 10 to 20 tumor cells or clumps of tumor on cut section that measure more or equal to 0.2mm in diameter. These cell clusters indicate that tumor cells have entered a node and replicated and are not merely isolated dormant cells. Although these micrometastases may be designated as N1mi, it may be better to consider these as standard positive nodes with the corresponding number, as pathologists likely have considered these to be positive in the past.

N1c — Tumor Deposits

Tumor deposits are defined as discrete tumor nodules within the lymph drainage area of the primary carcinoma without identifiable lymph node tissue or identifiable vascular or neural structure. The shape, contour, and size of the deposit are not considered in these designations. If the vessel wall or its remnant is identifiable on H&E, elastin, or any other stain, the lesion should be classified as lymph-vascular invasion (LVI) present (a CAP-required data element). If neural structures are identifiable, the lesion should be classified as a perineurial invasion. One to four individual tumor deposits of five or more deposits without the involvement of lymphatic, venous, or neural structures within the lymph drainage area of the primary carcinoma should be recorded. In the evaluation of tumors pretreated with radiation and/or chemotherapy, it is important for the pathologist to assess whether tumor nodules represent tumor deposits as defined earlier or discontinuous eradication of the original tour so that he or she can record the appropriate ypT and ypN categories.

In cases with tumor deposits but no identified lymph node metastases, the N1c category is used and is applicable to all T categories. The presence of tumor deposits does not change the primary tumor T category but does change the node status (N) to N1c if all regional lymph nodes are pathologically negative. The number of tumor deposits is not added to the number of positive regional lymph nodes if one or more lymph nodes contain cancer.

Metastasis

Metastasis to only one site/solid organ (e.g., liver, lung, ovaries, non-regional lymph node) should be recorded as M1a. Multiple metastases within only one organ, even if the organ is paired (e.g., the ovaries or lungs), is still M1a disease. Metastases to multiple sites or solid organs distant from the

primary site are M1b, excluding peritoneal carcinomatosis. Peritoneal carcinomatosis with or without blood-borne metastasis to visceral organs is designated as M1c, because recent studies suggest that the prognosis for the peritoneal disease is worse than that for visceral metastases to one or more solid organs. The pathologist should not assign pM0 because M0 is a global designation referring to the absence of detectable metastasis anywhere in the body.

Lymphovascular Invasion (LVI)

Invasion of either small or large vessels by the primary tumor is an important poor prognostic factor. Small vessel invasion is involvement by tumor of thin-walled structures lined by endothelium, without an identifiable smooth muscle layer or elastic lamina. These thin-walled structures include lymphatics, capillaries, and post-capillary venues. Large vessel invasion is defined by tumor involving endothelium-lined spaces that have an elastic lamina and/or smooth muscle layer. Circumscribed tumor nodules surrounded by an elastic lamina on H&E or elastic stain also are considered a venous invasion and may be extramural (beyond the muscularis propria) or intramural (submucosa or muscularis propria).

Perineural Invasion (PNI)

Invasion of the nerves within or adjacent to the primary tumor by colorectal carcinoma is a negative prognostic factor that may be as important as an invasion of lymphatics or blood vessels. However, it often is overlooked and may be present in as many as 20% of primary colonic or rectal carcinomas. Carcinoma invasion of peripheral nerves, including prineural spaces within the regional drainage area of the primary tumor, is an adverse prognostic factor. If present, PNI usually is apparent on standard H&E staining of formalin-fixed tissues.

A summary of the TNM-AJCC staging system and the definition of the various stages of the disease are available in Appendix A.

2.3.2 Japanese (JSCCR) grading system[90]

This classification applies to primary carcinomas of the colon and rectum and does not apply to recurrence or metastasis.

Anatomical division of the colon

The large intestine comprises the cecum; a pouch-like region extending caudally to the upper lip of the ileocecal valve. The boundary with the ascending colon is the height of the upper lip of the ileocecal valve. The ascending colon extends from the cecum to the right colic flexure. The transverse colon is the segment that extends between the left and right colic flexures. The descending colon is the segment fixed to the retroperitoneum extending from the left colic flexure to the root of the sigmoid colon (approximately at the height of the iliac crest). The sigmoid extends from the descending colon to the height of the sacral promontory. The rectum is divided in a) rectosigmoid — the segment from the height of the sacral promontory to the inferior border of the second sacral vertebra, b) upper rectum — the segment from the height of the inferior border of the second sacral vertebra to the peritoneal reflection, c) lower rectum — the segment from the peritoneal reflection to the superior border of the puborectal sling.

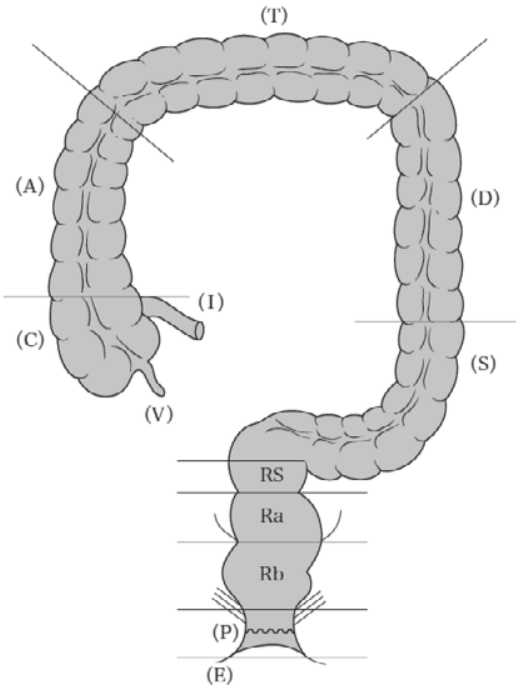


Fig. 3 Anatomical division of the large intestine

In this classification, the intestinal tract from the sacral promontory to the inferior border of the second sacral vertebra is treated as the rectum.

Depth of tumor invasion (T)

Tx: Primary tumor cannot be assessed

T0: No evidence of primary tumor

Tis: Tumor is confined to the mucosa (M) and does not invade the submucosa (SM)

T1: Tumor is confined to the SM and does not invade the muscularis propria (MP)

T1a: Tumor is confined to the SM, and invasion is within 1000μm

T1b: Tumor is confident to the SM, and invasion is 1000μm or more, but it does not extend to the MP

T2: Tumor invasion to, but not beyond, the MP

T3: Tumor invades beyond the MP. In sites with serosa, the tumor grows into the subserosa (SS). In sites with no serosa, the tumor grows into the adventitia (A)

T4: Tumor invades or perforates the serosa (SE) or directly invades other organs or structures (SI/AI)

T4a: Tumor invades or perforates the serosa (SE)

T4b: Tumor directly invades adjacent organs or structures (SI/AI)

Note 1: The extent of invasion of the primary tumor is recorded according to the T classification. Invasion into each layer of the bowel wall and into the adjacent organs is denoted using the letters M, SM, MP, SS, A, and SI/AI. SI indicates invasion through the serosa into adjacent organs in sites with serosa, and AI indicates invasion into adjacent organs in sites with no serosa.

Note 2: In the portion without the serosa, the advent (A) describes the pericolic/perirectal tissues equivalent to the SS of the portion with the serosa.

Note 3: The prefixes "c" (clinical findings) and "p" (pathological findings) are only used for the T classification and not for M-SI/AI (pathologically diagnosed mucosal cancer is recorded as pTis, and not pM).

Note 4: Tis conventionally refers to carcinoma in situ without invasion into the lamina propria; however, in colorectal cancer, Tis refers to cancer not extending beyond the lamina propria (i.e. intramucosal carcinoma) regardless of invasion.

Note 5: Regardless of the existence of metastasis. Tis and T1 are designed as "early cancers", and cancers that have grown into the MP or beyond are designated as "advanced cancers". The globally used terms "early stage colorectal cancer" and "advanced colorectal cancer" refer to stages I-III colorectal cancers and unresectable colorectal cancers, respectively, which define stages differently from T classification.

Note 6: For pT4b, the organ invaded is also noted, e.g., pT2b (prostate).

