MICROSATELLITE INSTABILITY – CLINICAL RECOMMENDATION (ESSENSE) AND TESTING

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ABSTRACT
Hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome is an autosomal dominantly inherited cancer syndrome characterized by early onset epithelial cancers.
At present 4 genes encoding proteins are integrally involved in DNA mismatch repair (MMR): *MLH1*, *MSH2*, *MSH6* and *PMS2*. The DNA mismatch repair system works as a "spell checker", that identifies and then corrects the mismatched base pairs in the DNA. MSI is a situation in which a germline microsatellite allele has gained or lost repeated units and has thus undergone a somatic change in length. MSI can be detected in tumor cells. The criteria of diagnostics of Lynch syndrome are discussed. Lynch syndrome

Key words: microsatellite instability, Lynch syndrome

Hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome (MIM 120435) accounts for approximately 2% of all diagnosed colorectal cancers (CRCs). Lynch syndrome is an autosomal dominantly inherited cancer syndrome characterized by early onset epithelial cancers. Patients with Lynch syndrome have an increased risk of developing malignancies during their lifetime at a mean age of disease onset that is significantly lower than that observed in the general population. In addition, Lynch syndrome patients are also at risk of developing malignancies in a variety of organs that include the uterus, small bowel, stomach, ovary, bladder, pancreas and the urinary tract. At present 4 genes encoding proteins are integrally involved in DNA mismatch repair (MMR): *MLH1* (MIM 120436), *MSH2* (MIM 609309), *MSH6* (MIM 600678) *PMS2* (MIM 600259). MMR provides several genetic stabilization functions; it corrects DNA biosynthesis errors, ensures fidelity of genetic recombination and participates in the earliest steps of cell cycle checkpoint/control and apoptotic responses (1).

Endometrial cancer and defective mismatch repair. EC demonstrates 3 distinct features, that support the idea that this malignancy shares biological properties with CRC. First, the histopathological data suggest that EC evolve in a manner similar to colon cancers, starting from hyperplasias trough adenoma, referred to as a benign tumor. Second, the sequential inactivation of several genes such as Ki-ras, p53 and deleted in colorectal cancer (DCC) in CRC and EC, suggests that the same oncogenic molecular pathway may be shared, at least partly. Third, EC often occurrs in women with HNPCC, for whom the risk of developing EC is about 40% and 20% for their kindred. Indeed, patients with HNPCC and accompaning different cancers such as endometrial, ovarian, ureter, kidney, pancreas, small bowel, and stomach cancers are clasified as Lynch II Syndrome. Microsatellite instability (MSI) among ECs varies from 17% to 32% in sporadic cancers. Moreover, hypermethylation of the hMLH1 promoter region has been detected in sporadic endometrial cancer (2).

Microsatellites and genomic instability
Microsatellites are found in great number spread out over the whole DNA sequence. The most common microsatellite in humans is a dinucleotide repeat of cytosine and adenine which occurs in several thousand locations throughout the human germline (3).

MSI is a situation in which a germline microsatellite allele has gained or lost repeated units and has thus undergone a somatic change in length. This type of alteration can be detected only if many cells are affected by the same change, and it is thus an indicator of the clonal expansion typical of a neoplasm.

