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

Screening Studies (Requires Pathological Tissue)

Mismatch Repair by Immunohistochemistry 0049302
Method: Qualitative Immunohistochemistry
- 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
Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR

- Sensitivity/specificity
  - Clinical sensitivity – 90% (NCCN, 2018)
  - Analytical sensitivity/specificity – >99%

- 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

- 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

If no BRAF variant is detected, MLH1 promoter methylation is evaluated

Mismatch Repair by Immunohistochemistry with Reflex to MLH1 Promoter Methylation 2005270
Method: Qualitative Immunohistochemistry/Qualitative Real-time Polymerase Chain Reaction

- Preferred reflex screening test for LS in non-CRC tumors (eg, 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
**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
  - MLH1 promoter methylation detected
    - Presence in an MSI CRC indicates the tumor is probably sporadic and not associated with LS
    - Further germline testing not typically indicated
  - No variants detected
    - In MSI-H tumors with loss of MLH1 protein by IHC, MLH1 germline testing indicated

**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% (Miyaki 1997; Berends 2002; Petomaki 2003)
    - PMS2 – <5% (Senter 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 exons 1-7 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