The Pathologist's Role in Rectal Cancer Patient Assessments

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ABSTRACT

Since Cuthbert Dukes fundamental work linking cancer stage to prognosis, the pathologist has had an ever-expanding role in the multidisciplinary management of rectal cancer. Gross dissection techniques and histologic evaluation are reviewed. The evolving tumor size, node status, metastasis classification (TNM) staging system is outlined. The pathologist's critical role in correctly assessing circumferential margins and establishing resection adequacy and staging accuracy via lymph node assessment is emphasized.

KEYWORDS: Rectal cancer, total mesorectal excision, tumor size, node status, metastasis (TNM) classification, pathological evaluation

Objectives: Upon completion of this article, the reader will have an understanding of staging systems, margin importance, lymph node harvest, and the pathologist's critical role in the multidisciplinary management of rectal cancer.

The history of evolving standards for the pathologic examination of rectal carcinoma specimens mirrors the advance in our understanding of colorectal carcinomas. Cuthbert Dukes initially perceived the fundamental principle of tumor staging as a predictor of patient outcome in the 1930s.¹ This groundbreaking concept linking the two variables of extent of invasion of the primary tumor and the presence of lymph node metastases now forms the basis for prognostication in most patients suffering from cancer.

The intensive study of cancer resection specimens has improved the ability of pathologists to assess patient outcome by standardization of histological tumor classification; acceptance of minimal standards in pathological dissections, and the investigation of tumor specific morphologic and molecular features.

Since Dukes' original description, multiple attempts have been made to improve on the prognostic value of staging systems for colorectal carcinomas.² The most widely accepted standard for reporting colorectal

carcinomas is the tumor size, node status, metastasis (TNM) classification scheme published in *TNM Classification of Malignant Tumours*, which is currently in its sixth edition.³ The TNM system is organized by the International Union against Cancer and since 1987 has been recognized by the American Joint Committee on Cancer. The TNM system is used for clinical as well as pathologic staging with appropriate nomenclature designations to facilitate this process. The term *TNM stage* in the context of the staging process means pTNM or pathologic stage as opposed to the clinical stage—the cTNM. Importantly, it must be recognized that the TNM reporting system is an evolving process whereby standards are changed to reflect advances in understanding of prognostic factors in tumor analyses.⁴ Thus, what classifies as a cancer of a particular stage in one edition may have a different stage designation in a subsequent edition.

For obvious reasons, many of the important aspects of reporting and management of patients with

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rectal carcinoma overlap with colon carcinoma. Nevertheless, significant differences occur because of the anatomic location of these cancers, which allows for unique forms of therapeutic intervention. Tumor location also is associated with specific challenges in diagnosis and management. In addition, rectal carcinoma stage for stage has a higher mortality rate than nonrectal colon carcinomas.

GROSS ASSESSMENT OF A RECTAL CANCER EXCISION

The rectal excision specimen on receipt to the pathology department should be unopened. This allows for proper inspection of the circumferential and distal margins in particular. An annotation in the pathology report as to whether the circumferential margin is intact should be made. Ideally, this margin should be a smooth uniform surface of glistening adipose tissue (Fig. 1). The circumferential margin should be inked before opening. (I prefer to use India ink and Bouin's fixative as a mordant.)

Figure 1 Smooth outer surface of a total mesorectal excision.

It is also important not to compromise assessment of the distal margin. If a scissor cut is made through the distal margin, especially if done prior to inking the outer surface of the specimen, it may not be possible to subsequently establish if tumor on a "cut edge" represents a positive margin. Cutting a 1–2 cm "donut" of approximately three quarters of the circumference of the distal rectal margin initially will help to avoid this dilemma (Fig. 2). The rectum is then opened from distal to proximal avoiding the tumor if possible. The distance of the distal extent of the tumor from the distal margin should be documented at this time. This should be performed as quickly as possible after excision to prevent errors associated with tissue retraction.⁵ If the tumor is near or at the distal margin, histological sections taken perpendicular to this margin are often helpful in making the final interpretation of adequacy of excision.

