Microsatellite Instability Testing in Colorectal Carcinoma: A Practical Guide

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In this month’s “Road Ahead” article, we continue last month’s theme that focused on the role of pathology in the care of our GI patients. Many gastroenterologists have successfully incorporated pathology into their core practices either within a business infrastructure or as part of a larger health care system. Dr. Gibson and her colleagues at Yale University School of Medicine have helped inform us about a particularly difficult management problem: how to handle the genetically high-risk patients we see frequently in our hospitals and endoscopy units. New comprehensive practice guidelines concerning management of hereditary colon cancer syndromes are in development, but this short article provides a clear and concise guide for the practicing gastroenterologist.

John I. Allen, MD, MBA, AGAF
Special Section Editor

As health care reform progresses, the pressure to more closely integrate clinical service lines such as colorectal cancer (CRC) management has intensified. The practicing gastroenterologist may find that they are not equipped to understand pathology information required for coordinated team-based care of their patients. This is especially true in the case of molecular classification of colorectal cancer, something that has become a standard component of comprehensive oncologic care and has been incorporated into many gastroenterology (GI) practice pathology services. Molecular characterization and classification of colorectal cancer not only provides insight into the pathogenesis of cancer but has prognostic and therapeutic implications and is important for the gastroenterologist to understand and manage well. This review is a practical guide to the most common molecular tests used in what has become standard GI practice.

Molecular Classification of Colorectal Cancer

The molecular classification of colon cancer is based on the cumulative study of precursor lesions (such as adenomas and sessile serrated polyps), inherited colon cancer syndromes (such as familial adenomatous polyposis syndrome and Lynch syndrome/hereditary non-polyposis colon cancer), and molecular profiling of colorectal cancers. Broadly, colorectal cancers are divided into 2 general groups based on genomic differences: chromosomal instability, accounting for 75% to 80% of all colorectal cancers, and microsatellite instability (MSI), accounting for 15% to 20% of all colorectal cancers.1,2 Inherited colorectal susceptibility syndromes are estimated to account for approximately 1% to 2% of the MSI cancers and less than 1% of chromosomal instability cancers.

Microsatellite Instability Pathway

MSI is defined by changes of microsatellite length (repetitive noncoding DNA sequences) resulting from deficient mismatch repair (dMMR) during DNA replication.1,3,4 The protein complex responsible for mismatch repair function is a tetramer composed of 2 heterodimers: MLH1/PMS2 and MSH2/MSH6.4 The expression of each protein in a heterodimer is dependent on

Abbreviations used in this paper: CRC, colorectal cancer; dMMR, deficient mismatch repair; GI, gastroenterology; IHC, immunohistochemistry; MSI, microsatellite instability; MSS, microsatellite stable; PCR, polymerase chain reaction.

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its partner, such that if one protein is absent, the partner protein consequently is degraded. When this occurs, the heterodimer is not available to form a functional tetramer and dMMR, as manifested by MSI, is the result.

Most dMMR/MSI cancers occur sporadically and are associated with the loss of MLH1 expression owing to epigenetic silencing of the MLH1 gene promoter via CpG island methylation. The precursor lesion of sporadic dMMR/MSI cancers is believed to be the sessile serrated polyp, an epithelial proliferation characterized by the V600E BRAF mutation. Therefore, sporadic dMMR/MSI cancers also frequently harbor the V600E BRAF mutation.5

Approximately 1% to 2% of dMMR/MSI cancers occur in the setting of Lynch syndrome as a result of a hereditary gene defect in 1 of the 4 MMR genes.6,7 The most frequently mutated gene in Lynch syndrome patients is MSH2 (40%), followed by MLH1 (30%). MSH6 and PMS2 are mutated at lesser frequencies, approximately 15% each. In contrast to the sporadic setting, dMMR/MSI cancer in Lynch syndrome patients arises from adenomas without BRAF mutations. Therefore, cancers in Lynch syndrome patients will have a wild-type BRAF gene.5

### Technical Aspects of Testing

**Methods of Deficient Mismatch Repair/ Microsatellite Instability Detection**

dMMR is detected by immunohistochemistry (IHC) and MSI is detected by polymerase chain reaction (PCR).8 PCR involves extraction of DNA from a tumor followed by DNA amplification of microsatellite markers, and determination of the amplified microsatellite lengths as compared with nontumor DNA from the same patient. Although laboratories vary with regard to the number of microsatellites tested, most use a standard set of 5 microsatellite markers. A tumor is classified as MSI-high if 2 or more of the 5 microsatellite markers show instability, as MSI-low if only 1 of 5 markers is unstable, and as microsatellite stable (MSS) if the microsatellite markers show no expansion.1,8