Mismatches of nucleotides occur when DNA polymerase inserts the wrong bases in newly synthesized DNA **(Figure 1).** Normally, when two strands of DNA replicate, nucleotide mismatches occurr, but almost all such errors are quickly corrected by a molecular proofreading mechanism. The DNA mismatch repair system works as a "spell checker", that identifies and then corrects the mismatched base pairs in the DNA. However, defects in the mismatch repair mechanisms (mutated genes) lead to MSI (3, 4, 5).
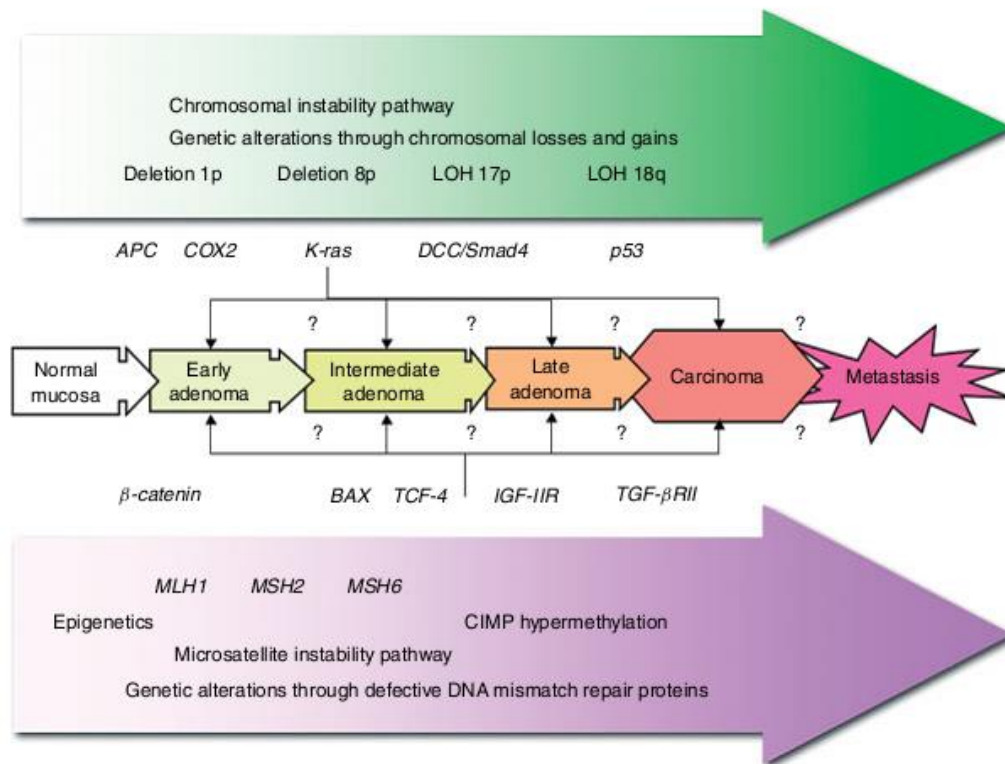

Figure 1. Genetic alterations in the different stages of colorectal carcinogenesis.

Mismatch repair genes expression and genetic instability. Mismatches might be introduced in DNA by spontaneous deamination of a cytosine to form an uracyl residue, by incorporation of modified nucleotides such as 8-hydroxy-dGTP, or as a result of DNA recombination. However, the main pool of mismatches result from misincorporation by the DNA polymerase of a normal nucleotide, which forms a mispair with the template base. These mispairs are processed by a specific DNA repair system (mismatch repair, MMR) **(Figure 2).** In humans, six MMR genes have been identified (hMSH2, hMSH3, hMSH6 [GTBP], hMLH1, hPMS2, and probably

hPMSI). It is, however, considered to process through 3 essential steps:
1) recognition of the mismatch and assembly of the repair protein complex;
2) degradation of the DNA strand containing the misincorporated nucleotide(s);
3) repair synthesis.

