Screening for Microsatellite Instability in Colorectal Cancer and Lynch Syndrome - A Mini Review

Minhua Wang, MD, PhD;1 Kazunori Kanehira, MD;2 Frank Chen, MD, PhD3*

1Department of Pathology, Buffalo General Medical Center, SUNY at Buffalo, Buffalo, NY
2Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY
3Quest Diagnostics, Clinical Lab, Medina Hospital, Medina, NY

Colorectal cancers with high frequency microsatellite instability (MSI-H) account for 15-20% of all colorectal cancers (CRC). The familial form of MSI-H CRC is Lynch syndrome, caused by germline mutation in mismatch repair (MMR) genes, accounting for 3-5% of all CRC. Universal screening in newly diagnosed colorectal cancers is recommended by many experts. MSI status is a marker of prognosis and a predictive factor of response to chemotherapy and immunotherapy. MSI DNA testing and immunohistochemical study for MMR proteins are commonly used screening tools, both with high sensitivity and specificity. IHC is an easily accessible and cost effective approach with the advantage over MSI testing of being able to pinpoint the mutated gene. It is widely used as an initial primary test for detection of MSI-H tumors. [N A J Med Sci. 2016;9(1):5-11. DOI: 10.7156/najms.2016.0901005]

Key Words: colorectal cancer, microsatellite instability, MSI, Lynch Syndrome, MLH1, MSH2, MSH6, PMS2

INTRODUCTION
Colorectal cancer is the second leading cause of death from cancer in the United States and the third most common cancer.1 It is now recognized that colorectal cancer arises through three different molecular pathways: chromosomal instability (CIN), microsatellite instability (MSI) caused by mutation in DNA mismatch repair (MMR) genes, and CpG island methylator phenotype (CIMP), an epigenetic change by methylation at CpG-rich sequences (CpG island). In this mini-review, we will focus on MSI caused by mutation in DNA MMR genes and its screening.

MSI-H COLORECTAL CANCERS
Microsatellites are short repetitive segments of DNA sequences, which are prone to mismatch during replication. DNA mismatch errors are normally repaired by MMR protein complexes. Functional loss of the MMR system results in accumulation of DNA errors, a condition of genetic hypermutability i.e. MSI. The high-frequency MSI (MSI-H) phenotype occurs in 15-20% of all colorectal cancers (CRC).2,5 MSI-H CRCs exhibit a number of clinical characteristics, including synchronous and metachronous colonic cancers, prominent lymphoid infiltrate and Crohn-like peritumoral lymphoid reaction, mucinous and signet ring cell histology, an undifferentiated component and medullary growth pattern, a proclivity of occurrence in the proximal colon, and lesser propensity to nodal and systemic metastasis.6-8

The MSI-H CRC can be categorized into familial and sporadic pathways. Sporadic MSI-H colorectal cancers account for 12-15% of all colorectal cancers, which are generally affected by the somatic inactivation of MLH1 gene through hypermethylation at the MLH1 promoter, i.e. CIMP pathway.5 The mutation of BRAF V600E gene is commonly associated with MLH1 hypermethylation, which is distinct in sporadic MSI-H tumors and not observed in tumors with germline mutations.5 The familial form of MSI-H colorectal cancer is Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer syndrome (HNPCC), accounting for 3-5% of all colorectal cancers.1,3,5 In Lynch syndrome patients, failure of MMR genes is caused by inherited germline mutation in one allele followed by inactivation of wild-type MMR gene in the other.5 Lynch syndrome CRC arises almost exclusively within adenomatous precursor lesions, in contrast with sporadic MSI-H CRC where the cancers tend to develop in sessile serrated polyps.10,11

LYNCH SYNDROME
Lynch syndrome is an autosomal dominant disorder, caused by a germline mutation in one of the DNA MMR genes: MLH1,12,13 MSH2,14 MSH615,16 and PMS2.17,18

Constitutional 3’ end deletions of epithelial cellular adhesion molecule (EPCAM) gene is also to cause Lynch syndrome by
epigenetic silencing of MSH2.19,20 The majority of Lynch syndrome can be attributed to the mutation of MLH1 or MSH2, accounting for about 90% of the cases identified. The mutation of MSH6 only accounts for a small portion of Lynch syndrome. Isolated loss of PMS2 is rare in patients with Lynch syndrome (Table 1).21,25

Patients with Lynch syndrome have a high risk of developing many different cancers, including colorectal cancer, endometrial carcinoma, ovary, small bowel, stomach, bladder, ureter, urethra, brain, kidney, biliary tract and gallbladder tumors, and sebaceous adenomas/carcinomas.21,26,27 In Lynch syndrome patients, the cumulative risk by age 70 years for colorectal cancer ranges from 37% to 45% for men, and 22% to 38% for women. For endometrial cancer, the cumulative risk by 70 years is between 32% to 42%. Colorectal cancer is the most common form of cancers seen in Lynch syndrome patients.25

Table 1. Percentages of germline mutations in characteristic patients.

