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TOPIC HIGHLIGHT

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Controversies in the pathological assessment of colorectal cancer

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Abstract

Pathologic assessment of colorectal cancer specimens plays an essential role in patient management, informing prognosis and contributing to therapeutic decision making. The tumor-node-metastasis (TNM) staging system is a key component of the colorectal cancer pathology report and provides important prognostic information. However there is significant variation in outcome of patients within the same tumor stage. Many other histological features such as tumor budding, vascular invasion, perineural invasion, tumor grade and rectal tumor regression grade that may be of prognostic value are not part of TNM staging. Assessment of extramural tumor deposits and peritoneal involvement contributes to TNM staging but there are some difficulties with the definition of both of these features. Controversies in colorectal cancer pathology reporting include the subjective nature of some of the elements assessed, poor reporting rates and reproducibility and the need for standardized examination protocols and reporting. Molecular pathology is becoming increasingly important in prognostication and prediction of response to targeted therapies but accurate morphology still has a key role to play in colorectal cancer pathology reporting.

Key words: Colorectal cancer; Staging; Prognosis; Histopathology; Tumor-node-metastasis

Core tip: Pathologic assessment plays a key role in management of colorectal cancer. Tumor-node-metastasis staging of colorectal cancer provides prognostic information but some morphological features not included in the staging system also have prognostic value. However some of these elements lack agreed definitions, are subjective and poorly reproducible. We discuss controversial areas of colorectal cancer histopathology reporting including tumor budding, tumor grade, tumor deposits, tumor regression grade, vascular invasion, perineural invasion and peritoneal involvement.

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INTRODUCTION

Pathologic assessment of the colorectal cancer (CRC) resection specimens plays a central role in patient management. The pathology report informs prognosis and contributes to decisions regarding adjuvant therapy. Currently, the primary method for assessing prognostic differences among patients is the tumor-node-metastasis (TNM) staging system, developed by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC)[1]. However there is sig-

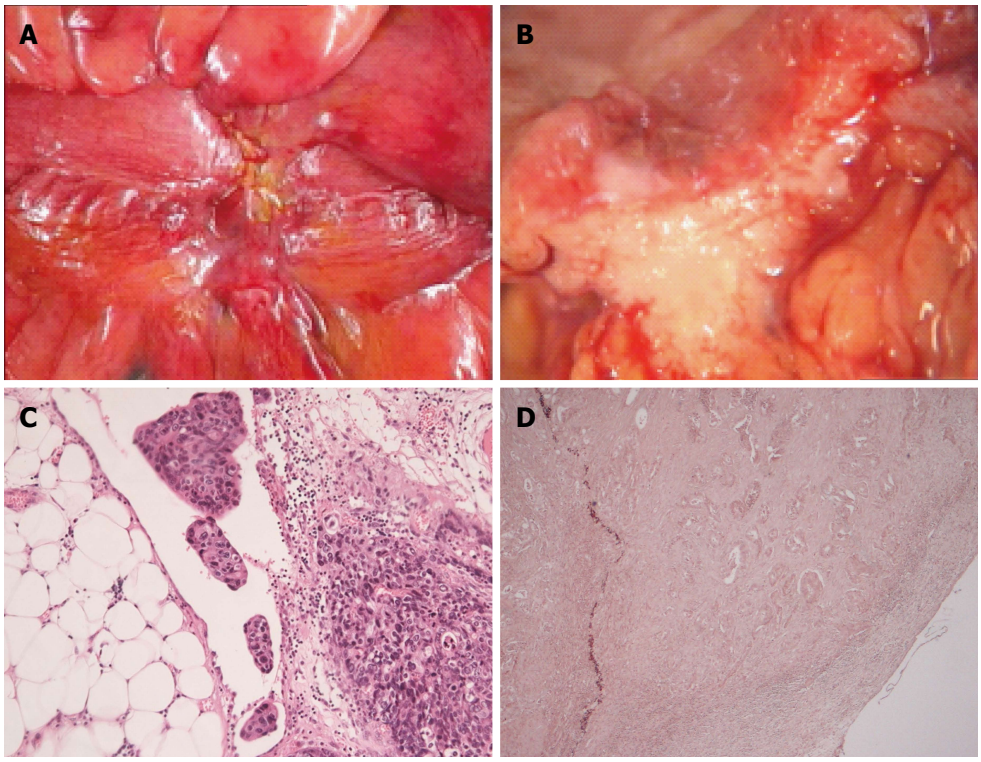

Figure 1 Macroscopic and microscopic features of peritoneal involvement. A: Peritoneal puckering; B: Area with peritoneal puckering correlates with the invasive edge of the tumor on sectioning; C: Adenocarcinoma in a peritoneal cleft in a pT4a case; D: Invasion through peritoneal elastic lamina highlighted with an elastic stain.

