GROSS ASSESSMENT OF COLORECTAL CANCER SPECIMENS

PRACTICAL ASPECTS

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Outline

1. radial margins in colon cancer
2. serosal assessment
3. Quirke method for rectal cancer grossing
4. TME assessment
5. lymph node retrieval
6. venous invasion
7. blocking summary
Radial Margins in the Colon

2 types, depending on extent of peritoneum investment, which varies with anatomical site:

• retroperitoneal bare area where colon not invested by peritoneum
• mesocolic vascular ties where colon is invested by peritoneum

Importance of Assessing Radial Margins in Colon Cancer

• local recurrence occurs in 10% of adenocarcinoma treated by right hemicolectomy alone
  Kopelson G. Cancer 83;52:633

• 100 right hemicolectomy specimens removed for adenocarcinoma of the proximal ascending colon
• radial margin involvement in 7%

• 10% margin involvement
  Quirke P et al. J Pathol 06;208:30A
Radial Margins

Key points

- differentiate between bare area type margin and vascular ties – and document which is present
- sections to show relationship to radial margin (at least 3)
- document whether closest tumor is:
  - direct tumor extension
  - tumor in a lymph node or a tumor nodule

Importance of Serosal Penetration

independent predictor of recurrence and poor prognosis

- 579 stage III colorectal cancer resection specimens
  - 16% of patients with (vs 44% without) serosal penetration alive after 5 years follow-up
  

- postsurgical outcomes of 467 stage II colon cancer patients
  - serosal spread independently associated with reduced 5-year survival
  

- 412 colon cancer patients
  - 19% of patients with tumor cells on the serosal surface experienced peritoneal recurrences
  

- 120 colon cancer patients
  - 15% of cancers with cytologic and/or histologic evidence of peritoneal penetration progressed to involve the peritoneal cavity
  
1. tumor well clear of closest peritoneal surface
2. mesothelial inflammatory and/or hyperplastic reaction with tumor close to, but not at, the peritoneal surface
3. tumor present at peritoneal surface with inflammatory reaction, mesothelial hyperplasia, and/or "ulceration"
4. tumor cells shown free in peritoneum and evidence of adjacent "ulceration"

Assessment of the Serosa

Diagnosis of serosal involvement is made by MICROSCOPIC examination BUT requires meticulous GROSS assessment and sampling

How?
- 2 blocks from areas where tumor closest to serosa or areas suspicious for serosal involvement
  → retraction/puckering, prominent vessels, granularity, exudate, loss of shiny surface

Ink?
- may obscure microscopic evaluation
- use a different color than for resection margins

When?
- ALL colon cancers
- UPPER rectal cancers
Serosa and Radial Margins

All rectal cancers above the anterior peritoneal reflection: serosa AND radial margin must be assessed.

Histologic Features and Cytologic Techniques That Aid Pathologic Stage Assessment of Colonic Adenocarcinoma


- evaluated cytology in determining stage: compared serosal scrape cytology with histologic stage in 120 resections – correlated findings with presence and type of inflammatory changes near the serosa to determine if any are reliable indicators of peritoneal penetration

- frequency of positive serosal cytology

  - 0% - pT3 tumors >1mm from serosa
  - 2.6% → peritoneal carcinomatosis
  - 46% - pT3 tumors located ≤ 1 mm from serosa with histologic changes e.g. fibroinflammatory reaction, vascular proliferation, hemorrhage or fibrin deposition, reactive mesothelial cells
  - 11% → peritoneal carcinomatosis
  - 55% - pT4a tumors
  - 18% → peritoneal carcinomatosis

- pT4a category should be expanded to include deeply invasive colon cancers that elicit a fibroinflammatory reaction, mesothelial hyperplasia, or hemorrhage and/or fibrin deposition at the peritoneal surface, despite the absence of tumor cells on the serosa
Serosal Assessment

- serosal involvement is an important adverse prognostic feature
- always look for evidence of serosal involvement
- colon AND rectal cancers
- at least 2 sections to show tumor and closest serosa
Total Mesorectal Excision

TME = surgical removal of this soft tissue envelope down to the pelvic floor - using sharp instruments under direct vision, dissecting the potential space ("holy plane") between the visceral and parietal pelvic fascia