<table>
<thead>
<tr>
<th>Reference Papers</th>
<th>MLH1</th>
<th>PMS2</th>
<th>MSH2</th>
<th>MSH6</th>
</tr>
</thead>
<tbody>
<tr>
<td>21*</td>
<td>51</td>
<td>1</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>22**</td>
<td>42</td>
<td>-</td>
<td>41</td>
<td>5</td>
</tr>
<tr>
<td>23**</td>
<td>34</td>
<td>-</td>
<td>42</td>
<td>-</td>
</tr>
<tr>
<td>24***</td>
<td>-</td>
<td>4.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25*</td>
<td>46.2</td>
<td>-</td>
<td>41.7</td>
<td>6.1</td>
</tr>
<tr>
<td>64*</td>
<td>49</td>
<td>4</td>
<td>45</td>
<td>1</td>
</tr>
</tbody>
</table>

* Patients with Lynch syndrome
** Patients fulfilled the Amsterdam criteria
*** Patients with MSI-H tumors

Figure 1. Typical histopathological features of MSI-H colorectal cancers. A. Tumor with increased numbers of infiltrating lymphocytes and mucinous components. B. Tumor with signet-ring cells. C and D. Poorly differentiated tumor with a medullary growth pattern and prominent infiltrating lymphocytes. Original magnifications X100 (A), X400 (B), X40 (C), and X200 (D).
CLINICAL SIGNIFICANCE OF MSI-H COLORECTAL CANCERS
The identification of MSI-H in patients with colorectal cancer is of clinical importance for several reasons. It is well established that MSI status is a marker for better prognosis and also a predictive factor for the response to fluorouracil (FU) chemotherapy treatment. Many studies show that patients with MSI-H colorectal cancers have better prognosis than those with microsatellite stable (MSS) or low-frequency of microsatellite instability (MSI-L) CRCs. Ribic et al in their study indicated that MSI-H tumors do not respond to fluorouracil (FU) chemotherapy as much as MSS or MSI-L tumors. A recent review by Des Guetz G et al also reports that MSI-H CRC patients do not benefit from FU chemotherapy. In a recent study, Le et al. found that pembrolizumab, an anti–programmed death 1 (PD-1) antibody is more effective in mismatch repair–deficient CRCs. Patients with MSI-high CRC had higher rates of immune-related objective response and higher rates of immune-related progression-free survival than those with MSS CRC. In addition, as MSI-H is a hallmark of MMR function deficiency, the detection of MSI-H is effective in screening potential Lynch syndrome patients who need further germline testing. The diagnosis of Lynch syndrome is essential since the patient has a high risk for developing many other cancers and needs appropriate surveillance. It is also important to identify family members carrying an MMR gene defect because they are at increased risk of developing cancers as well.

![Figure 2. Immunostaining with antibodies against MLH1 (A), MSH2 (B), PMS2 (C), and MSH6 (D) on a colorectal tumor. A, C, absence of MLH1 and PMS2 staining in tumor cells. Normal nuclear staining in stromal cells serves as internal positive control (shown by arrow heads). B, D, nuclear staining is observed for MSH2 and MSH6 in both tumor and stromal cells. BRAF V600E test was subsequently performed, and the patient was found to have BRAF V600E mutation, which exclude the germline mutation. Original magnifications X200 (A-D).](image_url)

SCREENING FOR LYNCH SYNDROME
Screening for Lynch syndrome draws extensive attention due to its significant clinical importance.