Note 7: The extent of histopathological depth is evaluated using the deepest area of cancer invasion. In case the deepest area is vascular/nerve invasion, it should be noted.

Note 8: The definition of the extent of primary tumors has been determined to be consistent with that of other digestive system tumors.

Note 9: TNM classifications take no account of vascular invasion into T classification; consequently, the extent of primary tumor in the TNM classification and the current JCCRC may not agree in a low number of cases.

Lymph node metastasis

Lymph node groups are classified and numbered according to their anatomical relationship to the superior mesenteric, Inferiore mesenteric, and iliac arteries (see Appendices C).

The lymph node station numbers of the large intestine are indicated with 3-digit numbers in the 200's. For lymph nodes of the superior and inferior mesenteric arteries, the first digit represents the group, with pericolic lymph nodes denoted by "1", intermediate lymph nodes by "2" and main lymph nodes by "3". The second digit represents the main artery, with the ileocolic artery denoted by "0", right colic artery by "1", middle colic artery by "2", left colic artery by "3", sigmoid artery by "4", and inferior mesenteric artery, along with the superior rectal artery by "5".

We would like to emphasize the fact that in Japanese culture reading takes place from the right to the left, this difference may create some confusion with the numbers assigned by the JSCCR. The digits representing the group are in fact the third digits, and the second digits which represent the main artery remain in place.

Lymph nodes are divided into regional lymph nodes and others. The presence or absence of regional lymph node metastasis and the degree of metastasis are recorded using the classification N0-N3.

Regional lymph nodes are classified into 3 groups pericolic, intermediate, and main lymph nodes. In addition, lateral lymph nodes are included in the lower rectum.

The specific range of regional lymph nodes is individually defined according to the anatomical relationship between the location of the tumor and its main feeding artery/arteries.

The main arteries of the colon are the ileocolic, right colic, middle colic (right and left branches), left colic, and sigmoid arteries. The range of pericolic lymph nodes in the colon can be classified

into the following 4 types on the basis of the positional relationship with the tumor and feeding artery.

NX: Lymph node metastasis cannot be assessed

N0: No evidence of lymph node metastasis

N1: Metastasis in 1-3 pericolic/perirectal or intermediate lymph nodes

N1a: Metastasis in 1 lymph node

N1b: Metastasis in 2-3 lymph nodes

N2: Metastasis in 4 or more pericolic/perirectal or intermediate lymph nodes

N2a: Metastasis in 4-6 lymph nodes

N2b: Metastasis in 7 or more lymph nodes

N3: Metastasis in the main lymph node (s). In the lower rectal cancer, metastasis in the main and/or lateral lymph nodes (s).

Note 1: Lymph node metastasis beyond the regional lymph nodes is classified as distant metastasis (M1).

Note 2: Of extramural cancer deposits without lymph node structures (EX), tour deposits other than vascular/perineural invasion (tumor nodules: ND) are classified as metastatic lymph nodes.

Note 3: The number of dissected and metastatic lymph nodes is described according to the lymph node metastasis ratio (number of metastatic lymph nodes/number of dissected lymph nodes) for each station of the lymph node. The tumor of ND is integrated into that of directed lymph nodes and the number of ND is described.

Distant Metastasis (M)

M0: No distant metastasis

M1: Distant metastasis

M1a: Distant metastasis confined to one organ. Peritoneal metastasis not present.

M1b: Distant metastasis in more than one organ. Peritoneal metastasis not preset.

M1c: Presence of peritoneal metastasis.

M1c1: Metastasis to the peritoneum only

M1c2: Metastasis to the peritoneum with other distant metastasis

Note 1: All metastases (lymphogenous, hematogenous, and peritoneal), except for metastasis for the regional lymph nodes, are classified as M1.

Note 2: Ovarian metastasis is now classified as distant metastasis (M1).

Note 3: In the event of hepatic, pulmonary, and peritoneal metastases, the extent of metastasis noted is recorded.

Note 4: In the event of distant metastasis (M1), the site of metastasis is recorded in parentheses. When recording the site of metastasis, the following abbreviations can be used:

Liver: H

Peritoneum: P

Lung: PUL

Bone: OSS

Skin: SKI

Pleura: PLE

Extraregional lymph nodes: LYM

Ovaries: OVA

Other: OTH

Note 5: With regard to the pathological findings of distant metastasis (pM), “pM0” indicates the absence of distant metastasis confirmed by autopsy, and “pM1” indicates histologically confirmed distant metastasis. Accordingly, the diagnosis of distant metastasis based on clinical findings, intraoperative palpation, and/or image findings without histological confirmation are recorded as “cM0” and “cM1”. When the pathological findings of distant metastasis are unclear, “pMX” is not used.

Clinical and Pathological Classification for Stage Grouping

Stage is divided into clinical and pathological classifications, which are denoted by the letters "c" and "p" placed before each respective staging (cStage and pStage).

cStage is based on pretreatment clinical findings and not on surgical findings.

pStage is based on pathological findings. However, clinical and/or surgical findings can be used to determined distant metastasis (M) (see Appendinx D).

2.3.3 Similarities and Differences between the TNM-AJCC and JSCCR grading systems

After an analysis of both grading systems, we have individualized the main similarities and differences between both grading systems (see Tab.1).

Main Differences Between TNM-AJCC and JSCCR

	TNM-AJCC	JSCCR
Anatomical division of the colon in different sections	7	5
Definition of T1: subdivision of T1 in T1a (tumor is confided to the SM, and invasion is within 1000μm) and T1b (tumor is confined to the SM, and invasion is 1000μm or more, but it does not extend to the MP).	Absent	Present
Presence of N3, metastasis in the main lymph node(s)	Absent	Present
Peritoneal metastasis (M1c) is divided into: M1c1 -metastasis of the peritoneum only. M1c2 - metastasis to the peritoneum with other distant metastasis.	Absent	Present
Lymph node harvest of at least 12 lymph nodes regardless of their distribution	Present	Absent
Designate a topographic location to each positive lymph node	Absent	Present
Considers tumor deposits as N+	Absent	Present
Tumor deposits are reported but do not modify the N stage	Present	Absent
Appoints a tumor ration to each lymph node station	Absetn	Present

Tab. 1

The JSCCR system takes into consideration N3 while defining the IIIb and IIIc stages, while the TNM-AJCC system considers N1c to define the IIIa and IIIb stages (see Tab. 2).

Differences in Stage

STAGE	TNM-AJCC	JSCCR
STAGE IIIA	T1-T2;N1/**N1c**;M0 T1;N2a;M0	T1-T2;N1;M0 T1;N2a;M0
STAGE IIIB	T1-T2;N2b;M0 T2-T3;N2a;M0 T3-T4;N1/**N1c;**M0	T1-T2;N2b-**N3;**M0 T2-T3;N2a;M0 T3-T4a;N1;M0
STAGE IIIC	T4a;N2a;M0 **T3**-T4a;N2b;M0 T2b;N1-N2;M0	T2-T4a;N2b-**N3**;M0 T4a;N2a;M0 T4b;N1-N2-N3;M0

Tab. 2

CHAPTER THREE

3.1 Rationale of the PhD project

The rationale for this study is that the differences between the TNM-AJCC and the JSCCR grading systems could lead to a difference in staging in the same patient. The JSCCR grading system gives a more detailed report of the lymph node status, reporting not only the number of positive LNs but also their topographic distribution. It is well known that a positive LN in the main lymph node chain has a worse prognosis than a positive LN in the marginal pericolic lymph nodes. The JSCCR grading system could allow us to stratify "topographic subtypes" of risk within the same stage (carrying a change in prognosis). Which could lead to a change in patient oncological management. This study's purpose is to apply both staging systems in each enrolled case.

Primary Aims

Evaluate:

1) The applicability of the JSCCR classification in western culture.

- The outcome is described as the percentage of cases in which this system results completely applicable (Note: for the TNM-AJCC systems, by definition is 100%)

2) The agreement between disease stages, defined after the application of both staging systems.

- Outcome: number of cases in which the TNM-AJCC system and the JSCCR system define the same stage of the disease.

