A Review of Current Challenges in Colorectal Cancer Reporting

Heather Dawson, MD; Richard Kirsch, MBChB, PhD; David Messenger, MBChB; David Driman, MBChB

• Context.—Pathologic assessment of colorectal cancer resection specimens plays an important role in postsurgical management and prognostication in patients with colorectal cancer. Challenges exist in the evaluation and reporting of these specimens, either because of difficulties in applying existing guidelines or related to newer concepts.

Objective.—To address challenging areas in colorectal cancer pathology and to provide an overview of the literature, current guidelines, and expert recommendations for the handling of colorectal cancer resection specimens in everyday practice.

Data Sources.—PubMed (US National Library of Medicine, Bethesda, Maryland) literature review; reporting protocols of the College of American Pathologists, the Royal College of Pathologists of the United Kingdom, and classification manuals of the American Joint Committee on Cancer and the Union for International Cancer Control.

Conclusions.—This review has addressed issues and challenges affecting quality of colorectal cancer pathology reporting. High-quality pathology reporting is essential for prognostication and management of patients with colorectal cancer.


Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. Although the modern approach to patient management takes place in a multidisciplinary setting, pathologists play a central role through the accurate detection and reporting of factors that determine prognosis and dictate the further course of treatment.

The tumor, node, metastasis (TNM) classification system, currently in its 8th edition, is the most widely used staging system in CRC, but is not always easily translated into daily practice. In our experience, certain issues seem to pose recurring challenges and some updates within the past decade have introduced controversial changes that have not always been adopted in current practice. In addition, a recent survey of US and Canadian pathologists has highlighted significant practice variations in reporting of CRC resection specimens.

Current classification systems do not always account for the varying biological behavior of tumors of the same stage. For instance, patients with stage II tumors may or may not receive adjuvant chemotherapy, depending on the presence or absence of certain prognostic factors. With the expansion of screening colonoscopy programs, the proportion of patients diagnosed with early CRC may increase. Therefore, additional reporting categories that better stratify prognosis in these patients are important. This review addresses new concepts and challenges in the pathologic assessment and reporting of CRC.

GROSS ASSESSMENT OF TOTAL MESORECTAL EXCISION SPECIMENS

For patients with rectal cancer, total mesorectal excision (TME) is regarded as the standard of surgical care. Since the popularization of this surgical technique in the 1980s, local recurrence rates for rectal cancer have dropped from as high as 30% to 40% using conventional surgical techniques to 5% to 15%, and 5% or less when high-quality TME surgery is combined with neoadjuvant therapy. The TME technique involves removal of the rectum and the complete perirectal soft tissue envelope (the mesorectum) using sharp dissection, under direct vision, in the plane between the visceral and parietal pelvic fascia. Proper assessment of the TME specimen involves paying close attention to 2 factors in particular: the (surgical) quality of the mesorectal excision and the status of the radial margin (as defined below). In the Dutch TME trial, an incomplete mesorectal excision was associated with a higher risk of local recurrence when compared with complete mesorectal excision (28.6% versus 14.9%) in patients with a negative circumferential resection margin (CRM). It is important for the pathologist to assess the quality of the TME specimen prior to dissection of the specimen (Figures 1, A through D, and 2). The following parameters are assessed: (1) bulk of the mesorectum, (2) measurement of defects if present, (3) regularity and...
smoothness of the CRM, and (4) presence of coning (Table 1). Coning refers to the tendency of the surgeon to cut toward the muscularis propria during distal dissection, rather than staying within the mesorectal plane, and is an indicator of suboptimal surgical quality.12

Although TME assessment is a nonmandatory data element in the protocol of the College of American Pathologists (CAP), the latest edition of the Royal College of Pathologists of the United Kingdom (RCPath[UK]) data set includes TME assessment as a mandatory element and additionally requires assessment of the plane of excision of the levator/sphincter area around the anal canal in abdomino-perineal resection specimen.13 The surgical technique used in traditional abdominoperineal resections yields specimens with a surgical “waist” (Figure 3), whereas with extralevator abdominoperineal resections, there is no waist and a greater volume of tissue surrounding the low rectal tumor, with decreased CRM involvement and local recurrence.

Involvement of the radial margin is the single most important factor in predicting local recurrence and is predictive of distant metastases (37% versus 15%) and shorter survival (2-year survival 90% versus 70%).13,14 Rectal tumors below the peritoneal reflection have a CRM, whereas rectal tumors above the peritoneal reflection have a noncircumferential radial margin with variable amounts of anterior and lateral aspects of the rectum covered by peritoneum.