Two sets of clinical criteria are used to predict the presence of MMR gene defects. The Amsterdam criteria are clinical guidelines formulated by the international Collaborative Group on HNPCC to help identify families who are likely to have Lynch Syndrome. The Bethesda guidelines were developed by the National Cancer Institute (NCI) to identify patients with CRC who should have supplementary MSI test.
The Bethesda guidelines were revised in 2004 to include family history and characteristic pathologic features of MSI-H CRC.\textsuperscript{34-37} The valuable histologic features include signet-ring cells, lack of dirty necrosis, mucinous features, undifferentiated components, a medullary growth pattern, Crohn-like reaction and prominent lymphocytic infiltration (Figure 1). An accelerated development from adenoma to colorectal cancer also occurs in MSI-H patients.\textsuperscript{37,38} Among these aforementioned features, prominent tumor infiltrating lymphocytes are the single best morphologic predictor of MSI-H.\textsuperscript{39}

Both the Amsterdam criteria and Bethesda guidelines have limited sensitivity and specificity for predicting a germline mutation in patients.\textsuperscript{40} One of the notable deficiencies of these two guidelines is associated with the small size of modern families and inadequate history. Therefore, they are generally no longer recommended to be used as the criteria to exclude individuals with newly diagnosed CRC, as screening with age, family history or histopathologic features will miss a number of patients with Lynch syndrome.\textsuperscript{41,42} Due to the significant clinical importance of identifying patients with MMR germline deficiency, it has been recommended by the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group and Centers for Disease Control and prevention (CDC) that genetic testing for Lynch syndrome has been recommended by the National Cancer Institute (NCI). These 5 markers include 2 quasimonomorphic markers, BAT25 and BAT26, and 3 dinucleotide repeat markers, D2S123, DSS346, and D17S250. Instability observed in two or more out of the 5 markers is designated as MSI-H; with one out of the 5 markers classified as MSI-L, and 0 out of 5 markers as MSS.\textsuperscript{44} In clinical practice, more complete panels of up to 10 markers may be used to determine MSI status. MSI-H is defined as instability at more than 30% of the examined markers, with MSI-low as 10-30% of the markers showing instability, and MSS as less than 10% of the markers showing instability. It should be noted that MSS and MSI-L require analysis of more than five markers. However, the distinction between MSS and MSI-L may not be necessary in clinical setting since MSS and MSI-L tumors display similar phenotypes in most cases.\textsuperscript{35} Caution needs to be taken for tumors with MSH6 deficiency, as they do not show microsatellite instability in dinucleotide markers and can be categorized as MSS or MSI-L phenotype when examined with the NCI panel.\textsuperscript{46,47} A pentaplex panel consisting of five mononucleotide repeats has been recommended to determine the tumor MSI status as recent studies have shown that the sensitivity and specificity of the mononucleotide markers are higher than those of the dinucleotide markers.\textsuperscript{48,49} Another advantage is that the pentaplex panel is highly sensitive to detect MSI-H phenotype in MSH6-deficient tumors which often show MSS/MSI-low using the NCI panel.\textsuperscript{50,51}

| Table 2. Immunohistochemistry staining pattern and affected MMR gene. |
|---------------------------------|------------------|
| IHC staining pattern           | Likely defected gene |
| MLH1+/PMS2-                    | MLH1             |
| MLH1+/PMS2-                    | PMS2             |
| MSH2+/MSH6-                    | MSH2             |
| MSH2+/MSH6-                    | MSH6             |

| Table 3. Immunohistochemistry staining patterns in MSI-H CRC. |
|-----------------|---------------------|---------------|-----------------|---------------|
| Reference Papers | MLH1/PMS2          | Isolated PMS2 | MSH2/MSH6       | Isolated MSH6 |
| 65              | 39                  | 4             | 42              | 18            |
| 60              | 51                  | 4             | 25              | 6             |
| 58              | 66.7                | 1.8           | 12.3            | 17.5          |
| 9               | 85.6                | 1.0           | 10.6            | 1.0           |

**IMMUNOHISTOCHEMISTRY (IHC) FOR THE DETECTION OF MMR PROTEINS**

Immunohistochemical analysis is widely used to identify the loss of one or more of the mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2). These MMR proteins are usually expressed in normal tissue and show positive nuclear staining on IHC. The absence of specific staining suggests an underlying inactivation of one or more MMR genes. The understanding of the roles of MMR proteins in DNA repair is helpful for the interpretation of immunohistochemical staining results. The MMR proteins form heterodimers as a part of the base-excision repair complex, with MLH1 dimerizing...
with PMS2 forming the functional complex, MutSa, and MSH2 dimerizing with MSH6, forming MutLa. MLH1 and MSH2 are obligatory components in the MutSa and MutLa complexes respectively. Therefore, the inactivation of MLH1 or MSH2 protein leads to destabilization of the PMS2 or MSH6 proteins, respectively. Consequently, the inactivation of MLH1 can lead to the concurrent absence of MLH1 and PMS2 in IHC. Likewise, when MSH2 is inactivated, both MSH2 and MSH6 are absent in IHC. However, the reverse is not true. The loss of PMS2 or MSH6 expression does not accompany MLH1 or MSH2 loss. Accordingly, when PMS2 or MSH6 is inactivated, IHC staining for MLH1 or MSH2 most likely remains positive.

