Endometrial Cancers in Lynch Syndrome (HNPCC)

Screening, histologic subtype(s), & evaluation of the risk-reducing hysterectomy specimen

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**Background:** Lynch Syndrome (HNPCC)

- Autosomal dominant mode of inheritance
- Predisposes to numerous malignancies – not just colon
- Often early age of onset
- One defective allele is inherited; 2\textsuperscript{nd} “hit” happens during patient’s lifetime
Background: Lynch Syndrome (HNPCC)

- Colorectal & endometrial
- Ureter and renal pelvis
- Ovarian
- Pancreas
- Stomach
- Biliary tract
- Brain (usually glioblastoma as seen in Turcot syndrome) tumors
- Sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome
- Small bowel
Background: Lynch Syndrome (HNPCC)

• Due to germline mutations in mismatch repair (MMR) genes*
  – 4 genes have been identified:
    • MSH2, MSH6, MLH1, and PMS2
  – Epigenetic methylation of *MLH1* can also lead to dysfunction - not part of Lynch Syndrome (HNPCC)***

• Microsatellites are repetitive sequences that are particularly vulnerable to error without functioning MMR system
  – Microsatellite instability (MSI) can serve as a proxy for impaired MMR
Background: The MMR system

- During DNA replication, insertions or deletions of one or more nucleotides and single nucleotide mismatches may occur.
- MSH2 and MSH6 form a heterodimer and recognize the mismatch.
- MLH1 and PMS2 dimerize and bind to the MSH2-MSH6 complex.
- The complex of four proteins activates an exonuclease to perform the DNA repair.

Background: Microsatellite Instability

- **Microsatellite stable (MSS) tumor:**
  - The size of the microsatellite is the same in DNA isolated from normal and tumor cells

- **Microsatellite unstable (MSI) tumor:**
  - The size of the microsatellite can change (usually becomes shorter) when comparing normal to tumor DNA
Microsatellite Instability (MSI)

- Five mononucleotide microsatellite loci (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) (Promega fluorescent multiplex assay)
- Allelic profiles from the normal and malignant tissue are compared
- MSI-H = 2 or more abnormal profiles
- MSI-L = 1 abnormal profile
- MSS = no abnormal profile
- MSI-H vs MSI-S/MSI-L
**Background: Microsatellite Instability**

- If no mutations in 5 microsatellite sequences=MSS
- If mutation in 1 of 5 microsatellite sequences=MSS-L
- If mutations in 2 or more of 5 microsatellite sequences=MSS-H
Background: MSI PCR Problems

- Insufficient tumor cell nuclei
- Excess mucin – esp problematic with colloid colorectal carcinomas
- May not identify MSH6 MMR protein deficient cases (MSS or MSI-L)
Background: Mismatch Repair Protein (IHC)

• MLH1 and PMS2 dimer: MLH1 is dominant
• MSH2 and MSH6 dimer: MSH2 is dominant
• Four possible patterns of expression on IHC
• All 4 intact = MMR proficient (pMMR)
• Loss of 1 or 2 = MMR deficient (dMMR)
Background: Patterns of Loss

- MLH1/PMS2
- MSH2/MSH6
- MSH6
- PMS2
Background: MMR IHC Problems

- Numerous TILs may create false impression of intact MMR expression in tumor nuclei
- MSH6 may be heterogeneous – need to evaluate entire tumor
- Absence of internal positive control – if tumor nuclei negative, test can only be interpreted as equivocal
- NOTE: MMR IHC is fixation dependent
Epigenetic Methylation in Colorectal Cancer: MLH1

- Common in colorectal carcinomas
- Occurs in left and right sided tumors
- Trend for older individuals
- Trend for females
- May show differential response to standard (5-FU) chemotherapy
- Can be detected by \textit{BRAF} mutation
Epigenetic Methylation in Endometrial Cancer: MLH1

- Common in endometrial cancer
- Endometrioid & mixed endometrioid/mucinous histology
- Average age: 65 years (range: 42-88)
- Majority (86%, 44/51) located in the uterine fundus
- Cannot be detected by BRAF mutation

