Challenges in Colorectal Cancer

Wendy L Frankel, MD
Chair of Pathology
Director of GI/Liver Pathology
Fellowship

Outline- Colorectal Cancer
- Tumor T staging and serosal involvement
- Neoadjuvant treatment and staging
- Lymph node N staging and tumor deposits
- Molecular and ancillary studies

TNM Staging
- Developed by UICC (Europe) and AJCC (NA)
- Predictive of outcome, data driven, evidence based
- Updated frequently
  - CAP protocol (K Washington, 2009)
  - CAP Checklist, Jan 2016
- Standardized pathologic assessment vital to
  - Determine extent of disease
  - Decisions on adjuvant therapy, clinical trials
  - Prognostic and predictive factors

Deepest Extent of Tumor is Shown. All Lymph Nodes are Negative. What Would You do?
A. Stage as T3
B. Cut deeper sections
C. Stage as T4a
D. Complain about AJCC

Deeper level; T4a
T4- Serosal Involvement

- Associated with decreased survival
- May need additional treatment
  - Adjuvant chemotherapy recommended by ASCO for Stage III and IV not II unless high risk features (i.e. T4)
- Significant variability in reporting serosal involvement
  - Studies with meticulous sampling 59%
  - Other studies <10%
- Underdiagnosed likely due to inadequate sampling and not recognizing serosal penetration (up to 20%)


CAP Cancer Staging Protocol- T4

- Absence of standard guidelines for assessing peritoneal involvement may contribute to underdiagnosis
- The following findings are considered to represent serosal involvement by tumor
  - Tumor at serosal surface with inflammatory reaction, mesothelial hyperplasia, and/or erosion/ulceration
  - Free tumor cells on serosal surface with underlying ulceration of visceral peritoneum
- Both associated with decreased survival

T4a- Serosal Surface

Deeper Sections

T3- close

Deeper Sections, T4a
Serosal Clefts- T4a

Cytokeratin 7 is not helpful in most cases

Cleft- Mesothelial Hyperplasia

Cancer → Mesothelium

Tumor less than 1 mm; Serosal Reaction T3 vs. T4a

- Serosal penetration under-recognized
- Serosal scrape cytology 128 cases colon cancer
- Peritoneal cytology + in 19% T3 (46% in T3 within 1 mm of serosal reaction) and 55% T4
- Tumor < 1 mm with reaction; T4a?
  - Fibroinflammatory, granulation tissue
  - Peritumoral abscess that communicates to surface
  - Hemorrhage, fibrin
  - Reactive mesothelial cells

Serosal Surface T3 or T4a?

If gross perforation, T4

Elastic Stain- Why Consider it?

- T3 vs. T4a can be challenging
  - Clinically important Stage II
  - Possible surrogate for serosal invasion
- Elastic in lung cancer for invasion visceral pleura, AJCC 7th ed TNM
- Subserosal elastic lamina colon
  - Located just deep to peritoneum
  - May be retracted toward front of carcinoma because of fibrosis
  - Not present in all cases

Elastic Stain- Helpful or Not?

- Studies variable results; different stains and # slides
- Challenges
  - Not all cases contain EL, particularly right colon
  - EL incomplete in many; need to ‘draw a line’
  - Not practical if necessary to stain several slides
- Reporting results (if you find EL+ deep T3)
  - Upstage or add comment?
- I currently do not use it

Deepest Extent of Tumor and Elastic Stain are Shown. What Would You do?