The IHC method uses antibodies directed against each MMR protein to detect the expression of the proteins in the tumor cells. In cancers with dMMR/MSI, loss of nuclear expression of MMR proteins is seen in the cancer cells. In contrast, non-neoplastic cells, such as lymphocytes or adjacent colonic mucosa, show preserved nuclear expression of the MMR proteins, irrespective of the hereditary or sporadic setting. The non-neoplastic cells therefore serve as an important internal control for the IHC procedure. Most laboratories test each of the 4 MMR proteins. The majority of dMMR/MSI cancers show loss of expression of both MMR proteins in a heterodimer (either MLH1/PMS2 or MSH2/MSH6) in the cancer cells, with preserved expression of the other heterodimer. In sporadic dMMR/MSI cancers, loss of MLH1/PMS2 expression is characteristic, whereas in Lynch syndrome either heterodimer may be lost.1,6 Occasionally, unusual IHC patterns exist, usually in the setting of Lynch syndrome, such as isolated loss of MSH6 in 10% of cancers or isolated loss of PMS2 in approximately 5% of cancers.6

**Polymearse Chain Reaction vs Immunohistochemistry**

The results obtained from PCR and IHC studies are complementary but provide different information.3,7 The PCR method does not detect which protein in the mismatch repair tetramer is deficient. Therefore, PCR cannot distinguish between sporadic or Lynch syndrome associated dMMR/MSI cancer. IHC, on the other hand, provides specific mismatch repair protein expression data and can suggest etiology. Loss of MSH2/MSH6 suggests Lynch syndrome, whereas loss of MLH1/PMS2, although seen in Lynch syndrome, is characteristic of the more common sporadic dMMR/MSI cancer.6 When present, abnormal IHC results also can be used to guide gene sequencing in patients with a high risk of Lynch syndrome. If the nuclear protein expression of all 4 MMR proteins is intact, the tumor is assumed to be MSS, with rare exceptions, and PCR may not be needed except in patients at high risk for Lynch syndrome.

IHC is inexpensive, is widely available in most pathology laboratories, and can be performed on both biopsy specimens and resection specimens, usually within 1 to 2 days. In the majority of cases, interpretation of IHC expression is straightforward and requires little training, with false-negative results rarely occurring. The latter occurs in less than 10% of Lynch syndrome patients with mutations that lead to protein dysfunction with preserved immunoreactivity.4 PCR analysis is performed on tissue removed from a tissue block containing an adequate tumor sample (at least 30% of the tissue within the block consisting of tumor) for DNA extraction, as well as accompanying normal tissue for comparison. Biopsy samples may not contain sufficient tumor volume for PCR, whereas most resections are sufficient. The turnaround time for PCR is 5 days to 2 weeks.
When to Test and Which Specimen

It now generally is accepted that all patients with colorectal cancer should be tested for MSI/dMMR using either IHC, PCR, or both at some point during the evaluation and treatment of their cancer, regardless of their age. Timing of testing and which specimen is used for molecular testing vary widely among medical centers and GI pathology services. Published guidelines, such as the 2004 Revised Bethesda guidelines and the Revised American College of Gastroenterology 2008 guidelines, are aimed at detecting Lynch syndrome patients post-operatively. However, testing of biopsy material before surgical resection has been advocated by some and has become standard practice in many institutions. At least 2 updated guidelines are in process, one by the US Multi-Society Task Force on Colorectal Cancer and one by the American Gastroenterological Association Institute (John I. Allen, MD, personal communication).

Testing of cancer in the setting of neoadjuvant chemoradiation treatment can be challenging because marked therapy responses limit the amount of cancerous tissue available for DNA extraction. Residual tumor volume may be sufficient for IHC analysis. Pitfalls exist; neoadjuvant therapy has been reported to induce MSH6 loss in 20% of colon cancer. In this setting, comparison with PCR results or prior biopsy samples may be needed.

In addition to testing cancer tissue, dMMR/MSI testing also can be performed on adenomatous tissue in patients with a high risk of having Lynch syndrome based on clinical criteria. However, in this setting, the interpretation of intact MMR expression by IHC should not be used as evidence against the possibility of Lynch syndrome because MMR loss is speculated to occur as a late event in the adenoma-carcinoma sequence.