Secondary Aims

1) Determine if the JSCCR system is able to highlight "topographic-subtypes" with a different risk of disease recurrence (local or metastatic disease) with a mean follow-up period of 3 years.

- Outcome: time to local recurrence and time to metastatic disease.

2) Evaluate if the JSCCR system highlights "topographic-subtypes" with different mortality rates.

- Outcome: overall survival

CHAPTER FOUR

4.1 Materials and Methods

4.1.2 Study Design

This is a prospective monocentric study that confronted the TNM-AJCC and the JSCCR staging systems, applying both systems to the same population. No modifications were applied to the diagnostic and therapeutic procedures, from this point of view, this study can be considered an observational study.

This study was designed in a two-phase manner. The first phase consisted of the enrollment phase, which lasted 12 months. At the end of the enrollment phase, we were able to answer our primary aims. The second phase consists of a 3-year follow-up period after which we will be able to answer our secondary aims.

This study was coordinated by Chirurgia ad Indirizzo Oncologico-AUSL-IRCCS dell'Azienda USL-IRCCS di Reggio Emilia after approval of the ethics committee (Comitato Etico di Area Vasta Emilia Nord) ID number CST:68/2020.

4.1.2 Population

Inclusion criteria:

- Patients >18 years old who expressed written informed consent.
- Patients with a diagnosis of colon cancer (right colon, transverse colon, left colon, or sigma) with a negative CT scan for metastatic disease.
- Patients who agree to continue the follow-up period at our Institution.

Exclusion criteria:

- Patients with a diagnosis of rectal cancer were excluded from this study, due to their different metastatic patterns, furthermore, patients with rectal cancer often undergo neoadjuvant chemotherapy (meanwhile in Japan these patients undergo radical surgical resection with D3 lymphadenectomy and adjuvant chemotherapy).
- Patients with a diagnosis of synchronous solid tumors or oncohematological diseases.
- Patients who underwent neoadjuvant chemotherapy.
- Patients with metastatic/residual disease.

The exclusion criteria were evaluated during the enrollment phase. The findings encountered during the development of the study were reasons for early withdrawal of the study and/or exclusion from the data analysis phase.

4.1.3 Study flow-chart

PROCEDURE	PRE-HOSPITALIZATION	SURGICAL PROCEDURE	FOLLOW-UP (3 YERAS)
Patient selection	X		
Inclusion/exclusion criteria	X		
Informed consent		X	
Surgical resection		X	
Pathologist report		X	
Oncological treatment and FU			X
Disease recurrence			X

4.1.4 Surgical Phase

After the specimen was surgically removed, a careful dissection of all the lymph node stations was executed according to the JSCCR classification. Each lymph node station was sent to the pathologist separately and coded according to the number assigned by the JSCCR classification.

Only the fatty tissue surrounding the tumor was left in place, to avoid any damage to the visceral wall nearby the tumor (see Appendix E).

4.1.5 Processing of the specimen by the Pathologist

The specimen once in the Pathology department remained in formalin for a 12-hour period. Then the specimen was cut open to describe the macroscopic findings, to ensure an adequate fixation and a correct tissue withdrawal. The organ was cut longitudinally avoiding the tumor. The tumor was measured in size and the distance between the section margin and the tumor was recorded. In the case of bulky tumors, the tumor was sectioned into thin slices for better fixation. The tissue was processed by automatic processors and then covered with paraffin. Afterward, the specimen was cut into thin slices of 3-4 microns that were put in thermostatic baths before being colored with Hematoxylin-eosin.

PATHOLOGIST'S REPORT

The pathologist's report contained the following information:

- Tumor size
- Histotype
- The number of lymph nodes present in every single station and its relative positive/total lymph node ratio.
- Tumor deposits (if present)
- Lymphovascular invasion (if present)
- Tumor grade of differentiation
- Tumor budding (when preset, it was reported as low-grade or high-grade)
- Poorly-differenciated clusters (if present)

4.1.6 Follow-up Phase

After discharge, patients were given an appointment with the Oncologist who took care of the following therapy and/or management of the follow-up period, following our institution's protocols.

CHAPTER FIVE

5.1 Results

We enrolled 91 patients with a diagnosis of colon cancer in a 12-month period who met our inclusion/exclusion criteria during the enrollment phase, from which 6 patients were withdrawn from the study. Three patients presented intraoperative findings of hepatic metastatic disease, one patient was classified as sigma adenocarcinoma during the preoperative staging with an intraoperative finding of rectal carcinoma, and in one patient the dissection of the specimen according to the JSCCR classification wasn't performed due to OR timing issues, and in another patient, there was an intraoperative finding of renal synchronous carcinoma.

Thirty-four patients were women and 57 were men. The mean age was 71,62 years old. Clinical presentation ad diagnosis is summarized in Tab. 3

CLINICAL PRESENTATION

Symtom	*N. Cases*
Anemization	35 (38.5%)
Intestinal Subocclusion	3 (3.3%)
Macroscopic bleeding	14 (15.4%)
Screening test	33 (36.3%)

Tab.3

The topographic distribution of the disease is illustrated in Tab. 4 and the surgical procedures performed are illustrated in Tab. 5

ANATOMICAL DISTRIBUTION OF THE DISEASE

SITE	N.
Right colon	53 (58.2%)
Right flexure	6 (6.6%)
Transvers colon	5 (5.5%)
Left flexure	7 (7.7%)
Left colon	2 (2,2%)
Sigma	17 (18.7%)
Right colon and sigma	1 (1.1%)
TOTAL	91

Tab. 4

SURGICAL PROCEDURES

Surgical Procedure	N.
Right Hemicolectomy	60 (65.9%)
Left Hemicolectomy	7 (7.7%)
Transverse colon resection	4 (4.4%)
Segmentary resection of the colon	8 (8.8%)
Sigmoidectomy	11 (12.1%)
TOTAL	91

Tab. 5

5.2 Statistical Analysis

Continuous variables were characterized by median and range. Categorical data were summarized as absolute and relative frequencies. For the analysis of our primary aims, the JSCCR classification was defined as applicable when it was able to deliver a disease stage for each patient and it was calculated as a percentage of cases in which both systems appointed the same stage for each patient with a CI of 95% according to Wilson. The degree of agreement was determined by Cohen's Kappa coefficient with a CI of 95%. Statistical analysis was performed with R 4.0.4 software.

Primary aim 1.1: applicability of the JSCCR classification to our population. The outcome is described as the percentage of cases in which this classification proves to be completely applicable. The JSCCR classification was applicable in 100% of cases (85/85) with a CI:95%.

- Results: the JSCCR classification proved to be applicable in 100% of cases

Primary aim 1.2: the agreement between disease stages applying both staging systems.

- Results: complete agreement between both grading systems, the $\varkappa$ coefficient being 1 is a confirmation of the complete agreement between both systems. Tab.6

DEGREE OF AGREEMENT

Agreement table, Cohen's Kappa (linearly weighted) = 1.000 (0.95 CI: 1.000 to 1.000)										
	TNM: 0	TNM: I	TNM: IIA	TNM: IIB	TNM: IIC	TNM: IIIA	TNM: IIIB	TNM: IIIC	TNM: IVA	Tot
JSCCR: 0	**1**	0	0	0	0	0	0	0	0	1
JSCCR: I	0	**26**	0	0	0	0	0	0	0	26
JSCCR: IIA	0	0	**25**	0	0	0	0	0	0	25
JSCCR: IIB	0	0	0	**8**	0	0	0	0	0	8
JSCCR: IIC	0	0	0	0	**1**	0	0	0	0	1
JSCCR: IIIA	0	0	0	0	0	**1**	0	0	0	1
JSCCR: IIIB	0	0	0	0	0	0	**19**	0	0	19
JSCCR: IIIC	0	0	0	0	0	0	0	**5**	0	5
JSCCR: IVA	0	0	0	0	0	0	0	0	**1**	1
Tot	1	26	25	8	1	1	19	5	1	87

Tab. 6

5.3 Discussion

Given the important role in prognosis that the lymph node status carries, an accurate grading method has been researched over the years. Even though colon cancer has a sequentially spread pattern through the regional lymphatic bed, skip metastases occur in 1% to 3% of cases.