By convention, the CRM is considered positive if the distance between any tumor cells and the CRM is 1 mm or less.13,15,16 A positive CRM can be due to direct tumor extension or tumor within lymph nodes, veins, or lymphatics or around nerves.13,16 However, not all positive margins are equivalent with respect to local recurrence risk. For example, Nagtegaal and Quirke12 found that a positive CRM due to an involved lymph node was associated with a lower risk of local recurrence than a positive CRM due to direct extension (12.4% versus 22.1%, respectively) and a risk no different from that of CRM-negative tumors. Therefore, the type of affected tissue leading to a positive CRM should be specified in the pathology report, and the significance may

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* Data derived from Parfitt and Driman14 and Smith et al.17
be discussed in a multidisciplinary setting. In the case of a positive CRM based solely on intranodal tumor contained by the lymph node capsule, we recommend a comment indicating that the risk of recurrence might not differ significantly from that of CRM-negative tumors.

In order to optimize assessment of the CRM, the Quirke technique (summarized in Table 2) was developed; it involves fixation of the unopened tumor-containing segment followed by cross-sectional slicing. This method preserves the anatomy of the specimen, facilitates correlation with radiology findings, and eases the evaluation of TME completeness in cross-sectional slices; it does require longer fixation times. Although the Quirke technique is the method of choice in many laboratories, accurate assessment of key parameters can also be achieved in traditionally opened specimens with careful examination and blocking. Regardless of the grossing technique used, the area of tumor closest to the CRM should be adequately sampled (at least 2 tumor blocks showing relationship to the radial margin).

**RADIAL MARGINS IN COLON CANCER**

The importance of radial margin involvement in colon cancer has not been as carefully studied compared with rectal cancer. In colon cancer resection specimens, the radial margin refers to the soft tissue margin created by surgical resection, and not to the serosal surface. The radial margin can be of 2 types, depending on anatomical location. The ascending and descending colon are covered by peritoneum on the anterior surface, leaving the posterior retroperitoneal aspect (bare area) as the radial margin. In the transverse and sigmoid colon, and often the cecum, the peritoneum usually completely envelopes these segments, leaving the vascular ties as the de facto radial margin. The 1-mm rule used to define CRM involvement in rectal cancers is also applied to radial margin involvement in colon cancers. Petersen et al found radial margin involvement using the 1-mm cutoff to be an independent predictor of adverse outcome in stage II colon cancer.

Analogous to the concept of TME in rectal cancer, complete mesocolic excision with central vascular ligation has been popularized for the treatment of colonic tumors in recent years, although a resultant reduction in radial margin involvement is yet to be demonstrated and grading the quality of mesocolic excision is not generally performed. Increasing evidence for the oncologic benefit of complete mesocolic excision and central vascular ligation may prompt its inclusion in future pathology reporting protocols.

**SEROSAL INVOLVEMENT IN CRC**

**Gross Assessment of Serosal Involvement**

The accurate identification of serosal involvement depends on meticulous gross assessment and adequate sampling. Grossly suspicious areas may show retraction or puckering, granularity, increased vascularity, or exude (Figure 4, A and B). Of note, serosal involvement occurs commonly in fat-lined crevices (serosal clefts) on the mesenteric aspect of the bowel or where the peritoneum is reflected at an acute angle from the bowel surface to the adjacent mesentery. Serosal involvement in these areas can be missed; therefore, careful inspection and sampling, particularly for tumors on the mesenteric aspect of the colon, is needed. Widening and discoloration of the clefts are useful clues to serosal involvement but are not invariably present. A minimum of 2 sections demonstrating the closest relationship of tumor to serosa is recommended. Inking of the serosa is not advised because this may obscure morphology and limit histologic assessment. It is also important to remember that serosal involvement can occur in upper rectal cancer, as the anterior portion of the proximal rectum is covered by peritoneum.

**Histologic Assessment of pT3 versus pT4 CRC**

Peritoneal involvement is a well-established adverse prognostic factor in stage II and stage III disease. Several studies suggest that it is underreported. For example, a review of 80 stage II colon cancers by Stewart et al
demonstrated serosal involvement in 20 cases (25%) that had originally been reported negative. Underreporting may be the result of several factors, including inadequate gross examination and sampling (see earlier section), practical difficulties associated with histologic assessment, and controversies surrounding diagnostic criteria. Although the assessment of serosal involvement is straightforward in most cases, it may be challenging in certain instances, for example, when (1) the mesothelial layer overlying tumor is disrupted or denuded during specimen handling, making it difficult to distinguish true serosal breach from artifact; (2) a prominent fibroinflammatory reaction to tumor obscures/effaces normal anatomic structures including peritoneum, making it difficult to confirm serosal breach; or (3) peritoneal involvement occurs in deep serosal clefts (as is frequently the case), which can be easily overlooked or difficult to recognize, particularly when distorted by tumor or fibroinflammatory reaction (Figure 5, A through D).