nificant variation in outcome of patients even within the same tumor stage[2]. Many promising prognostic and/or predictive molecular and immunohistochemical biomarkers are emerging but morphological parameters are still important predictors of patient outcome.

PERITONEAL INVOLVEMENT

Peritoneal involvement or serosal invasion is an important adverse prognostic factor in CRC associated with intraperitoneal recurrence and decreased overall survival[3-7]. Patients with stage Ⅱ CRC and peritoneal involvement (pT4a according to TNM 7[1]) and invasion into other structures/organs (pT4b according to TNM 7[1]) may be considered for adjuvant chemotherapy.

It should be noted that the classification of peritoneal involvement is different in TNM 5 and TNM 7. The Royal College of Pathologists (RCPath) in the United Kingdom still recommends use of the TNM5 staging system[8], while TNM 7 has been adopted in many other jurisdictions. In TNM 5 tumor directly invading other organs is staged as pT4a while tumor involving the visceral peritoneum is staged as pT4b[9].

The assessment of peritoneal involvement and the distinction of pT3 tumors from pT4a tumors can be particularly challenging for pathologists and there is a wide variation in the reported incidence of peritoneal involvement, ranging from 5% to 43% in studies of stage Ⅱ CRC[3,6,10-15]. A recent review by Stewart *et al*[16] highlighted the practical difficulties in diagnosis and interpretation of criteria for peritoneal involvement. Peritoneal involvement may be difficult to identify both at gross examination and microscopy and extensive sampling and sectioning of blocks may be required to detect it. The serosal surface of the CRC resection specimen should be carefully examined macroscopically. Block selection may be focused in areas where there is peritoneal abnormality such as puckering (Figure 1A and B), inflammation or fibrosis. Detection of peritoneal involvement may be enhanced with careful sampling of areas with peritoneal clefts (Figure 1C) and where the peritoneal lining reflects off the bowel wall on to the mesentery.

There is currently no universally accepted pathologic definition of peritoneal involvement and this contributes to the difficulty in making the diagnosis. The current AJCC Cancer Staging Manual and the RCPath Dataset for Colorectal Cancer regards tumor penetration of the peritoneum as either colonic perforation by tumor or histological detection of cancer cells on the serosal surface or free in the peritoneal space (Figure 1C)[8,17]. Shepherd *et al*[5,18] classified different histological patterns of local peritoneal involvement (LPI). LPI type 1 was defined as tumor well clear of the closest peritoneal surface; LPI type 2, mesothelial inflammatory reaction with tumor near but not at the peritoneal surface; LPI type 3, tumor at the peritoneal surface with inflammatory reaction, mesothelial hyperplasia, and/or ulceration; and LPI type 4 as tumor cells free in the peritoneum. LPI types 3 and 4 were associated with adverse patient outcomes whereas types 1 and 2 were not, and so only types 3

and 4 were considered to represent "true" peritoneal involvement. Many pathologists consider tumors associated with a pericolic abscesses that communicate with the peritoneum as pT4a even if malignant cells are not identified on the peritoneal surface[14,19,20]. Some authors draw attention to a group of "occult" pT4 CRCs that have already breached the serosal surface and are associated with a "cap" of fibro-inflammatory tissue, which may inadvertently be classified as negative for peritoneal involvement[21,22].

