Colon Cancer – Lynch Syndrome Tumor Testing

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Outline

- Lynch syndrome/CRC overview
- Genetics of LS
- Clinical characteristics/cancer risks
- Management of risk
- Tumor testing
  - MSI
  - IHC
- Case examples

Colorectal Cancer

- 3rd most common cancer in the U.S.
  - 145,000 new cases per year
- 3rd most common cause of cancer-related death
- Most common form of hereditary CRC is Lynch Syndrome (LS)
- Stepwise progression
  - Benign mucosa → polyp → cancer
- Effective screening → prevention

Epidemiology of Colorectal Cancer

- Sporadic: 60%
- Familial/Multifactorial: 30%
- Lynch Syndrome: ~2-4%
- Rare Syndromes: ~4%

LYNCH SYNDROME REVIEW

Lynch Syndrome
(formerly HNPPC: Hereditary Nonpolyposis Colorectal Cancer)

KEY FEATURES

- CRC (usually dx <50 y)
  - Usually RT-sided, signet ring, mucinous, poorly differentiated, microsatellite instability high (MSI-H)
  - Endometrial cancer <50 y
  - Can present prior to CRC dx in women

Other cancers:
- Ovarian, stomach, urinary tract, pancreatic, small bowel, bile duct, brain, sebaceous cysts

- AD inheritance
- 0 to very few polyps

- GENES: MLH1, MSH2, MSH6, PMS2 → "mismatch repair" genes

Slides Courtesy of WBH Cancer Genetics Program
Lynch Syndrome

- Amsterdam I Criteria, 1991
  - Three or more relatives with CRC, which one case is a first degree relative of the other two
  - At least two successive generations
  - One or more cancers diagnosed before age 50
  - FAP excluded

**Revised in 1999 – Amsterdam II**

Colorectal cancers in LS

- Distinct histologic features
  - Tumor infiltrating lymphocytes
  - “Crohn’s-like” lymphocytic infiltrate
  - Mucinous histology
- Adenoma to carcinoma – rapid progression
- Metachronous cancer risk – 16% at 10 yrs, 41% at 20 yrs
- Defect in mismatch repair (MMR) system

Mismatch Repair Genes

- MSH2 (40%)
- MLH1 (45%)
- PMS2 (5%)
- MSH6 (10%)

MISMATCH REPAIR SYSTEM AND LS

- DNA MMR system maintains genomic integrity by correcting DNA errors during replication
- Recognizes base-pair mismatches and repairs them
- Failure to repair DNA mismatches leads to genomic instability
- Occurs in regions of repetitive nucleotide sequences - microsatellites

EPCAM (TACSTD1)

- TACSTD1 lies upstream of MSH2 promoter
  - Included in LS testing schema
- Deletion in the 3' exons of TACSTD1 gene
  - Leads to hypermethylation of MSH2 promoter
- Leads to silencing of MSH2
  - Accounts for 25% of cases in which MSH2 absent
- Absence of MSH2 expression on IHC can be caused by either: MSH2 mutation or EPCAM mutation (leading to methylation of MSH2)
Red Flags for Lynch Syndrome

- **Lynch Syndrome**
  - Colon/uterine cancer under age 50
  - Presence of one LS-associated cancer in same individual
  - Specific pathological features
  - 3-2-1-0 Amsterdam Criteria
    - 3: 3 relatives with a LS associated tumor
    - 2: Two successive generations affected
    - 1: At least one diagnosed under age 50
    - 0: Polyposis syndrome ruled out

*LS cancers: colorectal, endometrial, gastric, ovarian, urothelial pelvis, biliary tract, small bowel, pancreas, brain, sebaceous adenoma

**CLINICAL PRESENTATION**

Lynch Syndrome - Cancer Risks

- **EC Avg age dx = 47-55**
  - *range of age for endometrial cancer differs by gene

Lynch Syndrome - Cancer Risks

- **Avg age dx = 42-61**
  - *range of age for colorectal cancer differs by gene

**Muir-Torre Syndrome:**

A Variant of HNPCC

Associated with MSH2 or MLH1 mutations

- Has typical features of HNPCC and
  - Sebaceous gland tumors
  - Keratoacanthomas
**Lynch Syndrome: Management**