The handling of tumors that are close to the serosal surface (<1 mm) with a hyperplastic/fibroinflammatory serosal response has been the subject of debate and controversy. Shepherd et al originally considered this finding to be negative for serosal involvement, which was subsequently supported by studies from other groups. However, a study of 120 CRC resection specimens reported that 46% of pT3 cancers with tumor cells less than 1 mm from the serosal surface and involving tumor tissue reaction (ie, fibroinflammatory reaction, hemorrhage/fibrin deposition, and/or mesothelial reaction) had tumor cells in cytologic preparations from the serosa, comparable with the rate for pT4a tumors (55%). None of the pT3 tumors more than 1 mm from the serosal surface had positive cytologic smears. Peritoneal carcinomatosis occurred in 11% of pT3 tumors less than 1 mm from a serosal tissue reaction, compared with 18% for pT4a tumors and 3% for the remaining pT3 tumors. It was concluded from this study that tumors less than 1 mm from a serosal fibroinflammatory tissue reaction are likely associated with peritoneal involvement, and it was suggested that the pT4a category be expanded to include such tumors.

Given the potential clinical implications of detecting serosal involvement, it is helpful that the most recent update of the CAP CRC protocol has clarified this issue. The protocol states that the significance of tumors less than 1 mm from the serosal surface with a serosal or fibroinflammatory reaction is unclear (with some but not all studies indicating a higher risk of peritoneal recurrence) and suggests that multiple level sections and/or additional section(s) of tumor should be examined in such cases. If serosal involvement is not present after additional evaluation, the tumor should be assigned to the T3 category.

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**Table 2. Quirke Technique for Macroscopic Handling of Total Mesorectal Excision Specimens**

| Before fixation: | • Documentation of specimen quality (Table 1). Photo documentation is an additional useful tool, especially in cases with incomplete mesorectum |
|                | • Ink nonperitonealized bare areas (CRM) of the specimen |
|                | • Open specimen anteriorly, leaving the bowel intact about 2 cm above and below the tumor |
|                | • Place loose, formalin-soaked gauze into the unopened segment of the specimen |
|                | • Fix for at least 48 h, preferably 72–96 h, ideally pinned to prevent shrinkage |

| After fixation: | • Cut through unopened rectum at 3–5-mm intervals, maintaining their order and orientation, with photo documentation if appropriate, recording: |
|                | • Extent of tumor; measure the closest distance of tumor to the CRM |
|                | • Any grossly positive nodes; measure the distance of these to the CRM |
|                | • Closest distance of tumor to the CRM, including orientation (anterior, posterior or lateral) |

| Block submission: | • 3 blocks of tumor in relation to the closest CRM |
|                  | • 2 blocks of tumor in relation to the lumen |
|                  | • 2 blocks of tumor with serosa, where appropriate |
|                  | • All lymph nodes |
|                  | • Polyps, if seen |
|                  | • Proximal and distal resection margins (ensuring that distal resection margin includes both mucosa and mesorectum) |

| Abbreviation: CRM, circumferential resection margin. | a Data derived from Parfitt and Driman. |

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Figure 4. Macroscopic identification of serosal involvement. A, Fresh specimen where the serosa is slightly puckered and has a dull, granular appearance (arrowhead). B, Fixed specimen where there is serosal puckering deep within a cleft (arrowheads).
From an audit perspective, the RCPath(UK) recommends serosal involvement be detected in at least 20% of colon cancers (10% for rectal cancers); some centers in the UK report serosal involvement in more than 50% of colon cancers after meticulous examination.18 Although useful, these audit guidelines should change as CRC screening leads to the detection of lower-stage cancers.

**Elastic Lamina Invasion**

Given the challenges associated with assessment of serosal involvement, there has been recent interest in the potential of peritoneal elastic lamina involvement (ELI) to provide a more objective measure of advanced local spread, analogous to pleural elastic lamina invasion in the lung, where, if present, it is considered as pleural involvement.30,31 In the normal colon, the peritoneal elastic lamina runs mainly parallel to, and just beneath, the serosal surface, but may be retracted toward the leading edge of the tumor because of the associated fibroinflammatory response.32

Several studies29,31–35 have evaluated peritoneal elastic lamina invasion as a potential prognostic marker in CRC. In 5 of these studies,31–35 pT3 tumors breaching the peritoneal elastic lamina (ELI+) were associated with a significantly worse survival than pT3 tumors without ELI. In 2 studies,31,33 ELI+ pT3 tumors had a similar survival to pT4a tumors in stage II CRC. A sixth study36 found no significant differences in survival between ELI+ and ELI− pT3 tumors in stage II CRC. However, studies have varied with respect to the stains used, the number of sections stained, and the proportion of cases in which the elastic lamina could be identified. In 4 studies, the peritoneal elastic lamina could be identified in all34,35 or nearly all cases (98% and “almost all”33 with the mean number of elastin stains per case ranging from 1 to 4.6 (not stated in one study),31,33–35 whereas in 2 studies29,32 it was not evaluable in a substantial proportion of specimens (18% and 59%, with a mean of 1.5 and 1 elastin stains per case). Failure to detect the elastic lamina may also be the result of anatomic variations, extensive tumor infiltration, or an associated fibroinflammatory response.