Ancillary techniques may aid in pathologic diagnosis of peritoneal involvement. Cytological examination of serosal scrapings has been explored as a means of detection of peritoneal involvement, revealing malignant cells in 19% to 26% of tumor specimens staged as pT3 by histological examination alone[20,23]. The peritoneal lining or serosa is composed of a mesothelial cell layer supported by a basement membrane with an underlying elastic lamina just beneath the subserosal layer. Immunohistochemical stains such as cytokeratin 7 highlight the mesothelial cells on the peritoneal surface and there are varied opinions as to their usefulness in the detection of peritoneal involvement[16,24]. Elastic stains have been used to aid in the diagnosis of pleural involvement by lung cancer and have recently been applied in CRC to improve visualization of the peritoneal elastic lamina (Figure 1D). Four recent studies investigated the use of elastic stains to detect peritoneal elastic lamina invasion (ELI). Three studies have found that ELI is associated with adverse prognosis in pT3 CRCs[6,24,25]. Conversely, ELI was not an adverse prognostic factor in pT3 CRC in the fourth study by Grin *et al*[15]. Despite the recognized limitations associated with this approach such as variability in the detection and continuity of the peritoneal elastic lamina, ELI has been shown to be a useful means of risk stratification in pT3 CRC in some studies. Some pathologists have proposed that subdivision of pT3 tumors based on presence or absence of ELI should be considered for inclusion in future staging systems[21,24].

TUMOR DEPOSITS (DISCONTINUOUS EXTRAMURAL EXTENSION)

Tumor deposits (TDs) are focal aggregates of adenocarcinoma located in the pericolic or perirectal fat discontinuous with the primary tumor. Studies investigating the clinical significance of TDs in CRC have found that their presence is associated with a poorer prognosis and lower survival rate[26-29]. Their origin has been shown to be heterogeneous. Studies using enhanced pathological assessment such as multiple step sections and histochemical stains have shown that some TDs represent venous invasion, lymphatic invasion, nerve sheath infiltration, and continuous growth[29,30]. A recent classification assigns different prognostic significance to different types of deposits[31]. TDs associated with large veins or nerves may represent "in transit" metastasis and are associated with a poor prognosis and distant metastasis while a second type is likely to represent tumor in lymphatic channels and is associated with nodal metastasis and a better prognosis.

Whether or not TDs should be considered lymph node metastases or satellite tumor nodules for the purposes of staging has been a topic of debate for many years, with changes in the approach to TD classification in the last three editions of the TNM staging system. In the TNM 5 classification, extramural deposits of tumor with no lymph node structure were regarded as lymph node deposits if they measured > 3 mm in diameter and were staged as pN1[9]. This rule was changed in TNM 6, when the contour of the deposit became the diagnostic feature. Deposits with a round contour were classified as lymph node metastases (pN1) and deposits with an irregular outline were classified as venous invasion[32]. There was criticism of the TNM 6 approach and the changes were not considered to be evidence-based or reproducible by some authors[26,33]. In the United Kingdom, the Royal College of Pathologists (RCPath) recommended that TNM 5 should be used for the staging of CRC resection specimens instead of TNM 6[8]. The TNM 7 classification proposed a new pN1c category for tumor deposits in the absence of lymph node metastases[1].

TUMOR GRADE

Tumor grade is another important variable shown to be a stage independent prognostic factor on multivariate analysis[34,35]. One drawback of CRC tumor grading is that it is largely subjective. The WHO grading system is the most widely used and defines the histological grade of CRC based on the percentage of gland formation[36]. Well differentiated tumors have > 95% glandular structures and are designated grade 1 (G1), moderately differentiated tumors with 50% to 95% gland formation are grade 2 (G2), poorly differentiated tumors with 5% to 50% gland formation are grade 3 (G3) and undifferentiated tumors with less than 5% gland formation are grade 4 (G4). The WHO also suggests dividing CRCs into low grade (G1 and G2) and high grade (G3 and G4) categories. The diagnosis of G3 and G4 is relatively consistent, but differentiation between G1 and G2 is associated with a more significant degree of inter-observer variability[37].

In an attempt to develop a more objective CRC grading system Ueno *et al*[38] recently proposed a method based on the number of poorly differentiated clusters. This group defined poorly differentiated clusters as clusters of ≥ 5 cancer cells in the stroma, lacking a gland-like structure. The authors studied five hundred stage Ⅱ and Ⅲ CRCs. Poorly differentiated clusters were counted under a 20X objective lens in a field where they appeared to be concentrated. Tumors with < 5, 5 to 9, and ≥ 10 clusters were classified as G1, G2, and G3, respectively. The study showed that grading based on this method is more reproducible and provides more significant prognostic information compared with grading based on the extent of the glandular component in the tumor. Barresi *et al*[39] found that this method is also more reproducible and

