Lynch Syndrome/Hereditary Nonpolyposis Colorectal Cancer

Colorectal cancer (CRC) exhibits the characteristics of familial clustering in ~10-15% of cases. The most common cause of hereditary CRC is Lynch syndrome (LS), also known as hereditary nonpolyposis colorectal cancer (HNPCC). LS is caused by a germline mutation in one of the genes within the DNA mismatch repair (MMR) system.

INDICATIONS FOR ORDERING

Microsatellite instability (MSI) and/or immunohistochemistry (IHC) tumor analyses
- Universal screening for LS in all individuals with newly diagnosed CRC
- Universal screening for LS in individuals with endometrial cancer

Germline MMR gene testing
- Order following abnormal screening test result
- Confirms diagnosis of LS

DISEASE OVERVIEW

Incidence
- Accounts for 2-4% of CRC and ~1-2% of endometrial cancers
- Most common hereditary CRC syndrome
- Most common cause of hereditary endometrial cancer

Risk Estimates
The following lifetime risk estimates apply to individuals with MLH1 and MSH2 pathogenic variants; risks for variants in MSH6 or PMS2 may be lower.
- CRC – 52-82%
- Endometrial – 25-60%
- Prostate – ~30%
- Ovarian – 11-24%
- Gastric – 6-13%
- Hepatobiliary tract – 1-4%
- Urinary tract – 1-7%
- Small bowel – 3-6%
- Brain/central nervous system – 1-3%
- Sebaceous neoplasms – 1-9%
- Pancreatic – 1-6% (MLH1 and MSH2 only)

GENETICS

Genes Tested
MLH1, MSH2, MSH6, PMS2, and EPCAM
Inheritance – autosomal dominant

TEST INTERPRETATION

Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR
- Sensitivity/specificity
  - Clinical sensitivity – 90%
  - Analytical sensitivity/specificity – >99%
BRAF Codon 600 Mutation Detection with Reflex to MLH1 Promoter Methylation
- Analytical sensitivity
  - Methylation levels >10% are reported as positive
- Results
  - BRAF V600E detected

Mismatch Repair by IHC
- Clinical sensitivity
  - 90% 1,2
- Results
  - Normal - MMR proteins are normally expressed
    - MMR deficiency is unlikely
    - LS unlikely
  - Abnormal - MMR protein expression is abnormal
    - Loss of expression of 1 or more proteins is highly predictive of MMR deficiency
    - Absence of both MLH1 and PMS2
      - MLH1 germline pathogenic variant is possible
        - Consider MLH1 methylation ± BRAF V600E studies
        - If methylation and BRAF studies are negative, follow with MLH1 germline genetic testing
    - Absence of PMS2 only
      - PMS2 germline pathogenic variant likely
        - Consider PMS2 germline testing
        - If PMS2 testing does not identify a germline pathogenic variant, consider MLH1 germline testing
    - Absence of both MSH2 and MSH6
      - MSH2 germline pathogenic variant likely
        - Consider MSH2 germline testing
    - Absence of MSH6 only
      - MSH6 germline pathogenic variant likely
        - Consider MSH6 germline testing
        - If MSH6 testing does not identify a germline pathogenic variant, consider MSH2 germline testing
- Limitations
  - ~10% of individuals with LS will have IHC tests that show normal staining of the MMR proteins
  - Because the correlation of MSI with IHC is not 100%, direct testing of MSI by PCR may be helpful
  - Screens for LS only and does not evaluate other hereditary causes of CRC or endometrial cancer

Limitations
- 10-15% of sporadic CRCs are also MSI-H 1,2
- Preoperative chemoradiation of rectal cancer
  - May complicate IHC interpretation and/or decrease tumor mass
  - May make MSI testing difficult
  - Evaluation of pretreatment biopsies will avoid this limitation
- Screens for LS only and does not evaluate other hereditary causes of CRC or endometrial cancer

Results
- High MSI (MSI-H)
  - MMR deficiency, either sporadic or LS
- Indeterminate MSI (MSI-I)
  - Instability in even 1 mononucleotide repeat can be associated with LS
  - Follow-up IHC studies are recommended
- Microsatellite stable (MSS)
  - LS unlikely

Mismatch Repair by Immunohistochemistry with Reflex to MLH1 Promoter Methylation
- Recommended reflex test to differentiate between LS and sporadic CRC in tumors showing loss of MLH1
- If no BRAF variant is detected, MLH1 promoter methylation is evaluated