Quirke Method

- permit optimal assessment of 2 important factors
  - status of the radial margin
  - quality of the mesorectal excision procedure
Assessment of the Radial Margin

Radial margin status = single most important factor for predicting risk of local recurrence

- involvement of radial margin associated with higher rates of local recurrence, distal metastases and poorer survival
- the closer the tumor to the radial margin, the worse the prognosis
- radial margins <1 mm vs >1 mm:
  - increased risk of distant metastases (37% vs 15%)
  - shorter survival (70% vs 90%)

Quirke P et al. Histopathol 07;50:103
Nagtegaal ID et al. Eur J Cancer 02;38:964
Birbeck KF et al. Ann Surg 02;235:449
Dexter SP et al. Gut 02;48:667

Assessment of the Quality of Mesorectal Excision Surgery

- operative plane of surgery predicts margin positivity, local recurrence and survival
- incomplete mesorectal excision related to:
  - increased recurrence rates
    28.6% vs 14.9%, p=0.03 (incomplete vs. complete) with negative CRM
  - increased incidence of margin positivity
    44% vs 24%, p<0.05 (incomplete vs. complete)

Quirke P et al. Histopathol 07;50:103
Quirke P et al. J Clin Oncol 06;24:A3512
Nagtegaal ID et al. J Clin Oncol 02;20:1729
Wibe A et al. Br J Surg 02;89:127
Step 1 = Define Anatomical Landmarks

Serosa and Radial Margins

All rectal cancers above the anterior peritoneal reflection: serosa AND radial margin must be assessed
Step 2 = Grade Quality of Surgery

- grade the quality of the mesorectal excision procedure
- final grade is based on the “worst” parameter
- initial assessment is best done prior to fixation and inking, but can be done after fixation if necessary

<table>
<thead>
<tr>
<th>Bulk</th>
<th>Defects</th>
<th>Coning</th>
<th>Radial Margin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>bulky smooth</td>
<td>no deeper than 5 mm</td>
<td>none</td>
</tr>
<tr>
<td>Complete</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nearly complete</td>
<td>moderate bulk irregular</td>
<td>deeper than 5 mm</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>no visible muscularis propria (except where levator muscles insert)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incomplete</td>
<td>little bulk irregular</td>
<td>down to muscularis propria</td>
<td>moderate-marked</td>
</tr>
</tbody>
</table>

Examples of Mesorectal Excisions

- intact, bulky mesorectum
- normal “rectal buttocks”
- no defects > 5 mm
- no coning
- wisps of fascia on surface

- little bulk to mesorectum
- irregular, ragged mesorectum
- defects down to visible muscularis propria (arrow)

Images courtesy Dr. R. McLean, Royal Alexandra Hospital, Edmonton, Alberta
Types of TME

- **waisted specimen**
- **cylindrical specimen**

More Examples

- **Bill Heald**: Good surgery with complete removal of mesorectum
- **Lars Pahlman**: Moderate surgery with incomplete removal of mesorectum
- **images courtesy Dr. P. Quirke**: Poor surgery with little mesorectum

Images courtesy Dr. P. Quirke
Step 3 = Ink & Partially Open Specimen

- ink the bare areas (non-peritonealized margins) below the peritoneal reflections

![Image of specimen](image)

Step 4 = Partially Open Specimen

- open the specimen along the anterior aspect from the top and the bottom, leaving the bowel intact at a level just above and just below the tumor

- place loose gauze (not paper towel) wicks – soaked in formalin – into the unopened ends of the bowel

- fix specimen for at least 72 hours (96 hours is ideal)

*Image courtesy Dr. R. McLean, Royal Alexandra Hospital, Edmonton, Alberta*
Step 5 = Section

- 96 hours fixation \(\rightarrow\) slice through the unopened bowel at 3-5 mm intervals
- assess
  - radial margin: smooth, regular vs moderately irregular vs very irregular
  - extent of tumor and the closest distance of tumor to the radial margin
  - obviously positive nodes and distance of any positive node to margin
  - record site of closest tumor
  - examine fat for lymph nodes
  - assess for polyps, other lesions