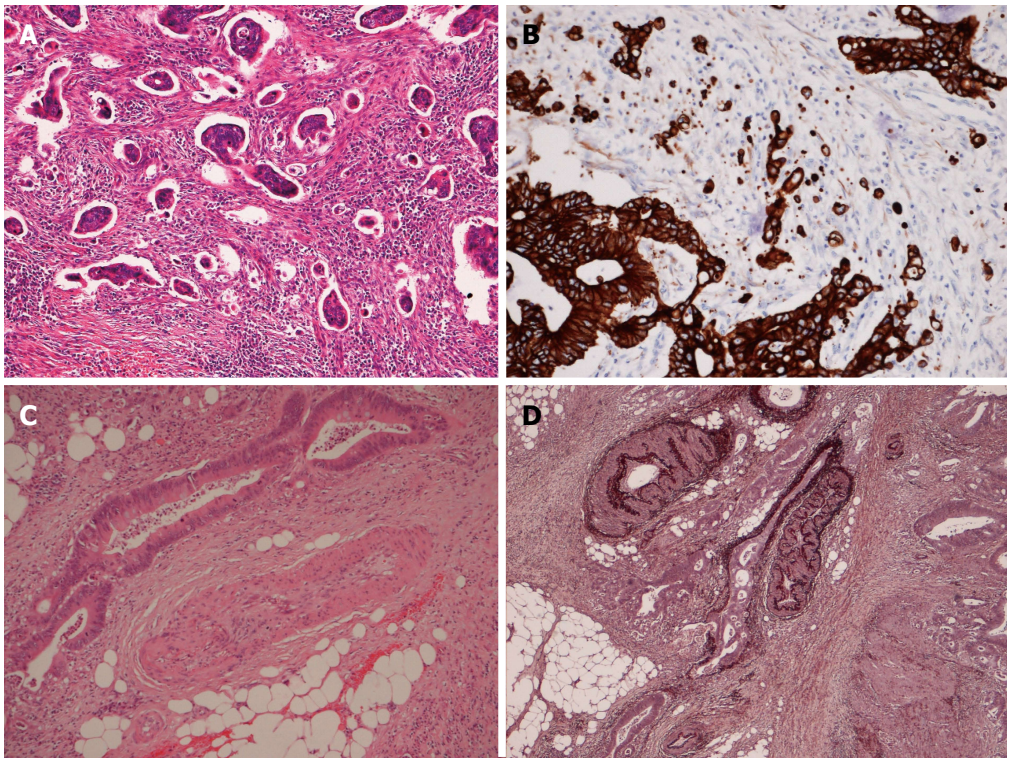

Figure 2 Histological appearances of tumor budding and vascular invasion. A: Tumor budding; B: Cytokeratin immunohistochemical stain highlights tumor buds; C: "Orphan" artery sign - an elongated tumor profile is identified adjacent to an artery with no visible accompanying vein; D: Elastic stain highlights elastic fibres in the walls of arteries and an adjacent vein filled with tumor.

provides better prognostic stratification of stage I CRC patients than conventional grading.

TUMOR BUDDING

Tumor budding is observed at the invasive tumor front, where isolated or small clusters of tumor cells (up to five cells) become detached from the neoplastic epithelium and migrate a short distance into a desmoplastic stroma (Figure 2A)[40]. It is thought to represent epithelial-mesenchymal transition and to be an early step in tumor invasion and metastasis. Epithelial-mesenchymal transition is a process whereby cells undergo morphologic changes characterized by a transition from an epithelial to a mesenchymal phenotype, leading to increased migratory capacity and invasiveness[41]. The aim of tumor buds appears to be the degradation of the peritumoral connective tissue, evasion of host response and finally the invasion of the lymphatic and blood vessels with the consequence of local and distant metastasis[42]. Loss of membranous expression of the cell adhesion molecule ecadherin facilitates detachment of buds from the main tumor. Up-regulation of proteins involved in extracellular membrane degradation, migration and angiogenesis, in tumor buds, enhances their ability to migrate and invade[43].

The majority of CRCs display some degree of tumor budding. Published scoring systems have attempted to identify a prognostically significant degree of budding, commonly termed "high-grade" budding or "high budding"[44]. The identification of tumor budding is facilitated by immunostaining with cytokeratin and it is particularly useful in cases where buds are obscured by peritumoral inflammatory cell infiltrates on H and E (Figure 2B).