Merrie *et al*., demonstrated that the anatomic distribution of metastases did not always follow the assumed sequential anatomic pattern spread.[91] Several studies support the notion that the location of the metastasized lymph node has prognostic value.[53,92,93] For example, the presence of inferior mesenteric (IMA) lymph node metastasis has been shown to predict para-aortic nodal recurrence in patients with cancer of the sigmoid colon or rectum.[53] Other studies have shown the distribution of lymph node metastasis to be an independent predictor of overall survival in sigmoid colonic and rectal cancer. [92,93] A cohort study of patients with stage III colonic cancer including patients from 71 hospitals across Japan suggests that identification of main lymph node metastasis by anatomical classification may provide additional prognostic value for risk stratification of cancer-specific death in patients with colonic cancer. [94] According to these findings the Japanese Society for Cancer of the Colon and Rectum incorporated in their grading system the determination of the anatomical distribution of the lymph nodes harvested during surgical resection. Arabiki M *et al*., stated the superiority of the JSCCR 9th compared to the 8th AJCCR system regarding the risk stratification power. According to the authors, the 8th AJCC grading system may have missed the importance of the stratification capacity of "tumor deposits".[95] This statement is also supported by Kitamura *et al*, who concluded that the 9th JSCCR classification accounts for the presence of TD and N3 disease, which were both significant predictors of poor prognosis.[96]

Another study reported that the JSCCR 9th edition, which further divides M1c based on the presence or absence of other organ involvement, was superior to the TNM 8th in predicting OS in stage IV colorectal cancer patients. [97]

All these studies reporting the superiority of the 9th JSCCR edition Japanese classification compared to the TNM-AJCC 8th edition are all studies performed in the Japanese population. To our knowledge no studies validating the 9th edition JSCCR classification have been performed in the Western population. It is most important to keep in mind the anatomical implications during lymph node harvest and the fact that the Western population carries a well know obesity burden, whereas the Japanese population tends to be more fit.

As a first step, we designed this study to determine whether or not the Japanese JSCCR classification is applicable to the Western population. According to our results, the JSCCR grading system is applicable to the Western population with a complete agreement between both staging systems. Since there was no upgrading in stage disease while applying the Japanese classification to our population we do not expect any modification in disease recurrence or mortality rates on a 3-year follow-up period.

We acknowledge the limitations of our study since it is a monocentric study with a small cohort. A bigger sample might be necessary to better assess any variation between both grading systems.

It is our belief that more studies applying the JSCCR classification in the Western population are needed to more accurately evaluate its role in the Western population.

5.4 Conclusions

According to our findings, the JSCCR grading system proved to be 100% applicable to our population with a CI of 95%.

We found a complete agreement between the TNM-AJCC and the JSCCR grading systems, therefore we didn't observe changes in disease stage while applying the JSCCR grading system in comparison to the TNM-AJCC grading system.

The fact that the TNM-AJCC and the JSCCR grading systems showed complete agreement could be due to the small sample size. A bigger sample size would be needed to prove the differences

between both grading systems. Nonetheless, the need for a bigger sample to prove a hypothetical change in disease stage made us question the real advantage of the JSCCR grading system, taking into consideration that the JSCCR system is time-consuming. The specimen dissection time is a phase that requires a surgeon and an OR nurse that take care of the dissection and accurate labeling of each lymph node station. In this study, the specimen dissection time was not recorded.

At the present moment, this study entered the follow-up phase. After the follow-up phase, we will be able to answer our secondary aims. With the data at our disposal, our preliminary reflection is that since there was no difference in disease stage between both grading systems, no differences in overall survival are expected nor the identification of risk subcategories based on the topographic distribution of positive LNs.

References

1. Labianca R, Beretta GD, Kıldan B et al. Colon Cancer. Crit Rev Oncol Hematol. 2010;74: 106-133
2. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. Ca Cancer J Clin. 2021;71:209-249
3. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Diet, nutrition, physical activity and colorectal cancer. Available at dietandcancerreport.org.
4. Cappell MS. Pathophysiology, clinical presentation, and management of colon cancer. Gastroenterol Clin N Am. 2008;37: 1-24.
5. Tamas K, Walenkamp AME, de Vries EGE, et al. Rectal and colon cancer: Not just a different anatomic site. Cancer Treat Rev. 2015;41: 671-679
6. Alitalo A and Detmar M. Interaction of tumor cells and Lymphatic vessels in cancer progression. Oncogene. 2012;31:4499-4508.
7. Knijn N, Van Erning FN, Overbeek LI, et al. Limited effect of lymph node status on the metastatic pattern in colorectal cancer. Oncotarget. 2016; 7;22: 31699-707. doi: 10.18632/oncotarget.9064.
8. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. Ca Cancer J Clin. 2017; 67:7-30
9. Wu X, Cokkinides V, Chen VW, et al. Association of subside-specific colorectal cancer incidence rates and stage of disease at diagnosis with county-level poverty, by race and sex. Cancer. 2006; 107 (Supply):1121-7. Doi:10.1002/cnrc.22009.
10. Siegel RL, Miller KD; Fuchs HE, et al. Cancer Statistics. Ca Cancer J Clin. 2021;71: 7-33
11. Berrino F, Krogh V, Riboli E. Epidemiology studies on diet and cancer. Tumori. 2003; 89(6):581-5
12. Jon D
13. Mihalko SL. Implementation of Colonoscopy for mass screening for colon cancer and colonic polyps: Efficiency with high quality care. Gastroenterol Clin N Am. 2008;37:117-128. Doi: 10.1016/j.gtc.2007.12.011
14. Macken E, Moreels T, Vannoote J, et al. Quality Assurance in colonoscopy for colorectal cancer diagnosis. EJSO. 2011;37:10-15. Doi: 10.1016/j.ejso.2010.09.013
15. Rex DK, Schoenfeld PS, Cohen J, et al. Quality indicators for colonoscopy. Gastrointest Ends. 2015;81(1):31-53. Doi: 10.1016/j.gie.2014.07.058
16. Jerome DW. The new view of colon cancer screening: Forwards and backwards. Gastrointest Endoscopy Clin N Am. 2013 (23): 647-661. Doi: http://dx.doi.org/10.1016/j.giec.2013.03.013
17. Dighe S, Swift I, Brown G. CT staging of colon caner. Clinical Radiol. 2008;63:1372-1379. Doi: 10.1016/j.crad.2008.04.021
18. Nerad E, Lambregts DMJ, Kersten ELJ, et al. MRI for local staging of colon cancer: Can MRI Become the optimal staging modality for patients with colon cancer?. Dis Colon Rec. 2017;60:385-392