Some variation after this step will occur depending on patient-specific and institutional-specific characteristics. Involvement of adjacent structures may be a concern during the surgical procedure. A frozen section of a specific margin may be indicated to assess whether further surgery (or intraoperative radiation therapy—if available) is indicated. Frozen sections on fatty tissue are, however, sometimes technically challenging.⁶

Subsequent handling of the specimen is performed to allow for assessment of prognostic information needed to guide further patient care. Inevitably, variations in the handling of these specimens occur between institutions. The specimens should be processed only after a period of fixation in 10% neutral buffered formalin.^{7,8} The specimen is opened longitudinally and the tumor bisected. Standard permeable gauze is placed in the center of the tumor to facilitate formalin penetration. A similar technique is used on the mesorectum and mesentery. This is important as formalin is an aqueous solution that permeates only slowly into fatty tissues. At our institution, all colorectal specimens are processed after overnight fixation. A careful dissection

Figure 2 Total mesorectal excision with ulcer crater at site of irradiated rectal cancer (short arrows), "donut section" at distal margin (long arrows).

for nodules that may be either lymph nodes or metastatic nodules in the mesorectum and mesentery is performed. This is the most technically challenging component of the gross dissecting process. Some institutions have had variable success with the use of an assortment of processes to help identify lymph nodes in colorectal cancer resection specimens.⁹⁻¹¹

Preferably all of the tumor should be submitted for histological review. Often this is impracticable; optimally, at least five sections should be submitted.¹² It is also of vital importance to take several sections of the circumferential margin at its closest approximation to the primary tumor. Histological verification of adequacy of the distal margin—especially in the era of preoperative chemoradiation therapy where gross assessment can be inadequate—should be performed. Variations in the dissection should take into account other findings—such as the presence of concomitant polyp(s), colitis, etc.

HISTOLOGICAL ANALYSIS OF RECTAL CARCINOMA RADICAL EXCISIONS

Review of histological sections of the primary tumor reveals information relating to four separate categories: tumor type (including grade), pT status, completeness of excision, and other prognostic factors.

The most common carcinoma of the rectum is a moderately differentiated adenocarcinoma. This is characterized by an adenocarcinoma with greater than 50% tubular formation. The tubules generally have an irregular growth pattern characterized by variable tubular size and shape, classically showing necrosis in the tumor lumens. Nuclei are significantly larger than those that are found in adjacent colonocytes with hyperchromasia and irregularity indicative of increased nuclear DNA content and disorganization (Fig. 3). Virtually all these carcinomas elicit a prominent desmoplastic response.

Figure 3 Moderately differentiated adenocarcinoma with characteristic luminal necrosis (H&E $\times 20$).

The most recent World Health Organization classification scheme for colorectal carcinoma is listed in Table 1. Tumor type classification has not been identified as an independent prognostic factor of patient outcome in most instances of colorectal carcinoma.¹³⁻¹⁵ The notable exceptions are signet ring carcinomas and small cell carcinomas. Signet ring carcinoma is defined as an adenocarcinoma with $> 50\%$ of tumor cells having prominent intracytoplasmic mucin accumulation.¹⁶ Commonly, these tumors present with a higher stage than non-signet ring carcinoma. Nonetheless, when matched stage for stage to non-signet ring carcinoma patients, these patients have a worse 5-year survival rate. The reasons for this are probably multifactorial, but peritoneal seeding and angiolymphatic invasion are more common in these tumors than non-signet ring adenocarcinomas.^{17,18} There is still an open debate as to whether mucinous carcinoma has a worse prognosis than standard-type adenocarcinoma. Mucinous carcinomas are defined as such if $> 50\%$ of the lesion is composed of mucin.¹⁶ The problem arises with the morphologic overlap of this tumor with signet ring carcinomas. It is likely that the associated poor prognosis of mucinous carcinoma is related, in many instances, to its subcomponent of signet ring cells.¹³ Small cell carcinoma of the rectum, like the remaining colon, is a rare entity. The world's documented experience is mostly related to individual case reports. Overall, the 5-year survival rate of these patients is unfortunately dismal—even when presenting with early-stage lesions.¹⁹⁻²¹ If a concomitant adenoma or adenocarcinoma is also present at the same site in the gastrointestinal tract, the diagnosis of a primary carcinoma can be made with relative certainty.

Multiple studies have identified tumor grade as an independent factor in determining patient outcome in colorectal carcinoma.^{14,22-25} The consensus of these studies is that the presence of a high-grade carcinoma is a factor predicting an adverse outcome. There is significant interobserver variability in attempts to subclassify “well” versus “moderately differentiated” adenocarcinomas. This is similar to pathological classification schemes involving neoplasia generally²⁶ and relates to differences in criteria used for classification, as well as common intratumoral variations.^{13,27} The College of American Pathologists, in an effort to help standardize

Table 1 World Health Organization Classification of Carcinoma

Adenocarcinoma
Mucinous adenocarcinoma
Signet ring adenocarcinoma
Small cell carcinoma
Adenosquamous carcinoma
Medullary carcinoma
Undifferentiated carcinoma

reporting of colorectal carcinomas, has recommended the use of a two-tier classification scheme:

Low Grade—Well-differentiated and moderately differentiated carcinomas

High Grade—Poorly differentiated and undifferentiated carcinomas

In this scheme, the proportion of gland formation by the tumor, i.e., greater or less than 50% is the sole discriminator of categorization.⁸

The extent to which colorectal carcinoma extends through the bowel wall is a useful measure of subsequent patient outcome. This is in marked contrast to the lack of utility of the size of the tumor or its gross configuration.^{8,28-31}

The current pT classification for colorectal carcinomas is summarized as follows:

TX—Primary tumor cannot be assessed.