MLH1 Promoter Methylation, Paraffin
- Method: Real-Time Polymerase Chain Reaction/Fluorescence Resonance Energy Transfer
- Recommended test to distinguish between LS and sporadic non-CRC tumors with loss of MLH1

Diagnostic Germline Genetic Studies
- Specimen – peripheral blood
- Germline genetic testing is available for all 4 MMR genes known to cause LS, either separately or as part of the hereditary gastrointestinal (GI) cancer panel (see Related Tests)

HNPCC/Lynch Syndrome (MLH1)
- Sequencing and Deletion/Duplication
- Method: Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification
- Detect germline MLH1 variants
- Use in MMR-deficient carcinoma with suggestive IHC results (loss of MLH1 and PMS2 proteins), negative for the BRAF codon 600 pathogenic variant, and with normal MLH1 promoter methylation studies

HNPCC/Lynch Syndrome (MSH2)
- Sequencing and Deletion/Duplication
- Method: Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification
- Detect germline MSH2 variants
- Use in MMR-deficient carcinoma with suggestive IHC results (loss of MLH1 and PMS2 proteins), negative for the BRAF codon 600 pathogenic variant, and with normal MLH1 promoter methylation studies

Results
- High MSI (MSI-H)
  - MMR deficiency, either sporadic or LS
- Indeterminate MSI (MSI-I)
  - Instability in even 1 mononucleotide repeat can be associated with LS
  - Follow-up IHC studies are recommended
- Microsatellite stable (MSS)
  - LS unlikely

Mismatch Repair by IHC
- Clinical sensitivity
  - 90% 1,2
- Results
  - Normal - MMR proteins are normally expressed
    - MMR deficiency is unlikely
    - LS unlikely
  - Abnormal - MMR protein expression is abnormal
    - Loss of expression of 1 or more proteins is highly predictive of MMR deficiency
    - Absence of both MLH1 and PMS2
      - MLH1 germline pathogenic variant is possible
        - Consider MLH1 methylation ± BRAF V600E studies
        - If methylation and BRAF studies are negative, follow with MLH1 germline genetic testing
    - Absence of PMS2 only
      - PMS2 germline pathogenic variant likely
        - Consider PMS2 germline testing
        - If PMS2 testing does not identify a germline pathogenic variant, consider MLH1 germline testing
    - Absence of both MSH2 and MSH6
      - MSH2 germline pathogenic variant likely
        - Consider MSH2 germline testing
    - Absence of MSH6 only
      - MSH6 germline pathogenic variant likely
        - Consider MSH6 germline testing
        - If MSH6 testing does not identify a germline pathogenic variant, consider MSH2 germline testing
- Limitations
  - ~10% of individuals with LS will have IHC tests that show normal staining of the MMR proteins
  - Because the correlation of MSI with IHC is not 100%, direct testing of MSI by PCR may be helpful
  - Screens for LS only and does not evaluate other hereditary causes of CRC or endometrial cancer

BRAF Codon 600 Mutation Detection with Reflex to MLH1 Promoter Methylation
- Analytical sensitivity
  - Methylation levels >10% are reported as positive
- Results
  - BRAF V600E detected
Germline Genetic Studies (MLH1, MSH2, MSH6, PMS2, or EPCAM)

- Sensitivity/specificity
  - Proportion of LS attributed to pathogenic variants in specific MMR gene
    - MLH1 – 50% 4
    - MSH2 – 40% 4
    - MSH6 – 7-10% 5,6,7
    - PMS2 – <5% 8
    - EPCAM – ~1-3% 9
  - Analytical sensitivity/specificity – 99%

- Results
  - Positive – 1 pathogenic variant detected
    - Predicted to be causative for LS
  - Negative – no pathogenic variants detected
    - Diagnosis of LS unlikely, but not excluded
  - Inconclusive – variant detected, but whether it is benign or pathogenic is unknown

- Limitations
  - Not evaluated
    - Regulatory region and deep intronic variants
    - Sequence variants and large deletion/duplications in genes other than MLH1, MSH2, MSH6, and PMS2
    - Sequence variants in EPCAM
    - Large deletions/duplications in EPCAM, other than exon 9
    - Large gene inversions, other than the MSH2 10 Mb exons 1-7 inversion
    - Causes of hereditary CRC or endometrial cancer other than LS
  - Diagnostic errors can occur due to rare sequence variations

REFERENCES