Tumor budding has been found to be an independent adverse prognostic factor in CRC and is a strong predictor of lymph node involvement, venous and lymphatic invasion, local recurrence, metastases and poor disease free survival[45-51]. Tumor budding is of particular clinical interest in two subgroups of patients. Budding is an independent predictor of lymph node metastasis in patients with submucosal invasive or early pT1 CRC[52-55]. In the setting of polyp cancer, evaluation of tumor budding in combination with other prognostically significant clinical and pathological features aids in risk stratification and identification of patients who may need segmental resection with lymphadenectomy. Tumor budding has been associated with a poorer outcome in Stage II CRC[45,48,49] and may guide decisions regarding the use of adjuvant chemotherapy in these patients.

Two recently published studies investigated the value of assessing intra-tumoral budding in biopsies. Rogers *et al*[56] retrospectively evaluated tumor budding in diagnostic rectal biopsies from patients who had neodjuvant chemoradiotherapy and found intra-tumoral budding at diagnosis of rectal cancer to be a poor prognostic marker and a predictor of poor response to neoadjuvant treatment. A 2012 study from Switzerland found that high intra-tumoral budding in preoperative CRC biopsies predicted high peri-tumoral budding at the invasive margin and lymph node metastasis in the subsequent resection

specimens as well as distant metastasis[57]. Assessment of tumor budding in the preoperative setting shows some potential as a prognostic and predictive marker and if prospective studies confirm the value of this approach it may become routine practice in the future.

Despite the proven prognostic significance of tumor budding it has not yet become part of standard pathology reporting of CRC. The reasons for this are manifold. Although a large number of individual studies have demonstrated the association with adverse outcome, no clinical trials have assessed the contribution of tumor budding in the prospective setting, and in particular its potential impact among stage Ⅱ patients[42]. Application of this promising parameter is hampered by the lack of a standardized scoring system and sufficient evidence of reproducibility. Several different methods of assessment (at least seven) have been published and there are currently no consensus criteria for quantitative and qualitative evaluation of tumor budding. Development of an internationally accepted scoring system to rapidly and reproducibly identify CRC specimens with prognostically significant levels of tumor budding is challenging. Finding the right balance between accuracy, reproducibility and practicality is crucial. Recent multicentre studies have begun to address these issues[58,59].

VASCULAR INVASION

Vascular invasion is associated with poor outcome in CRC[60-62]. Accurate assessment of vascular invasion is of particular importance in stage Ⅱ CRC, identifying a high-risk group who may benefit from adjuvant treatment[14,63,64]. Vascular invasion in endoscopically resected pT1 cancer is seen in patients at higher risk of lymph node metastasis and may influence the decision to proceed to surgical resection[51,65].

Vascular invasion has two distinct elements: blood vessel invasion (usually venous, rarely arterial) and lymphatic vessel invasion. Differentiating venous and lymphatic invasion is important as they have different clinical implications. Venous invasion is associated with the presence of visceral metastases[65-67]. Presence of lymphatic invasion has been shown to correlate well with lymph node metastasis[68-70]. There is also debate in the literature about the importance of the site of vascular invasion, that is, whether it is extramural or intramural. Vascular invasion in the submucosal and/or muscular layer is considered to be intramural invasion, and that beyond the muscularis propria is extramural invasion. Whilst extramural venous invasion is a well established predictor of adverse outcome[71], incorporated in CRC reporting datasets, the clinical significance of intramural venous invasion is less clear. Some investigators have demonstrated an association between intramural venous invasion and distant metastases[14,62,64], indicating that the presence of venous invasion may be more important than its site.

The reporting of vascular invasion is highly variable[72]; the incidence of venous invasion reported varies between 11% and 89.5%. Venous invasion is widely under-reported[73]. Interobserver variability also poses problems with only low to moderate agreement on reporting vascular invasion in CRC among pathologists[74,75].