**Lynch Syndrome (HNPCC): Endometrial Cancer**

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<th>Endometrial Cancer</th>
<th>Colorectal Cancer</th>
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<tr>
<td><strong>Age at presentation</strong></td>
<td>45-55 years</td>
<td>35-45 years</td>
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<tr>
<td><strong>DNA MMR mutations</strong></td>
<td>$MSH6 &gt; MSH2 &gt; MLH1$</td>
<td>$MLH1 = MSH2 &gt; MSH6$</td>
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<td><strong>MSI-H</strong></td>
<td>70%</td>
<td>95%</td>
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Lynch Syndrome (HNPCC): Endometrial Cancer [Population Based Data]

- MSH2/MSH6 (24) >> MSH1: (2)
- Almost 75% >50 years of age
- Approx 10% LUS
- Approx 33% TILs
- Grade 1 (48%) > Grade 2 (26%) or Grade 3 (26%)

Lynch Syndrome (HNPCC): Endometrial Cancer [Population Based Data]

- Endometrioid (21)
- Mixed (1)
- Serous (1)
- Clear (1)
- Mucinous (1)
- Carcinosarcoma (1)

Uterine Endometrioid Tumors in Lynch Syndrome (HNPCC)

- Composite low grade and high grade undifferentiated carcinoma
- Tumor infiltrating lymphocytes
- Trend to origin in lower uterine segment
Nonendometrioid Carcinoma Types

- Clear cell carcinoma
- Undifferentiated carcinoma
- Mucinous carcinoma
- Carcinosarcoma
- Serous carcinomas
Lynch Syndrome (HNPCC): Ovarian Cancer [Population-Based Data < 50 Years Of Age] (n=54)

- MSH2 > MLH1
- Clear cell (3/4)
- Undifferentiated (1/4)
- Unilateral, organ-confined (3/4)
- Synchronous endometrial cancer (1/4)

Lynch Syndrome (HNPCC): Ovarian Cancer [Registry Data]

- Most < 50 years of age
- MSH2 > MLH1
- Unilateral, organ-confined (7/15)
- Synchronous endometrial cancer (4/15)

Lynch Syndrome (HNPCC): Ovarian Cancer [Registry Data]

- Clear cell
- Endometrioid
- Serous
- Mucinous
- Mixed

Lynch Syndrome: What About Cervix?

- No reported association
- Stanford study: 60 cervical adeno, incl 13 in situ & 10 minimal deviation*
- Anecdotal experience: cervical adenocarcinoma in LS patient
  All 4 mismatch repair proteins intact

Lynch Syndrome: What About Lower Uterine Segment (LUS)?

- Prevalence of LS much higher (29%) in patients with tumors localized to LUS when compared to the general endometrial cancer population (1.8%)*

- In this location, distinction from endocervical primary may be difficult, particularly when:
  - atypical morphology
  - IHC stains are inconclusive
  - evidence of HPV-infection is lacking or absent

- So, loss of mismatch repair protein in LUS tumor almost certainly uterine corpus primary

*JCO, 26:2008; 5965-5971
Screening For Lynch Syndrome (HNPCC)

- Clinical
- Pathological
- Clinical & pathological
- All endometrial cancers
### Amsterdam Criteria I

1. Three or more family members with a confirmed diagnosis of colorectal cancer, one of whom is a first degree (parent, child, sibling) relative of the other two.
2. Two successive affected generations.
3. One or more colon cancers diagnosed under age 50 years.
4. Familial adenomatous polyposis (FAP) has been excluded.
<table>
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<th>Amsterdam Criteria II</th>
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<tbody>
<tr>
<td>➢ Three or more family members with HNPCC-related cancers, one of whom is a first degree relative of the other two</td>
</tr>
<tr>
<td>➢ Two successive affected generations</td>
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<tr>
<td>➢ One or more of the HNPCC-related cancers diagnosed under age 50 year</td>
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<tr>
<td>➢ Familial adenomatous polyposis (FAP) has been excluded</td>
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Bethesda Criteria

- Colorectal cancer diagnosed in a patient who is less than 50 years of age
- Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors, regardless of age
- Colorectal cancer with the MSI-H histology diagnosed in a patient who is less than 60 years of age
- Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age of 50 years.
- Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age
SGO Criteria (20-25% Risk)

- Patients with endometrial or colorectal cancer who meet the revised Amsterdam criteria
- Patients with synchronous or metachronous endometrial and colorectal cancer with the first cancer diagnosed prior to age 50
- Patients with synchronous or metachronous ovarian and colorectal cancer with the first cancer diagnosed prior to age 50
- Patients with colorectal or endometrial cancer with evidence of a mismatch repair defect (i.e. MSI or dMMR)
- Patients with a first or second degree relative with a known mismatch repair gene mutation
SGO Criteria (5-10% Risk)