In summary, a breach of the elastic lamina by tumor appears to indicate a worse outcome. However, the elastic lamina may not be identifiable in a substantial proportion of cases when only a single elastin stain is performed. Routine staining for ELI is probably not justified at this time, especially because peritoneal ELI is not included as a reportable element in most CRC protocols.

**LYMPH NODES**

**Lymph Node Yield**

Lymph node status is highly predictive of patient outcome and a major determinant of postoperative treatment, with patients who have node-positive cancers (stage III disease) generally being offered adjuvant chemotherapy.5 With regard to the minimum acceptable lymph node yield in CRC, most guidelines consider 12 lymph nodes to be the minimum standard.36–38 Nevertheless, it should be noted that for an individual case, there is no minimum acceptable number of lymph nodes, and the number of nodes found may vary owing to numerous causes. The 12-node standard

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**Figure 5.** Microscopic identification of serosal involvement on hematoxylin-eosin. A, Serosal clefts (arrow) are a frequent site of serosal infiltration and can extend deep into the pericolic tissue. B, Appreciating the cytologic atypia of the tumor cells (arrow) can help distinguish from the adjacent mesothelial cell layer (arrowhead). C, Areas with tumor (arrow) very close to, but not directly at, the surface eliciting a fibroinflammatory response (arrowhead) should be carefully examined to demonstrate serosal involvement either on deeper levels or in other areas (D, arrow) (original magnifications ×5 [A], ×20 [B and C], and ×15 [D]).
should only apply to a series of cases, that is, be used as a quality benchmark.

Lymph node metastases frequently involve small lymph nodes (<5 mm)\(^4\) and care should be taken to ensure these are submitted for histologic examination. The factors that influence the number of nodes detected in a resection specimen can be divided into 3 general categories:

1. Surgeon or specimen-related factors: The number of nodes removed is directly proportional to the length of the specimen.\(^4\)
2. Pathologist/prosector–related factors: The skill, diligence, and methods used by the prosector in retrieving lymph nodes from the specimen are among the most important factors influencing lymph node yield.
3. Patient-associated factors: Right-sided specimens, microsatellite-instable tumors, and female sex are factors associated with higher lymph node yield.\(^3,14,42\) Fewer lymph nodes tend to be found in the obese, the elderly,\(^43,44\) and patients who have received neoadjuvant therapy.\(^46,48\)

Large population-based studies\(^49-51\) have shown that the likelihood of finding positive lymph nodes tapers off and actually decreases when more than 17 nodes are evaluated. Calculations by Denham et al\(^51\) estimate that 12 to 15 negative lymph nodes have a negative predictive value of roughly 80% for lymph node metastasis in colon cancer, and Hernanz et al\(^52\) demonstrated an error probability of only 0.01 in staging if 10 lymph nodes are examined. In a study by Baxter et al,\(^49\) patients with pT3 tumors and 7 nodes identified as likely to be node positive as those with 30 nodes or more.

The relationship between low lymph node yield and decreased survival is well established.\(^53-55\) Initially, this phenomenon was attributed to understaging of tumors, based on the assumption that positive lymph nodes are missed in a proportion of stage II tumors when the yield is low. Thus, a subset of stage II tumors with low lymph node yields would represent “missed” stage III tumors and behave as such. These patients would also have been inappropriately denied adjuvant chemotherapy unless other high-risk features were present. However, more recent studies suggest that this phenomenon cannot be attributed to stage migration alone. For example, a systematic review\(^66\) described improved survival in patients with higher lymph node yields in both stage II (16 of 17 studies) and stage III disease (4 of 6 studies). Stage migration cannot explain the improved survival associated with higher lymph node yield in stage III disease. Furthermore, lymph node sampling past a certain point appears not to be correlated with a significant change in disease staging.\(^51,57-59\) Several mechanisms to explain these observations have been suggested. On the one hand, increased survival may be due to unmeasured indicators of higher quality patient care. On the other hand, and perhaps more plausibly, biological tumor-host interactions could lead to more favorable survival outcomes in patients with a larger number of nodes, and a higher lymph node yield may reflect a stronger host immune response.

