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BIOLOGY OF NEOPLASIA

Clinical Relevance of Microsatellite Instability in Colorectal Cancer

Albert de la Chapelle and Heather Hampel

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ABSTRACT

Microsatellite instability (MSI) is a clonal change in the number of repeated DNA nucleotide units in microsatellites. It arises in tumors with deficient mismatch repair due to the inactivation of one of the four mismatch repair genes: *MSH2, MLH1, MSH6*, and *PMS2*. In order to determine the MSI status of a tumor, microdissection and polymerase chain reaction–based detection strategies are required. For practical purposes, MSI is equivalent to the loss of staining by immunohistochemistry (IHC) of one of the mismatch repair genes since both signify an abnormality in mismatch repair. Of all colorectal cancers (CRCs), 15% to 20% display MSI or abnormal IHC (often referred to as microsatellite instability [MIN] pathway). The remaining 80% to 85% of CRCs are microsatellite stable but most are characterized by chromosomal instability (CIN pathway). Almost all Lynch syndrome tumors have MSI or abnormal IHC and they account for up to one third of all MIN CRCs (3% to 5% of all CRCs). The remaining MIN tumors are sporadic as a result of somatic inactivation of the *MLH1* gene caused by methylation of its promoter. Thus, the presence of a MSI/IHC abnormality prompts further investigations to diagnose Lynch syndrome, whereas its absence excludes Lynch syndrome. We recommend screening all CRC tumors for IHC or MSI. MIN tumors have a more favorable outcome than CIN tumors, and fluorouracil-based adjuvant chemotherapy does not improve the outcome of stage II or stage III MIN tumors. More data are needed to determine how best to treat patients with stage II and stage III MIN CRCs.

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DEFINITION OF MICROSATELLITES: OCCURRENCE

Microsatellites are stretches of DNA sequence where a single nucleotide (mononucleotides) or units of two or more nucleotides (eg, di-, tri-, tetra-, or pentanucleotides) are repeated in the genome. Currently, there is no general consensus on the minimum number of repeated nucleotide units necessary to define a microsatellite, the maximum number of repeated nucleotide units allowed to still be considered a microsatellite, or the minimum number of repeated nucleotide units necessary to define a minisatellite. Repeats with units as few as three and as many as several hundred have been classified as microsatellites. Examples of typical microsatellites are presented in Table 1.

There are at least 500,000 microsatellites in the human genome. They can occur in intergenic regions as well as in genes. They are commonly located in the introns of genes but there are numerous examples of microsatellites in promoters, untranslated terminal regions, and in the coding exons themselves. When exonic microsatellites are translated into proteins, this can occur with or without repeated amino acids, depending on the particular reading frame situation. Examples of coding microsatellites in the cancer literature include a GCG trinucleotide repeat in exon 1 of the *TGFBR1* gene that encodes multiple alanines in the signal peptide, a mononucleotide $(A)_{10}$ repeat in *TGFBR2*, and a $(G)_8$ repeat in *BAX*.[1-3] In many microsatellites the monotonous repetition of the unit is occasionally broken (eg, ...CACACACAACACACACACACA).

MONOMORPHIC VERSUS POLYMORPHIC MICROSATELLITES

A monomorphic microsatellite is one in which all individuals share the same number of repeat units. The BAT26 marker (Table 1) is comprised of 26 adenines in more than 99% of ethnic Europeans, whereas alleles with different numbers of adenines at this location (eg, 15, 20, 22, 23) are seen in up to 25% of ethnic Africans, including African Americans.[4] As a result, BAT26 has been called quasimonomorphic. It might be more accurately described as monomorphic in Europeans and polymorphic in Africans.

Table 1. Examples of Microsatellites

Repeat Type	Sequence	Name and Location
Mononucleotide	AGGTAAAAAAAAAAAAAAAAAAAAAAAAAAGGGT $(A)_{26}$ shown	*BAT26*, Intron 5 of *MSH2* gene Chromosome 2
Dinucleotide	TGTACACACACACACATCGA $(CA)_6$ shown	D5S346 Chromosome 5
Tetranucleotide	ATATTCTATCTATCTATCTATCTATCTG $(TCTA)_5$ shown	D14S608 Intergenic region chromosome 14

A polymorphic microsatellite is one in which more than 1% of the population display heterozygosity for the number of repeat units. Another often used definition is that the minor allele frequency exceeds 0.01 or 1%. In fact, many microsatellites include alleles with repeat sizes that occur with heterozygosity frequencies of 50% or higher. These are highly informative markers for mapping, allelic association, and loss of heterozygosity studies.