19. Jhaveri KS and Safad A. Role of MRI for staging of rectal cancer. Expert Rev Anticancer There. 2009;9(4):469-481
20. Kijima S, Sasaki T, Nagata K, et al. Preoperative evaluation of colorectal cancer using CT colonography, MRI, and PET/CT. World J Gastroenterol. 2014;20(45):16964-16975. Doi: 10.3748/wjg.v20.i45.16964
21. Veit P, Kuhle C, Beyer T, et al. Whole body positron emission tomography/computed tomography (PET/CT) tumour staging with integrated PET/CT colonography: technical feasibility and fist experiences in patients with colorectal cancer. Gut. 2006;55:68-73. Doi: 10.1136/gut.2005.064170
22. Buczacki SJA and Davies RJ. Colon resection. Is standard technique adequate?. Surg Oncol Clin N Am. 2014;23:25-34.
23. Salem JF, Gummadi S, Marks JH. Minimally invasive surgical approches to colon cancer. Surg Oncol Clin N Am. 2008;27:303-318. Doi: https://doi.org/10.1016/j.soc.2017.11.005
24. Hiuranyakas A and Ho YH. Surgial treatment for colorectal cancer. Int Surg. 2011;96:120-126.
25. Jabos M, Verdeja JC, Goldstein HS. Minimally invasive colon resection (Laparoscopic Colectomy). Surg Laparosc Endosc. 1991;1(3):144-150.
26. COLOR Study Group. COLOR: a randomized clinical trial comparing laparoscopic and open resection for colon cancer. Dig Surg. 2000;17:617-622.
27. Fleshman J, Sargent DJ, Green E, et al. Laparoscopic colectomy for cancer is not inferior to open surgery based on 5-year data from the COST Study Group Trial. Ann Surg. 2007;246:655-664. Doi: 10.1097/SLA.0b013e318155a762.
28. Jayne DG, Guillou PJ, Thorpe H, et al. Randomized trial of laparoscopic-assisted resection of colorectal carcinoma: 3-year results of the UK MRC CLASSIC Trial Group. J Clin Oncol. 2007;25:3061-3068. Doi: 10.1200/JCO.2006.09.7758.
29. Deijen LD, Vasmel JE, de Lange-de Klerk ESM, et al. Ten-year outcomes of randomized trial of laparoscopic versus open surgery for colon cancer. Surg Ends. 2017;31:2607-2615. Doi: 10.1007/s00464-016-5270-6.
30. Cameron JL and Cameron AM. Current Surgical Therapy. 12ª Ed. Philadelphia. ELSEVIER. 2017.
31. Fazio VW, Church JM, Delaney CP et al. Current therapy in colon and rectal surgery. 3° edizione. ELSEVIER. 2017.
32. Gallot D, Colectomie per tumore del colon discendente, iliaco e del trasverso. EMC (Elsevier Masson SAS, Paris), Technique Chirurgiche-Addominale, 40-570, 2007.
33. Thirion P, Michiels S, Pignon JP, et al. Meta-Analysis Group in Cancer. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: an updated meta-analysis. J Clin Oncol. 2004 Sep 15;22(18):3766-75. do: 10.1200/JCO.2004.03.104. Erratum in: J Clin Oncol. 2005 Feb 20;23(6):1337-8. PMID: 15365073.
34. Wu C. Systemic Therapy for colon cancer. Surg Oncol Clin N Am. 2018;27:235-242. Doi: https://doi.org/10.1016/j.soc.2017.11.001.

35. Cremolini C, Loupakis F, Antoniotti C, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol. 2015;16:1306-1315. Doi: http://dx.doi.org/10.1016/S1470-2045(15)00122-9
36. Jung HS, Ryoo SB, Lim HK, et al. Tumor Size >5 cm and Harvested LNs <12 Are the Risk Factors for Recurrence in Stage I Colon and Rectal Cancer after Radical Resection. Cancers. 2021;13:5294. Doi: https//doi.org/10.3390/cancers13215294
37. Sheperd NA, Baxter KJ, Love SB. The prognostic importance of peritoneal involvement in colonic cancer: a prospective evaluation. Gastroenterology 1997; 112: 1096-102
38. Lennon AM, Mulchay HE, Hyland JM, et al. Peritoneal involvement in stage II colon cancer. AM J Clin Patrol. 2003;119:108-113
39. Keshava A, Chapuis PH, Chan C, et al. The significance of involvement of a free serosal surface for recurrence and survival following resection of clinicopathological stage B and C rectal cancer. Colorectal Dis. 2007;9:609-618. Doi:10.1111/j.1463-1318.2006.01136.x
40. Saha S, Shaik M, Johnston G, et al. Tumor size predicts long-term survival in colon cancer: an analysis of the National Cancer Data Base. Am J Surg. 2015;209:570-574. Doi: http://dx.doi.org/10.1016/j.amjsurg.2014.12.008
41. Nelson H, Petrelli N, Carlin A, et al. Guidelines 2000 for Colon and Rectal Cancer Surgery. J Natl Cancer Inst. 2001;93:583-596.
42. Lee YTM. Local and regional recurrence of carcinoma of the colon and rectum: II. Factors relating to operative technique. Surg Oncol. 1996;5:1-13.
43. Rocha R, Marinho R, Aparicio D, et al. Impact of bowel resection margins in node negative colon cancer. SpringerPlus. 2016;5:1959. Doi: 10.1186/s40064-016-3650-y
44. Tang XY, Huan MX, Han SQ, et al. The circumferential resection margin is a prognostic predictor in colon cancer. Front Oncol. 2020;26 (10):927. Doi: 10.3389/fonc.2020.00927
45. Healy MA, Peacock O, Hu CY, et al. High rate of positive circumferential resection margin in colon cancer: A national appraisal and call for action. Ann Surg. Epub ahead of print. Doi: 10.1097/SLA.0000000000004682.
46. Fielding LP, Arsenault PA, Chaps PH, et al. Clinicopathological staging for colorectal cancer: an International Documentation System (IDS) and an International Comprehensive Anatomical Terminology (ICAT). J Gastroenterol Hepatol. 1991;6:325-344.
47. Vather R, Sammour T, Kahokehr A, Connolly A, Hill A. Quantitative lymph node evaluation as an independent marker of long-term prognosis in stage III rectal cancer. ANZ J Surg. 2011;81:883-888.
48. Jin M and Frankel WL. Lymph node metastasis in colorectal cancer. Surg Oncol Clin N Am. 2018;27:401-412. Doi: https://doi.org/10.1016/j.soc.2017.11.011

49. Sjo OH, Merok MA, Svindland A, Nesbakken A. Prognostic impact of lymph node harvest and lymph node ratio in patients with colon cancer. Dis Colon Rectum. 2012;55:307-315.
50. Wang LP, Wang HY, Cao R, Zhu C, Wu XZ. Proposal of a new classification for stage III colorectal cancer based on the number and ratio of metastatic lymph nodes. World J Surg. 2013;37:1094-1102.
51. Lykke J, Roikjaer O, Jess P. The relation between lymph node status and survival in Stage I-III colon cancer: results from a prospective nationwide cohort study. Colorectal Dis. 2013;15:559-565.
52. Lu YJ, Lin PC, Lin CC, Wang HS, Yang SH, Jiang JK, Lan YT, Lin TC, Liang WY, Chen WS. The impact of the lymph node ratio is greater than traditional lymph node status in stage III colorectal cancer patients. World J Surg. 2013;37:1927-1933.
53. Arrichiello G, Pirozzi M, Facchini BA, et al. Beyond N staging in colorectal cancer: Current approaches and future perspectives. Front Oncol. 2022; 12:937114. Doi:10.3389/fonc.2022.937114.
54. Kang J, Min BS, Lee KY, et al. Prognostic impact of the lymph node ratio in rectal cancer patients who underwent preoperative chemoradiation. J Surg Oncol. 2011;104:53-58.
55. Occhionorelli S, Andreotti D, Vallese P, et al. Evaluation on prognostic efficacy of lymph nodes ratio (LNR) and log odds of positive lymph nodes (LODDS) in complicated colon cancer: the first study in emergency surgery. World J Surg Oncol. 2018;16:186. Doi: https//doi.org/10.1186/s12957-018-1483-6
56. Kataoka K, Beppu N, Shiozawa M, Ikeda M, Tomita N, Kobayashi H, Sugihara K, Ceelen W. Colorectal cancer treated by resection and extended lymphadenectomy: patterns of spread in left- and right-sided tumours. Br J Surg. 2020 Jul;107(8):1070-1078. doi: 10.1002/bjs.11517. Epub 2020 Apr 4. PMID: 32246469.
57. Yamaoka Y, Kinugasa Y, Shiomi A, Yamaguchi T, Kagawa H, Yamakawa Y, Furutani A, Manabe S. The distribution of lymph node metastases and their size in colon cancer. Langenbecks Arch Surg. 2017 Dec;402(8):1213-1221. doi: 10.1007/s00423-017-1628-z. Epub 2017 Oct 5. PMID: 28983781.
58. Huh JW, Kim YJ, Kim HR. Distribution of lymph node metastases is an independent predictor of survival for sigmoid colon and rectal cancer. Ann Surg. 2012 Jan;255(1):70-8. doi: 10.1097/SLA.0b013e31823785f6. PMID: 22133895.
59. Al Sahaf O, Myers E, Jawad M, et al. The prognostic significance of extramural deposits and extra capsular lymph node invasion in colon cancer. Dis Colon Rectum. 2011;54:982-988. Doi: 10.1097/CDR.0b013e31821c4944.
60. Zheng P, Chen Q, Li J, et al. Prognostic Significance of Tumor Deposits in Patients With Stage III Colon Cancer: A Nomogram Study. J Surg Research. 2020;245:475-482. Doi:https://doi.org/10.1016/j.jss.2019.07.099
61. Shen F and Hong X. Prognostic value of N1c in colorectal cancer: a large population-based study using propensity score matching. Int J Colorectal Dis. 2019;34(8):1375-1383. Doi: 10.1007/s00384-019-03328-9.