The association between the number of examined nodes and survival in rectal cancers after neoadjuvant treatment is less clear. Although node positivity after preoperative therapy is a poor prognostic factor,\(^47,60-62\) several studies\(^47,61,62\) reported the number of nodes in rectal cancer in these patients not to be predictive of either overall or disease-free survival. However, results from more recent studies\(^63-65\) on large patient databases showed a negative impact on survival in rectal cancer patients with low lymph node counts after neoadjuvant treatment.

**Visual Enhancement Techniques**

Several fat-clearing and node-revealing (enhancing) techniques have been used to increase lymph node yield. These include fat-clearing solutions such as xylene or acetone (which are toxic and unpleasant to use), dyes such as methylene blue injection, or nonnoxious node-enhancing solutions such as alcohol or GEWF solution (glacial acetic acid, ethanol, water, and formalin). Most of these solutions are readily available, low in cost, and easily implemented.

Virtually all published single-center studies\(^56-79\) have demonstrated a significant increase in lymph node retrieval when node-enhancing or fat-clearing methods are used, but the impact on accurate staging is more difficult to assess. Although many studies\(^57-60,71,72,80\) have compared enhancing/fat-clearing methods and conventional techniques in different cohorts, only a few have studied the impact of enhancing techniques on staging following initial manual dissection on the same specimen (the before-and-after study design being more in line with recommended practice). In these studies, reported rates in upstaging of CRC from node negative (pN0) to node positive (pN1–2) have varied between 1 of 104 (0.96%) after the addition of GEWF\(^70,72\) and 8 of 30 (27%) after a similar lymph node–revealing solution.\(^76\) However, some of these studies are limited by type II error due to small sample size and the 2 studies\(^77,79\) that performed statistical analysis both yielded P values >.05.

In studies comparing node-enhancing/fat-clearing techniques with conventional manual dissection in different cohorts, the impact on accurate staging is more difficult to assess. It has been suggested that fat clearance may lead to increased detection of positive lymph nodes in resection specimens after neoadjuvant therapy.\(^80\) However, 2 studies\(^76,77\) were not able to detect significant upstaging in their study groups (GEWF; n = 423 and n = 51, respectively) versus control groups (n = 1050 and n = 59, respectively). Newell et al\(^80\) found lymph node metastases in 16 of 35 (41%) of their study group (GEWF) and 10 of 32 (31%) of their control group.

Therefore, although visual enhancement techniques can increase lymph node yield and ensure an adequate lymph node harvest, there is insufficient evidence to suggest that these measures have a significant impact on tumor staging when used initially or after conventional manual dissection.\(^81\)

**Isolated Tumor Cells and Micrometastases**

According to the 8th edition of the American Joint Committee on Cancer staging manual,\(^62\) all nodal deposits 0.2 mm or larger are regarded as positive nodes, whereas single tumor cells or small clusters of tumor cells less than 0.2 mm are recorded as isolated lymph node metastases (ITCs) and are considered node negative and staged as pN0(i+), irrespectively of the method of detection (ie, hematoxylin–eosin [H&E] or immunohistochemistry). The term *micrometastasis* is applied to nodal deposits 0.2 to 2 mm in greatest dimension, but these are staged as for larger deposits (ie, as node positive). The clinical significance of ITCs remains unclear.
Occult tumor cells are defined as micrometastases or ITCs that are not evident on H&E but not detected by other methods (mostly immunohistochemistry). The impact of occult tumor cells on survival has been difficult to evaluate, because many studies have used the term occult tumor cells interchangeably for ITCs and micrometastases or have encompassed both categories. In a meta-analysis of 30 studies, only 8 separated these 2 categories for outcome events. Pooled data from 5 of these 8 studies showed disease recurrence to be significantly increased in patients with micrometastases in comparison with those without (odds ratio, 5.63; 95% CI, 2.4–13.1) whereas the presence of only ITCs had no impact on recurrence. However, a more recent multicenter trial including almost 200 stage I and stage II tumors found significantly higher rates of recurrence in patients with pN0i+ detected by immunohistochemistry versus the pN0i− group. Further evidence is required to support the use of additional measures to detect occult tumor cells that are not evident on H&E.

Acellular Mucin in Lymph Nodes

After neoadjuvant treatment, tumor cells in lymph nodes and the rectal wall can be replaced by acellular mucin. This finding represents tumor regression, should not be used to alter T or N stage, and does not appear to affect patient outcome. Acellular mucin may also rarely be identified in regional lymph nodes draining CRC that have not been treated with adjuvant therapy. This raises the question as to whether this represents a lymph node metastasis or whether acellular mucin can reach the lymph node via efferent lymphatics. Although neither the CAP CRC protocol nor the American Joint Committee on Cancer section on CRC currently provides any recommendations as to how such nodes should be handled or classified, the Union for International Cancer Control states that acellular mucin pools in lymph nodes are considered positive for tumor if there has been no neoadjuvant therapy. Nonetheless, data from a survey of North American pathologists indicate that the vast majority (>90%) of pathologists would report such lesions as pN0 if examination of additional levels does not identify tumor. When encountering acellular mucin pools in lymph nodes, the use of step sections with or without immunohistochemistry to detect occult tumor cells is recommended, especially in otherwise pN0 specimens or in therapy-naive patients.