DESCRIPTION OF MICROSATELLITE INSTABILITY

The number of repeats contained in any one particular microsatellite is, in principle, the same in every cell of the body. Microsatellite instability (MSI) is said to occur when some cells display one or two alleles with different numbers of repeats. For this to be detectable (ie, present in enough cells to be identified by the common detection methods), the aberrant cells must be clonal. Hence, MSI is typically seen in tumors, which are almost always monoclonal or oligoclonal.

As an example, if a typical (CA)n dinucleotide microsatellite is polymorphic in the population, it might display a total of five different alleles, for example, $(CA)_{22}$, $(CA)_{20}$, $(CA)_{17}$, $(CA)_{16}$, and $(CA)_9$. Any given individual will have two of these alleles. If homozygous, an individual might have two copies of the $(CA)_{20}$ allele; if heterozygous, the individual might have one copy of $(CA)_{20}$ and one of $(CA)_9$.

If MSI is present, the $(CA)_{20}$ homozygous individual's tumor might display $(CA)_{20}$ and $(CA)_{24}$. If both alleles were changed, the tumor might show $(CA)_{24}$, $(CA)_{20}$, and $(CA)_{11}$. The remaining $(CA)_{20}$ allele would most likely emanate from noncancer tissue contained in the sample, which occurs almost always. Likewise, the tumor of a heterozygous individual could display three or four different alleles with different sized repeats.

It is important to recognize that defective mismatch repair (MMR) does not by any means affect all microsatellites in the genome, but in a given tumor often affects only a proportion of them. Moreover, a low frequency of MSI can be seen in tumors without MMR deficiency.[5,6] Such occasional MSI can be tentatively viewed as background noise and disregarded.

METHODS TO DETECT MSI

By far the most common method to detect MSI is to measure the length of a polymerase chain reaction amplicon containing the entire microsatellite. This requires DNA, a pair of primers of which one is fluorescently end labeled, a sequencer, and suitable software. Alternatively, if the amplicon is sequenced, one can simply count the number of repeat units. Examples of MSI analyses in colorectal cancer (CRC) are shown in Figures 1 and 2.

