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Detecting Microsatellite Instabilities to Diagnose Lynch Syndrome in Colorectal Cancer Patients

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Abstract

Detection of microsatellite instabilities (MSI in colorectal cancer (CRC) patients aids in the diagnosis of Lynch Syndrome. Early diagnosis allows the patient and their family to take preventative measures. Lynch Syndrome is a hereditary disease caused by the loss-of-function germline mutations in genes that encode mismatch repair (MMR) factors and is one of the most common cancer susceptibility syndromes. Individuals with Lynch syndrome have a 50-70% lifetime risk of colorectal cancer, 40-60% risk of endometrial cancer, and increased risk for several other malignancies. Lynch Syndrome has led to CRC in patients beginning as early as 20 years old, with the average age of affected individuals being 46 years old. Microsatellite instabilities was the first DNA marker available to identify a hereditary CRC and led to the realization that MSI are a result of defects in the MMR system. Screening for Lynch Syndrome is either carried out by polymerase chain reaction (PCR) of MSI or immunohistochemistry (IHC) testing. There is some debate about which method is most effective, and this literature review will look more closely at this debate about testing methodology. Regions of the MMR genes are also sequenced to look for mutations that may help in diagnosis. There has been a steady increase in Lynch Syndrome tumor screening programs since 2000 and institutions are rapidly adopting a universal screening approach to find the best methodology for testing and interpreting results from CRC patients.

What is Lynch Syndrome

Lynch Syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), is the most common cause of hereditary colorectal (colon) cancer (CRC). Patients with Lynch syndrome are susceptible to several other types of cancers such as uterine (endometrial), stomach, liver, kidney, brain, and certain types of skin cancer. Lynch Syndrome is caused by loss-of-function germline mutations in genes that code for the DNA mismatch repair (MMR) factors. There are four genes that make up the MMR system: MLH1, MSH2, MSH6, AND PMS2. The normal function of the MMR proteins is to proofread the nucleotide sequence for potential base-base errors that occur during DNA synthesis. A defect in any of the four genes can cause microsatellite instabilities (MSI). Microsatellites are short repetitive sequences that are distributed throughout the human genome. MSI consists of a gain or loss in repeat length depending on the defective gene from the MMR. The focus on MSI in studies of CRC was aimed at Lynch Syndrome because of its inherited and unique features. Patients with Lynch Syndrome develop tumors at early ages, often between 20 and 30 years old. These patients frequently have multiple tumors, including those in the colon, rectum, endometrium, stomach, ovary, urinary tract, small intestine, and several other sites. Although Lynch Syndrome accounts for up to 5% of CRCs, affected individuals carry up to 80% lifetime risk for developing CRC and other cancers at an average age of 46.

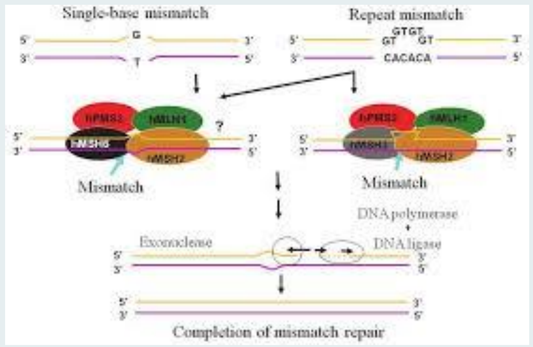
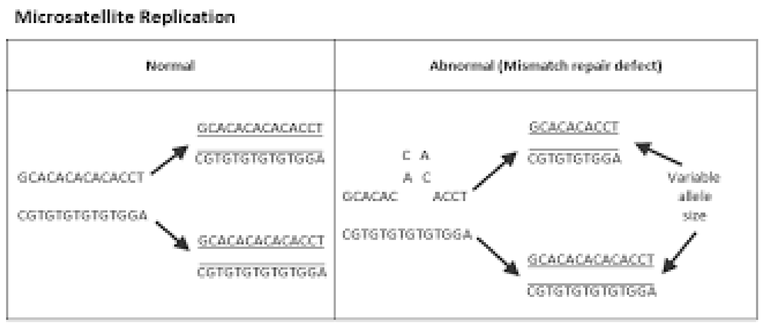


Fig. 1 (left): The DNA mismatch repair system is comprised of four definitive genes, MLH1, MSH2, MSH6, AND PMS2. These four genes search for a single-base mismatches and repeat mismatches that arise during DNA synthesis.