62. Mayo E, Llanos AAM, Yi X, et al. Prognostic value of tumor deposit and perineurial invasion status in colorectal cancer patients: a SEER-based population study. Histopatology. 2016;69(2):230-238. Doi:10.1111/his.12936.
63. Delattre JF, Cohen R, Henriques J, et al. Prognostic value of tumor deposits for disease-free survival in patients with stage III colon cancer: A post hoc analysis of the IDEA France Phase III Trial (PRODIGE-GERCOR). J Clin Oncol. 2020;38(15):1702-1710. Doi: 10.1200/JCO.19.01960
64. Yamano T, Sembra S, Noda M, et al. Prognostic significance of classified extramural tumor deposits and extra capsular lymph node invasion in T3-4 colorectal cancer: a retrospective single-center study. BMC Cancer. 2015;15:859. Doi: 10.1186/s12885-015-1885-6.
65. Veronese N, Nottegar A, Pea A, et al. Prognostic impact and implications of extracapsular lymph node involvement in colorectal cancer: a systematic review with meta-analysis. Ann Oncol. 2016;27(1):42-48. Doi: 10.1093/annonc/mdv494.
66. Koebrugge B, Bosscha K, Liefers GJ, et al. Can micrometastases be used to predict colon cancer prognosis? Hopes for the EnRoute+ study. 2011;5(5):559-561. Doi 10.1586/egh.11.56
67. Bilchik AJ, Hoon DSB, Saha S, et alo. Prognostic impact of micrometastases in colon cancer. Interim results of a prospective multi center trial. Ann Bug. 2007;246:568-577. Doi: 10.1097/SLA.0b013e318155a9c7.
68. Hogan J, Chang KH, Duff G, et al. Lymphovascular invasion: A comprehensive Appraisal in Colon and Rectal Adenocarcinoma. Dis Colon Rectum. 2015;58:547-555. Doi: 10.1097/DCR.0000000000000361.
69. Prugmahachaikul A and Sanpavat A. Prognostic significance of lymph-vascular invasion detected by D2-40 in low-risk stage II colon cancer. 2021;13(11):e19825. Doi: 10.7759/cureus.19825.
70. Bianchi G, Annicchiarico A, Morini A, et al. Three distinct outcomes in patients with colorectal adenocarcinoma and lymph-vascular invasion: the good, the bad, and the ugly. Int J Colorectal Dis. 2021;36:2671-2681. Doi: https://doi.org/10.1007/s00384-021-04004-7.
71. Koelzer VH, Zlobec I, and Lugli A. Tumor budding in colorectal cancer -ready for diagnostic practice?. Human Pathology. 2016;47:4-19. Doi: http://dx.doi.org/10.1016/j.humpath.2015.08.007.
72. Mehta A, Goswani M, Sinha R, et al. Histopathological significance and prognostic impact of tumor budding in colorectal cancer. Asian Pac J Cancer Prev;19(9):2447-2453. Doi: 10.22034/APJCP.2018.19.9.2447.
73. Lugli A, Kirsch R, Ajioka Y, et al. Recommendations for reporting tumor budding in colorectal cancer bassed on the international Tumor Budding Consensus Conference (ITBCC). Modern Pathology. 2017;30:12.
74. Argilés G, Tabernero J, Labianca R, et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020;31(10):1291-1305. Doi: https://doi.org/10.1016/j.annonc.2020.06.022

75. Konishi T, Shimada Y, Lee LH, et al. Poorly differentiated clusters predict colon cancer recurrence: an in-depth comparative analysis of invasive-front prognostic markers. Am J Surg Pathol. 2018;42(6):705-714. Doi: 10.1097/PAS.0000000000001059.
76. Lee VWK and Chan KF. Tumor budding and poorly-differentiated cluster in prognostication in stage II colon cancer. Pathol Research Practice. 2018;214:402-407.
77. Smit MA, van Pelt GW, Terpstra V, et al. Tumour-stroma ratio outperforms tumor budding as biomarker in colon cancer: a cohort study. Int J Colorectal Dis. 2021;36(12):2729-2737. Doi:10.1007/s00384-021-04023-4.
78. Hansen TF, Kjaer-Frifeldt S, Lindebjerg J, et al. Tumor-stroma ratio predicts recurrence in patients with colon cancer treated with neoadjuvant chemotherapy. Acta Oncologica. 2018;57(4):528-533. Doi:https//doi.org/10.1080/0284186X.2017.1385841.
79. Chen TM, H YT and Wang GC. Outcome of colon cancer initially presenting as colon perforation and obstruction. World J Surg Oncol. 2017;15:164. Doi: 10.1186/s12957-017-1228-y.
80. Pei JP, Zhang CD, Fan YC, et al. Comparison of different lymph node staging systems in patients with resectable colorectal cancer. Frontiers in Oncology. 2019;8:671. Doi: 10.3389/fonc.2018.00671
81. Yang KM, Jeong MJ, Yoon KH, et al. Oncologic outcome of colon cancer with perforation and obstruction. BMC Gastroenterology. 2022;22:247. Doi: https://doi.org/10.1186/s12876-022-02319-5.
82. NIH. National Cancer Institute SEER Training Modules. https://training.seer.cancer.gov/staging/systems/schemes/duke.html
83. Kehoe J and Khatri VP. Staging and prognosis of colon cancer. Surg Oncol Clin N Am. 2006;15:129-146. Doi:10.1016/j.soc.2005.08.006.
84. Jass JR and Morsen BC. Reporting colorectal cancer. J Clin Pathol. 1987;40:1016-1023.
85. Akkoka AN, Yanik S, Ozdemir ZT, et al. TNM and modified Dukes staging along with the demographic characteristics of patients with colorectal carcinoma. Int J Clin Exp Med. 2014;7(9):2828-2835.
86. Henson DEm, Hueman MT, Chen D, et al. The anatomy of the TNM for colon cancer. J Gastrointest Oncol. 2017;8(1):12-19. Doi:10.21037/jgo.2016.11.10.
87. Markl Bruno. Stage migration vs immunology: The lymph node count story in colon cancer. World J Gastroenterol. 2015;21(43):12218-12233. Doi:10.3748/wjg.v21.i43.12218.
88. Feinstein AR, Sosin DM and Wells Ck. The Will Rogers phenomenon. Stage migration and new diagnostic techniques as a source of misleading statistics for survival in cancer. N Engl J Med. 1985;312(25):1604-1608. Doi:10.1056/NEJM198506203122504.
89. Jessup JM, Golberg RM, Aware EA, et al. Colon and Rectum. In: AJCC Cancer Staging Manual, 8th ed. Amin MB (Ec), AJCC, Chicago 2017. P 251. Corrected at 4th printing, 2018.
90. Japanese Society for Cancer of the Colon and Rectum. Japanese Classification of Colorectal, Appendices, and Anal Carcinoma: the 3d English Edition [Secondary Publication]. J Anus Rectum Colon. 2019;3(4):175-195. Doi:10.23922/jarc.2019-018.