Initiation of dMMR/MSI testing is best achieved through a coordinated effort between clinicians (gastroenterologists, oncologists, surgeons), pathologists, and genetic counselors. Decisions regarding which test to use (IHC, PCR, or both) are center-dependent. There is a trend at many centers to begin with IHC for dMMR, reserving PCR analysis for specific situations (Figure 1).

Figure 1. MMR IHC algorithm.

*PCR testing for MSI status may be performed at any step to provide additional clarification as to whether genetic counseling is advisable, to provide prognostic information, or to guide chemotherapeutic options. See text for specific information.
Clinical Aspects of Testing

Indications for Mismatch Repair/Microsatellite Instability Testing

Tissue testing for dMMR/MSI serves 2 clinically important functions: to screen for Lynch syndrome and to provide prognostic information regardless of syndrome status. In addition, although beyond the scope of this review, MSI testing is also increasingly used in other investigative contexts, such as to predict therapeutic response to 5-fluorouracil. For these reasons, and because the Amsterdam and Bethesda criteria have failed to serve as effective screens for Lynch syndrome, determination of dMMR/MSI now is recommended in all colorectal tumors.

Relevance to Lynch Syndrome

dMMR/MSI detection serves solely as a screen for Lynch syndrome, and are not by themselves diagnostic. In addition, each test will miss 5% to 15% of all cases of Lynch syndrome. Therefore, a negative tumor screen should not negate a referral to genetic counseling if the personal and/or family history is suggestive of a hereditary cancer syndrome. If IHC shows loss of MLH1 and PMS2 expression, BRAF analysis should be performed. If a V600E BRAF mutation is present and there are no other risk factors (Supplementary Table 1), the patient most likely does not have Lynch syndrome. Outside of this scenario, patients whose tumors are MSI-high and/or show abnormal IHC should be referred to a certified genetic counselor for informed consent to undergo diagnostic germline testing for mutations in the Lynch genes (Figure 1). Informed consent before tumor testing has been raised as an ethical issue; however, it is not currently required because tumor testing is simply a screen for Lynch syndrome. It is advised that clinicians prepare patients for the possibility that if their tumor screen is positive, they will then be referred for genetic counseling and testing.

Tumor screening and genetic testing for Lynch syndrome is critical in preventing additional primary malignancies in the patient, and in testing and providing appropriate surveillance and risk reduction to family members. However, a recent study showed that surgeons referred fewer than half of their patients with high-risk tumors for genetic testing. This represents not only a substantial liability risk for clinicians and their institutions, but a waste of health care dollars and a potentially life-saving lost opportunity for patients and their families. At some centers genetic counselors review all MSI testing, including follow-up methylation/BRAF testing, to ensure correct interpretation and to increase the likelihood that patients will receive the necessary genetic counseling and testing they need.

The cost of dMMR/MSI testing is variable and depends on methods used (IHC and/or PCR). With the decreasing prices of germline gene panels that include not only Lynch syndrome genes but many other genes associated with hereditary cancer syndromes, it soon may be less expensive and more accurate to offer all patients diagnosed with CRC at age 50 years or younger, and those with a personal or family history suggestive of a hereditary cancer syndrome, genetic counseling and testing and to reserve routine tumor testing for those diagnosed with CRC at older than age 50 with no risk factors. In fact, with the cost of genome-wide analysis expected to decrease to less than $1000 within a few years, gastroenterologists may be increasingly confronted with a patient who brings their tumor analysis, showing an alteration in MMR genes. Thus, education about the implications of these findings will become increasingly important.

Relevance of Mismatch Repair/Microsatellite Instability Testing to Prognosis

The diagnosis of dMMR/MSI colorectal cancer has important clinical implications regarding prognosis, and has emerged as an essential component of the evaluation and management of patients with colorectal cancer, especially those with stage II colon cancer.

Retrospective studies have shown that dMMR/MSI colorectal cancer is associated with a favorable prognosis independent of classic clinical prognostic factors, including stage. Patients with dMMR/MSI tumors are less likely to have lymph node and distant metastatic disease than patients with MMR-proficient tumors, and the prevalence of the dMMR/MSI phenotype decreases with advancing stage at diagnosis from more than 20% in stage II to less than 4% in stage IV. In one of the largest pooled analyses of more than 7600 colorectal cancer cases, of which 16.7% were dMMR/MSI, the hazard ratio for overall survival associated with dMMR/MSI was 0.65, and this benefit was confirmed in all stages. In patients with stage II and III colon cancer, recurrence-free survival and overall survival are increased significantly in patients with dMMR/MSI tumors compared with those with MMR-proficient/MSS tumors. These observations, in the aggregate,
support the hypothesis that dMMR/MSI tumors have reduced metastatic potential and a favorable biology compared with MMR-proficient/MSS tumors.