The RCPath use the definition originally proposed by Talbot *et al*[61] in their CRC reporting dataset. This group defined venous invasion as "a rounded mass of tumor in an endothelium-lined space either surrounded by a rim of smooth muscle or containing red blood cells." Venous invasion may also be suspected when a rounded or elongated tumor profile is identified adjacent to an artery, especially when no separate accompanying vein can be identified (the "orphan" artery sign), or where smooth tongues of tumor extend into pericolic/perirectal fat ("protruding tongue" sign) (Figure 2C and D). Diagnosis of vascular invasion can be difficult on H and E alone. Strategies to improve detection of venous invasion in particular have been the subject of many recent studies. Increasing the number of tumor blocks taken has been shown to increase rates of detection[62]. Tangential as opposed to perpendicular sectioning of the peritumoral mesocolic/mesorectal fat has also been proposed to facilitate detection of extramural venous invasion[76]. Ancillary techniques that aid identification of vascular invasion are used with increasing frequency. Immunohistochemical markers of endothelial cells such as CD31 and CD34 help in identification of lymphatic and small blood-vessel invasion[68] but are less helpful for identifying venous invasion as the endothelium of many involved veins is destroyed. Specific lymphatic markers such as D2-40 and LYVE-1 can distinguish invasion of lymphatics from invasion of capillaries and small veins. Histochemical elastic stains highlight elastic fibres in the walls of veins (but not lymphatic vessels), allowing much more accurate identification of venous invasion. Studies have shown that use of elastic stains result in a 25%-53% increase in the proportion of cases with venous invasion compared with routinely stained sections[66,72,77-81], and also improve inter-observer agreement[77]. Many pathologists now advocate the use of elastic stains in the routine pathological assessment of CRC. Others warn that over-diagnosis of venous invasion may occur with the use of elastic stains, highlighting the potential for misinterpretation of other histological features as venous invasion, *e.g.*, tangentially sectioned subserosal elastic lamina, mucosal protrusion into the submucosa, periganglionic, perineural and perinodal elastic fibres or periglandular and perimuscular elastosis[82].

PERINEURAL INVASION

Perineural invasion (PNI) is an important prognostic marker in CRC and has been shown to be an independent poor prognostic factor on multivariate analysis in several studies[83-87]. Identification of PNI in CRC is variable with rates between 6% and 31% reported[88,89]. It is an under-reported parameter[83]. PNI is more frequent in the non-peritonealized rectum and colon and this is thought to be due to the relative abundance of nerve

plexuses in the retroperitoneum[90]. PNI is associated with other pathological markers of poor prognosis such as lymphovascular invasion, poor differentiation and tumor budding[83,85,87].

There is no agreed definition of PNI. The most widely used definition of PNI is broad, including invasion of tumor cells in, around and through the nerves[91]. Others have defined PNI according to the layers of nerve sheath involved by tumor. The nerve sheath is composed of 3 connective tissue layers; the outer epineurium, the perineurium and the inner endoneurium[92]. Liebig *et al*[92] defined PNI as the presence of tumor cells within any of the 3 layers of the nerve sheath (epineurium, perineurium and endoneurium) or tumor foci outside of the nerve with involvement of 33% of the nerve's circumference. Some authors report PNI only when tumor cells are observed inside the perineurial layer[84,93].

Studies have evaluated the significance of the localization of PNI both within the neural structure itself and within the bowel wall. A German group developed a "Neural Invasion Severity Score" based on invasion of tumor cells into the epineurium, perineurium or endoneurium, with invasion of endoneurium being assigned the highest score. These authors found that increasing "Severity Scores" were associated with a worse prognosis in both rectal and colon cancer[89,93]. The Japanese Society for Cancer of the Colon and Rectum conducted a multi-institutional study involving 2845 patients. This group proposed a 3-tiered PNI grading system based on location of PNI within the bowel and classified cases as Pn0 (no PNI), Pn1a (intramural PNI only), and Pn1b (extramural PNI)[87]. Using this grading system the investigators determined 5-year disease-free survival as 88%, 70%, and 48%, for the three different categories. Multivariate analysis identified PNI grade as a significant prognostic marker independent of T or N stage.