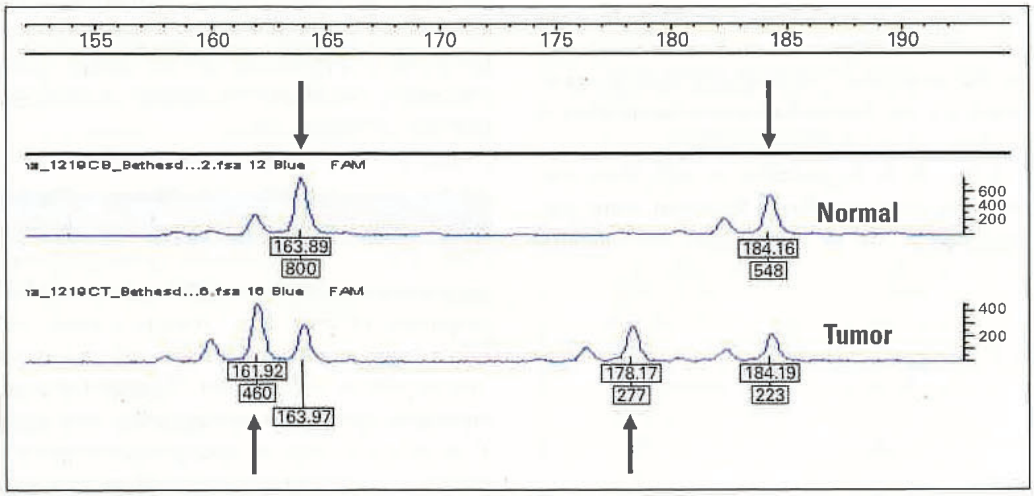


Fig 1. Tracings of polymerase chain reaction (PCR) amplicons containing microsatellite D2S123, a highly polymorphic (CA)n dinucleotide. One PCR primer was end labeled with fluorescent dye, the amplicon was run on a sequencer, and it was analyzed by the Genotyper software. Lengths of the amplicons containing the microsatellite and the strength of the signal are indicated. The upper tracing is from blood DNA displaying heterozygosity, with one allele measuring 164 bp and the other allele measuring 184 bp in length (arrows). The lower tracing is from the same patient's colorectal tumor showing two new alleles, which measure 162 bp and 178 bp in length (arrows). This means that both of the germline alleles were mutated in the tumor. The persistence of the germline alleles most likely emanates from noncancerous tissue present in the tumor specimen (eg, tumor-infiltrating lymphocytes, stroma, blood vessels).

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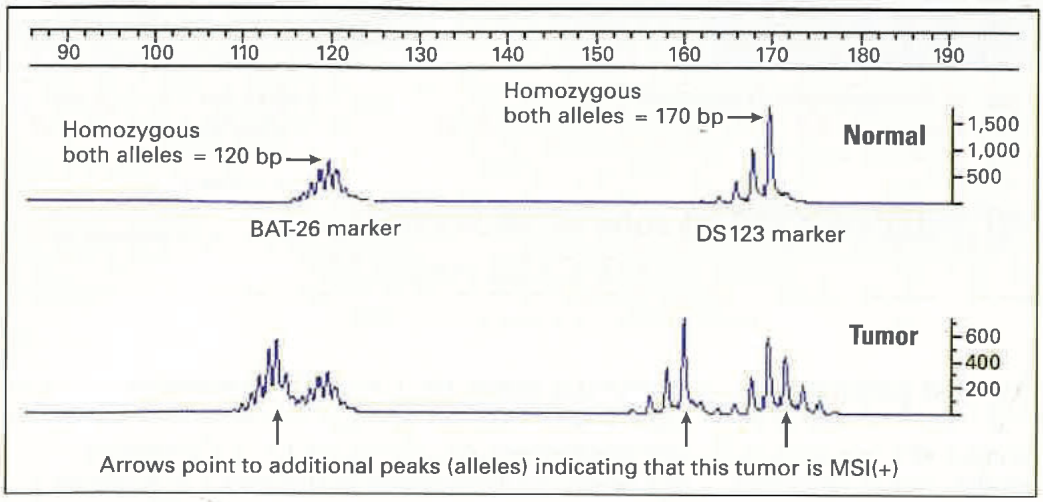


Fig 2. Microsatellite instability (MSI) determination of two microsatellites in the same, multiplexed run. The upper tracing is from blood DNA showing (A)n mononucleotide marker BAT26 at 120 bp and (CA)n dinucleotide marker D2S123 at 170 bp. The patient is homozygous for both markers. The lower tracing is from the same patient's tumor DNA. Arrows point to the new alleles in BAT26 at 114 bp and in D2S123 at 172 and 160 bp. The fact that D2S123 displays two novel alleles suggests that the tumor consists of at least two different clonal expansions. Note the "stutter" bands that occur both in the dinucleotide marker and, more strongly, in the mononucleotide marker. These are believed to be produced during the polymerase chain reaction and do not occur in vivo.

DEFINITION OF MSI: NUMBER AND CHOICE OF MARKERS

Because defective MMR does not affect all microsatellites in a given tumor, it is important to study more than one microsatellite and to study microsatellites that are frequently affected by instability. In the early days of MSI study (from 1993 onward), researchers began to choose markers based on their own empirical experience. This led to considerable variation in the number and choice of markers. A conference was held in Bethesda, MD, to discuss the issues and make suggestions to promote consistency across studies. This resulted in a recommendation that has stood the test of time.[7] A panel consisting of three dinucleotide repeats (D2S123, D5S346, D17S250) and two mononucleotide repeats (BAT26, BAT25) was proposed as a standard test for MSI. Moreover, it was proposed that MSI would be called if 40% or more of the markers tested were unstable. When using the five-marker panel, this means that MSI is called when at least two of them are positive; however, often four or all five are positive in tumors with MSI.

While most tumors show either a high degree of instability or no unstable markers, a minority of tumors display instability in fewer than 20% of the markers studied (or just one unstable marker in the case of the five-marker Bethesda panel). Although the biologic significance of this observation was not clear at the time, a classification of MSI low (MSI-L) was suggested, as presented in Table 2.

