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 Lynch syndrome (LS) in all individuals with newly diagnosed colorectal cancer (CRC) (NCCN, 2018)
- Screening for LS in individuals with endometrial cancer (ASCO, 2015)
Germline mismatch repair (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 (NCCN, 2018; Chadwick, 2001)
- 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 (NCCN, 2018)
- 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% (NCCN, 2018)
  - Analytical sensitivity/specificity – >99%

TESTS TO CONSIDER

Screening Studies (Requires Pathological Tissue)

Mismatch Repair by Immunohistochemistry 0049302
Method: Qualitative
- First-line screening test for LS
- Highly recommended prior to ordering germline MMR gene testing
- Directs subsequent genetic diagnostic testing

Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR 0051740
Method: Polymerase Chain Reaction/Fragment Analysis
- First-line screening test for LS
- Directs subsequent genetic diagnostic testing for LS

Mismatch Repair by Immunohistochemistry with Reflex to BRAF Codon 600 Mutation and MLH1 Promoter Methylation 2002327
Method: Qualitative
- Immunohistochemistry/Qualitative Real-time Polymerase Chain Reaction
  - Preferred screening test for LS in individuals with CRC
  - If MLH1 IHC is abnormal, evaluations of BRAF codon 600 and, possibly, MLH1 methylation are performed
  - Definitive diagnosis of LS requires additional targeted MMR germline molecular studies
  - Do not use in endometrial cancer

BRAF Codon 600 Mutation Detection with Reflex to MLH1 Promoter Methylation 0051750
Method: Polymerase Chain Reaction/Pyrosequencing
- 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
• Results
  • High MSI (MSI-H)
    • MMR deficiency, either sporadic or LS
  • Indeterminate MSI (MSI)
    • Instability in even 1 mononucleotide repeat can be associated with LS
    • Follow-up IHC studies are recommended
  • Microsatellite stable (MSS)
    • LS unlikely

• Limitations
  • 10-15% of sporadic CRCs are also MSI-H (NCCN, 2018)
  • 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

Mismatch Repair by IHC
• Clinical sensitivity
  • 90% (NCCN, 2018)

• 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
    • Presence in MMR-deficient CRC indicates the tumor is probably sporadic and not associated with LS
    • Further germline testing not typically indicated

Mismatch Repair by Immunohistochemistry with Reflex to MLH1 Promoter Methylation 20025270
Method: Qualitative Immunohistochemistry/Quantitative Real-time Polymerase Chain Reaction
• Preferred reflex screening test for LS in non-CRC tumors (e.g. endometrial carcinoma)
• If MLH1 expression is lost, MLH1 methylation is performed

MLH1 Promoter Methylation, Paraffin 2002499
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 0051650
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 0051654
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 MSH2 and MSH6 proteins), includes evaluation of EPCAM exon 9 deletions and 10 Mb inversion of MSH2 exons 1-7

HNPCC/Lynch Syndrome (MSH6) Sequencing and Deletion/Duplication 0051656
Method: Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification
• Detect germline MSH6 variants
• Use in MMR-deficient carcinoma with suggestive IHC results (isolated loss of MSH6 protein)

HNPCC/Lynch Syndrome (PMS2) Sequencing and Deletion/Duplication 0051737

**Germline Genetic Studies (MLH1, MSH2, MSH6, PMS2, or EPCAM)**

- **Sensitivity/**specificity:
  - Proportion of LS attributed to pathogenic variants in specific MMR gene
  - MLH1 – 50% (Smith 2016)
  - MSH2 – 40% (Smith 2016)
  - MSH6 – 7-10% (Myaki 1997; Berends 2002; Petomaki 2003)
  - PMS2 – <5% (Senters 2008)
  - EPCAM – ~1-3% (Kuiper 2011)
  - 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 inversion
  - Causes of hereditary CRC or endometrial cancer other than LS
  - Diagnostic errors can occur due to rare sequence variations

**REFERENCES**


**RELATED TESTS**

**Hereditary Gastrointestinal Cancer Panel, Sequencing and Deletion/Duplication 2013449**

Method: Massively Parallel Sequencing/Exonic Oligonucleotide-based CGH Microarray/Sequencing/Multiplex Ligation-dependent Probe Amplification