LYMPH NODES

Briefly, lymph node metastasis is highly predictive of outcome for CRC patients and those with lymph node involvement are likely to be offered adjuvant treatment[94]. This topic has been the subject of a recent comprehensive review in the *World Journal of Gastroenterology*[94]. An association between lymph node yield and survival has also been demonstrated[95-97]. Adequate lymph node evaluation is crucial for accurate staging. Guidelines state that the minimum acceptable lymph node harvest is 12 nodes[1,8], but the number of nodes retrieved from CRC specimens is variable and often falls short of this recommendation[98]. Factors such as age of the patient, body mass index, location of the tumor, neoadjuvant therapy, surgical technique, and pathologist's handling of the specimen may influence the lymph node yield[94,99]. Manual lymph node dissection is the standard technique used in most institutions. After formalin fixation the soft tissue around the specimen is serially sectioned and nodes sought by visual inspection and palpation. However, the identification of small nodes is limited by this approach[100,101]. Alternative techniques to improve lymph node detection have been proposed and a recently published meta-analysis and systematic review compared different pathological methods of lymph node retrieval from gastrointestinal cancer surgical specimens[102]. Meta-analysis showed that fat clearing and methylene blue staining increased the mean lymph node yield from gastrointestinal cancer specimens. Despite an improved lymph node harvest these authors concluded that there was insufficient evidence to suggest that use of these techniques led to upstaging.

TUMOR REGRESSION GRADING

Preoperative neoadjuvant chemoradiotherapy (CRT) has become a standard treatment of locally advanced rectal cancer (clinically T3-4 or node positive rectal cancers). Rectal tumors from these patients may undergo regression. Careful macroscopic assessment of the resection specimen is necessary as tumors with a significant response to treatment may be difficult to recognize. Confirmation of the original site of the tumor will help in selecting tissue for histological examination. Many regressed tumors are firm, white and fibrotic, resembling mucosal ulcers or scars (Figure 3A). There are different approaches to tumor sampling. Some pathologists will submit the entire fibrotic area for histology upfront while others will take 5 blocks in the first instance and submit further tissue for histology if there is no residual tumor on initial sections. Post-treatment histological changes include replacement of neoplastic glands by fibrosis or fibro-inflammatory change, presence of acellular mucin pools, necrosis, foamy macrophages, haemosiderin and calcification[103]. For tumor staging following neoadjuvant therapy, only the presence of tumor cells in the surgical specimen is taken to determine the stage and cases with complete regression are staged as ypT0[1,8]. Tumor regression grading (TRG) systems aim to measure response to neoadjuvant CRT. TRG systems are generally based on assessing the ratio of histological changes induced by CRT to residual tumor in the resected specimen. Several grading systems, some 5-tiered and some 3-tiered, have been published[104-107]. The 3-tier system used in our own department is illustrated in Figure 3B-D.

Pathological complete response (pCR) is reported in 9%-27% of patients and is associated with improved clinical outcomes[108-111]. A meta-analysis including 3105 patients treated with neoadjuvant CRT followed by surgery found that the group with pCR had improved disease free and overall survival, lower risk of local recurrence or distant metastasis, compared to those without pCR[112]. The multicenter prospective MERCURY study designed to assess magnetic resonance imaging (MRI) and pathologic staging after neoadjuvant therapy for rectal cancer, found that a ypT0 resection following neoadjuvant CRT was associated with increased disease-free and overall survival, and decreased rates of local recurrence[113].