Therefore, immunohistochemistry results for MLH1, MSH2, MSH6 and PMS2 in MSI-H tumor most likely show four staining patterns: MLH1-/PMS2-, MLH1+/PMS2-, MSH2-/MSH6- and MSH2+/MSH6- (Table 2). Concordant MLH1-/PMS2- is the most commonly seen pattern, accounting for 39-85.6% of MSI-H cases, and concordant MSH2-/MSH6- accounting for 10.6-42% of cases (Table 3). Isolated PMS2- or MSH6- is infrequent (Table 3). Other anomalous patterns (such as MLH1-/MSH2-/MSH6-, MLH1-/MSH6-, PMS2-/MSH6) are rare, with the combined rate less than 5%. With the exception of MLH1, the absence of PMS2, MSH6 or MSH2 on IHC indicates the mutation of the corresponding genes. Because of the characteristic binding features, the concordant loss of MSH2 and MSH6 suggests a MSH2 mutation. Isolated loss of PMS2 or MSH6 protein on IHC is suggestive of a germline PMS2 or MSH6 mutation respectively. In these circumstances, the next step is to perform genetic testing to confirm the germline mutation. Unlike the other three patterns, the concordant loss of MLH1 and PMS2 reflects the inactivation of MLH1 caused by either hypermethylation or germline mutation. MLH1 hypermethylation is likely the most common cause of this staining pattern. Therefore, if MLH1 expression is absent on IHC, the next step is to do BRAF V600E testing and/or test for MLH1 hypermethylation by methylation-specific PCR for MLH1HM. The sequence of the two tests is not mandated. If the BRAF V600E mutation or MLH1 hypermethylation is confirmed, the MLH1-/PMS2- result on IHC is most likely from the methylation of MLH1 and almost completely excludes the possibility of germline mutation. Only in cases where BRAF V600E is wild-type, further genetic testing needs to be performed.

In a recent study, a two antibody panel comprising of PMS2 and MSH6 has been proposed to replace the 4 panel antibody test for immunohistochemical screening. The two panel antibody test showed the same sensitivity and specificity as the four panel antibody test. The strategy in this study is similar to the 4 panel test: 1. If PMS2 is negative, BRAF V600E test should be performed. If BRAF V600E is mutated, methylation of MLH1 is likely the cause; if not mutated, germline mutation of MLH1 followed by PMS2 should be tested. 2. If MSH6 is negative, germline mutation of MSH2 followed by MSH6 should be tested.

Easy availability and cost effectiveness are two of the advantages of IHC. IHC analysis is helpful in identifying the defective genes within the tumor. The limitation of IHC is mostly due to the confusing staining patterns that produce interpretation difficulty. Three major patterns causing confusion include focal heterogeneous staining, lack of convincing positive internal control and cytoplasmic staining. However, with experience and careful analysis, the interpretation of IHC can be accurate and consistent. The overall sensitivity and specificity of IHC in predicting a germline mutation can be as high as 90%, virtually comparable to that of MSI testing.

The concordance rate between IHC and MSI testing is about 92%. To increase the detection rate, these two tests can be performed synergistically to detect cases that maybe missed by either test alone.

CONCLUSION

Screening for MSI-H colorectal tumors is clinically significant in predicting prognosis and determining the application of adjuvant chemotherapy and immunotherapy in colorectal cancers. Identifying patients with Lynch syndrome has a profound impact on their management, which can lead to significantly reduced morbidity and mortality. Immunohistochemical analysis is a cost effective and valuable tool in detecting MSI-H colorectal tumors and an aid to identifying mutated genes for further genetic tests.

In the future, detection of germline mutation via DNA sequencing may be feasible and affordable and eventually replace current screening tools and diagnostic algorithm.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

REFERENCES