The publication of these Bethesda guidelines brought about considerable standardization of procedures all over the world, many centers applying these guidelines as such. The guidelines also suggested that researchers might wish to use other panels of markers and other definitions. Numerous reports have been published about the possible superiority and ease of studying (eg, quasimonomorphic markers that in principle obviate the need to study unaffected tissue or panels containing tetra- or pentanucleotides that may be more sensitive to MMR than mono- and dinucleotide repeats).[8-12] There is evidence that the sensitivity of MSI can be improved by including three or more mononucleotide markers; from 80% to 91% for tumors from patients with *MLH1* mutations, from 84% to 87% for tumors from patients with *MSH2* mutations, and from 55% to 77% for tumors from patients with *MSH6* mutations.[13] It is important to note that MSI is less strong in *MSH6* mutation carriers possibly because mutations in this gene seem more likely to affect mononucleotide markers than dinucleotide markers.[14] The inclusion of additional mononucleotide markers improves the detection rate for these tumors, but it is important to remember that these patients may occasionally present as microsatellite stable (MSS) or MSI-L. We are aware of the fact that some commercial laboratories routinely apply other markers, more markers, and fewer markers, but with the exception of data favoring the inclusion of three or more mononucleotide markers, we are not aware of systematic studies unequivocally favoring such panels.[15] Remarkably, it appears that the five-marker Bethesda panel, perhaps with slight modifications, remains a verifiable and reproducible means of testing for MSI.[7]

Table 2. Proposed Classification of MSI[7]

Description	Proportion of Unstable Markers	Positive Markers in the Bethesda Panel
MSI high	≥ 40%	≥ 2/5
MSI low	< 40%; at least one	1/5
Microsatellite stable	0%	0/5

NOTE. After extensive debate the Bethesda Workshop did not precisely define the distinction between MSI low and microsatellite stable, except in cases where the recommended five markers are used.
Abbreviation: MSI, microsatellite instability.

CLINICAL SIGNIFICANCE OF MSI-L

Around 15% of unselected CRC tumors are MSI high (MSI-H). The proportion of MSI-L CRC tumors is lower, often 3% to 10%.[16-19] While almost all MSI-H tumors are MMR deficient, most or all MSI-L tumors have no MMR defect.[20,21] Only few studies have directly addressed the question of whether MSI-L ever signals MMR deficiency. This can best be done by testing random tumors for MSI followed by molecular analysis of the four *MMR* genes in both MSI-H and MSI-L tumors in search of mutations or promoter methylation. In one such study of 1,566 unselected patients with CRC, 199 tumors (12.7%) were found to be MSI-H and 107 tumors (6.8%) were found to be MSI-L.[19,22] By mutational analysis of *MSH2, MLH1, MSH6,* and *PMS2,* 44 cases had deleterious germline mutations; all but one were in

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the MSI-H group. In several smaller studies, Lynch syndrome has been excluded or considered unlikely in MSI-L cases.[21] By mathematical modeling it was suggested that most low-level MSI in CRC occurs without requiring an elevated slippage rate during neoplastic development.[23] The very existence of a MSI-L group can be questioned based on the existence of a background mutation frequency.[6] Hypothesizing that if many markers are studied, many if not most CRCs would show MSI in occasional markers, Laiho et al[24] studied 90 CRC samples with 377 microsatellite markers. All cases were non–MSI-H based on stability at BAT26. As expected, as many as 71 (79%) of the cases displayed MSI at 1 to 11 loci. The authors concluded that there were no convincing differences between MSI-L and MSS.

A considerable number of researchers have used the Bethesda criteria to delineate MSI-L and asked whether MSI-L patients show distinguishing clinical or molecular features. This is complicated by the uncertain distinction between MSI-L and MSS.[25] In a study of 183 patients with stage C CRC, the 51 MSI-L patients had significantly poorer outcome compared with both the 42 MSI-H and the 90 MSS cases.[26] In a study of 657 CRCs from Korea, 30 MSI-L cases more often had poorly differentiated histology, mucinous appearance, and large tumor size in comparison with 574 MSS cases. In addition, MSI-L (n = 30) was accompanied by less frequent lymph node metastases and less advanced tumor stage than MSS.[27] In contrast, Wright et al[28] also studying stage C CRCs (n = 255) suggested that the MSI-L and MSS cases had comparable clinicopathologic features.