hMSH2, hMSH3 and hMSH6 share homology with *E. coli* MutS, and associate in 2 possible c omplexes: hMutSα (hMSH2/hMSH6), that recognize either a single mismatch and +1 insertions/deletions loops; and hMutSβ (hMSH2/hMSH3), that binds to +1 or larger insertions/deletions (2).
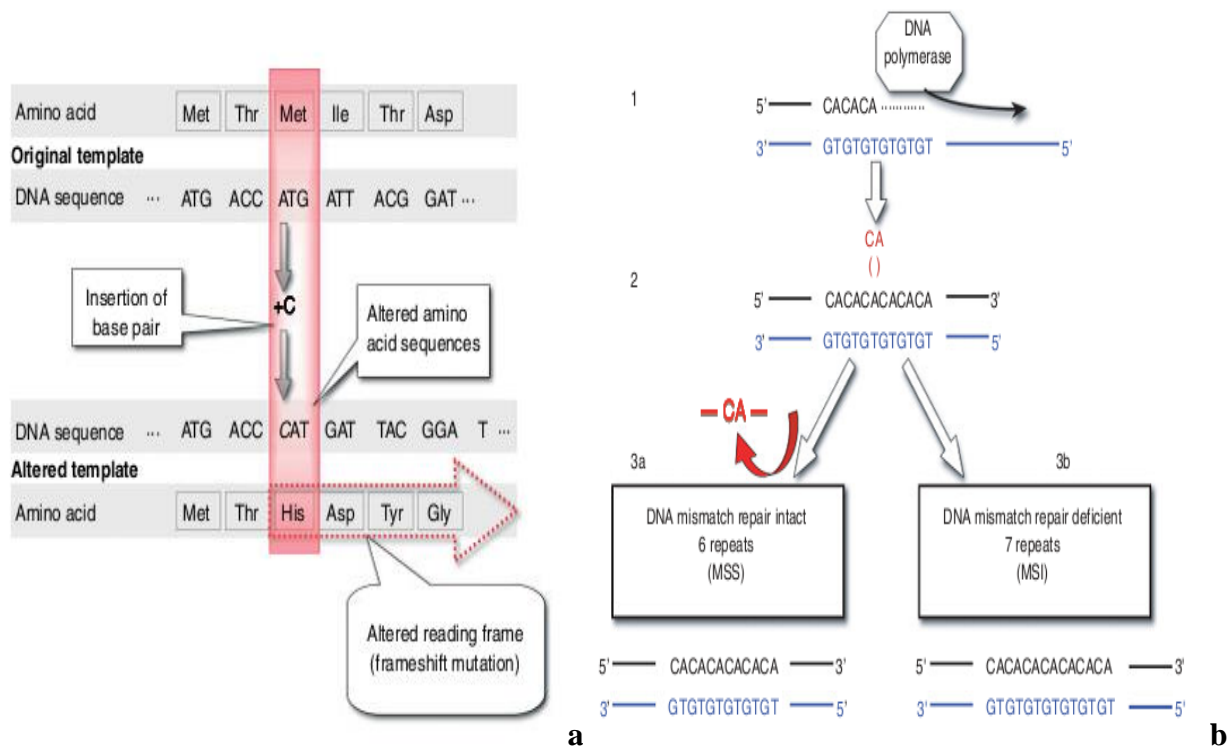
Figure 2. a. The mechanism of a frameshift mutation; **b.** Mismatched DNA repair.

Inactivation of some of these genes has a dramatic effect on genome stability and results in an accumulation of mutations as illustrated in HNPCC, which represents 6% of all colorectal cancers (6). Affected patients, who represent an elevated risk of colon tumor, as well as other cancers of the gastrointestinal and urogenital tract, inherited a defect in one of the MMR genes, essentially hMSH2, hMLH1 and hMSH6 (about 35%, 55% and 7% of all mutations

analysed, respectively), although few cases of mutations in hPMS1 and hPMS2 were reported (7).

Lynch syndrome I (hereditary sitespecific nonpolyposis colonic cancer, LSI) is characterized by inherited susceptibilty to nonpolupous CRC, developing at early age, localized in the proximal colon and is multiple primarily. Lynch syndrome II (cancer family syndrome, LSII) has the same features like LSI but is also associated with extra-colonic cancers, especially endometrial cancer (8).

Twenty years ago, in 1993 MSI was established (3) and the Amsterdam Criteria for identification of Lynch syndrome were defined (9).

Amsterdam Criteria I
- at least three(3) relatives need to have histologically verified colorectal cancer (CRC);
- one needs to be a first degree relative of the other two;
- At least two successive generations need to be affected;
- At least one of the relatives with CRC needs to have received the diagnosis before age 50;
- Familial adenomatous polyposis needs to have been ruled out.

Amsterdam Criteria II
- at least three (3) relatives need to have a cancer associated with hereditary nonpolyposis colorectal cancer (colorectal, endometrial, stomach, ovary, ureter or renal-pelvis, brain, small bowel, hepatobilliary tract, or skin[sebaceous tumors]);
- one needs to be a first degree relative of the other two;
- At least two successive generations need to be affected;
- At least one of the relatives with CRC needs to have received the diagnosis before age 50;
- Familial adenomatous polyposis should have been ruled out in any relative with CRC;
- Tumors should be verified whenever possible.

In 1996 and 2002 the National Cancer Institute in Bethesda the workshop creates **Bethesda guidelines** for identification of Lynch syndrome (10). It is recommended MSI testing, if the patient fulfils one of the following criteria:

Modified Bethesda criteria

Tumors from individuals should be tested for microsatellite instability in the following situations:

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors,* regardless of patient age.
3. Colorectal cancer with the microsatellite instability-H-like histology diagnosed in a patient who is less than 60 years of age.
4. Colorectal cancer diagnosed in a patient with one or more first-degree relatives with an HNPCC-related tumor, with one of cancers being diagnosed before the age of 50.
5. Colorectal cancer diagnosed in a patient with one or more first- or second-degree relatives with HNPCC-related tumors, regardless of patient age.