TUMOR DEPOSITS

Discontinuous tumor deposits usually represent completely replaced lymph nodes, foci of vascular invasion or less frequently, perineural invasion (PNI). Careful pathologic examination (eg, evaluating deeper levels and/or elastin stains) can often elucidate the nature of these deposits (see below).

In the current versions of the American Joint Committee on Cancer/TNM manuals, all deposits are now included in the N category. The TNM 7th edition created a new subcategory, pN1c, for tumors without lymph node metastases but with at least one tumor deposit present. pN1c as soon as one lymph node metastasis is present, the number of deposits should still be recorded. Therefore, from a patient management point of view, discriminating between involved lymph nodes and tumor deposits is not crucial because both are indications for adjuvant therapy. If a vessel wall is identifiable on H&E or other stains it should be classified as venous invasion (VI) or lymphatic invasion, and if neural structures are identified, the lesion should be classified as pN1.

The rationale for the pN1c category was originally to enable data collection and analysis of patient outcome to better understand the biological significance of tumor deposits. Indeed, their presence appears to indicate poorer survival, with diminishing significance in advanced tumors. The prognostic relevance of tumor deposits may be in part explained by the fact that a significant proportion of these have originated from foci of extramural VI (EMVI), a well-established prognostic indicator in node-positive and node-negative disease (see below).

After neoadjuvant therapy, discontinuous islands and isolated clusters of tumor cells are commonly found as a result of tumor regression. For this reason, some authors advise against reporting tumor deposits after neoadjuvant therapy. Moreover, the data regarding prognostic significance of tumor deposits in the postadjuvant setting are limited. A study by Song et al suggests the category ypN1c may not have prognostic relevance, as tumor deposits had no significant impact on disease-free or overall survival in patients with node-negative rectal cancer after neoadjuvant therapy. Gopal et al reported an association between tumor deposits in rectal tumors following neoadjuvant treatment and the presence of lymph node and distant metastases, although tumor deposits were not an independent predictor of survival. This study used a cutoff of more than 1 cm from the edge of the primary tumor to define tumor deposits.

Practical questions that remain unanswered include: What is the minimum distance from the main tumor required for the designation of a discontinuous tumor deposit? To what lengths should the pathologist go to attempt to classify the origin of a tumor deposit? What is the minimum size? Ueno et al demonstrated a stage-independent prognostic effect by using a cutoff of more than 2 mm from the main tumor body. However, some aspects of this study reflecting ongoing controversies have been challenged. Others suggest that tumor deposits are best abandoned entirely, because several studies suggest superior risk stratification can be achieved by simply counting all tumor deposits as positive lymph nodes or adding the number of tumor deposits to the number of lymph node metastases to derive a final pN stage. If tumor deposits are seen, efforts to determine their origin may be justified in certain instances, for example when a tumor deposit is suspicious for EMVI in a cancer that is otherwise stage II.

RISK STRATIFICATION OF STAGE II CRC

With the expansion of screening colonoscopy programs, the proportion of patients diagnosed with stage II disease (T3/T4, N0) is set to increase; at least 70% of screening-detected CRCs are currently considered to be node negative. Risk stratification is becoming increasingly important in this growing subset of stage II CRC, because these patients are not offered adjuvant chemotherapy unless “high-risk” features are present. Such high-risk features include poorly differentiated histology (excluding microsatellite-unstable cancers), lymphovascular invasion (considered by the US-based National Comprehensive Cancer Network to include lymphatic/vascular invasion and by the UK-based National Institute for Health and Care Excellence to include only EMVI), PNI, localized perforation.
Venous invasion is a well-established independent indicator of hematogenous spread and survival in CRC. Although its prognostic importance has been recognized since the late 1930s, its assessment remains one of the most poorly performed aspects of CRC reporting. Venous invasion was originally defined as “tumor present within an endothelial-lined space either surrounded by a rim of muscle or containing red blood cells,” but more recently, this definition has been expanded to include the demonstration of convincing elastin staining around rounded or elongated tumor profiles, usually adjacent to an artery.