91. Merrie AE, Phillips LV, Yun K, McCall JL. Skip metastases in colon cancer: Assessment by lymph node mapping using molecular detection. Surgery. 2001;129(6):684-691. Doi: 10.1067/msy.2001.113887
92. Kang J, Hur H, Min BS, et al. Prognostic impact of inferior mesenteric artery lymph node metastasis in colorectal cancer. Ann Surg Oncol. 2011;18:704-710.
93. Kobayashi H, Ueno H, Hashiguchi Y, Mochizuki H. Distribution of lymph node metastasis is a prognostic index in patients with stage III colon cancer. Surgery. 2006; 139:516-522.
94. Kawada H, Kurita N, Nakamura F, et al. Incorporation of apical lymph node status into the seventh edition of the TNM classification improves prediction of prognosis in stage III colonic cancer.
95. Arabiki M, Shimada Y, Nakano M, et al. Verification of the Japanese staging system for rectal cancer, focusing on differences with the TNM classification.
96. Kitamura K, Shida D, Sekine S, et al. Comparison of model fit and discriminatory ability of the 8th edition of the tumor-node-metastasis classification and the 9th edition of the Japanese classification to identify stage III colorectal cancer. Int J Clin Oncol. 2021;26(9):1671-1678. Doi: 10.1007/s10147-021-01955-3.
97. Shida D, Boku N, Nakamura Y, et al. Comparison of model fit and discriminatory ability of M category as defined by the 7th and 8th editions of the tumor-node-metastasis classification of colorectal cancer and the 9th edition of the Japanese classification. Cancer Med. 2021;10(20):6937-6946. Doi: 10.1002/cam4.3972.

Aknowledgements

I would like to thank my tutor Luisa Savoldi for her support not only in the development of this project but also for sustaining me during the difficult times of this process.

I want to thank my mother Silvia Ruiz Viveros for being the wind beneath my wings, for being the person who always inspires me to do better, and who by example pushes me to grow as a person and professionally.

I would like to thank my Italian family and friends who have supported me over the years, during this journey. Thank you so much for your kind words and constant presence.

Last but not least, I want to thank my fellow PhD students, who have always supported me. I want to thank them for creating a safety net where we could rely on each other.

APPENDIX A

Colorectal cancer TNM staging AJCC UICC 8th edition

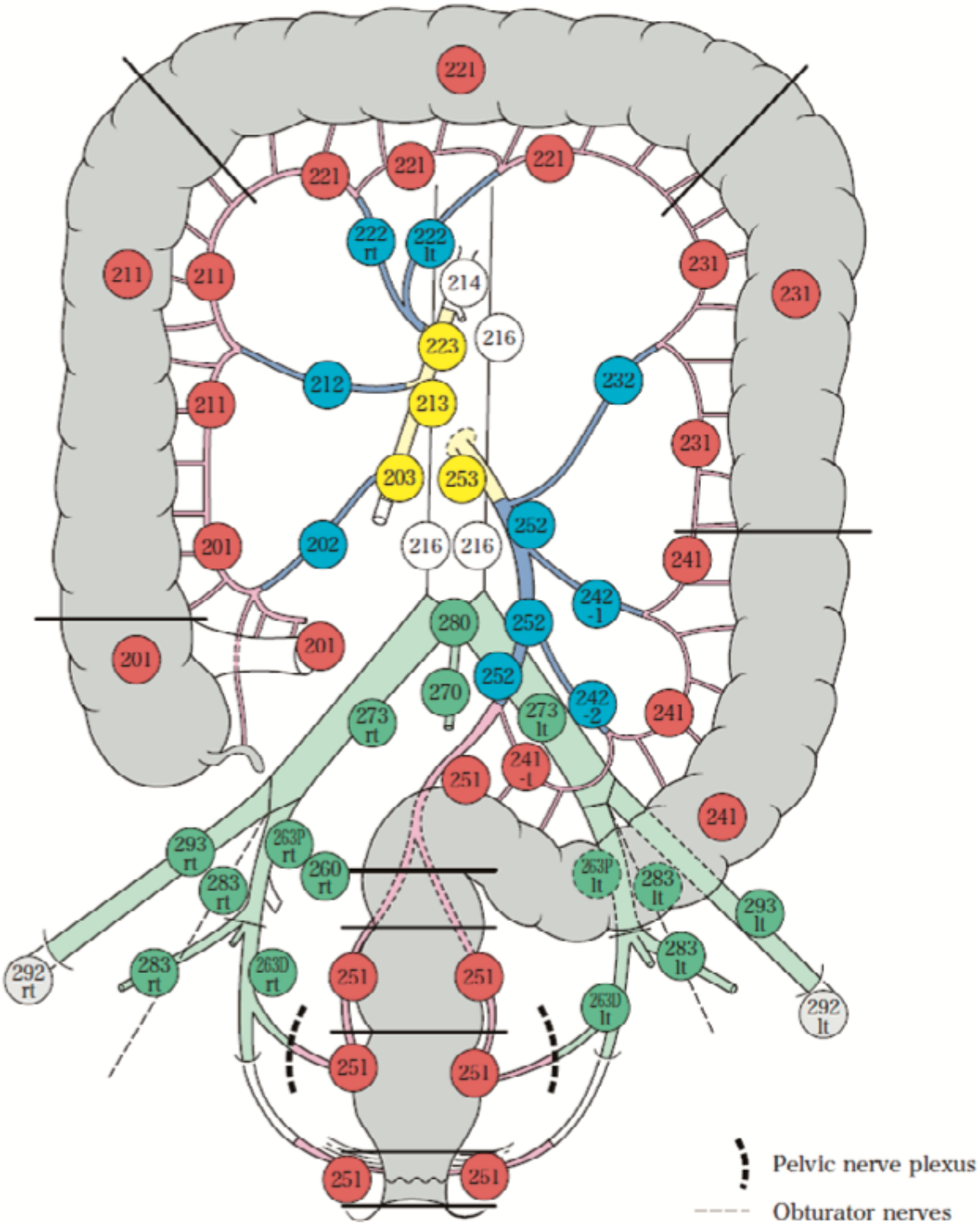
Primary tumor (T)	
T category	**T criteria**
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ*, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
T1	Tumor invades the submucosa (through the muscularis mucosa but not into the muscularis propria)
T2	Tumor invades the muscularis propria
T3	Tumor invades through the muscularis propria into pericolorectal tissues
T4	Tumor invades* the visceral peritoneum or invades or adheres¶ to adjacent organ or structure
T4a	Tumor invades* through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
T4b	Tumor directly invades* or adheres¶ to adjacent organs or structures
* Direct invasion in T4 includes invasion of other organs or other segments of the colorectum as a result of direct extension through the serosa, as confirmed on microscopic examination (for example, invasion of the sigmoid colon by a carcinoma of the cecum) or, for cancers in a retroperitoneal or subperitoneal location, direct invasion of other organs or structures by virtue of extension beyond the muscularis propria (ie, respectively, a tumor on the posterior wall of the descending colon invading the left kidney or lateral abdominal wall; or a mid or distal rectal cancer with invasion of prostate, seminal vesicles, cervix, or vagina). ¶ Tumor that is adherent to other organs or structures, grossly, is classified cT4b. However, if no tumor is present in the adhesion, microscopically, the classification should be pT1-4a depending on the anatomical depth of wall invasion. The V and L classification should be used to identify the presence or absence of vascular or lymphatic invasion whereas the PN prognostic factor should be used for perineural invasion.	
Regional lymph nodes (N)	
N category	**N criteria**
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	One to three regional lymph nodes are positive (tumor in lymph nodes measuring ≥0.2 mm), or any number of tumor deposits are present and all identifiable lymph nodes are negative
N1a	One regional lymph node is positive
N1b	Two or three regional lymph nodes are positive
N1c	No regional lymph nodes are positive, but there are tumor deposits in the: ▪ Subserosa ▪ Mesentery ▪ Nonperitonealized pericolic, or perirectal/mesorectal tissues
N2	Four or more regional nodes are positive
N2a	Four to six regional lymph nodes are positive
N2b	Seven or more regional lymph nodes are positive
Distant metastasis (M)	
M category	**M criteria**
M0	No distant metastasis by imaging, etc; no evidence of tumor in distant sites or organs. (This category is not assigned by pathologists.)
M1	Metastasis to one or more distant sites or organs or peritoneal metastasis is identified
M1a	Metastasis to one site or organ is identified without peritoneal metastasis
M1b	Metastasis to two or more sites or organs is identified without peritoneal metastasis
M1c	Metastasis to the peritoneal surface is identified alone or with other site or organ metastases

Prognostic stage groups			
When T is...	**And N is...**	**And M is...**	**Then the stage group is...**
Tis	N0	M0	0
T1, T2	N0	M0	I
T3	N0	M0	IIA
T4a	N0	M0	IIB
T4b	N0	M0	IIC
T1-T2	N1/N1c	M0	IIIA
T1	N2a	M0	IIIA
T3-T4a	N1/N1c	M0	IIIB
T2-T3	N2a	M0	IIIB
T1-T2	N2b	M0	IIIB
T4a	N2a	M0	IIIC
T3-T4a	N2b	M0	IIIC
T4b	N1-N2	M0	IIIC
Any T	Any N	M1a	IVA
Any T	Any N	M1b	IVB
Any T	Any N	M1c	IVC

TNM: Tumor, Node, Metastasis; AJCC: American Joint Committee on Cancer; UICC: Union for International Cancer Control.