Serosal vs Radial Margin Involvement

Differentiate between tumor extending to the serosal surface and tumor extending to the radial margin (need to correlate location of slice relative to peritoneal reflection)
Examples of Incomplete Specimens

Surgical impingement → intramesorectal plane

Surgical impingement → muscularis propria plane

Images courtesy Prof. P. Quirke, University of Leeds, UK

Step 6 = Block

- tumor
  - at least 6 blocks, to include:
    - 3 sections showing deepest point of invasion
    - NB: include closest radial margin in all if feasible; if closest radial margin is further away, include 1 additional section of closest radial margin
    - if closest tumor to radial margin is a positive lymph node or tumour nodule, submit node/nodule with margin (and designate in block legend)
    - for tumors above the peritoneal reflection, 2 sections showing closest overlying serosa, especially areas of puckering/retraction
    - 1 section showing adjacent uninvolved mucosa (omit if included in other sections)
    - 1 section showing perforation site (if present)
    - relationship with other organs, as appropriate
    - entire abnormal area in post-treatment rectal tumours if little or no tumour apparent
  - lymph nodes
    - be careful not to double-count nodes present in more than one slice
  - polyps
  - proximal and distal resection margins
    (NB: include both mucosa and mesorectum in the distal margin)

Image courtesy Dr. R. McLean, Royal Alexandra Hospital, Edmonton, Alberta
Reasons for Not Using Quirke Method

- often opened in OR at surgeon request (assessment of distal margin)
- obtaining tissue for banking
- not recommended by CAP
- lack of awareness/knowledge/familiarity/experience with the method
- poor fixation of tumor
- extended fixation time affects TAT and patient care
- doesn’t allow proper assessment of tumor gross type
- makes assessment of tumor size more difficult
- makes finding lymph nodes more difficult
- lack of good reason for doing so
- other methods can achieve the same results

http://philquirke.com/

http://philquirke.weebly.com/
Quirke Method

Key points

- preferred method for grossing rectal cancers
- optimizes assessment of TME quality and relationship of tumor to radial margins

History of 12 Lymph Nodes

- 1989 and 1991: suggested 12 or 13 lymph nodes may serve as the appropriate minimum recovery number

- 1991: Working Party Report to the World Congresses of Gastroenterology; recommended that at least 12 lymph nodes must be sampled to adequately stage a patient

- 2001: National Cancer Institute; recommended that at least 12 lymph nodes be harvested and examined to properly define a colorectal cancer as node negative

- 2007: National Quality Forum: endorsed harvest of at least 12 lymph nodes as a standard quality indicator for segmental colorectal cancer resection specimens

- AJCC staging manual 7th edition: important to obtain at least 10-14 lymph nodes in radical colon and rectum resection in patients without neoadjuvant therapy

- subsequent studies found it difficult to find a number above which the detection of lymph node metastasis plateaus
  Swanson RS, Compton CC, Stewart AK, Bland WF. The prognosis of T3N0 colon cancer is dependent on the number of lymph nodes examined. Ann Surg Oncol 2003; 10: 655–61
  Goldstein NS. Lymph node recoveries from 2477 pT3 colorectal resection specimens spanning 45 years: recommendations for a minimum number of recovered lymph nodes based on predictive probabilities. Am J Surg Pathol 2002; 26: 179–189
A certain amount of patience is necessary in carrying out the gland dissection. In some of our perineoabdominal excision specimens we have found as many as 60 glands, and the average has been 28. The exact position and size of each gland was marked on a natural-size drawing, using calipers to ensure accuracy in measurement.

Factors Affecting LN yield

**Patient**
- age: fewer in older patients for every 10-year incremental increase in age, 9% reduction in harvest
- more in females
- fewer with increased BMI

**Tumor**
- size: for every 1 cm increase in size, 2-3% increased nodes
- location: right > left by about a third
- MSI status: MSI-H have higher counts

**Surgeon**
- size
- complete mesocolic excision → higher numbers (also depends on no of vascular pedicles resected)
- experience

**Pathologist**
- several studies shown differences amongst pathologists or PAs
- highlighting and clearing

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**Lymph Node Numbers in Practice**

*there is no unacceptably low number of lymph nodes for an individual dissection*