- Patients with endometrial or colorectal cancer diagnosed prior to age 50
- Patient with endometrial or ovarian cancer with a synchronous or metachronous colon or other Lynch/HNPCC-associated tumor at any age
- Patients with endometrial or colorectal cancer and a first degree relative with a Lynch/HNPCC-associated tumor diagnosed prior to age 50
- Patients with colorectal or endometrial cancer diagnosed at any age with two or more first or second degree relatives† with Lynch/HNPCC-associated tumors, regardless of age
- Patients with a first or second degree relative† that meets the above criteria
Combined Pathology & Clinical Screening: Endometrial Cancer

- Nonendometrioid carcinoma in young women
- Endometrioid carcinoma with numerous tumor infiltrating lymphocytes
- Lower uterine segment localization
- Synchronous ovarian clear cell carcinoma

Memorial-Sloan Kettering Protocol
How Many TILs?

• Few data
• M-S-K: More than 42 per 10 HPF
• Increased TILs not specific for LS (HNPCC): (33% of sporadic methylated endometrial cancer have increased TILs)
IHC testing for loss of MMR protein expression for all endometrial carcinomas

MMR intact per IHC but clinical suspicion of LS

Order LS microsatellite instability by PCR

- Instability at ≥ 2/5 of microsatellite markers
  - High
    - Consider germline testing of mismatch repair genes
  - Indeterminate
    - No instability present

- Instability 1 microsatellite marker
  - Low
    - Methylation present
      - Likely sporadic endometrial carcinoma*
    - Methylation absent
      - Genetic mutation testing for LS: recommend LS MSH1 sequencing and deletion/duplication as first test

Loss of MMR per IHC

- Abnormal staining for MLH1 & PMS2
  - Test for MLH1 promoter methylation
    - Methylation present
      - Genetic mutation testing for LS: recommend LS MSH2 sequencing and deletion/duplication as first test
  - Methylation absent
    - Genetic mutation testing for LS: recommend LS MSH6 sequencing and deletion/duplication as first test

- Abnormal staining for MSH2 and MSH6
  - Genetic mutation testing for LS: recommend LS MSH6 sequencing and deletion/duplication as first test

- Abnormal staining for MSH6
  - Genetic mutation testing for LS: recommend LS MSH6 sequencing and deletion/duplication as first test

- Abnormal staining for PMS2
  - Genetic mutation testing for LS: recommend LS MSH1 sequencing and deletion/duplication as first test

*If strong clinical suspicion for LS, consider MLH1 promoter methylation analysis of non-neoplastic tissue/peripheral blood to evaluated for germline epigenetic MLH1 promoter methylation
2 Antibody Approach

- PMS2 & MSH6
  - Both Intact
  - Loss of PMS2
  - Loss of MSH6

  - Stop
  - MLH1
  - MSH2

Mod Pathol 2011; April 15 [Epub]
2 Antibody Approach

- Effective for colon, endometrial, cutaneous, & sebaceous lesions
- Requires unequivocal positive internal control
- May not be reliable in small samples due to heterogeneity in protein expression, esp. MSH6
- Despite promising results, most labs still performing 4 antibody panel

Mod Pathol 2011; April 15 [Epub]
Risk Reducing Hysterectomy

• Despite reports in literature of dMMR in atypical hyperplasia (or colorectal adenoma), this is rare in Stanford experience

• As with early adenomatous lesions in the colon, the value of MSI & IHC in identifying LS in atypical hyperplasia (or EIN) is not firmly established
Risk Reducing Hysterectomy (RRH)

- Clinical significance of early-onset hyperplasia in known LS (HNPCC) patients unknown No standardized approach
- Section entire endometrium – including entire LUS
- Focus on lower uterine segment
- Limited experience, but small, isolated LUS cancer (no assoc hyperplasia) has been identified in RRH from LS patients
Risk-Reducing Oophorectomy

• Serial section and microscopically examine entire ovaries
• No documented tubal involvement in LS, but we submit entire fallopian tubes using SEE-FIM protocol
Future Directions

• Efficacy of screening
• Significance of early onset precursor lesions
• Identification of dietary and/or chemo-preventative measures
• Identification of rare germline carriers not detected with current tests
• Improved patient counseling
Thank you

Stanford University