A. Stage as T3
B. Stage as T4a
✔ C. Cut deeper sections
D. Complain about both AJCC and Elastic stains

Neoadjuvant Chemoradiation

- For advanced rectal cancer; T3-T4 and/or LN+
- Improved resectability/reduced local recurrence
- Associated with significant tumor response, downstaging, better prognosis
- Several grading systems, modified Ryan suggested
- Evaluate in tumor
  - Not LN or other metastatic site
- Acellular mucin likely represent treated cancer
  - Do not use to classify T or N

Rectal Resections

- Radial margin most critical factor local recurrence in rectal cancer (< 1mm)
- No 4a in non-serosalized rectum

Tumor Regression Grade

<table>
<thead>
<tr>
<th>Description</th>
<th>Tumor Regression Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No viable cancer cells</td>
<td>0 (complete response)</td>
</tr>
<tr>
<td>Single cells or small groups of cells</td>
<td>1 (near complete response)</td>
</tr>
<tr>
<td>Residual cancer with evident tumor regression, but more than single cells or small groups of cells</td>
<td>2 (partial response)</td>
</tr>
<tr>
<td>Extensive residual cancer with no evident regression</td>
<td>3 (poor or no response)</td>
</tr>
</tbody>
</table>
Tumor Regression Grade

- 0 (Complete Response)
- 1 (Near Complete Response)
- 2 (Partial Response)
- 3 (Poor Response)

Mucin Pools

- Mucin Pools Deeper than Malignant Cells
- Still ypT2, Radial Margin - R0
- Don’t upstage T or call + radial margin

All Other 14 Lymph Nodes are not Involved, What is the N Stage?

- A. N0
- B. N1
- C. N1a
- D. N1 with comment

Mucin in perirectal fat

Still ypT2

Mucin at radial margin

ypT2

57%  
14%  
16%  
13%
Muddy the Waters

Tumor Deposits
What Counts as a Lymph Node?

- Nodules without residual nodal tissue (recognized 1935)
    - Size matters, 3mm
    - Shape matters (contour)
      - Round smooth
      - Irregular
    - Count all separately
      - LN, LVI or discontinuous tumor
      - N1c

Classify this Metastasis:

A. Involved lymph node
B. Tumor deposit
C. Lymphovascular invasion
D. Indirect spread of tumor

- There are identifiable LN < 3mm; data not confirmed
- Poor reproducibility
- Still subjective, residual LN
Lymph Node (pN) Issues in Staging - Tumor Deposit

- Discrete foci tumor in pericolic/perirectal fat or in adjacent mesentery away from leading edge of tumor and no evidence of residual LN but within lymph drainage area of primary carcinoma
- TD - Discontinuous spread, LV with extravascular extension, or totally replaced LN
- Identifiable LVI/LN is not TD

Tumor Deposit and N1c

- TD can be diagnosed when
  - No residual LN is found
  - Do not add TD to positive LN number
  - Do not use N1c if any positive LN
  - Do not use N1c if N1(mic) - 0.2 to 2 mm
  - N1c is not worse, by definition, than N1a or b
  - N1c appears to be at least as bad as N1
  - Does not change the T stage even if tumor is T1/T2, and TD is in pericolonic tissue

Interobserver Study LN vs. TD: 25 Metastasis Reviewed by 7 Pathologists


Tumor Deposit (7/7)


Rock, Arch Path Lab Med, 2013
Challenging- TD or LN?

Tumor Deposit (4/7)  
Lymph Node (4/7)

Moderate agreement
Useful features: round, peripheral lymphocytes/follicles, thick capsule, possible subcapsular sinus

Classify this Metastasis:

A. Involved lymph node
B. Tumor deposit
C. Lymphovascular invasion
D. Indirect spread of tumor

No definite residual LN or vessel

14% 70% 11% 5%

Classify this Metastasis; If there is Already 1 Positive LN, What N Stage?

A. Involved lymph node, N1a
B. Tumor deposit, N1a
C. Involved lymph node, N1c
D. Tumor deposit, N1c

No definite residual LN so TD; Do not use N1c since another positive LN; Do not add to LN count (so not N1b)

48% 32% 11% 5%

Lymph Nodes

- Minimum number? The more the better
- Fat clearing helps, not standard practice
- AJCC TNM 7, at least 10-14; CAP, <12, regross
- No definite minimum rectum after neoadjuvant
- Many factors affect recovery
  - Pathologist/surgeon experience and diligence
  - Patient age, sex, obesity, immune response
  - Length colon, procedure, site, size
- Future- lymph node ratio?