**Recommendations for the reporting of surgically resected specimens of colorectal carcinoma**

Jeremy R. Jass MD*, Michael J. O’Brien MD, Robert H. Riddell FRCPath, Dale C. Snow MD, on behalf of the Association of Directors of Anatomic and Surgical Pathology

Human Pathology (2007) 38, 537–545

“mean number of nodes in a series of dissections gives an indication of practice quality (goal = 12-15)”

“if fewer than 12 lymph nodes are found, re-examining the specimen for additional lymph nodes, with or without visual enhancement techniques, should be considered”

“median number of lymph nodes examined should be > 12”

“These are minimum standards with many good centres in the UK finding 18 lymph nodes as a median count.”

**GEWF Solution**

- an effective lymph node **highlighting** solution

  - 6.8 \( \rightarrow \) 10.2
  - additional 39 nodes in 11 cases (breast)
  - Colon 9 \( \rightarrow \) 16, rectum 10 \( \rightarrow \) 17
- Gregurek SF & Wu HH. Arch Pathol Lab Med 2009;133:83
  - 18.3 \( \rightarrow \) 19.9
- Li Chang H et al.
  - 153 resections (with GEWF) vs 169 (without)
  - 21 vs 13 lymph nodes

**RECIPES**

- absolute ethanol 10L
- distilled water 3.4L
- formaldehyde 40% 1.6L
- glacial acetic acid 1L
Lymph Node Retrieval

- there is no minimum of lymph nodes
- use of dissection aids can be helpful

Intramural and Extramural Vascular Invasion in Colorectal Cancer

Prognostic Significance and Quality of Pathology Reporting

Johannes Beigel, Helion J. Pollock, MD; Richard A. Lindner, MD; Peter Kompolti, MD
Andreas Schreiber, MD; Peter Menke, MD; Michael Vahl, MD; Gerald Hartler, MD; and Cord Langenau, MD

Cancer 2012;118:626-38.

- 381 tumors retrospectively reviewed
- VI in 23%
- stage II disease
  - venous invasion = independent prognostic variable
  - EMVI > IMVI
The Clinical Utility of the Combination of T Stage and Venous Invasion to Predict Survival in Patients Undergoing Surgery for Colorectal Cancer

Campbell T. D., Rothman, PhD; Donald C. McDowell, PhD; Colin H. Richards, MBChB; Manal Alexanian; John H. Anderson, MD; Tim Harvey, MS; Paul G. Horgan, PhD; and Alan K. Findlay, MD

(Am Surg 2014;239:1156–1165)

- 631 stage I to III CRC specimens
- VI detected in 56%
- multivariate analysis
  - VI associated with shorter survival duration, independent of other features
    (all cases \( P < 0.001 \)); node-negative cases \( P < 0.001 \)
  - with the exception of T stage, no other pathology features associated with survival time
- examined combination of T stage and VI on survival
  - all cases: TVI had similar predictive value as T stage and node status
  - node-negative cases: TVI had superior predictive value

92 CRC cases reviewed

- 50 VI negative H&E alone → VI positive with Movat in 22 (44%, \( p < 0.001 \))
- 25 equivocal for VI H&E alone → VI positive with Movat in 19 (76%)

- 46/69 cases with VI: 61% metastases
- 26/69 cases without VI: 35% metastases \( p < 0.03 \)

- 2 clues in H&E sections:
  - “unaccompanied artery” sign
  - “protruding tongue” sign

Use of an elastic stain to show venous invasion in colorectal carcinoma: a simple technique for detection of an important prognostic factor