Despite its prognostic value, MMR status is not incorporated into widely used calculators for the assessment of risk of recurrence in stage II and III colorectal cancer and does not figure into colonoscopy surveillance recommendations after cancer resection. Nonetheless, MMR/MSI status should be assessed routinely and considered in risk assessment in all patients with stage II colon cancer because the favorable prognosis of the dMMR/MSI phenotype is a key determinant in the decision to use adjuvant chemotherapy in these patients.

Although BRAF mutation confers a worse prognosis compared with BRAF wild type in dMMR/MSI colorectal cancer, the prognosis of dMMR/MSI colorectal cancer remains superior to MMR-proficient/MSS colorectal cancer, irrespective of BRAF status.5 However, BRAF status in dMMR/MSI colorectal cancer is not yet incorporated routinely into risk assessment or treatment decisions.

Conclusions

In a previous article in this “Practice Management: The Road Ahead” section of Clinical Gastroenterology and Hepatology, the authors described how gastroenterologists might develop a seamless CRC clinical service line including risk assessment for hereditary CRC syndromes.22 In addition, with rapid movement to value-based reimbursement and the potential to create a bundled colonoscopy payment methodology,22 careful consideration of how molecular testing and analysis will be incorporated into our standard CRC prevention practice will be important and challenging. Hopefully, this article will add to the knowledge of practicing gastroenterologists in a direct and useful way.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of Clinical Gastroenterology and Hepatology at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2013.11.001.

References


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Conflicts of interest
The authors disclose no conflicts.
**Supplemental Table 1.** Risk Factors for Lynch Syndrome

<table>
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<th>Risk Factor</th>
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<tr>
<td>CRC diagnosed at age ≤50 y</td>
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<td>Multiple CRC primaries</td>
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<td>A personal and/or family history of the following cancers: uterine, ovarian, colon-rectal, pancreatic, endometrial, gastric, ureter, renal pelvis, biliary tract, brain, small intestine, sebaceous adenoma, and/or carcinoma, especially in families with a history of CRC</td>
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<tr>
<td>Multiple family members in the same blood line with CRC</td>
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<tr>
<td>Abnormal MSI and/or IHC testing</td>
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<tr>
<td>Known familial mutation for hereditary colon cancer</td>
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1. Microsatellite instability (MSI):
   a. reflects a deficient mismatch repair process
   b. is diagnostic for Lynch Syndrome
   c. Indicates variability in the length of repetitive non-coding DNA sequences
   d. Is not found in sporadic colon neoplastic lesions

2. A 51 year old man has colon cancer, it tests positive for MSI, IHC shows absence of MLH1/PMS2, family history is negative.
   a. This would be more typical for sporadic colon cancer
   b. The presence of a BRAF mutation excludes Lynch
   c. He is at high risk for metastatic disease
   d. If the tumor is BRAF negative, there is no need for genetic counseling

True or False

3. All patients with colon cancer should be tested for MSI
4. A dMMR/MSI colon tumor is more aggressive and metastasizes earlier.
5. PCR testing for MSI can differentiate sporadic colon cancer from Lynch syndrome.
6. MSI in Lynch syndrome is most commonly due to a mutation in MSH2
7. MSI in sporadic colon cancer is usually due to loss of MLH1 expression
8. Microsatellite instability accounts for approximately 15% to 20% of all colorectal cancers
9. Sporadic colon cancers with MSI are characterized by the absence of the BRAF mutation
10. The presence of a BRAF gene mutation in a tumor that has MSI excludes Lynch Syndrome if there is no suggestive family history
11. Negative PCR for MSI excludes Lynch
12. Immunostaining for mismatch proteins (IHC) requires normal colonic tissue for comparison
13. A patient with adenomas and a clinical suspicion for Lynch syndrome who tests negative by IHC can be assured of not having Lynch.
14. Tumor testing for MSI requires informed consent by a certified geneticist
15. An MSI (+)/BRAF (+) cancer is more aggressive than a microsatellite stable cancer