T0—No evidence of primary tumor.

Tis—Carcinoma in situ, intraepithelial or invasion of the lamina propria or muscularis mucosa.

T1—Tumor invades the submucosa.

T2—Tumor invades the muscularis propria.

T3—Tumor invades through the muscularis propria into the subserosa or into the nonperitonealized pericolic or perirectal tissues. (This is then divided into subsections based on distance of tumor extent from the lower border of the muscularis propria.)

T4—Tumor directly invades other organs or structure (T4a) or perforates the visceral peritoneum (T4b).

There are several concepts that when expanded upon make this table easier to incorporate into everyday practice.

First, the term *Tis* is confusing to many. This is because in other sites of neoplasia the term *carcinoma in situ* refers to neoplastic proliferations accompanied by marked nuclear irregularities that do not invade into its adjacent stroma—conventionally speaking, the tumors are confined to their basement membranes. However, the terms of *Tis* and carcinoma in situ when used in the context of TNM staging of colorectal carcinomas are used to describe a different concept. This concept is based on the knowledge that unless a tumor invades into the submucosa—it is a pT1 carcinoma; there is no risk of lymph node metastasis. These are then not considered “true cancers” from a patient outcomes’ point of view. This reality then leads to a nomenclature issue that the TNM system tries to address. Thus, *Tis* describes neoplastic proliferations confined to the mucosa of the colon and rectum that have marked cytological features and that may or may not extend into the stroma of the mucosa (the lamina propria and muscularis mucosa). To promote a better reporting mechanism, subdivision of

these lesions into *high-grade dysplasia/intraepithelial carcinoma* (if no stromal invasion is identified) or *intramucosal carcinoma* (if stromal invasion is identified) has been recommended. (Despite this subclassification, there is no evidence to date that therapy or follow-up should differ between each type of *Tis* because as long as complete removal is achieved the risk associated with the specific neoplasm will have been alleviated. Subsequent patient management will then center on screening for and removal of new intestinal neoplasms.)

Direct extension of a carcinoma into an adjacent organ is regarded as a T4a lesion, for example, into the prostate. However, intramural spread of a carcinoma from one gastrointestinal organ to another, for example, to the anal canal, does not change the pT classification. T4b classification is limited to carcinomas that extend to the visceral peritoneal surface. There is a demonstrated marked worsening of outcome between in colorectal carcinoma patients with pT4b versus pT4a lesions.^{32,33}

Multiple studies have investigated a wide variety of histological parameters seen on colorectal cancer resection specimens to improve patient prognostication. These efforts have vastly improved our overall understanding of tumor classification, given insights into how tumors progress, and how host factors interact and modify tumor progression.

The most important histological finding after tumor type and grade is the presence of blood vessel invasion. The most significant form of blood vessel invasion identified in resection specimens is extramural large vessel invasion. This pertains to involvement of large blood vessels, virtually always large veins, in the perirectal soft tissues outside of the muscularis propria. This finding is significantly associated with a predisposition to development of hepatic metastases.^{15,34,35} The significance of blood vessel invasion outside of the above context is best understood in carcinomas with angiolymphatic invasion arising in polyps diagnosed at polypectomy. Most authors³⁶⁻³⁹ (although not all authors⁴⁰) agree that this feature identifies patients at increased risk of lymph node metastasis. The presence or absence of angiolymphatic invasion should be documented in every case. The finding of blood vessel invasion is best qualified as to anatomic site and type of blood vessel involved. Distortion of connective tissue during routine processing can uncommonly make definitive diagnosis of this feature difficult.

The presence of perineural invasion is associated with a worse outcome for patients with rectal carcinomas.^{38,40} agree that this feature identifies patients at increased risk of lymph node metastasis. The presence or absence of angiolymphatic invasion should be documented in every case. The finding of blood vessel invasion is best qualified as to anatomic site and type of blood vessel involved.

The presence of perineural invasion is associated with a worse outcome for patients with rectal carcinomas.^{38,41,42} The presence or absence of this finding should be reported on every resection specimen.