**Colon Cancer**
- **Surveillance**
  - Colonoscopy – every 1-2 y starting at age 20-25 y

**Surgery**
- Prophylactic Colectomy
  - Consider if:
    - Cancer diagnosis
    - Large polyp burden
    - Pt unwilling to undergo surveillance

**Endometrial/Ovarian Cancer**
- **Surveillance**
  - (no clear evidence to support)
  - Transvaginal U/S
  - Endometrial sampling
  - Starting at age 30-35 y

**Surgery**
- Hysterectomy
- RRSO

**Chemoprevention of LS**
(Burn et al Lancet 10/12)
- CAPP2 Trial – largest LS intervention trial
- 861 pts with LS (UK)
- ASA 600 mg vs placebo
- > 4 y f/u (mean 56 mos)
- 63% reduction in CRC (HR 0.41, p=0.02)
- Also decrease in non-CRC LS cancers
- No protection if < 2 y of intervention
- No increase in ulcers, GI bleed, anemia

**Lynch Syndrome Evaluation**
- **Germline Testing**
  - Molecular genetic testing of the germline genes MLH1, MSH2, MSH6, PMS2 for a deleterious mutation
- **Tumor screening**
  - Microsatellite Instability (MSI)
  - Immunohistochemistry (IHC)

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Germline Testing

- MLH1 and MSH2 (EPCAM) ~90%
- MSH6 ~7-10%
- PMS2 <5%
- Tumor screening (MSI/IHC) allows for targeted germline testing

Lynch Syndrome Testing

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Microsatellite Instability (MSI)

- MSI-H is associated with Lynch syndrome
- MSI-stable and MSI-low is not

Malignant Repair Genes

- MLH1 and MSH2 (EPCAM) ~90%
- MSH6 ~7-10%
- PMS2 <5%
- Tumor screening (MSI/IHC) allows for targeted germline testing

Tumor Tests to Screen for Lynch Syndrome

- Microsatellite Instability (MSI) testing
  - Performed on DNA extracted from tumor and normal tissue
  - 15% of sporadic CRC cases are MSI-H
  - Test is positive (MSI-H) in 77-89% of LS cases
  - 5-10% false negative rate
- Immunohistochemistry staining
  - Abnormal (absence of MMR protein) in 10-15% of sporadic CRC
  - 5-10% false negative rate

MSI testing in LS
Lynch Syndrome

- Revised Bethesda Guidelines, 2004

Tumors from individuals should be tested for MSI in the following situations:

1. CRC dx < 50 y.
2. Presence of synchronous or metachronous LS-associated tumors, regardless of age.
3. CRC with MSI-H histology in pt < 60 y.
4. CRC in pt with one or more first-degree relatives with a LS-related tumor, with one of the cancers < 50 y.
5. CRC dx in pt with two or more first- or second-degree relatives with a LS-related cancers, regardless of age.

Immunohistochemistry (IHC)

- Patient 1: Absent MLH1/PMS2
- Patient 2: Absent MSH2/MSH6

Five Possible Results of IHC test:

1. Normal – All 4 Stains Present
   - 80% of the time will get this result
   - May need genetics evaluation if suspect LS, patient dx <45, patient has had multiple CRC primaries (meets Amsterdam II criteria)

2. Abnormal – MLH1 & PMS2 Absent
   - 80% acquired methylation of MLH1
   - BRAF testing
   - 20% will be LS

IHC results – Interpretation

- Normal: “staining present” or “positive staining of MMR protein”
- Abnormal: “Staining absent” or “negative staining for MMR protein”
3. Abnormal – MSH2 & MSH6 Absent

- Most likely LS due to either MSH2 or MSH6 gene mutation
- Possible EPCAM mutation

4. Abnormal – MSH6 Absent

- Most likely LS due to an MSH6 gene mutation

5. Abnormal – PMS2 Absent

- Most likely LS due to an PMS2 gene mutation
- Older age of cancer

LS is under-recognized

- Amsterdam criteria too restrictive
- ~25 - 50% of individuals with LS do not meet Amsterdam or Bethesda criteria
- Accuracy of family history poor
- Estimated 1.2% of all individuals with LS are aware of their LS diagnosis
  