We conclude from these studies that the apparently contradictory results may be best explained by the lack of a conclusive cutoff between MSI-L and MSS described above. Convincing molecular or physiological differences do not appear to have been found between MSI-L and MSS tumors. Thus, after more than 15 years of research, the question about a clinically relevant distinguishable MSI-L phenotype is unanswered. It tentatively follows that if a difference exists, it is minor. For simplicity we shall hereafter refer to MSI-H simply as MSI.

CLINICAL SIGNIFICANCE OF MSI

MSI occurs in around 15% of all CRC tumors in white populations.[16-19] It arises as a result of defective MMR caused by the failure of one of the four main MMR genes, *MSH2, MLH1, MSH6,* or *PMS2*. On the cellular level, the mechanism is recessive.[29] There are two different types of *MMR* gene failure: caused by an inherited germline mutation in one allele followed by somatic inactivation of the wild-type allele in a colonic mucosa cell (these individuals have Lynch syndrome and account for 3% to 5% of all CRCs), or failure caused by somatic inactivation of both alleles. These account for 10% to 15% of all CRCs. We shall discuss separately the diagnostic and prognostic implications of MSI (Fig 3).

Diagnostic: Lynch Syndrome

Lynch syndrome is the most common inherited cause of CRC. Making the diagnosis of Lynch syndrome in a patient with CRC is important to his future management since he will have a high risk for developing second primary cancers. It is also important to his family members since many of them will have also inherited Lynch syndrome and the attendant cancer risks. It has been shown that cancers and deaths can be prevented in individuals with Lynch syndrome through the appropriate management. More than 80% of tumors from patients with Lynch syndrome display MSI. This conclusion is based on the systematic study of tumors from patients belonging to families diagnosed with Lynch syndrome based on mutational analysis.[13,30] There are two possible explanations for the absence of MSI in a small number of tumors from individuals with Lynch syndrome. The most common cause of this finding is that the MSI determination is false negative resulting from either an inadequate number of microsatellite markers or an inadequate proportion of tumor cells in the sample (Table 3). A less common cause of this finding is that the tumor is a phenocopy, that is a spontaneous (sporadic) tumor in an individual with Lynch syndrome. In practice, unless there is a strong family history of Lynch syndrome–associated cancer, patients whose tumors

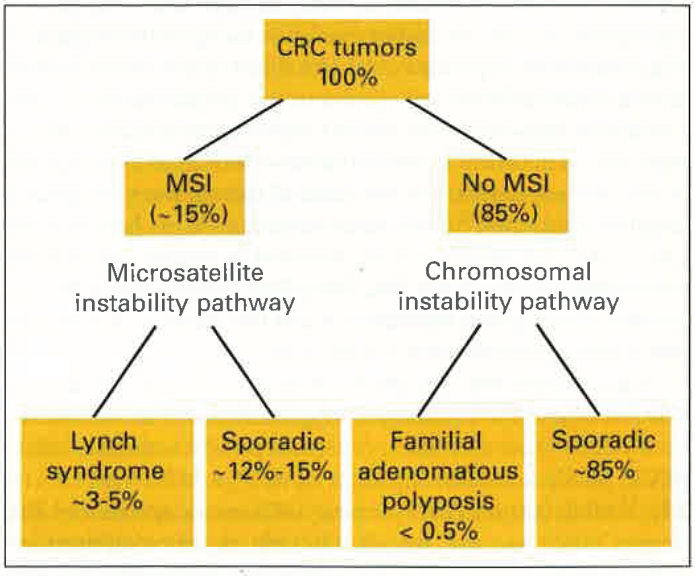


Fig 3. Schematic classification of colorectal cancers (CRCs). MSI, microsatellite instability.