2003; Chang GJ, JNCI, 2007; Dilman RD, Cancer, 2009; Govindarajan, J Clin Onc, 2011; de
Tumor Budding

- Currently not recommended AJCC or CAP checklist
- Groups of up to 5 cells at the invasive front of tumor
- Associated with aggressive behavior
- Most evidence where it may affect therapy
  - Polypectomy (?resection), Stage II CRC (?adjuvant)
- Variability in criteria, high and low grade, cytokeratin, intratumoral budding (ITB)
- Many studies published and ongoing


Molecular Tests and Biomarkers

- +Not required; may be clinically important
- Histologic features suggestive of microsatellite instability (MSI) and Lynch
- CAP biomarker template- December 2014
  - MSI status
  - Immunohistochemistry for MMR proteins
  - BRAF V600E analysis
  - KRAS mutational analysis
  - MLH1 promoter methylation, NRAS, PIK3CA, PTEN
Why is MSI Important?

- All MSI CRC patients better prognosis (sporadic and germline/Lynch)
- MSI CRC do not respond to 5FU-based chemotherapy (predictive/treatment)
- Identification Lynch Syndrome (LS) helps patients/families
  - Colonoscopic screening ↓ CRC & death
  - LS patients risk 2nd primary (CRC & others)
  - LS patients' relatives benefit from testing
- MSI predictive of response to PD-1 inhibitors (immune checkpoint blockade with pembrolizumab)

Lynch Syndrome

- Most common hereditary CRC syndrome
- 2-4% of CRCs, 1 in 35 CRC patients
- Autosomal dominant, penetrance 80%
- Early, variable age at CRC diagnosis, 45 y/o
- Susceptibility to CRC & extracolonic cancers
- Germline mutation in genes belonging to DNA MMR family- MLH1, MSH2, MSH6, PMS2, EPCAM
- Mutations lead to defective DNA repair & MSI

Histology MSI CRC

Histology and History are not Enough to Identify LS

Screening CRC recommended by EGAPP, NCCN, ASCO, EuSMO, US Multi-Society Task Force on CRC
Adoption of Universal Tumor (UTS) Screening for LS

<table>
<thead>
<tr>
<th>Type of Institution</th>
<th>% Performing UTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-Comprehensive Cancer Centers</td>
<td>71%</td>
</tr>
<tr>
<td>COS-Accredited Community Hospital Comprehensive Cancer Programs</td>
<td>36%</td>
</tr>
<tr>
<td>Community Hospital Cancer Programs</td>
<td>15%</td>
</tr>
</tbody>
</table>

Cost effective in US to screen- Universal IHC → BRAF → Directed MMR gene

Are you screening?


Mismatch Repair Deficiency Immunohistochemistry

- IHC Identifies MMRP
- Normally present
- If protein absent, gene not being expressed (mutation/methylation)
- Helps direct gene testing by predicting likely involved gene
- If abnormal IHC (absent), MSI

Shia, Am J Surg Pathol, 2009

MLH1 & PMS2 Absent

- 15% of the time
- CRC is MSI
- Better prognosis
- 80% sporadic, acquired methylation MLH1
- Up to 20% will be LS
- Test BRAF or methylation MLH1 promoter

MSH2 & MSH6 Absent

- 3% of the time
- CRC is MSI
- Better prognosis
- May be LS due to MSH2 (MSH6 less likely) gene mutation
- Always refer to Genetics
- MSH6 and PMS2 only similar

MLH-1, MSH-2, MSH-6, PMS-2

MLH1, MSH2, MSH6

MLH1 & PMS2 Absent

MLH1 & PMS2 Absent

MLH-1, MSH-2, MSH-6, PMS-2

MSH2 & MSH6 Absent

MLH-1, MSH-2, MSH-6, PMS-2

MSH2 & MSH6 Absent
OSU Universal Screening Algorithm

- All proteins present (80%)
- MLH1 and PMS2 absent (15%)
- MSH2 and/or MSH6 absent; PMS2 only absent (5%)

