Microsatellite Instability and Immunohistochemistry Testing for Lynch Syndrome and Sporadic Colorectal Cancers

Background

Microsatellites are short, repetitive DNA sequences that are prone to errors during DNA replication, particularly in the setting of abnormal DNA mismatch repair (MMR). Microsatellite instability (MSI) is characterized by alterations in the length of these sequences. It indicates a failure to repair certain errors during DNA replication and serves as a marker of a deficiency in at least one of the DNA mismatch repair proteins. Combined analysis of MSI and immunohistochemistry (IHC) for DNA MMR protein expression provides a sensitive and specific method for identifying tumors with defects in the DNA mismatch repair complex.

Clinical Indications

Lynch Syndrome Screening

Lynch syndrome (LS), which is also known as hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant hereditary cancer syndrome that predisposes patients to colorectal, uterine, gastric, ovarian and other tumors. It is caused by germline mutations in the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2*, as well as germline deletions in *EPCAM*. At least 90% of Lynch syndrome patients can be detected through MMR testing (MSI or IHC).

In 1999, the Amsterdam II Criteria were published (see below) to identify individuals who may have Lynch syndrome[1]:

- Three or more family members diagnosed with an HNPCC-related cancer (colorectal, endometrial, stomach, small intestine, hepatobiliary, renal pelvic or ureteral), one of whom is a first-degree relative of the other two
- Two successive affected generations
- One or more of the HNPCC-related cancers diagnosed before age 50 years
- Familial adenomatous polyposis has been excluded

In 2009, the Evaluation of Genomic Applications in Practice and Prevention recommended that all colorectal cancers undergo MSI and/or IHC testing.[2] At Cleveland Clinic, all surgically resected colorectal cancers (except those related to inflammatory bowel disease or polyposis) undergo MSI testing.

Prognostic/Predictive Role

In the landmark study by Ribic *et al*[3], patients with stage II and stage III colorectal carcinomas were stratified according to MSI status and randomized to 5-FU chemotherapy or surgery alone. As the table below shows, there is significantly better five-year survival in MSI-High carcinoma. It also reveals loss of this survival advantage when patients with MSI-High carcinomas receive 5-FU.

Survival in Colorectal Carcinoma According to MSI and 5-FU treatment

	No 5-FU 5-year survival	5-FU 5-year survival
MSI-High	88%	71%
MSI-Stable/MSI-Low	68%	76%
P-value	0.004	0.66

Accordingly, the National Comprehensive Cancer Care Network (NCCN) recommends MMR testing should also be considered for all patients with stage II disease.[4]

Interpretation

MSI-high (MSI-H): The neoplasm exhibits instability in at least two of the five microsatellites analyzed.

MSI-low (MSI-L): The neoplasm exhibits instability at only one microsatellite locus.

Microsatellite stable (MSS): The neoplasm exhibits no alterations in any of the microsatellites tested.

Note: All lesions classified as MSI-H undergo immunohistochemistry for MLH1 and MSH2 because one of these two proteins are implicated in the vast majority of MSI-H carcinomas. If MLH1 is lost, then *BRAF* V600E mutation testing is undertaken; see *BRAF* Technical Brief for additional information. If MLH1 and MSH2 are normally expressed in the carcinoma nuclei, then IHC for PMS2 and MSH6 are tested.

Limitations of the Assay

Both MSI and IHC are imperfectly sensitive for the detection of LS/HNPCC; however MSI is slightly more sensitive (≈ 5%) than IHC because not all proteins in the DNA MMR complex can be tested by IHC and non-functional proteins may be antigenically detectable by IHC.

MSI requires a relatively pure sample of tumor, and we aim for at least 50% tumor cells to be confident in the results of the analysis. A pathologist screens the slide(s) and carefully marks the most dense area of tumor for microdissection in the lab. Borderline cases (about 25-50%) will be backed up by IHC, and cases with scant carcinoma cells (less than about 25%) will only receive IHC testing.

IHC in the evaluation of MMR suffers from capricious staining. Both false positive and false negative results are well-documented in the literature. Loss of expression is only interpreted if the internal positive control cells (non-lesional inflammatory cells, normal epithelium or mesenchyme) retain their normal nuclear expression. In order to reduce opportunities for diagnostic errors as well as because of the slightly reduced sensitivity of IHC, Cleveland Clinic prefers to use the IHC tests as a followup to MSI testing or a backup to technically challenging cases.

Methodology

DNA is extracted from the paraffin-embedded neoplastic tissue sections and from separate non-neoplastic paraffin-embedded tissue sections or a blood sample from the patient. PCR amplification is performed using an MSI multiplex system, which includes five mononucleotide markers (*BAT 25*, *BAT 26*, *NR 21*, *NR 24*, and *MONO 27*) in addition to two penta-nucleotide markers to confirm sample identity (*Penta C* and *Penta D*). The PCR products are analyzed by capillary gel electrophoresis. By comparing the sizes of the PCR products from normal and abnormal samples, the presence of MSI is determined by the appearance of new alleles in the lesional sample that are not present in the corresponding normal sample.

This system meets the recommendations proposed at the 2002 National Cancer Institute's workshop on HNPCC and MSI testing.[6]

References

1. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. *Gastroenterology*. 1999;116:1453–6.
2. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med*. 2009;11:35-41.
3. Ribic CM, Sargent DJ, Moore MJ, *et al*. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med*. 2003;349:247-257.
4. National Comprehensive Cancer Network, NCCN Clinical Practice Guidelines in Oncology, Version 3.2013 Colon Cancer, COL-D, 2012. Available at http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site.
5. Sargent DJ, Marsoni S, Monges G, *et al*. Defective mismatch repair as a predictive maker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J. Clin Oncol*. 201-;28:3219-3226. Available at http://www.ncbi.nlm.nih.gov/pubmed/20498393.
6. Bacher JW *et al*. Development of a fluorescent multiplex assay for detection of MSI-High tumors. *Dis Markers.* 2004; 20:237-250.

Cleveland Clinic

Cleveland Clinic Laboratories

Test Overview

Test Name	Microsatellite Instability (MSI)
Reference Range	Microsatellite stable (MSS): No instability detected in five microsatellites analyzed. MSI-Low: Instability detected in one of five (<40%) microsatellites analyzed. MSI-High: Instability detected in two or more (at least 40%) microsatellites analyzed.
Specimen Requirements	Tumor tissue: 5 -10 unstained sections of formalin-fixed, paraffin-embedded tissue on charged, unbaked slides (1-2 cm^2 total tumor area is ideal) OR 1 formalin-fixed paraffin block containing representative tumor tissue. Normal control tissue: 5-10 unstained sections of formalin-fixed, paraffin-embedded tissue on charged, unbaked slides (1-2 cm^2 total tumor area is ideal) OR 1 formalin-fixed paraffin block containing representative tumor tissue OR 5 mL peripheral blood in EDTA.
Test Ordering Information	Submit specimens with an Anatomic Pathology request form. Indicate Microsatellite instability (MSI)
Billing Code	82447
CPT Codes	83891 x 2; 83901 x 2; 83894 x 2; 83912

Technical Information Contacts:

Christine Dziekan, MT(ASCP)
216.444.8444
dziekac@ccf.org

Wendy Nedlik, MT(ASCP)
216.444.8410
nedlikw@ccf.org

Scientific Information Contact:

Thomas Plesec, MD
216.636.9707
plesect@ccf.org