***HNPCC-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain tumors; sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome; and carcinoma of the small bowel.**

Detection of MSI
Currently MSI is detected indirectly, by demonstrating absence of expression of MMR proteins by immunohistochemistry or directly by PCR-based amplification of specific microsatellite repeats.

IHC of MMR proteins
The principle of using IHC of MMR proteins to indirectly indicate the presence of MSI is that the absence of one or more of the MMR proteins can cause MSI. Antibodies against MMR proteins are MLH1, PMS2, MSH2 and MSH6. Loss of expression of one or more of these proteins suggests deficient MMR, and indicates which gene harbors a germline mutation or has been inactivated by hypermethylation.

Eukaryotic MMR proteins form functional heterodimers. MSH2 dimerizes with either MSH6 or MSH3, and then recruits heterodimers of MLH1 and PMS2 or MLH1 and PMS1 to excise the mismatched nucleotides. Loss of expression of MSH6 or PMS2 alone is typically observed with germline mutations in each of these respective genes but with retained positive staining of corresponding MSH2 or MLH1.

PCR-based MSI testing
The principle of using PCR-based testing is to detect the presence of different lengths of specific microsatellite repeats in tumor cells comparing to normal tissues caused by

mismatches due to the absence of one or more of the MMR proteins.

- In 1997, National Cancer Institute (NCI) workshop established a reference panel of microsatellites for clinical and research testing, and aldso defined the criteria for diagnosing MSI. The core panel consists of two mononucleotide repeats (*BAT25, BAT26*) and three dinucleotide repeats (*D5S346*, *D2S123*, *D17S250*). Three categories of MSI, have been established based on the following criteria:

= MSI-high (MSI-H), indicating instability at two or more loci (or > 30% of loci if a larger panel of markers is used);

= MSI-low (MSI-L) indicating instability at one locus (or in 10% - 30% of loci in larger panels);

= MSS indicating no loci with instability (or <10% of loci in larger panels) (11).

MSI-L CRCs do not appear to differ clinically or pathologically from MSS CRCs, and generally MSI-L CRCs are categorized as group of MSS CRCs (31). MSI-L CRCs usually only show instability for dinucleotide markersq, so the mononucleotide markers are more precise.

- In 2002, NCI workshop (the revised Bethesdfa guidelines) addended guidelines with recommendations of testing additional mononucleotide markers in tumors with instability at only the dinucleotide loci, as mononucleotide markers are more reliable in the identification of MSI-H tumors (12).

Comparison of IHC and PCR-based MSI testing

The results of MMR IHC and PCR-based MSI testing have been shown to be largely concordant (97.80% concordance) (13). Studies have shown that IHC for MMR proteins MLH1, PMS2, MSH2 and MSH6 provides a rapid, cost-effective, sensitive and highly specific method for screening CRC with MSI. Reviewing the IHC results of 16 series representing 3494 cases (14). 2003 demonstrated that the following performances of IHC in assessing MSI: sensitivity, 92.4%; specificity, 99.6%; positive predictive value, 98.5%; and negative predictive value, 97.8%, which are comparable to PCR-based molecular MSI testing. Lindor et al. (15) 2002 showed that IHC in CRCs, for MLH1 and MSH2 provided a rapid, cost-effective, sensitive

(92.3%), and extremely specific (100%) method for screening for DNA MMR defects. The predictive value of normal IHC for MSS/MSI-L phenotype was 96.7%, and the predictive value of abnormal IHC was `100% for an MSI-H phenotype (15). Advantages of IHC:

= widely available in general pathology laboratories;

= tumors with MSH6 germline mutations sometimes lack MSI in PCR-based MSI testing, owing to a functional redundancy in the MMR system, but demonstrate loss of MSH6 expression by IHC (16);

= however, rare missense mutations in *MLH1* and *MSH6* gene, affect protein function other than protein translation and antigenicity. IHC will still show positive staining despite MSI (17, 16). In these cases, PCR-based MSI testing should be performed. Therefore, IHC determines subsequent PCR-based MSI testing.

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