While it is now widely accepted that pCR predicts a

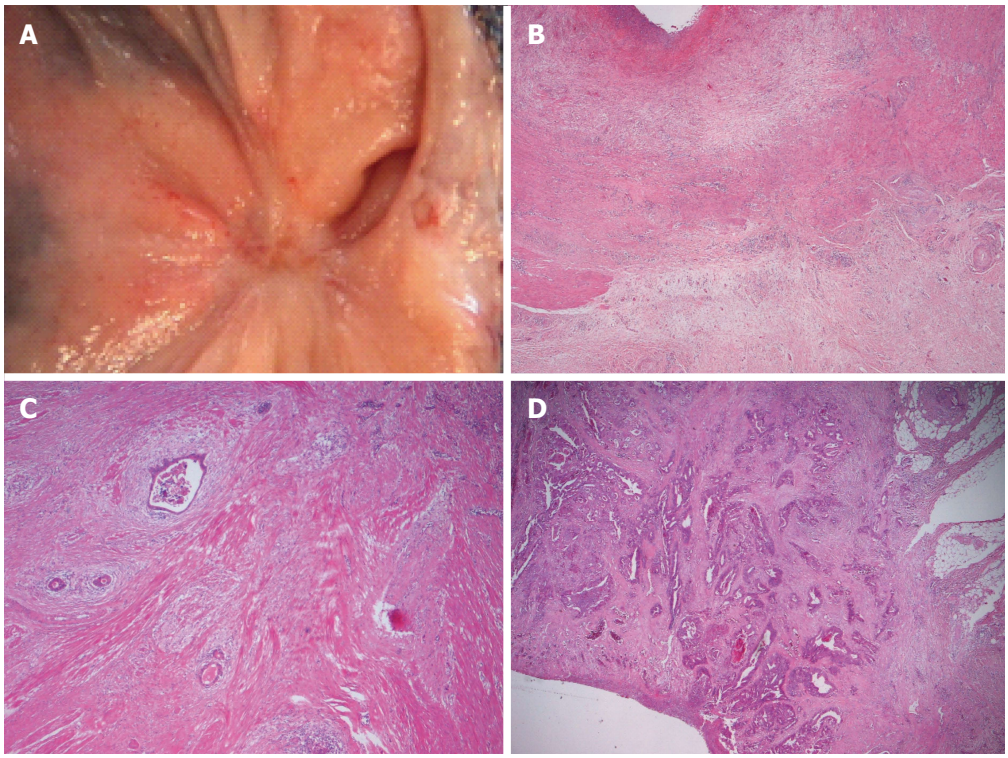

Figure 3 Pathological appearances of tumor regression. A: Regressed tumor with the appearance of a mucosal scar; B: No residual tumor seen in tumor regression grading 1 (TRG1); C: Fibrosis outgrows tumor in TRG2; D: Extensive residual tumor in TRG3.

better prognosis, the clinical significance of "incomplete" or "partial" regression is not clear. Two recent studies from Asia have found lesser degrees of tumor regression to be prognostic factors on multivariate analysis[114,115], but further investigation is warranted. MacGregor *et al*[116] emphasized the importance of addressing this important clinico-pathological question in future research and stressed the need for a standardized approach to the analysis of post neoadjuvant CRT rectal cancer resection specimens and a universally accepted TRG reporting system. An "International Study Group on Rectal Cancer Regression Grading" also recognizes the need for standardization in order to elucidate the clinical importance of "partial regression". The group demonstrated that there is a lack of consensus on pathological sampling of post treatment specimens and choice of TRG system[117]. Disappointingly, they also found that 17 experienced GI pathologists could not reach good concordance on TRG using 3 systems, with only fair kappa values (0.28-0.38) for all 3 systems[118]. These authors advocate the introduction of an internationally accepted, simplified and reproducible TRG system with well-validated correlates to clinical outcomes.

MOLECULAR PATHOLOGY

Molecular pathology can provide prognostic and predictive information for CRC patients and also aids in identification of hereditary CRC syndromes such as Lynch syndrome. In recent years there have been significant advances in our understanding of CRC biology, contributing to the development of targeted CRC therapies. The recognition that activating mutations of the KRAS oncogene can predict resistance to anti-epidermal growth factor receptor agents[119], has turned the spotlight on the clinical value of biomarkers in CRC. Investigation of the clinical utility of emerging biomarkers such as mutations of BRAF, PIK3CA and PTEN deletion is ongoing. CRC genomic profiling and the development of gene expression signature profiles such as ColoPrint® and Oncotype DX Colon Cancer Assay may also contribute to treatment planning decisions. There have been interesting developments in relation to biomarkers and the use of aspirin in CRC. A large 2012 molecular pathological epidemiology study analyzed data from 964 patients and found that regular use of aspirin after CRC diagnosis was associated with longer survival among those with mutated-*PIK3CA* tumors, but not among patients with wild-type *PIK3CA* tumors. These findings suggest that the *PIK3CA* mutation, present in 15% to 20% of CRCs, may be a useful predictive biomarker for adjuvant aspirin therapy in CRC patients[120].

Molecular pathology testing is a dynamic area with new biomarkers regularly reported. Many of the biomarkers require validation and are not yet clinically applicable. A National Comprehensive Cancer Network Task Force publication provides an overview of the role of molecular testing in oncology, an assessment of clinical and analytic validity of some of the tests available, and serves as a useful molecular biomarker guideline for healthcare providers[121].

The role of the surgical pathologist in the manage-

ment of CRC is evolving and while high quality morphology remains central to CRC pathology reporting it needs to be integrated with results of molecular pathology testing. It is essential that pathologists are involved in molecular testing to ensure proper tissue selection and interpretation of results in the context of the pathological findings.

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