Fig. 2 (below): Here is a comparison between normal satellite replication and abnormal caused by a mismatch repair defect. Normal satellite replication is shown on the left where these repeated sequences are replicated from the original strand of DNA during synthesis. When there is a MMR defect, as shown on the right, we see a loss of repeat length and results in a variance of allele size during DNA synthesis.



References

1) Boland, R., Goel, A., (2010), **Microsatellite Instability in Colorectal Cancer**, *Gastroenterology.* 2010 June; 138(6): 2073-2087.e3. doi:10.1053/j.gastro.2009.12.064
2) Rishabh Sehgal, Kieran Sheahan, Patrick R. O'Connell, Ann M. Hanly, Sean T. Martin, and Desmond C. Winter, (2014), **Lynch Syndrome: An Updated Review**, *Genes* **2014**, 5, 497-507; doi: 10.3390/genes5030497

Table 2. Revised Bethesda Guidelines and Amsterdam II Criteria

Revised Bethesda Guidelines[20]
≥ 1 of the following:
• Colorectal cancer (CRC) diagnosed in a patient < 50 years old
• Presence of synchronous or metachronous HNPCC-related tumors
• CRC tumor with MSI-high histology in a patient < 60 years old
• CRC in a patient with ≥ 1 first-degree relative with an HNPCC-related tumor diagnosed before age 50
• CRC in a patient with ≥ 2 first- or second-degree relatives with an HNPCC-related tumor
Amsterdam II Criteria[79]
≥ 3 relatives with HNPCC-related tumors, *and*:
• ≥ 1 is a first-degree relative of the other 2 relatives
• ≥ 2 successive generations affected
• ≥ 1 relative diagnosed before age 50
• FAP excluded
• Tumors verified whenever possible

FAP = familial adenomatous polyposis; HNPCC = hereditary nonpolyposis colorectal cancer; MSI = microsatellite instability.

Fig. 3 (above): This comparison shows the similarities and differences in 2 popular revised screening methods used in diagnosing individuals with Lynch Syndrome, the Revised Bethesda Guidelines and Amsterdam II Criteria.

Screening for Lynch Syndrome

In the 1990's, research began on Lynch syndrome. Research groups have attempted to develop a universal screening process to identify Lynch syndrome in individuals within families with clusters of CRC. In the late 1990's, the Amsterdam criteria and Bethesda guidelines for screening and diagnosing patients was developed along with many other proposed guidelines. Despite meeting the criteria and guidelines, germline mutations are only detected in up to 80% of Lynch syndrome instances. Other studies have also shown that guidelines may miss 6%-25% of mutation carriers. Because universal screening has not developed a complete set of guidelines that fits every instance, many clinicians make a major effort to document detailed family histories in order to identify families with Lynch Syndrome. Once a patient is determined to fit this criteria, the next step is to test them for Lynch Syndrome.

Testing for Lynch Syndrome

Once a patient has been determined by a clinician that they fit the criteria to be tested for Lynch syndrome, they move on to the testing phase which gets them a few steps closer to a diagnosis. There are two types of tests used for detecting Lynch syndrome—pathology tests and genetic blood tests.

Pathology tests are tests that are performed on a tumor. When tumors are removed, most hospitals store tissue samples for many years. If Lynch syndrome is suspected, special pathology tests can be used to detect characteristics in tumors that may be caused by Lynch syndrome and can identify which gene may be responsible for Lynch syndrome in the family. There are two pathology tests used to evaluate the possibility of Lynch syndrome:

-Microsatellite instability (MSI) testing: Microsatellites are sequences of cellular DNA. In people with Lynch syndrome, tumors will show changes in the microsatellites and these changes are called microsatellite instability or MSI. Tumors with this instability are referred to as MSI-positive. Approximately 95 percent of colorectal and uterine cancers in Lynch syndrome patients are MSI-positive.