A significant lymphocyte predominant immune response is associated with a more favorable patient outcome in rectal cancer patients.^{14,15,43} The morphologic features of this response can be divided into two broad categories: increased tumor-infiltrating lymphocytes and an extratumoral lymphocytosis. The prognostic significance of tumor-infiltrating lymphocytes is related, in part, to its association with microsatellite instability.^{44,45} Extratumoral lymphocytosis has two broad patterns—infiltration in the stroma in the region of the tumor and prominent lymphoid nodules often in the region of the tumor edge or at the depth of adjacent muscularis propria, analogous to that seen in Crohn's disease.^{14,15}

Other features such as tumor growth pattern and tumor budding at the leading edge of the tumor,^{14,27,46} and prominence of desmoplasia¹⁵ are not reported routinely.

CIRCUMFERENTIAL RESECTION MARGIN

The surgical community has understood the problem of recurrence of rectal carcinomas in the pelvis after radical excision for decades.^{1,47-49} Approximately 20 years ago, Quirke et al⁵⁰ intensively sampled 52 rectal cancer resections and found 14 of these had positive circumferential resection margins [CRM]; 12 of these 14 patients developed local pelvic recurrences.

The introduction of radiation therapy—either in the preoperative or postoperative setting—as well as the introduction of the total mesorectal excision (TME) have significantly lowered the incidence of pelvic recurrences in these patients. The decrease in rates of pelvic recurrence after the introduction of TME has been dramatic, from 30 to 40% to 5 to 15%.⁵¹ A cumulative benefit with combined preoperative chemoradiation therapy and TME has also been documented.⁵²

The exact definition and significance of a positive CRM in the current era is open to some debate. A recent report from a large multicenter trial from the Netherlands reviewed TME resection specimens from 656 patients treated in one arm of a study without preoperative radiation therapy.⁵³ They demonstrated that positive CRM, as defined by carcinoma ≤ 2 mm from the inked surface, is associated with a local recurrence risk of 16% compared with 6% in patients with greater distance between tumor and inked CRM. As would be expected patients with carcinoma ≤ 1 mm had a significantly increased risk of pelvic recurrence—38%.

Clearly, the status of the CRM is of vital importance to planning further management of patients

after resection for rectal carcinoma. For this assessment, the closest tumor can be either the primary lesion or a metastatic focus.¹³ Unfortunately, in what is probably an insight into current practice, a study of three rectal cancer protocols revealed that this margin was evaluated in only 21% of cases.⁵⁴ The status of this margin should be explicitly stated in all radical rectal cancer excisions and not incorporated in the catch-all phrase “all margins.”

LYMPH NODE STATUS

The importance of lymph node status in the management of cancer patients has been long established. As with any organ staging system the lymph nodes referred to are the regional lymph nodes. The regional lymph nodes for the rectum are the perirectal, sigmoid mesenteric, inferior mesenteric, lateral sacral, presacral, internal iliac, sacral promontory, superior rectal, middle rectal, and inferior rectal. Lymph node metastasis outside of these sites, for example, other portions of the large bowel mesentery and external iliac or common iliac nodes are considered distant metastases.^{32,55}

The current pN classification for colorectal carcinomas is summarized as follows:

NX—Regional lymph nodes cannot be assessed.

N0—No regional lymph node metastasis.

N1—Metastasis in one to three lymph nodes.

N2—Metastasis in four or more lymph nodes.

Although lymph node status is the main prognostic marker for long-term survival in patients without distant metastases, it is only relatively recently that standardization of lymph node reporting has been addressed. Although rectal cancers have unique properties compared with nonrectal colon cancers, the literature concerning lymph node status assessments, for the most part, group these together. The main focus of these reports is to quantify the number of lymph nodes that should be harvested from these specimens.⁵⁶ The underlying concern is understaging patients who thus may lose an opportunity for postoperative therapies from which they may derive benefit.^{57,58} This is based on the finding that colorectal cancer patients classified as having stage 2 disease have a prognosis inversely related to the number of lymph nodes harvested; the fewer lymph nodes harvested the worse the prognosis.^{59,60}

The appropriate number of lymph nodes needed to adequately predict patient outcome is open to some debate. Various authors have suggested numbers ranging from 6 to 20 lymph nodes per colorectal resection specimen.⁶⁰⁻⁶⁵ The higher number relates to a study which suggested that 20 lymph nodes are needed in pT1 resection specimens to identify nodal involvement with a 95% confidence interval.⁶¹