Table 3. Sample Preparation for MSI Study and Sources of Uninformative Results

For MSI to be readily detectable the proportion of tumor cells in the sample used to extract DNA must be sufficient; estimates for the minimum proportion vary greatly between laboratories (10% to 50%); as can be seen in Figures 1 and 2 even in clean tracings of the relevant amplicons, "stutter bands" occur that may make interpretation difficult
To accomplish as high a proportion of tumor cells as possible, most laboratories practice some form of "microdissection"; the pathologist examines a section of the paraffin block and marks the area with most tumor cells with a pen directly on the block; the tissue is collected from this area by "coring," that is digging out a piece of tissue with the tip of a needle; alternatively, sections are cut, and the tissue outside the marked area is scraped off and discarded
One common reason for false negative MSI results is the low tumor cellularity found in mucinous tumors (which is a typical feature in CRCs with deficient mismatch repair) making the study vulnerable to false-negative results
If there are reasons to question a MSI result the best way of resolving the issue is to repeat the determination using cells from another tissue block or another microdissection of the original block or consider IHC for the four mismatch repair proteins; in cases where more than one family member might be affected, samples from as many patients as possible should be studied

Abbreviations: MSI, microsatellite instability; CRC, colorectal cancer; IHC, immunohistochemistry.

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are MSS based on the five-marker Bethesda panel or another panel of high quality, do not need further evaluation for a possible diagnosis of Lynch syndrome. In patients with CRC, if their tumors are found to be MSS this excludes both Lynch syndrome and somatic inactivation of a *MMR* gene. In those 10% to 20% of Lynch syndrome tumors that are MSS, IHC of the MMR proteins sometimes shows the absence of one or two of the proteins, and the cause of this apparent discrepancy usually is not known.[19,31] In a study of 500 cases where both MSI and IHC of all four proteins was done, there were 64 patients with MSI and 71 patients with abnormal IHC (including 56 with MSI). One case showing a discrepancy between MSI and IHC had Lynch syndrome (the tumor was MSI but IHC was normal).[19]

Increasingly, IHC is replacing MSI as a screening method for deficient MMR. The sensitivity of MSI was discussed above. IHC has been found to have a sensitivity of 83% for patients with mutations in *MSH2*, *MLH1*, or *MSH6*.[13] Specificity is 90.2% for MSI and 88.8% for IHC.[13] While both tests have similar sensitivities and specificities, IHC is more convenient and cheaper. IHC only requires equipment and expertise that are available in most pathology departments. In addition, IHC has the major advantage of pinpointing directly the *MMR* gene that is likely to be mutated. As outlined in detail by Boland et al,[32] the MMR proteins function in heterodimer pairs with MSH2 associating with MSH6 and some other MMR proteins while MLH1 associates with PMS2 and some other MMR proteins. MSH6 can only form a heterodimer with MSH2 and PMS2 can only form a heterodimer with MLH1. As a result, when MSH2 is inactivated IHC usually shows absence of both MSH2 and MSH6 protein. In contrast, when MSH6 is lost, IHC staining for MSH2 remains positive. Likewise, IHC loss of MLH1 and PMS2 signals mutational or epigenetic inactivation of MLH1, while IHC loss of PMS2 alone signals a mutation in PMS2 (Table 4).

Diagnostic: Sporadic CRC

As indicated, 60% to 80% of MMR-deficient tumors are caused by somatic events affecting both alleles, and therefore not inherited. The overwhelming majority of these are due to hypermethylation of the *MLH1* promoter.[33,34] The methylation-specific PCR method (MSP) designed by Herman et al[35] is a relatively simple way of testing for methylation. When a patient's tumor does not stain for *MLH1* by IHC or is found to have MSI, it is important to distinguish between the hereditary form (Lynch syndrome) and the sporadic form (methylation of the promoter). To exclude Lynch syndrome without resorting to mutational analysis, testing for mutations in the *BRAF* gene is advocated by some. The common somatic V600E mutation in *BRAF* is present in 40% to 60% of MSI positive tumors and in 69% of tumors with absence of MLH1 on IHC but virtually never in Lynch syndrome.[13,36] Finding the mutation allows Lynch syndrome to be excluded; however, a negative result has no predictive value. Another distinguishing test should be to directly assess MLH1 promoter methylation (eg, by MSP). While a positive MSP test is highly suggestive of a sporadic tumor, it is only approximately 80% specific so it cannot completely rule out a diagnosis of Lynch syndrome since some MLH1 methylation has been reported in up to 46% of Lynch syndrome tumors.[37] Ultimately, by mutation analysis of the *MLH1* gene the presence or absence of germline mutations signaling Lynch syndrome can be determined.