**STOP**

Problems in Interpretation

- MMRP present but 40yo, family hx, suspicious features
  - Genetics consult, MSI and/or mutation screen
  - If MSI+ and MMR mutation found
    - Possibly protein present but not functional (missense)
- MMRP lost, gene mutation not found
  - Large rearrangement (insertion or deletion) in or near gene (EPCAM is upstream of MSH2)
  - Lynch-like
    - Biallelic somatic mutation in tumor (no need screen family)
      - 67% with MSH2/MSH6 loss, germline mutation found
      - 33% none found- 68% acquired double somatic tumor mutation
    - Others possibly LS, limited by technology (inversions,..)
    - Other germline defects
    - Incorrect interpretation MMR stains

Haraldsdottir, Gastroenterol, 2015

IHC MMRP- Problems in Interpretation

- Variability
- Weak nuclear staining
- Cytoplasmic staining
- Tissue and fixation
- Controls are important

IHC MMRP Result? Next Best Step?

- A. Cannot tell if LS, do BRAF
- B. Cannot tell if LS, test specific germline genes
- C. Microsatellite stable, stop
- D. Likely sporadic MSI, stop
PCR Mutation Test (and/or IHC) for BRAF is Positive, Next Best Step?

A. Likely sporadic, stop
B. Likely LS, stop
C. Likely LS, test specific germline genes
D. Still cannot tell, do molecular MSI test

IHC MMRP Result? Next Best Step?

A. Cannot tell if LS, do BRAF
B. Likely sporadic MSI, stop
C. Likely LS, stop
D. Probably LS, test specific germline gene

OSU Universal Screening Algorithm

All proteins present (80%)
MLH1 and PMS2 absent (15%)
MSH2 and/or MSH6 absent; PMS2 only absent (5%)

BRAF mutation analysis (or MLH1 methylation)
BRAF mutation present (10-12%)
BRAF mutation absent (3-5%)
Sequence and large rearrangements for MLH1 (or MLH1 methylation)
Sequence and large rearrangements for absent one(s)
No germline mutation in MLH1, MSH2, MSH6, PMS2
Consider family history, MSI analysis, tumor somatic testing

IHC MMRP s/p Neoadjuvant Therapy for Rectal Cancer Result? Next Best Step?

A. Likely LS (MSH6 mutation), specific germline gene test
B. Likely sporadic MSI, do BRAF
C. Likely microsatellite stable, stop or test biopsy
D. Likely microsatellite stable, do BRAF
**Challenge- Rectal Cancer Post Neoadjuvant**

**MMRP Post-Treatment**

<table>
<thead>
<tr>
<th>MSH2, MLH1, PMS2 present</th>
<th>MSH6 absent/ equivocal 15-20%</th>
</tr>
</thead>
</table>

Bao, Am J Surg Pathol, 2010; Bellizzi, Mod Pathol (ab), 2010; Radu, Hum Pathol, 2011; Shia, Mod Pathol, 2013

**LS- Other Issues**

- Testing on biopsies or resections?
  - IHC works well on both
  - Advantage biopsy- may change operation if LS
  - Disadvantage- treatment elsewhere and no follow-up
  - At OSU, we test biopsies only by request

- Testing adenomas?
  - If MMR protein lost helpful to predict
  - If all MMR proteins present does not exclude LS
  - At OSU, we test adenomas by request and explain pitfall

- Testing serrated polyps?- not precursor for LS
  - Not helpful to distinguish SSA/P vs. HP

- Testing metastasis or primary is OK


**Next Generation Sequencing**

- Will likely replace single gene assays- tests for mutations, translocations, copy number changes
- Will be less expensive than single gene tests soon
- Likely make LS screening not necessary
- Many panels already available
Summary and Take Home Message

- T4a underdiagnosed, may impact additional treatment; deeper or more sections helpful
- Post neoadjuvant acellular mucin not upstage
- Nodal issues- document TD
  - Do not add TD to LN count
  - Use N1c for TD if no positive LN
- Literature on tumor budding and LN ratio, no change in AJCC yet
- Tumor screening for MSI and LS recommended, NGS likely in future

Thanks for Your Attention Questions?