The International Union Against Cancer and the American Joint Committee on Cancer have set guidelines to standardize the minimal optimal number of negative lymph nodes needed for adequate staging at 12.^{66,67} This recommendation has been seconded by a National Cancer Institute-sponsored panel of experts for the assessment of rectal cancer patients.⁶⁸ Unfortunately, as of 2001, the majority of colorectal cancer resections in the United States failed to meet this standard.⁶⁹ Several variables interplay to affect the final lymph node count including patient factors, surgical factors, and pathology-related factors.⁵⁶ Nonetheless, there appears to be a growing awareness of this problem with increasing numbers of lymph nodes being harvested from colorectal specimens over time.^{65,69}

The current policy at our institution is to identify at least 15 lymph nodes per colorectal cancer resection. If the lymph node count at the initial dissection was deemed inadequate for this purpose a further dissection is performed and documented. We use an internal benchmark of at least one lymph node measuring 2 mm in size as an indirect assessment of the adequacy of the dissection. Because the majority of lymph node metastases occur in lymph nodes < 5 mm in size,⁷⁰ a dissection containing 15 large lymph nodes is probably insufficient for accurate staging purposes. Neoadjuvant therapy for rectal cancers can reduce the number of lymph nodes harvested in a specimen.^{71,72}

The vexing problem of how to interpret isolated extranodal tumor nodules in the supporting extramural mesenchymal tissues (commonly referred to as mesenteric deposits) has been addressed in the last two versions of the *TNM Classification of Malignant Tumours*. In the 1997 fifth edition,⁶⁶ any histological confirmed focus of tumor that measured 3 mm or less was classified as a discontinuous extension of the main tumor—a pT3 lesion. If the nodule was > 3 mm this was characterized as equivalent to a lymph node metastasis and staged as a pN1. Contrastingly, the sixth edition of the *TNM Classification of Malignant Tumours*³ classifies all mesenteric nodules that have smooth contours as nodal metastases regardless of size. Irregular nodules in the pericolonic or perirectal fat are considered variants of vascular invasion and do not warrant a nodal metastasis designation.

Small nodal metastases, those measuring < 2 mm but > 0.2 mm identified by routine H&E sectioning are classified as micrometastases, which carry the same significance as nodal metastases. These carry a pN1(mi) [or pN2(mi) depending on the number found] designation. Isolated tumor cells, single cells, or small clusters measuring ≤ 0.2 mm should be reported, although currently they carry the designation of pN0.

Several specialized techniques such as immunohistochemistry for tumor antigens or PCR-based assays for tumor RNA and DNA are currently available, which highlight the presence of micrometastases. However, the significance of positive results obtained with these methods must be interpreted with significant caution. These assays may upstage up to 50% of pN0 patients.⁷³ Conflicting data have been presented in the literature as regards the utility of these assays.¹³ The clear worry is to potentially over assess the risk of recurrent disease leading to overtreatment in patients who are cured by standard surgical protocols. Data are currently insufficient to recommend routine implementation of these methods.⁸

PREOPERATIVE CHEMORADIATION THERAPY

Preoperative chemoradiation therapy (CRT) for locally advanced rectal cancer is a widely accepted clinical practice.⁷⁴ This modality has been reported to reduce local recurrence rates and has been associated with improved survival.^{52,75,76}

Pathological assessment of these specimens must take into account some unique features. As with patients treated by resection alone, the assessment of the primary tumor is important in predicting local recurrence. After CRT, the findings are classified using the nomenclature *yTNM*.

Most tumors regress to some degree with CRT, although the spectrum of change can be quite varied.⁷⁷ Standard regimes are associated with tumor downstaging in between 28 to 86% of treated patients.^{78–80} Radiation dose and time elapsed from radiation to surgery are also important.^{78,81} The pathological response to CRT has been studied with a view to identifying independent predictors of patient outcome.^{82–85} Greater than 95% tumor regression (Fig. 4) is associated with a better patient outcome.^{77,83} It is still unclear however, whether tumor regression is independent of

Figure 4 Pathological regression. Small amounts of residual carcinoma (arrows and inset) in a background of extensive fibrosis (H&E × 4, Inset × 20).

the yT status.⁸⁶ Patients with complete regression (which ranges from 9 to 30%) demonstrate significant survival advantage in some,⁸⁷ although not all series.⁸⁵

THE FUTURE

Similar to most areas of medicine, technological and biomedical advances continue to improve our care of cancer patients. Multiple studies endeavoring to find ways to predict patient response to CRT have been performed. Although no assay is currently available to help triage these patients, hopefully this situation will dramatically improve in a relatively short period. Advances in information technologies such as synoptic reporting^{88,89} will allow for better and more timely communication between clinicians and pathologists to improve patient care.

As important as these technological advances are to the treatment of future rectal cancer patients, understanding and implementing current standards of pathology significantly improves our ability to understand and manage this common cancer today.

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