-Immunohistochemistry (IHC) testing: Immunohistochemistry testing uses special dyes to stain tissue samples in order to determine whether the proteins made by the Lynch syndrome genes are present or absent. In patients with a Lynch syndrome gene mutation, the protein will be absent in the tumor. IHC testing can help identify which of the four Lynch syndrome genes to test for.
The results of IHC or MSI tests can indicate that Lynch syndrome might be present, but they don't provide definitive information because some people can develop these gene mutations only in their cancer cells. The suspicion of Lynch syndrome will be confirmed by blood tests.

Genetic tests are tests that look for changes in your genes that indicate that you have Lynch syndrome. In order to undergo genetic testing, you will need to provide a blood sample. Genetic professionals will then perform a special laboratory analysis on your blood to look at specific gene mutations that cause Lynch syndrome.

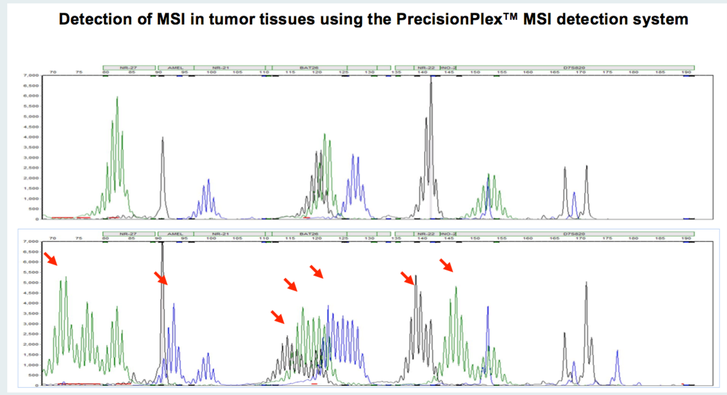
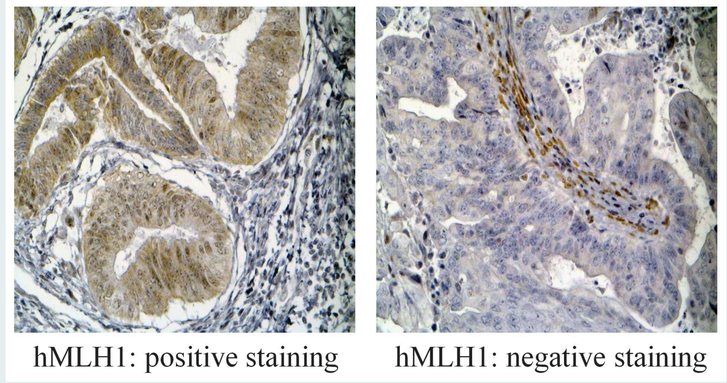


Fig. 4 (above): To diagnose Lynch Syndrome, MMR genes are analyzed by PCR to detect point mutations and small insertion-deletion mutations within DNA sequences. The figure shows a comparison of the DNA sequences in cells from healthy tissue (top) against the DNA sequence from the cells of a tumor from a biopsy (bottom). The red arrows indicate either point mutations or small insertion-deletion mutations.

Fig. 5 (below): This is an example between the positive and negative IHC staining for a biopsied tumor for the MLH1 gene, one of the four genes that makes up the MMR system. A positive stain indicates a defect in the MHL1 gene whereas a negative stain indicates no defect in the MLH1 gene.



Using MSI as a Predictor of Chemotherapy Response

Tests for MSI can be used to predict how a patient responds to adjuvant chemotherapy, although this practice is controversial because many studies are still in the clinical trial phase. In one study, MMR genes were identified in bacteria; inactivating mutations created a mutant phenotype and allowed tolerance to DNA-damaging agents. A similar phenotype has been identified in mammalian cell lines. The stable correction of MMR activity in cell lines restored the cytotoxic response to several chemotherapeutic agents. This indicated that tumors with MSI might be resistant to some chemotherapeutic regimens. Some studies have shown little to no effect on survival times of MSI associated CRC while others have been successful in their clinical trials. In one noted study by Fallik et al associated the inclusion of the topoisomerase-I inhibitor, irinotecan, in the chemotherapeutic regimen increased survival times of patients with MSI associated CRC. A similar finding has been supported in another study by Bertagnolli et al. It is possible that a combination of drugs without excessive immunotoxicity is best for patients with MSI associated CRC. Patents with MSI associated CRC should continue to be included in clinical trials until an appropriate treatment regimen is identified.

Acknowledgments

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