Extramural VI (tumor in veins outside of the muscular wall of the colorectum), in particular, is a strong predictor of adverse outcome. There is accumulating evidence to suggest that intramural VI (tumor in veins in the submucosa or muscularis propria) is also prognostic, prompting its inclusion alongside EMVI as a mandatory data element in the RCPath(UK) CRC data set. In contrast to VI, the role of lymphatic invasion (also called small vessel invasion) as an independent prognostic indicator is uncertain at this point, and the RCPath(UK) still considers the evidence insufficient for the inclusion of lymphatic invasion as a mandatory data element.

In contrast to previous CAP protocols, where all forms of blood and lymphatic invasion were grouped under a single term, lymph-vascular invasion, the updated protocol (version 4.0.0.1 and later) includes separate categories for small vessel invasion (including lymphatics, capillaries, and postcapillary venules) and large vessel (venous) invasion, and for EMVI and intramural VI. Although recommended, the separate reporting of small and large vessel invasion as well as intramural VI and EMVI is not currently mandatory.

Accurate detection of VI can be challenging on H&E slides, particularly when the muscular wall of the vein is effaced/distorted beyond morphologic recognition or altered by the effects of neoadjuvant chemoradiation. In such circumstances, VI is frequently missed unless key morphologic clues are carefully sought (Table 3). These include the orphan-artery sign (a circumscribed tumor nodule adjacent to a muscularized artery without an obvious accompanying vein; Figure 6, A) and the protruding-tongue sign (a smooth bordered protrusion of tumor into pericolic fat). The finding of either of these morphologic clues should prompt the use of an elastin stain (Figure 6, B), which will resolve the vast majority of equivocal cases.

Elastin staining can enhance VI detection. Several studies have demonstrated the superiority of elastin staining compared with an H&E stain on serial sections. Most have reported a 2- to 3-fold increase in VI detection rates at the time of gross sampling, elastin stains are associated with only minor additional costs and no increase in turnaround time.

Table 3. Recommendations for Optimal Detection of Venous Invasion (VI)

| When submitting tumor blocks, areas of linear spiculation at the infiltrating edge of the tumor should be targeted for histologic examination. Close attention should be paid to morphologic clues for VI, including the orphan-artery and protruding-tongue signs. These findings should prompt elastin stains to confirm or refute the diagnosis. In addition, elastin stains should be performed on any block where findings are equivocal for VI. Individual departments should monitor VI detection rates as a quality indicator. Those with VI detection rates consistently below the Royal College of Pathologists of the United Kingdom minimum standard of 30% should consider adoption of routine elastin staining. When ordered at the time of gross sampling, elastin stains are associated with only minor additional costs and no increase in turnaround time. |
Reporting of tumor budding is recommended in pT1 and stage II colorectal cancer. The hot-spot method (in a field measuring 0.785 mm² at the invasive front) is recommended.

1. Select the slide with the highest amount of tumor budding at the invasive front.
2. Scan 10 fields at medium power (∗∗×10 objective) to identify the area of highest budding (hot spot) at the invasive front (note: for pT1 endoscopic resections there are usually <10 individual fields available; in such cases scan all fields).
3. Count the number of buds in the selected hot spot at ×20.
4. Divide the bud count by the normalization factor (Table 5) to adjust to 0.785 mm².

A three-tier system should be reported along with the budding count in order to facilitate risk stratification, for example:

- Bd1 (≥5 buds/hotspot, 0.785 mm²; low: 0–4 buds)
- Bd2 (∗× buds/hotspot, 0.785 mm²; intermediate: 5–9 buds)
- Bd3 (∗∗× buds/hotspot, 0.785 mm²; high: ≥10 buds)

Note: In pT1 colorectal cancer, Bd2 and Bd3 are associated with an increased risk of lymph node metastasis, whereas in stage II colorectal cancer, Bd3 is associated with an increased risk of recurrence and mortality.¹²

Abbreviation: Bd, budding.

¹ Data derived from Lugli et al.¹³⁷
² References 137, 141, 147, 153–159, 161, 167.
³ References 137, 142, 144–146, 148, 149, 163–166.

TUMOR BUDDING

Tumor budding in CRC is defined as the presence of single tumor cells or clusters of up to 4 tumor cells at the invasive tumor front.¹³⁷ High-grade tumor budding (as defined below) is a well-established, independent predictor of recurrence and mortality in CRC.¹³⁸–¹⁵¹ The potential for tumor budding to influence clinical decision making has been studied in 2 scenarios:¹⁵²

- Early-stage (pT1) CRC: Tumor budding is an independent predictor of lymph node metastasis in early-stage (pT1) CRC (malignant polyps and endoscopic resections).¹⁴¹,¹⁴⁷,¹⁵³–¹⁶¹ In the absence of tumor budding or other risk factors for nodal metastasis (e.g., lymphovascular invasion and a poorly differentiated component), the risk of lymph node metastasis is very low (<1%), so that polypectomy alone is considered appropriate treatment in most cases, provided local excision is complete. However, the presence of tumor budding (≥5 buds/×20 objective field), or any of the above-mentioned high-risk features, has been reported to increase the risk of lymph node metastasis to approximately 20% (and up to 35% if 2 or more high-risk features are present).¹⁴¹ Therefore, the finding of tumor budding might prompt consideration for surgical resection with lymphadenectomy, provided the surgical risk does not outweigh that of metastatic disease.