C. J. Howlett, E. J. Tweedie, D. K. Driman

Studies of Elastin Stains and VI

<table>
<thead>
<tr>
<th>Year</th>
<th>H&amp;E (%)</th>
<th>Elastic Stain (%)</th>
<th>Stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoue et al 1992</td>
<td>17</td>
<td>47</td>
<td>Elastic von Gieson</td>
</tr>
<tr>
<td>Vass et al 2004</td>
<td>27</td>
<td>57</td>
<td>Miller</td>
</tr>
<tr>
<td>Abdulkader et al 2006</td>
<td>36</td>
<td>46</td>
<td>Elastic von Gieson</td>
</tr>
<tr>
<td>Kingston et al 2007</td>
<td>10</td>
<td>48</td>
<td>Elastic von Gieson</td>
</tr>
<tr>
<td>Howlett et al 2009</td>
<td>18</td>
<td>62</td>
<td>Movat</td>
</tr>
<tr>
<td>Roxburgh et al 2010</td>
<td>18</td>
<td>58</td>
<td>Miller</td>
</tr>
<tr>
<td>Sejben et al 2010</td>
<td>18</td>
<td>71</td>
<td>Orcein</td>
</tr>
<tr>
<td>Kirsch et al 2013</td>
<td>20</td>
<td>46</td>
<td>Movat</td>
</tr>
</tbody>
</table>

average 34% increase


Venous Invasion in Colorectal Cancer
Impact of an Elastin Stain on Detection and Interobserver Agreement Among Gastrointestinal and Nongastrointestinal Pathologists

Richard Kirsch, MRCPath, PhD,* David E. Messenger, MRCPath, Robert H. Riddell, MD,* Aaron Pollett, MD,* Megan Cook, MS,* Sabah Al-Haddad, MRCPath*

Catherine J. Stavroulakis, MD† Dimitrios X. Drouva, MRCPath‡ Rajani Pandi, MD§ Ken J. Newell, MD, PhD, Jinjin Liu, MD, Russell G. Price, MD, Sharyn Smith, MD** Jeremy R. Purvis, MD†† and David K. Driman, MRCPath††


interobserver agreement
H&E: k=0.23 vs. Movat: k=0.41
• EMVI in 50/79 (63%) cases
• metastases: 26% EMVI positive vs 3.5% EMVI negative

How many blocks?

• more blocks stained → more VI detected
• suspicious cases: 1 block is sufficient

USCAP 2015 abstract submitted
Kirsch R, Juda A, Assarzadegan N, Messenger D, Riddell RH, Driman DK.
The impact of routine elastin staining on venous invasion detection in colorectal cancer
  • no routine elastin staining (n=145): 20% VI detection rate
  • routine elastin staining (≥ 5 tumor containing blocks/case (n=138): 42% VI detection rate [p<0.001]

LHSC: Audit of cases Jan-Sep 2014:
  • EMVI detection rate = 44.9%
  • mean 1.9 blocks/case
Recommendations

1. **grossing**
   - at least 3 blocks of deepest invasion of tumor
   - target areas of linear spiculation at the advancing edge of the tumor
   - consider tangential sections

2. **elastin stains**
   - routinely on all tumor blocks or on blocks showing deepest invasion OR on equivocal blocks on H&E

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**Assessment of Venous Invasion**

- take at least 3 blocks of deepest invasion of tumor
- target areas of linear spiculation at the advancing edge of the tumor
- consider tangential sections
- consider routine elastin stains up front on blocks showing deepest invasion

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*Developments in the assessment of venous invasion in colorectal cancer: Implications for future practice and patient outcome*

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Human Pathology (2012) 43, 965–973
Blocking Summary

Blocking Protocol

**Tumor:** At least 6 sections or 1 section per cm, to include:
- 3 sections showing deepest point of invasion (include closest radial margin in all if feasible)
- if closest radial margin is further away, include 1 additional section of closest radial margin
- if closest tumor to radial margin is a positive lymph node/ tumor nodule, ATE node/nodule with margin (and designate in block legend)
- 2 sections showing closest overlying serosa, especially areas of puckering/retraction (including in upper rectal cancers)
- 1 section showing adjacent uninvolved mucosa (omit if included in other sections)
- 1 section showing perforation site (if present)
- relationship with other organs, as appropriate
- entire abnormal area in post-treatment rectal tumors if little or no tumor apparent

**Lymph nodes:** All lymph nodes as follows:
- submit in their entirety
- describe whether nodes are bisected or trisected in one block or if more than one block represents a single large node
- if <12 found, place fat in GEWF solution overnight and re-examine the following day

**Polyps:**
- if <5: submit all; if 5 or more: section the largest 5
- in general, one section from polyps <1 cm, 2 or more sections from larger polyps

**Proximal and distal resection margins:** 1 longitudinal section from each unless tumor < 1 cm from margin