  Solution – Universal Tumor Screening for LS

"The Search for Unaffected Individuals with LS: Do the Ends Justify the Means?" (Hampel '11)

- Population incidence of LS: 1 in 370
- LS: 2.8% of all newly dx CRC
- Estimated 829,747 of the 307,006,550 people in U.S. could have LS
- < 10,000 LS cases currently diagnosed in US
- 1.2% (10,000/829,747) of all individuals with LS are aware of their diagnosis

Universal Tumor Screening

- Reduce the morbidity and mortality from LS-related cancer in at-risk affected and unaffected relatives
- Sufficient evidence to recommend offering Lynch syndrome tumor screening to all individuals with newly diagnosed colorectal cancer
  - Justified from a national health care system perspective
  - ICER is $22,522 (anything below $50K considered cost-effective)
  - The Evaluation of Genomic Applications and Prevention Practice (EGAPP) Guidelines, 2009
EGAPP Guidelines

- Endorsed tumor testing of all patients with CRC (cost-effective)
- Many (71%) NCI designated Cancer Centers have adopted reflex IHC/MSI to screen for LS
- Community hospitals less often (15%)
- LSSN – Lynch syndrome screening network
  - Beaumont a member
  - Beamer et.al. JCO 4/1/12

Lynch Syndrome Testing

The most cost effective strategy involves IHC testing first and subsequent targeted gene testing (Mvundura et al 2010)

- MLH1, MSH2, MSH6, and PMS2 analysis = $4000-4500
- MSI & IHC = $900-1000
- IHC alone = $300-400
- Single Gene Analysis = $1000-1400

Cost-effectiveness data

- Universal tumor testing of all CRC
- Preferred approach:
  - IHC followed by BRAF mutation testing
  - Incremental cost-effectiveness ratio (ICER)
    - $36,200 per life-year gained
  - Requires participation of at-risk relatives (3 or more)

Universal Tumor Screening for LS:
The Beaumont Experience

The Beaumont Experience

IHC for the four Lynch associated proteins initiated January 1, 2012 for ALL newly diagnosed colorectal cancer resections ONLY
- Biopsies may not provide enough normal tissue for internal control
- Infrastructure in place to handle screening results and insure communication and proper follow-up (Cancer Genetics)

The Beaumont Testing Schematic
Beaumont Data 1/1/12 – 6/1/12

- Reflex IHC for MMR proteins (MLH1, MSH2, MSH6, PMS2)
  - 109 IHC performed on colorectal cancer resections
  - 10 currently pending
- 75 of 99 IHCs were normal (76%)
  - Expected 80-85%
- 24 / 99 IHCs were abnormal (24%)
  - Expected 15-20%

IHC Results

CASE EXAMPLES

Case Example 1

- 21-year-old male presented with a bowel obstruction due to carcinomatosis
- Found to have adenocarcinoma of the colon with liver metastases
  - Treatment included a left hemicolectomy and palliative chemotherapy
- Patient was referred to cancer genetics due to his early age of diagnosis of CRC

Expected Observation

CASE EXAMPLES

- Abnormal results:
  - 22 showed absent MLH1 and PMS2 (22%)
    - Expected 15%
  - 20% of those expected to have LS
  - 80% Sporadic etiology (somatic hypermethylation MLH1/BRAF mutation)
  - 2 showed absent MSH6 and MSH2 (2%)
    - Expected 3%
  - Most will likely have LS
  - 0 MSH6 or PMS2 only absent
    - Expected 2%
Case Example 2

- 51-year-old male presented for a routine screening colonoscopy
  - Polyp in right colon
  - Pathology revealed a high grade adenocarcinoma
  - Tumor screening performed following colon resection:
    - IHC loss of MSH6 expression
    - MSI-H 3/6 markers
  - Patient presented to cancer genetics
Summary

- Lynch Syndrome is the most common inherited colorectal cancer syndrome
- Intense cancer surveillance and/or prophylactic surgery decreases morbidity and mortality from LS-related cancers
- Family history and testing criteria alone are insufficient in identifying individuals at risk
- Tumor screening is a cost-effective approach to increasing the detection rate of Lynch Syndrome