- Stage II CRC: High-grade tumor budding (≥10 or more buds/×20 objective field) identifies a subset of stage II CRC that behaves very aggressively,¹⁴²,¹⁴⁴–¹⁴⁶,¹⁶²–¹⁶⁵ with similar outcomes to stage III.¹⁴⁴,¹⁶⁶ As such, patients with stage II CRC with high-grade tumor budding might benefit from adjuvant chemotherapy, as might patients with stage II cancers with other high-risk features.

Despite the established prognostic importance of tumor budding, its inclusion as a mandatory element in most CRC cancer protocols has been held back by a lack of universally accepted criteria. However, recent international consensus on a standardized, evidence-based method for tumor budding assessment (the International Tumor Budding Consensus Conference 2016), which has been included as an optional element in the most recent version of the CAP CRC protocol, may pave the way for wider and more consistent reporting of tumor budding (Tables 4 and 5; Figure 7, A through D).

The assessment of tumor budding on H&E may occasionally present challenges. For example, reactive stromal cells or histiocytes may be difficult to distinguish from buds, marked inflammation can obscure tumor buds, and on occasion it can be difficult to know whether small clusters of cells represent true budding or mechanical fragmentation of...
a larger (typically inflamed) gland. In such cases, a cytokeratin immunostain can be performed to confirm the impression of tumor buds, although the tumor buds themselves should be counted on H&E.

Interobserver agreement for the assessment of tumor budding has ranged from moderate to substantial in most studies (κ values from 0.41 to 0.938). However, it has been suggested that immunohistochemistry might improve interobserver agreement. However, further studies demonstrating the superior reproducibility (and at least equivalent prognostic power) of immunohistochemistry-based methods are required before their use can be justified in routine practice.

**PERINEURAL INVASION**

Perineural invasion, defined as tumor cells within the perineural space or in direct contact with nerve fascicles, is an important mode of regional spread in CRC. There is wide variation in the reported prevalence of PNI in CRC (9%–42%), possibly reflecting differences in sampling, skill/diligence of histologic examination, and the characteristics of the cohorts studied. As with VI, PNI appears to be underreported, with one study showing detection rates increasing from 0.5% on initial review to 22% following expert review. Tumors with PNI frequently show a distinct pattern of intramural spread dotted along the Auerbach plexus zone (referred to as myenteric spread; Figure 8, A and B), which can be readily identified at low power. It has been suggested that this pattern of myenteric spread can be used as evidence of PNI (even when residual nerve fibers are not identified on H&E), because S100 staining highlights residual nerve fibers in almost all such cases.

Perineural invasion occurs more frequently in rectal cancers, especially in more advanced tumors. This is likely due to the presence of dense neural plexuses surrounding the rectum in the pelvis. Retroperitoneal segments of the colon (ascending and descending colon) also show a higher rate of PNI, likely because of the proximity of tumors to the densely innervated retroperitoneum.

In a recent meta-analysis, PNI was found to be an independent predictor of local recurrence and survival, with a similar impact on disease-free survival as for T stage, N stage, and vascular invasion. Currently included as a mandatory element in the CAP and a noncore data set.
item in the RCPPath(UK) guidelines, PNI is considered a high-risk-for-recurrence feature by the National Comprehensive Cancer Network, with potential implications for adjuvant chemotherapy.²⁶

Although data on interobserver agreement for PNI are limited, κ values range²¹⁶,²¹⁷,²¹³ from 0.50 to 0.94. Very high rates of interobserver agreement were reported in a small pilot study²¹⁷ comparing 2 consultant gastrointestinal pathologists from the same institution, but were much lower in a study²²⁶ involving a larger heterogeneous group of gastrointestinal and nongastrointestinal pathologists.

The role of immunohistochemistry in the detection of PNI remains unclear. In a pilot study by White et al.,²²⁶ which included only 44 rectal cancers, S100 immunohistochemistry led to an increase in PNI detection (31% to 60%) but there was no difference between S100-detected PNI and HE-detected PNI in ability to predict clinical outcome; however, the study was clearly underpowered to detect such differences.

CONCLUSIONS

This review has addressed common challenges, misconceptions, and new concepts in pathology assessment of CRC. Accurate and standardized reporting of CRC specimens is essential for conveying important prognostic information and guiding patient management. It is hoped that this review will help to improve the consistency and quality of CRC pathology reporting.

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