Table 4. Loss of Immunohistochemistry Staining in Relation to Affected Mismatch Repair Gene

Immunohistochemical Loss of Staining	Affected Gene			
	MSH2	*MSH6*	*MLH1*	*PMS2*
MSH2 and MSH6	+	Occasional		
MSH6		+		
MLH1 and PMS2			+	Rare
PMS2				+

MSI in Colorectal Adenomas

Large numbers of colonic adenomas are endoscopically removed during routine surveillance. Provided MMR repair deficiency is an early event in CRC tumorigenesis, the screening of adenomas for deficient MMR should be a useful strategy to detect Lynch syndrome. In an early study of 402 adenomas from 378 randomly ascertained patients, six patients had at least one MSI positive adenoma. Five of the six patients had germline Lynch syndrome mutations.[38] This study suggested that MSI testing of adenomas might indeed be a useful way of diagnosing Lynch syndrome as had been suggested by others.[39]

Several smaller studies using various strategies and finding little or no MSI in adenomas have been published.[40,41] Various arguments against using MSI to screen adenomas for Lynch syndrome have been advanced, mainly based on the finding that some adenomas in patients diagnosed with Lynch syndrome are MSI negative.[42] Nevertheless, positive findings have recently been reported.[43,44,45] MSI testing is more accurate in larger adenomas, with high-grade dysplasia, particularly tubulovillous adenomas.[38] In summary, a reasonable proportion (50% or more) of adenomas from patients with Lynch syndrome show MSI and IHC evidence of MMR deficiency is highly suggestive of Lynch syndrome. There is little doubt that systematic analyses of all adenomas would lead to the detection of otherwise undetected Lynch syndrome. Whether comprehensive screening of this type would be deemed cost-effective should be evaluated in larger prospective trials. Obviously it will not be possible to study very small adenomas at all because of lack of material.

MSI AND CRC PROGNOSIS

The literature regarding the prognosis of patients with MSI CRC was reviewed in 2005 and the data was pooled.[46] There were 32 eligible studies including a total of 7,642 CRC cases; 16.7% (1,277) of these tumors were MSI. Notably, in all but one of these studies, MSI-L tumors were grouped with the MSS tumors for the analysis. The overall survival hazard ratio (HR) associated with MSI was 0.65 (95% CI, 0.59 to 0.71) with no evidence for heterogeneity or publication bias. Patients with MSI-H CRCs had a better prognosis even when the data were restricted to clinical trial patients and those with locally advanced CRC. This study also evaluated progression-free survival (PFS) in nine data sets from eight of the published studies. The pooled HR for PFS was 0.67 (95% CI, 0.53 to 0.83), however, there was evidence of heterogeneity and the data set was too small for subset analysis.

Some recent studies have not found a better prognosis for patients with MSI CRCs.[47,48] It has been noted that the differences in prognosis may be difficult to detect in more recent patient cohorts

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given the increased benefit from chemotherapy in the MSS group of patients.[47] In addition, the MSI group of patients has a bimodal age distribution; those with MSI due to MLH1 promoter hypermethylation are older and may have other comorbidities versus those with MSI due to germline *MMR* gene mutations who are younger but less numerous and this may affect the survival data.[49]

Conversely, a meta-analysis of the association between CRC with chromosome instability (CIN) and prognosis was recently performed.[50] This analysis of 63 studies included outcome data from 10,126 patients of whom 60% had CIN + CRC tumors. The overall HR for survival associated with CIN was 1.45 (95% CI, 1.35 to 1.55; $P < .001$). In patients with stage II and stage III CRCs, the HR was also 1.45 (95% CI, 1.27 to 1.65; $P < .001$). A similar effect was seen for progression-free survival (HR, 1.71; 95% CI, 1.51 to 1.94; $P < .001$). It is not clear whether the positive prognosis associated with MSI+ tumors is independent of the negative prognosis related to CIN+ tumors. In addition, the prognosis associated with CIN-/MSS CRCs or CpG island methylator phenotype CRCs is not yet known.