APPENDIX B

LYMPH NODE GROUPS AND STATION NUMBERS



Red: pericolic/perirectal lyphm nodes
Blue: intermediate lymph nodes
Yellow: main lymph nodes
Green: lateral lymph nodes
Gray: downward lymph nodes
White: lymph nodes proximal to the main lymph nodes

APPENDIX C

Lymph Node Groups and Station Numbers

Pericolic/ perirectal lymph nodes	Lymph nodes along the marginal arteries and near the bowel wall • Pericolic lymph nodes (201, 211, 221)	Lymph nodes along the marginal arter- ies, near the bowel wall, and along the terminal sigmoid artery • Pericolic lymph nodes (231, 241: 241- 1, 241-2, 241-t) Lymph nodes along the superior rectal artery • Perirectal lymph nodes (251)	Lymph nodes medial to the pelvic nerve plexus along the middle rectal artery. • Perirectal lymph nodes (251)
Intermediate lymph nodes	Lymph nodes along the ileocolic, right colic, and middle colic arteries. • Ileocolic nodes (202) • Right colic nodes (212) • Right middle colic nodes (222-rt) • Left middle colic nodes (222-lt)	Lymph nodes along the left colic and sigmoid arteries and the inferior mesen- teric artery between the origin of the left colic artery of the terminal sigmoid ar- tery • Left colic nodes (232) • Sigmoid colic nodes (242: 242-1, 242- 2) • Inferior mesenteric trunk nodes (252)	
Main lymph nodes	Lymph nodes at the origin of the ileoco- lic, right colic, and middle colic arteries • Ileocolic root nodes (203) • Right colic root nodes (213) • Middle colic root nodes (223)	Lymph nodes along the inferior mesen- teric artery from the origin of the inferior mesenteric artery to that of the left colic artery • Inferior mesenteric root nodes (253)	
Lateral lymph nodes			Lymph nodes along the internal iliac ar- teries and along the obturator vessels and nerves • Proximal internal iliac nodes (263P) • Distal internal iliac nodes (263D) • Obturator nodes (283) Lymph nodes along the common iliac external iliac, and median sacral arteries • Common iliac nodes (273) • External iliac nodes (293) • Lateral sacral nodes (260) • Median sacral nodes (270) • Aortic bifurcation nodes (280)
Downward lymph nodes			• Inguinal nodes (292)
Lymph nodes proximal to the main lymph nodes	Lymph nodes at the origin of the superi- or mesenteric artery and along the aorta • Superior mesenteric arterial root nodes (214) • Para-aortic nodes (216)	Lymph nodes along the aorta • Para-aortic nodes (216)	
Other lymph nodes	• Sub-pyloric nodes (206) • Gastroepiploic nodes (204) • Splenic hilar nodes (210)		

Note 1: The sigmoid artery commonly comprises the first, second, and terminal arteries, with pericolic lymph nodes recorded as 241-1, 242-2, and 241-t, respectively, and intermediate lymph nodes recorded as 242-1 and 242-2.

Note 2: Iliac arterial lymph nodes are recorded according to whether they are to the left or right (right = rt and left = lt); e.g., right distal internal iliac lymph nodes are recorded as rt263D.

Note 3: In anal cancer, 292 is treated as intermediate lymph nodes.

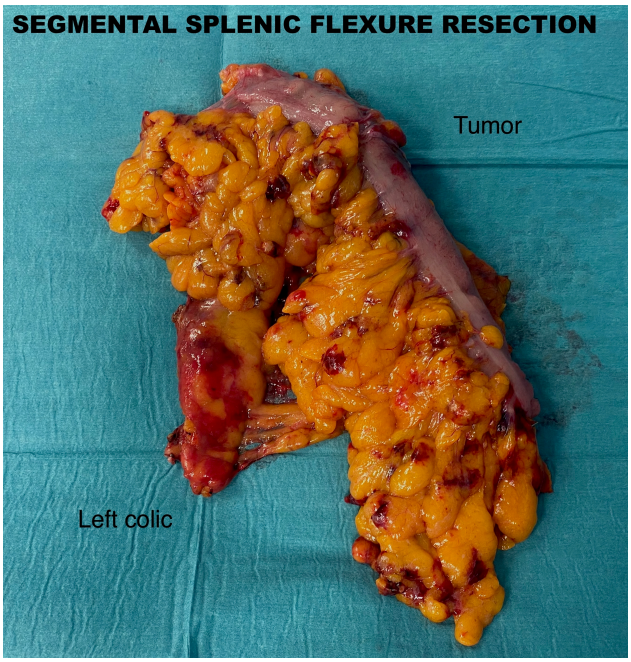
APPENDIX D

STAGE GROUPING OF THE JSCCR CLASSIFICATION

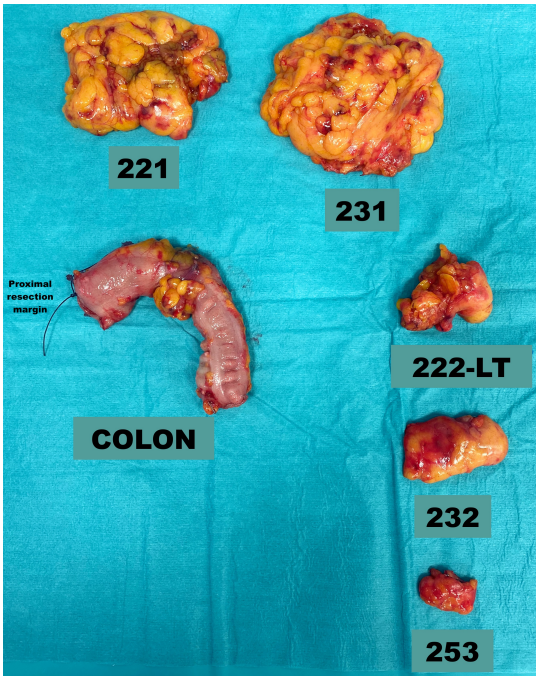
STAGE	T	N	M
Stage 0	Tis	N0	M0
Stage I	T1,T2	N0	M0
Stage II	T3,T4	N0	M0
Stage IIa	T3	N0	M0
Stage IIb	T4a	N0	M0
Stage IIc	T4b	N0	M0
Stage III	Any T	N0	M0
Stage IIIa	T1,T2 T1	N1 N2a	M0
Stage IIIb	T1,T2 T2,T3 T3,T4a	N2b, N3 N2a N1	M0 M0 M0
Stage IIIc	T3,T4a T4a T4b	N2b,N3 N2a N1,N2,N3	M0 M0 M0
Stage IV	Any T	Any N	M1
Stage IVa	Any T	Any N	M1a
Stage IVb	Any T	Any N	M1b
Stage IVc	Any T	Any N	M1c

APPENDIX E

Example of the postoperatory dissection of the specimen.



Specimen surgically removed



Specimen after dissection of each lymph node station