MSI AND CRC TREATMENT

While it has been relatively well-established that the prognosis is better for patients with MSI-H CRC, whether or not MSI status predicts response to adjuvant chemotherapy has been more controversial. Some of the controversy stems from the fact that early studies showing a better prognosis for patients with MSI CRCs did not include a control group of nontreated patients for comparison.[51,52,53] Without untreated controls, it was not clear if the survival benefit was the result of the chemotherapy or the result of the inherent better survival for patients with MSI CRCs. On a molecular level, there is in vitro data supporting the fact that patients would need an intact MMR system to induce apoptosis of fluorouracil (FU) -modified DNA.[54,55,56,57]

The first major study indicating that patients with stage II or stage III MSI CRCs did not benefit from FU-based adjuvant therapy while patients with stage II or stage III MSS or MSI-L CRC did was Ribic et al[58] in 2003. This study indicated that the overall survival for patients with MSI tumors who received FU was worse than for those who did not (HR, 2.17; 95% CI, 0.84 to 5.55; $P = .10$).[58] In addition, when given FU, the prognosis for patients with MSI CRCs became indistinguishable from the patients with MSS or MSI-L CRCs (HR, 1.07; 95% CI, 0.62 to 1.86; $P = .80$).[58] In the 2005 pooled data analysis by Popat et al,[46] the authors also attempted to determine whether or not MSI status predicted benefit from adjuvant FU-based chemotherapy. Only two of the studies analyzed provided data that allowed for the benefit of FU-based chemotherapy to be assessed by MSI status. These studies confirmed a benefit in overall survival for patients with MSS CRCs who received FU adjuvant chemotherapy (HR, 0.72; 95% CI, 0.57 to 0.91; $P = .007$). However, the 184 patients with MSI CRCs did not appear to benefit from FU-based chemotherapy (HR, 1.24; 95% CI, 0.72 to 2.14) when compared with patients with MSI CRCs who did not receive chemotherapy. The numbers were small and not statistically significant at the time. Since then, several studies have been published including two prospective cohorts and a new meta-analysis of the data was performed in 2009.[59]

This most recent meta-analysis identified seven eligible studies which included 3,690 patients.[59] Of these patients, 14% (454) were MSI-H, 810 were stage II, 2,444 were stage III, and 1,444 received FU-based chemotherapy. The MSI-H patients did not have a significant difference in recurrence-free survival whether or not they received chemotherapy (HR, 0.96; 95% CI, 0.62 to 1.49; $P = .86$). The MSI-H patients also did not have a signification difference in overall survival whether or not they were treated with adjuvant chemotherapy (HR, 0.70; 95% CI, 0.44 to 1.09; $P = .12$). No significant heterogeneity was found between the studies. There was not enough data to perform a subset analysis for the patients with stage II and stage III CRC separately.

There was a significant interaction on survival between MSI status and chemotherapy status. This means that MSI-H CRC patients do not benefit from FU-based adjuvant chemotherapy while MSS CRC patients do benefit from FU-based chemotherapy. The HR for recurrence-free survival among the patients with MSS CRC was 0.77 (95% CI, 0.68to 0.87; $P < .001$).

It appears that MSI status is a marker of nonresponse to FU-based chemotherapy in CRC patients. Currently, all patients with stage III CRC and some patients with high-risk stage II CRC receive FU-based chemotherapy. Based on these data, it seems clear that patients with stage II MSI CRC should not receive FU-based chemotherapy. Given that most patients with stage II CRC already do not receive chemotherapy, this is a reasonable approach clinically. It also appears that patients with stage III MSI CRC should not receive FU-based chemotherapy, however, many clinicians may feel uncomfortable withholding treatment for these patients in the absence of any alternative therapy options so this approach has not been widely adopted at present. Finally, given the expense of genetic testing, most studies have not assessed whether or not the prognostic or treatment response findings differ among patients with MSI CRC tumors due to Lynch syndrome versus those due to acquired hypermethylation of the MLH1 promoter.

SIGNIFICANCE

Given the implications of MSI for the prognosis, treatment, and diagnosis of Lynch syndrome, it has been recommended by us[19,23] and others that all patients with newly diagnosed CRC should be screened for MSI either directly through MSI testing or indirectly through IHC of the four MMR proteins. This could have a significant public health impact in the reduction of CRC diagnoses and deaths due to CRC over time as at risk individuals are identified and receive appropriate surveillance.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Albert de la Chapelle, Heather Hampel
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